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Low site diversity but high diversity across sites of depauperate Crustacea and Annelida communities in groundwater of urban wells in Kraków, Poland

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Abstract

Crustaceans and annelids are key components of groundwater communities, influenced by both abiotic conditions and biotic interactions. This study assessed their diversity in urban groundwaters accessed via 91 dug/drilled wells in Kraków, southern Poland, subject to chronic anthropogenic disturbance. Invertebrates were recorded in 47 wells, with 19 species-group taxa identified from 28 wells, including eight annelid and 11 crustacean taxa (Ostracoda: 3; Copepoda: 6; Bathynellacea: 1; Amphipoda: 1). Six stygobiontic taxa were detected in 10 wells: *Trichodrilus cernosvitovi*, *Trichodrilus* sp., *Typhlocypris* cf. *eremita*, *Diacyclops languidoides*, *Bathynella natans*, and *Niphargus* cf. *tatrensis*. Due to some taxonomic uncertainties, open nomenclature was used where necessary. Species accumulation did not reach saturation, but extrapolation suggested the sampling was near-complete. Alpha diversity was low (1–3 species per well, mean = 1.4), while beta diversity was high (Whittaker index = 12.3), indicating substantial species turnover, a typical feature of groundwater ecosystems. No clear seasonal trends were observed, consistent with previous studies in Kraków. Four main community types were identified. One, dominated by *Enchytraeus* gr. *buchholzi*, may indicate degraded conditions, another, with *Bathynella natans* and *Aeolosoma* spp., suggests transitional states; a third, dominated by *Trichodrilus* spp., likely reflects relatively undisturbed groundwater; and a fourth, more heterogeneous type dominated by surface copepods, was ecologically ambiguous. Despite generally low richness and dominance by surface taxa, the presence of six stygobiontic species suggests that at least 20% of the surveyed wells retain relatively good ecological conditions.

Keywords: subterranean waters, municipal wells, stygobionts, expected species richness

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1. Introduction

Urban wells, constructed for various purposes, provide convenient access to groundwater and offer valuable opportunities for studying subterranean aquatic fauna. Urban expansion is a global phenomenon, with cities continuously increasing in area (Kraków in southern Poland, for instance, has expanded by 42% since 1950), often encompassing regions of high conservation value. In urban settings, groundwater is frequently exposed to various types of pollution, including chemical, organic, and thermal contaminants (Kim, 1992; Burri et al., 2019; Becher et al., 2022), which pose significant risks to subterranean fauna. These threats are increasingly recognized, prompting frequent assessments of water chemistry and microbial communities. Consequently, the monitoring of groundwater quality has become a standard practice in many European countries under the EU Water Framework Directive (WFD, 2000) and the Groundwater Directive (GWD, 2006).

Despite the ecological significance of urban groundwater ecosystems, the invertebrate fauna of urban wells has historically been studied in only a limited number of European cities. Some of the earliest and most extensive research was conducted in Prague, Czech Republic, by Vejdovský (1882), Sládeček and Řehačková (1952), Řehačková (1953), and Ertl (1957). Additional historical studies include those by Jaworowski (1893) in wells of Lviv (Ukraine) and Kraków, Chappuis (1924) in Basel, and Vornatscher (1972) on crustaceans in Vienna. After a long hiatus, interest in urban groundwater fauna has recently resurged, with studies by Koch et al. (2021), Becher et al. (2022, 2024), Englisch et al. (2024), and Meyer et al. (2024). Only very recently have systematic efforts to monitor groundwater fauna in cities been initiated (Johns, 2024), underscoring the growing importance of this field. Earlier publications focused primarily on species inventories, whereas recent studies have increasingly addressed the impacts of urbanization and patterns of biodiversity.

The most commonly encountered invertebrate groups in urban wells are annelids and crustaceans. In Poland, annelids have been relatively well studied in various subterranean habitats, including rural wells, and additional records have been provided in the course of other ecological research. These data were comprehensively reviewed by Dumnicka et al. (2020). A checklist of Polish groundwater crustaceans was compiled by Pociecha et al. (2021), and subsequently complemented by Karpowicz et al. (2021) and Karpowicz and Smolska (2024). The distribution of the amphipod genus *Niphargus* in Poland has been particularly well documented, primarily through the work of Skalski (e.g., 1970, 1981), with a

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synthesis provided by Dumnicka and Galas (2017) and additional records recently reported (pers. comm. A. Górny).

Despite this progress, the subterranean aquatic fauna of urban areas in Poland remains insufficiently explored. In Kraków, local geology and contamination patterns have been shown to influence species presence and distribution (Dumnicka et al., 2025).

The aim of this study was to assess the taxonomic diversity of annelid and crustacean communities inhabiting groundwater from 91 urban wells in Kraków, estimate total species richness, evaluate seasonal variation, and identify major community types. These insights may help determine whether groundwater in at least some of these wells still retains a reasonably good ecological state.

2. Material and Methods

2.1. Study area and sampling sites

Kraków is located at the junction of three tectonic units: the Silesian-Kraków Monocline, the Miechów Basin, and the Precarpathian Depression, which results in a highly complex geological and hydrogeological structure of this area (Kleczkowski et al., 2009). The studied urban wells are distributed across the city, from the historic Old Market Square to the outer residential districts (Fig. 1). Most wells are situated in Quaternary sediments composed of gravel and sand, with occasional inclusions of peat and silt. In the city center, these are overlain by a few-meter-thick layer of anthropogenic deposits. Other wells are located in Neogene (Miocene) sediments composed of various lithologies, such as marls, shales, and gypsum (gypsum-salt formations) (Rutkowski, 1989). The Vistula River flows through Kraków center and is fed by several tributaries (Fig. 1). Holocene fluvial sediments, mainly sands and gravels, occur within the river valleys.

According to the list obtained from the Kraków Water Company, approximately 350 bored/dug, driven or drilled wells were constructed in the area between the early 19th century and the 1980s. Currently, only about half of these are operational. For the present study, 83 such relatively shallow bored/dug wells were selected. Their depths range from 2.3 to 30.0 meters, with a typical water column height of 2–4 meters. These wells are primarily fed by percolating water from Quaternary aquifers; however, hydraulic connections with deeper Jurassic or Cretaceous layers have been identified in some locations (Chowaniec et al., 2007). Each well is equipped with a piston pump and is fully sealed at the surface (Fig. 2, left).

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In addition, 11 deep artesian wells are present in the area. These wells reach the Jurassic aquifer at depths of 80–100 meters and discharge groundwater to the surface without mechanical pumping due to artesian pressure (Rajchel, 1998). The emerging water is directed through pipes (Fig. 2, right). Five of these artesian wells were included in the study.

Finally, tap water samples were collected from three separate municipal water intakes. Although well water is not potable, it is occasionally used for purposes such as plant irrigation.

2.2. Sampling, sample processing and invertebrate identification

Sampling was conducted in two consecutive years, 2019 (59 wells) and 2020 (32 wells), with two sampling events per year: spring (April–June) and autumn (September–October). At each of the 91 sampling sites (see Supplementary Table 1 and Fig. 1) and during each sampling event, 100 L of groundwater were filtered using a plankton net with a mesh size of 50 µm. Invertebrates were sorted live under a stereoscopic microscope and subsequently preserved in 95% ethanol. Crustaceans and annelids were identified to the species level whenever possible, based on available literature (e.g., Meisch, 2000; Timm, 2009; Błędzki and Rybak, 2016). All taxa were analyzed in samples from both sampling years, with the exception of copepods, which were identified to species level only in the samples collected during the second year.

Water properties were measured concurrently with biological sampling. Temperature and specific conductivity (at 25°C) were recorded *in situ* using a portable multimeter (Elmetron CX-401). Ion concentrations were determined in the laboratory of the Institute of Geography and Spatial Management, Jagiellonian University, Kraków, Poland, using ion chromatography following the methods described in Dumnicka et al. (2025).

2.3. Ecological and statistical analyses

To assess whether the sampling effort was sufficient to capture the theoretical species richness of the studied area, species accumulation curves were generated using all 182 samples collected from the 91 wells. These curves illustrate how the number of detected species increases with the number of accumulated samples. Four standard non-parametric richness estimators based on abundance data (Chao 1, Jackknife 1, Bootstrap, and Michaelis-Menten) were also calculated to predict the total expected species richness. The mean (and standard deviation for Chao 1) of both observed and estimated species richness were calculated from 9,999 permutations, with samples added in random order, using PAST v. 4.10 (Hammer et al.,

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2001) and the Species-Accumulation Plot routine in PRIMER 7 software (Clarke and Gorley, 2015).

To evaluate biodiversity across the wells, several diversity metrics were computed using PAST v. 4.10 (Hammer et al., 2001) and in PRIMER 7 (Clarke and Gorley, 2015). These included: a) Alpha diversity (α) – species richness per site, b) species frequency – number of wells in which each species was present, c) Gamma diversity (γ) – total species richness across all sites, and d) Beta diversity (β) – computed as Whittaker's species turnover index ($\beta = \gamma / \alpha - 1$). For both alpha diversity and species frequency, mean values, ranges, standard deviations, and and 95% confidence intervals were estimated using the bias-corrected and accelerated (BCa) bootstrap method with 9,999 replicates. These diversity metrics were calculated separately for three datasets: 1) all species, 2) stygobiontic species, and 3) non-stygobiontic species. Differences in mean alpha diversity and mean species frequency between stygobiontic and non-stygobiontic groups were tested using one-way permutational analysis of variance (PERMANOVA) with 9,999 permutations in PRIMER 7 with the PERMANOVA+ add-on (Anderson et al., 2008).

To assess seasonal differences in the composition and structure of crustacean and annelid communities, non-metric multidimensional scaling (nMDS) was performed based on Bray-Curtis similarity matrices derived from species abundance data (with all-zero samples excluded). Seasonal differences were further tested using PERMANOVA (9,999 permutations), implemented in PRIMER 7 with the PERMANOVA+ add-on.

Finally, Principal Coordinates Analysis (PCoA) based on Bray-Curtis similarity of relative abundance (percentage) data was used to visualize differences in community structure and composition of crustaceans and annelids among wells. Pearson correlation vectors (threshold > 0.2) were overlaid on the PCoA plot to highlight taxa contributing most strongly to observed patterns. This analysis was also conducted using PRIMER 7 with the PERMANOVA+ add-on.

3. Results

3.1. Groundwater physical and chemical properties

A summary of the variation in physical and chemical water properties across the studied wells is provided in Fig. 3, with detailed data available in Dumnicka et al. (2025).

Water temperature in all wells was relatively high, ranging from 9.7 to 16.2°C, while pH values were predominantly circumneutral (6.5–8.2). Electrical conductivity ranged from 314 to

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3641 µS/cm, with a mean of 1276 µS/cm, indicating soft to moderately hard water and generally low to moderate dissolved ion concentrations. Some wells exhibited elevated concentrations of sulphates and/or chlorides (> 250 mg / L), with values varying depending on well location and local pollution sources (Dumnicka et al., 2025). Nitrogen concentrations, in the form of nitrates and ammonium, also varied widely. A considerable proportion of wells were nitrate-enriched (> 50 mg NO_3^- / L) or ammonium-enriched (> 0.5 mg NH_4^+ / L). Additionally, relatively high concentration of phosphates (> 0.1 mg PO_4^{-3} / L) and of fluorides (> 1.5 mg F^- / L) were recorded in some wells (see Dumnicka et al., 2025; Fig. 3 here).

3.2. Taxonomic richness and diversity

Crustaceans or annelids were recorded in 47 out of 91 wells (52%), but these could be identified to species level in 28 wells (31%), resulting in 19 species-group taxa (gamma diversity) belonging in Annelida and in four crustacean groups: Ostracoda, Copepoda, Bathynellacea, and Amphipoda, and representing both stygobiontic and non-stygobiontic ecological groups. Interestingly, annelids and crustaceans occurred largely allopatrically (Table 1). Other invertebrate taxa, including Microturbellaria, Nematoda, Rotifera, Collembola, and Diptera larvae, were also found but were excluded from further analysis as they were not identified to species level (see Dumnicka et al., 2025).

As expected given the rarity of many species, the species accumulation curve of the observed number of annelid and crustacean species based on all 182 samples from 91 wells did not reach an asymptote (Fig. 4), indicating incomplete sampling. Extrapolated species richness estimates ranged from 23.2 (Bootstrap) to 31.5 (Chao 1), suggesting that between 60.3% and 81.9% of the estimated total species pool of the studied area was captured (Table 2).

Annelids were found in 17 wells (Table 1), and conservatively assigned to at least eight species. Many specimens were immature and were therefore left in open nomenclature. The semi-aquatic family Enchytraeidae was considered as represented by two species: one identified as *Enchytraeus* gr. *buchholzi* and one taxon of the genus *Achaeta*. Other recorded annelids included surface-dwelling *Rhynchelmis* (Lumbriculidae), treated as a single species due to immature material, two *Aeolosoma* species-group taxa, one identified as *A. hyalinum*, and stygobiontic *Trichodrilus*, conservatively treated as two species-group taxa, one of which (*T. cernosvitovi*) was confirmed by a single mature individual.

Ostracods were recorded only in three wells and they were represented by at least three species of the family Candonidae, one stygobiontic *Typhlocypris* cf. *eremita*, and two other

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surface dwelling species left in open nomenclature since the collected juvenile specimens were not identified down to the species level (representatives of the genus *Pseudocandona*) and neither at the species nor at the genus level (representatives of the family Candonidae) (Table 1).

Copepods were the most frequently recorded group, present in 37 wells. However, only specimens from the second sampling year were identified to species level and considered in further analyses. Six species were identified, most typical of small, astatic surface waters. Only one stygobiontic species, *Diacyclops languidoides*, was recorded in a single well (Table 1).

Two additional stygobiontic crustaceans were recorded: *Bathynella natans* (Bathynellacea) and *Niphargus* cf. *tatrensis* (Amphipoda) (Table 1).

Among the 28 wells where crustaceans and/or annelids were identified to species level, the most frequent species were *Enchytraeus* gr. *buchholzi* (found in 10 wells, 36%) and *Trichodrilus* spp. (4 wells, 14%). Notably, 10 species (53%) were found in only one well (Table 1). The mean species occurrence frequency across the full dataset was 2.1 ± 2.0 wells (mean ± standard deviation SD), with no significant difference between stygobionts (2.0 ± 1.3) and non-stygobionts (2.2 ± 2.5) (PERMANOVA: F = 0.020, P = 0.953; Table 3).

Alpha diversity averaged 1.4 ± 0.7 species per well, ranging from 1 to 3. Stygobiontic species exhibited significantly lower mean alpha diversity (0.4 ± 0.7) compared to non-stygobionts (1.0 ± 0.7) (PERMANOVA: F = 4.874, P = 0.026). Despite generally low alpha diversity, beta diversity (Whittaker index) was high for the entire dataset (12.3) and for both stygobionts (13.0) and non-stygobionts (12.0), indicating high species turnover among wells (Table 3). This is consistent with the PCoA results based on Bray-Curtis similarity for each pair of wells (see below).

3.3. Seasonal variation in community structure and composition

Non-metric Multidimensional Scaling (nMDS) ordination revealed overlap between samples collected in spring and autumn (Fig. 5), suggesting no significant seasonal differences in the structure and composition of crustacean and annelid communities. This was supported by PERMANOVA (F = 1.30, P = 0.171).

3.4. Major community types

Principal Coordinate Analysis (PCoA) revealed four distinct community types, with the first two axes explaining 35.7% of the total variance (Fig. 6). Eight species/taxa exhibited

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the strongest correlations with the first two PCoA axes and contributed most to the observed pattern (Table 4). The first PCoA axis separated seven wells dominated by nearly monospecific community of *Enchytraeus* gr. *buchholzi* (with minor contribution from Candonidae ostracods), positioned on the right side of the plot, from three additional community types on the left. The lower-left quadrant grouped four wells dominated by stygobiontic *Trichodrilus* spp. and *Niphargus* cf. *tatrensis* (with minor enchytraeid presence). The upper-left quadrant contained five wells with the community type characterized by *Bathynella natans* and *Aeolosoma* spp. Between these two community types were 12 wells representing a fourth, less clearly structured community type, distinguished primarily by the surface-dwelling copepods *Acanthocyclops venustus* and *Paracyclops imminutus*, along with various copepods, ostracods, amphipods, and annelids, including stygobionts.

4. Discussion

Although invertebrates have been recorded in 74 of the 91 wells examined in this study, representing 81.3% of the total (Dumnicka et al., 2025), which is comparable to the 81.6% colonization rate observed in 201 wells in Munich (Becher et al., 2024), crustaceans and/or annelids were detected in only about half of these wells (52%). Given the absence of significant differences in the main environmental variables between wells with and without invertebrates (Dumnicka et al., 2025), the absence of crustaceans and annelids in some wells is likely attributable to other natural factors or methodological constraints. These may include the limited dispersal capacity of these taxa in groundwater, the isolation of some aquifers, low population densities leading to non-detection during sampling, or unexamined environmental factors known to influence groundwater fauna (e.g., Marmonier et al., 2023; Hotèkpo et al., 2025), particularly in urban settings. Potential anthropogenic stressors include chemical pollution, oxygen depletion, and thermal disturbances (Becher et al., 2022). Notably, groundwater temperatures in Kraków wells were approximately 3°C higher than those in rural wells located 30–40 km from the city (Dumnicka et al., 2017, 2025).

The availability of organic matter and dissolved oxygen, largely dependent on surface-subsurface water exchange, is essential for sustaining groundwater faunal communities. In urban environments, such exchange is often impeded be extensive built-up areas, impervious surfaces, and drainage infrastructure (Becher et al., 2022). Other water chemistry parameters of Kraków urban wells, such as mineralization and nutrient levels, shaped in part by the complex geology of the region (Kleczkowski et al., 2009; Gradziński and Gradziński, 2015),

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as well as local contamination, may also affect the faunal presence (Chowaniec et al., 2007; Dumnicka et al., 2025). Similar variability in groundwater chemistry has been reported from other European cities (Koch et al., 2021; Becher et al., 2022; Englisch et al., 2022; Meyer et al., 2024). Unfavourable chemical conditions, such as low oxygen or high salinity, may explain the absence of invertebrates in certain wells. Furthermore, the complete sealing of some wells and their location in “urban desert” areas likely inhibit colonization by surface-dwelling species, which can otherwise enhance local groundwater biodiversity. Given this isolation, colonization pathways for surface annelids and crustaceans remain difficult to trace – entry into groundwater likely occurs by chance, and populations may or may not persist. Surface water proximity and rainwater infiltration probably facilitate invertebrate access. In the wells studied in Kraków, a substantial proportion of the annelid and crustacean communities consisted of surface-water taxa, a pattern also observed in other Polish wells (Dumnicka et al., 2020; Karpowicz et al., 2021; Pociecha et al., 2021) and those in other countries (e.g., Vejdovský, 1882; Řehačkova, 1953; Dalmas, 1973; Bozkurt, 2023).

The frequencies of annelids (19%) and crustaceans (46%) observed in this study fall within the broad range reported for other large European and North African cities, where annelids and crustaceans have been found in 7–58% and 3–75% of wells, respectively (Vejdovský, 1882; Jaworowski, 1893; Řehačkova, 1953; Vornatscher, 1972; Koch et al., 2021; El Moustaine et al., 2022; Dumnicka et al., 2025).

We found no clear pattern of seasonal variation in the composition of crustacean and annelid communities. This aligns with previous findings in the urban wells in Kraków showing no significant differences in total or group-specific abundances, although taxa richness and the Shannon–Wiener diversity index were significantly higher in autumn than in spring (Dumnicka et al., 2025). Seasonal dynamics in invertebrate communities in urban wells remain unexplored. Bozkurt (2023), for example, found only minor seasonal differences in copepods, cladocerans and rotifers in 29 wells in Kilis, southern Turkey, although these were not statistically tested.

The gamma diversity of crustaceans and annelids in Kraków wells was relatively high, with 19 species recorded, including six stygobionts, although some uncertainty remains to whether all juvenile *Trichodrilus* specimens represent true stygobiont species. However, the species accumulation curve did not reach saturation. The low mean species frequency (2.1 wells), with no significant differences between stygobionts and non-stygobionts, suggests that additional sampling would likely yield further species. Extrapolation metrics indicate,

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however, that 4 to 13 additional species might be detected, implying that the current sampling effort approached completeness.

Alpha diversity was relatively low, ranging from one to three species per well, with an average of 1.4 ± 0.7. Notably, the alpha diversity was significantly lower for the stygobiontic species (on average < 1 species) than for surface-dwelling species (on average 1 species). Similar alpha diversity (mean 1.3, range 1–2) for crustaceans and annelids has been historically reported in urban wells in Lviv, Ukraine as well as in Kraków (Jaworowski, 1893), while more diverse communities of these invertebrate groups have also been reported in wells in other cities. For instance, El Moustaine et al. (2022) documented alpha diversity ranging from 1 to 6 species (mean 2.8) in eight well in Meknes, Morocco, although some taxa were identified only to genus or family level. Comparative analyses remain limited due to a paucity of detailed taxonomic studies in urban well fauna in Europe and the tendency of some to report only higher taxonomic groups (e.g., Cyclopoida, Amphipoda, Oligochaeta) (Koch et al., 2021).

Copepods were the most frequently encountered in Kraków wells, with six species identified, including only one stygobiont: *Diacyclops languidoides*, previously recorded in Poland from five caves, a well and an interstitial habitat (Pociecha et al., 2021). Copepods are key components of groundwater fauna, often comprising true stygobionts and taxa adapted to subsurface habitats (Galassi et al., 2009). Previous surveys of Polish groundwater habitats have reported 51 copepod species, with only four true stygobionts (Karpowicz et al., 2021; Pociecha et al., 2021; Karpowicz and Smolska, 2024). In wells 37 species have been recorded (including three stygobionts), primarily Cyclopoida (30 species) plus one Calanoida and six Harpacticoida. Most non-stygobiontic copepods in our study likely originated from surface waters in the Kraków area, as suggested by the presence of *Acanthocyclops venustus* and *A. vernalis*, known from local surface habitats (see Ślusarczyk, 2003; Kur, 2012; Pociecha and Bielańska-Grajner, 2015; Żurek, 2000 and Żurek et al., 2019 for surface water copepods in Kraków).

Despite the relatively high total number of ostracod species recorded in Polish groundwater environments (38 species, including nine stygobionts) and specifically from wells (22 species, including eight stygobionts), only three species were found in present study: one stygobiont (*Typhlocypris* cf. *eremita*) and two surface dwelling juvenile candonids. *Typhlocypris eremita* is a most common representative of the stygobiontic genus *Typhlocypris*, with representatives found mainly in the interstitial habitats of alluvial aquifers, in the hyporheic zone along rivers and in cavernicolous habitats of central and south-eastern

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Europe (Namiotko and Danielopol, 2004; Namiotko et al., 2004, 2014). In Poland *T. eremita* is a most common stygobiontic ostracod (Sywula, 1981; Pociecha et al., 2021), occasionally collected in surface waters connected to groundwater (Namiotko, 1990; Namiotko and Sywula, 1993).

Two additional crustacean stygobionts were recorded: the bathynellacean *Bathynella natans* and the amphipod *Niphargus* cf. *tatrensis*. The former has been found in one well and five interstitial sites in southern Poland (Sywula, 1989; Pociecha et al., 2021), while the latter is the most widespread Polish stygobiont amphipod, commonly found in caves, wells, and interstitial habitats (Dumnicka and Galas, 2017; Pociecha et al., 2021).

Subterranean waters in Poland are known to host 111 annelid species (Dumnicka et al., 2020), with Enchytraeidae being particularly diverse, including soil-dwelling or semi-aquatic species. In Kraków, surface water oligochaetes have been rarely studied (Szarski, 1947, Dumnicka, 2002), with *Nais elinguis* being the most common taxon, mainly in lotic habitats. Previous surveys found Enchytraeidae to be relatively rare in Polish wells, with more common Tubificidae and Naididae including several stygophiles (Dumnicka et al., 2020). Similarly, our study found relatively few annelid species. Beside semi-aquatic taxa, also occasional individuals of surface-dwelling genera *Rhynchelmis* and *Aeolosoma*, were unexpectedly observed in the wells in the Kraków centre. Stygobiontic *Trichodrilus* spp. were found in four wells, potentially indicating higher water quality.

Despite low alpha diversity, beta diversity among wells was high – a pattern characteristic of groundwater ecosystems (Hahn and Fuchs, 2009; Malard et al., 2009; Stoch and Galassi, 2010; Zagmajster et al., 2014; Hose et al., 2022). Crustaceans and annelids generally occurred allopatrically, forming four main community types. One type, dominated by *Enchytraeus* gr. *buchholzi* and observed in seven wells may reflect degraded conditions according to the German groundwater ecosystem status index (GESI) (Koch et al., 2021). Another type, dominated by *Bathynella natans* with *Aeolosoma* spp. which was observed in five studied wells may indicate transitional ecological conditions, while the third type dominated in four wells by *Trichodrilus* spp. suggest relatively unaffected conditions. The forth, more heterogeneous community, distinguished primarily by the surface copepods *Acanthocyclops venustus* and *Paracyclops inminutus* is difficult to interpret ecologically. In conclusion, although species richness and abundances of annelids and crustaceans were relatively low and dominated by surface water taxa, the occurrence of six stygobiontic species in 10 of 47 wells with crustaceans and/or annelids (or of 28 wells with species-level identifications) suggests that 1/5 to 1/3 of wells in Kraków may offer relatively good

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ecological conditions. Even in urban environments, groundwater fauna play a vital ecological role and may serve as bioindicators, reflecting environmental changes over multiple time scales. Accordingly, the development of a biomonitoring framework for subterranean waters, as proposed by Johns (2024), is warranted.

Conclusion

This study reveals that despite the relatively low alpha diversity of annelids and crustaceans in urban wells of Kraków, their beta and gamma diversity indicate a heterogeneous and partially natural subterranean ecosystem. The occurrence of stygobiontic species in a notable proportion of wells suggests that some groundwater habitats in the city retain ecological integrity. These findings highlight the importance of including urban groundwater fauna in biodiversity assessments and support the need for long-term biomonitoring systems to track environmental changes and protect subterranean ecosystems in urban areas.

Author Contributions

E. D.: conceptualization, investigation, methodology (collection, analysis, and interpretation of data), formal analysis, writing – original draft, writing – review and editing, project administration; J. G.: investigation, methodology (collection, analysis of data), writing – original draft, writing – review and editing, project administration; T. N.: conceptualization, investigation, methodology (analysis and interpretation of data), formal analysis, writing – original draft, writing – review and editing; A. P.: conceptualization, investigation, methodology (analysis, and interpretation of data), formal analysis, writing – original draft, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors confirm that data supporting the findings of this study are available in the article. Readers interested in other materials can request this information from the corresponding author.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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Table 1. Occurrences of annelids and crustaceans in the studied urban wells in Kraków, Poland. Stygobiontic species are shown in bold. Asterisks (*) indicate identifications based on samples collected only in 2020. Addresses of the numbered wells are provided in Supplementary Table S1. Complete data on water properties and the occurrences of other invertebrate groups are available in Dumnicka et al. (2025).

Taxon	Number of individuals	Wells
Annelida		
Aeolosoma hyalinum Bunke, 1967	2	3
Aeolosoma spp.	4	21, 73, 82
Achaeta sp. juv.	1	35
Enchytraeus gr. *buchholzi* Vejdovský, 1879	3	61, 68
Enchytraeidae gen. sp. juv. (mainly *Enchytraeus*)	8	18, 29, 32, 44, 55, 56, 68, 77, 82
Marionina argentea (Michaelsen, 1889)	1	3
Rhynchelmis sp. juv.	1	57
***Trichodrilus cernosvitovi* Hrabě, 1938**	1	28
***Trichodrilus* spp. juv.**	11	12, 28, 29, 55
Oligochaeta gen. spp. juv.	2	18, 35
Crustacea Ostracoda		
Pseudocandona sp. juv.	61	19, 48
***Typhlocypris* cf. *eremita* (Vejdovský, 1882)**	5	48
Candonidae gen. sp. juv.	1	18
Crustacea Copepoda		
Acanthocyclops robustus (Sars, 1863) *	6	84
Acanthocyclos venustus (Norman & Scott, 1906) *	9	69, 84, 85
Acanthocyclops vernalis (Fischer, 1853) *	108	84
Diacyclops crassicaudis (Sars, 1863) *	3	83
***Diacyclops languidoides* (Lilljeborg, 1901) ***	4	84a
Paracyclops imminutus Kiefer, 1929 *	14	86, 91
Cyclopoida copepodites *	9	69, 82, 84, 85, 86, 88, 91
Cyclopoida nauplii *	4	84, 91
Copepoda not identified to species	ca. 500	2, 3, 6, 9, 12, 21, 23, 25, 26, 27, 29, 31, 32, 35, 37, 40, 48, 49, 50, 52, 56, 57, 58, 59, 60, 61, 62
Crustacea Bathynellacea		
***Bathynella natans* Vejdovský, 1882**	38	64, 73, 75
Crustacea Amphipoda		
***Niphargus* cf. *tatrensis* Wrześniowski, 1888**	7	48, 50

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Table 2. Estimated total number of species predicted by four extrapolation estimators based on abundance data for 19 crustacean and annelid species from 182 samples collected in 91 urban wells in Kraków. SD = standard deviation.

Estimator	Expected species richness
Chao 1 ± SD	31.5 ± 17.1
Jackknife 1	28.9
Bootstrap	23.2
Michaelis-Menten	31.4

Table 3. Diversity measures for crustaceans and annelids found in the 28 out of 91 studied urban wells in Kraków, shown for the full dataset and separately for stygobiontic and non-stygobiontic species subsets. Gamma diversity = total species richness, Beta diversity = global Whittaker species turnover index, Alpha diversity = average species richness per well, Species frequency = number of wells in which species occurred. SD = standard deviation, BCa = bias-corrected and accelerated bootstrap method. Differences in mean alpha diversity and species frequency between stygobiontic and non-stygobiontic datasets were tested using one-way permutational analysis of variance PERMANOVA: F = permutation-based test statistic, P = probability based on 9,999 permutations. Statistically significant value is bolded.

	Full species dataset	Stygobiontic species subset	Non-stygobiontic species subset	Statistical difference
Gamma diversity	19	6	13	
Beta diversity	12.3	13.0	12.0	
Alpha diversity				
Range	1–3	0–2	0–3	
Mean	1.43	0.43	1.00	F = 4.874 **P = 0.026**
SD	0.69	0.69	0.67	
BCa 95% Lower limit	1.18	0.14	0.71	
BCa 95% Upper limit	1.64	0.64	1.21	
Species frequency				
Range	1–10	1–4	1–10	
Mean	2.11	2.00	2.15	F = 0.020 P = 0.953
SD	2.13	1.26	2.48	
BCa 95% Lower limit	1.26	1.00	1.08	
BCa 95% Upper limit	3.05	2.83	3.38	

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Table 4. Pearson correlation coefficients between the first two axes of Principal Coordinate Analysis (PCoA) and relative abundance of eight crustacean and annelid species showing correlation values > 0.2 with at least one axis. Stygobiontic species are shown in bold.

Species	PCoA1	PCoA2
Enchytraeus spp.	0.979	0.061
***Trichodrillus* sp. juv.**	-0.130	-0.903
Bathynella natans	-0.303	0.419
Trichodrilus cernosvitovi	-0.130	-0.412
Aeolosoma spp.	-0.090	0.339
Acanthocyclops venustus	-0.210	0.085
Paracyclops imminutus	-0.203	0.080
Candonidae juv.	0.247	0.021

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Captions of Figures

Fig. 1. Map of the study area showing the locations of urban wells sampled within the Kraków metropolitan area for the study of annelids and crustaceans (modified from Dumnicka et al. 2025). Wells where stygobionts were detected are marked as follows: B = *Bathynella natans*, D = *Diacyclops languidoides*, N = *Niphargus* cf. *tatrensis*, T = *Typhlocypris* cf. *eremita*, Tr = *Trichodrilus* spp. juv. + *T. cernosvitovi*.

Fig. 2. Photographs of representative surveyed wells: left – bored/dug well with piston pump; right – artesian deep well with tap.

Fig. 3. Boxplots summarizing the chemical and physical characteristics of groundwater in the studied urban wells in Kraków, Poland. Boxes represent the mean ± standard deviation, with whiskers indicating full range (minimum to maximum) of values.

Fig. 4. Mean cumulative species richness of the 19 studied invertebrate species (including stygobiontic and non-stygobiontic annelids, ostracods, copepods, bathynellaceans, and amphipods) plotted against the number of 182 samples from 91 urban wells in Kraków. Whiskers represent ±1 standard deviation.

Fig. 5. Non-metric Multidimensional Scaling (nMDS) ordination plot showing no significant differences in annelid and crustacean community structure between groundwater samples collected in spring (light green circles) and autumn (brown diamonds) from the studied urban wells in Kraków, Poland.

Fig. 6. Principal Coordinate Analysis (PCoA) of annelid and crustacean community composition in groundwater from the studied urban wells in Kraków, Poland. Points represent individual wells (well numbers correspond to Table 1 and Supplementary Table 1). Pearson correlation vectors with values > 0.2 on at least one axis are overlaid.

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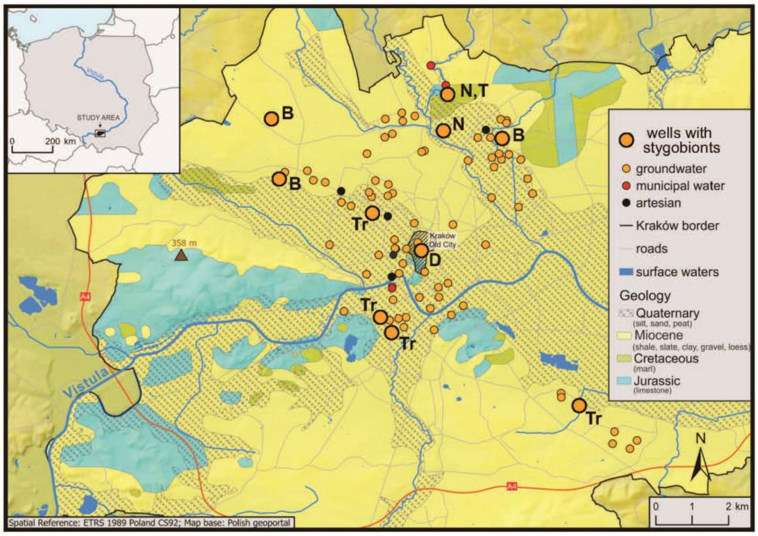

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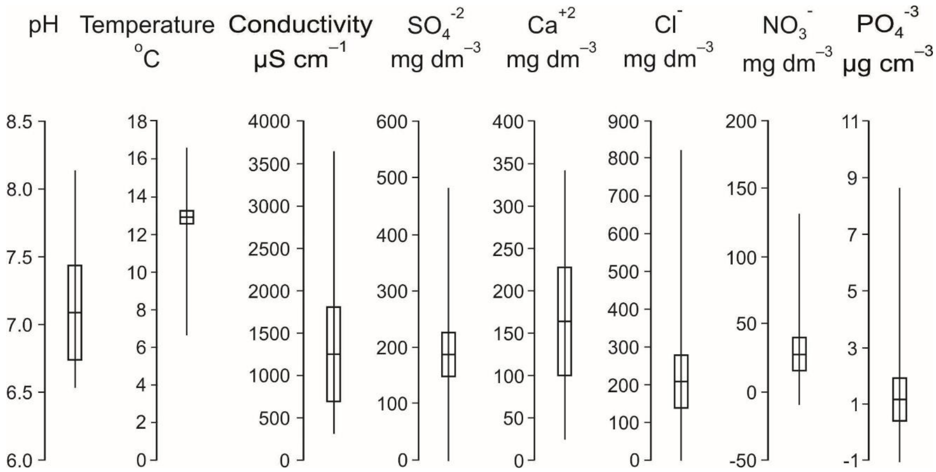

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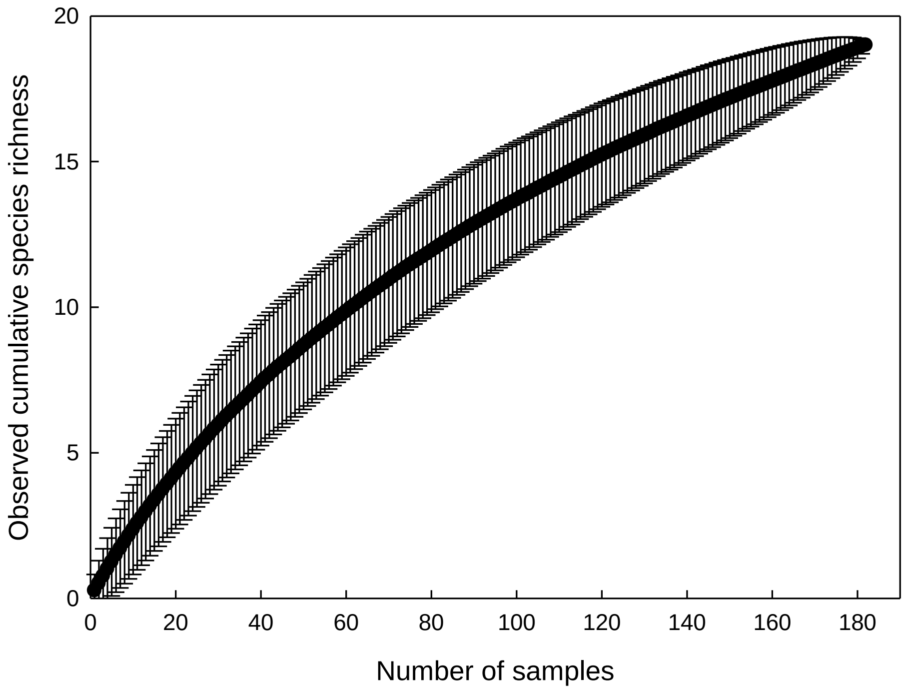

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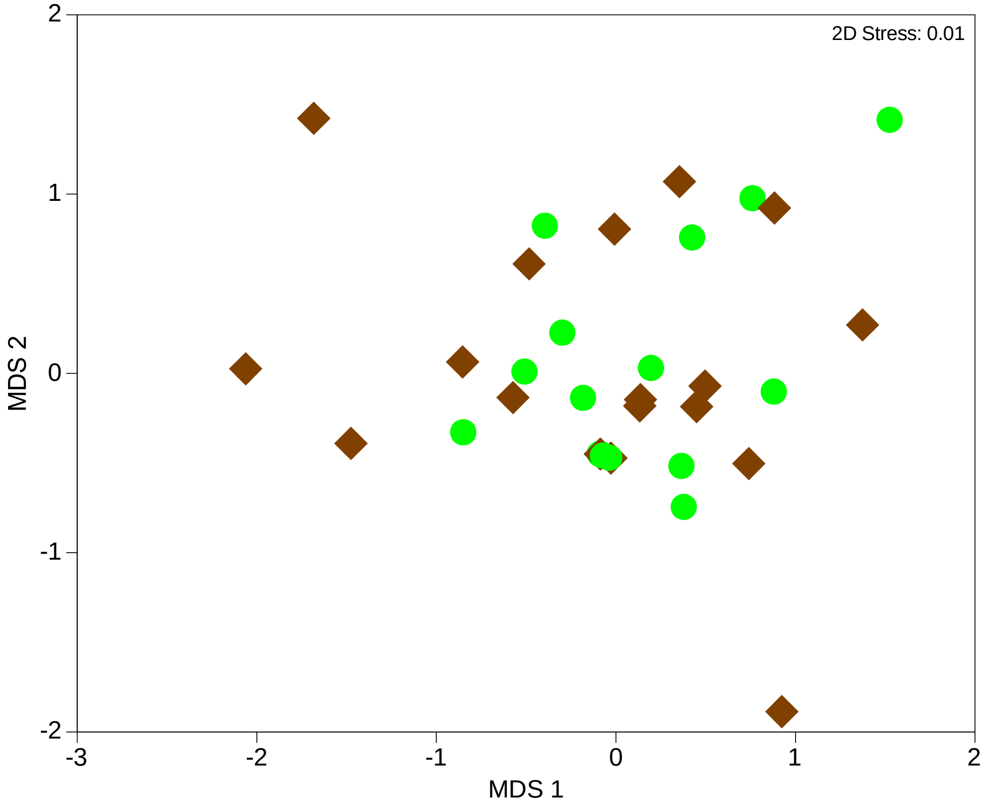

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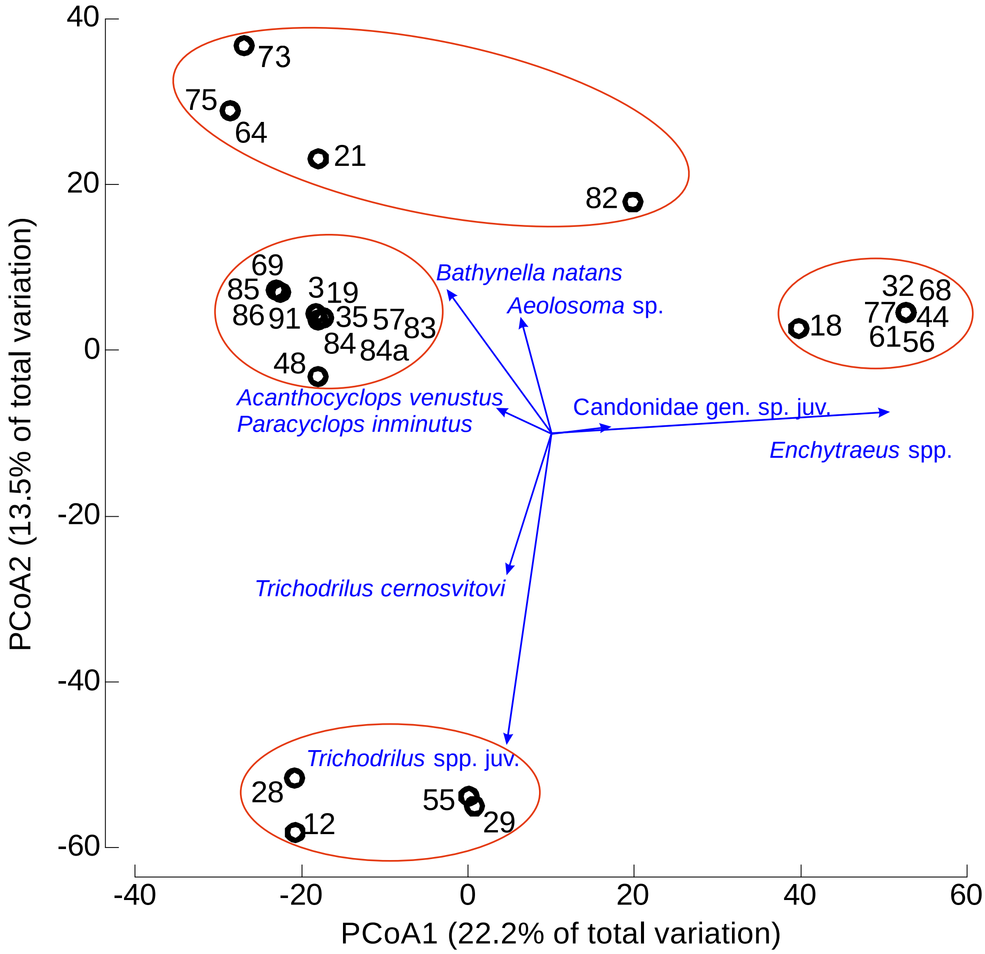

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