DOI: 10.7343/as-2017-307

Ammonium natural attenuation in complex hydrogeological settings: insights from a multi-isotope approach

Attenuazione naturale dell'ammonio in contesti acquiferi complessi: approfondimenti da un approccio multi-isotopico

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Riassunto: Questo studio riporta una metodologia completa che include un approccio multi-isotopico accoppiato ad un campionamento delle acque sotterranee profondità dipendente, con l'obiettivo di indagare i processi di attenuazione naturale dell'ammonio.

Tre i contesti idrogeologici, rappresentativi di diversi aspetti ambientali in relazione all'inquinamento di nitrati e ammonio: un sistema settico in cui l'effluente che afferisce ad un acquifero superficiale vede l'ammonio come elemento contaminante (SSP); un ecosistema dipendente dalle acque sotterranee, dove le acque superficiali sono impattate dall' ammonio antropogenico e naturale che incide negativamente sulla funzionalità dell'ecosistema (GDE), ed un acquifero costiero alluvionale affetto da concentrazioni anomale di ammonio come anche elevati contenuti in altri composti indesiderati (ACA). Le acque sotterranee sono state campionate secondo la modalità profondità- dipendente tramite multi-level nested wells, straddle packers system, e tramite i long screen wells. Tali tecniche di campionamento sono state accoppiate ad un approccio multi-isotopico. Gli isotopi ambientali ($\delta^{18}O$ e δD in H_2O) sono stati utilizzati per ottenere approfondimenti sul sistema di flusso idrogeologico mentre altri isotopi stabili $\delta^{15}N_{NH_4}$, $\delta^{15}N_{NO_3}$, $\delta^{18}O_{NO_3}$ sono stati appositamente applicati per l'identificazione della sorgente, del destino e dei processi dei composti azotati. Inoltre, $\delta^{34}S_{SO_4}$, $\delta^{18}O_{SO_4}$ e $\delta^{13}C_{DIC}$ sono stati proposti come strumenti per la comprensione dei processi di attenuazione potenziale di altri cicli biogeochimici correlati. I dati di Trizio invece sono stati utilizzati per la stima del tempo di ricarica e del tempo di residenza delle acque sotterranee.

L'approccio adottato ha contribuito a raggiungere approfondimenti sui modelli di circolazione idrica sotterranea sito-specifici come anche del modello concettuale idrogeochimico.

Nel sito SSP, la prova isotopica della presenza di anammox insieme a processi di denitrificazione e nitrificazione, risulta responsabile del 60-80% della rimozione totale di azoto nella porzione distale del plume; nel sito GDE, ammonio e nitrato, provenienti principalmente da pratiche agricole, influenzano i sistemi di flusso profondo e superficiale e dai risultati si ricava che l'ammonio è attenuato localmente da diluizione e da nitrificazione; nel sito ACA, il pool di ammonio deriva in massima parte dall'aquitard sottostante ed è di natura biogenica. Inoltre sono stati identificati input minori di natura antropica (NH_4^+ e NO_3^-) che appartengono ad una circolazione idrica superficiale. L'NH_4^+ è risultato attenuato dai processi di trasporto e da processi di nitrificazione parziale. I dati isotopici dimostrano inoltre la presenza di processi di ossidazione anaerobica del metano accoppiati a processi di solfato riduzione.

La panoramica mostrata dai casi di studio proposti pongono l'attenzione nell'importanza di identificare dei modelli concettuali affidabili, che includono elementi fisici (come la circolazione delle acque sotterranee) insieme a dati chimici ed isotopici, rappresentando uno step fondamentale per l'integrazione del ciclo dell'acqua con quello dell'azoto, e quindi di elevato interesse per la gestione sostenibile dell'acqua e relative linee guida.

Abstract: *A full methodology including a multi-isotopic approach coupled with depth-dependent groundwater sampling is here reported to investigate the occurrence of ammonium natural attenuation.*

Examples from three hydrogeological settings, representative of different environmental issues in relation to nitrate and ammonium pollution are here reported: a septic system plume (SSP), where the discharging effluent composition into a shallow aquifer is dominantly ammonium-based; a Groundwater Dependent Ecosystem (GDE), where river waters are impacted by upwelling of anthropogenic and natural ammonium negatively affecting the ecosystem's functionality, and an alluvial coastal aquifer (ACA) affected by abnormal natural ammonium concentrations along with a high content of other undesired compounds. Several sampling methodologies were applied for depth-dependent groundwater sampling such as multi-level nested wells, straddle packers system, and common long screen wells. Such detailed sampling techniques were coupled with a multi-isotopic approach. Environmental isotopes ($\delta^{18}O$ and δD in H_2O) were used to gain insights into the hydrogeological flow system while other stable isotopes $\delta^{15}N_{NH_4}$, $\delta^{15}N_{NO_3}$, $\delta^{18}O_{NO_3}$ were purposefully applied for the identification of N compounds' source(s), fate and processes. In addition, $\delta^{34}S_{SO_4}$, $\delta^{18}O_{SO_4}$, and $\delta^{13}C_{DIC}$ were proposed as tools for understanding the potential attenuation processes in relation to the other biogeochemical cycles. Tritium data was also applied for recharge rate and groundwater residence time estimation.

Keywords: *ammonium, natural attenuation, isotopes, groundwater.*

Parole chiave: ammonio, attenuazione naturale, isotopi, acque sotterranee.

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Ricevuto/*Received*: 01 October 2017-Accettato/*Accepted*: 29 November 2017

The adopted approach helped reaching site-specific insights into the hydrogeological and geochemical conceptual models. In SSP, the isotopic evidence of anammox occurrence, together with denitrification and nitrification processes, result to be responsible of the 60-80% of the total N removal at the distal portion of the plume; in GDE, ammonium and nitrate, coming mainly from agricultural practices, affect the deep and the shallow flow systems. Ammonium results to be locally attenuated by dilution and nitrification. In ACA, the major ammonium pool derives from the low-lying aquitard as a biogenic source while anthropogenic minor inputs (NH_4^+ and NO_3^-) from the shallow circulation in the aquifer were also identified. NH_4^+ resulted attenuated by transport processes and partial nitrification processes. Moreover, by the use of a multi-isotope approach sound evidence of the anaerobic oxidation of methane coupled with SO_4^{2-} reduction has been arisen.

The overview provided by the proposed case studies put emphasis on the importance of the identification of reliable conceptual models which cluster physical patterns (e.g. groundwater flow) together with chemical and isotopic data, representing a fundamental step in the integration of the groundwater and the nitrogen cycles, thereby of high concern for sustainable water management and best practices.

Introduction

Nitrogen (N) compounds, which are found naturally at moderate concentrations in many freshwater environments, may often reach very high concentrations due to anthropogenic activities (Mariotti et al. 1981). To date, nitrate (NO_3^-) is one of the most frequently detected contaminants in water resources, to the extent that it is now regulated and monitored worldwide (Böhlke 2002). Notably, other N compounds such as nitrite (NO_2^-) and ammonium (NH_4^+) can also represent a severe environmental issue. The presence of N compounds in aquifers can have detrimental effects on groundwater quality and usability, thereby potentially threatening surface water and the related ecosystems (Böhlke et al. 2006). Conversely to nitrate, NO_2^- and NH_4^+ are not considered in most legislation cases (Nitrates Directive OJEC, 1991), nor in environmental studies (Sutton et al. 2011).

Opposite to nitrate - which has been extensively studied out over the last decades - much less is known about the fate and transport of ammonium in the sub-surface. NH_4^+ can naturally occur in groundwater, having originated from mineralization of sedimentary N-rich organic matter (Jiao et al. 2010) or released from sediments by cation exchange under anoxic conditions (Lewandowski and Nützmann 2010). Instead, anthropogenic ammonium is commonly associated with organic waste disposal and septic systems; for such cases NH_4^+ represents the dominant compound in the generated contaminant plumes (Robertson et al. 2012). Agricultural and domestic settings can act as another source for NH_4^+ in groundwater, for example by the dissociation of ammonium nitrate fertilizers or by mineralization of released urea (Groeschke et al. 2016).

Redox-dependent transformations strictly control NH_4^+ transport, particularly removal processes (Spiteri et al. 2008). Depending on the aquifer geochemistry, NH_4^+ transport may be influenced by numerous physical- chemical processes. In general, mixing and dispersion dilute the NH_4^+ load in groundwater whereas chemical sorption - including cation-exchange - induces relevant retardation on dissolved NH_4^+ transportation (van Breukelen et al. 2004). In addition, NH_4^+ can undergo significant volatilization processes which are favored by high pH, although restricted to the interface of the saturated-unsaturated zone (Christensen et al. 2001).

With regards to microbiological degradation, NH_4^+ can be naturally attenuated in groundwater by several well- known reaction pathways. In aerobic environments, ammonium transformation is mediated dominantly by microbial-induced transformation such as nitrification which results in the production of NO_2^-, and subsequently of NO_3^-. Alternatively, under anaerobic conditions ammonium can be oxidized in combination with reduction of NO_2^-, to produce N_2 through what is called the ANAMMOX process (ANaerobic AMMonium Oxidation; Thamdrup and Dalsgaard 2002).

Despite the major role of NH_4^+ in contaminated groundwater, its multiple behaviors are still only sparsely documented. In fact, constraining the cause of such N contamination is often complicated, especially in hydrogeological settings where multiple contributions of diffuse and point sources may overlap. In this light, stable isotope fractionations can provide valuable insights into sources and processes affecting ammonium and other N compounds, thereby contributing to the understanding of all processes which contribute or remove nitrogen within a hydrogeological system. Several research studies have demonstrated the potential of stable isotopes in evaluating the processes affecting NO_3^- and NH_4^+ in groundwater (Aravena and Mayer 2010; among others). Denitrification processes have been identified by the use of combined $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$ at many field sites (Otero et al. 2009; Hosono et al. 2014). Recently, Robertson et al. (2012) demonstrated the occurrence of N removal by anaerobic NH_4^+ oxidation in a septic system plume by measuring both $\delta^{15}N_{NO_3}$ and $\delta^{15}N_{NH_4}$.

Only through a correct understanding of the origin together with the assessment of the fate - including natural attenuation processes and particularly biodegradation - will water practitioners be enabled to successfully manage water resources, intended also as a surface/groundwater continuum, and to put in place prevention and/or mitigation measures to avoid additional deteriorations.

In this framework, examples from three case studies are herein reported. To study N compounds sources, fate, and related transformations in groundwater, depth-dependent sampling technique, and stable isotopes application represent the applied methodological approach. Evidences of N attenuation, particularly for the NH_4^+, are presented from an anthropogenic impacted alluvial coastal aquifer (ACA) and a septic system plume (SSP) as indicative of a diffuse and a point source contamination, respectively. The third case study concerns the investigation of the potential impact of N compounds with regards to ammonium in a groundwater dependent ecosystem (GDE) to evaluate the N detrimental effect on the ecosystem's functionality (EC, 2015). The main intention of this paper is to highlight the great potential of the multi-isotope approach combined to depth-dependent sampling technique in hydrogeological studies, particularly to untangle nitrogen attenuation processes that naturally occur at field scale. To achieve this purpose, examples from three case studies are herein reported, covering quite a comprehensive range of hydrogeological settings, contaminant sources, and potential target receptors. Thought the manuscript, a summary the specific data for each case study are reported, addressing to the cited publications for detailed information. Therefore, the reader will benefit of an overall picture based on the emphasis given to reliable conceptual models, ad-hoc scientific approaches and unique insights, representing relevant progresses compared to previous investigations for each field site.

Analytical approach

This section describes the methodology used for characterization of field sites, particularly the methodology developed via straddle low pressure packers for a depth-dependent groundwater sampling as well as the multi-isotope isotope application. The advance of a vertical high resolution characterization of an aquifer will be explained, as will the laboratory procedures used to perform the isotopic analyses.

Appling the proposed approach, groundwater sites with the occurrence of both NH_4^+ and NO_3^- contamination were preferred. Three field sites were selected as representative of both diffuse and point source contaminations same as different hydrogeological settings (Fig. 1):

i. A septic system where the discharging effluent composition was dominantly ammonium-based (SSP); here hydrogeological investigations regard the effluent infiltration into a porous media (Fig. 1; Caschetto et al. submitted).

ii. A GDE where river waters, particularly those in correspondence to the hyphoreic zone, are impacted by upwelling of anthropogenic and natural ammonium which negatively affect the ecosystem's functionality (GDE); the major hydrogeological concern about this site is the enhanced connectivity between the multilayer aquifer and the surface river water, due to human activity along the river stretches (Fig. 1; Caschetto et al., 2014; 2017b).

iii. An alluvial coastal aquifer affected by abnormal natural ammonium concentrations along with high content of other undesired compounds (ACA); the main hydrogeological issues for this site result from the upward flux of palaeo seawater from the low-lying aquitard into the alluvial aquifer, intended by return-flow infiltrating from irrigation practices (Fig. 1; Caschetto et al., 2017a).

Depth-dependent sampling technique

A depth-dependent sampling is an invaluable method which, by sampling at multiple intervals of an aquifer, allows the reconstruction of three-dimensional distribution of groundwater parameters within aquifer systems (Mastrocicco et al. 2012). Depth-dependent sampling is routinely applied in contaminated sites to map contaminant vertical spreading (Prommer et al. 2006; Colombani et al. 2009; Henderson et al. 2009; Palau et al. 2014), and is also used in monitoring salinization in coastal areas (Einarson and Cherry 2002).

Depth-dependent sampling methodology can rely on the use of systems already emplaced in-situ, such as (i) multilevel nested wells, (ii) multiple completion, (iii) continuous multi-channel tubing, and (iv) the Waterloo multi-levels systems, among others. If not available at the site, depth-dependent sampling is still possible by the use of unique systems that allow for discrete sampling within a well, thus collecting samples at precise intervals. Removable packers are most frequently

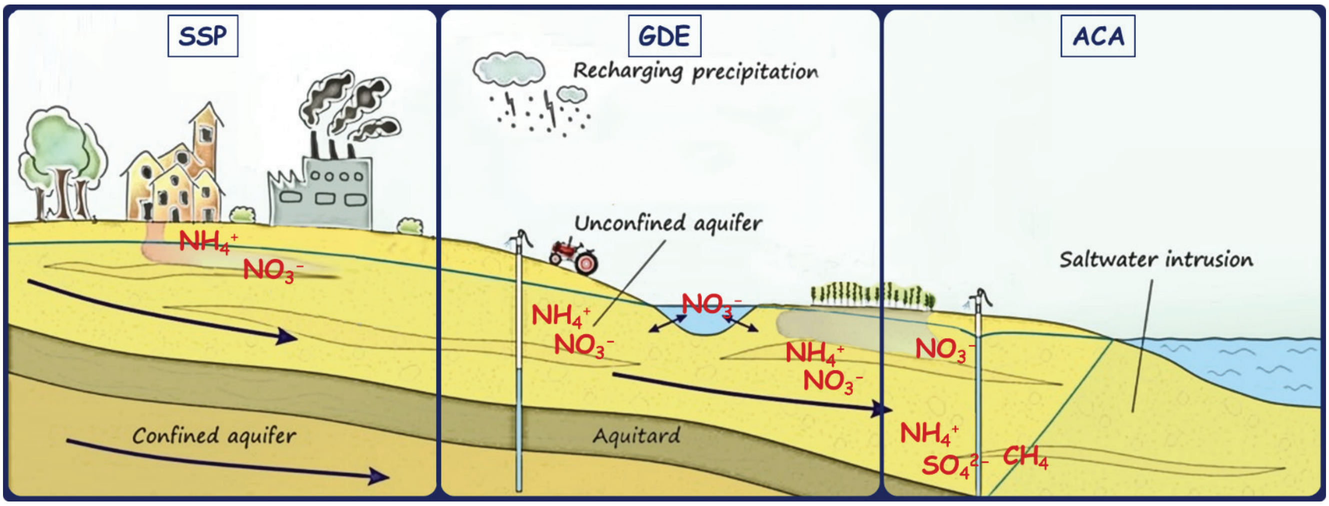

Fig. 1 - Graphical summary of the three hydrogeological conceptual models with respect to the nitrogen sources.

Fig. 1 -Schema dei tre modelli idrogeologici in relazione alle sorgenti di nitrato.

used for such purpose. They are usually made of natural rubber and are inflated pneumatically (or hydraulically) by pressurizing the interior. After inflating, this system isolates a portion of the casing from which the sample will be collected. On average, the costs in terms of piezometer installation and sampling can be higher than the cost of common long screen wells. However, the higher costs cannot obscure the need for a dedicated depth-dependent groundwater monitoring, which is necessary for a proper vertical estimation of the chemical composition of groundwater. Such detailed information can enhance the predictability of contaminated site to regional scale management models, with particular reference to the infiltration of pollutants deriving from anthropogenic sources.

Ad hoc depth-dependent sampling was performed within the three selected field sites as follow:

i. in SSP, groundwater samples were obtained from multilevel bundle piezometers (Waterloo multilevel system). These consisted of three sampling tubes set at varying depths b.g.l.: shallow from 1.2 to 1.4 m, mid-depth from 1.5 to 1.7 m, and deep intervals (-d) from 1.8 to 2.0 m.
ii. continuous multi-channel tubing and multilevel nested wells with a 10 m window were adopted in GDE, together with water collected from springs, surface flowing waters and hyporheic zones (Caschetto et al. 2014; 2017b).
iii. monitoring wells screened from 1 m below ground level (b.g.l.) to a maximum of 15 m b.g.l. to fully penetrate the unconfined aquifer were adopted in ACA study site (Caschetto et al. 2017a).

Laboratory procedures

Stable and radioactive isotopes have proven to be a valuable tool in hydrogeological investigations. Among the wide areas of interests, isotopes are particular useful in studying flows and interconnections between aquifers, in the identification of recharge patterns, and constraining the mechanism of pollution. Particularly, multi-isotopes approach are commonly used to trace pathways and to predict spatial distribution and temporal changes in pollution, same as to assess pollution migration scenarios for suited remediation plans. Comparing multiple isotope ratios (e.g. $\delta^{15}N_{NH_4}$ and $\delta^{15}N_{NO_3}$) is crucial for sources and fate identification of contaminants (e.g. NH_4^+), as several processes can lead to isotopic fractionations, not only related to a target contaminant (e.g. NH_4^+) but also the potential by-products. These fractionation processes may influence the accuracy of the interpretation if not properly understood. Moreover, isotopic analysis of SO_4^{2-} ($\delta^{34}S$ and $\delta^{18}O$) and dissolved inorganic C ($\delta^{13}C_{DIC}$) represents an efficient corollary tool to better constrain the origin of contaminants and the potential transformation in relation to the other biogeochemical cycles (Clark and Fritz 1997; Otero et al. 2009). With the exception of $\delta^{18}O_{H_2O}$ and $\delta^2H_{H_2O}$, analysis, performed at the Isotope Geochemistry Laboratory at the University of Parma, all other isotope analyses were processed at the Environmental Isotope Laboratory at the University of Waterloo, Canada. Data are processed according the analytical procedures reported by the following authors: Ryabenko et al. (2009) for $\delta^{15}N_{NO_3}$ and $\delta^{15}N_{NH_4}$; Mayer and Krouse (2004) for $\delta^{34}S_{SO_4}$ and $\delta^{18}O_{SO_4}$; Matthews et al. (2005) for $\delta^{13}C_{DIC}$; and Longinelli and Selmo (2003) for $\delta^{18}O_{H_2O}$, $\delta^2H_{H_2O}$. Below, Table 1 shows which isotope tool was adopted for the selected field sites.

Results

The tested methodological approaches provided unique insights, representing relevant progresses compared to previous investigations for each field site. Major findings for each case study are hereinafter presented.

The septic system plume (SSP)

Hydrogeological investigations at this site are related to the septic effluent infiltration into a porous media. The groundwater monitoring network is comprised of 30 monitoring points in 12 multilevel sampling tubes (nests) used for groundwater sampling activities. Concentration data helped characterizing N species distributions along the groundwater flowpath, revealing an approximate 60-80% of N removal at the distal areas of the well- characterized septic system plume (Caschetto et al., submitted). $\delta^{15}N_{NH_4}$, $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$ data highlight the occurrence of consistent attenuation processes: in the uppermost zone ammonium is oxidized to nitrate, whereas in the anoxic main body of the plume anammox process plays a major role. Significant $\delta^{15}N_{NH_4}$ enriched values (up to +62.7‰) confirm a reactive loss of ammonium at the distal areas deeper in the plume. The degree of enrichment increases at lower NH_4^+ concentrations and it is consistent with the Rayleigh-type distillation with an overall isotopic enrichment factor (ε) of -5.1‰, which is in line with recent literatures documenting the occurrence of anaerobic oxidation of ammonium (Clark et al. 2008; Robertson et al. 2012). The detected NO_3, is probably a product of aerobic ammonium oxidation, as supported by the observed isotopically enriched NH_4^+ and depleted $\delta^{15}N$ values. A positive correlation between

Tab. 1 - Summary of the isotope analyses processed at each field site.

Tab. 1 - Riassunto delle analisi isotopiche svolte per ogni caso di studio.

ID	$\delta^2H_{H_2O}$	$\delta^{18}O_{H_2O}$	^3H	$\delta^{15}N_{NO_3}$	$\delta^{15}N_{NH_4}$	$\delta^{18}O_{NO_3}$	$\delta^{34}S_{SO_4}$	$\delta^{18}O_{SO_4}$	$\delta^{13}C_{DIC}$
SSP				*x*	*x*	*x*			
GDE	*x*	*x*		*x*	*x*	*x*			
ACA	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*	*x*

$\delta^{15}N_{NH_4}$ and $\delta^{15}N_{NO_3}$ is observed, meaning that both nitrate and ammonium are consumed through anammox reactions. Nitrate isotopic composition ($\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$) show a positive trend through denitrification, remaking the fact that nitrate is concomitantly produced and degraded along the flowpath (Fig. 2). Moreover, isotopic patterns clarify NO_3^- transformations along the plume, which are consistent with varying stages of denitrification coupled with anammox. Clear evidence of anammox reaction together with denitrification and nitrification processes were provided (Fig. 2). In this case study it was demonstrated that anammox is naturally active in groundwater, even at low pH and in rich organic carbon environments (Caschetto et al. submitted).

The river basin (GDE)

The Sagittario River basin presents a complex hydrogeological framework, with groundwater coming from a multilayer aquifer-aquitard system, and surface water affected by morphological and discharge regime disturbances (Caschetto et al. 2014). Besides, severe nitrogen pollution coming from indiscriminate land use is also impacting this GDE. Hydrogeological investigations constrained elevated connectivity between the Sagittario River and the multilayer aquifer within the basin. Gaining and losing stretches were identified, occurring mainly through the exchange of river water and shallow groundwater. Environmental stable isotope $\delta^{18}O_{H_2O}$ and δD data provided evidence of different recharge areas and showed that the groundwater system is composed of both a shallow and a deeper flowpath (Caschetto et al. 2014). Chemical and isotopic data showed that both ammonium and nitrate affect the deep and the shallow flow systems, respectively. Ammonium is locally attenuated by dilution and nitrification, whereas denitrification is negligible in nitrate attenuation (Fig. 2) (Caschetto et al. 2017b). Additionally, in correspondence with the gaining stretches of the Sagittario River, it was demonstrated how the hyporheic zone can receive ammonium-enriched water upwelling from the deep aquifer through the high permeability layers. The isotopic characterization of nitrate ($\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$) indicates that manure is one of the main sources of nitrate in the basin, especially in the western part of the study area, with minor inputs associated to inorganic fertilizers while ammonium originates mainly from sewage and synthetic fertilizers (characteristics values of $\delta^{15}N_{NH_4}$). The shallow groundwater flow system is under aerobic conditions in contrast to the deepest anaerobic flow system, which explains the presence of high ammonium concentrations and very low nitrate concentrations at the river reach scale. From the upper areas in the catchment to the area near the river, nitrogen load follows both the shallow and the deep flowpath and is consequently becoming a noteworthy concern for the ecological status of the hyporheic zone and spring habitats. Additionally, in correspondence with the gaining stretches of the Sagittario River, it was demonstrated how the hyporheic zone can receive ammonium-enriched water upwelling from the deep aquifer through the high permeability layers. Results from this study highlighted the impact of the multiple stressors such as nitrogen pollution within the basin, indicating severe risks for the GDE functionality (Caschetto et al. 2017b).

The alluvial coastal aquifer in a river floodplain (ACA)

Recharge rates, residence time, groundwater flow patterns and aquifer salinization processes were investigated at urban and agricultural settings by the use of ^3H, δ^2H, and $\delta^{18}O_{H_2O}$: high recharge rates are preventing complete salinization of the shallow aquifer in the urban setting, whereas the seepage from the underlying hypersaline aquitard salinize the coastal aquifer in the agricultural setting (Caschetto et

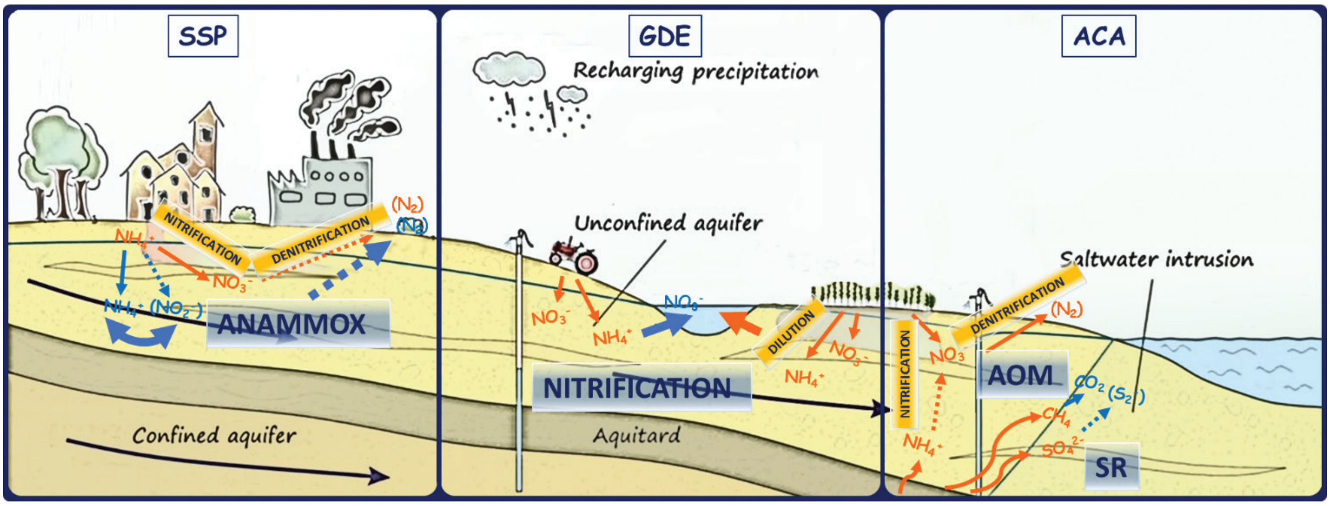

Fig. 2 - Graphical summary of the three hydrogeological conceptual models with respect to the biogeochemical processes identified by the proposed methodological approach. AOM and SR stand for anaerobic oxidation of methane and sulphate reduction, respectively.

Fig. 2 - Schema dei tre modelli idrogeologici in relazione ai processi biogeochimici identificati grazie all'approccio metodologico proposto. AOM e SR si riferiscono all'ossidazione anaerobica di metano e alla solfato riduzione, rispettivamente.

al. 2016). Regarding ammonium, $\delta^{15}N_{NH_4}$ results confirm that the major pool derives from the low-lying aquitard as a biogenic source while anthropogenic minor inputs (NH_4^+ and NO_3^-) from the shallow circulation in the aquifer were also identified. $\delta^{15}N_{NO_3}$ data suggest that NO_3^- derives from septic effluent leaching behaving as a point-source contamination at the urban setting (Caschetto et al. 2017a). Besides, NH_4^+ resulted attenuated by transport and partial nitrification processes (Fig.2; Caschetto et al. 2017a). Stable isotopes gave insights into attenuation processes as well as for other compounds such NO_3^-, SO_4^{2-} and methane (Fig. 2). Strong reducing conditions and elevated DOC content indicate favorable conditions for attenuation processes, while the dual isotope approach ($\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$) confirms that NO_3^- is attenuated by denitrification (Fig. 2; Caschetto et al. 2017a). With regards to SO_4^{2-}, $\delta^{34}S$ and $\delta^{18}O$, they indicate a marine origin deeper in the aquifer, whereas a soil/fertilizers and sewage origins in agricultural and urban settings respectively characterize the shallow portion of the aquifer. $\delta^{34}S_{SO_4}$ signatures distinguish two biogeochemical processes: bacterial SO_2^- reduction and sulphide oxidation (pyrite) for the deeper and shallow portions of the aquifer, respectively. $\delta^{13}C_{DIC}$ isotopic composition resulted related to a methane pool, relying in the under-lying aquitard as a biogenic source. This situation represents an additional independent proof of the upconing from the aquitard, which salinizes the unconfined aquifer. Moreover, the upwardly diffusing methane can be oxidized as suggested by the strongly depleted $\delta^{13}C_{DIC}$ values, highlighting the occurrence of anaerobic methanotrophy (Fig. 2; Caschetto et al. 2017a). By the use of a multi-isotope approach, sound evidence of the anaerobic methanotrophy coupled with bacterial SO_4^{2-} reduction has been arisen (Fig. 2). For this case study, it was postulated how the reminiscent effect of the paleo-marine environment still has a pivotal role in nitrogen (N), sulphur (S), and carbon (C) biogeochemical cycle's evolution (Caschetto et al. 2017a).

Conclusions

Proper knowledge of the hydrogeological systems, the origin, and the natural attenuation processes affecting contaminants is essential for the correct management of water resources. In this light, the identification of a reliable conceptual model, as demonstrated by the reported case studies represents a fundamental step in hydrogeochemical investigations.

Only a comprehensive understanding of the hydrogeological framework for the saline coastal aquifer and for groundwater-surface water continuum at the river basin (GDE) allowed a correct understanding of the dynamics of contaminants in relation to the groundwater flowpaths. Environmental isotopes were shown to be a powerful tool for hydrogeological investigations including water recharge areas, regimen, and flowpath. The ammonium detected for the three case studies resulted in their association with different sources - both natural and anthropogenic. Likewise, the use of stable isotopes was confirmed as a suitable tool in tracing investigations regarding the sources. Most significantly, regardless of the differences in scale and environmental settings, all three case studies reported the importance of natural attenuation processes in containing/removing nitrogen compounds within the water resource. Unfortunately, natural attenuation processes do not receive proper attention by water managers, practitioners, or regulators. Natural attenuation is often not even considered in water contamination studies, while results from the present study showed that high content of N contaminants can be attenuated by a variety of potential biodegradation processes depending on the redox conditions (Fig. 2).

In this light, stable isotopes were shown to be an appropriate tool for "directly" demonstrating the occurrence of such natural attenuation processes. The multi-isotopic approach turned out to be effective also in the identification of biogeochemical reactions related to N compounds attenuation as well as S and C compounds, thereby contributing innovative insights to the hydrogeological and geochemical characterization for each case study.

However, each case study highlights how the degree of accuracy and the reliability of concentration and isotopic data are undoubtedly dependent on the high resolution vertical sampling that is performed. Depth-dependent data helped discretize groundwater chemical composition in relation to the variation of stratigraphic aquifer deposits and geometry, but also in relation to groundwater flow systems which have different recharge patterns. From catchments to local scales, the proposed high resolution vertical sampling and stable isotope tool approach successfully constrained relevant attenuation processes, such as anammox naturally contributing to the ammonium removal in groundwater (SSP case study) or anaerobic oxidation of methane coupled with sulphate reduction at the ACA study site.

The proposed methodological approach represents a valuable asset for a wide spectrum of hydrogeological and hydrochemical applications. The outcomes of this paper emphasize the fact that a detailed understanding of nitrogen attenuation processes occurring naturally in aquifers is possible only through an ad-hoc methodological approach and it is much needed, particularly for design groundwater detection/monitoring programs and for performing remediation/mitigation actions. The approaches proposed through this paper may be of high relevance for proper management of water resources, best practices, and future actions.

Acknowledgment: I am greatly indebted to Prof. Marco Petitta and Prof. Ramon Aravena, my tutor and co-tutor during my Ph.D. program. I also would like to acknowledge Prof. Micòl Mastrocicco, Dr. Nicolò Colombani and Prof. William Roberson for providing access to the field sites and for their scientific support, and to Dr. Alessandro Lacchini for his help in editing the manuscript. Lastly, I would like to acknowledge the two anonymous reviewers for the useful comments that improved the quality of this manuscript.

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