Integrated Analysis of Ammonium Origins in a Serbian Anoxic Alluvial Aquifer: Insight from Physicochemical, Isotopic, Microbiological data

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Abstract

The significance of examining groundwater quality is highlighted by the fact that 75% of the European Union population relies on groundwater for their water supply. In the Republic of Serbia, over 50% of the groundwater used for public water supply comes from alluvial aquifers, with 80-90% of this water originating from river water infiltration. This study investigates the origin of increased ammonium concentrations (up to 4.7 mgN/l) in the Danube alluvial aquifer near Kovin-Dubovac area, Serbia, a potential future regional water supply source surrounded by intensive agricultural production and an open coal mine. Comprehensive research involved statistical processing of physicochemical data (13 parameters from 33 sampling sites), isotopic analyses (δ^{15}N-NH_4 – 12 samples; δ^{34}S-SO_4, δ^{18}O-SO_4 in 5 samples), and microbiological tests (denitrifying, sulphate-reducing, iron-related, and heterotrophic aerobic bacteria in 15 samples). Factor analysis revealed significant positive loadings (>0.5) among indicators of autochthonous organic matter origin (Fe^{2+}, Fe_{tot}, and TOC), geological matrix components (Na, H_2S, Cl), and groundwater state parameters (pH, Eh, and NH_4^+). This multifaceted approach and the spatial concentration gradients of parameters associated within extracted principal components revealed the predominance of two NH_4^+ sources. The riparian zone is characterized by sediment organic matter mineralization, increased iron content, and natural ammonium origin (δ^{15}N-NH_4^+ from +4.82‰ to +6.93‰) accompanied by conditions suitable for DNRA and sulphate reduction. Approaching to the hinterland lower iron and total organic matter content, accompanied by increased redox values revealed the signature of fertilizers application (δ^{15}N-NH_4^+ -0.84‰ and -0.33‰), associated with denitrification influence. This multifaceted approach reduces ambiguity, providing a clearer interpretation of results when discerning the origin of nitrogen and aquifer potential for nitrogen loss or conservation in a reducing groundwater environment.

Keywords ammonium, BART tests, groundwater, nitrogen isotopes

1 Introduction

The chemical species which show the wide variety of potential forms with different abilities of movement and sorption, pose a unique research challenge. Although the perception of nitrogen origin in water and its health effects are long-standing and quite well documented, the behaviour and fate of nitrogen in anoxic groundwater is poorly understood. Typical slow groundwater flow velocities ranging from 1 m/day to 1 m/year, encourage the occurrence of comprehensive, intricate, and specific processes of dissolution, sorption, dispersion, convection, and redox reactions, within the self-purifying potential of the aquifer. There is a prominent difference between the aquifers formed in the upper and lower river flow alluvium. The oxidation of organic carbon and low oxidation state metals, sorbed on fine-grained materials within alluvial aquifers of lower river basins, induces oxygen depletion in the groundwater environment. Higher oxygen content is observed in alluvial aquifers formed in the upper parts of river basins due to the influence of high river flow energy and coarse-grained material in the riverbed (Perović and Dimkić, 2021). The concentration of oxygen remains the principal determinant influencing the progression of oxidation-reduction reactions. Factors like pH, temperature, oxidant concentration, and organic matter reactivity influence this process. Aerobic bacteria predominantly oxidize organic matter, depleting dissolved oxygen reserves. When oxygen is depleted by aerobic organisms, facultative anaerobes switch to 'utilizing' nitrates, the subsequent energetically favourable electron acceptors. With diminishing oxygen levels in the water, obligate anaerobes initiate the utilization of alternative electron acceptors. Reduction reactions occur sequentially, involving Mn^{4+} and Fe^{3+} consumption, followed by SO_4^{2-}, H^+, and CO_2, ultimately leading to methane production (Dimkić et al., 2008; Perović and Dimkić, 2021). Main dissolved nitrogen compounds in water are nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+) (Zhang et al., 2020). NH_4^+ in groundwater usually originate from anthropogenic sources such as fertilizers, manure, sewage, and wastewater discharged from treatment plants. It can indicate concurrent presence of pathogens and/or pesticides, especially if it comes from manure or sewage. NH_4^+ nitrification, under favourable conditions, depletes oxygen, producing health-hazardous NO_2^-. NH_4^+ present in aquifers, significantly influence interactions within the aquifer matrix, and might present a substantial nitrogen source in surface waters receiving groundwater discharge (Bohlke et al., 2006). In specific cases, elevated NH_4^+ levels in groundwater can also originate from rainwater and the presence of organic matter in the soil (Hinkle et al., 2007). Zhang et al., 2020 revealed that high-nitrate and high-ammonium groundwater in urbanized areas were nearly or more than twice those in non-urbanized areas (south China). Glessner and Roy, 2009 pointed out that naturally increased ammonium concentrations in groundwater in the USA originated from glacially buried paleosols of pre-Holocene age. Ammonium in the groundwater of Michigan (USA) was also of natural origin, resulting from the in-situ degradation of organic matter in a confined glacial drift aquifer (Lingle et al., 2017). Research on nitrate origins predominantly targeted aquifers beneath agricultural lands (Zhang et al., 2020). Burrow et al., 2010 showed that nitrate concentrations were highest in shallow oxic groundwater beneath agricultural surfaces of well-drained soil, while lowest in deep reduced groundwater (USA). The study of Benson et al, 2006 analysed how land use affects groundwater nitrate levels in Prince Edward Island (PEI), Canada. Rivett et al., 2007 examined nitrate levels in the major aquifers of England and Wales, and found widespread, rising nitrate concentrations and limited denitrification, significant only in confined, oxygen-depleted zones. Nitrogen fertilizer fate and nitrate transport in the Seine basin, France, was evaluated by Ledoux, et al 2007. The study of Thorburn et al., 2003 revealed that the main source of nitrate contamination in groundwater in coastal Northeastern Australia, was fertilizer

with some contamination from organic sources, underscoring the need for improved nitrogen fertilizer management. In recent years, multivariate statistical methods (Perović et al., 2024; Zhang et al., 2015; Zhang et al., 2020) and stable isotope ratio of nitrogen ($\delta^{15}N\text{-}NO_3$ and $\delta^{15}N\text{-}NH_4$) (Bohlke et al., 2006; Li et al., 2007; Lingle et al., 2017; Maeda et al., 2016; Mayer, 2005; Norman et al., 2015) have become a highly utilized tools for determining the sources of nitrogen compounds in water. Multivariate statistical techniques, such as principal component analysis (PCA), are invaluable for uncovering hidden patterns in groundwater chemistry. These methods reveal the origins and interactions among chemical components, helping to interpret factors influencing water chemistry and group sampled sites based on their similarities (Huang et al., 2013; 2018a; 2022; Hou et al., 2020; Perović et al., 2021; 2024). PCA reduces the dimensionality of hydrochemical data, grouping chemical components with similar geochemical behaviours into the same principal component (PC), as demonstrated by Hou et al., 2020. PCA reduces the dimensionality of hydrochemical data by grouping chemical components with similar geochemical behaviours into the same principal component (PC), simplifying the data (Hou et al., 2020; Zhang, 2015; 2020). PCA can distinguish between anthropogenic impacts on groundwater quality and natural background concentrations in various aquifer types (Huang et al., 2013; Huang et al., 2018). Additionally, PCA analysis can facilitate the identification of natural sources contributing to increased groundwater ammonium levels through organic nitrogen mineralization (Huang et al., 2018; Zhang et al., 2019; Hou et al., 2020). Zhang et al. (2020) employed principal component analysis (PCA) to investigate the distribution of nitrate, nitrite, and ammonium in aquifers in China, aiming to identify their sources using hydrochemical and socioeconomic data.

Since the isotopic signature of nitrogen can change due to various complex biogeochemical transformation mechanisms such as volatilization, nitrification, and denitrification, it cannot be solely relied upon for source identification (Pasten-Zapata et al., 2014). Therefore, a comprehensive understanding of the conditions under which specific processes occur is crucial, along with an examination of the associated physicochemical changes in the groundwater environment that serve to validate the conclusions drawn. Combining the study of stable nitrogen isotopes with tracking the movement patterns of conservative tracers can contribute to a more accurate determination of nitrogen sources and transformation processes in groundwater. Halides are often used for these purposes as comparative tracers (Pasten-Zapata et al., 2014). Misinterpretation in tracing the ammonium sources in groundwater, based on $\delta^{15}N\text{-}NH_4^+$ values can occur due to fractionation effects, with volatilization leading to isotopic enrichment of remaining NH_4^+. The initial $\delta^{15}N\text{-}NH_4^+$ values in pig manure fall within the range of +8‰ to +10‰, as reported by Vitòria et al. 2003. In the case of cow manure, Maeda et al. (2016) found slightly lower values, approximately +7.4‰ ± 3.8‰. It has been documented that $\delta^{15}N$ values observed in animal excrement exhibit a 3-4‰ increase in $\delta^{15}N$ values with each trophic level (Vittoria et al., 2003). Unlike manure, mineral fertilizers usually have $\delta^{15}N\text{-}NH_4^+$ values of 0‰ or less (Kendall, 1998; Nikolenko et al., 2018). Other results from diverse sources encompass $\delta^{15}N\text{-}NH_4^+$ values as follows: Kendall (1998) noted a value of -0.91‰ (±1.88‰), Choi et al. (2007) documented a value of -3.9‰ (±0.3‰), and Li et al. (2007) indicated a range spanning from +2.7‰ to +5.1‰. Various studies of $\delta^{15}N\text{-}NH_4^+$ in untreated sewage, have shown the $\delta^{15}N\text{-}NH_4^+$ in the range from +5‰ to +9‰ (Cole et al., 2006), +5.3‰ in China (Liu et al., 2006) and +4.4‰ ± 4.6 ‰ in the USA and Canada (Robertson et al., 2012). Decomposition of soil or sediment organic matter can be the source of ammonium in groundwater. The fractionation that occurs as organic matter decomposes into soil ammonia is minimal (0±1‰). If the final product of mineralization is nitrate, fractionation can be large and variable (Kendal, 1998). Per the findings of Norrman et al. (2015), NH_4^+ in Vietnamese groundwater, exhibiting an isotopic signature spanning from +2.4 to +4.1‰, was determined to have its source in the surface layer of peat. According to the research conducted by Hinkle et al. (2007), the elevated NH_4^+

levels in groundwater in the USA, coupled with a $\delta^{15}N\text{-}NH_4^+$ isotopic signature ranging from +2.5 to +3.9‰, are likely attributed to the mineralization of organic nitrogen. Furthermore, Lingle (2013) has documented a range of $\delta^{15}N\text{-}NH_4^+$ values in deep aquifers, extending from +2.5 to +3.9‰, and these values are thought to result from the mineralization of organic nitrogen. The fractionation of NH_4^+ sorption on negatively charged soils, clays and artificial cation exchange resins leaves depleted NH_4^+ in groundwater solution (Bohlke et al., 2006). On the other hand, nitrification of NH_4^+ produces products with a lower $\delta^{15}N$ value, typically leading to a significant rise in the $\delta^{15}N$ value of the remaining NH_4^+ (Bohlke et al., 2006; Nikolenko et al., 2018).

The geochemical properties and sources of SO_4^{2-} impact not only the carbon cycle via sulphuric acid weathering of carbonate rocks but also influence the nitrate cycle through sulphur oxidation and reduction (Aravena & Robertson, 1998; Humez et al., 2019; Kendall, 1998). The considerable diversity in terrestrial sulphur isotope compositions arises from the broad spectrum of valence states (+6 to -2) associated with the S element, as well as its numerous functional groups. As per the predictions of statistical thermodynamics, higher valence states of sulphur are expected to exhibit an enrichment of heavier isotopes (Krouse and Mayer, 2000). Sulphate found in rivers typically originates from atmospheric deposition, the dissolution of sulphide minerals and evaporites, leaching from inorganic fertilizers, and industrial activities. In the soil, sulphur is found within organic compounds, partially bound to carbon. It is also produced through sulphur assimilation by plants and microorganisms and, consequently, by assimilatory sulphate reduction (Mayer et al., 2005). According to Long et al., 2021 the negative values of $\delta^{34}S$ from -30 ‰ to 5 ‰ and $\delta^{18}O$ from -10 ‰ to +4 ‰ characterize sulphates derived from reduced inorganic sulphur compounds. Elevated sulphate levels and positive sulphur ($\delta^{34}S$) and oxygen ($\delta^{18}O$) isotopic compositions suggest the subsurface dissolution of evaporitic sulphate. Conversely, low sulphate concentrations and less positive or negative $\delta^{34}S$ and $\delta^{18}O$ values indicate a significant contribution of sulphate sulphur from the oxidation of sulphides in the crystalline basement rocks and sedimentary cover rocks. In the process of sulphate reduction, the decrease in sulphate concentration is accompanied by an enrichment of the remaining sulphates with the heavier isotope, where the isotopic signature of residual sulphates can reach up to +30‰ according to the studies by Bottrell et al. (2008) and Moncaster et al. (2000). Research by Hosono et al. (2014) indicates an increase in $\delta^{34}S$ of up to 20‰ during sulphate reduction. Anthropogenic sulphate exhibited $\delta^{34}S$ values of +2.5‰ (Moncaster et al., 2000).

To ascertain the origin of water pollutants, it is crucial to discern the direction of water flow, the concentration change patterns, and their isotopic signatures. Furthermore, monitoring the prevailing conditions in the groundwater environment, such as oxygen levels, redox potential, and pH, can provide insights into the groundwater potential for pollutant transformation processes, which may lead to alterations in the isotopic source signature through fractionation processes. Simultaneous monitoring of changes in pH value, concentrations of NO_3^-, SO_4^{2-} and reduced and total arsenic in groundwater can indicate the process of denitrification by pyrite oxidation and the progress of the Feammox process. Simultaneous monitoring of changes in concentration levels of B, Cl^-, and Na usually indicate anthropogenic influence (untreated sewage inflow, fertilizer/manure application), although in certain geological settings this can imply an autochthonous influence of the geological substratum (Nikolenko et al., 2018).

The complex nitrogen chemistry in reducing groundwater environment is directly related to the sulphur and iron cycling, which are interconnected through reactions presented in Table 1.

Table 1 Selected nitrogen transformation processes, required conditions and expected changes in groundwater

Process		Reaction		Essential requirements and followed changes (approximate)	Fractionation Degree (approximate)
Denitrification (DN) - an anaerobic respiration process; terminal electron acceptors nitrate and nitrite anions are reduced resulting in gases production as nitrogen(II)oxide (NO), nitrogen(I)oxide (N_2O) and diatomic gas (N_2).		$5CH_2O + 4NO_3^- \rightarrow 2N_2 + 4HCO_3^- + CO_2 + 3H_2O$		Dissolved oxygen concentration of <2.0 mg/l standard redox potential Eh<250 mV; pH raises, high TOC, nonsulfidic, low C:N; low TOC, low Fe, high C:N	Residual nitrate becomes enriched, by 5-40‰
Autotrophic denitrification by reduced iron oxidation - nitrate can be converted to nitrite involving oxidation by Fe^{2+} or Mn^{2+}, followed by a rapid transformation of nitrite to N_2 gas.		$10\ Fe^{2+} + 2\ NO_3^- + 14\ H_2O \rightarrow 10\ FeOOH + N_2 + 18H^+$		Low TOC, high Fe,	
Autotrophic denitrification coupled with (metal)sulphide oxidation.		$5HS^- + 8NO_3^- + 3H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O$ $5\ FeS_2 + 14\ NO_3^- + 4\ H^+ \rightarrow 7\ N_2 + 10\ SO_4^{2-} + 5\ Fe^{2+} + 2\ H_2O$		Sulfphidic waters, FeS, S^0 high TOC	
Dissimilatory nitrate reduction to ammonium (DNRA)	coupled with the oxidation of organic matter;	C>>N	$2H^+ + NO_3^- + 2CH_2O \rightarrow NH_4^+ + 2CO_2 + H_2O$	High TOC, nonsulphidic waters, high C:N- fermentative DNRA;	Residual nitrate enriches by approximately +10‰, generating isotopically depleted NH_4^+.
	heterotrophic with the oxidation of reduced sulphur compounds;		$4HS^- + 4NO_3^- + 4H_2O + 4H^+ \rightarrow 4SO_4^{2-} + 4NH_4^+$	Sulphidic waters, H_2S, high TOC,	
Anaerobic ammonium oxidation –(Anammox) - anaerobic process converting NH_4^+ to N_2 using NO_2^- or NO_3^- as electron acceptors, reducing total nitrogen in the system.		$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$		Low TOC, low Fe, low C:N (N>C), respiratory DN to NO_2^- followed by anammox	Moderately enriches residual NH_4^+ by 4-8‰ due to higher NH_4^+ adsorption on solid aquifer particles, which buffers and reduces the isotopic enrichment ($\delta^{15}N$) in dissolved NH_4^+
Anaerobic ammonium oxidation using Fe^{3+} as the electron acceptor (Feammox), reducing Fe^{3+} to Fe^{2+} and converting NH_4^+ to either NO_2^- or N_2.		$3Fe(OH)_3 + 5H^+ + NH_4^+ \rightarrow 3Fe^{2+} + 9H_2O + 0.5N_2$			

For adequate groundwater management in terms of quality maintenance two steps are required - identifying the pollution source and recognising potential transformation reaction that polluting substances might undergo in certain conditions. The presented research investigated the origin of increased ammonium concentrations in groundwater, hypothesizing that both agricultural and anthropogenic activities contribute to these elevated levels. The study's novelty lies in its integrated analytical approach to identifying ammonium origin and groundwater potential for nitrogen transformation, revealing the interrelation of nitrogen and sulphur transformation cycles in an anoxic alluvial aquifer through comprehensive, simultaneous physico-chemical, isotopic, and microbiological analysis. This research is particularly significant for the Kovin-Dubovac area, earmarked as a potential future regional water supply source for northern and central Banat, as designated by the Spatial Plan and Water Management Strategy of the Republic of Serbia until 2034.

2 Study area

The study area encompasses an alluvial aquifer situated beneath the Kovin-Dubovac drainage system, located in the lower part of the Danube alluvium within the Republic of Serbia (Fig. 1). It surrounds the southern region of Banat, covering an area of 172 km^2, bordered by the localities of Kovin, Dubovac, and Gaj. The northern, eastern, and western boundaries of the study area consist of a lower river terrace of the Danube and a cover of low sand dunes. The southern boundary of the considered area is formed by the Danube River, stretching from Kovin to Dubovac. The existing drainage system was established after the construction of the Đerdap I Hydroelectric Power Plant (HEPP) with the purpose of maintaining adequate groundwater levels, achieved through the operation of a network of pump stations and associated canal systems. Drainage system consists of three drainage lines from the Danube, heading inland. The aquifer is hydraulically connected to the Danube, which serves as its main recharge source. In the examined area there is the coexistence of the open coal mine, arable land, and potential regional water supply system (Majkić-Dursun et al., 2018). Kovin holds significant energetic and economic importance as a major coal basin in Serbia (Upper Miocene). Vertical mixing of both shallow and deeper groundwater provided confirmation of post-sedimentary faulting and erosion affecting coal seams in the central portion of the study area (Majkić-Dursun et al., 2018). In the central Kovin depression, post-sedimentary faulting results in an antiform formation in lignite seams, leading to partial layer erosion. Conversely, in the western and eastern regions, seams either lie at greater depths or are depleted (Jevtić and Zorić, 2012; Matenco L and Radivojević D., 2012).The investigation of groundwater quality in the Kovin-Dubovac area is highly significant, as this region has been earmarked as a potential future regional water supply source of northern and central Banat in the Republic of Serbia.

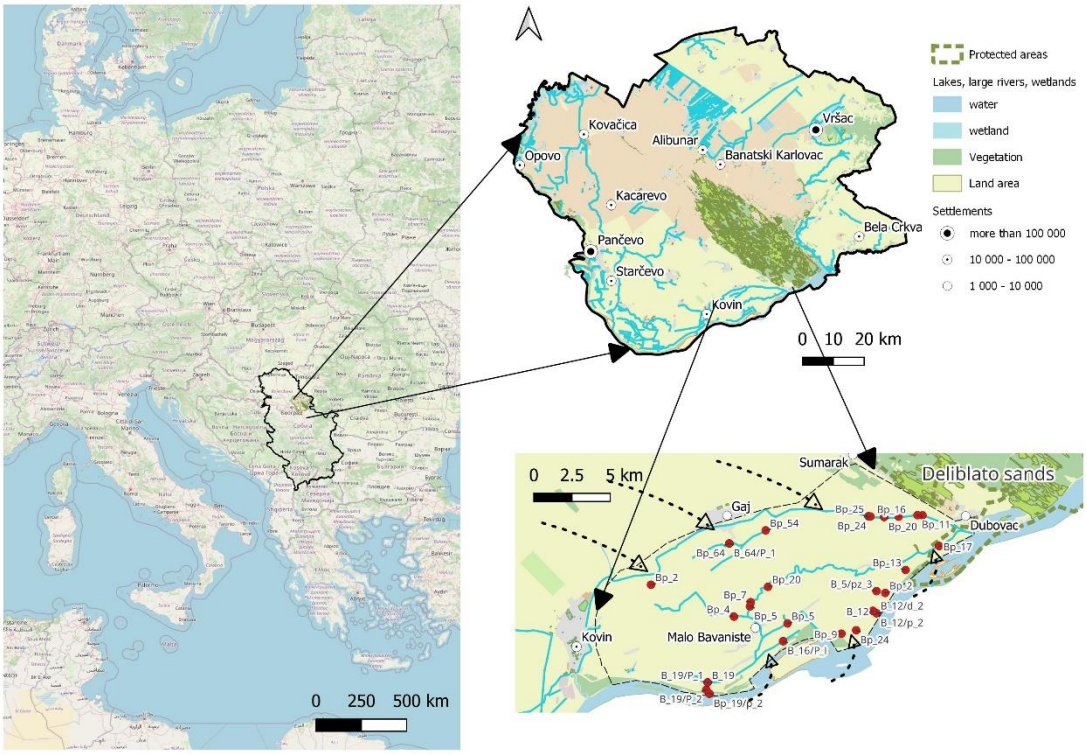

Fig. 1 Position of examined area

2.1 Land use

Intensive agricultural practices and high groundwater levels (2 to 4 meters below the surface) characterise this area (Perović et al. 2017). Most of the arable land, 66.2% is covered by maize. Sunflower and soybean are the next most cultivated cereals, with 14.5% and 14.3% of the covered area, respectively. The reported average application rate of N (in form of mineral fertilizers) is 110 kgN/ha, mostly in form of NPK (nitrogen-phosphorous-potassium), AN (ammonium-nitrate), KAN (Calcium ammonium nitrate) and UREA. The average, calculated N plant uptake rate of 108 kgN/ha, equals the mineral fertilizer N application rate, theoretically leaving no surpluses for eventual N leaching. At the examined site, no significant impact from either residential areas or industrial activities can be considered.

2.2 Hydrogeological characteristics

This segment of the Danube's alluvial plain extends approximately 2 to 7.5 kilometres in width. The lower regions primarily consist of irregularly granulated gravels and sandy gravels, containing cobbles exceeding 120 mm in size (Perović et al., 2017). Deposited above are medium-sized gravels, sandy gravels, and gravel sands. The lowermost layers are composed of more recent Quaternary deposits, featuring 'productive coal series' including siltstones, fine-grained, and medium-sized coal sand layers, and clay where coal layers are present (Perović et al., 2017). The water-bearing layer exhibits a filtration coefficient of $K=1.0x10^{-4}$ - $1.2x10^{-3}$ m/s, while the upper layers are characterized by $K=(1\text{-}5)x10^{-7}$ m/s. By measured groundwater levels (Fig. 1) the first drainage line mainly collects water from the Danube River and includes channels along the dam for the protection from the Danube direction. The second drainage line includes channels for regulating the level of groundwater in the interior of the

area. The third drainage line regulates water coming from the hinterland. The Deliblato Sands, the largest sandy terrain in Europe, part of a vast prehistoric desert, having originated from the withdrawal of the Pannonian Sea, forms the hinterland.

3 Materials and method

3.1 Physico-chemical analysis

The assessment of groundwater quality involved the sampling of 33 piezometers and wells between the years 2010 and 2018 (Fig.1). Monitored parameters obtained: O_2, Eh, pH, Fe^{2+}, Fe_{tot}, TOC, NH_4^+, NO_3^-, Cl^-, SO_4^{2-}, H_2S, Na and Ca (Table 2 and Table S1 Supplementary Material). *In-situ* parameters such as pH, redox potential, electrical conductivity, and dissolved oxygen content were assessed using a WTW 197i multiparameter probe (Germany). This instrument was meticulously calibrated on-site and subsequently lowered to the screen level for measurements. Water samples were extracted using a bailer or submersible pump. Glass bottles were immediately screw with no head space. Samples were collected in quantity that enabled performing analysis in triplicates. N-component (NO_2^-, NO_3^- and NH_4^+) was quantified by spectrophotometric method. Turbidimetric method was applied for SO_4^{2-} detection (WTW TURB 430IR, Germany) and volumetric method was utilized for Cl^- and H_2S determination. Fe^{2+} was analysed by spectrophotometer from samples where previously 4 ml of 1 M sodium acetate and 2.5 ml of 0.1% phenantroline (1,10 – phenanthroline monohydrate) was added. Total Fe, Na and Ca were quantified by Inductively Coupled Plasma (Spectra Genesis ICP instrument, Germany). TOC was analysed by Hiper TOC analyser Thermo Scientific (United Kingdom). Chemical analyses were conducted following procedures outlined in APHA, 2005. The quality assurance and quality control were applied in sampling and analysis procedures, according to ISO 17025 general requirements for the competence of testing and calibration laboratories. Samples were collected in triplicates.

3.2 Microbiological analysis

In addition to the physico-chemical parameters obtained through monitoring from 2010 to 2018, 15 sampling sites underwent extensive microbiological analyses between 2010 and 2013 (Fig. 3 and Fig. 4) using biological activity reaction tests (BART tests). Microbiological analyses were conducted before isotopic composition research. Together with physicochemical indicators, they were used to evaluate the potential contributions of different sources, providing possible explanations for the pathways of transformation and origin of nitrogen components in the Danube alluvium. The construction of biological activity reaction tests (BART tests) is based on the column principles of Winogradsky (Cullimore 2007, 2010; Dworkin 2012). The gradient of conditions in the biotests, including oxygen, redox potential, and specially designed nutrient mediums for each type, enables the recovery, growth, and activity of various groups of microorganisms found in groundwater (Cullimore 2007, 2010). These biotests visualize biochemical reactions by forming noticeable biofilms, gases, precipitates, or coloured products. They also allow the simultaneous cultivation and monitoring of aerobic, facultatively aerobic, and anaerobic microorganisms, providing qualitative and semi-quantitative results. The time lag (TL) of the initial reaction indicates biochemical activity or bacterial aggressiveness. QuicPop software 1.3 (Cullimore 2010) was used to quantify the correlation between the day, reaction type, and number of active cells. By defining environmental bacterial groups through average values of triplicate tests, we assessed the microbiological groundwater status and identified the potential for nitrogen transformation processes. To explore the intricate interplay between the

nitrogen cycle in groundwater and the transformative cycles of organic matter, iron, and sulphur, a series of microbiological tests were conducted, encompassing the Sulphate reducing (SRB BART), Denitrifying (DN BART), Iron related bacteria (IRB BART), and Heterotrophic aerobic bacteria (HAB BART). Presented bacteria were quantified and qualified using the Nitrate-peptone medium (DN BART), Postgate Medium "B" (SRB BART), Winogradsky medium (IRB BART), and Carbohydrate peptone medium with methylene blue (HAB BART). The DN BART biotest identifies bacteria that reduce nitrates to gaseous nitrogen. A positive result is indicated by foam from N_2 gas bubbles, forming around the testers ball within three to five days. Absence of foam, regardless of turbidity, indicates a negative result for denitrifying bacteria. The SRB BART test detects sulfate-reducing bacteria by their production of H_2S, which reacts with iron to form black FeS precipitate. A positive result shows black slime at the bottom or surface. The IRB cultivation medium, uses iron ammonium citrate as the primary iron source. Upon adding the sample, the crystallized substrate at the vial's base dissolves, triggering complex reactions influenced by the sample's chemical and biological composition and the redox and nutrient gradients in the BART test. The dominant part of biodegradation under aerobic conditions is due to the activity of heterotrophic aerobic bacteria (HAB). A unique characteristic of the HAB BART test is its use of a carbohydrate-peptone medium with methylene blue, acting as an indicator of respiratory activity. Methylene blue serves as a substitute for oxygen, being reduced from blue to colourless by electrons generated during microbial oxidation of nutrients. This dye, a redox indicator traditionally used to gauge bacterial activity, evaluates microbial load in a liquid medium, with faster colour disappearance indicating higher microbial activity due to increased oxygen demand and decreased oxygen concentration (Nandy and Venkatesh 2010). Given that applied cultivation techniques (BART analyses) detect only a small fraction of the microbial population that is culturable and the metabolic activity of consortia, future research should employ more sensitive metagenomic analyses (e.g., Next-Generation Sequencing - NGS) and molecular identification based on 16S rRNA/rDNA genes, as well as biochemical (enzymatic) analyses, for a more comprehensive understanding of total biodiversity and metabolic capabilities. Number and distribution of sampling sites were conditioned by financial constraints and the requirement that samples represent all three drainage lines, respectively. Specialized software BART-SOFTv6_1_0_0_8 was employed to calculate the correlation between the time of day, the type of reaction, and the quantity of active cells based on the BART test results.

3.3 Isotopic composition

In addition to the regular parameters obtained through monitoring from 2010-2018, in 2018, 12 samples were collected for $\delta^{15}N\text{-}NH_4^+$ stable isotope analysis and 5 samples for $\delta^{18}O\text{-}SO_4$ and $\delta^{34}S\text{-}SO_4$ analysis. Samples were selected to ensure representation from all three drainage lines, taking into account financial constraints that limited the number of samples available for analysis. $\delta^{15}N$ was also determined in soil sample from the first drainage line, river proximity, (the vicinity of Bp-12 well). Due to the consistently low average nitrate concentrations in groundwater sampling sites (below 1 mgN/l as shown in Table 2 and Table 5), attributed to the reducing environment, nitrate isotopes could not be determined in groundwater samples. The sole sample for nitrate isotopes ($\delta^{15}N\text{-}NO_3$ and $\delta^{18}O\text{-}NO_3$) was collected from surface water at the Kovinski Most profile on the Danube in 2018. The stabile isotopes signatures were quantified applying elemental analyzer (EA) and continuous-flow

isotope-ratio mass spectrometer (CF-IRMS).Nitrate isotope analysis was carried out by the denitrifier method (Sigman et al., 2001; Casciotti et al., 2002), whereby nitrate was converted to N_2O and an isotope ratio mass spectrometer (GasbenchII/delta V plus, Thermo Fisher Scientific®, USA) is used for simultaneous determination of $\delta^{15}N–NO_3$ and $\delta^{18}O–NO_3$.

Samples for N analysis of ammonium was prepared based on a modified Kjeldahl method that is suitable for sample volumes smaller than 50 mL. The produced ammonium sulfate was homogenized and weighed for combustion in an elemental analyzer (vario isotope cube, Elementar) connected to an isotope ratio mass spectrometer (IsoPrime100). Any dissolved sulfate was recovered by precipitation as $BaSO_4$. Sulphur isotopic compositions were measured after the conversion of $BaSO_4$ to SO_2, using an elemental analyser (continuous flow flash combustion technique) coupled with an isotope-ratio mass spectrometer (delta S, ThermoFinnigan, Bremen, Germany). Nitrogen, sulfur and oxygen isotope results are reported in delta notation ($\delta^{15}N$, $\delta^{34}S$, $\delta^{18}O$) as part per thousand (‰) deviation relative to the standards AIR for nitrogen, VCDT for sulfur and VSMOW for oxygen (equation (1)), where R is the ratio of the heavy to light isotopes (e.g. $^{15}N/^{14}N$; $^{34}S/^{32}S$; $^{18}O/^{16}O$).

$$\delta_X\ (‰) = [(R_{sample} - R_{standard}) / R_{standard}] \times 1000 \quad (1)$$

The standard deviation of the nitrogen and sulfur isotope analysis is ±0.4‰. Oxygen isotope analysis of nitrate and sulfate is conducted with a precision of better than ±0.8‰.

3.4 Multivariate statistical techniques

PCA proved invaluable in identifying common origins and interactions among chemical components in groundwater (Hou et al., 2020; Huang et al., 2013; Huang et al., 2018; Huang et al., 2020; Huang et al., 2020a; Huang et al., 2022; Perović 2021; Perović 2024; Zhang et al., 2019; 2020;). Parameters in the same PC with positive loadings mean a same origin or similar geochemical behaviour (Zhang et al., 2019). In this study, PCA was employed to explore the relationships between groundwater NH_4^+ and parameters indicative of its origin or transformation processes. The physico-chemical parameters which can indicate the occurrence or potential for nitrogen transformation processes in groundwater: Eh, pH, Fe^{2+}, Fe_{tot}, TOC, NH_4^+, Cl^-, $SO4^{2-}$, H_2S, Ca and Na, were processed by PCA. Only principal components (PCs) with eigenvalues greater than 1 were retained for analysis, and the Oblimin Rotation with Kaiser Normalization were adopted (Huang et al., 2022). Factor loading indicated the importance, the share of participation of an individual variable in defining the factor, and essentially represented the interrelationship between each variable and the factor. Values of factor loadings over ±0.50 are considered significant, while values over ±0.70 are considered an indication of a firmly formed structure. Based on strong positive loadings identified through PCA, coloured maps are used to visually illustrate spatial concentration gradients of parameters associated within PCs highlighting clear interdependencies between sampling sites. The data was statistically processed using the IBM SPSS Statistics v. 23. To facilitate a more in-depth analysis of the principal components, the open-source software QGIS Desktop 3.32.1 Lima was utilized, and Thin Plate Spline interpolation techniques were implemented to visualise extracted PCs.

4 Results and discussion

4.1 Physico-chemical results

The prevalence of reductive conditions was confirmed by consistently low concentrations of the most energetically favourable electron acceptors O_2 and NO_3^-; Oxygen content ranged from <0.1 mg/l to 2.35 mg/l, with three-quarters of the samples exhibiting dissolved oxygen concentrations at or below 0.13 mg/l. Nitrate levels vary from <0.05 mgN/l to 2.62 mgN/l. The lowest recorded standard redox potential (Eh) is -177.5 mV, with 75% of samples measuring below 115.5 mV. Groundwater quality results are presented in Table 2, while average values of selected groundwater quality parameters by sampling site (2010-2018) can be found in the Table S1 in Supplementary material.

Concentrations of reduced forms, potential electron donors (Fe^{2+}, NH_4^+, and TOC) are elevated, occasionally accompanied by the appearance of sulphides (maximum value 4.17 mg/l). Ammonium concentration levels exhibit a predominant increase in objects situated along the first drainage line which dominantly recharges from the Danube (Bp-2, B-12, Bp-13, Bp-24, and connected piezometers) (Fig 2.). Intermittent increase of NH_4^+ level was observed in Bp-7z, Bp-16z, B-16 and Bp-16/P1, from the inland areas. Deeper into the inland area, the wells accumulate water infiltrated from the terrain surface and the Deliblato Sands region. According to official data from Serbian Environmental Protection Agency monitoring, average nitrate and ammonium levels in Danube are 0.81 mgN/l and 0.17 mgN/l respectively (profile Smederevo, 2015-2019). In the groundwater from the first drainage line, the ratio of these ions is reversed, with a higher proportion of ammonium ions compared to nitrate ions (average NH_4^+ of 1.32 mgN/l and NO_3^- of 0.15 mgN/l).

Table 2 Statistical overview of selected groundwater quality parameters in Kovin-Dubovac from 2010 to 2018

Parameter	Unit	No. Data	Min.	Avg.	Max.	Median	Std. Deviation	Percentiles			Measurement range
								25	50	75	
O_2	mg/l	140	<0.1	0.12	2.35	<0.1	0.27	<0.1	<0.1	0.13	0.1-20
Eh	mV	134	-177.5	97.95	336.6	87.71	68.92	64.04	87.71	115.5	-1999 - +1999
pH	/	142	6.68	7.32	8.28	7.3	0.34	7.1	7.3	7.55	2-12
ϰ	µS/cm	147	367	625.03	1191	551	208.34	478	551	722	0.1-10000
NH_4^+	mgN/l	142	0.01	0.83	4.7	0.62	0.77	0.24	0.62	1.36	≥ 0.01
NO_3^-	mgN/l	143	<0.05	0.22	2.62	0.1	0.34	0.05	0.1	0.22	≥ 0.05
Cl^-	mg/l	147	<5	21.41	128.2	18.1	17.95	16.3	18.1	20.5	≥ 5
SO_4^{2-}	mg/l	145	<2.00	20.05	157	6.8	31.02	2.01	6.8	21.12	2-40
H_2S	mg/l	109	<0.02	0.1	4.17	0.01	0.47	<0.02	<0.02	0.03	> 0.02
TOC	mg/l	143	<1.0	2.38	9.20	2.22	1.19	1.54	2.22	2.90	≥ 0.5
Na	mg/l	142	3.71	22.01	96.41	19.43	13.68	14.2	19.43	25.29	≥ 0.5
Ca	mg/l	143	21.31	79.99	256.12	72.13	38.34	57.92	72.13	90.67	≥ 0.5

4.2 Statistical data processing

The four extracted factors explain 68.93% of total variance. The first, PC1, explains 24.34 % and shows the strong positive loadings between Na, Cl and H_2S (Table 3, Fig. 2, PC1). Scree plot is presented within Supplementary material. In the context of anoxic groundwater environment, this factor can be regarded as an indicator of an autochthonous-geological influence on groundwater quality. The addition to this interpretation is reflected within factor PC3, which demonstrated a strong negative loading between SO_4^{2-} and Ca (Fig. 2). The dissolution of calcium-sulphate minerals in anoxic groundwater leads to reduction of sulphates followed by Ca sorption onto matrix particles. Second extracted factor (PC2) explains 20.12% of variance and shows strong positive loadings between Fe^{2+}, Fe_{tot} and TOC indicating mutual natural origin. The presumed common source of organic carbon and ferrous ions is associated with alluvial aquifers in the lower parts of the river basin, where an abundance of potential electron donors, such as organic carbon and Fe^{2+}, leads to the prevalence of anoxic alluvial conditions, as indicated by Dimkić et al., 2008 and Perović and Dimkić, 2021. The oxidation of organic matter leads to a decrease in pH and Eh, resulting in the creation of anoxic conditions in which Fe^{3+} is transformed into its reduced form, the mobile Fe^{2+} ion. The observed ammonium cation fraction in the groundwater in the river proximity can be attributed to the oxidation of organic matter. The fourth extracted factor (PC4) explains 11.61% of the variance and reveals that pH and Eh increase is followed by corresponding decrease in ammonium concentration. Fig 2 PC4, shows that the areas of increased pH and Eh (hinterland) likely have a lower organic matter content, comparing to the river proximity (first drainage line). Observing the terrain from the river towards the hinterland, approaching to Deliblato sands, sand cover layer becomes thicker, decreasing the ratio of organic matter, and increasing redox conditions. PC4 indicates that approaching the hinterland conditions favourable for mineralization and eventually DNRA in a river proximity, give way to conditions suitable for denitrification and sporadic nitrification (with an increase in pH and Eh). The concentration pattern of H_2S distribution, which acts as a denitrification inhibitor (Burgin and Hamilton, 2007), is consistent with these assumptions (PC1 vs PC4).

Table 3 Principal component loadings (based on data from Perović and Dimkić, 2021)

	Factor loadings			
	PC1	PC2	PC3	PC4
Eh				0.853
pH				0.584
Fe^{2+}		0.768		
Fe_{tot}		0.848		
TOC		0.657		
NH_4^+				-0.574
Cl^-	0.709			
SO_4^{2-}			-0.864	
H_2S	0.942			
Ca			-0.913	
Na	0.792			

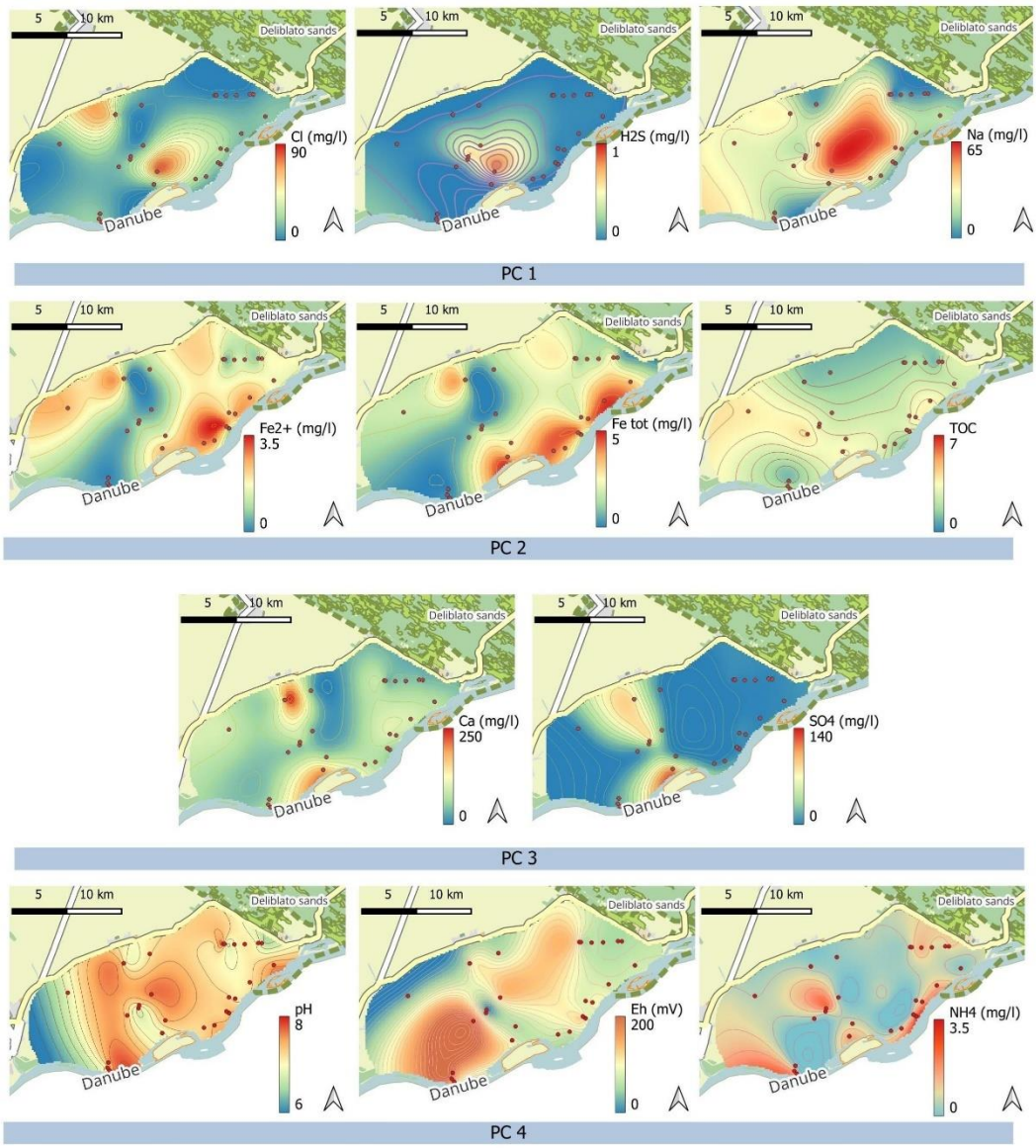

Fig. 2 Spatial gradient of the concentration levels of the parameters extracted within the PC1: Cl^- (mg/l) H_2S (mg/l) Na (mg/l); PC2: Fe^{2+} (mg/l), Fe_{tot} (mg/l) and TOC (mg/l); PC3: SO_4^{2-} (mg/l) and Ca (mg/l); PC4: pH, Eh (mV) and NH_4^+ (mg/l)

4.3 Microbiological analysis results

Analysing the reduction of methylene blue in the HAB BART biotest within the heterogeneous hydrogeological continuum of Kovin-Dubovac groundwater revealed the dominance, abundance, and biochemical activity of facultative anaerobic heterotrophic bacteria. These bacteria, including both anaerobes and facultative anaerobes, actively mineralize organic matter. Methylene blue reduction serves as an indicator of their respiratory activity and microbial oxidation of nutrients in the sampled water. Both anaerobes and facultative anaerobes actively mineralize organic matter. Their high abundance and biochemical aggressiveness indicated a significant content of organic matter and a postoxic/anoxic status in the Kovin-Dubovac groundwater. Biological activity tests (Table 4) confirmed a prevalence of reductive-anoxic conditions, where dominant fermentation reactions prevail, intermittent denitrification occurs, and substantial sulphate reduction potential is concentrated in specific areas.

The community structure of groundwater microbiome depends on the oxygen content, as well as other alternative electron acceptors and available carbon sources. The low oxygen content and Eh values suggest that the oxidation of many reduced chemical species is likely microbiologically mediated. In terms of abundance and aggressiveness, iron-associated and fermentative bacteria emerged as co-dominant groups in the analysed groundwater, particularly in the first drainage line (Fig. 3 and Fig. 4). These bacteria were accompanied by potential denitrifying and sulphate-reducing communities. An aggressive population of sulphate-reducing bacteria, producing hydrogen sulphide, was detected in the groundwater in the coastal zone of the Danube (Fig.4). Iron-oxidizing bacteria dominated in the groundwater closer to the riparian zone of the Danube (around B-19/p2 near Kovin, Fig. 4), and downstream from the Danube branch around Bp-9, Bp-2, and Bp-17. It is considered that the transition from aerobic to anaerobic respiration occurs when the oxygen concentration falls below approximately 0.5 mg/L. For the initiation of nitrate reduction (denitrification), the oxygen concentration threshold may be around 2.0 mg/L, indicating a certain degree of overlap with aerobic respiration. This phenomenon has been detected in several sites (Table 4, Fig. 3, and Fig. 4) and aligns with the observed growth of heterotrophic microorganisms as facultative anaerobes, utilizing both aerobic and anaerobic respiration. In cases where the organic carbon content is low or unavailable, various lithotrophs that oxidize inorganic electron donors, such as sulphide or bivalent iron, also participate in nitrate reduction.

Table 4 BART test results by sampling location (p.a.c/ml) (in the period 2010-2013)

Sampling site	**x**	**y**	**IRB**	**SRB**	**HAB**	**DN**
			p.a.c/ml			
B-19/P-2	7504741.23	4953197.50	35300	850	454000	26
B-19/P-1	7504805.47	4953534.42	35300	6133	318600	82093
B-19	7504807.53	4953533.66	30243	1131	319333	5133
B-16	7508321.98	4955450.14	22000	50600	500000	
B-12/P-2	7512663.00	4956758.00	88150	254	454000	122070
B-12	7512501.10	4956863.81	23009	4701	229204	5519
Bp-17	7515570.79	4959874.44	13780	2069	183863	6705
Bp-5	7508522.53	4956272.77	3950	23900	2330000	6667
Bp-20	7507612.07	4957970.23	14640	6700	252000	66035
Bp-25	7512280.06	4961246.01	8955	104	199267	6807
Bp-64	7505806.06	4959978.83	22075	850	151000	6669
Bp-9	7511033.28	4955801.62	26402	477	352450	5044
Bp-13	7514015.49	4958759.06	14697	3752	192966	9239
Bp-24	7511719.20	4955960.92	15440	18000	169747	8809
Bp-2	7513069.73	4957694.19	35300	604	183200	5911

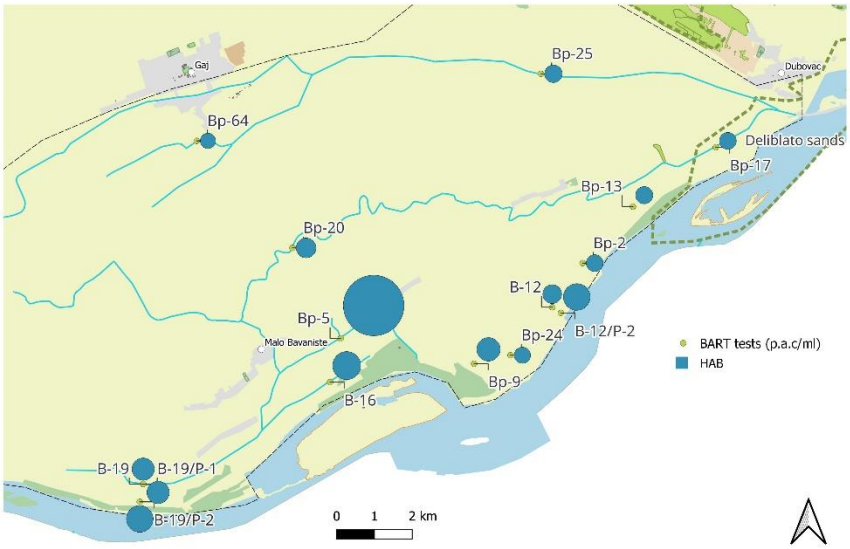

Fig. 3 The spatial distribution of the HAB BART test results (scaled by value)

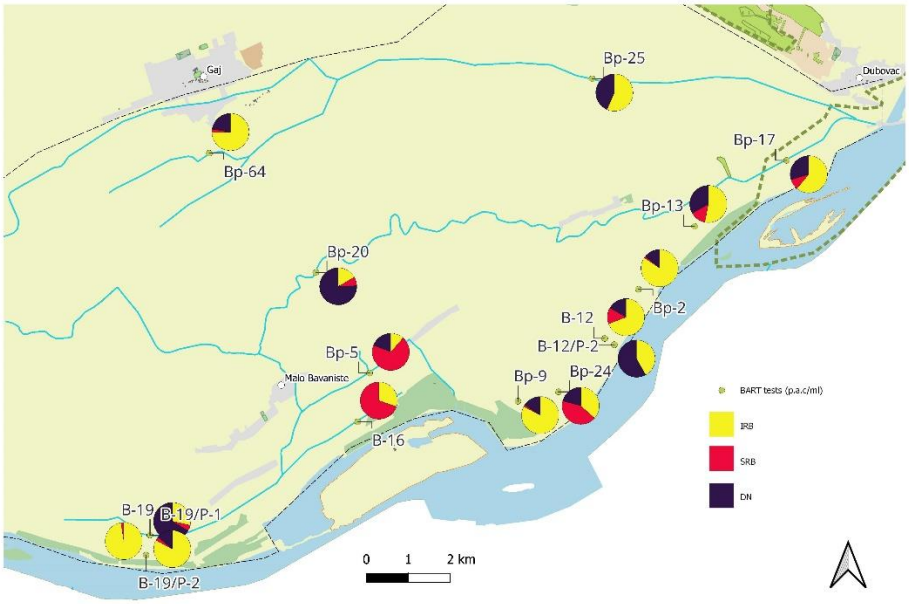

Fig. 4 The spatial dispersion of the BART test results ratio (IRB, SRB, DN)

When considering chemically redox-sensitive species, the coastal zone exhibits higher average concentrations of reduced iron and organic carbon (TOC) in riparian areas, along with elevated concentrations of ammonium ions. This suggests anaerobic degradation of organic matter associated with the utilization of ferric ions as electron acceptors. Toward hinterland, sporadically higher Eh and pH values indicate intensive oxidation processes, with lower organic carbon content and, on average, lower concentrations of Fe and NH_4^+.

In the riparian zone, sulphate-reducing activity (aggressiveness) was occasionally very high (zone Bp-5), while in the groundwater of the central alluvial area of Kovin-Dubovac, there was an overall aggressive and abundant population of denitrifying bacteria, with sulphate-reducing bacteria present but, on average, with lower abundance and aggressiveness.

The detected inverse relation between SRB and DN BART test results likely reflects the influence of seasonal fluctuations in the local hydrological conditions and the availability of electron acceptors. These fluctuations can lead to a transition from conditions favouring NH_4^+ production via DNRA under low groundwater recharge circumstances to conditions facilitating respiratory denitrification and N_2 loss under conditions of high groundwater recharge (Perović et al., 2017; Rivett et al., 2008, Burgin and Hamilton, 2007). Zones of intensive hydrodynamic exchange are characterized by a wide spectrum of microorganism species and fluctuating redox conditions.

4.4 Isotopic signature results

4.4.1 N isotopic signature

The isotopic signature of ammonium ($\delta^{15}N\text{-}NH_4^+$) was analysed for 12 piezometers samples: riparian zone (Bp-2, Bp-12, Bp-13, Bp-16), northeast part (Deliblato sand proximity: Bp-10z, Bp-11z, Bp-16z, Bp-20z, Bp-24z), central part of examined area (Bp-2z, Bp-5, and Bp-7) (Fig. 5, Fig. 7). $\delta^{15}N\text{-}NH_4^+$ values range from -0.8 ‰ to +6.9 ‰ (Table 5). The isotopic signature of ammonium ions, ranging from +4.8 to +6.9 ‰, roughly aligns with the range of organic matter origin (+2.4 ‰ to +4,1 ‰) and mineral fertilizers (-7.4 ‰ to +5.1 ‰) (Nikolenko et al., 2018). The isotopic signature of all samples is notably lower than the signature typically associated with manure (+8 ‰ - +11‰) and sewage water (+5‰ - +9 ‰). The $\delta^{15}N\text{-}NH_4^+$ value on the first drainage line is in the narrow range of +4.82 ‰ to +6.93 ‰. $\delta^{15}N\text{-}NH_4^+$ from north-east (Deliblato sand proximity) expresses the more depleted range of -0.84‰ to +4.49 ‰. Bp-16z and Bp-24z in the hinterland exhibited the lowest $\delta^{15}N$ values, recording -0.84 ‰ and -0.33 ‰, respectively. The central part of the area demonstrates the variety of the narrow range of +4,45 ‰ to +5,63 ‰. The highest $\delta^{15}N\text{-}NH4^+$ value of +6.93 ‰ was detected in Bp-16, which belongs to the first drainage line (Danube proximity). The composite soil sample of the first meter of soil from the first drainage line of Kovin (surroundings Bp-12, V-I-I) has a $\delta^{15}N$ isotopic signature value of +3.65 ‰.

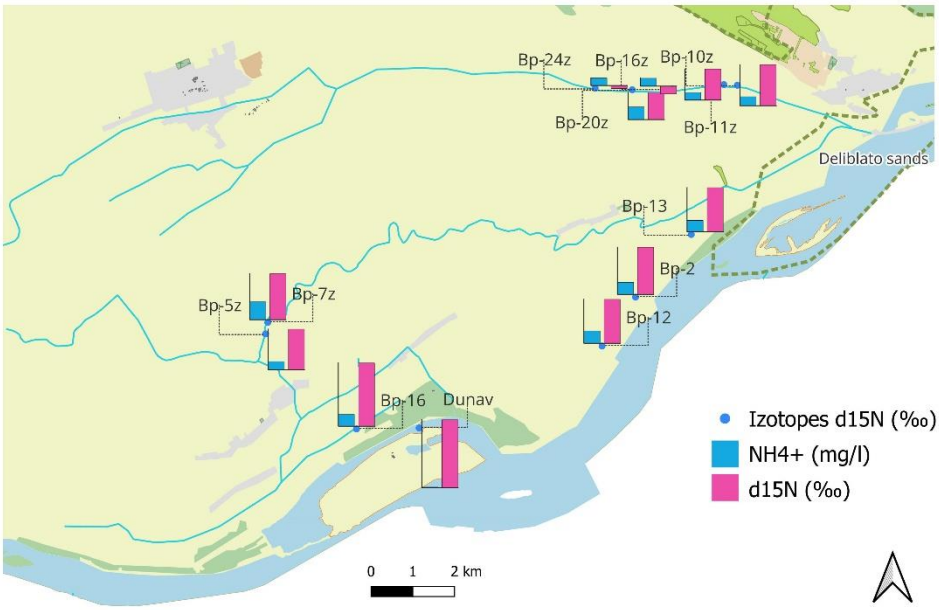

Fig. 5 The ratio of ammonium concentration and δ^{15}N-NH4^{+} value

The elevated nitrogen values in the riparian zone, can be attributed to the higher presence of electron donors, such as TOC and Fe^{2+}. These electron donors create predominantly reducing conditions, which are the most prominent in the river proximity and can be differentiated using extracted principal components (PC2 and PC4). The infiltration of oxygen-rich water from the Danube creates conditions conducive to the oxidation of mineralized organic matter, ammonification, and nitrification. The isotopic signature δ^{15}N in nitrates in the Danube was +7.45‰ and the nitrate concentration was 1.6 mgN/l in the Danube at the Kovin Bridge. Nitrification, due to the rapid consumption of oxygen (in an environment rich in organic matter and bivalent iron), is not highly intensive, and the assumption is that its effect on enriching the remaining NH_4^+ with the heavier isotope δ^{15}N is small (1-3 ‰). The nitrates from the Danube and from the agricultural surface of the field could be reduced to ammonium ions by dissimilatory reduction (due to elevated concentrations of Fe and TOC), which would result in a more depleted ammonium cation, compared to the initial ^{15}N originating from nitrates (min. fertilizers δ^{15}N-NO_3^- from -8 to +7‰) (Lindebaum 2012). The discernible influence of agricultural production on groundwater quality has already been noted by the presence of detected pesticides in the wells situated along the first drainage line in Kovin-Dubovac (Živančev, 2019).

Appearing of the isotopic signature of mineral fertilizers, as it moves towards the inland areas, suggests that despite aquifer confinement and the low permeability of the top layer (K= (1-5) x10^{-7} m/s), shallow groundwater might be influenced by prolonged agricultural practices and the application of fertilizers.

The interdependence of the sulphur and nitrogen cycles holds significant importance, especially evident in anoxic groundwater environments. As Kovin-Dubovac drainage system is characterized by an absence of oxygen, accompanied by prominent fermentation and sulphate reduction reactions that exhibit an inverse relationship with the recorded denitrification process, it is essential to acknowledge the significance of the sulphate reduction process and the associated fractionation during this reduction process. The sulphate concentration in the

groundwater across the Kovin-Dubovac area varies between 2 and 110 mg/l. Due to reducing conditions, sulphate concentrations are only sporadically noted in Bp-16 and Bp-64, as well as in Bp-4z, Bp-5z, Bp-7z, with average sulphate concentrations of 93.24 mg/l, 81.40 mg/l, 8.9 mg/l, 41.81 mg/l, and 71.16 mg/l, respectively. The average sulphate concentration in the Danube at the Smederevo profile for five years (2015-2019) was 23.6 mg/l. The spatial distribution of average sulphate concentrations is shown in Fig. 2. In these areas, a pronounced loading and interconnection exist between elevated concentrations of SO_4^{2-} and Ca^{2+} ions, as indicated by factor PC3 (Fig.2).

4.4.2 S isotopic results

The sulphate isotopic analysis was conducted for five objects (Bp-4z, Bp-7z, Bp-16, Bp-64, Bp-5z) and are presented in Table 5. The measured $\delta^{34}S\text{-}SO_4$ range was from +5.45 ‰ to +24.92 ‰, and $\delta^{18}O\text{- }SO_4$ from +1.80 ‰ to +13.50 ‰.

Table 5 Selected parameter values by sampling objects

Sampling site	NO_3^- (mgN/l)	NH_4^+ (mgN/l)	$\delta^{15}N\text{-}NH_4$ (‰)	$\delta^{15}N\text{-}NO_3$ (‰)	$\varkappa$ (µS/cm)	SO_4^{2-} (mg/l)	$\delta^{34}S\text{-}SO_4^{2-}$ (‰)	$\delta^{18}O\text{-}SO_4^{2-}$ (‰)	$\delta^{18}O\text{-}NO_3$ (‰)
Bp-2 V-I-I	0.13	1.33	5.2		634.3	<2.0			
Bp-12 V-I-I	0.1	1.36	4.8		573.1	<2.0			
Bp-13 V-I	0.21	1.25	4.8		719.9	<2.0			
Bp-16 B-I-II	0.27	1.3	6.9		1006.8	157	15.7	8.8	
Bp-4z B-I-III	0.18	0.28			817	8.9	14.7	1.8	
Bp-7z B-I-III-I	0.13	1.96	5.0		376	71.2	24.7	13.1	
Bp-64 D-I-I	0.13	0.38			1005.8	105	5.4	8.5	
Bp-5z B-I-III-I	0.16	0.92	4.4		919	41.8	24.9	13.5	
Bp-2z B-I-III-II	0.13	0.5	5.6		905	<2.0			
Bp-10z	0.15	0.99	4.5		378	<2.0			
Bp-11z	0.35	0.84	3.4		387	<2.0			
Bp-16z	0.2	0.88	-0.8		410	<2.0			
Bp-20z D-I-I	0.32	1.39	3.0		405	<2.0			
Bp-24z D-I-I	0.21	0.82	-0.3		376	<2.0			
Danube Ban.Palanka (average 2018)	1.64	0.08		7.4	462.4				3.5

Conducted SRB BART test analyses, show significant activity of sulphate-reducing bacteria (Fig. 4). The isotopic signature of $\delta^{34}S\text{-}SO_4^{2-}$ for two out of the 5 examined wells (Bp-5 and Bp-7 (B-I-III-I) ≈ +25 ‰), which are in the central part of the investigated terrain, is in the range of sulphate originating from evaporites (Fig. 8). The $\delta^{34}S\text{-}SO_4^{2-}$ and $\delta^{18}O\text{-}SO_4^{2-}$ for Bp-16 (B-I-II), are in the overlapping range of sulphates originating from atmospheric deposition and evaporites (Fig. 8). The isotopic signature in Bp-64 (D-I-I), which is on the third drainage line is in the overlapping range of sulphate originating from atmospheric deposition and anthropogenic influence. In the central part of the terrain, an eroded coal layer was registered, where the quality of groundwater from the shallow aquifer is affected by the inflow of groundwater from deeper aquifers (Jevtić and Zorić, 2012). There is an indication that the detected sulphates may originate from coal, exploited in Kovin. Given the reducing conditions and the presence of nonreduced sulphates only in the central part of the surveyed area, significant enrichment, and a shift of $\delta^{34}S$ of original sulphates toward higher values must be considered.

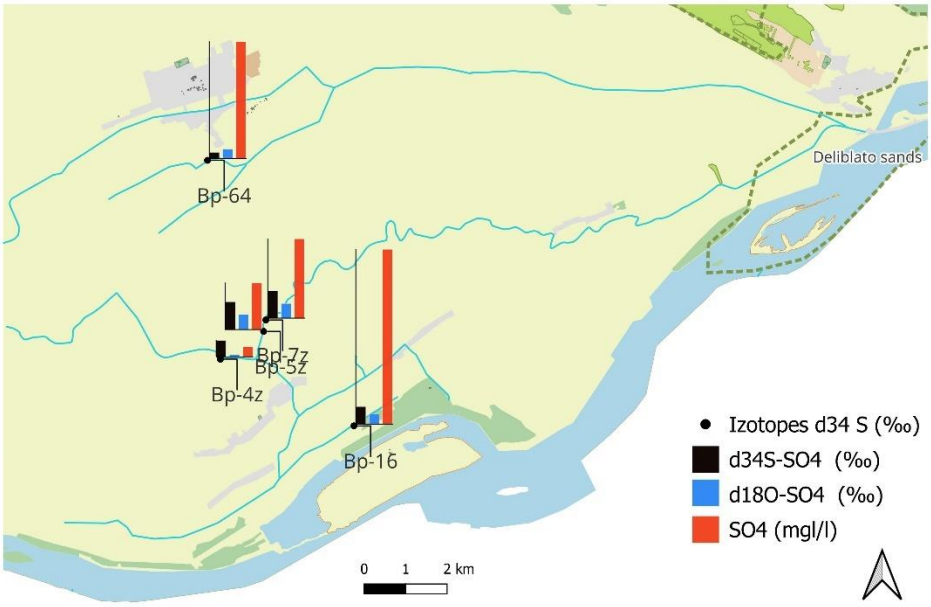

Fig. 6 The ratio of sulphate concentration and $\delta^{34}S\text{-}SO_4^{2-}$ and $\delta^{18}O\text{-}SO_4^{2-}$ value

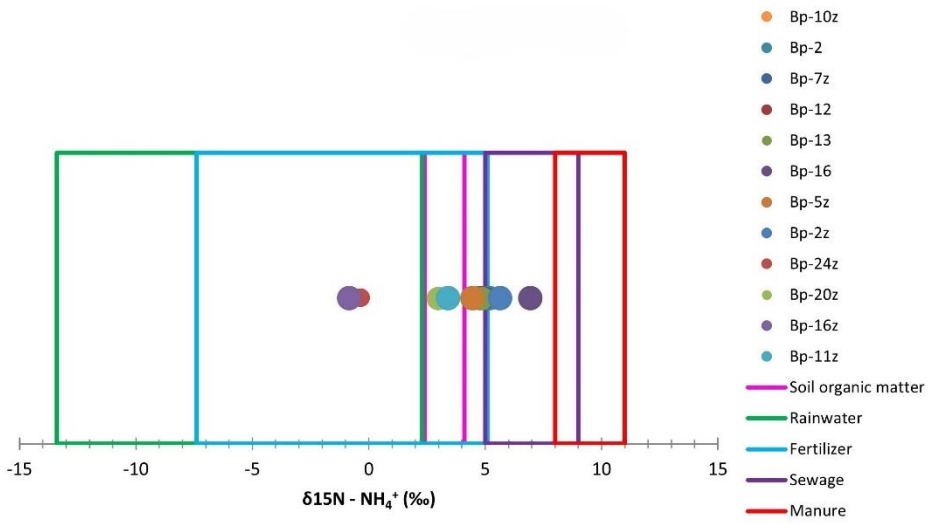

Fig. 7 Observed δ^{15}N-NH_4 and distinctive ranges of various NH_4^+ sources

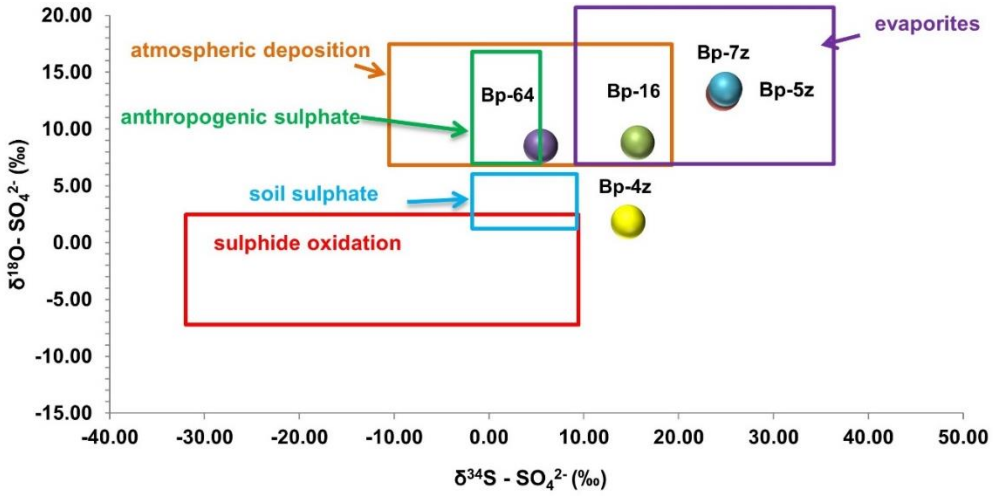

Fig.8 Schematic of typical ranges of the δ^{34}S and δ^{18}O values from different SO_4 sources with plotted data from conducted research (modified from Perović 2019)

5 Conclusions

This study integrated groundwater flow data, physico-chemical analysis, isotopic signatures, and microbiological tests, yielding the following insights: the presence of ammonium ions in riparian groundwater is attributed to the mineralization of soil organic matter, influenced to some extent by the application of mineral fertilizers. Increased concentration of ammonium ions coincides with higher levels of Fe^{2+} and the absence of sulphates and sulphides. The lack of sulphates is caused by sulphate reduction in an organic-rich and Fe^{2+}-abundant environment. Toward hinterland the heightened thickness of the sandy layer results in increased permeability, lower TOC content, enabling mineral fertilizers to reach the groundwater with less fractionated signature. The unfolding of denitrification process is supported by the positive loading between pH and NH_4^+ concentration, which is inversely related to the Eh value. Denitrification, along with the hydrolysis of the generated ammonium carbonate, slightly increases the pH. Observed significant loadings and groundwater composition indicate that nitrates entering the

groundwater in the hinterland are most likely reduced through the process of autotrophic denitrification. The identified sulphates in the Kovin-Dubovac area arise from the dissolution of calcium-sulfate minerals and coal mining activities in the region, influenced by sulphate reduction. The conducted research highlights the complexity of determination the origin of nitrogen compounds in the groundwater environment, where electron donors overlap, and different transformation processes simultaneously unfold. Gained results give a unique and novel approach to trace the sources of ammonium by determination of reductive transformation processes. The integrated approach utilized in this research provides guidance that can be applied to other alluvial aquifers for tracing nitrogen sources and solving the complex transport and transformation processes of nitrogen compounds.

Acknowledgment

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