



UNIVERSITAT DE BARCELONA

Optimization of induced denitrification strategies in water resources contaminated by agricultural and urban sources

Abu Alex Tiewin

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PHD THESIS

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CIÈNCIES DE LA TERRA

MAY 2024

OPTIMIZATION OF INDUCED DENITRIFICATION STRATEGIES IN WATER RESOURCES CONTAMINATED BY AGRICULTURAL AND URBAN SOURCES.

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OPTIMIZATION OF INDUCED DENITRIFICATION STRATEGIES IN WATER RESOURCES CONTAMINATED BY AGRICULTURAL AND URBAN SOURCES.

Tesi doctoral presentada per Alex Tiewin Abu per optar al títol de doctor per la
Universitat de Barcelona

Thesis carried out within the framework of the Earth Sciences doctorate program under
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This thesis was conducted with the aid of grants for the recruitment of pre-doctoral research staff (2019 FI_B 01059) provided by the Agency for Management of University and Research Grants of the Catalan Government. Additional support was received from the Fundació Bosch i Gimpera of the University of Barcelona. This research received funding from various sources, including REMEDIATE (TED2021-131005B-C31), the PACE-ISOTEC (CGL2017-87216-C4-1-R), and the Consolidate Research Group MAGH (2021-SGR-00308) of the Catalan Government.

Aquesta tesi es va dur a terme amb l'ajuda de beques per a la contractació de personal investigador predoctoral (2019 FI_B 01059) proporcionades per l'Agència per a la Gestió de Subvencions Universitàries i Investigació de la Generalitat de Catalunya. A més, es va rebre suport addicional de la Fundació Bosch i Gimpera de la Universitat de Barcelona. Aquesta recerca va rebre finançament de diverses fonts, incloent REMEDIATE (TED2021-131005B-C31), PACE-ISOTEC (CGL2017-87216-C4-1-R) i el Grup de Recerca Consolidat MAGH (2021-SGR-00308) de la Generalitat de Catalunya.

Esta tesis se llevó a cabo con la ayuda de becas para la contratación de personal investigador predoctoral (2019 FI_B 01059) proporcionadas por la Agencia para la Gestión de Subvenciones Universitarias e Investigación del Gobierno de Cataluña. Además, se recibió apoyo adicional de la Fundació Bosch i Gimpera de la Universidad de Barcelona. Esta investigación recibió financiamiento de diversas fuentes, incluyendo REMEDIATE (TED2021-131005B-C31), PACE-ISOTEC (CGL2017-87216-C4-1-R) y el Grupo de Investigación Consolidado MAGH (2021-SGR-00308) del Gobierno de Cataluña.

ACKNOWLEDGEMENTS

I am profoundly grateful to the numerous individuals who have provided unwavering support and encouragement throughout my journey to completing my PhD thesis. Your guidance and support have played a crucial role in making this research possible.

First and foremost, I want to express my heartfelt gratitude to God for guiding me through this academic journey and providing me with strength and perseverance throughout this challenging endeavor.

I am deeply thankful to my tutor and director, Dra. Cristina Domènech Ortí, and my co-director, Dr. Dídac Navarro-Ciurana, for their invaluable mentorship, patience, and insightful guidance. I sincerely acknowledge my former co-director, Dr. Raúl Carrey Labarta, whose contributions are duly recognized. Your collective efforts, expertise and dedication have been instrumental in shaping the direction of my research and improving the quality of this thesis. Beyond academia, your influence has positively impacted my entire life, and I am truly fortunate and blessed to have had such exceptional mentors. Your understanding, patience, and encouragement have been instrumental in shaping my life and helping me pursue my academic aspirations.

I would like to express my appreciation to the members of my thesis monitoring committee, Luis Gibert, José Ignacio Canudo, and Maite Garcia Valles, for your time, expertise, and valuable contributions. Your critical evaluation, constructive feedback, and valuable insights have significantly refined and clarified my research. Additionally, I would like to thank Prof. Dr. Alberto Sáez, the head of doctoral studies of the faculty, and Dr. Luis Rivero Marginedas, head of the department, for their unwavering support and guidance throughout this endeavor.

My sincere appreciation goes to the MAIMA Research Group, the Departament de Mineralogia, Petrologia i Geologia Aplicada i Facultat de Ciències de la Terra de la Universitat de Barcelona for providing an enriching academic environment and access to valuable resources that have significantly contributed to the success of this research. The teaching staff, researchers and all non-academic staff are duly acknowledged for fostering a collaborative spirit, creating a sound atmosphere, and enhancing the depth of my research.

I am deeply grateful to Prof. Dr. Albert Soler (Dean of the Faculty of Earth Sciences), Prof. Dra. Neus Otero, Dr. Jesús Causapé, Dra. Cristina Valhondo, Dra. Rosanna Margalef-Martí, Dra. Sara Herrero Martín, Dra. Clara Torrentó Aguerri, Dra. Mònica Rosell Linares, Albert Fernandez-Lagunas and Pastor Kwadwo Osei Boateng, who have immensely contributed to this endeavor

Acknowledgements

through insightful discussions, technical assistance, and diverse support. Your incredible support, guidance and contribution are valuable and deeply appreciated.

I express gratitude to the dedicated staff of the laboratory of the MAiMA-UB research group, the Scientific and Technological Centres of the University of Barcelona (CCiT-UB) and the Environmental Unit (UMA) of the CCiT-UB for the analytical support. Special appreciation goes to all co-authors and reviewers of my papers for their immense support throughout the review process. I extend sincere acknowledgements to Dra. Lurdes Martínez-Landa, Dra. Silvia Díaz-Cruz, Dr. Jesús Carrera and Dr. Robert Benaiges-Fernandez for their significant contributions.

I wish to acknowledge my PhD colleagues and all my friends, who have constantly motivated and supported me. Your motivation and support have been instrumental in making this achievement possible.

To my family, I am profoundly indebted for your unconditional love, unwavering belief in me, and continuous encouragement throughout this long and demanding journey. Your love and support have been the foundation of my strength.

I extend my profound gratitude to the Agència de Gestió d'Ajuts Universitaris i de Recerca de la Generalitat de Catalunya for the PhD grant that made this research possible. Additionally, I express my gratitude to the Fundació Bosch i Gimpera and L'Institut de Recerca de l'Aigua (IdRA) de la Universitat de Barcelona for the financial support.

Finally, I am deeply thankful to all the participants and individuals who graciously devoted their time and expertise to contribute to this study. Their involvement has been instrumental in the successful completion of this thesis.

Although the list of names here is limited, I am keenly aware of the countless others who have played a part in shaping my academic and personal growth. To everyone who has been a part of my journey, big or small, I extend my heartfelt appreciation.

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ABSTRACT

Nitrogen is essential for sustaining life on Earth; however, excessive nitrogen levels have become a pressing environmental concern with far-reaching consequences. Nitrogen pollution arises primarily from agricultural and urban activities, such as the excessive use of synthetic fertilizers, inappropriate waste disposal, industrialization, and increased tourist activities. One of the most concerning groundwater pollutants is nitrate, a compound formed by the oxidation of excess nitrogen in aerobic conditions. The consequences of global nitrate pollution pose significant threats to human health and the ecosystem. Excessive nitrate levels in drinking water have been linked to several human health issues, such as methemoglobinemia in children and a prolonged effect of gastric, colorectal and bladder cancer in adults, and deplete oxygen levels in aquatic systems, deteriorating aquatic life.

Addressing the global nitrate pollution problem requires remediation interventions to preserve the quality of water resources and safeguard clean water use. This thesis focuses on understanding the pathways of nitrate decontamination and on developing cost-effective, environmentally eco-friendly treatment and removal strategies in water resources. This thesis addresses the characterization of groundwater's prevailing nitrate removal processes using chemical and isotopic tools coupled with numerical modeling for successfully designing innovative techniques for nitrate removal from groundwater.

Denitrification stands as the primary process for nitrate reduction in groundwater. Denitrifying bacteria are widely distributed in both surface and subsurface water, and their activity is influenced mainly by the availability of electron donors, which act as the main limiting factor for denitrification. In light of this, the potential of using cheap and easily accessible electron donor sources aligning with the principles of circular economy, supporting the objectives of United Nations Sustainable Development Goals 6 and 7, focusing on clean water, waste reduction, and resource efficiency, were investigated. This study utilized wine residues as electron donors to remove nitrate from field nitrate-contaminated water in laboratory batch and column experiments. Two types of wine residues, named *lías* and *vínico*, were examined to assess their ability to induce denitrification by providing external organic carbon. *Lías* exhibited high efficiency in removing N compounds, as NO_3^- was completely removed within two days and NO_2^- in four days, whereas *vínico* showed limited efficiency, achieving only 35% NO_3^- removal paralleled with high NO_2^- concentration in the batch experiments. The distinct organic compound composition of the wine residues accounts for the differing N attenuation efficiencies observed in the batch experiments, whereas *lías* is mainly characterized by polysaccharides, *vínico* is

predominantly composed of humic and fulvic acids with less labile organic carbon. N and O isotope analysis of NO_3^- and NO_2^- confirmed biological denitrification as the primary mechanism responsible for NO_3^- attenuation in the conducted batch experiments.

In a column experiment using *lías*, controlled daily injection of the wine waste resulted in complete NO_3^- removal within three days. The injection was stopped in four days, and NO_3^- recovery began after seven days and reached the initial concentration by day ten. Nitrate supply to the column, variations in biomass, mixing, flow velocity, and preferential flow path did not significantly affect nitrate attenuation in the column. Nitrite did not significantly accumulate during biostimulation. Similar to the batch experiments, the NO_3^- and NO_2^- isotopologues analyses confirmed denitrification as the dominant process. Moreover, both experiments, biostimulated with *lías*, exhibited similar isotopic fractionation, indicating the same nitrate degradation processes. The laboratory-controlled isotope fractionation values obtained are reliable for quantifying denitrification efficiency for field studies, as they mitigate the variability associated with field conditions. The column experimental findings show that controlled injection with *lías* wine waste is effective for wetland applications, providing flexibility in adjusting injection parameters to achieve high denitrification at specific times of heightened denitrification activity. *Lías* also does pose a pollution risk, as it was not linked with heavy metals and could be used at the field-scale as an eco-friendly electron donor product to remediate NO_3^- .

A conceptual and numerical geochemical model that describes the coupled nitrate reduction and organic matter oxidation reactions in a fully saturated system was developed, incorporating biomass growth and decay, nitrate and nitrite degradation kinetically controlled, changes in NO_3^- and NO_2^- isotopic composition, aqueous speciation in equilibrium and the possibility of calcite precipitation (in equilibrium), assuming a constant temperature (25 °C). Nitrate and nitrite respiration rates were quantified using the dual Monod kinetics. Numerical simulations conducted for batch experiments were used to determine the reaction rate parameters with experimental results, and the obtained values were within the reported literature values.

Combining ferrous iron and organic carbon in nitrate treatment systems can lead to the simultaneous occurrence of chemodenitrification and biological denitrification processes, thereby enhancing nitrate removal. Ferrous iron, found abundantly in nature, plays a crucial role in optimizing denitrification and prolonging the effectiveness of organic carbon donors for sustainable, long-term nitrate treatment, especially in iron-rich environments. Therefore, the influence of the coupled nitrite (NO_2^-) reduction-Fe(II)oxidation has been investigated by

considering a solution of high nitrite concentration in contact with siderite (FeCO_3), dissolved Fe(II) and siderite plus dissolved Fe(II) in laboratory batch experiments, designed to evaluate the nitrite removal efficiency and the $\text{N}_2\text{O(g)}$ production. Although nitrite reduction capacity was low in the experiments with only siderite (only 3% NO_2^- removal), in the experiment with dissolved Fe(II) , up to 54% of nitrite was removed. However, the nitrite removal rate increased to 84% in the experiment where siderite plus dissolved Fe(II) was used. Nitrous oxide production was observed in all the experiments, but $\text{N}_2\text{O(g)}$ recovery in the headspace did not match the expected values. The study revealed that $\text{N}_2\text{O(g)}$ was the final product formed and was not further reduced to $\text{N}_2\text{(g)}$. $\text{N}_2\text{O(g)}$ also exhibited no leakage through the butyl rubber stoppers. Consequently, the low N_2O recovery was associated with low diffusion of aqueous N_2O into the headspace, as 50-98% of the generated N_2O remained dissolved in water. The diffusion of $\text{N}_2\text{O(g)}$ was unaffected by kinetic isotope fractionation. The isotopic analyses revealed that the same mechanism degraded NO_2^- in all experiments. A kinetic equation has been obtained for the coupled nitrite-reduction Fe(II) -oxidation reaction.

The Rayleigh distillation equation using the experimentally calculated $\epsilon^{15}\text{N}$ values, coupled with NO_2^- isotopic data, N_2O site preference values, and N_2O in the liquid phase according to Henry's Law confirmed that chemodenitrification was the primary NO_2^- attenuation pathway in the experiments. A geochemical model coupling chemodenitrification, kinetic isotope fractionation, aqueous speciation in equilibrium and precipitation and dissolution of calcite was implemented and reproduced the experimental results. These models could help predict the efficiency of abiotic nitrite reduction in bioremediation treatments using Fe(II) and distinguishing biotic and abiotic reactions in the field.

Partially treated effluents from wastewater treatment plants and untreated wastewater frequently exhibit high concentrations of inorganic nitrogen. Ensuring the safe use of this treated wastewater for groundwater aquifer recharge and domestic applications necessitates the remediation of inorganic nitrogen to alleviate the elevated inorganic nitrogen levels. Permeable reactive barriers have proven to be effective and sustainable solutions for the remediation of inorganic nitrogen in groundwater. In this thesis, the migration of secondary wastewater treatment effluent in two subsurface infiltration systems has also been studied: one with a reactive barrier (SAT-PRB) and the other without (SAT-Control). Chemical and isotopic analyses were conducted to assess the spatial and temporal variability of N species (mainly NH_4^+ and NO_3^-) at different sections and depths along the systems. The chemical and isotopic results revealed contemporaneous nitrification-denitrification as the systems' main N transformation and distribution pathway. The nitrification rate increased along the systems and with depth.

Temperature and microbial community growth and adaptation influenced N attenuation efficiency in the systems. High temperatures favored denitrification in the systems during summer periods. Other mechanisms, including sorption and plant assimilation, were identified as potential contributors to nitrogen (N) removal.

At the end of the study period, the SAT-PRB system successfully removed 66% of the total N in the solution. In comparison, the SAT-Control system achieved a removal rate of 69%, measured at the outlet of both systems. NO_3^- was the dominant species in the SAT-Control system, with 27% NO_3^- and 4% NH_4^+ , whereas the SAT-PRB system exhibited a more balanced distribution, with approximately equal proportions of NO_3^- and NH_4^+ , at 18% and 16%, respectively. The isotopic data revealed complete NO_3^- degradation in the systems during the warmer periods, indicating the possible mixing of newly generated NO_3^- with residual NO_3^- in the substrate pool.

This thesis provides valuable insights into effective nitrate removal strategies using sustainable electron donors through the application of carbon injection systems, ferrous iron, and permeable reactive barriers. The findings offer potential solutions for mitigating nitrate pollution of water resources, preserving their quality and ensuring a sustainable supply of clean water. It provides environmentally friendly methods to safeguard human health and the environment from the adverse impacts of nitrate pollution. However, further research and practical implementation of these strategies are essential to achieve lasting positive impacts on aquatic ecosystems and secure a healthier future for all.

RESUM

El nitrogen és essencial per mantenir la vida a la Terra. Tot i això, els nivells excessius de nitrogen s'han convertit en una preocupació ambiental urgent amb conseqüències de gran abast. La contaminació per nitrogen prové principalment de les activitats agrícoles i urbanes, com l'ús excessiu de fertilitzants sintètics, l'eliminació inadequada de residus, la industrialització o l'augment de l'activitat turística. En concret, el nitrat, format per l'oxidació de l'excedent de nitrogen en condicions aeròbiques, ha esdevingut un dels contaminants més preocupants de les aigües subterrànies. La contaminació global per nitrats és una amenaça important i significativa per a la salut humana i els ecosistemes. Els nivells excessius de nitrats a l'aigua s'han relacionat amb diversos problemes de salut, com la metahemoglobinèmia en nens i un efecte prolongat de càncer gàstric, colorectal i de bufeta en adults, a més de reduir els nivells d'oxigen en els sistemes aquàtics, deteriorant la vida aquàtica.

Abordar el problema global de la contaminació per nitrats requereix intervencions per preservar la qualitat dels nostres recursos hídrics i garantir l'ús d'aigua neta. Aquesta tesi es centra en comprendre les vies de descontaminació de nitrats i en desenvolupar estratègies de tractament i eliminació de nitrats en els recursos hídrics, que siguin al mateix temps, respectuoses amb el medi ambient i econòmicament viables. Aquesta tesi aborda la caracterització dels processos dominants en la degradació de nitrats a les aigües subterrànies utilitzant eines químiques i isotòpiques combinades amb models numèrics amb l'objectiu de poder dissenyar amb èxit tècniques innovadores per eliminar-ne els nitrats.

El procés principal per a la reducció de nitrats a les aigües subterrànies és la desnitrificació. Les bacteris desnitrificants estan àmpliament distribuïts en les aigües superficials i subterrànies, i la seva activitat depèn en gran mesura de la disponibilitat de donadors d'electrons, que ha esdevingut el principal factor limitant del procés. En aquest treball s'ha investigat l'ús de fonts donadores d'electrons econòmiques i fàcilment accessibles, d'acord amb els principis de l'economia circular, i amb els Objectius de Desenvolupament Sostenible de les Nacions Unides 6 i 7, centrats en aigua neta, reducció de residus i eficiència dels recursos. En concret, aquest estudi estudia residus de vi com a donants d'electrons en processos de desnitrificació en experiments de tipus batch i columna al laboratori. S'han fet servir dos tipus de residus, de vi, anomenats *lías* i *vínico*, i s'ha avaluat la seva capacitat d'induir la desnitrificació proporcionant carboni orgànic extern al sistema. El *lías* ha demostrat ser molt eficient en la remoció de compostos de nitrogen, perquè el NO_3^- es va eliminar completament en dos dies i el NO_2^- en quatre dies, mentre que l'eficiència del *vínico* ha estat més limitada i, aconseguint només el 35%

de la remoció de NO_3^- i mantenint una concentració elevada de NO_2^- als experiments en batch. Aquesta diferència en l'eficiència d'atenuació s'explica per la diferent composició dels residus de vi, ja que mentre que el *lías* està compost principalment per polisacàrids, el *vínico* està predominantment compost per àcids húmics i fúlvics amb menys carboni orgànic làbil. L'anàlisi isotòpica de N i O de NO_3^- i NO_2^- ha confirmat la desnitrificació com el mecanisme principal responsable de l'atenuació de NO_3^- en els experiments en batch.

En l'experiment de columna realitzat on s'ha utilitzat *lías*, la injecció diària de *lías* ha aconseguit l'eliminació completa del NO_3^- en tres dies. Després d'aturar la injecció al quart dia, la concentració de nitrat es va anar recuperant i va arribar a la concentració inicial al desè dia. S'ha observat que l'aportació de nitrats a la columna, les variacions en la biomassa, la barreja, la velocitat del flux i la ruta preferent no han afectat significativament l'atenuació dels nitrats a la columna. El nitrit no s'ha acumulat significativament durant la biostimulació. Com als experiments batch, les anàlisis dels isòtops de NO_3^- i NO_2^- han confirmat que la desnitrificació és el procés dominant. A més, els dos experiments biostimulats amb *lías*, han exhibit una fraccionament isotòpic similar, indicant que estan ocorrent els mateixos processos de degradació dels nitrats. Els valors de fraccionament isotòpic obtinguts en el laboratori seran útils per quantificar l'eficiència de la desnitrificació en estudis de camp. Els resultats dels experiments de columna mostren que la injecció controlada de *lías* seria efectiva per a la desnitrificació en llacunes o aiguamolls, i no presentaria un risc de contaminació important, perquè no està associada amb metalls pesats. Podria doncs, utilitzar-se a a escala de camp com a producte donador d'electrons respectuós amb el medi ambient.

S'ha desenvolupat un model geoquímic conceptual i numèric que descriu les reaccions acoblades de reducció de nitrat i oxidació de la matèria orgànica en un sistema completament saturat, incorporant el creixement i la descomposició de la biomassa, la degradació cinèticament controlada dels nitrats i nitrits, els canvis en la composició isotòpica de NO_3^- i NO_2^- , i l'especiació aquosa i la precipitació de calcita en equilibri, a una temperatura constant de 25 °C. Les velocitats de degradació del nitrat i del nitrit s'han quantificat assumint l'equació cinètica dual de Monod. Les simulacions numèriques realitzades per als experiments de batch i columna s'han utilitzat per determinar els paràmetres de reacció, obtenint valors similars als valors de la literatura.

La combinació de ferro fèrric i carboni orgànic en sistemes de tractament de nitrats pot conduir a l'aparició simultània de processos de quimodenitrificació i desnitrificació biològica, millorant així l'eliminació de nitrats. El ferro ferro, que es troba abundantment a la natura, té un paper crucial en l'optimització de la desnitrificació i la perllongació de l'eficàcia dels donants de

carboni orgànic per al tractament de nitrats sostenible i a llarg termini, especialment en entorns rics en ferro. Per tant, en aquest estudi s'ha investigat la influència de la reducció del nitrit (NO_2^-) acoblada a l'oxidació del Fe(II) , en tres tipus d'experiment batch, considerant 1) una solució amb una concentració elevada de nitrit en contacte amb siderita (FeCO_3), 2) una solució amb una concentració elevada de nitrit en contacte amb Fe(II) dissolt i 3) una solució amb una concentració elevada de nitrit en contacte amb Fe(II) dissolt i siderita, dissenyats per avaluar l'eficiència de l'eliminació de nitrats i la producció de $\text{N}_2\text{O(g)}$. Tot i que la capacitat de reducció del nitrit ha resultat ser baixa en els experiments amb només siderita (només el 3% d'eliminació de NO_2^-), en l'experiment amb Fe(II) dissolt, s'ha eliminat fins al 54% del nitrit i fins el 84% en l'experiment amb siderita més Fe(II) dissolt. S'ha observat la producció de òxid nítric en tots els experiments, però la recuperació de $\text{N}_2\text{O(g)}$ a l'espai de cap no ha coincidit amb els valors esperats. L'estudi ha confirmat que el $\text{N}_2\text{O(g)}$ és el producte final i que no s'ha reduït fins a $\text{N}_2\text{(g)}$. Donat que les pèrdues de $\text{N}_2\text{O(g)}$ a través dels taps de goma també s'han descartat, la baixa recuperació de $\text{N}_2\text{O(g)}$ s'ha associat a la baixa difusió del N_2O aquós a l'espai de cap, ja que el 50-98% del N_2O generat s'ha mantingut dissolt a l'aigua. La difusió del $\text{N}_2\text{O(g)}$ no està afectada pel fraccionament isotòpic cinètic. Les anàlisis isotòpiques han revelat que en tots els experiments ha actuat el mateix mecanisme per degradar el NO_2^- . S'ha obtingut una equació cinètica de segon ordre per a la reacció de reducció del nitrit acoblada a l'oxidació del Fe(II) .

L'equació de destil·lació de Rayleigh utilitzant els valors $\epsilon^{15}\text{N}$ calculats experimentalment, juntament amb dades isotòpiques de NO_2^- , valors de preferència pel lloc de N_2O , i concentracions aquoses de N_2O obtingudes amb la llei de Henry, han confirmat la quimiodesnitrificació com la principal via de degradació del NO_2^- en els experiments. S'ha implementat un model numèric geoquímic que considera quimiodesnitrificació, fraccionament isotòpic cinètic, especiació aquosa i precipitació i dissolució de calcita en equilibri que reproduïx els resultats experimentals. Aquest tipus de model podria ajudar a predir l'eficiència de la reducció abiòtica de nitrit que s'observa en tractaments de biorremediació on s'utilitza Fe(II) i facilitaria la distinció entre accions biòtiques i abiòtiques al camp.

Els efluent que surten de les plantes de tractament d'aigües residuals mostren sovint altes concentracions de nitrogen inorgànic. Garantir l'ús segur d'aquestes aigües residuals tractades per a la recàrrega d'aqüífers subterranis i per a aplicacions domèstiques requereix la reduir la concentració d'aquest nitrogen inorgànic. En aquest sentit, s'ha provat que les barreres permeables reactives són solucions efectives i sostenibles per a aconseguir la degradació del nitrogen inorgànic. En aquesta tesi, també s'ha estudiat el comportament d'un efluent d'una planta de tractament d'aigües residuals al creuar dos sistemes d'infiltració: un amb una barrera

reactiva (SAT-PRB) i l'altre sense (SAT-Control). S'han realitzat anàlisis químiques i isotòpique per avaluar la variabilitat espacial i temporal de les espècies aquoses de nitrogen (principalment NH_4^+ i NO_3^-) en diferents seccions i profunditats dels sistemes. Els resultats químics i isotòpics han mostrat que la nitrificació i la desnitrificació es donen de manera simultània i que són la principal via de transformació i distribució de nitrogen als sistemes. La nitrificació va augmenta al llarg dels sistemes i amb la profunditat. La temperatura i el creixement i adaptació de la comunitat microbiana han influït en l'eficiència de l'atenuació del nitrogen en els sistemes. Les temperatures altes han afavorit la desnitrificació als sistemes durant els períodes estivals. S'han identificat altres mecanismes, com ara la sorció i l'assimilació per part de les plantes, com a possibles contribuents a l'eliminació del nitrogen (N).

Al final del període d'estudi, el sistema SAT-PRB va eliminar amb èxit el 66% del N total de la solució. En comparació, el sistema SAT-Control va assolir una taxa d'eliminació del 69%, mesurada a la sortida dels dos sistemes. El NO_3^- és l'espècie dominant en el sistema SAT-Control, amb un 27% de NO_3^- i un 4% de NH_4^+ , mentre que el sistema SAT-PRB ha presentat una distribució més equilibrada, amb proporcions aproximadament iguals de NO_3^- i NH_4^+ , del 18% i el 16%, respectivament. Les dades isotòpiques han revelat la degradació completa de NO_3^- en els sistemes durant els períodes més càlids, indicant la possible mescla de NO_3^- de nova generació amb el NO_3^- residual del substrat.

Aquesta tesi aporta nous coneixements, valuosos per al disseny d'estratègies efectives d'eliminació de nitrats mitjançant donadors d'electrons sostenibles a través de la injecció de compostos orgànics, Fe(II) i barreres reactives permeables. Les troballes ofereixen solucions potencials per mitigar la contaminació per nitrats de les fonts d'aigua, preservant-ne la qualitat i assegurant un subministrament sostenible d'aigua neta. Proporciona mètodes respectuosos amb el medi ambient per salvaguardar la salut humana i el medi ambient dels impactes adversos de la contaminació per nitrats. No obstant això, la realització d'investigacions addicionals i la implementació pràctica d'aquestes estratègies són essencials per aconseguir impactes positius duradors en els ecosistemes aquàtics i garantir un futur més saludable per a tothom.

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1. GENERAL INTRODUCTION

1.1. State of the art

1.1.1. Groundwater nitrate pollution

Groundwater is an essential consumptive water source that provides drinking water to about 50% of the world's population and contributes 40% of irrigation water (Abascal et al., 2022; Canter, 2019). Goal 6 of the Sustainable Development Goals (SGD: Clean water and sanitation) set out in the 2030 agenda by the United Nations is to provide high-quality drinking water (United Nations, 2022). However, groundwater pollution has enormously increased over the past three decades, impacting life and the environment (Li et al., 2021). For instance, the WHO (2022) has reported that poor drinking water quality is the leading cause of diseases and death worldwide.

Nitrogen is an essential nutrient required for the growth and survival of all living organisms. Nitrogen fertilizers are an important anthropogenic nitrogen source that contributes 40% of the world's dietary protein (Smil, 2001) and has increased 40% crop production in the past century (Mosier, 2008). Excess nitrogen from nitrogen-based fertilizers is oxidized in aerobic conditions into nitrate (NO_3^-), as a significant and one of the most prevalent groundwater pollutants. Excessive fertilizer use, therefore, represents the largest NO_3^- pollution source globally (Arauzo, 2017). Moreover, domestic and industrial wastewater mostly contain organic nitrogen, and as wastewater leaves the septic system and percolates through the unsaturated zone, organic nitrogen is oxidized to nitrate plumes within the aquifer. Consequently, septic system discharge also contributes significantly to nitrate and contaminants of emerging concern.

The ingestion of water containing high NO_3^- levels ($> 50 \text{ mg NO}_3^- \text{ L}^{-1}$) affects human well-being and causes health problems, including methemoglobinemia in children and a prolonged effect of gastric, colorectal and bladder cancer in adults (Schullehner et al., 2018; WHO, 2022). High nitrate concentration in groundwater dependent bodies promotes algae growth, causing eutrophication, which depletes aquatic life (Withers et al., 2014). Therefore, the European Union nitrate directive has set a threshold limit of $50 \text{ mg NO}_3^- \text{ L}^{-1}$ for the safe consumption of water (EU parliament, 2020; Massarelli et al., 2021; WHO, 2022).

Nitrate concentrations have gradually increased in many parts of the world in the last few decades above this threshold value and have sometimes doubled over the past 50 years in some countries. Figure 1 shows the global nitrate concentration pattern compiled from reported studies from 2013 to 2020 by Abascal et al. (2022).

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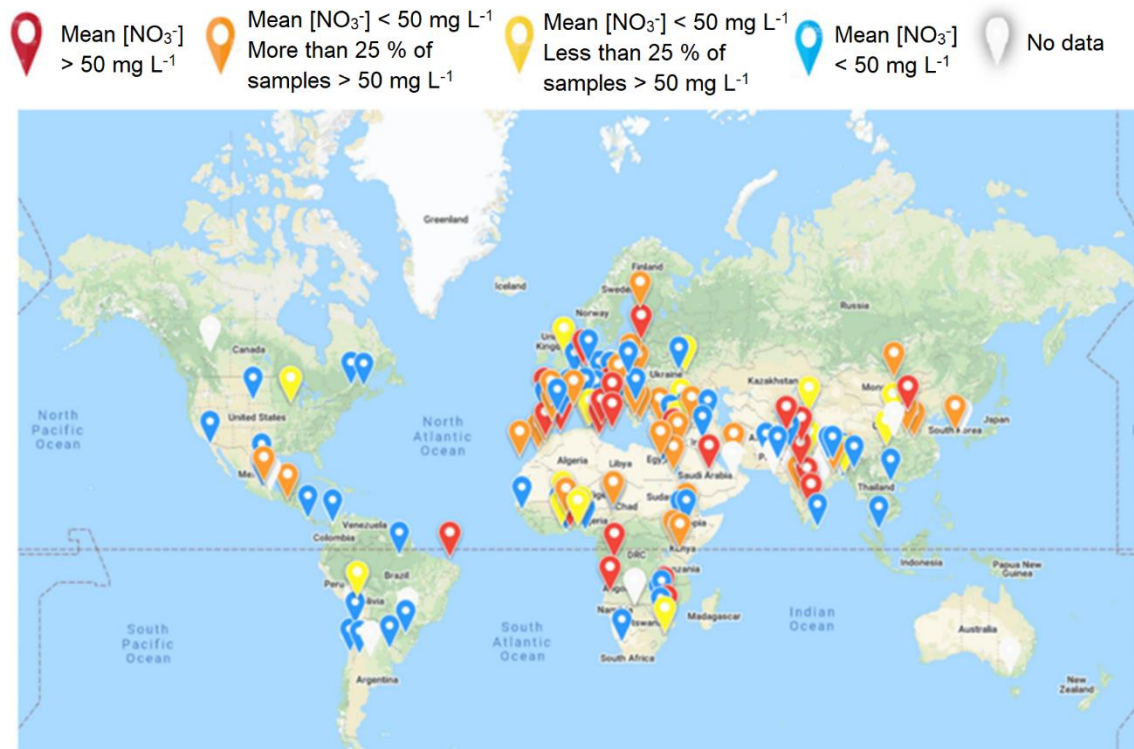


Figure 1. Global groundwater nitrate pollution (Abascal et al., 2022).

It is, therefore, imperative to mitigate groundwater nitrate to regulatory limits to render water safe for human consumption. Nitrate can be transformed into other N compounds depending on the aquifer and environmental conditions. Integrated groundwater management and protection against nitrate contamination require a systematic and scientific understanding of the biogeochemical processes and reactions that control nitrate transformation.

Nitrogen, depending on its source, enters the groundwater in an organic or inorganic form. N-based compounds, such as amino acids, proteins, humic substances, and organic fertilizers, provide organic nitrogen, whereas chemical fertilizers and partially or untreated wastewater discharge mostly contain inorganic nitrogen sources, usually in the form of NH_4^+ (Canter, 2019; Grzyb et al., 2021; Hatfield and Follett, 2001; Larramendy, 2016). The form that nitrogen exists in groundwater depends on environmental conditions, such as Eh, pH, O_2 concentration and temperature. Nitrate is produced mainly by nitrification and transformed by denitrification, dissimilatory nitrate reduction to ammonia (DNRA) and anaerobic ammonium oxidation (anammox) reactions (Figure 2).

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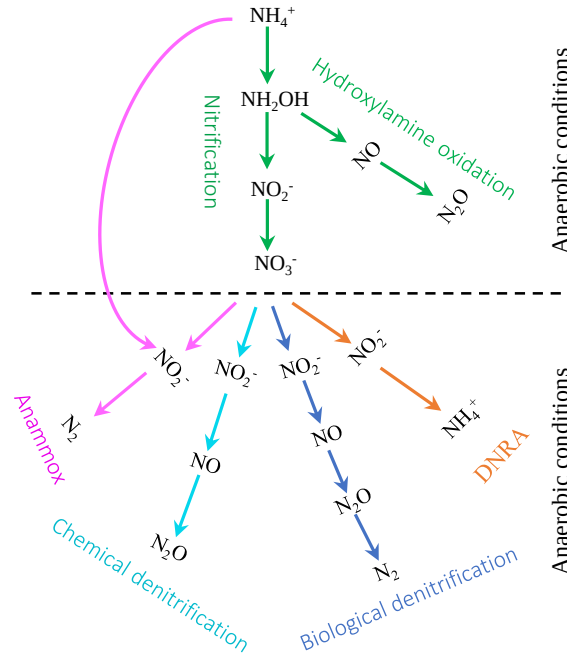
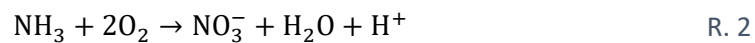
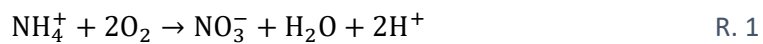


Figure 2. Nitrate production and degradation pathways.

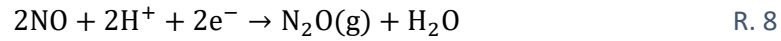
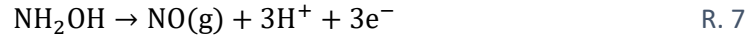
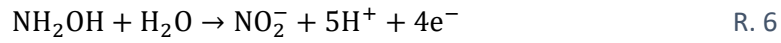
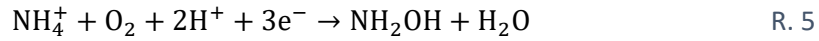
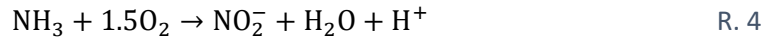
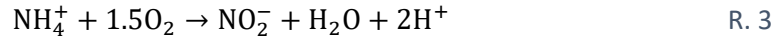
1.1.2. Nitrification, the primary nitrate production pathway

Biochemical nitrogen mineralization of organic matter in the environment transforms organic nitrogen into aqueous NH_4^+ . Nitrification is the oxidation of this ammonium or free ammonia, which is ubiquitous in the environment, to nitrate (R. 1 and R. 2) through intermediate steps (Figure 2), mainly driven by chemolithoautotrophic nitrifying bacteria.



Nitrification plays a crucial role as it serves as the only recognized source of nitrate production, facilitated by two distinct groups of microorganisms. During the first step of nitrification, ammonia or ammonium is oxidized to nitrite (R. 3 and R. 4, respectively). This conversion process involves ammonia-oxidizing bacteria (AOB), which utilize oxygen as the electron acceptor through reactions R. 5 and R. 6 (Soler-Jofra et al., 2021; Teske et al., 1994). The intermediary hydroxylamine (NH_2OH) produced in R. 5 is chemically reactive and can also be chemically oxidized to nitric oxide (R. 7), which is further reduced to nitrous oxide, according to R. 8 (Figure 2). Consequently, not all the NH_2OH produced during nitrification is converted to nitrite, but it can result in the formation of $\text{NO}(\text{g})$ and $\text{N}_2\text{O}(\text{g})$ as important greenhouse gases that can escape into the atmosphere and cause depletion of the ozone (O_3) layer.

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During the second step of nitrification, nitrite is oxidized to nitrate mediated by nitrite-oxidizing bacteria (NOB), according to reaction R. 9.



Nitrification occurs in varying environmental conditions and is influenced by factors regulating the ammonium supply, such as nitrogen mineralization, immobilization and plant assimilation. High ammonium immobilization by heterotrophs and plant uptake decreases available NH_4^+ content, as well as the nitrification rate. On the other hand, nitrification is promoted by elevated O_2 concentration because all the known nitrifiers are obligate aerobes, and different types of obligate aerobes have been reported in surface and subsurface waters (Kampschreur et al., 2008; Robertson and Groffman, 2007). Nonetheless, some nitrifiers can use O_2 from nitrite as an electron acceptor during respiration (Robertson and Groffman, 2007). Nitrification in groundwater is often limited because of the low dissolved O_2 content of most groundwater and is active only in oxidized zones around rhizospheres and sediment-water interface. Nitrification is highly sensitive to pH and requires a neutral to slightly alkaline optimal pH of 7.0 to 8.0 (Amatya et al., 2011).

Nitrification provides H^+ to the solution that tends to lower pH in poorly buffered environments, inhibiting nitrifying bacteria's enzymatic activity and reducing the nitrification rate (Robertson and Groffman, 2006). Nitrification efficiency increases with increasing temperatures and is inhibited at temperatures below 5 °C and higher than 45 °C, with optimal temperatures between 28 °C and 32 °C (Fdiz-Polanco et al., 1994; Gerardi, 2003).

1.1.3. Groundwater nitrate degradation pathways

1.1.3.1. Denitrification

Denitrification is the primary nitrate removal process in groundwater aquifers and involves the stepwise reduction of nitrate to nitrite and gaseous nitrogen products (Figure 2). Biological denitrification (R. 10) is the dissimilatory reduction of nitrate to dinitrogen gas through a set of

biochemical reduction steps (R. 11 - R. 14) (Table 1) catalyzed by reductive enzymes (Hiscock et al., 1991; Ni et al., 2016).

Table 1. Nitrate reduction steps during biological denitrification (*nar*, *nir*, *nor*, and *nos* are nitrate, nitrite, nitric oxide, and nitrous oxide reductase enzymes, respectively).

		GLOBAL DENITRIFICATION REACTION $\text{NO}_3^- + 12\text{H}^+ + 10\text{e}^- \rightarrow \text{N}_2 + 6\text{H}_2\text{O}$	R. 10
Step 1	<i>nar</i>	$\text{NO}_3^- + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$	R. 11
Step 2	<i>nir</i>	$\text{NO}_2^- + 2\text{H}^+ + \text{e}^- \rightarrow \text{NO} + \text{H}_2\text{O}$	R. 12
Step 3	<i>nor</i>	$2\text{NO} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{N}_2\text{O} + \text{H}_2\text{O}$	R. 13
Step 4	<i>nos</i>	$\text{N}_2\text{O} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{N}_2(\text{g})$	R. 14

Electron donors are the main factor controlling groundwater biological denitrification and are classified into organic substrates (mainly organic carbon sources) and inorganic substrates, which comprise inorganic carbon, sulfur-based and iron-based substrates and composite compounds (Faisal et al., 2018; Liu et al., 2013; Ma et al., 2020; Zhao et al., 2022). Most denitrifying microorganisms are facultative anaerobic heterotrophs that utilize organic substrates as electron donors and molecular oxygen or nitrogen oxides as electron acceptors to generate energy for cell synthesis and growth (Robertson and Groffman, 2007). These denitrifying bacteria synthesize denitrifying enzymes for denitrification at oxygen concentrations below 0.2 mg L^{-1} (Hiscock et al., 1991; Seitzinger et al., 2006). Autotrophic denitrifying bacteria, on the other hand, use inorganic carbon sources, such as carbon dioxide and bicarbonate, as oxidizable substrates (autotrophic denitrification). These bacterial are also capable of using hydrogen (hydrogenotrophic biological denitrification), reduced iron (II) metals and minerals, such as zero valent iron (ZVI), pyrite, siderite, olivine and magnetite (chemolithoautotrophic denitrification), or reduced sulfur compounds, such as S^0 , H_2S , S^{2-} and SO_3^{2-} (sulfur autotrophic denitrification), as oxidizable electron donors (Liu et al., 2013).

Temperature is an essential factor that influences the growth of the microbial community and, consequently, the biogeochemical processes that control groundwater nitrate transformation. Many denitrifying bacteria can adapt to changes in temperature, and denitrification has been observed over a wide range of temperatures (12 to 45 °C), but it is optimal at temperatures between 25 and 30 °C (Qu et al., 2022). Low temperatures below 15 °C limit denitrifying microbial activities and slow down oxidation-reduction reactions, reducing the electron transfer for nitrate removal in groundwater (Rivett et al., 2008).

The pH for heterotrophic denitrification ranges between 5.5 and 8.0, with optimum nitrate removal often observed at 7.0 to 7.5, but the optimal pH is site-specific due to the

acclimatization and adaptation of the microbial community (Rivett et al., 2008). Sulfur-driven autotrophic denitrification can occur in slightly acidic conditions at a pH range of 4.0 to 8.0 (Ma et al., 2020).

The hydraulic retention time, defined as the contact period of the nitrogen contaminant with the electron donor substrate, significantly influences the denitrification rate. In favorable conditions, the slow flow of contaminant plumes prolongs the contact of microorganisms with nitrates, facilitating nitrate removal. Longer retention times may also cause secondary reactions and, consequently, lead to the generation of secondary contaminants. Pesticides, including aldrin, atrazine, lindane, and captan, and heavy metals ($\text{Cd} > \text{Cu} > \text{Zn} > \text{Pb}$, in order of toxicity) have been observed to affect the performance of soil and groundwater microorganisms and the reactions mediated by these microorganisms (Bååth, 1989; Sáez et al., 2003).

Besides biological denitrification, chemical denitrification also contributes to significant nitrate loss, but in this case, step 3 of Table 1 (R. 13) is the last reaction step. Because the chemical reduction of N_2O to N_2 requires a significant amount of energy, N_2O reduction is very slow, and it is mostly unfavorable in the groundwater environment, making N_2O the end-product. Chemical denitrification (Figure 2) is the chemical redox coupling between nitrogen oxide species and reduced metals, mainly Fe(II). Iron is the most prevalent redox active metal on the earth as particulate ferric Fe(III) or ferrous Fe(II) iron-bearing minerals or as dissolved ions (Kappler and Straub, 2005). Iron is ubiquitous in most groundwater environments, and the redox transformation of iron and nitrogen are often coupled, affecting the fate of nitrogen oxides (Kampschreur et al., 2011). Nitrate has relatively high chemical stability, and biological processes dominate its attenuation. However, the intermediate nitrite generated during biological nitrate transformation is chemically reactive and can be reduced simultaneously by biological and chemical means. The chemical reduction of nitrite has been found to occur in acidic conditions at $\text{pH} < 6$ in groundwater (Dhakal et al., 2013). Nitrite reduction by zerovalent iron in groundwater treatment is quick, requires low cost and maintenance and is acid driven with optimal reduction occurring at pH less than 4 and leads to the production of ammonium, which requires further treatment to remove nitrogen from water (Huang et al., 1998; Young et al., 1964; Zhu and Getting, 2012).

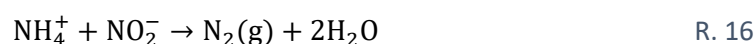
1.1.3.2. Dissimilatory nitrate reduction to ammonium (DNRA)

DNRA is the dissimilatory reduction of nitrate to ammonium by facultative and fermentative bacteria in anaerobic conditions, generating nitrite as an intermediate product (Luvizotto et al., 2019; Mohan and Cole, 2007) (Figure 2). During DNRA, nitrate is reduced to ammonium,

conserving N in groundwater, and further treatment is required to remove the ammonium. DNRA occurs in environmental conditions similar to biological denitrification and competes for nitrate in groundwater (Giblin et al., 2013; Rütting et al., 2011). However, the electron donor to nitrogen ratio, often expressed as the molar C/N ratio, is the primary factor controlling nitrate reduction partitioning between DNRA and biological denitrification. At limiting nitrogen oxides (high C/N ratio), DNRA may outcompete denitrification (Friedl et al., 2018; Pan et al., 2020; Tiedje, 1988; Van den Berg et al., 2016). The C/N ratio favorable for DNRA or denitrification depends on the type of organic substrate, the experimental method, the bacteria type, and the oxygen concentration. Different authors have proposed various C/N ratios for DNRA and denitrification. For instance, Yin et al. (1998) identified DNRA at C/N ratios greater than 12 in soils (Van den Berg et al., 2016). DNRA has shown importance at C/N ratios greater than 5 in groundwater studies (Burgin and Hamilton, 2007); however, in batch and column denitrification experiments, C/N ratios ≥ 1.6 were identified to favor DNRA (Carrey et al., 2014). DNRA has been found to occur in neutral to slightly alkaline conditions (Jahangir et al., 2017; Kraft et al., 2014; Yoon et al., 2015).

1.1.3.3. Anaerobic ammonium oxidation (Anammox)

Ammonium contamination often occurs due to surface activities of composting and landfilling (Erskine, 2000), animal waste disposal (Umezawa et al., 2008), fertilization (Barcelona and Naymlk, 1984), and septic system effluent (Aravena and Robertson, 1998). During anammox reactions (Figure 2), ammonium is anaerobically oxidized to nitrogen gas by anammox bacteria using nitrate and nitrite as an oxidant and contributes to the loss of a large portion of ammonium nitrogen in low-oxygen marine environments and aquifers (Wang et al., 2020). A mix of partial nitrogen products (NH_4^+ , NO_2^- and NO_3^-) tends to cause anammox activity in anaerobic conditions and involves a two-step process: the oxidation of NH_4^+ to NO_2^- by ammonium-oxidizing bacteria (nitrification, R. 15) and the subsequent reduction of NO_2^- to $\text{N}_2(\text{g})$ by anammox bacteria (R. 16) (Cho et al., 2020).



Anammox bacteria responsible for these transformations are primarily autotrophic and are widely distributed in the subsurface aquifer, which provides anammox bacteria with an ideal environment for growth and N-remediation (Wang et al., 2020, 2019; Xing and Clark, 2011). The anammox process is strongly influenced by temperature, and a wide range of temperatures have been reported for anammox reactions, as low as -5°C and as high as 80°C , but the optimum

temperature for anammox bacteria growth is between 30 and 40°C (Cho et al., 2020; Egli et al., 2001; Jin et al., 2012; Tomaszewski et al., 2017). Water pH can also influence anammox bacteria, causing an accumulation of inhibitory compounds, which slow down or impede anammox reactions. Anammox reactions have been observed for pH between 6.5 and 9.0, with an optimum pH of 7.0 to 8.0 (Carvajal-Arroyo et al., 2014; Egli et al., 2001; Tomaszewski et al., 2017). At low pH, nitrite reduction slows down, raising nitrite levels, which inhibits anammox reactions, but the level of inhibition varies between treatment systems and with different inoculation sources. Like denitrification, high levels of heavy metals (Cu, Zn, Hg, Cd, Ag, and Pb) inhibit anammox processes as anammox bacteria cannot degrade and accumulate the heavy metals in their cells, disrupting cell metabolism (Val del Río et al., 2017).

1.1.4. Nitrate pollution mitigation

Understanding groundwater nitrate degradation processes is crucial for designing effective techniques to remediate nitrate contamination. In this section, the pathways of groundwater nitrate attenuation, both through natural processes and induced methods applied in situ or ex situ, are discussed. Nitrate pollution mitigation is challenging due to the prolonged presence of nitrate and the diffuse and continuous nature of its pollution (Rave et al., 2006; Rivett et al., 2008). The widespread hazards of nitrate pollution have sparked global research interest in decontaminating groundwater.

Various mitigation measures are employed to address nitrate pollution, including natural attenuation and induced attenuation techniques. Biostimulation treatments are used to enhance the activity of denitrifying bacteria by introducing organic and/or inorganic electron donors into the aquifer. Laboratory biostimulation studies exploring the potential of different electron donors, such as whey (Margalef-Martí et al., 2019b), glucose (Carrey et al., 2014), methanol, ethanol, and acetate (Peng et al., 2007), and compost (Grau-Martínez et al., 2017), in removing nitrates have been investigated. Although laboratory-scale research has been promising, successfully upscaling these treatments to field conditions requires pilot-scale studies, which often resemble field conditions. Acetate and ethanol (Martin et al., 2009) have shown success in pilot-scale studies. However, pure organic carbon sources like glucose, acetate, methanol, and ethanol are expensive and seldom used for long-term treatments. Some biostimulation nitrate treatments that have yielded positive results at the laboratory and pilot scale have been successfully applied in the field, which includes the use of fumarate (Park et al., 2019), formate (Killingstad et al., 2002), hydrogen and carbon dioxide (Evans et al., 2011), acetic acid (Margalef-Martí et al., 2019c) and ethanol (Vidal-Gavilan et al., 2014, 2013).

Furthermore, sulfur and iron mineral compounds, like pyrite, have been utilized to enhance denitrifying bacterial activity (Straub et al., 1996; Till et al., 1998; Torrentó et al., 2011). However, some of these compounds can lead to secondary contamination issues, such as the release of toxic metals or increased sulfate concentration in water.

1.1.4.1. Natural nitrate attenuation

Natural nitrate attenuation is the physical and biochemical process that causes groundwater nitrate removal without changes to the natural state of the aquifer conditions. The physical nitrate attenuation process includes phytoremediation, and the biochemical processes include denitrification and anammox. Phytoremediation involves the direct uptake of nitrates by vegetation and immobilization by microorganisms, and it is applicable in areas with shallow groundwater and low nitrate concentration, where groundwater nutrients are reachable by plant roots (Zhao et al., 2022). As explained in section 1.1.2, chemical and biological denitrification and anammox reactions tend to remove nitrate from groundwater, whereas DNRA only alters the nitrogen form, and these processes can occur alone or in combination (Green and Bekins, 2010; Kazumi and Capone, 1994; Singleton et al., 2007; Vitòria et al., 2008). The microbial communities responsible for nitrate degradation are ubiquitous in groundwater aquifers, but the limited bioavailable electron donors only support a low denitrification rate and, thus, limit natural denitrification (Hiscock et al., 1991; Jacinthe et al., 1998).

1.1.4.2. Induced nitrate attenuation

The induced nitrate attenuation approach has become a strategy to create optimized conditions by providing external organic substrates to supplement the inherent aquifers' organic carbon to overcome the natural limitation of organic carbon in groundwater aquifers. The organic carbon in aquifers is the main bacterial energy-generating electron donor source in the subsurface. Facultative aerobic bacteria preferentially oxidize organic carbon with O_2 , and nitrogen oxides become the energetically favorable electron acceptors when the O_2 is consumed to low levels. Facultative and obligate anaerobes then use the remaining organic carbon to oxidize the nitrogen oxides in anaerobic respiration.

Induced nitrate attenuation methods could be applied as in-situ or ex-situ remediation, depending on the mode of application. Ex-situ remediation is applied above the ground in treatment systems, whereas in-situ remediation is a contaminant site treatment often applied below the groundwater level. Enhanced in-situ bioremediation technique has emerged as a significant and widely adopted technology to remove groundwater nitrate using engineered technology that causes physical, chemical, and biological changes to the subsurface

environment to create the necessary conditions for microorganisms to transform contaminants (EPA, 2013; Rave et al., 2006). During bioremediation, microbial reagent strains may be added to facilitate contaminant removal, or nutrients may be supplied to enhance the activity of the existing microorganisms to degrade contaminants (biostimulation). Bioremediation techniques are economical and can be adapted to the conditions of the contaminated site (EPA, 2013).

In the design, implementation and operation of in-situ biostimulation systems, it is essential to characterize the contaminant site in detail, considering the aquifer type, baseline geochemistry, and lithology (EPA, 2013). They must be carefully designed to avoid generating secondary contaminants such as hydrogen sulfide gas from dissimilatory sulfate reduction in the presence of excess organic carbon during denitrification. The successful design of in-situ biostimulation systems encompasses laboratory-scale (micro-scale) and pilot-scale (meso-scale) treatability studies to evaluate the design criteria and its efficiency before the fully-fledged field-scale study. The design criteria include the electron donor feeding strategy and sustainability. In-situ biostimulation techniques depend on the delivery method of organic carbon into the subsurface environment. These include permeable reactive barriers (PRB) (Beganskas et al., 2018; Gibert et al., 2019), constructed wetlands (Adhikari et al., 2015; Beutel et al., 2009; Luo et al., 2018; Rampuria et al., 2020; Vymazal et al., 2020) and underground injection systems (Critchley et al., 2014; Margalef-Marti et al., 2019c).

1.1.4.2.1. Permeable Reactive Barriers (PRBs)

Permeable Reactive Barriers (PRBs) provide a dynamic and efficient approach to remediating contaminated groundwater and have successfully been applied to remove nitrates, dissolved metal and petroleum hydrocarbons (Guerin et al., 2002; Powell et al., 1995; Zhang et al., 2012). They are passive methods, require no external energy consumption during operation, and can be used for long periods. PRBs consist of a reactive media emplaced vertically or horizontally in the subsurface perpendicular to the groundwater flow path to intercept and transform nitrates into environmentally acceptable forms downstream of the reactive barrier by chemical or biological reactions (Faisal et al., 2018). The horizontal installation configuration is often preferred during soil aquifer recharge due to the advantage of initiating contaminant attenuation before the recharge water infiltrates the aquifer. In contrast, vertical PRBs are mainly used to treat horizontal flow nitrate contaminant plumes. PRB remediation mechanisms depend on the composition of the reactive media, which must be appropriate to the subsurface environment and not create secondary contaminants. The reactive material must be low-cost, sustainable for an extended period, readily available, and near the contamination site to achieve

nitrate remediation at a minimal economic cost. Organic substrates reactive media such as plant-based compost and woodchips (Beganskas et al., 2018; Hellman et al., 2022; Su and Puls, 2007; Valhondo et al., 2020a, b), sawdust (Robertson et al., 2008), mulch (Gibert et al., 2019), and activated carbon (Ghaeminia and Mokhtarani, 2018), have successfully been employed on meso-scale and field-scale biological denitrification treatments. Zerovalent iron has been used as an inorganic reactive material in PRBs to induce chemical denitrification (Geranio, 2007; Su and Puls, 2007). Chemical denitrification induced by the ZVI often leads to the generation of ammonium, and in anaerobic conditions, ZVI may corrode to generate hydrogen, which can be used as an electron donor by autotrophic denitrifiers. Applying mixed organic-inorganic reactive materials (spongy iron and pine bark: Huang et al., 2015) helps overcome the limitation of a single reactive material and improves the efficiency of nitrate remediation. In the design of PRBs, adsorbent materials, such as activated carbon, zeolites and limestones, can be implemented to sorb intermediate products such as ammonium produced during nitrate transformation.

1.1.4.2.2. Constructed wetlands (CWs)

CWs for wastewater treatment are engineered systems designed to attenuate wastewater contaminants in a controlled environment using natural processes such as wetland vegetation, soil, and microorganisms. In terms of the position of the water surface, CWs are described as either surface flow or subsurface flow CW. In surface flow CWs, water flows horizontally above the wetland substrate (soil and/or gravel) and is exposed to the atmosphere. In subsurface flow CWs, water flows horizontally and/or vertically through the wetland substrate below its surface. Surface flow CWs are designed to treat surface wastewater, including agricultural runoff, industrial and urban wastewaters, sewage, and landfill leachate, before infiltrating the groundwater aquifer.

CWs are easy to operate and require low installation and maintenance costs, providing a sustainable and efficient alternative to removing nitrate in water resources. Biological processes, including phytoremediation and bioremediation, mainly contribute to nitrate attenuation in CWs. Vegetation assimilates and accumulates nitrate in roots, shoots, and leaves. Microorganisms attached to the surfaces of submerged wetland vegetation, plant litter, and the wetland-substrate degrade or sorb organic substances. Macrophytes provide organic carbon from plants' biomass and root exudates to biologically enhance denitrification. However, these natural organic carbon compounds are insufficient to maintain the electron donor demands for long-term nitrate removal to statutory levels. External electron donors are, therefore, needed to meet the electron donor demands for high nitrate removal (Gagnon et al., 2007; Ma et al.,

2020). Diverse electron donor sources including wheat hay, corn stubble, and animal compost (Margalef-Martí et al., 2019a), elemental sulfur, sulfur-based limestones and natural sulfur ores (Bezbaruah and Zhang, 2003; Ge et al., 2019; Sierra-Alvarez et al., 2007; Yiping Zhang et al., 2019; Yongwei Zhang et al., 2019), iron (Till et al., 1998), and H_2 (Kurt et al., 1987), high nitrates retention plants, such as Lemna, Phragmites and Cattail (Adhikari et al., 2015; Ceschin et al., 2019; García et al., 2020; Saeed and Sun, 2017), have been used in CWs to enhance biological denitrification. External electron donors in surface flow CWs are easily degraded and must be frequently replaced (Li et al., 2014).

1.1.4.2.3. *Underground injection systems (UISs)*

Underground injection systems involve the emplacement of electron donors through injection wells into the subsurface to facilitate nitrate attenuation. Solid, liquid or gaseous electron donors are injected into groundwater aquifers to degrade contaminants chemically or biologically (Evans et al., 2011; Hosseini and Tosco, 2015; Park et al., 2013, 2019). The type of injection well depends on the aquifer's depth and properties and electron donor type. It ranges from low-technology constructions, such as shallow dug wells and natural sinkholes with surface modifications, to highly engineered constructions (EPA, 2013). Injection wells must be appropriately sited, constructed and operated to maintain environmental safety conditions. The efficiency of nitrate attenuation during electron donor injection depends on the electron donor feeding strategy, including the electron donor to nitrogen ratio and the electron donor injection periodicity, which are often determined from laboratory experiments (Margalef-Martí et al., 2019b; Vidal-Gavilan et al., 2014).

1.1.4.3. *Monitoring nitrate transformations in groundwater*

Chemical and multi-isotopic tools are useful methods to monitor nitrate transformation processes. During nitrification, the unreacted NH_4^+ is often characterized by an enrichment of the heavy isotope of nitrogen, ^{15}N (Fukada et al., 2003; Knöller et al., 2011). ^{14}N of NH_4^+ is preferentially incorporated into the generated NO_3^- , decreasing ^{15}N of NO_3^- (Figure 3) and accumulating ^{15}N in the residual NH_4^+ . $\delta^{15}N$ of NH_4^+ values can also be influenced by the volatilization of NH_3 in vadose zones, which tends to increase $\delta^{15}N$ of the residual NH_3 .

In NO_3^- , the initial $\delta^{15}N$ is derived from $\delta^{15}N$ of NH_4^+ , and $\delta^{18}O$ has been experimentally determined to come from two-thirds of the oxygen atoms of water and one-third from the oxygen atoms of atmospheric oxygen during nitrification according to Eq. 1 (Andersson and Hooper, 1983).

$$\delta^{18}\text{O}_{\text{NO}_3} = \frac{1}{3} \delta^{18}\text{O}_{\text{O}_2} + \frac{2}{3} \delta^{18}\text{O}_{\text{H}_2\text{O}} \quad \text{Eq. 1}$$

During NO_3^- transformation, the initial $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isotopic composition of NO_3^- is altered. The unreacted NO_3^- is characterized by an enrichment of the heavy isotopes (^{15}N and ^{18}O) during denitrification, DNRA or plant uptake (Fukada et al., 2003; Knöller et al., 2011) as the light isotopes preferentially react faster, accumulating heavy isotopes in the residual substrate and increasing $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ of the unreacted NO_3^- (Figure 3).

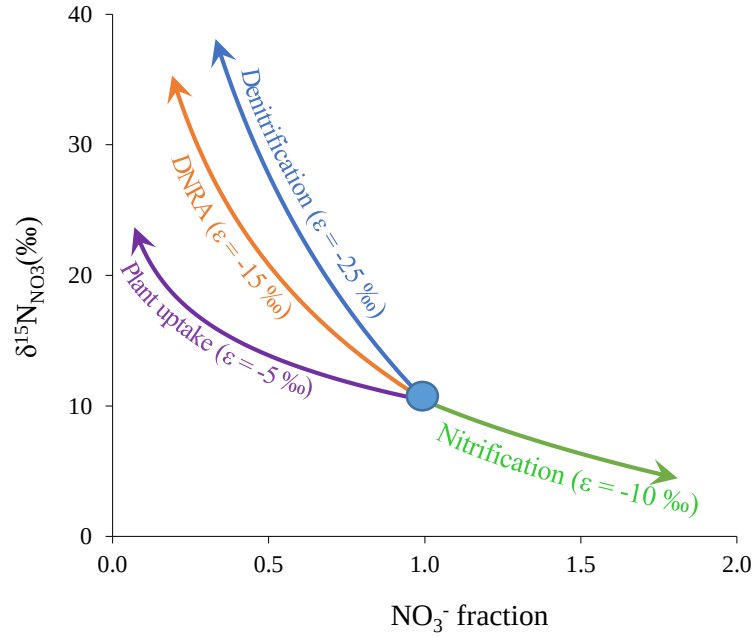


Figure 3. The effect of nitrate transformation on $\delta^{15}\text{N}_{\text{NO}_3}$ (modified after Ryabenko, 2013).

The kinetic isotope effects of nitrogen transformation processes are presented in terms of their fractionation factors (ϵ), showing the isotope enrichment of a transformation product relative to the substrate, which, in a closed system, follows the Rayleigh equation (Eq. 2), where R_0 and R_t are the isotope ratio of the original and residual substrate at time zero and time t , respectively, C_0 is the initial analyte concentration at time zero in mol L^{-1} , C_t is the analyte concentration at time t in mol L^{-1} , and ϵ is the kinetic isotope fractionation in ‰ (Mariotti et al., 1981).

$$\ln (R_t/R_0) = \epsilon \cdot \ln (C_t/C_0) \quad \text{Eq. 2}$$

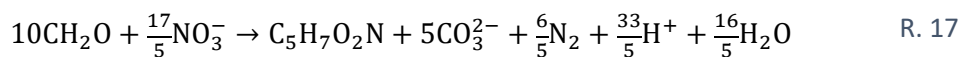
The magnitude of fractionation associated with nitrification, denitrification, anammox and DNRA reactions is influenced by environmental conditions, such as the substrate concentration, the availability of electron donors, concentration of dissolved oxygen, temperature, pH and residence time (Böttcher et al., 1990; Nikolenko et al., 2018). Therefore, isotope fractionation is site-specific and helps in quantifying the efficiency of nitrate degradation in field-scale studies.

1.1.5. Geochemical modeling

Geochemical modeling is a useful tool in groundwater contaminant remediation studies. It helps to evaluate complex processes and to predict the extent and consequences of geochemical reactions beyond the range of laboratory experiments. It addresses the challenges related to measuring gaseous denitrification products (NO , N_2O and N_2) and the low concentrations of soluble components (NO_3^- and NO_2^-) using analytical methods. Therefore, the utilization of geochemical models offers a more effective solution for addressing such complexities (Zhu, 2012). There is a great number of computer programs and codes for geochemical modeling, such as EQ3/6 (Wolery, 1992), SOLMINEQ.88 (Kharaka et al., 1988), PHREEQC (Parkhurst and Appelo, 2013) and MINTEQA2 (Allison et al., 1991), among others. PHREEQC is one of the most used codes in modeling hydrogeochemical processes; it can cope with 1D transport calculations, is compatible with several open-source databases and is easy to use.

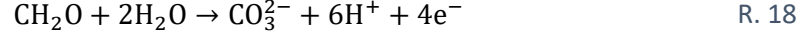
Computer models have been used to simulate and quantify denitrification at laboratory scales and field conditions to test remediation strategies. Denitrification models have become essential tools for integrating the current understanding of the processes that control denitrification with measurements of rate-controlling properties for field-scale application. Models of varying scope and complexity have been developed, ranging from simple empirical equations to complex simulation models to estimate how environmental conditions such as the Eh, pH and temperature interact to control denitrification. The representation of various denitrification models varies widely among approaches.

Complex simulation approaches have been developed based on how the organic and inorganic species are linked. In one-step process models, both the electron donor and the acceptor are treated kinetically in a single step (R. 17), where the electron donor (e.g. CH_2O) reacts with a known electron acceptor (NO_3^-) to produce one mole of biomass (expressed as $\text{C}_5\text{H}_7\text{O}_2\text{N}$) (Lensing et al., 1994; Schäfer et al., 1998a, 1998b).



Two-step process models are based on the Partial Equilibrium Approach (PEA), which assumes that the degradation of organic compounds occurs in two steps (Jakobsen and Postma, 1996; McNab and Narasimhan, 1994). First, oxidation of the organic compound releases electrons by R. 18 and the subsequent consumption of the electrons by the electron acceptors, according to R. 10, and both processes co-occur. During reaction R. 10, microorganisms consume the organic compounds as the energy source for biomass growth, producing electrons. The

second reaction, R. 18, balances the production of electrons using nitrate as the electron acceptor.



In the context of the PEA, various simplifying assumptions are made to facilitate the use of mass action equations in describing equilibrium between all aqueous phases. These assumptions are tailored to the specific geochemical system being studied, and the key assumptions include the rate-limiting character of the oxidation of organic matter (R. 18), the absence of internal NO_3^- production source, and limited occurrence of aqueous complexation reactions involving electron acceptors (Brun and Engesgaard, 2002). The nitrate degradation pathway discussed in section 1.1.2 is influenced by the geochemical conditions of the environment, which ultimately dictate its energetic favorability (Jin and Bethke, 2003). Several authors have modeled biological denitrification in groundwater in two steps (Breukelen et al., 2004; Kinzelbach et al., 1991; Rodríguez-Escales et al., 2016a, 2014).

Nitrogen compounds degradation pathways are primarily redox reactions driven by microorganisms, and their respiration rate depends on the electron donor and acceptor concentration. The microbial respiration rate in groundwater studies is qualified mainly by the dual Monod kinetics. The Monod kinetics, according to Eq. 3, is a mathematical model used to describe the growth rate of the denitrifying microorganisms in a controlled environment with a limiting nutrient concentration where R_{EA} is the rate of electron acceptor degradation (R_{NO_3} for NO_3^- degradation and R_{NO_2} for NO_2^- degradation in $\text{mol L}^{-1} \text{ s}^{-1}$). $k_{\text{max}}[\text{X}_{\text{EA}}]$ is the maximum respiration rate being, k_{max} the maximum rate of nutrient utilization (s^{-1}), and $[\text{X}_{\text{EA}}]$ the biomass concentration (mol L^{-1}). The electron donor kinetic factor depends on the electron donor concentration $[\text{ED}]$ (mol L^{-1}) and K_{ED} , the electron donor half-saturation constant (mol L^{-1}), whereas the electron acceptor kinetic factor depends on the electron acceptor concentration $[\text{EA}]$ (mol L^{-1}) and K_{EA} , electron acceptor half-saturation constant (mol L^{-1}).

$$R_{\text{EA}} = -k_{\text{max}} \cdot [\text{X}_{\text{EA}}] \cdot \left(\frac{[\text{EA}]}{[\text{EA}] + K_{\text{EA}}} \right) \cdot \left(\frac{[\text{ED}]}{[\text{ED}] + K_{\text{ED}}} \right) \quad \text{Eq. 3}$$

When the energy in the environment is the same or slightly higher than the microbial cells' energy, the microbial respiration rate becomes zero, and when the energy in the environment is lower than the cells' energy, the respiration rate becomes negative. Therefore, the respiration rate of the microbial community influences the growth rate and decay of the microbial population. The growth rate of the bacterial community is linked with the respiration rate according to Eq. 4, where $d[\text{X}_{\text{EA}}]/dt$ is the microbial growth rate ($\text{mol L}^{-1} \text{ s}^{-1}$), Y is the bacterial growth yield (moles C cells/moles C DOC) and b is the decay constant (s^{-1})

$$\frac{d[X_{EA}]}{dt} = -Y \cdot R_{EA} - b \cdot [X_{EA}] \quad \text{Eq. 4}$$

Reactive metals, primarily ferrous iron, can serve as catalysts for the abiotic reduction of oxidized nitrogen compounds in groundwater environments. Nitrite is highly chemically reactive and undergoes reduction to form nitrous oxide, a potent greenhouse gas. This reduction process is linked to the oxidation of the reactive metals, and the degradation rate of the nitrogen compounds is commonly quantified using second-order kinetics, reflecting the complex interplay between chemical reactions and environmental factors.

1.2. Scope and objectives of the thesis

Nitrate contamination is Spain's most significant groundwater pollution problem (Figure 1). For instance, in Catalonia (NE Spain), 46% of the groundwater is in a bad status ($\text{NO}_3^- > 50 \text{ mg NO}_3^- \text{ L}^{-1}$), mainly from agricultural activities (Micola Colomer et al., 2018).

Many areas in Catalonia (and Spain at large) have recorded nitrate concentrations above the safe drinking water threshold value. Consequently, these areas have been declared NO_3^- vulnerable zones (European Commission, 2021; Micola Colomer et al., 2018). This declaration necessitates the implementation of treatment measures to ensure the provision of clean water for both residential and industrial purposes. Figure 4 shows the current state of groundwater nitrates in Catalonia.

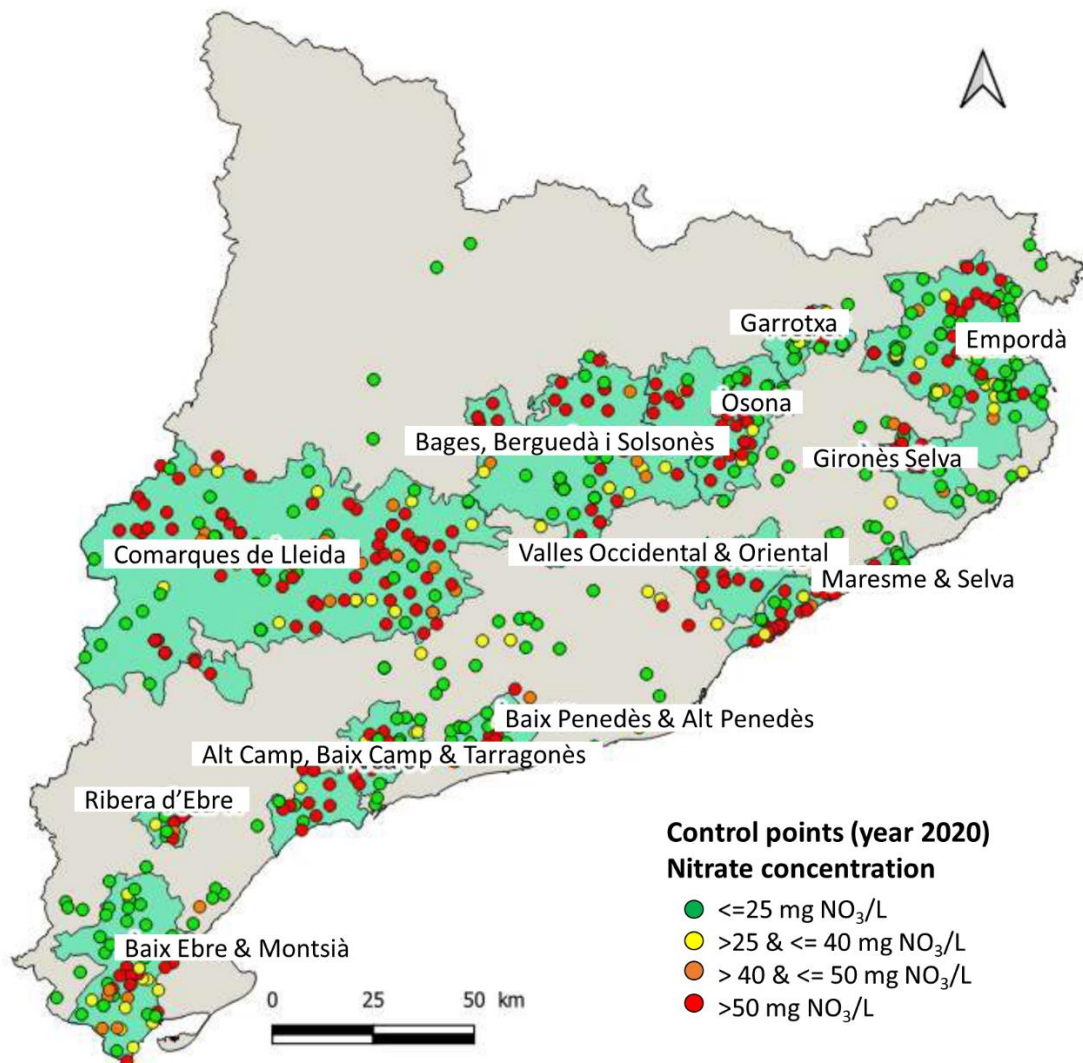


Figure 4. Groundwater nitrate vulnerable areas and location of sampling points in Catalonia (modified after Agència Catalana de l'Aigua, 2021).

The dry, hot summers and the high touristic development of the northeastern coast of Spain (Mediterranean Spain) cause recurring draught along the coastal communities (Gabarda-Mallorquí et al., 2016; Rodríguez-Villanueva and Sauri, 2021). Consequently, water reuse has become an important alternative to the water stress problem in Mediterranean Spain. Therefore, effluent from wastewater treatment systems is used to recharge the aquifers to supplement the groundwater and improve its quality. However, these effluents contain high levels of nitrogen compounds, mainly ammonium, which oxidizes into nitrate in aerobic conditions, contaminating the groundwater aquifers. Treatment options are, therefore, required during aquifer recharge to remove nitrogen compounds.

In this context, it is necessary to continue researching alternative sources of low-cost electron donors that represent an economic and environmental benefit and allow the evaluation of industrial waste products or minerals through their use in biostimulation (circular economy). At the same time, bioremediation strategies must continue to be improved to optimize induced attenuation performance. The generation of adverse by-products (such as NO_2 and H_2S) and the emission of gases into the atmosphere (such as $\text{N}_2\text{O(g)}$ and $\text{CO}_2\text{(g)}$) should be avoided since decontamination should not transfer pollution from one compartment to another. For this reason, it is necessary to make a detailed hydrochemical and isotopic characterization of nitrogen compounds to understand the reactions that affect contaminant attenuation and to characterize N_2O emissions into the atmosphere during denitrification processes. The analysis of the isotopic composition of N_2O and its isotopomers is being used to determine the origin of this compound (Well et al., 2012; Wunderlin et al., 2013), as N_2O presents different isotopic values depending on its production source. The generation of N_2O during biostimulation processes is not usually monitored, and therefore, its characterization during induced attenuation processes could help define the attenuation processes.

The main objective of the doctoral thesis is to advance the knowledge of the mechanisms of remediation of groundwater contaminated by agricultural and urban activities based on biostimulation of denitrification by means of chemical, isotopic and numerical modeling tools.

Taking this into account, the specific objectives derived from the main objective are:

- Study the denitrification potential of industrial wine wastes as organic electron donor sources in batch and column experiments.
- Study of the chemodenitrification potential of aqueous Fe(II) and siderite (Fe(II) mineral) in laboratory batch experiments with particular emphasis on the dynamic behavior of nitrite reduction and nitrous oxide gas production.

General introduction

- Use chemical and multi-isotopic tools to quantify the treatment efficiencies of nitrogen compounds attenuation and determine the isotopic fractionation for field application in all the studied cases.
- Study the biochemical processes that control the fate of N compounds (NH_4^+ , NO_3^- and NO_2^-) transformation using plant-based compost as a permeable reactive barrier in soil aquifer treatment operations.
- Develop conceptual and numerical geochemical models able to reproduce the evolution of NO_3^- , NO_2^- , non-purgeable dissolved organic carbon (NPDOC) concentrations, and $\delta^{15}\text{N}$ of NO_3^- , NO_2^- and N_2O of the denitrification processes studied to serve as a tested tool for potential future field-scale applications.

1.3. Thesis outline

This thesis comprises of three international peer-reviewed papers: two have been published, one requires minor revisions, and it also includes seven conference abstracts (four international and three national). Table 2 provides a comprehensive overview of these contributions.

Table 2. Summary of the various papers and the contributions to conferences from this thesis.

Paper/ Conference	Title	Journal/conference name	Year
PAPER 1	Enhancing Nitrate Removal with Industrial Wine Residue: Insights from Laboratory Batch and Column Experiments using Chemical, Isotopic, and Numerical Modeling Tools.	Water Resources Research (Minor revisions)	2024
PAPER 2	Isotopic and geochemical modeling approach to evaluate abiotic nitrite reduction by ferrous iron.	Chemical Geology https://doi.org/10.1016/j.chemgeo.2024.121942	2024
PAPER 3	Pathways and efficiency of nitrogen attenuation in wastewater effluent through soil aquifer treatment.	Journal of Environmental Management https://doi.org/10.1016/j.jenvman.2022.115927	2022
Conference 1	Numerical modelling of enhanced biodenitrification in a laboratory flow-through experiment.	II Jornada de Jóvenes Investigadores SEM / SEM-Cat, Barcelona, Spain.	2021
Conference 2	Isotopic characterization of NO_2^- and N_2O during abiotic NO_2^- reduction by ferrous Fe(II).	3rd European Mineralogical Conference (emc2020), Cracow, Poland.	2021
Conference 3	The role of siderite on abiotic nitrite reduction by dissolved Fe(II).	XXXIX Reunión Científica de la Sociedad Española de Mineralogía, Baeza, Spain. (Best communication of young researchers)	2022
Conference 4	The potential of wine residue to induce biodenitrification in a groundwater flow constructed wetland.	The 12th International Geochemistry of the Earth's Surface Symposium (GES12), Zurich, Switzerland. https://zenodo.org/doi/10.5281/zenodo.6829032	2022
Conference 5	The efficiency of different electron donor sources in biological denitrification processes.	Goldschmidt2023, Lyon, France. https://doi.org/10.7185/gold2023.19118	2023
Conference 6	Denitrification biostimulation using wine industry by-products.	WRI-17 & AIG-14 (Water-Rock Interaction & Applied Isotope Geochemistry), Sendai, Japan.	2023
Conference 7	Nitrate attenuation potential using wine residues in laboratory experiments.	GeoSpeeches 2023, University of Barcelona, Spain.	2023

The first paper, PAPER 1, currently undergoing minor revisions, explores the utilization of wine wastes as electron donors in laboratory batch and column biological denitrification studies using nitrate-contaminated water from the Lerma basin (Spain). Additionally, this paper presents a conceptual and numerical model of biological denitrification based on the batch and column experiments.

PAPER 2 focuses on the use of inorganic electron donors, specifically ferrous iron, in a laboratory batch chemodenitrification study. This paper also includes conceptual and numerical modeling to enhance the understanding of the denitrification process.

In PAPER 3, the application of plant-based compost permeable reactive barriers for treating secondary wastewater effluent during aquifer recharge is evaluated. The study was conducted as a pilot study in Palamós, NE Spain.

Figure 5 is a summary of the research undertaken in the scope of this thesis.

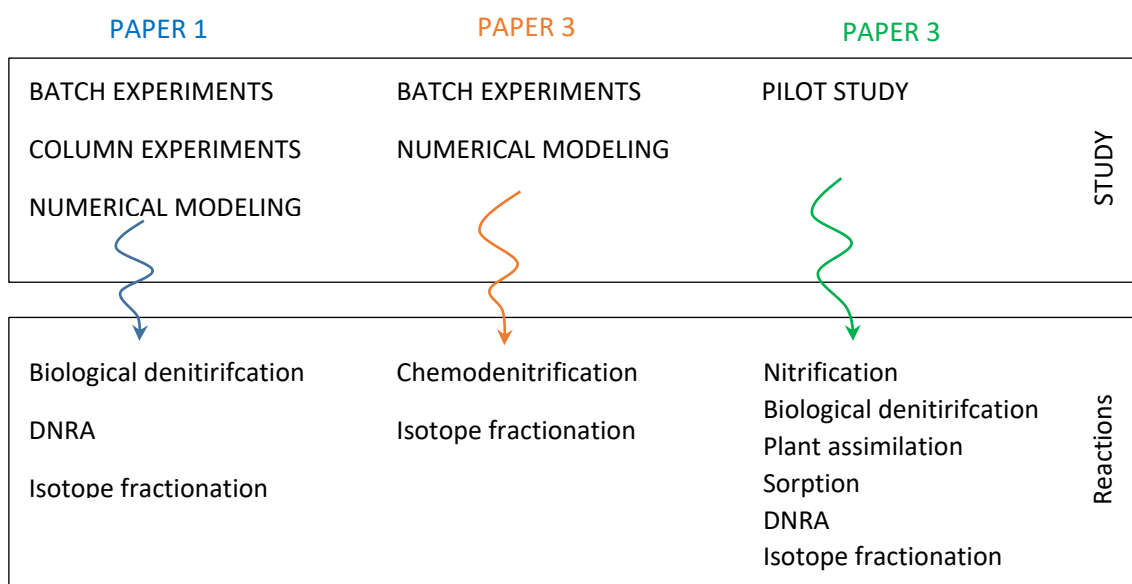


Figure 5. A scheme of the research areas and the relationship between the obtained publications.

1.3.1. PAPER 1: Laboratory treatability study of nitrate-contaminated water

This first paper aims to advance the knowledge on inducing denitrification using industrial waste products. It investigated the ability of industrial wine residues to induce denitrification in laboratory experiments using field water from the Lerma basin (Spain), a small hydrological basin covering 7.38 km² on the left bank of the Ebro basin in the municipality of Ejea de los Caballeros in northeastern Spain (Figure 6A).

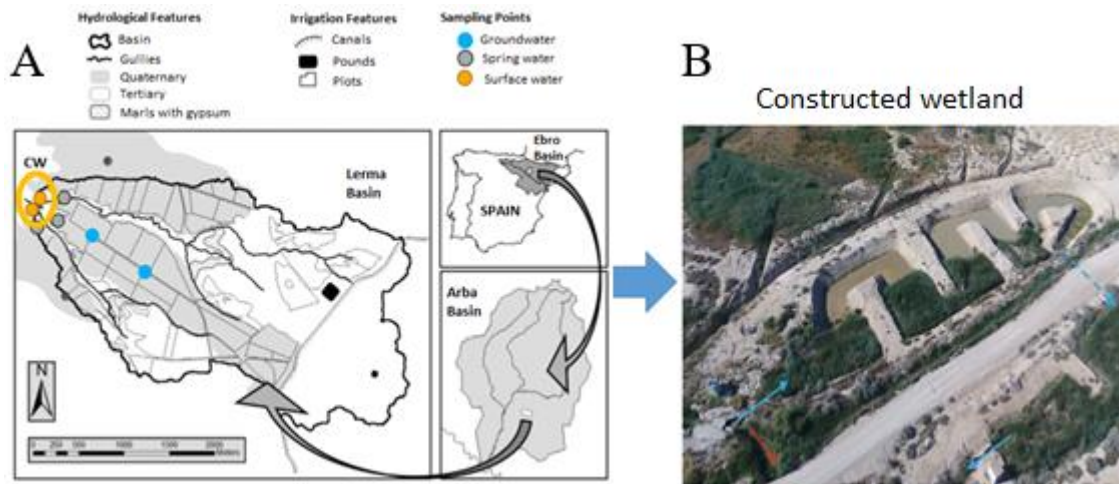


Figure 6. A). Location of the Lerma basin in the Arba and Ebro basins (Spain) and the simplified geology. B). Design of the surface flow constructed wetland located northwest of the Lerma basin.

The Lerma basin has a semi-arid Mediterranean climate with a temperature range of 5 to 40 °C and a mean annual temperature of 14 °C. The area experiences mean yearly rainfall of 468 mm and is characterized by alternating wet (spring and autumn) and dry (summer and winter) seasons (Merchán et al., 2013). Agricultural return flows from the Lerma Basin have increased N compounds concentration in the drainage basin, triplicating N exports to receiving water bodies (Merchán et al., 2015, 2014, 2013). In order to diminish the N exports to the surrounding water bodies, a surface flow CW covering an area of approximately 2500 m² has been constructed at the northwestern margin of the basin (Figure 6B). Surface water in the watershed is diverted to the CW, where nitrate-contaminated runoff is treated before discharge.

This paper aimed to search for alternative cheap and readily available industrial electron donor sources that can be applied in this CW to reduce nitrate concentration before discharging the agricultural runoff. To achieve this, laboratory batch and column experiments were performed to evaluate the potential of two industrial wine residues to provide an eco-friendly bioremediation solution to nitrate while providing waste recycling (circular economy) before future field-scale application. The industrial wine residues were obtained from Destileria San Valero Sociedad Cooperativa Limitada, Zaragoza (Spain), close to the study site. "Lías" was the residue produced during the first stage of fermentation of leece and pomace, and "vínico" during the second. Two batch experiments were performed, B-L and B-V, each biostimulated with *lías* and *vínico*, respectively (Figure 7). Based on preliminary batch treatments, 13 batch reactors were chosen for each set and biostimulated with the wine residues, and a column experiment, biostimulated with only "lías" (C-L), was chosen based on the results of the batch experiments.

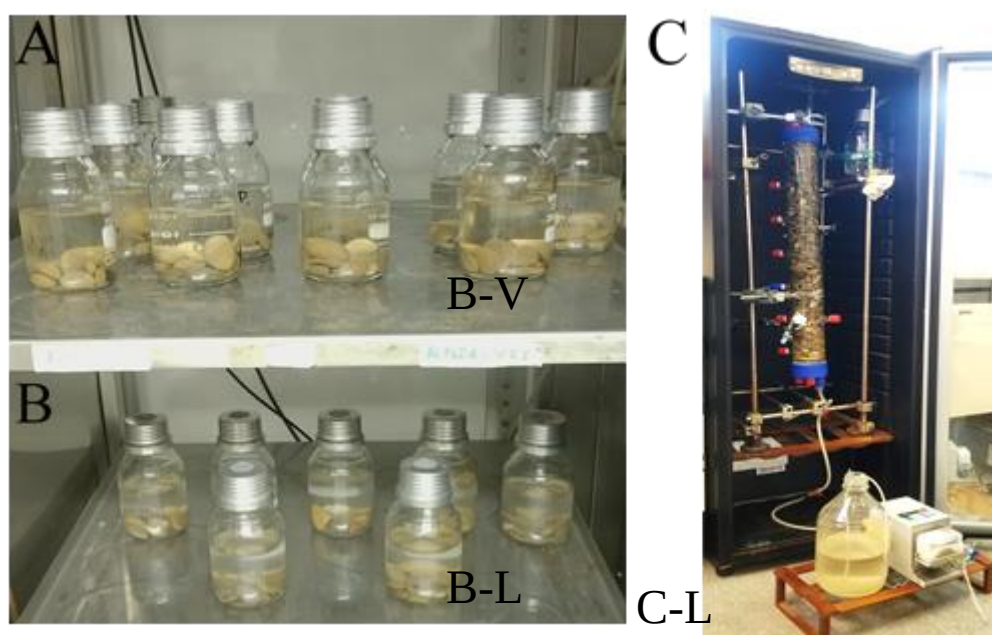


Figure 7. A) The experimental set-up of batch experiments biostimulated with A) *lías* and B) *vínico* C) experimental column set-up. B-L and B-V are batch experiments biostimulated with *lías* and *vínico*, respectively, and C-L is a column experiment biostimulated with *lías*.

Chemical and multi-isotopic tools were used to quantify the efficiency of the batch and column bioremediation treatments. Samples for chemical and isotopic analyses were prepared at the laboratory of the MAiMA research group and analysed according to the analytical methods and in the laboratories summarized in Table 3.

Table 3. A summary of the main analyses, the analytical method employed, and the laboratory used in PAPER 1.

Analyses	Analytical method	Laboratory
pH, Eh and conductivity	pH, Eh and conductivity meters	Laboratory of MAiMA-UB research group
Major Cations	Inductively coupled plasma optical emission spectroscopy (ICP-OES)	Parc Científic de Barcelona
Trace elements	Inductively Coupled Plasma Mass Spectrometry (ICP-MS)	Parc Científic de Barcelona
Anions	Ion chromatography (IC)	Parc Científic de Barcelona and UPC
NPDOC	Organic matter combustion method	CCiT-UB
HCO ₃	Titration method	CCiT-UB
$\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO ₃	Cadmium reduction method	CCiT-UB
$\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO ₂	Sodium azide reduction method	CCiT-UB

Isotopic analyses were carried out using the Finnigan MAT 253, Thermo Scientific, Isotope Ratio Mass Spectrometry (IRMS) and notation (δ) was expressed per mille of (‰), and $\delta^{15}\text{N}$ was

reported against international atmospheric N₂ (AIR) and $\delta^{18}\text{O}$ against the international Vienna Standard Mean Oceanic Water (V-SMOW). International and laboratory standards used to normalize the isotopic results are summarized in Table 4.

Table 4. International and laboratory (CCiT-UB) standards used for the normalization and reproducibility of the isotopic analysis in PAPER 1.

Analysis	Standard	Reference value [‰]	Reproducibility (1 σ)
$\delta^{15}\text{N}_{\text{NO}_3}$	USGS-32	+180.0	± 1.0 ‰
	USGS-34	-1.8	
	USGS-35	+2.7	
	CCiT-IWS	+16.9	
$\delta^{18}\text{O}_{\text{NO}_3}$	USGS-32	+25.3	± 1.5 ‰
	USGS-34	-27.9	
	USGS-35	+57.3	
	CCiT-IWS	+28.5	
$\delta^{15}\text{N}_{\text{NO}_2}$	CCiT-IWS-NO2	-28.5	± 1.0 ‰

To interpret the findings, chemical and isotopic results were integrated with geochemical numerical models, using the Phreeqc and GibbStudio codes (Nardi and de Vries, 2017; Parkhurst and Appelo, 2013) to simulate the experimental results and establish a foundation for future field studies.

Alex Tiewin Abu was involved in the methodology, preparation of samples for chemical and isotopic analyses, curation and formal analysis of the experimental data, numerical modeling, writing of the original draft of the paper, and managing the revisions.

1.3.2. PAPER 2: Abiotic nitrite reduction with ferrous iron

Because chemodenitrification often coexists with biological denitrification in the presence of reduced metals and Fe(II) minerals, this article focuses on the performance of nitrite reduction by ferrous iron in laboratory batch experiments. Nitrite reduction potential was tested in 3 scenarios using a NO_2^- bearing solution amended with: i) siderite (Sid), ii) synthetic Fe(II) solution (DFe) and iii) siderite mixed with synthetic Fe(II) solution (Sid+DFe) (Figure 8).

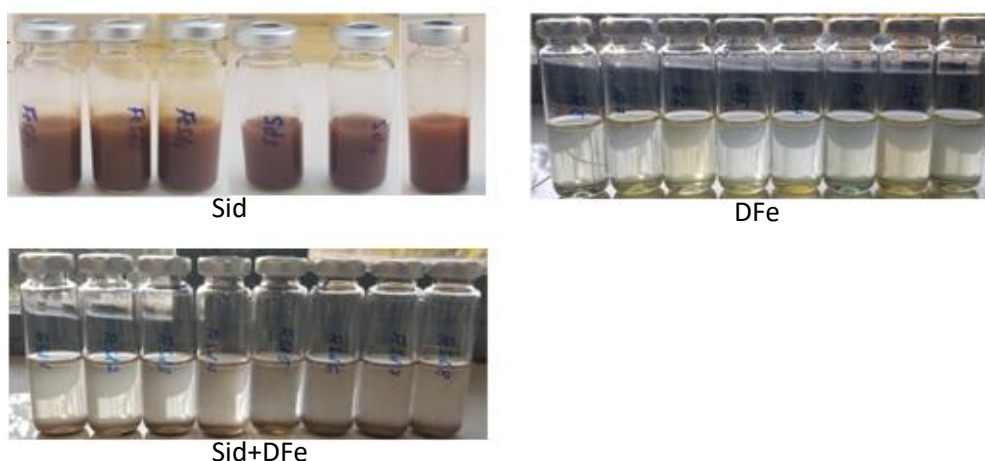


Figure 8. The experimental set-up for the experiments of Sid (A), DFe (B) and Sid+DFe (C).

This work was partly carried out at the Research Cluster on Environmental Chemistry and Microbiology (Pôle CME) of the Institute of Analytical Sciences and Physico-Chemistry for Environment and Materials (IPREM). Results of batch experiments carried out within the MAIMA research group were discussed with the Pôle CME research group, and new experiments were designed within the framework of a collaboration program between the two research groups to improve the previous methods.

The laboratory work, encompassing sample preparation and analysis, was conducted at the MAiMA research group laboratory and the environmental unit of the CCiT-UB (UMA), respectively. The analytical methods employed in this study and the laboratories used are presented in Table 5.

Table 5. A summary of the main analyses, the analytical method employed, and the laboratory used in PAPER 2.

Analyses	Analytical method	Laboratory
NO ₂ ⁻ concentration	Isotope Ratio Mass Spectrometry (IRMS)	CCiT-UB
N ₂ O concentration	Isotope Ratio Mass Spectrometry (IRMS)	CCiT-UB
$\delta^{15}\text{N}^{\text{bulk}}$ of N ₂ O	Isotope Ratio Mass Spectrometry (IRMS)	CCiT-UB
$\delta^{15}\text{N}^{\alpha}$ of N ₂ O	Isotope Ratio Mass Spectrometry (IRMS)	CCiT-UB
$\delta^{15}\text{N}$ of NO ₂	Refer to Table 3	Refer to Table 3

$\delta^{15}\text{N}^{\beta}$ and the site preference (SP) of N₂O were calculated according to Eq. 5 and Eq. 6, where $\delta^{15}\text{N}^{\text{bulk}}$ is the bulk $\delta^{15}\text{N}$ of N₂O(g) and $\delta^{15}\text{N}^{\beta}$ and $\delta^{15}\text{N}^{\alpha}$ are the $\delta^{15}\text{N}$ of the outer and central N of N₂O(g).

$$\delta^{15}\text{N}^{\text{bulk}}_{\text{N}_2\text{O}(\text{g})} (\text{‰}) = (\delta^{15}\text{N}^{\alpha}_{\text{N}_2\text{O}(\text{g})} + \delta^{15}\text{N}^{\beta}_{\text{N}_2\text{O}(\text{g})})/2 \quad \text{Eq. 5}$$

$$\text{SP} (\text{‰}) = (\delta^{15}\text{N}^{\alpha}_{\text{N}_2\text{O}(\text{g})} - \delta^{15}\text{N}^{\beta}_{\text{N}_2\text{O}(\text{g})}) \quad \text{Eq. 6}$$

International and internal working standards used to correct the isotopic results are summarized in Table 6.

Table 6. International and laboratory (CCiT-UB) standards used for the normalization and reproducibility of the isotopic analysis in PAPER 2.

Analysis	Standard	Reference value [‰]	Reproducibility (1 σ)
$\delta^{15}\text{N}_{\text{NO}_2}$	Refer to Table 3	Refer to Table 4	Refer to Table 4
$\delta^{15}\text{N}_{\text{N}_2\text{O}}$	USGS-51	+1.32	± 1.0 ‰
$\delta^{18}\text{O}_{\text{N}_2\text{O}}$	USGS-51	+41.23	± 1.0 ‰

Numerical modeling was performed using the GibbStudio code (Nardi and de Vries, 2017) and the Phreeqc code (Parkhurst and Appelo, 2013).

Alex Tiewin Abu contributed to the batch experimental design and sample preparation for chemical and isotopic analyses, the analysis and interpretation of the experimental data, the development of the conceptual and numerical model, and the writing of the original manuscript of the paper and also managed the revisions until the paper's final publication.

1.3.3. PAPER 3: Pilot soil aquifer treatment using PRBs

Partially treated wastewater effluents are recognized for having elevated levels of inorganic nitrogen, primarily ammonium. Ammonium is readily oxidized to other nitrogen compounds as a contaminant source, and the safe use of these effluents for domestic purposes and groundwater aquifer recharge necessitates ammonium nitrogen remediation. In order to address this concern, nitrogen compound treatment strategies must be employed to mitigate the high inorganic nitrogen levels in water treatment plant effluents.

Palamós is a coastal community located in northeastern Spain (Figure 9A) with a water stress problem that requires improving the quantity and quality of the groundwater resource through managed aquifer recharge. The work of this paper has been done in the frame of the RESTORA project (<https://restora.h2ogeo.upc.edu/>) to investigate the use of organic substrates to accelerate wastewater remediation during soil aquifer treatment (SAT) at the Palamós Waste Water Treatment Plant (WWTP). The WWTP covers an area of 3.3 Ha, with a design capacity of 33,000 m³/d for 165450 equivalent habitants (Figure 9B and C). The WWTP receives wastewater from 90000 inhabitants, which triples during the summer periods due to the touristic nature of Palamós, causing intensive seasonal variation of the plant influent, which may result in different effluent quality over the year (Gabarda-Mallorquí et al., 2016; Valhondo et al., 2020c). The WWTP employs the activated sludge and physical-chemical treatment processes going through

pre-treatment (waste retention), primary treatment, secondary treatment, and tertiary treatment (only applied for green irrigation areas). A pilot-scale experiment has been executed to assess the feasibility of enhancing the water quality during SAT by degrading nitrogen compounds.

The experimental pilot area, built within the WWTP facility, comprises six different tanks designed to receive secondary treatment effluent from the WWTP, simulating the natural recharge process (Figure 9D). PAPER 3 evaluates the behaviour of two of the tanks, T2 (SAT-Control) and T4 (SAT-PRB), to understand the biochemical processes that govern the fate and migration of the nitrogen compounds in the wastewater effluent. T4 was filled with sand, a small fraction of clay and plant compost as a PRB, while T2 was filled with only sand without the PRB. The Polytechnic University of Catalonia (UPC) designed and built the pilot experiment and performed four recharge episodes from February to December 2018. The first one was to condition the systems, and the remaining three were to study the performance of the systems.

General introduction

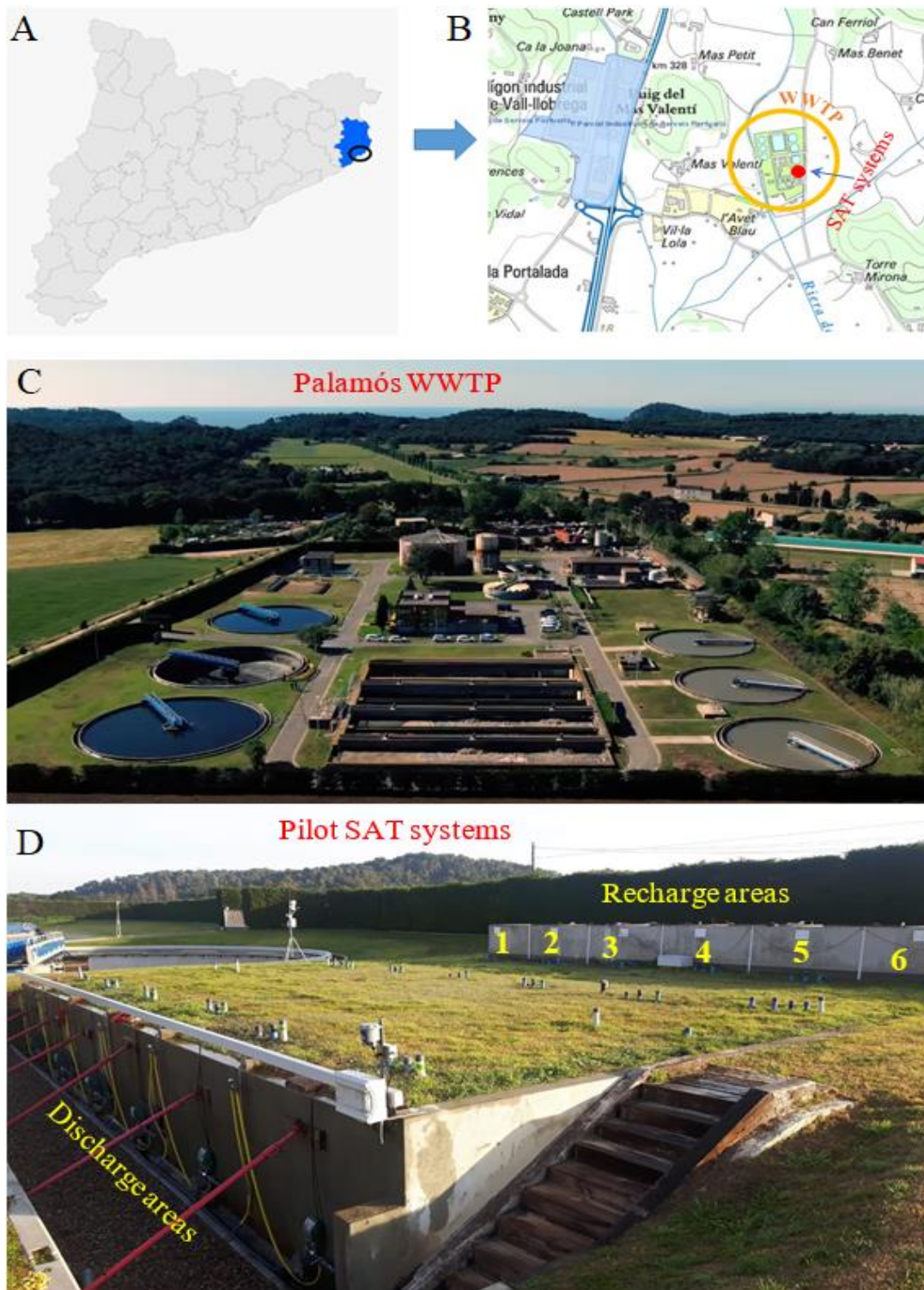


Figure 9. A) Location of Palamós (black circle). B). The topographical map shows the location of WWTP and the pilot SAT systems within Palamós. C). The general view of the WWTP. D). The general view of the SAT system showing the recharge and discharge areas of the tanks (1-6) and piezometers.

Field measurements were carried out at the experimental study site, where water samples were collected for laboratory analyses. The samples were then prepared at the MAiMA research group's laboratory for isotopic analyses. The analytical methods used in this study are

summarized in Table 7, and the international and laboratory standards used to normalize nitrate, nitrite and ammonium isotopic results are presented in Table 4 and Table 8, and ammonium isotopic results in Table 8.

Table 7. A summary of the main analyses, the analytical method employed, and the laboratory used in PAPER 3.

Analyses	Analytical method	Laboratory
Field O ₂ , pH, Eh, Conductivity and temperature	Multiparameter probe	UPC
NH ₄	ORION Ion-Selective Electrode	UPC
NPDOC	TOC-VCSH analyzer	UPC
Anions	Refer to Table 3	Refer to Table 3
$\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO ₃	Refer to Table 3	Refer to Table 3
$\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO ₂	Refer to Table 3	Refer to Table 3
$\delta^{15}\text{N}$ of NH ₄	sodium hypobromite and sodium azide reduction methods	CCiT-UB

Table 8. International and laboratory (CCiT-UB) standards used to correct isotopic results.

Analysis	Standard	Reference value [‰]	Reproducibility (1 σ)
$\delta^{15}\text{N}_{\text{NH}_4}$	USGS-25	-30.4	± 1.0 ‰
	IAEA-N2	+20.3	
	CCiT-IWS-NO2	-28.5	
	CCiT-IWS-NH4	-0.8	
$\delta^{15}\text{N}_{\text{NO}_3}$	Refer to Table 4	Refer to Table 4	Refer to Table 4
$\delta^{18}\text{O}_{\text{NO}_3}$	Refer to Table 4	Refer to Table 4	Refer to Table 4
$\delta^{15}\text{N}_{\text{NO}_2}$	Refer to Table 4	Refer to Table 4	Refer to Table 4

Alex Tiewin Abu was involved in sample preparation for laboratory isotopic analyses, data analyses, interpretation of the results, writing of the original manuscript and managed the revisions until the paper's final publication.

2. GLOBAL SUMMARY OF RESULTS

The groundwater nitrate remediation treatments studied in the framework of this thesis show a series of compelling findings in three distinct studies. In the first study investigating the potential of industrial wine wastes in the nitrate removal in laboratory batch and column experiments, the results show significant changes in NO_3^- , NO_2^- , NPDOC, and bicarbonate concentrations in both batch and column experiments, accompanied by significant changes in the isotopologues of NO_3^- and NO_2^- . The results of the second study about NO_2^- reduction with ferrous iron show intriguing nitrite attenuation dynamics accompanied by nitrous oxide generation. Results of the final study in the WWTP show the spatio-temporal variations in NH_4^+ , NO_3^- , NPDOC, and O_2 concentrations at different points along the groundwater flow path and at varying depths across different recharge episodes. These findings underscore the impacts of various nitrate remediation strategies for sustainable water management and isotopic composition dynamics, offering valuable information on nitrogen transformation and attenuation pathways.

2.1. PAPER 1: Laboratory treatability study of nitrate-contaminated water

The experimental results from batch and column studies demonstrate significant changes in various parameters following the injection of wine wastes despite the constant conductivity and pH in the experiments (Figure 10). In B-L and B-V, the injection of wine wastes led to a substantial increase in NO_3^- concentration, rising from 1.7 mM to 2.8 mM in B-L and to 2.6 mM in B-V within 12 hours (Figure 10a and c). Subsequently, B-L achieved complete NO_3^- removal within two days, while in B-V, it was reduced to 1.1 mM by day 5 (Figure 10a and c). No notable changes in NO_3^- concentration were observed in the control reactors during the study. Initially, NO_2^- slightly increased in B-L but was completely removed within three days, whereas in B-V, it increased to 1.3 mM and remained the same throughout the experiment (Figure 10a and c). C-L was biostimulated daily for four days, and NO_3^- was fully removed within three days and remained undetected in the outflow until day 7, after which it began recovering and reached full recovery by day 10 (Figure 10e). Unlike the batch experiments, C-L did not show an initial NO_3^- increase, and there was no significant NO_2^- accumulation in the outflow water (Figure 10e).

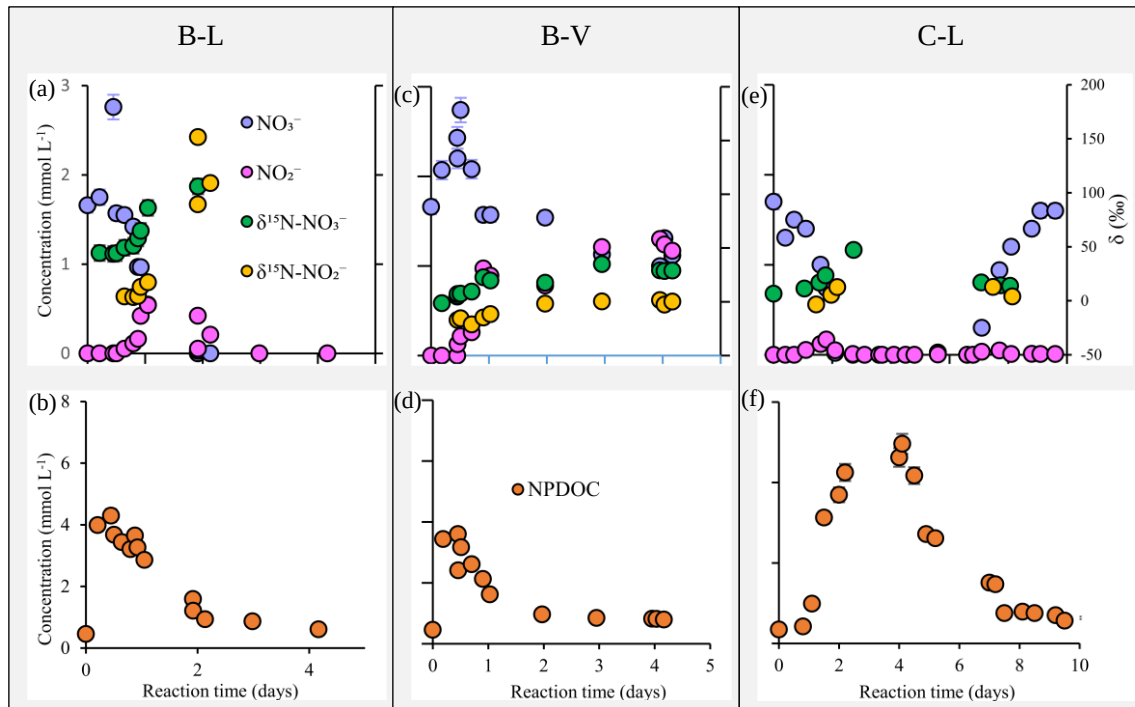


Figure 10. Changes in NO_3^- , NO_2^- and NPDOC concentration and the evolution of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- and NO_2^- in B-L, B-V and C-L. B-L and C-L represent batch and column experiments biostimulated with *lías*, and B-V is the batch experiment biostimulated with *vinico*.

The injection of the wine wastes caused NPDOC concentration to increase substantially in B-L (from 0.4 mM to 4.3 mM), B-V (from 0.4 mM to 3.6 mM), and C-L (from 0.4 mM to 5 mM) (Figure 10b, d and f). However, NPDOC levels gradually decreased towards the end of the experiments, reaching 0.6 mM (B-L), 0.8 mM (B-V), and 0.8 mM (C-L) (Figure 10b, d and f).

Global summary of results

Bicarbonate concentration also increased over time in response to organic carbon consumption, rising from 4.8 mM to 7.2 mM (B-L), 5.2 mM to 6.2 mM (B-V), and 2.3 mM to 6.1 mM (C-L).

The decrease in NO_3^- concentration in the experiments was linked with a progressive increase in the heavy isotopes of N and O atoms in both NO_3^- and NO_2^- . $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- exhibited notable enrichment, such as in B-L ($\delta^{15}\text{N}\text{-NO}_3^-$: +6.2 ‰ to +31.5 ‰; $\delta^{18}\text{O}\text{-NO}_3^-$: +4.2 ‰ to +23.2 ‰), B-V ($\delta^{15}\text{N}\text{-NO}_3^-$: -1.4 ‰ to +29.1 ‰; $\delta^{18}\text{O}\text{-NO}_3^-$: +6.9 ‰ to +32.2 ‰) and C-L ($\delta^{15}\text{N}\text{-NO}_3^-$: +6.6 ‰ to +23.5 ‰; $\delta^{18}\text{O}\text{-NO}_3^-$: +1.7 ‰ to +17.1 ‰) (Figure 10a, c and e). The $\delta^{15}\text{N}$ of NO_2^- also increased, from -18.5 ‰ to -10.2 ‰ in B-L, from -21.1 ‰ to -1.5 ‰ in B-V and -3.4 ‰ to +12.8 ‰ in C-L. Unlike $\delta^{15}\text{N}\text{-NO}_2^-$, the $\delta^{18}\text{O}\text{-NO}_2^-$ did not show a noticeable progressive increase in all the experiments due to its equilibration with $\delta^{18}\text{O}\text{-H}_2\text{O}$ during denitrification (Kool et al., 2007). These findings underscore the significant impact of wine waste injection on various parameters, particularly the removal of NO_3^- and the associated changes in isotopic compositions.

2.2. PAPER 2: Abiotic nitrite reduction with ferrous iron

The results showed that siderite alone had negligible nitrite reduction capacity, with only 3% removal observed in the Sid experiments. In those experiments performed with dissolved Fe(II), 54% of nitrite was removed. However, when siderite was combined with dissolved Fe(II) solution, the nitrite removal rate increased to 84%.

During nitrite attenuation, nitrous oxide gas was generated in the headspaces of the vials. Less to no $\text{N}_2\text{O}(\text{g})$ was detected in the Sid experiment. However, 0.187 mmol $\text{N}_2\text{O}(\text{g})/\text{L H}_2\text{O}$ and 0.194 mmol $\text{N}_2\text{O}(\text{g})/\text{L H}_2\text{O}$ of $\text{N}_2\text{O}(\text{g})$ were accumulated in DFe and Sid+DFe, respectively, by day 7. In the Sid+DFe experiments, the liquid-phase $\text{N-N}_2\text{O}$ constituted 50–96% of the transformed nitrogen in water and 68 to 98% in DFe experiments. Figure 11A, B and C show the transformed nitrogen and the recovered headspace $\text{N}_2\text{O}(\text{g})$ during nitrite reduction. The rate of $\text{N}_2\text{O}(\text{g})$ recovery in the headspace of the vials ranged from 19 to 46% (Figure 11D). Nitrite reduction was marked by an increase of $\delta^{15}\text{N-NO}_2^-$ over time, from -26.9 to -26.4 ‰ in Sid, -18.0 ‰ in DFe and -15.9 ‰ in Sid+DFe. On the other hand, $\delta^{18}\text{O-NO}_2^-$ values did not correlate with the residual NO_2^- concentration due to $\delta^{18}\text{O-NO}_2^-$ equilibration with $\delta^{18}\text{O-H}_2\text{O}$. The $\delta^{15}\text{N}^{\text{bulk}}$ values of the generated $\text{N}_2\text{O}(\text{g})$ also increased from -52.6 to -36.5 ‰ in DFe and from -57.1 to -38.6 ‰ in Sid+DFe.

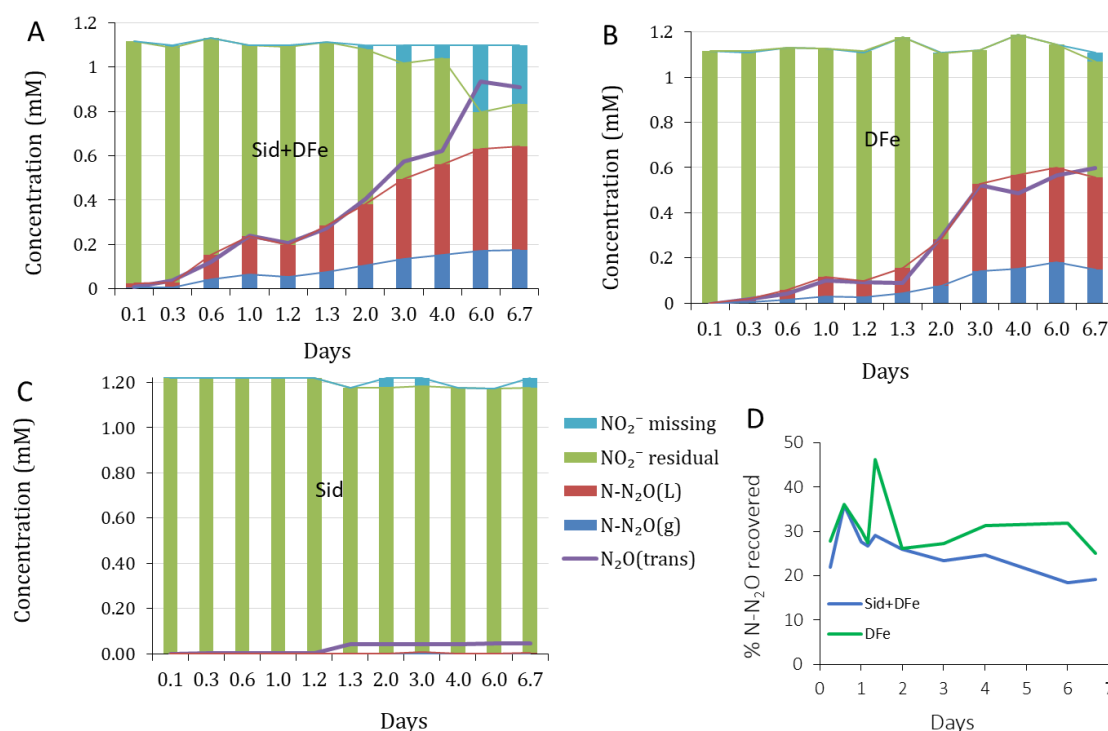


Figure 11. Bar graphs showing the evolution of residual NO_2^- , the recovered $\text{N-N}_2\text{O}(\text{g})$, $\text{N-N}_2\text{O}(\text{L})$ in the liquid phase, and the transformed $\text{N-N}_2\text{O}(\text{trans})$ in the experiments containing only siderite (Sid: A), only dissolved Fe(II) (DFe: B) and siderite plus dissolved Fe(II) (Sid+DFe: C). The percentage distribution of recovered N_2O (g) over time is also shown in D.

2.3. PAPER 3: Pilot soil aquifer treatment (SAT) using PRBs

During the soil aquifer recharge, the NH_4^+ , NO_3^- , NPDOC and O_2 concentrations were measured at the inflow (INF) and the monitoring points O, A, B, C and E (Figure 12) of pilot tanks 2 (SAT-PRB) and 4 (SAT-Control) from February to June 2018 (recharge episode 2: RE-2), July to September 2018 (RE-3) and October to January 2019 (RE-4).

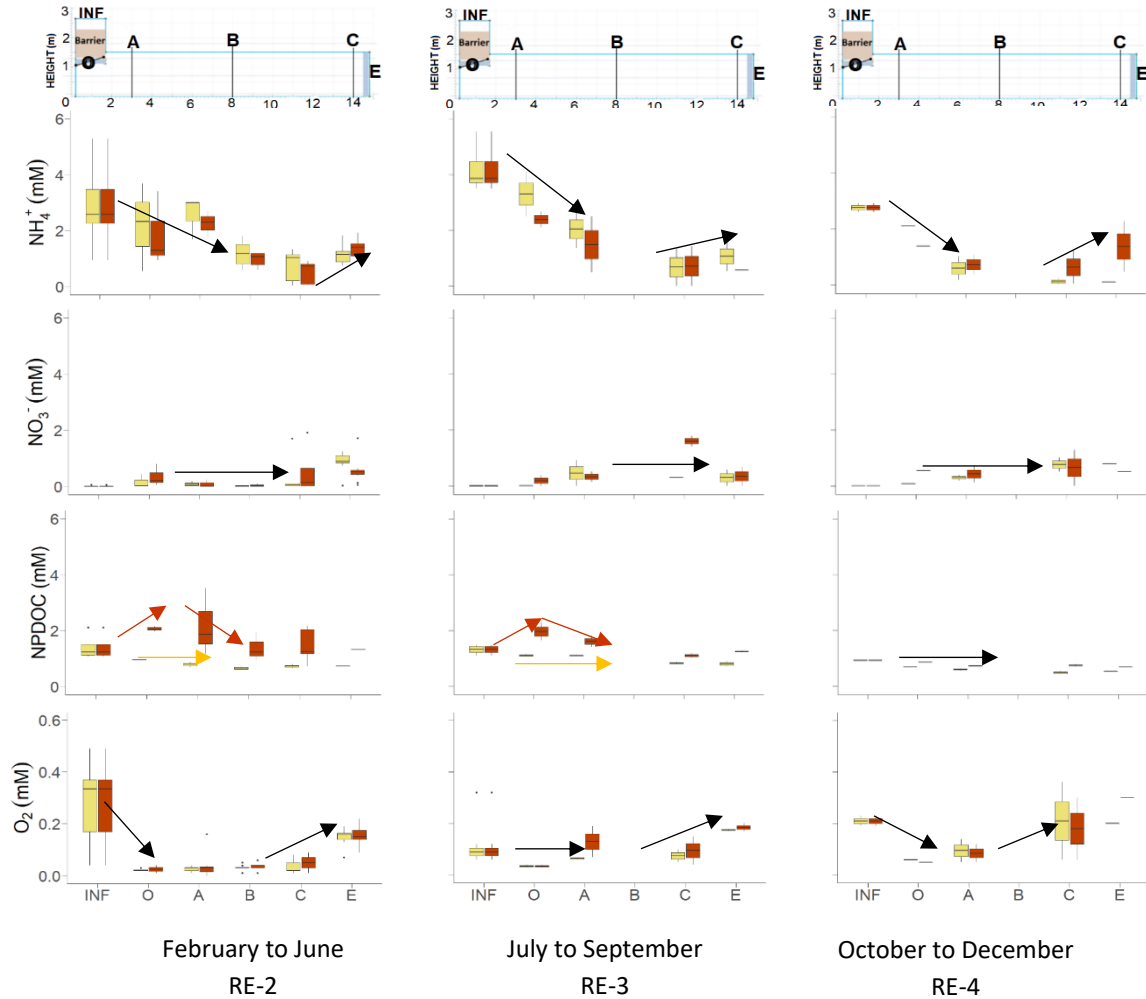


Figure 12. The general scheme of the pilot tanks (SAT-PRB and SAT-Control), showing the monitoring points O, A, B, C and E during RE-2, RE-3 and RE-4. The boxplots show NH_4^+ , NPDOC, O_2 and NO_3^- concentrations of the various recharge episodes along the flow path and outliers are represented by dots. Values in A, B, and C were calculated with the samples of the different depths at each monitoring point. INF =inflow water concentration and E is the outflow water concentration. First boxplot at each monitoring point (orange) = SAT-Control system, and the second boxplot (brown) = SAT-PRB system.

NH_4^+ concentration in the inflow water was high during RE-3 and low during RE-2 and RE-4. No significant NO_3^- was measured in the inflow water during all recharge episodes. NPDOC measured during RE-4 was slightly lower compared to RE-2 and RE-3 in the inflow water. O_2 concentration was highest during RE-2 and lowest during RE-3. NH_4^+ concentration progressively

decreased along the systems from O to C and slightly increased from C to E. NO_3^- concentration was very low at points O, A, and B but increased significantly from C to the outlet (E) in both SAT systems for all recharge episodes (Figure 12). In the SAT-Control system, NPDOC concentration slightly decreased from the inflow to the outlet across all recharge episodes. However, in the SAT-PRB system, NPDOC concentration significantly increased from the inflow to point O and gradually decreased afterwards. O_2 of the inflow water significantly decreased at O in both systems during all recharge episodes and remained consistently low from O to C, slightly increasing at E (Figure 12).

NH_4^+ , NO_3^- , NPDOC, and O_2 concentration at different depths (0.25 m, 0.85 m, and 1.35 m) were measured in sections A, B, and C. NH_4^+ concentration displayed an increasing profile with depth at section C of both systems and section A of the SAT-Control system. The concentration of NH_4^+ increased and decreased at section B in both systems, and the opposite was observed at section A for the SAT-PRB system. NO_3^- did not show significant accumulation at any depth in either system. NPDOC decreased with depth in the SAT-PRB system but remained relatively stable in the SAT-Control system. O_2 concentration slightly decreased with depth in all sampling points in both systems.

The isotopic composition of NH_4^+ ($\delta^{15}\text{N-NH}_4^+$) ranged from +8.4 ‰ to +29.4 ‰ in the SAT-Control system and from +9.6 ‰ to +34.7 ‰ in the SAT-PRB system. The $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ ranged from +4.6 ‰ to +27 ‰ and from +9.2 ‰ to +41.9 ‰, respectively. The highest values for $\delta^{15}\text{N-NH}_4^+$ and $\delta^{18}\text{O-NO}_3^-$ were observed in sample points O and A and decreased along the flow path.

3. SUMMARY OF DISCUSSIONS

3.1. PAPER 1: Laboratory treatability study of nitrate-contaminated water

The increase in nitrate concentration observed during the initial stages in B-L and B-V experiments aligns with previous studies suggesting that industrial liquid wastes can serve as a potential source of ions and denitrifying bacteria. In the case of C-L, however, no initial increase in NO_3^- concentration was observed in the outflow water, indicating that the NO_3^- introduced with *lías* was either not transported in the column or consumed before reaching the outflow reservoir.

Lías proved highly effective in removing nitrogen oxides, completely degrading NO_3^- and NO_2^- in batch and column experiments. Nitrate removal in *lías* biostimulated batch and column experiments occurred at similar time frames, indicating that nitrate supply, biomass variations, mixing, flow velocity, and preferential flow paths in the column had minimal impact on denitrification. Nitrate removal efficiency is attributed to the ability to provide organic carbon to the latent denitrifying bacteria in the field water and gravel. On the other hand, *vinico* showed only a slight NO_3^- removal efficiency, achieving 35% of NO_3^- removal. The disparity in the attenuation efficiencies between the two batch treatments is linked to the composition of the wine residues. *Vínico* mainly comprised of humic and fulvic acids with less labile organic carbon, which explains the lower efficiency of NO_3^- removal. The incomplete NO_3^- reduction in B-V inhibited NO_2^- reductase production, leading to the high accumulation of NO_2^- . Calcite was oversaturated in the outflow water in the experiments, although an equilibrium with amorphous calcite explains the buffering of pH in the experiments, keeping the pH constant over time.

The Rayleigh distillation equation effectively replicated the experimental data, treating it as a closed system, showing that the mixing of heavy isotopes of residual nitrate with fresh nitrate from wine waste was not significant. Consequently, the magnitude of fractionation was quantified using the Rayleigh distillation equation (Eq. 2), and the calculated ϵ values for $\epsilon^{15}\text{N-NO}_3^-$ and $\epsilon^{18}\text{O-NO}_3^-$ were determined for the conducted experiments. The resulting $\epsilon^{15}\text{N}_{\text{NO}_3}$ and $\epsilon^{18}\text{O}_{\text{NO}_3}$ were $-16.5 \pm 2.7 \text{ ‰}$ and $-12.1 \pm 1.8 \text{ ‰}$ in B-L and $-17.7 \pm 2.9 \text{ ‰}$ and $-17.9 \pm 1.6 \text{ ‰}$ in C-L, respectively (Figure 13). The ϵ values obtained in this study agree with the reported range of $+6.5 \text{ ‰}$ to -39.0 ‰ for $\epsilon^{15}\text{N}_{\text{NO}_3}$ and -1.0 ‰ to -30.4 ‰ for $\epsilon^{18}\text{O}_{\text{NO}_3}$ for biological denitrification (Grau-Martínez et al., 2017). The results showed that B-L and C-L had similar ϵ values, indicating that the same processes were responsible for NO_3^- degradation, likely attributed to using the same organic matter source (*lías*). On the contrary, B-V, despite exhibiting lower efficiency in

NO_3^- removal, yielded higher fractionation values (i.e., more negative $^{15}\epsilon$), with $\epsilon^{15}\text{N-NO}_3^-$ and $\epsilon^{18}\text{O-NO}_3^-$ equal to -32.0 ± 4.8 ‰ and -27.6 ± 3.3 ‰, respectively. The larger fractionation in B-V is attributed to its slower denitrification rates, which are consistent with findings reported for low denitrification rates (Mariotti et al., 1988). $\delta^{15}\text{N-NO}_3^-$ versus $\delta^{18}\text{O-NO}_3^-$ showed a higher enrichment effect on $\epsilon^{15}\text{N}_{\text{NO}_3}$ typical of denitrification at field-scale studies than at laboratory studies, which may be attributed to NO_2^- reoxidation to NO_3^- or the microbial community type. The isotopic analyses revealed denitrification as the primary NO_3^- attenuation process (Carrey et al., 2013; Kendall et al., 2007), ruling out DNRA due to the low C/N ratio in the experiments (Burgin and Hamilton, 2007).

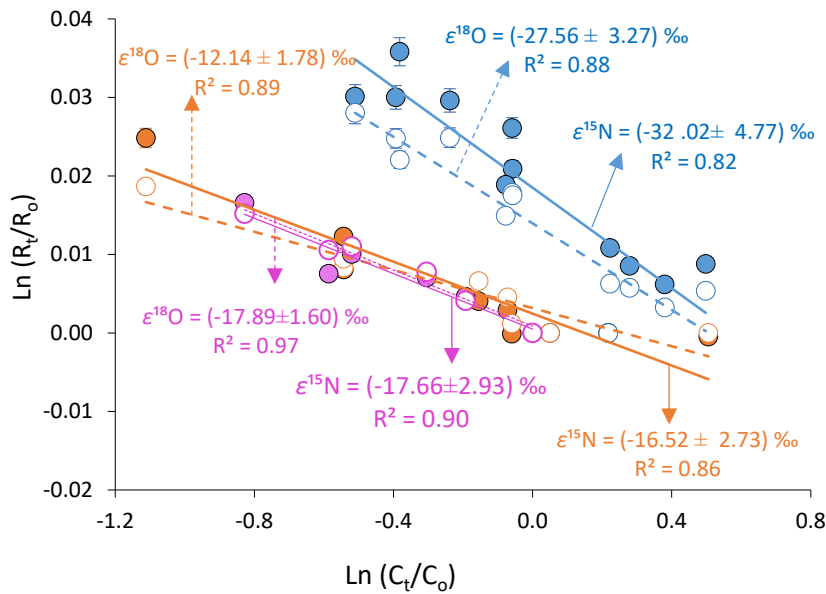


Figure 13. A plot of the natural logarithm of the substrate remaining fraction (C_t/C_0) against the ratio of the isotopes ratios of the residual and initial substrate (R_t/R_0) showing the isotope fractionation values (ϵ) for B-L (orange), C-L (pink) and B-V (blue).

According to laboratory results, *lías* can potentially remove nitrogen oxides from water through denitrification at a C/N ratio of 1.25. As a result, *lías* appears to be a suitable option for denitrification in water treatment, providing valuable insights into the efficiency, isotopic dynamics, and safety considerations for future field applications at the wetland.

A geochemical modeling study focusing on the coupled reduction of nitrate (NO_3^-) and the oxidation of organic matter linked to denitrifying bacterial growth and decay, as well as the evolution of the isotopic composition ($\delta^{15}\text{N}$) of NO_3^- and NO_2^- has been carried out. The study assumes a fully saturated system at a constant temperature of 25 °C. The model presents NO_3^- reduction as a two-step process, nitrate to nitrite and nitrite to dinitrogen gas, linked to the oxidation of the specific organic compounds for *lías* and *vínico*. Nitrate and nitrite reduction and organic carbon oxidation reactions are summarized in Figure 14.

Summary of discussions

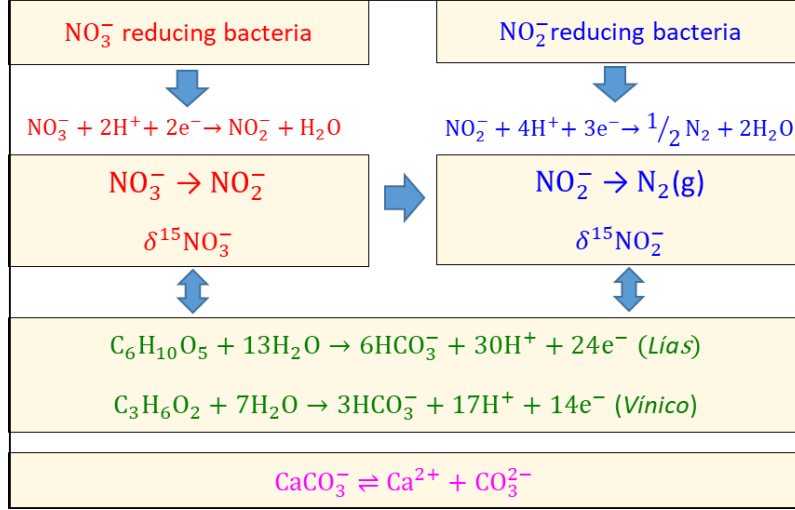


Figure 14. A scheme of the linked nitrate and nitrite reduction and organic carbon compounds oxidation reactions, bacterial growth and decay and calcite precipitation.

The kinetics of NO_3^- and NO_2^- reduction reactions are quantified using the dual Monod kinetics equation Eq. 3, which considers both the electron donor and acceptor, as well as the limitation of the degradation rates. The oxidation rate of organic compounds, expressed in Eq. 7, relies on the degradation rates of nitrate (R_{NO_3}) and nitrite (R_{NO_2}) determined using Eq. 3, where k is the specific rate of organic matter consumption (dimensionless), $[\text{ED}]$ is the concentration of *lías* or *vínico* (mol L^{-1}), and k_1 and k_2 are the C/N ratios of *lías* and *vínico* in a balanced chemical equation between the oxidation and reduction half-reactions.

$$R_{\text{OM}} = k \cdot [\text{ED}] \cdot [k_1 \cdot R_{\text{NO}_3^-} + k_2 \cdot R_{\text{NO}_2^-}] \quad \text{Eq. 7}$$

The isotopic fractionation of nitrogen during denitrification is determined according to the first-order degradation kinetic equation described in Van Breukelen and Prommer (2008) (Eq. 8), where $R_{\delta^{\text{H}}\text{EA}}$ is the change in the $\delta^{15}\text{N}$ of NO_3^- or NO_2^- in per mille (‰), R_{EA} is the degradation rate of NO_3^- or NO_2^- calculated according to Eq. 3, $\delta^{\text{H}}\text{EA}$ is $\delta^{15}\text{N}$ isotopic composition of NO_3^- or NO_2^- (‰), $\delta^{\text{L}}\text{EA}$ is $\delta^{14}\text{N}$ isotopic composition of NO_3^- or NO_2^- (‰) and ε is the experimentally calculated isotopic fractionation value (‰).

$$R_{\delta^{\text{H}}\text{EA}} = R_{\text{EA}} \cdot \frac{[\delta^{\text{H}}\text{EA}]}{[\delta^{\text{H}}\text{EA}] + [\delta^{\text{L}}\text{EA}]} \cdot \varepsilon \quad \text{Eq. 8}$$

The model assumes aqueous speciation in equilibrium and incorporates a constant partial pressure of CO_2 of $10^{-2.6}$ atm and the possibility of calcite precipitation when the saturation index exceeds the calculated saturation indices value (0.7 for *lías* and 0.6 for *vínico*). The column experiment is simulated in a one-dimensional system with specified velocity, dispersivity, and diffusion coefficient parameters determined after a conservative tracer test.

Summary of discussions

Initial values for reaction rate parameters $k_{\max}$, k_{ED} , K_{EA} , Y , k and b for Eq. 6, Eq. 7 and Eq. 8 were taken from Rodríguez-Escales et al. (2014) and adjusted afterwards with CeresStudy in GibbsStudio (Nardi and de Vries, 2017) to obtain the best-fit values shown in Table 9. The Levenberg–Marquardt algorithm yielded low least square errors (0.005 to 0.012), indicating a high level of accuracy in the models.

Table 9. Fitted reaction rate parameters for equations used in the present model estimated from GibbsStudio (batch and column experiments) and reported values in the literature.

Model Parameter	Range of values	Initial guess	Fitted values	
			B-L/C-L	B-V
K_{EA,NO_3^-} (mol·L ⁻¹)	$1.0 \cdot 10^{-6}$ - $1.7 \cdot 10^{-4}$ ^{a,b,c,d,e}	$1.4 \cdot 10^{-5}$	$5.0 \cdot 10^{-4}$	$5.0 \cdot 10^{-4}$
K_{EA,NO_2^-} (mol·L ⁻¹)	$1.0 \cdot 10^{-6}$ - $1.7 \cdot 10^{-4}$ ^{a,b,c,d,e}	$1.4 \cdot 10^{-5}$	$6.0 \cdot 10^{-5}$	$6.0 \cdot 10^{-5}$
K_{ED,NO_3^-} (mol·L ⁻¹)	$8.3 \cdot 10^{-6}$ - $1.0 \cdot 10^{-1}$ ^{a,b,c,d,e}	$2.2 \cdot 10^{-5}$	$2.2 \cdot 10^{-5}$	$2.2 \cdot 10^{-5}$
K_{ED,NO_2^-} (mol·L ⁻¹)	$8.3 \cdot 10^{-6}$ - $1.0 \cdot 10^{-1}$ ^{a,b,c,d,e}	$2.2 \cdot 10^{-5}$	$1.0 \cdot 10^{-6}$	$1.0 \cdot 10^{-6}$
$k_{\max,NO_3^-}$ (s ⁻¹)	$1.0 \cdot 10^{-3}$ - $3.9 \cdot 10^{-7}$ ^{a,b,c,d,e}	$3.9 \cdot 10^{-7}$	$5.0 \cdot 10^{-7}$	$5.0 \cdot 10^{-7}$
$k_{\max,NO_2^-}$ (s ⁻¹)	$5.2 \cdot 10^{-4}$ - $7.0 \cdot 10^{-8}$ ^{a,b,c,d,e}	$4.4 \cdot 10^{-7}$	$5.0 \cdot 10^{-7}$	$5.0 \cdot 10^{-7}$
Y, NO_3^- (moles C-cells/moles C-OC)	0.05 – 0.73 ^{a,b,c,d,e,f}	0.57	0.53	0.53
Y, NO_2^- (moles C-cells/moles C-OC)	0.05 – 0.73 ^{a,b,c,d,e,f}	0.57	0.53	0.53
b, NO_3^- (s ⁻¹)	0 - $5.8 \cdot 10^{-7}$ ^{a,b,c,d,e}	$1.2 \cdot 10^{-7}$	$3.0 \cdot 10^{-6}$	$3.2 \cdot 10^{-5}$
b, NO_2^- (s ⁻¹)	0 - $5.8 \cdot 10^{-7}$ ^{a,b,c,d,e}	$1.2 \cdot 10^{-7}$	$3.0 \cdot 10^{-6}$	$5.0 \cdot 10^{-5}$
k		210	950	1400

^aChen & MacQuarrie (2004). ^bKillingstad et al. (2002). ^cKinzelbach et al. (1991). ^dMastrocicco et al. (2011). ^eRodríguez-Escales et al. (2014). ^fParkhurst & Appelo, (2013).

The model results of the simulated pH and Eh well align with the experimental data for both the batch and column experiments. The model successfully reproduces NO_3^- , NO_2^- , $\delta^{15}N-NO_3^-$, and $\delta^{15}N-NO_2^-$ (Figure 15a, c, and e) along with NPDOC levels (Figure 15b, d, and f). The simulation captures the removal of nitrate and nitrite from the solution and the increase of $\delta^{15}N$ values of the remaining nitrate fraction and the generated nitrite. However, some discrepancies between the model and experimental results are noted, particularly in the case of NPDOC in the B-L model, which may be attributed to using the same reaction rate parameters for B-L and C-L. In the C-L model, the effect of variations in transport parameters over time and biofilm colonization was not considered, which can affect the outcome in B-L, contributing to these differences.

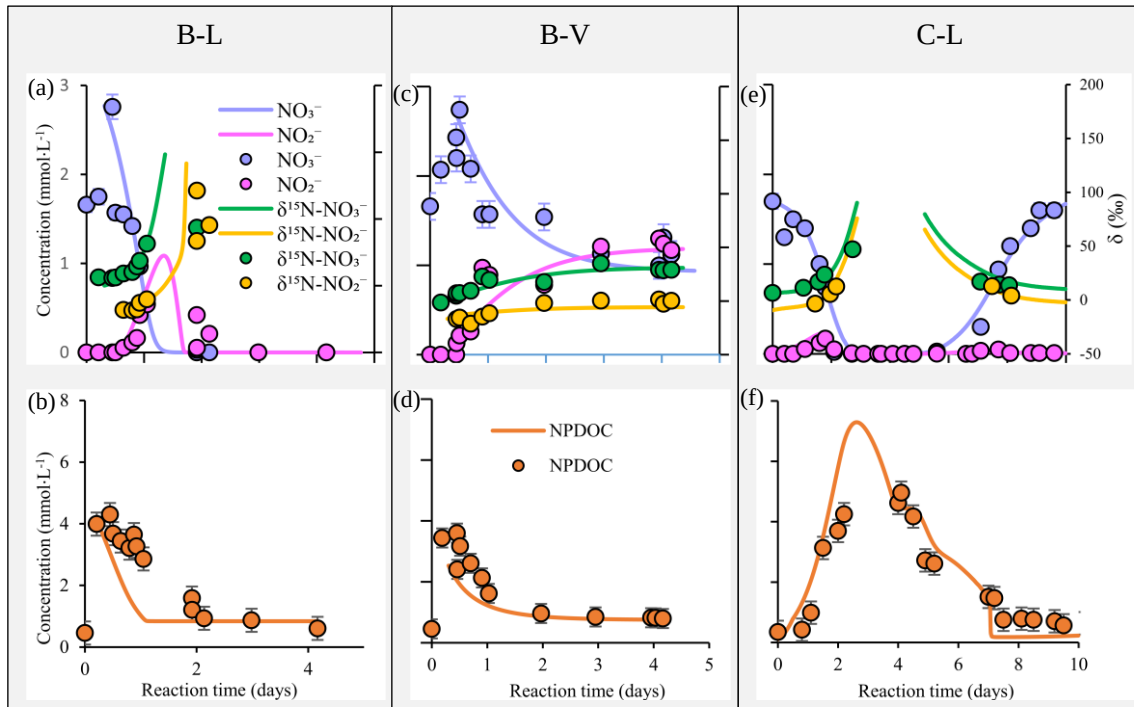


Figure 15. Results of reactive transport model of batch and column experiments. The dots represent experimental data, a 5% error of experimental data is indicated by error bars with non-visible error bars within the circles, and the solid lines represent the fitted model.

The obtained model goes beyond most typical denitrification models by incorporating nitrate and nitrite concentrations, dissolved organic carbon, biomass, and nitrogen isotope composition, which is less common in the literature. This model highlights the importance of considering multiple variables and parameters in geochemical modeling to understand better and predict complex environmental processes.

3.2. PAPER 2: Abiotic nitrite reduction with ferrous iron

The developed study supports recent findings that nitrite can be abiotically reduced by solely dissolved Fe(II) without a mineral catalyst. However, the nitrite removal rate significantly increased when siderite was mixed with dissolved Fe(II). This suggests that a portion of the dissolved Fe(II) could have been sorbed onto the siderite surface to create reactive sites, acting as a catalyst to increase the electron density of Fe(II) to improve nitrite reduction (Dhakal et al., 2021; Jones et al., 2014; Rakshit et al., 2016, 2008).

N-N₂O(g) recoveries during nitrite reduction did not match the N-NO₂⁻ transformed. N₂O concentration in the liquid and gas phases calculated with Henry's Law, complemented with the Rayleigh distillation equation, showed that N₂O was the final product formed. The absence of biological components in the experiments may have inhibited N₂O to N₂ reduction, and the transformed N-NO₂⁻ was always equal to the sum of N-N₂O in the liquid and gas phases, indicating the absence of N₂O leakage through the butyl rubber stoppers or the formation of NO. The study revealed that 50-98% of the generated N₂O remained dissolved in water, likely influenced by metal chlorides, contributing to low N₂O(g) recoveries in the headspace of the vials. The obtained N₂O recoveries were consistent with previous studies reporting less than 50% of N-NO₂⁻ recoveries during abiotic nitrite reduction production (G. Chen et al., 2020; Dhakal et al., 2021; Jones et al., 2015; Rakshit et al., 2016). The N₂O(g) recovery decreased from 36% to 19% in Sid+DFe and 46% to 25% in DFe, attributed to the formation of iron oxide minerals, known to influence N₂O(g) production (G. Chen et al., 2020; Jones et al., 2014, 2015).

NO₃⁻ was consistently below detection, and NH₄⁺ was not contained in the initially prepared water, ruling out the possibility of DNRA. The absence of biological components in the experiments also restricts the potential for biological processes.

Nitrite reduction was linked with $\delta^{15}\text{N}$ enrichment over time, as light isotopes are preferentially consumed. The isotopic data in this study ($\epsilon^{15}\text{N}_{\text{NO}_2} = -11.8 \pm 11.0$ ‰ for Sid, $\text{SP}_{\text{N}_2\text{O}} = 22.5 \pm 0.5$ ‰; $\epsilon^{15}\text{N}_{\text{NO}_2} = -12.0 \pm 1.4$ ‰ for DFe and $\text{SP}_{\text{N}_2\text{O}} = 23.5 \pm 0.7$ ‰; $\epsilon^{15}\text{N}_{\text{NO}_2} = -13.0 \pm 1.1$ ‰ for Sid+DFe) (Figure 16A) are consistent with previous findings for chemodenitrification ($\epsilon^{15}\text{N}_{\text{NO}_2} = -19.7$ to $+16.5$, $\text{SP}_{\text{N}_2\text{O}} = 0$ to 30.2 ‰) (Benaiges-Fernandez et al., 2020; Grabb et al., 2017; Li et al., 2022). The similar ϵ values obtained in all the experiments suggest a common nitrite degradation process. The $\delta^{15}\text{N}^{\text{bulk}}$ of the generated nitrous oxide became progressively enriched, and the $\delta^{15}\text{N}^{\text{bulk}}$ approached the initial $\delta^{15}\text{N}_{\text{NO}_2}$ (Figure 16B). Given the above factors, chemodenitrification was considered the main nitrite reduction process in the experiments, supported by isotopic data showing characteristic patterns.

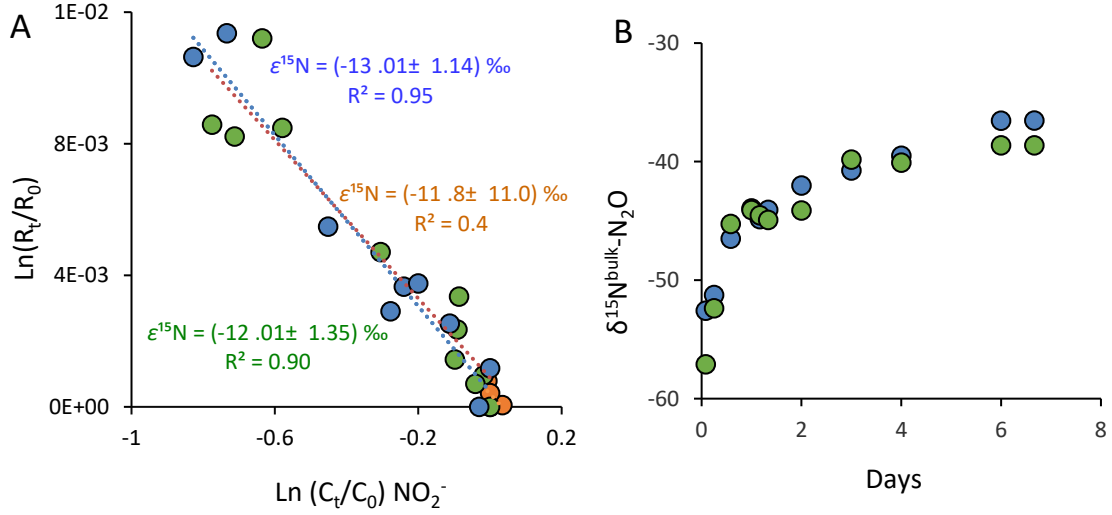
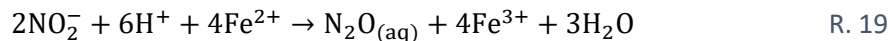


Figure 16. A plot showing the isotope fractionation values of the experiments of only siderite (orange), only dissolved Fe(II) (green) and siderite plus dissolved Fe(II) (blue). The graph of the bulk $\delta^{15}\text{N}$ of N_2O generated over time is presented in B.

Nitrite degradation followed a pseudo-first-order kinetics with respect to nitrite concentration, while the coupled nitrite reduction-Fe(II) oxidation followed a second-order kinetics according to Eq. 9, where $d[\text{NO}_2^-]/dt$ is the degradation rate of nitrite ($\text{mol L}^{-1} \text{ s}^{-1}$) and $(9.39 \pm 0.36) \cdot 10^{-4} \text{ L mol}^{-1} \text{ s}^{-1}$ is the second-order kinetic rate constant. The second-order kinetic rate constant is the slope obtained from the plot of the pseudo-first-order constants versus the total aqueous Fe(II) concentration in solution ($[\text{Fe(II)}]_T$) before the start of nitrite reduction. $[\text{Fe(II)}]_T$ was calculated with the Phreeqc software after equilibrating the prepared synthetic water (SW) with atmospheric $\text{CO}_2(\text{g})$ at a partial pressure of $p\text{CO}_2(\text{g}) = 10^{-3.5} \text{ atm}$, calcite and Fe-minerals before nitrite reduction starts. In all cases, amorphous Fe(III) hydroxide ($\text{Fe(OH)}_3(\text{am})$) and calcite were left to precipitate if oversaturated.

$$\frac{d[\text{NO}_2^-]}{dt} = (9.39 \pm 0.36) \cdot 10^{-4} \cdot [\text{NO}_2^-] \cdot [\text{Fe(II)}_T] \quad \text{Eq. 9}$$

The linked NO_2^- reduction and Fe(II) oxidation reaction (R. 19) was modeled in one step using equation Eq. 9.



The first-order degradation kinetics, according to Eq. 8, was used to model the isotopic evolution (Van Breukelen and Prommer 2008) using the experimentally calculated kinetic isotope fractionation (ϵ).

The initial water composition, the initial nitrite concentration of the prepared water, the siderite amount, and the amorphous Fe(III) hydroxide presented in Table 10 were used as the

Summary of discussions

initial conditions for calculations. Siderite, calcite, and amorphous Fe(III) hydroxide were allowed to precipitate or dissolve in equilibrium. The models were run for seven days in 70 equal steps, and sensitivity cases were also conducted to account for uncertainties in the rate constant.

Table 10. The composition of CO₂(g) equilibrated synthetic water (CESW) with Fe-minerals and mineral composition after equilibration for Sid, DFe and Sid+DFe cases.

Chemicals	pH	Eh V	Ca mM	Mg mM	K mM	Na mM	Cl mM	S mM	P mM	C mM	Fe(II) _T mM	Siderite mM	Fe(OH) ₃ mM
CESW-Sid	6.5	0.2	0.2	1.2	2.6	6.4	5.8	1.4	0.04	5.9	0.1	5.0	3.5
CESW-DFe	3.1	0.7	0.2	1.2	2.6	6.4	15.8	1.4	0.04	2.4	1.5	- -	2.4
CESW-Sid+DFe	5.5	0.3	0.2	1.2	2.6	6.4	15.8	1.4	0.04	4.4	3.5	6.6	3.5

The model well-reproduced nitrite concentration experimental results in Sid, DFe and Sid+DFe (Figure 17) and accurately simulated the general trends of the total aqueous nitrous oxide concentration resulting from nitrite reduction-Fe(II) oxidation, which does not fit the experimental gaseous N-N₂O(g) measured in the HS. The models project the total aqueous N-N₂O generated within the solution, whereas the experimental results measure the N-N₂O(g) diffused from the solution into the HS. However, the combined N-N₂O in the gas and liquid phases closely matches the total aqueous N-N₂O concentration predicted by the model (Figure 17). Geochemical calculation with the Phreeqc software (Parkhurst and Appelo, 2013) showed that amorphous Fe-oxyhydroxide was formed in all the batch experiments. Fe(OH)₃ slightly increased from 3.5 to 3.7 mmol L⁻¹ in Sid and significantly increased from 2.2 to 2.9 mmol L⁻¹ in DFe and 3.5 to 5.5 mmol L⁻¹ in Sid+DFe. 0.1 and 1.5 mmol L⁻¹ of siderite were dissolved in Sid and Sid+DFe, respectively, and calcite did not form in any of the models due to the low concentration of Ca ions in synthetic water used.

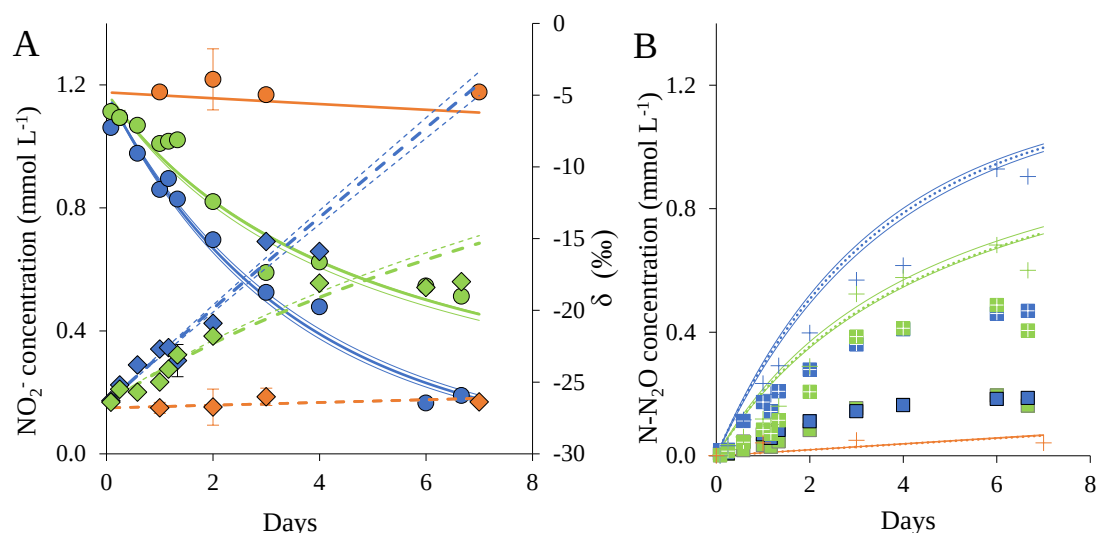


Figure 17. A) NO_2^- concentration and $\delta^{15}\text{N}_{\text{NO}_2}$ evolution over time for the three experiments. Orange, green, and blue colors represent Sid, DFe and Sid+DFe, whereas circles and diamonds indicate experimental $[\text{NO}_2^-]$, and $\delta^{15}\text{N}_{\text{NO}_2}$ and lines are model results. B) Recovered $\text{N-N}_2\text{O(g)}$ in the gas phase (squares), calculated $\text{N-N}_2\text{O}$ in the liquid phase (plus inside squares), transformed N-NO_2^- (plus) and modeled N_2O (lines) over time. The thin outer lines represent the margin of error.

Several chemical denitrification models have been created for nitrogen compound removal in wastewater treatment systems, primarily concentrating on concentration-based approaches (Chen et al., 2020; Dhakal et al., 2013; Liu et al., 2019). However, these models may not capture processes in which isotope effects have considerable influence, as seen in natural attenuation or biogeochemical reactions influenced by microbial activity. The developed model introduces a novel dimension by integrating concentration and isotopic data, enhancing its complexity compared to concentration-only models. Combining isotope and concentration offers a more comprehensive understanding by providing insights into the overall denitrification rates, whereas distinguishing between the distinct denitrification pathways based on the isotopic signatures of nitrogen compounds.

3.3. PAPER 3: Pilot soil aquifer treatment (SAT) using PRBs

The chemical and isotopic results indicate organic pollution sources, likely from urban wastewater and nearby hospitals. NH_4^+ concentration peaked in summer due to higher sewage input from increased tourism during these warm periods.

The main N-NH_4^+ transformation mechanism in the systems was nitrification. NH_4^+ concentration decreased along the flow path, linked with decreasing O_2 and NPDOC concentration levels and increasing NO_3^- concentration. However, the measured N-NO_3^- concentration did not match the N-NH_4^+ removed, suggesting other N-NH_4^+ removal processes such as sorption, plant assimilation, denitrification or anammox, supported by the microbial communities' genetic analyses (Hellman et al., 2022). Clay minerals in the PRB could offer cation exchange sites, yet $\epsilon^{15}\text{N-NH}_4^+$ results showed sorption's minor role in the long-term N removal, limiting its major impact to inflow fluctuation buffering. Plant assimilation likely played a more significant role in the recharge area, particularly during the initial stages of plant growth. The decomposition of plants might have contributed to organic nitrogen during the later phases of the study when the plants reached full maturity and subsequently decayed. Denitrification played a significant role in the systems, especially following infiltration when O_2 concentration was low, confirmed by the isotopic analysis of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- . A linear plot with a 1:1 relationship between $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ typical of denitrification was not observed, attributed to the simultaneous NO_3^- production and reduction. The isotopic results showed the mixing of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of newly generated NO_3^- and residual NO_3^- in the tanks, which hindered the modeling of $\epsilon^{15}\text{N-NO}_3^-$ and $\epsilon^{18}\text{O-NO}_3^-$ as a closed-system process using the Rayleigh distillation equation.

The high levels of NPDOC in the SAT-PRB system could have inhibited nitrification in the SAT-PRB system as NPDOC consumption of O_2 may outcompete nitrification, explaining the low NH_4^+ removal in the SAT-PRB system, which agrees with previous studies (Jie et al., 2009; Ling and Chen, 2005). The isotopic results of $\delta^{15}\text{N-NH}_4^+$ suggest that the NO_3^- produced in the SAT-PRB system was quickly denitrified, facilitated by the reducing conditions created at the reactive barrier base.

At different sections in the systems, the maximum accumulation of NH_4^+ was observed at the bottom, indicating a decrease in nitrification rate with depth. This decreasing tendency is attributed to the prevailing aerobic conditions in the shallower part of the systems, which is facilitated by the diffusion of atmospheric oxygen into the tanks. The increased nitrification rate at the top also resulted in greater O_2 consumption, leading to anoxic conditions for

denitrification, which explains the high NH_4^+ and low O_2 concentration observed at the bottom of the systems. However, despite the high nitrification rate at the top, maximum NO_3^- was not identified at the top due to the simultaneous production and consumption of NO_3^- . No significant NO_3^- accumulation was observed along both systems, except at the outlet (E), where a considerable increase was detected. This result may be attributed to an elevated nitrification rate promoted by the presence of grama grass, facilitating a flux of O_2 in the canal. The analysis of the isotopologues of NO_3^- along the systems displayed trends typical for denitrification and was linked to NPDOC consumption, providing energy for denitrifying bacteria.

Although the residual N at the outlets of both systems showed similarities, distinct differences in the dominant nitrogen species were noticed. In the SAT-Control system, the NH_4^+ concentration progressively decreased from 72% to 4% and NO_3^- reduced from 54% to 27% of the total N, whereas in the SAT-PRB system, NH_4^+ decreased from 67% to 16% and NO_3^- decreased from 75% to 18% during the study period at the outlet (E) (Figure 18). Consequently, NO_3^- emerged as the dominant species at the end of the study period in the SAT-Control system, with 27% NO_3^- and 4% NH_4^+ . In contrast, the SAT-PRB system exhibited a more balanced distribution, with approximately equal proportions of NO_3^- and NH_4^+ , with 18% NO_3^- and 16% NH_4^+ .

Temperature variations significantly impacted the growth and adaptation of the microbial community, thereby influencing nitrogen removal in the studied cases. Increasing temperatures in the tanks were marked by an increase in the N removal rate by up to 72% in the SAT-Control system and 65% in the SAT-PRB system (Figure 18).

An enrichment factor ($\epsilon^{15}\text{N-NH}_4^+$) of -5 ‰ was obtained in both systems. The lower $\epsilon^{15}\text{N-NH}_4^+$ value obtained in this work compared to literature values (-10 ‰ to -28 ‰: Sebilo et al., 2006) could be attributed to the influence of plant assimilation.

Summary of discussions

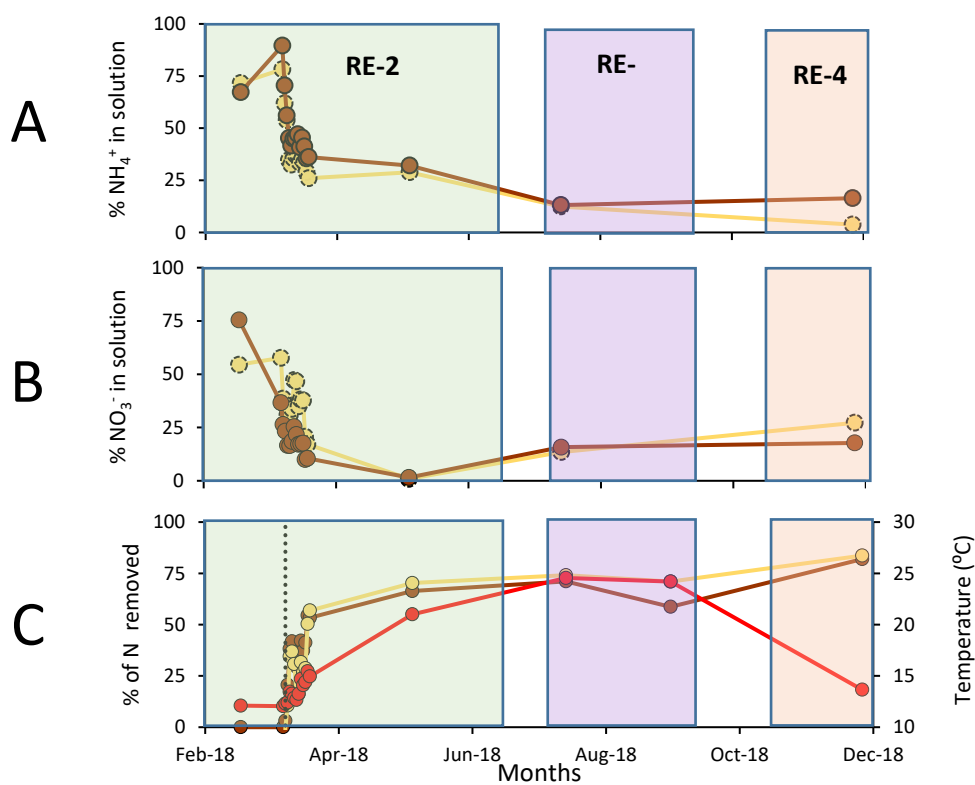


Figure 18. The % distribution of residual NH_4^+ and NO_3^- in solution and % of total N (NH_4^+ + NO_3^-) removed from the solution as a function of the average temperature in the tanks. Orange circles represent the SAT-Control system, brown circles represent the SAT-PRB system, and red circles represent the average temperature in the tanks. The dotted line represents the point below where no N is removed from the tanks.

3.4. General discussion

Nitrogen pollution, primarily from agricultural runoff, urban wastewater, and industrial activities, poses significant threats to aquatic ecosystems and human health. Understanding and managing nitrogen compounds attenuation and transformation is crucial for maintaining water quality and ecosystem integrity. In this thesis, the attenuation and transformation of nitrogen compounds, including ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-), have been investigated using a variety of innovative approaches. The research spans diverse environmental contexts and electron donor sources, shedding light on complex nitrogen cycling processes. This general discussion synthesizes the findings and insights gained from the specific discussions on nitrate attenuation using wine residues, nitrite reduction using ferrous iron and the transformation and attenuation of N-NH_4^+ using plant-based compost permeable reactive barrier during aquifer recharge.

Wine industry byproducts, particularly wine residues, hold substantial promise as a cost-effective and environmentally eco-friendly carbon source for advancing biological denitrification research. Repurposing these discarded materials aligns with the concept of circular economy and supports the United Nations Sustainable Development Goals related to clean water, waste reduction, and resource efficiency. Efficient use of wine waste can reduce greenhouse gas emissions associated with their disposal. These residues, derived from various winemaking stages, are gaining recognition for their rich organic content. They contain diverse organic compounds, such as sugars, polysaccharides, acids, and lignocellulosic materials, making them an ideal carbon source for denitrifying bacteria. Variability in residue composition underscores the need for characterization to optimize denitrification outcomes since wine residue composition impacts their potential to release carbon. Beyond acting as carbon sources, wine residues can provide essential nutrients such as nitrogen, phosphorus, cations (Ca, Mg, K, and Na), and trace elements. While these nutrients are valuable, their presence can pose challenges, potentially impeding denitrification. Due to this complexity, achieving the ideal composition of wine residues that perfectly aligns with microbial denitrification needs can be challenging. Careful management of nutrient ratios is also vital to avoid imbalances and nutrient deficiencies.

Despite challenges such as variations in organic matter composition and microbial adaptation, integrating ferrous iron-mediated chemical nitrite reduction into existing water treatment systems is feasible. The presence of both organic (organic carbon) and inorganic (ferrous iron) electron donors enables the coexistence of biological and chemical processes, ultimately enhancing denitrification. In groundwater, nitrite is primarily generated as an

intermediate product through nitrate reduction. The chemical reduction of nitrite to nitrous oxide is important in iron-rich environments, as it represents a crucial transformation of nitrogen compounds and contributes to controlling nitrogen availability and mobility. Ferrous iron is readily available and inexpensive, making it a cheap option for enhancing denitrification processes.

Nitrite molecules are sorbed onto the surface of ferrous iron minerals, such as ferrous hydroxides or Fe(II)-bearing minerals, making them more susceptible to reducing reactions. The adsorbed ferrous iron donates electrons to nitrite, reducing it to nitrous oxide gas via nitric oxide (NO) intermediate, which mainly does not accumulate in most environmental conditions. The primary end products of ferrous iron-mediated denitrification are nitrous oxide and Fe(III) compounds, but a greater portion of the generated nitrous oxide gas can be further reduced to nitrogen gas during biological denitrification.

The pH of the environment strongly influences the rate and extent of the nitrite reduction process. Low pH conditions generally promote the reduction process by enhancing the availability of reactive ferrous iron species. Concurrently, the concentration of ferrous iron significantly influences the reaction rate of nitrite; elevated concentrations enhance the nitrite reduction process, whereas lower concentrations have the opposite effect. Furthermore, halides and metal chlorides in water can affect the $\text{N}_2\text{O(g)}$ recoveries. Ferric iron minerals produced from nitrite reduction by ferrous iron tend to adsorb to intermediate reduction products like NO, thereby influencing the production and transformation of $\text{N}_2\text{O(g)}$. The presence of other chemical species, such as heavy metals, can inhibit nitrite reduction by influencing the electron transfer kinetics and adsorption properties.

The significance of abiotic nitrite reduction has implications for various environmental processes, including soil nitrogen cycling, groundwater quality, and nutrient dynamics in aquatic systems. It influences the release of nitrous oxide gas into the atmosphere, contributing to greenhouse gases.

Partially treated wastewater effluent contains high levels of inorganic nitrogen, and PRBs can be designed to attenuate nitrogen compounds by passing it through a reactive medium that promotes various physical, chemical, and biological processes to remove or transform these pollutants. In the current study, plant-based compost is used as an electron donor in a PRB to treat wastewater containing ammonium during aquifer recharge. A complex set of biological reactions can lead to the transformation and attenuation of ammonium nitrogen.

Ammonium-rich wastewater entering the PRB provides a source of ammonium ions, which are subjected to oxidation reactions driven by the microbial community in the wastewater and the compost. Ammonium-oxidizing bacteria (AOB) and/or archaea (AOA) oxidize ammonium to nitrite in ammonium oxidation. The nitrite produced from ammonium oxidation can undergo further oxidation to nitrate through the action of other bacteria groups, such as nitrite-oxidizing bacteria (NOB). The plant compost serves as a source of organic carbon for microbial activity. As the microorganisms in the compost break down the organic matter, they release carbon compounds that act as energy sources for various microbial processes, including denitrification, anaerobic ammonium oxidation and dissimilatory nitrate reduction to ammonium. In suitable conditions, such as limited oxygen availability, denitrifying bacteria utilize nitrate as an electron acceptor to perform denitrification. Denitrification cooccurs with nitrification, reducing the generated nitrate to nitrogen gas through microbial-mediated reactions. In the presence of concurrent nitrification-denitrification partial products (NH_4^+ , NO_3^- and NO_2^-), anammox bacteria convert ammonium into nitrogen gas using nitrite as the electron donor in anaerobic conditions. DNRA outcompetes denitrification in high organic matter levels, transforming nitrate to ammonium in anaerobic conditions. Other constituents of the PRB, such as clay minerals, play a significant role in nitrogen species transformation, which may act as sorption sites. Ammonium is one of the primary mobile nitrogen forms that plants can absorb alongside nitrate. Ammonium assimilation by vegetation is a crucial process in the nitrogen cycle, as plant roots take up ammonium ions.

The success of PRBs depends on various factors, including the composition of the reactive barrier, the microbial community present, environmental conditions (e.g., pH, temperature, and oxygen availability), and the specific design of the PRB. Monitoring and assessing nitrogen attenuation pathways are essential to ensure the efficient and effective removal of nitrogen compounds and other pollutants from wastewater.

Geochemical modeling is a powerful tool that helps us better understand the complex interactions between microbial activities, environmental conditions, and nitrogen cycling to predict the behavior of nitrogen compounds in various environmental systems. Geochemical modeling uses computer simulations to describe the interactions between chemical species and their transformations over time. Denitrification geochemical models simulate the rates at which denitrification reactions occur based on the concentration of reactants (nitrate, nitrite) and the environmental conditions (e.g., temperature, pH, and redox potential). Denitrifying microorganisms require a carbon source as an energy supply for metabolic activities. Geochemical models incorporate organic carbon availability and predict how carbon availability

changes influence denitrification rates. Nitrate, nitrite, and organic carbon concentrations are influenced by the transport of water and solutes in aquatic systems.

Different species of denitrifiers have varying metabolic capabilities and responses to environmental changes, and modeling microbial interactions provides insights into community shifts and their impacts on denitrification rates. Denitrification models can be applied at different temporal scales, allowing researchers to assess denitrification dynamics in various ecosystems, from small laboratory time scales to larger time scales. The Dual Monod kinetics have been considered in the present study, assuming that the denitrification rate depends on the availability of nitrate and organic carbon for the denitrifying microorganisms. It enhances the accuracy of biological denitrification modeling by considering the interplay between the electron donor and acceptor, providing a more comprehensive understanding of denitrification kinetics in various environments and aiding in optimizing denitrification processes for efficient nitrogen removal.

4. SUMMARY OF CONCLUSIONS

This thesis presents valuable insights into nitrogen compounds removal strategies and processes from various research approaches, including batch, column, and pilot studies, using chemical, isotopic and geochemical modeling tools.

One of the promising approaches investigated in this research is the use of liquid organic residues from wine production as a material to biostimulate denitrification in field study laboratory experiments. The effectiveness of the remediation method employed was examined using *lías*, which demonstrated efficient nitrate removal capacity in batch and column experiments. However, the *vínico* residue, with less labile organic carbon, showed lower nitrate removal efficiency and is not considered feasible for future nitrate removal treatments. The successful biostimulation of denitrification using *lías* suggests a promising strategy for the remediation of nitrate-polluted water bodies. The results of this study provide valuable insights for developing a sustainable and effective approach to mitigate nitrate pollution on a field-scale through biostimulation of denitrification and promote sustainable water management. This study also emphasizes the importance of using controlled laboratory-scale fractionation values for estimating denitrification efficiency, considering the challenges in applying field-scale values due to the inherent natural variability of field conditions.

The study employed isotopic tools to gain valuable insights into nitrate attenuation processes, contributing to a better understanding of nitrogen cycling during denitrification and facilitating the quantification of denitrification efficiency.

The research also incorporated geochemical numerical modeling, which supported the experimental findings, reproducing the identified changes in nitrate, nitrite, and organic carbon concentrations and the isotopic compositions of nitrate and nitrite isotopologues. This numerical modeling approach proved to be a valuable tool for predicting nitrogen compounds' evolution beyond laboratory treatments, which could be used for field applications in the near future.

Because both concurrent biotic-abiotic processes transform nitrogen compounds in groundwater in iron-rich environments, abiotic nitrite reduction by ferrous iron and its implications on nitrous oxide production in three distinct scenarios have been investigated. The study provides insights into the coupled nitrite reduction-Fe(II) oxidation reaction and the resulting isotopic composition changes. The findings contribute to the understanding of these coupled processes, emphasizing the significant role of mineral surfaces in influencing nitrite

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reactions. This study is essential in understanding nitrogen cycling in iron-rich environments and can be applied to further studies and field applications. The results showed that nitrite reduction occurred with a removal efficiency of up to 84% when siderite was combined with dissolved Fe(II). However, the reactivity of nitrite with siderite alone was negligible. Chemodenitrification, identified as the main nitrite attenuation process, was linked to the generation of gaseous nitrous oxide. Low recoveries (19-46%) were observed due to restricted diffusion of aqueous N_2O from water, resulting in 50-98% of the generated N_2O remaining in the liquid phase. The kinetic analysis showed that coupled nitrite reduction and Fe(II) oxidation followed second-order degradation kinetics. The developed conceptual and numerical models successfully reproduced the experimental results, offering a valuable tool for predicting nitrite reactivity over extended periods and understanding nitrite reduction in natural systems.

Addressing the presence of high NH_4^+ concentrations in partially treated water, the thesis provided a comprehensive investigation into the spatial and temporal variability of nitrogen compounds migration and transformations within two studied systems: one system installed with plant-based compost permeable reactive barrier and another controlled system with no reactive barrier. The results highlight the complexity of the nitrogen removal processes, influenced by various factors, including seasonal variations, temperature, microbial activity, and redox conditions.

Multiple processes, including nitrification, denitrification, sorption, and plant assimilation, contributed to the overall nitrogen attenuation in the systems. Nitrification was identified as the primary mechanism for inorganic NH_4^+ transformation along the tanks, and denitrification was crucial in reducing NO_3^- concentrations at low oxygen levels, supported by the isotopic signatures of $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$, leading to the concurrent production and reduction of NO_3^- along the systems. The temporal variability of N removal rates showed that the systems were more effective in removing nitrate at higher temperatures.

The isotopic fractionation of NH_4^+ and NO_3^- provided valuable information about the underlying microbial reactions. $\epsilon^{15}\text{N}\text{-NH}_4^+$ values indicated enrichment of the residual NH_4^+ , suggesting the preferential incorporation of lighter isotopes into the newly generated NO_3^- during nitrification, making it difficult to model the simultaneous NO_3^- production and reduction as a closed system process using the Rayleigh distillation equation. However, isotopic tools could not quantify the exact contributions of sorption and plant assimilation.

Finally, this thesis significantly contributes to the understanding of nitrogen removal dynamics and highlights the importance of considering multiple processes, microbial activities,

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and environmental factors in devising effective nitrogen management strategies. Continuing to address the challenges of nitrogen pollution, the knowledge gained from this research will serve as a valuable resource in improving environmental practices related to nitrogen pollution.

5. FUTURE WORK

Promising results have been obtained from the current laboratory work, demonstrating that *lías* effectively removes nitrate at a C/N ratio of 1.25. However, in the present study, it was observed that the organic carbon concentration did not support the occurrence of DNRA in the prevailing experimental conditions. In order to gain more robust insights into the absence of DNRA under these specific organic carbon concentrations, future investigations should diligently monitor ammonium levels to establish concrete evidence and identify the conditions conducive to DNRA. This comprehensive analysis will contribute significantly to the understanding of nitrogen removal pathways. Additionally, it would be intriguing to evaluate the denitrification efficiency of *lías* under diverse feeding strategies, varying C/N ratios, injection frequencies, and flow velocities. This exploration can uncover valuable insights into optimizing denitrification processes. Considering the scope of this thesis, experimental biomass analyses were not conducted. However, incorporating experimental biomass analyses and assessment of community dynamics of denitrifying bacteria in future experiments would be important, as it may be useful in model applications to refine the geochemical models and understand the role of denitrifying bacteria in nitrogen cycling processes.

To bridge the gap between controlled laboratory conditions and real-world applications of *lías*, it is essential to conduct field campaigns in the constructed wetland in the future. The field treatment campaigns will allow the assessment of the performance of *lías* at larger scales, advancing the understanding of their practical applications. The isotopic fractionation values calculated from laboratory experiments can serve as a valuable tool for estimating the efficiency of nitrate degradation at the field-scale. By utilizing these data, valuable insights can be gained into the potential of *lías* as a sustainable and eco-friendly approach for nitrate remediation.

The utilization of ferrous iron has shown promise in augmenting the removal of nitrogen compounds. It would be compelling to explore the combined effects of integrating Fe(II)-containing minerals with *lías* to evaluate their combined impact on nitrate attenuation during nitrate treatment in future laboratory experiments, advancing the current research endeavors. Previous studies have shown that solid organic compounds like pine bark with zero-valent iron can improve nitrate removal. However, there remains a lack of research into the treatability of nitrate with liquid organic waste and ferrous iron, both at the laboratory and field-scales. Exploring the combined efficacy of Fe(II) containing minerals and organic liquid waste would be particularly intriguing for evaluating their potential in nitrate remediation feasibility studies. This investigation will provide valuable insights into the potential synergy between organic and

inorganic wastes, which could enhance nitrate reduction and more sustainable electron donor sources.

The present study identified that a significant portion of the generated N_2O remained dissolved in water during nitrite reduction, and samples were not purged prior to the azide reduction method, which can lead to potential interferences. It will be important in future experiments to implement measures to ensure complete recovery of all N_2O produced from nitrite reduction in the headspace of the vials during the azide reduction method. A crucial step to achieve this will be to flush the vials with an inert gas after the azide reduction method to expel any dissolved N_2O from the water. This adjustment is vital for obtaining a sufficient quantity of gaseous N_2O in the headspace of the vials, particularly during the initial stages of the experiments when nitrite reduction is minimal for subsequent site preference analyses. Additionally, it is essential to avoid potential sources of interference, such as the mixing of N_2O generated from the coupled NO_2^- reduction-Fe(II) oxidation (first step) with N_2O produced through the sodium azide reduction method (second step) in future experiments. To mitigate this risk in future experiments, thorough purging of the headspace vials with inert gas is recommended before starting the second step, ensuring the removal of any residual N_2O from the first step.

In this study, the application of isotopic tools faced certain limitations in precisely quantifying the relative contributions of nitrate removal efficiencies linked to multifaceted processes. By employing more advanced tools, future experiments can comprehensively characterize various nitrate removal processes, ultimately leading to a deeper understanding of the underlying mechanisms.

Scientific research has given relatively limited attention to geochemical models describing biogeochemical processes, and future study bridges this gap by applying the numerical models developed in this thesis to previous experiments to study the behavior and transformation of NO_3^- , NO_2^- , N_2O and NPDOC concentrations and $\delta^{15}\text{N}$ of NO_3^- and NO_2^- to have a verified tool to upscale laboratory treatments to the field-scale study. To establish the reliability of the models for field-scale studies, it is essential to extend their application to more comprehensive studies conducted under varied experimental conditions. This expanded evaluation will contribute to more robust and thoroughly tested models, enhancing their suitability for future field applications. This study will include the coupled reactions involving nitrate and nitrite reduction and the oxidation of various organic carbon sources linked to biomass growth and decay for biostimulation treatments in the case of Margalef-Martí et al. (2019b) and the coupled nitrite

Future work

reduction-Fe(II) oxidation for the abiotic nitrite treatments for the case of Benaiges-Fernandez et al. (2020).

Another aspect of future work aims to investigate alternative organic matter injection methods in Phreeqc, which addresses the limitation of continuous organic matter injection, which may introduce errors, especially for daily injections in long-term groundwater nitrate treatments. Incorporating field measurements is also essential for future studies to further augment the models' accuracy and precision. Robust field measurements and the establishment of long-term monitoring protocols are necessary to accommodate the intricate and constantly evolving dynamics of natural systems. This step is crucial to ensuring the models' effectiveness in field-scale applications.

An intriguing avenue for advancing the models developed in this thesis also involves modeling the $\delta^{18}\text{O}$ (oxygen isotopic composition) of nitrogen oxides. Addressing the intricate nature of O_2 reactions with diverse compounds in natural settings poses challenges. However, efforts to refine the models and acquire valuable insights into the isotopic behavior of $\delta^{18}\text{O}$ in nitrate and nitrite in future models will carry considerable significance. This advancement will contribute to a better understanding of the isotopic dynamics within nitrogen cycling processes.

In conclusion, the proposed future work aims to expand upon the current thesis, addressing its limitations and exploring new avenues of research. By incorporating novel experimental approaches and advanced analytical tools, future investigations will improve nitrate removal in laboratory and field-scale biostimulation treatments and nitrate attenuation geochemical numerical models.

REFERENCES

- Abascal, E., Gómez-Coma, L., Ortiz, I., Ortiz, A., 2022. Global diagnosis of nitrate pollution in groundwater and review of removal technologies. *Sci. Total Environ.* 810, 152233. <https://doi.org/10.1016/j.scitotenv.2021.152233>
- Adhikari, U., Harrigan, T., Reinhold, D.M., 2015. Use of duckweed-based constructed wetlands for nutrient recovery and pollutant reduction from dairy wastewater. *Ecol. Eng.* 78, 6–14. <https://doi.org/10.1016/j.ecoleng.2014.05.024>
- Allison, J.D., Brown, D.S., Novo-Gradac, K.J., 1991. MINTEQA2/PRODEFA2, a geochemical assessment model for environmental systems: version 3.0 user's manual. Environmental Research Laboratory, Office of Research and Development, US Environmental Protection Agency.
- Amatya, I.M., Kansakar, B.R., Tare, V., Fiksdal, L., 2011. Role of pH on Biological Nitrification Process. *J. Inst. Eng.* 8, 119–125. <https://doi.org/10.3126/jie.v8i1-2.5102>
- Andersson, K.K., Hooper, A.B., 1983. O₂ and H₂O are each the source of one O in NO₂ produced from NH₃ by Nitrosomonas: 15N-NMR evidence. *FEBS Lett.* 164, 236–240. [https://doi.org/10.1016/0014-5793\(83\)80292-0](https://doi.org/10.1016/0014-5793(83)80292-0)
- Arauzo, M., 2017. Vulnerability of groundwater resources to nitrate pollution: A simple and effective procedure for delimiting Nitrate Vulnerable Zones. *Sci. Total Environ.* 575, 799–812. <https://doi.org/10.1016/j.scitotenv.2016.09.139>
- Aravena, R., Robertson, W.D., 1998. Use of multiple isotope tracers to evaluate denitrification in ground water: Study of nitrate from a large-flux septic system plume. *Ground Water* 36, 975–982. <https://doi.org/10.1111/j.1745-6584.1998.tb02104.x>
- Bååth, E., 1989. Effects of heavy metals in soil on microbial processes and populations (a review). *Water. Air. Soil Pollut.* 47, 335–379. <https://doi.org/10.1007/BF00279331>
- Barcelona, M.J., Naymlk, T.G., 1984. Dynamics of a Fertilizer Contaminant Plume in Groundwater. *Environ. Sci. Technol.* 18, 257–261. <https://doi.org/10.1021/es00122a009>
- Beganskas, S., Gorski, G., Weathers, T., Fisher, A.T., Schmidt, C., Saltikov, C., Redford, K., Stoneburner, B., Harmon, R., Weir, W., 2018. A horizontal permeable reactive barrier stimulates nitrate removal and shifts microbial ecology during rapid infiltration for managed recharge. *Water Res.* 144, 274–284. <https://doi.org/10.1016/j.watres.2018.07.039>
- Benaiges-Fernandez, R., Offeddu, F.G., Margalef-Martí, R., Palau, J., Urmeneta, J., Carrey, R., Otero, N., Cama, J., 2020. Geochemical and isotopic study of abiotic nitrite reduction coupled to biologically produced Fe(II) oxidation in marine environments. *Chemosphere* 260, 127554. <https://doi.org/10.1016/j.chemosphere.2020.127554>
- Beutel, M.W., Newton, C.D., Brouillard, E.S., Watts, R.J., 2009. Nitrate removal in surface-flow constructed wetlands treating dilute agricultural runoff in the lower Yakima Basin, Washington. *Ecol. Eng.* 35, 1538–1546. <https://doi.org/10.1016/j.ecoleng.2009.07.005>
- Bezbaruah, A.N., Zhang, T.C., 2003. Performance of a constructed wetland with a sulfur/limestone denitrification section for wastewater nitrogen removal. *Environ. Sci. Technol.* 37, 1690–1697. <https://doi.org/10.1021/es020912w>

References

- Böttcher, J., Strebel, O., Voerkelius, S., Schmidt, H.L., 1990. Using isotope fractionation of nitrate-nitrogen and nitrate-oxygen for evaluation of microbial denitrification in a sandy aquifer. *J. Hydrol.* 114, 413–424. [https://doi.org/10.1016/0022-1694\(90\)90068-9](https://doi.org/10.1016/0022-1694(90)90068-9)
- Breukelen, B.M. Van, Griffioen, J., Ro, W.F.M., 2004. Reactive transport modelling of biogeochemical processes and carbon isotope geochemistry inside a landfill leachate plume 70, 249–269. <https://doi.org/10.1016/j.jconhyd.2003.09.003>
- Brun, A., Engesgaard, P., 2002. Modelling of transport and biogeochemical processes in pollution plumes: Literature review and model development. *J. Hydrol.* [https://doi.org/10.1016/S0022-1694\(01\)00547-9](https://doi.org/10.1016/S0022-1694(01)00547-9)
- Buchwald, C., Grabb, K., Hansel, C.M., Wankel, S.D., 2016. Constraining the role of iron in environmental nitrogen transformations: Dual stable isotope systematics of abiotic NO₂-reduction by Fe(II) and its production of N₂O. *Geochim. Cosmochim. Acta* 186, 1–12. <https://doi.org/10.1016/j.gca.2016.04.041>
- Burgin, A.J., Hamilton, S.K., 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. *Front. Ecol. Environ.* 5, 89–96. [https://doi.org/10.1890/1540-9295\(2007\)5\[89:HWOTRO\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2007)5[89:HWOTRO]2.0.CO;2)
- Canter, L.W., 2019. Nitrates in groundwater. Routledge. <https://doi.org/10.1201/9780203745793>
- Carrey, R., Otero, N., Soler, A., Gómez-Alday, J.J., Ayora, C., 2013. The role of Lower Cretaceous sediments in groundwater nitrate attenuation in central Spain: Column experiments. *Appl. Geochemistry* 32, 142–152. <https://doi.org/10.1016/j.apgeochem.2012.10.009>
- Carrey, R., Otero, N., Vidal-Gavilan, G., Ayora, C., Soler, A., Gómez-Alday, J.J., 2014. Induced nitrate attenuation by glucose in groundwater: Flow-through experiment. *Chem. Geol.* 370, 19–28. <https://doi.org/10.1016/j.chemgeo.2014.01.016>
- Carvajal-Arroyo, J.M., Puyol, D., Li, G., Sierra-Álvarez, R., Field, J.A., 2014. The role of pH on the resistance of resting- and active anammox bacteria to NO₂-inhibition. *Biotechnol. Bioeng.* 111, 1949–1956. <https://doi.org/10.1002/bit.25269>
- Ceschin, S., Sgambato, V., Ellwood, N.T.W., Zuccarello, V., 2019. Phytoremediation performance of Lemna communities in a constructed wetland system for wastewater treatment. *Environ. Exp. Bot.* 162, 67–71. <https://doi.org/10.1016/j.envexpbot.2019.02.007>
- Chen, D., Yuan, X., Zhao, W., Luo, X., Li, F., Liu, T., 2020. Chemodenitrification by Fe(II) and nitrite: pH effect, mineralization and kinetic modeling. *Chem. Geol.* 541. <https://doi.org/10.1016/j.chemgeo.2020.119586>
- Chen, D.J.Z., MacQuarrie, K.T.B., 2004. Numerical simulation of organic carbon, nitrate, and nitrogen isotope behavior during denitrification in a riparian zone. *J. Hydrol.* 293, 235–254. <https://doi.org/10.1016/j.jhydrol.2004.02.002>
- Chen, G., Chen, D., Li, F., Liu, T., Zhao, Z., Cao, F., 2020. Dual nitrogen-oxygen isotopic analysis and kinetic model for enzymatic nitrate reduction coupled with Fe(II) oxidation by *Pseudogulbenkiania* sp. strain 2002. *Chem. Geol.* 534, 119456. <https://doi.org/10.1016/j.chemgeo.2019.119456>
- Cho, S., Kambey, C., Nguyen, V.K., 2020. Performance of anammox processes for wastewater treatment: A critical review on effects of operational conditions and environmental stresses. *Water (Switzerland)* 12. <https://doi.org/10.3390/w12010020>

References

- Clement, T.P., Peyton, B.M., Skeen, R.S., Jennings, D.A., Petersen, J.N., 1997. Microbial growth and transport in porous media under denitrification conditions: Experiments and simulations. *J. Contam. Hydrol.* 24, 269–285. [https://doi.org/10.1016/S0169-7722\(96\)00014-9](https://doi.org/10.1016/S0169-7722(96)00014-9)
- Critchley, K., Rudolph, D.L., Devlin, J.F., Schillig, P.C., 2014. Stimulating in situ denitrification in an aerobic, highly permeable municipal drinking water aquifer. *J. Contam. Hydrol.* 171, 66–80. <https://doi.org/10.1016/j.jconhyd.2014.10.008>
- Dhakal, P., Coyne, M.S., McNear, D.H., Wendroth, O.O., Vandiviere, M.M., D'Angelo, E.M., Matocha, C.J., 2021. Reactions of nitrite with goethite and surface Fe(II)-goethite complexes. *Sci. Total Environ.* 782, 146406. <https://doi.org/10.1016/j.scitotenv.2021.146406>
- Dhakal, P., Matocha, C.J., Huggins, F.E., Vandiviere, M.M., 2013. Nitrite reactivity with magnetite. *Environ. Sci. Technol.* 47, 6206–6213. <https://doi.org/10.1021/es304011w>
- Egli, K., Fanger, U., Alvarez, P.J.J., Siegrist, H., Van der Meer, J.R., Zehnder, A.J.B., 2001. Enrichment and characterization of an anammox bacterium from a rotating biological contactor treating ammonium-rich leachate. *Arch. Microbiol.* 175, 198–207. <https://doi.org/10.1007/s002030100255>
- EPA, 2013. Introduction to in situ bioremediation of groundwater. Epa 542-R-13-018 1–86.
- Erskine, A.D., 2000. Transport of ammonium in aquifers: retardation and degradation. *Q. J. Eng. Geol. Hydrogeol.* 33, 161–170. <https://doi.org/10.1144/qjegh.33.2.161>
- EU Parliament, 2020. Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption, Official Journal of the European Union. <https://doi.org/https://www.fao.org/faolex/results/details/en/c/LEX-FAOC201243/>
- European Commission, 2021. Report on the implementation of Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources based on Member State reports for the period 2016–2019, European Communication.
- European Union, 1991. Council directive of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources. *Off. J. Eur. Communities L* 375, 1–13.
- Evans, P.J., Fricke, R.A., Hopfensperger, K., Titus, T., 2011. In situ destruction of perchlorate and nitrate using gaseous electron donor injection technology. *Gr. Water Monit. Remediat.* 31, 103–112. <https://doi.org/10.1111/j.1745-6592.2011.01355.x>
- Faisal, A.A.H., Sulaymon, A.H., Khaliefa, Q.M., 2018. A review of permeable reactive barrier as passive sustainable technology for groundwater remediation. *Int. J. Environ. Sci. Technol.* 15, 1123–1138. <https://doi.org/10.1007/s13762-017-1466-0>
- Fdz-Polanco, F., Villaverde, S., Garcia, P.A., 1994. Temperature effect on nitrifying bacterial activity in biofilters: activation and free ammonia inhibition. *Water Sci. Technol.* 30, 121. <https://doi.org/10.2166/wst.1994.0552>
- Friedl, J., De Rosa, D., Rowlings, D.W., Grace, P.R., Müller, C., Scheer, C., 2018. Dissimilatory nitrate reduction to ammonium (DNRA), not denitrification dominates nitrate reduction in subtropical pasture soils upon rewetting. *Soil Biol. Biochem.* 125, 340–349. <https://doi.org/10.1016/j.soilbio.2018.07.024>

References

- Fukada, T., Hiscock, K.M., Dennis, P.F., Grischek, T., 2003. A dual isotope approach to identify denitrification in groundwater at a river-bank infiltration site 37, 3070–3078. [https://doi.org/10.1016/S0043-1354\(03\)00176-3](https://doi.org/10.1016/S0043-1354(03)00176-3)
- Gabarda-Mallorquí, A., Fraguell, R.M., Pavón, D., Ribas, A., 2016. Tourist development and wastewater treatment in the Spanish Mediterranean coast: The Costa Brava case study. *Int. J. Sustain. Dev. Plan.* 11, 245–254. <https://doi.org/10.2495/SDP-V11-N3-245-254>
- Gagnon, V., Chazarenc, F., Comeau, Y., Brisson, J., 2007. Influence of macrophyte species on microbial density and activity in constructed wetlands. *Water Sci. Technol.* 56, 249–254. <https://doi.org/10.2166/wst.2007.510>
- García, J., Solimeno, A., Zhang, L., Marois, D., Mitsch, W.J., 2020. Constructed wetlands to solve agricultural drainage pollution in South Florida: Development of an advanced simulation tool for design optimization. *J. Clean. Prod.* 258. <https://doi.org/10.1016/j.jclepro.2020.120868>
- Ge, Z., Wei, D., Zhang, J., Hu, J., Liu, Z., Li, R., 2019. Natural pyrite to enhance simultaneous long-term nitrogen and phosphorus removal in constructed wetland: Three years of pilot study. *Water Res.* 148, 153–161. <https://doi.org/10.1016/j.watres.2018.10.037>
- Geranio, L., 2007. Review of Zero Valent Iron and Apatite as reactive materials for Permeable Reactive Barrier. Term Pap. SS 07/08, major Biogeochem. Pollut. Dyn. ETH Zürich. <https://doi.org/10.2172/814389>
- Gerardi, M.H., 2003. Nitrification and denitrification in the activated sludge process, John Wiley & Sons, Inc. <https://doi.org/10.1002/0471216682>
- Ghaeminia, M., Mokhtarani, N., 2018. Remediation of nitrate-contaminated groundwater by PRB-Electrokinetic integrated process. *J. Environ. Manage.* 222, 234–241. <https://doi.org/10.1016/j.jenvman.2018.05.078>
- Gibert, O., Assal, A., Devlin, H., Elliot, T., Kalin, R.M., 2019. Performance of a field-scale biological permeable reactive barrier for in-situ remediation of nitrate-contaminated groundwater. *Sci. Total Environ.* 659, 211–220. <https://doi.org/10.1016/j.scitotenv.2018.12.340>
- Giblin, A.E., Tobias, C.R., Song, B., Weston, N., Banta, G.T., Rivera-Monroy, V.H., 2013. The importance of dissimilatory nitrate reduction to ammonium (DNRA) in the nitrogen cycle of coastal ecosystems. *Oceanography* 26, 124–131. <https://doi.org/10.5670/oceanog.2013.54>
- Grabb, K.C., Buchwald, C., Hansel, C.M., Wankel, S.D., 2017. A dual nitrite isotopic investigation of chemodenitrification by mineral-associated Fe(II) and its production of nitrous oxide. *Geochim. Cosmochim. Acta* 196, 388–402. <https://doi.org/10.1016/j.gca.2016.10.026>
- Grau-Martínez, A., Torrentó, C., Carrey, R., Rodríguez-Escales, P., Domènech, C., Ghiglieri, G., Soler, A., Otero, N., 2017. Feasibility of two low-cost organic substrates for inducing denitrification in artificial recharge ponds: Batch and flow-through experiments. *J. Contam. Hydrol.* 198, 48–58. <https://doi.org/10.1016/j.jconhyd.2017.01.001>
- Green, C.T., Bekins, B.A., 2010. Sustainability of Natural Attenuation of Nitrate in Agricultural Aquifers. US Dep. Inter. US Geol. Surv. 1–8. <https://doi.org/10.3133/fs20103077>
- Grzyb, A., Wolna-Maruwka, A., Niewiadomska, A., 2021. The significance of microbial transformation of nitrogen compounds in the light of integrated crop management. *Agronomy* 11. <https://doi.org/10.3390/agronomy11071415>

References

- Guerin, T.F., Horner, S., McGovern, T., Davey, B., 2002. An application of permeable reactive barrier technology to petroleum hydrocarbon contaminated groundwater. *Water Res.* 36, 15–24. [https://doi.org/10.1016/S0043-1354\(01\)00233-0](https://doi.org/10.1016/S0043-1354(01)00233-0)
- Hatfield, J.L., Follett, R.F., 2001. Nitrogen in the Environment: Sources, Problems and Management. <https://doi.org/10.1016/b978-0-444-50486-9.x5000-6>
- Hellman, M., Valhondo, C., Martínez-Landa, L., Carrera, J., Juhanson, J., Hallin, S., 2022. Nitrogen Removal Capacity of Microbial Communities Developing in Compost- and Woodchip-Based Multipurpose Reactive Barriers for Aquifer Recharge With Wastewater. *Front. Microbiol.* 13, 1–11. <https://doi.org/10.3389/fmicb.2022.877990>
- Hiscock, K.M., Lloyd, J.W., Lerner, D.N., 1991. Review of natural and artificial denitrification of groundwater. *Water Res.* 25, 1099–1111. [https://doi.org/10.1016/0043-1354\(91\)90203-3](https://doi.org/10.1016/0043-1354(91)90203-3)
- Hosseini, S.M., Tosco, T., 2015. Integrating NZVI and carbon substrates in a non-pumping reactive wells array for the remediation of a nitrate contaminated aquifer. *J. Contam. Hydrol.* 179, 182–195. <https://doi.org/10.1016/j.jconhyd.2015.06.006>
- Huang, C.P., Wang, H.W., Chiu, P.C., 1998. Nitrate reduction by metallic iron. *Water Res.* 32, 2257–2264. [https://doi.org/10.1016/S0043-1354\(97\)00464-8](https://doi.org/10.1016/S0043-1354(97)00464-8)
- Huang, G., Huang, Y., Hu, H., Liu, F., Zhang, Y., Deng, R., 2015. Remediation of nitrate-nitrogen contaminated groundwater using a pilot-scale two-layer heterotrophic-autotrophic denitrification permeable reactive barrier with spongy iron/pine bark. *Chemosphere* 130, 8–16. <https://doi.org/10.1016/j.chemosphere.2015.02.029>
- Jacinto, P.A., Groffman, P.M., Gold, A.J., Mosier, A., 1998. Patchiness in microbial nitrogen transformations in groundwater in a riparian forest. *J. Environ. Qual.* 27, 156–164. <https://doi.org/10.2134/jeq1998.00472425002700010022x>
- Jahangir, M.M.R., Fenton, O., Müller, C., Harrington, R., Johnston, P., Richards, K.G., 2017. In situ denitrification and DNRA rates in groundwater beneath an integrated constructed wetland. *Water Res.* 111, 254–264. <https://doi.org/10.1016/j.watres.2017.01.015>
- Jakobsen, R., Postma, D., 1996. Redox zonation: Equilibrium constraints on the Fe(III)/SO₄-reduction interface. *Geochim. Cosmochim. Acta* 60, 3169–3175. [https://doi.org/10.1016/0016-7037\(96\)00156-1](https://doi.org/10.1016/0016-7037(96)00156-1)
- Jie, H.U., Daping, L.I., Qiang, L.I.U., Yong, T.A.O., Xiaohong, H.E., Xiaomei, W., Xudong, L.I., Ping, G.A.O., 2009. Effect of organic carbon on nitrification efficiency and community composition of nitrifying biofilms 21, 387–394. [https://doi.org/10.1016/S1001-0742\(08\)62281-0](https://doi.org/10.1016/S1001-0742(08)62281-0)
- Jin, Q., Bethke, C.M., 2003. A New Rate Law Describing Microbial Respiration. *Appl. Environ. Microbiol.* 69, 2340–2348. <https://doi.org/10.1128/AEM.69.4.2340>
- Jin, R.C., Yang, G.F., Yu, J.J., Zheng, P., 2012. The inhibition of the Anammox process: A review. *Chem. Eng. J.* 197, 67–79. <https://doi.org/10.1016/j.cej.2012.05.014>
- Jones, A.M., Griffin, P.J., Collins, R.N., Waite, T.D., 2014. Ferrous iron oxidation under acidic conditions - The effect of ferric oxide surfaces. *Geochim. Cosmochim. Acta* 145, 1–12. <https://doi.org/10.1016/j.gca.2014.09.020>
- Jones, L.C., Peters, B., Lezama Pacheco, J.S., Casciotti, K.L., Fendorf, S., 2015. Stable isotopes and iron oxide mineral products as markers of chemodenitrification. *Environ. Sci. Technol.* 49, 3444–3452. <https://doi.org/10.1021/es504862x>

References

- Kampschreur, M.J., Kleerebezem, R., de Vet, W.W.J.M., Van Loosdrecht, M.C.M., 2011. Reduced iron induced nitric oxide and nitrous oxide emission. *Water Res.* 45, 5945–5952. <https://doi.org/10.1016/j.watres.2011.08.056>
- Kampschreur, M.J., van der Star, W.R.L., Wielders, H.A., Mulder, J.W., Jetten, M.S.M., van Loosdrecht, M.C.M., 2008. Dynamics of nitric oxide and nitrous oxide emission during full-scale reject water treatment. *Water Res.* 42, 812–826. <https://doi.org/10.1016/j.watres.2007.08.022>
- Kappler, A., Straub, K.L., 2005. Geomicrobiological cycling of iron. *Mol. Geomicrobiol.* 59, 85–108. <https://doi.org/10.2138/rmg.2005.59.5>
- Kazumi, J., Capone, D.G., 1994. Heterotrophic microbial activity in shallow aquifer sediments of Long Island, New York. *Microb. Ecol.* 28, 19–37. <https://doi.org/10.1007/BF00170245>
- Kendall, C., Elliott, E.M., Wankel, S.D., 2007. Tracing anthropogenic inputs of nitrogen to ecosystems, in: *Stable Isotopes in Ecology and Environmental Science*. pp. 375–449. <https://doi.org/10.1002/9780470691854.ch12>
- Kharaka, Y.K., Gunter, W.D., Aggarwal, P.K., Perkins, E.H., DeBraal, J.D., 1988. SOLMINEQ. 88, a computer program for geochemical modeling of water-rock interactions (Vol. 88, No. 4227). Department of the Interior, US Geological Survey. <https://doi.org/10.3133/wri884227>
- Killingstad, M.W., Widdowson, M.A., Smith, R.L., 2002. Modeling Enhanced In Situ Denitrification in Groundwater. *J. Environ. Eng.* 128, 491–504. [https://doi.org/10.1061/\(asce\)0733-9372\(2002\)128:6\(491\)](https://doi.org/10.1061/(asce)0733-9372(2002)128:6(491))
- Kinzelbach, W., Schäfer, W., Herzer, J., 1991. Numerical modeling of natural and enhanced denitrification processes in aquifers. *Water Resour. Res.* 27, 1123–1135. <https://doi.org/10.1029/91WR00474>
- Knöller, K., Vogt, C., Haupt, M., Feisthauer, S., Richnow, H.H., 2011. Experimental investigation of nitrogen and oxygen isotope fractionation in nitrate and nitrite during denitrification. *Biogeochemistry* 103, 371–384. <https://doi.org/10.1007/s10533-010-9483-9>
- Kool, D. M., Wrage, N., Oenema, O., Dolfing, J., & Van Groenigen, J. W. (2007). Oxygen exchange between (de) nitrification intermediates and H₂O and its implications for source determination of NO₃ and N₂O : a review. *Rapid Communications in Mass Spectrometry : RCM*, 21, 3569–3578. <https://doi.org/10.1002/rcm>
- Kornaros, M., Lyberatos, G., 1998. Kinetic modelling of *Pseudomonas dentrificans* growth and denitrification under aerobic, anoxic and transient operating conditions. *Water Res.* 32, 1912–1922. [https://doi.org/10.1016/S0043-1354\(97\)00403-X](https://doi.org/10.1016/S0043-1354(97)00403-X)
- Kraft, B., Tegetmeyer, H.E., Sharma, R., Klotz, M.G., Ferdelman, T.G., Hettich, R.L., Geelhoed, J.S., Strous, M., 2014. The environmental controls that govern the end product of bacterial nitrate respiration. *Science* (80-.). 345, 676–679. <https://doi.org/10.1126/science.1254070>
- Kurt, M., Dunn, I.J., Bourne, J.R., 1987. Biological denitrification of drinking water using autotrophic organisms with H₂ in a fluidized-bed biofilm reactor. *Biotechnol. Bioeng.* 29, 493–501. <https://doi.org/10.1002/bit.260290414>
- Larramendy, M.L., 2016. Organic Fertilizers - From Basic Concepts to Applied Outcomes. *Org. Fertil. - From Basic Concepts to Appl. Outcomes*. <https://doi.org/10.5772/61454>

References

- Lee, M.S., Lee, K.K., Hyun, Y., Clement, T.P., Hamilton, D., 2006. Nitrogen transformation and transport modeling in groundwater aquifers. *Ecol. Modell.* 192, 143–159. <https://doi.org/10.1016/j.ecolmodel.2005.07.013>
- Lensing, H.J., Vogt, M., Herrling, B., 1994. Modeling of biologically mediated redox processes in the subsurface. *J. Hydrol.* 159, 125–143. [https://doi.org/10.1016/0022-1694\(94\)90252-6](https://doi.org/10.1016/0022-1694(94)90252-6)
- Li, F., Lu, L., Zheng, X., Ngo, H.H., Liang, S., Guo, W., Zhang, X., 2014. Enhanced nitrogen removal in constructed wetlands: Effects of dissolved oxygen and step-feeding. *Bioresour. Technol.* 169, 395–402. <https://doi.org/10.1016/j.biortech.2014.07.004>
- Li, P., Karunanidhi, D., Subramani, T., Srinivasamoorthy, K., 2021. Sources and Consequences of Groundwater Contamination. *Arch. Environ. Contam. Toxicol.* 80, 1–10. <https://doi.org/10.1007/s00244-020-00805-z>
- Li, S., Wang, S., Ji, G., 2022. Influences of carbon sources on N₂O production during denitrification in freshwaters: Activity, isotopes and functional microbes. *Water Res.* 226, 119315. <https://doi.org/10.1016/j.watres.2022.119315>
- Ling, J., Chen, S., 2005. Impact of organic carbon on nitrification performance of different biofilters 33, 150–162. <https://doi.org/10.1016/j.aquaeng.2004.12.002>
- Liu, F., Huang, G., Fallowfield, H., Guan, H., Zhu, L., Hu, H., 2013. Study on Heterotrophic-Autotrophic Denitrification Permeable Reactive Barriers (HAD PRBs) for In Situ Groundwater Remediation. Springer briefs in water science and technology.
- Liu, T., Chen, D., Luo, X., Li, X., Li, F., 2019. Microbially mediated nitrate-reducing Fe(II) oxidation: Quantification of chemodenitrification and biological reactions. *Geochim. Cosmochim. Acta* 256, 97–115. <https://doi.org/10.1016/j.gca.2018.06.040>
- Luo, P., Liu, F., Zhang, S., Li, H., Yao, R., Jiang, Q., Xiao, R., Wu, J., 2018. Nitrogen removal and recovery from lagoon-pretreated swine wastewater by constructed wetlands under sustainable plant harvesting management. *Bioresour. Technol.* 258, 247–254. <https://doi.org/10.1016/j.biortech.2018.03.017>
- Luvizotto, D.M., Araujo, J.E., Silva, M.D.C.P., Dias, A.C.F., Kraft, B., Tegetmeyer, H., Strous, M., Andreote, F.E.R.N.A.N.D.O.D., 2019. The rates and players of denitrification, dissimilatory nitrate reduction to ammonia (Dnra) and anaerobic ammonia oxidation (anammox) in mangrove soils. *An. Acad. Bras. Cienc.* 91, 1–14. <https://doi.org/10.1590/0001-3765201820180373>
- Ma, Y., Zheng, X., Fang, Y., Xu, K., He, S., Zhao, M., 2020. Autotrophic denitrification in constructed wetlands: achievements and challenges. *Bioresour. Technol.* 123778. <https://doi.org/10.1016/j.biortech.2020.123778>
- Margalef-Martí, R., Carrey, R., Merchán, D., Soler, A., Causapé, J., Otero, N., 2019a. Feasibility of using rural waste products to increase the denitrification efficiency in a surface flow constructed wetland. *J. Hydrol.* 578. <https://doi.org/10.1016/j.jhydrol.2019.124035>
- Margalef-Martí, R., Carrey, R., Soler, A., Otero, N., 2019b. Evaluating the potential use of a dairy industry residue to induce denitrification in polluted water bodies: A flow-through experiment, in: *Journal of Environmental Management*. Elsevier, pp. 86–94. <https://doi.org/10.1016/j.jenvman.2019.03.086>
- Margalef-Martí, R., Carrey, R., Viladés, M., Jubany, I., Vilanova, E., Grau, R., Soler, A., Otero, N., 2019c. Use of nitrogen and oxygen isotopes of dissolved nitrate to trace field-scale induced denitrification efficiency throughout an in-situ groundwater remediation strategy. *Sci.*

References

- Total Environ. <https://doi.org/10.1016/j.scitotenv.2019.06.003>
- Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., Tardieux, P., 1981. Experimental determination of nitrogen kinetic isotope fractionation: Some principles; illustration for the denitrification and nitrification processes. *Plant Soil* 62, 413–430. <https://doi.org/10.1007/BF02374138>
- Mariotti, André, Landreau, A., Simon, B., 1988. 15N isotope biogeochemistry and natural denitrification process in groundwater: Application to the chalk aquifer of northern France. *Geochimica et Cosmochimica Acta*, 52(7), 1869–1878. [https://doi.org/10.1016/0016-7037\(88\)90010-5](https://doi.org/10.1016/0016-7037(88)90010-5)
- Martin, D., Salminen, J.M., Niemi, R.M., Heiskanen, I.M., Valve, M.J., Hellstén, P.P., Nystén, T.H., 2009. Acetate and ethanol as potential enhancers of low temperature denitrification in soil contaminated by fur farms: A pilot-scale study. *J. Hazard. Mater.* 163, 1230–1238. <https://doi.org/10.1016/j.jhazmat.2008.07.092>
- Massarelli, C., Losacco, D., Tumolo, M., Campanale, C., Uricchio, V.F., 2021. Protection of water resources from agriculture pollution: An integrated methodological approach for the nitrates directive 91–676-eeec implementation. *Int. J. Environ. Res. Public Health* 18. <https://doi.org/10.3390/ijerph182413323>
- Mastrocicco, M., Colombani, N., Salemi, E., Castaldelli, G., 2011. Reactive modeling of denitrification in soils with natural and depleted organic matter. *Water. Air. Soil Pollut.* 222, 205–215. <https://doi.org/10.1007/s11270-011-0817-6>
- McNab, W.W., Narasimhan, T.N., 1994. Modeling reactive transport of organic compounds in groundwater using a partial redox disequilibrium approach. *Water Resour. Res.* 30, 2619–2635. <https://doi.org/10.1029/94WR01305>
- Merchán, D., Causapé, J., Abrahão, R., 2013. Impact of irrigation implementation on hydrology and water quality in a small agricultural basin in Spain. *Hydrol. Sci. J.* 58, 1400–1413. <https://doi.org/10.1080/02626667.2013.829576>
- Merchán, D., Causapé, J., Abrahão, R., García-Garizábal, I., 2015. Assessment of a newly implemented irrigated area (Lerma Basin, Spain) over a 10-year period. I: Water balances and irrigation performance. *Agric. Water Manag.* 158, 277–287. <https://doi.org/10.1016/j.agwat.2015.04.016>
- Merchán, D., Otero, N., Soler, A., Causapé, J., 2014. Main sources and processes affecting dissolved sulphates and nitrates in a small irrigated basin (Lerma Basin, Zaragoza, Spain): Isotopic characterization. *Agric. Ecosyst. Environ.* 195, 127–138. <https://doi.org/10.1016/j.agee.2014.05.011>
- Micola Colomer, N., Garrido Martín, T., Ballesté, E., Blanch, A., Carrey, R., Fraile Maseras, J Lucena, F., Munne Torras, A., Otero Pérez, N., Solà Subiranas, J., 2018. Determinació de l'origen dels nitrats: tècniques isotòpiques, traçadors microbiològics i anàlisi de pressions.
- Mohan, S.B., Cole, J.A., 2007. The Dissimilatory Reduction of Nitrate to Ammonia by Anaerobic Bacteria, in: *Biology of the Nitrogen Cycle*. pp. 93–106. <https://doi.org/10.1016/B978-044452857-5.50008-4>
- Mosier, A.R., 2008. Exchange of Gaseous Nitrogen Compounds Between Terrestrial Systems and the Atmosphere, in: *Nitrogen in the Environment*. pp. 291–309. <https://doi.org/10.1016/B978-0-12-374347-3.00013-5>
- Nardi, A., de Vries, L.M., 2017. GibbsStudio, Barcelona Science Technologies SL, Barcelona,

References

- Spain. <https://doi.org/https://gibbsstudio.io/>
- Ni, B.J., Pan, Y., Guo, J., Virdis, B., Hu, S., Chen, X., Yuan, Z., 2016. Denitrification Processes for Wastewater Treatment, in: *Metalloenzymes in Denitrification*. The Royal Society of Chemistry, pp. 368–418. <https://doi.org/10.1039/9781782623762-00368>
- Nikolenko, O., Jurado, A., Borges, A. V., Knöller, K., Brouyère, S., 2018. Isotopic composition of nitrogen species in groundwater under agricultural areas: A review. *Sci. Total Environ.* 621, 1415–1432. <https://doi.org/10.1016/j.scitotenv.2017.10.086>
- Pan, H., Qin, Y., Wang, Y., Liu, S., Yu, B., Song, Y., Wang, X., Zhu, G., 2020. Dissimilatory nitrate/nitrite reduction to ammonium (DNRA) pathway dominates nitrate reduction processes in rhizosphere and non-rhizosphere of four fertilized farmland soil. *Environ. Res.* 186, 109612. <https://doi.org/10.1016/j.envres.2020.109612>
- Park, K.Y., Maeng, S.K., Ahn, K.H., Lee, K., Choi, O.K., Lee, J.W., 2013. Carbon dioxide injection method for enhancing hydrogenotrophic denitrification of secondary wastewater effluent in fixed bed reactor. *Biotechnol. Bioprocess Eng.* 18, 326–332. <https://doi.org/10.1007/s12257-012-0537-9>
- Park, S., Kim, H.K., Kim, D.H., Lee, G.M., Yoon, J.H., Choi, H., Kim, M., Han, K., Kim, Y., Chung, H.M., 2019. The effectiveness of injected carbon sources in enhancing the denitrifying processes in groundwater with high nitrate concentrations. *Process Saf. Environ. Prot.* 131, 205–211. <https://doi.org/10.1016/j.psep.2019.08.029>
- Parkhurst, D.L., Appelo, C.A.J., 2013. Description of input and examples for PHREEQC version 3: a computer program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical calculations, U.S. Geological Survey. <https://doi.org/10.1097/00000446-195210000-00005>
- Peng, Y. zhen, Ma, Y., Wang, S. ying, 2007. Denitrification potential enhancement by addition of external carbon sources in a pre-denitrification process. *J. Environ. Sci.* 19, 284–289. [https://doi.org/10.1016/S1001-0742\(07\)60046-1](https://doi.org/10.1016/S1001-0742(07)60046-1)
- Powell, R.M., Puls, R.W., Hightower, S.K., Sabatini, D.A., 1995. Coupled Iron Corrosion and Chromate Reduction: Mechanisms for Subsurface Remediation. *Environ. Sci. Technol.* 29, 1913–1922. <https://doi.org/10.1021/es00008a008>
- Qu, W., Suo, L., Liu, R., Liu, M., Zhao, Y., Xia, L., Fan, Y., Zhang, Q., Gao, Z., 2022. Influence of Temperature on Denitrification and Microbial Community Structure and Diversity: A Laboratory Study on Nitrate Removal from Groundwater. *Water (Switzerland)* 14, 1–15. <https://doi.org/10.3390/w14030436>
- Rakshit, S., Matocha, C.J., Coyne, M.S., 2008. Nitrite Reduction by Siderite. *Soil Sci. Soc. Am. J.* 72, 1070–1077. <https://doi.org/10.2136/sssaj2007.0296>
- Rakshit, S., Matocha, C.J., Coyne, M.S., Sarkar, D., 2016. Nitrite reduction by Fe(II) associated with kaolinite. *Int. J. Environ. Sci. Technol.* 13, 1329–1334. <https://doi.org/10.1007/s13762-016-0971-x>
- Rampuria, A., Gupta, A.B., Brighu, U., 2020. Nitrogen transformation processes and mass balance in deep constructed wetlands treating sewage, exploring the anammox contribution. *Bioresour. Technol.* 314, 123737. <https://doi.org/10.1016/j.biortech.2020.123737>
- Rave, J., Hodge, V., Acheson, C.M., Lutes, C., Liles, D., USEPA, 2006. In Situ and Ex Situ Biodegradation Technologies for Remediation of Contaminated Sites. *Epa* 625, 015.

References

- Rivett, M.O., Buss, S.R., Morgan, P., Smith, J.W.N., Bemment, C.D., 2008. Nitrate attenuation in groundwater: a review of biogeochemical controlling processes. *Water Res.* 42, 4215–32. <https://doi.org/10.1016/j.watres.2008.07.020>
- Robertson, G.P., Groffman, P.M., 2007. Nitrogen Transformations, in: Eldor, P.A. (Ed.), *Soil Microbiology, Ecology, and Biochemistry*. pp. 341–364. <https://doi.org/10.1016/B978-0-08-047514-1.50017-2>
- Robertson, G.P., Groffman, P.M., 2006. Nitrogen transformations, 4th ed, *Soil Microbiology, Ecology and Biochemistry: Third Edition*. Elsevier Inc. <https://doi.org/10.1016/b978-0-12-415955-6.00014-1>
- Robertson, W.D., Vogan, J.L., Lombardo, P.S., 2008. Nitrate removal rates in a 15-year-old permeable reactive barrier treating septic system nitrate. *Gr. Water Monit. Remediat.* 28, 65–72. <https://doi.org/10.1111/j.1745-6592.2008.00205.x>
- Rodríguez-Escales, P., Breukelen, B.M. Van, Vidal-gavilan, G., Soler, A., Folch, A., 2014. Integrated modeling of biogeochemical reactions and associated isotope fractionations at batch scale : A tool to monitor enhanced biodenitrification applications. *Chem. Geol.* 365, 20–29. <https://doi.org/10.1016/j.chemgeo.2013.12.003>
- Rodríguez-Escales, P., Folch, A., Breukelen, B.M. Van, Vidal-gavilan, G., Sanchez-vila, X., 2016. Modeling long term Enhanced in situ Biodenitrification and induced heterogeneity in column experiments under different feeding strategies 538, 127–137. <https://doi.org/10.1016/j.jhydrol.2016.04.012>
- Rodríguez-Villanueva, P., Sauri, D., 2021. Wastewater treatment plants in Mediterranean Spain: An exploration of relations between water treatments, water reuse, and governance. *Water (Switzerland)* 13. <https://doi.org/10.3390/w13121710>
- Rütting, T., Boeckx, P., Müller, C., Klemetsson, L., 2011. Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. *Biogeosciences* 8, 1779–1791. <https://doi.org/10.5194/bg-8-1779-2011>
- Ryabenko, E., Altabet, M.A., Wallace, D.W.R., 2009. Effect of chloride on the chemical conversion of nitrate to nitrous oxide for $\delta^{15}\text{N}$ analysis. *Limnol. Oceanogr. Methods* 7, 545–552. <https://doi.org/10.4319/lom.2009.7.545>
- Saeed, T., Sun, G., 2017. A comprehensive review on nutrients and organics removal from different wastewaters employing subsurface flow constructed wetlands. *Crit. Rev. Environ. Sci. Technol.* 47, 203–288. <https://doi.org/10.1080/10643389.2017.1318615>
- Sáez, F., Pozo, C., Gómez, M.A., Rodelas, B., González-López, J., 2003. Growth and nitrite and nitrous oxide accumulation of *Paracoccus denitrificans* ATCC 19367 in the presence of selected pesticides. *Environ. Toxicol. Chem.* 22, 1993–1997. <https://doi.org/10.1897/02-351>
- Schäfer, D., Schäfer, W., Kinzelbach, W., 1998a. Simulation of reactive processes related to biodegradation in aquifers 1. Structure of the three-dimensional reactive transport model. *J. Contam. Hydrol.* 31, 167–186. [https://doi.org/10.1016/S0169-7722\(97\)00060-0](https://doi.org/10.1016/S0169-7722(97)00060-0)
- Schäfer, D., Schäfer, W., Kinzelbach, W., 1998b. Simulation of reactive processes related to biodegradation in aquifers. 2. Model application to a column study on organic carbon degradation. *J. Contam. Hydrol.* 31, 187–209. [https://doi.org/10.1016/S0169-7722\(97\)00061-2](https://doi.org/10.1016/S0169-7722(97)00061-2)
- Schullehner, J., Hansen, B., Thygesen, M., Pedersen, C.B., Sigsgaard, T., 2018. Nitrate in drinking

References

- water and colorectal cancer risk: A nationwide population-based cohort study. *Int. J. Cancer* 143, 73–79. <https://doi.org/10.1002/ijc.31306>
- Sebilo, M., Aloisi, G., Mayer, B., Perrin, E., Vaury, V., Mothet, A., Laverman, A.M., 2019. Controls on the Isotopic Composition of Nitrite ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) during Denitrification in Freshwater Sediments. *Sci. Rep.* 9, 1–14. <https://doi.org/10.1038/s41598-019-54014-3>
- Seitzinger, S., Harrison, J.A., Böhlke, J.K., Bouwman, A.F., Lowrance, R., Peterson, B., Tobias, C., Van Drecht, G., 2006. Denitrification across landscapes and waterscapes: A synthesis. *Ecol. Appl.* 16, 2064–2090. [https://doi.org/10.1890/1051-0761\(2006\)016\[2064:DALAWA\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2006)016[2064:DALAWA]2.0.CO;2)
- Sierra-Alvarez, R., Beristain-Cardoso, R., Salazar, M., Gómez, J., Razo-Flores, E., Field, J.A., 2007. Chemolithotrophic denitrification with elemental sulfur for groundwater treatment. *Water Res.* 41, 1253–1262. <https://doi.org/10.1016/j.watres.2006.12.039>
- Singleton, M.J., Esser, B.K., Moran, J.E., Hudson, G.B., McNab, W.W., Harter, T., 2007. Saturated zone denitrification: Potential for natural attenuation of nitrate contamination in shallow groundwater under dairy operations. *Environ. Sci. Technol.* 41, 759–765. <https://doi.org/10.1021/es061253g>
- Smil, V., 2001. Enriching the earth: Fritz Haber, Carl Bosch, and the transformation of world, Chemical and Engineering News. The MIT Press Cambridge, Massachusetts.
- Soler-Jofra, A., Pérez, J., van Loosdrecht, M.C.M., 2021. Hydroxylamine and the nitrogen cycle: A review. *Water Res.* 190, 116723. <https://doi.org/10.1016/j.watres.2020.116723>
- Straub, K.L., Benz, M., Schink, B., Widdel, F., 1996. Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. *Appl. Environ. Microbiol.* 62, 1458–1460. <https://doi.org/10.1128/aem.62.4.1458-1460.1996>
- Su, C., Puls, R.W., 2007. Removal of added nitrate in the single, binary, and ternary systems of cotton burr compost, zerovalent iron, and sediment: Implications for groundwater nitrate remediation using permeable reactive barriers. *Chemosphere* 67, 1653–1662. <https://doi.org/10.1016/j.chemosphere.2006.09.059>
- Teske, A., Alm, E., Regan, J.M., Toze, S., Rittmann, B.E., Stahl, D.A., 1994. Evolutionary Relationships among Ammonia- and Nitrite-Oxidizing Bacteria. *Journal of Bacteriology* 176, 6623–6630. <https://doi.org/10.1128/jb.176.21.6623-6630.1994>
- Tiedje, J.M., 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium. *Environ. Microbiol. Anaerobes* 179–244.
- Till, B.A., Weathers, L.J., Alvarez, P.J.J., 1998. Fe(O)-supported autotrophic denitrification. *Environ. Sci. Technol.* 32, 634–639. <https://doi.org/10.1021/es9707769>
- Tomaszewski, M., Cema, G., Ziemińska-Buczyńska, A., 2017. Significance of pH control in anammox process performance at low temperature. *Chemosphere* 185, 439–444. <https://doi.org/10.1016/j.chemosphere.2017.07.034>
- Torrentó, C., Urmeneta, J., Otero, N., Soler, A., Viñas, M., Cama, J., 2011. Enhanced denitrification in groundwater and sediments from a nitrate-contaminated aquifer after addition of pyrite. *Chem. Geol.* 287, 90–101. <https://doi.org/10.1016/j.chemgeo.2011.06.002>
- Umezawa, Y., Hosono, T., Onodera, S. ichi, Siringan, F., Buapeng, S., Delinom, R., Yoshimizu, C., Tayasu, I., Nagata, T., Taniguchi, M., 2008. Sources of nitrate and ammonium

References

- contamination in groundwater under developing Asian megacities. *Sci. Total Environ.* 404, 361–376. <https://doi.org/10.1016/j.scitotenv.2008.04.021>
- United Nations, 2022. The Sustainable Development Goals Report 2022, United Nations publication issued by the Department of Economic and Social Affairs.
- Val del Río, Á., da Silva, T., Martins, T.H., Foresti, E., Campos, J.L., Méndez, R., Mosquera-Corral, A., 2017. Partial Nitrification-Anammox Granules: Short-Term Inhibitory Effects of Seven Metals on Anammox Activity. *Water. Air. Soil Pollut.* 228. <https://doi.org/10.1007/s11270-017-3628-6>
- Valhondo, C., Carrera, J., Martínez-Landa, L., Wang, J., Amalfitan, S., Levantesi, C., Díaz-Cruz, M.S., 2020a. Reactive Barriers for Renaturalization of Reclaimed Water during Soil Aquifer Treatment. *Water* 12, 10. <https://doi.org/https://doi.org/10.3390/w12041012>
- Valhondo, C., Martínez-Landa, L., Carrera, J., Díaz-Cruz, S.M., Amalfitano, S., Levantesi, C., 2020b. Six Artificial Recharge Pilot Replicates to gain Insight into Water Quality Enhancement Processes. *Chemosphere* 240. <https://doi.org/10.1016/j.chemosphere.2019.124826>
- Valhondo, C., Martínez-Landa, L., Carrera, J., Díaz-Cruz, S.M., Amalfitano, S., Levantesi, C., 2020c. Six artificial recharge pilot replicates to gain insight into water quality enhancement processes. *Chemosphere* 240. <https://doi.org/10.1016/j.chemosphere.2019.124826>
- Van Breukelen, B.M., Prommer, H., 2008. Beyond the Rayleigh equation: Reactive transport modeling of isotope fractionation effects to improve quantification of biodegradation. *Environ. Sci. Technol.* 42, 2457–2463. <https://doi.org/10.1021/es071981j>
- Van den Berg, E.M., Boleij, M., Kuenen, J.G., Kleerebezem, R., van Loosdrecht, M.C.M., 2016. DNRA and denitrification coexist over a broad range of acetate/N-NO₃⁻ ratios, in a chemostat enrichment culture. *Front. Microbiol.* 7, 1–12. <https://doi.org/10.3389/fmicb.2016.01842>
- Vidal-Gavilan, G., Carrey, R., Solanas, A., Soler, A., 2014. Feeding strategies for groundwater enhanced biodenitrification in an alluvial aquifer: Chemical, microbial and isotope assessment of a 1D flow-through experiment. *Sci. Total Environ.* 494–495, 241–251. <https://doi.org/10.1016/j.scitotenv.2014.06.100>
- Vidal-Gavilan, G., Folch, A., Otero, N., Solanas, A.M., Soler, A., 2013. Isotope characterization of an in situ biodenitrification pilot-test in a fractured aquifer. *Appl. Geochemistry* 32, 153–163. <https://doi.org/10.1016/j.apgeochem.2012.10.033>
- Visser, A.N., Wankel, S.D., Frey, C., Kappler, A., Lehmann, M.F., 2022. Unchanged nitrate and nitrite isotope fractionation during heterotrophic and Fe(II)-mixotrophic denitrification suggest a non-enzymatic link between denitrification and Fe(II) oxidation. *Front. Microbiol.* 13, 1–23. <https://doi.org/10.3389/fmicb.2022.927475>
- Vitòria, L., Soler, A., Canals, À., Otero, N., 2008. Environmental isotopes (N, S, C, O, D) to determine natural attenuation processes in nitrate contaminated waters: Example of Osona (NE Spain). *Appl. Geochemistry* 23, 3597–3611. <https://doi.org/10.1016/j.apgeochem.2008.07.018>
- Vymazal, J., Sochacki, A., Fučík, P., Šereš, M., Kaplická, M., Hnátková, T., Chen, Z., 2020. Constructed wetlands with subsurface flow for nitrogen removal from tile drainage. *Ecol. Eng.* 155, 105943. <https://doi.org/10.1016/j.ecoleng.2020.105943>
- Wang, S., Zhu, G., Zhuang, L., Li, Y., Liu, L., Lavik, G., Berg, M., Liu, S., Long, X.E., Guo, J., Jetten,

References

- M.S.M., Kuypers, M.M.M., Li, F., Schwark, L., Yin, C., 2020. Anaerobic ammonium oxidation is a major N-sink in aquifer systems around the world. *ISME J.* 14, 151–163. <https://doi.org/10.1038/s41396-019-0513-x>
- Wang, Y., Xu, L., Wang, S., Ye, F., Zhu, G., 2019. Global Distribution of Anaerobic Ammonia Oxidation (Anammox) Bacteria – Field Surveys in Wetland, Dryland, Groundwater Aquifer and Snow. *Front. Microbiol.* 10, 1–12. <https://doi.org/10.3389/fmicb.2019.02583>
- Well, R., Eschenbach, W., Flessa, H., von der Heide, C., Weymann, D., 2012. Are dual isotope and isotopomer ratios of N₂O useful indicators for N₂O turnover during denitrification in nitrate-contaminated aquifers? *Geochim. Cosmochim. Acta* 90, 265–282. <https://doi.org/10.1016/j.gca.2012.04.045>
- WHO, 2022. Guidelines for drinking-water quality: fourth edition incorporating the first and second addenda, Fourth. ed.
- Withers, P.J.A., Neal, C., Jarvie, H.P., Doody, D.G., 2014. Agriculture and eutrophication: Where do we go from here? *Sustain.* 6, 5853–5875. <https://doi.org/10.3390/su6095853>
- Wolery, T.J., 1992. EQ3/6, a software package for geochemical modeling of aqueous systems: package overview and installation guide (version 7.0) (No. UCRL-MA--110662-PT. 1). Lawrence Livermore National Lab. <https://doi.org/10.2172/138894>
- Wunderlin, P., Lehmann, M.F., Siegrist, H., Tuzson, B., Joss, A., Emmenegger, L., Mohn, J., 2013. Isotope signatures of N₂O in a mixed microbial population system: Constraints on N₂O producing pathways in wastewater treatment. *Environ. Sci. Technol.* 47, 1339–1348. <https://doi.org/10.1021/es303174x>
- Xing, Y., Clark, I.D., 2011. Anaerobic Ammonium Oxidation in Waste Water -An Isotope Hydrological Perspective. *Waste Water - Treat. Reutil.* 89–108. <https://doi.org/10.5772/16154>
- Yin, S., Shen, Q., Tang, Y., Cheng, L., 1998. Reduction of nitrate to ammonium in selected paddy soils of China. *Pedosphere* 8, 221–228.
- Yoon, S., Cruz-García, C., Sanford, R., Ritalahti, K.M., Löffler, F.E., 2015. Denitrification versus respiratory ammonification: Environmental controls of two competing dissimilatory NO₃⁻/NO₂⁻-reduction pathways in *Shewanella loihica* strain PV-4. *ISME J.* 9, 1093–1104. <https://doi.org/10.1038/ismej.2014.201>
- Young, G.K., Bungay, H.R., Brown, L.M., Parsons, W.A., 1964. Chemical reduction of nitrate in water. *J. Water Pollut. Control Fed.* 36, 395–398.
- Zhang, Yiping, Douglas, G.B., Kaksonen, A.H., Cui, L., Ye, Z., 2019. Microbial reduction of nitrate in the presence of zero-valent iron. *Sci. Total Environ.* 646, 1195–1203. <https://doi.org/10.1016/j.scitotenv.2018.07.112>
- Zhang, Yongwei, Wei, D., Morrison, L., Ge, Z., Zhan, X., Li, R., 2019. Nutrient removal through pyrrhotite autotrophic denitrification: Implications for eutrophication control. *Sci. Total Environ.* 662, 287–296. <https://doi.org/10.1016/j.scitotenv.2019.01.230>
- Zhao, B., Sun, Z., Liu, Y., 2022. An overview of in-situ remediation for nitrate in groundwater. *Sci. Total Environ.* 804, 149981. <https://doi.org/10.1016/j.scitotenv.2021.149981>
- Zhu, C., 2012. Geochemical Modeling in Environmental and Geological Studies, in: *Encyclopedia of Sustainability Science and Technology*. pp. 4094–4104. <https://doi.org/10.1007/978-1-4419-0851-3>

References

Zhu, I., Getting, T., 2012. A review of nitrate reduction using inorganic materials. *Environ. Technol. Rev.* 1, 46–58. <https://doi.org/10.1080/09593330.2012.706646>

ANNEX 1: PAPER 1

LABORATORY TREATABILITY STUDY OF NITRATE-CONTAMINATED WATER

Enhancing Nitrate Removal with Industrial Wine Residue: Insights from Laboratory Batch and Column Experiments using Chemical, Isotopic, and Numerical Modeling Tools.

Submitted to Water Resource Research

First submission: 15th June 2023

Second submission after revisions: 31st October 2023

Status: Minor revisions

Enhancing Nitrate Removal with Industrial Wine Residue: Insights from Laboratory Batch and Column Experiments using Chemical, Isotopic, and Numerical Modeling Tools.

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Key Points:

- Wine industry residues induced nitrate attenuation in laboratory batch and column experiments.
- Denitrification efficiency varied with the wine residue type, showing $\epsilon^{15}\text{N}$ between -16.5 – -32.0 ‰ and $\epsilon^{18}\text{O}$ between -12.1 – -27.6 ‰.
- A geochemical model describing the trends of the experimental results has been developed and can be used for field applications.

Abstract

Agricultural run-off exposes recipient water bodies to nitrate (NO_3^-) pollution. Biological denitrification is a suitable method for removing NO_3^- in water resources that can be induced by the use of industrial organic liquid waste as an electron donor source. In light of this, batch and column experiments and chemical and isotopic tools were used in the laboratory to assess the potential of two industrial wine residues (*lías* and *vínico*) to induce biological denitrification of NO_3^- contaminated water from a constructed wetland and to evaluate the efficiency of these treatments. In batch experiments (performed at a C/N ratio of 1.25), *vínico* was not efficient enough in removing N species, attenuating only 35% NO_3^- and was not used in column experiments. In similar experimental conditions, *lías* completely removed N species from water in both batch and column experiments. The calculated isotope fractionation ($\epsilon^{15}\text{N}_{\text{NO}_3}$ and $\epsilon^{18}\text{O}_{\text{NO}_3}$) was the same in both batch and column experiments biostimulated with *lías* and differed from those for *vínico*. The isotopic data confirmed that denitrification was the principal NO_3^- attenuation pathway in all the experiments. The isotopic fractionation can be later applied to field studies to quantify the efficiency of biologically enhanced denitrification. A numerical geochemical model that accounts for the changes in nitrate, nitrite concentration and isotopic composition due to the degradation of *lías* and *vínico*, including transport in the case of the column experiment, was performed to simulate the experimental results and can be up-scaled in field treatments.

Plain Language Summary

Groundwater nitrate pollution is a significant global concern resulting from excessive fertilizer use in agriculture. This pollution poses health risks to humans and ecosystems by contaminating drinking water supplies and aquatic ecosystems. Sustainable remediation of nitrate is necessary to safeguard human health and the environment. Successful field-scale remediation requires laboratory feasibility studies to find the appropriate compounds to reduce nitrate (electron donors) and the best application measures to remove nitrate at a minimum cost. In our research, laboratory experiments were carried out using two industrial wine wastes as electron donors to evaluate their potential to remove nitrate from nitrate-polluted water. Chemical, isotopic, and numerical modeling tools have been used to quantify the amount of nitrate removed. The results indicate that one product successfully removed nitrate and can be implemented in the field as a mitigation strategy, while the other was ineffective and cannot be used. The isotopic fractionation from the laboratory experiments and the numerical model would be subsequently applied in the field to quantify the efficiency of nitrate removal.

1 Introduction

Nitrogen from anthropogenic activities has been identified as one of the primary constituents of agricultural run-off and municipal and industrial wastewater (Lu et al., 2009). Leachates of N from these sources are oxidized to nitrate (NO_3^-) in aerobic conditions. In many agricultural areas, NO_3^- is a common N water resources pollutant that poses a significant challenge because elevated NO_3^- concentrations may cause environmental impacts such as eutrophication, harmful algal blooms, and associated human health hazards such as stomach cancer and blue-baby syndrome (WHO, 2017). To render water safe for human consumption, the European Union Nitrate Directive established a NO_3^- concentration threshold value of 50 mg $\text{NO}_3^- \cdot \text{L}^{-1}$ (WHO, 2017). Many agricultural basins globally have recorded NO_3^- concentrations above this limit. The treatment of agricultural run-off before discharging it into the environment is essential to reduce NO_3^- loads in receiving water bodies.

Constructed Wetlands (CWs) are horizontal-flow artificial ponds specially designed to remediate wastewater in a controlled environment and are often used to treat agricultural run-off and industrial and municipal wastewater. Numerous studies have demonstrated the potential of CWs to efficiently remediate N-rich (NO_3^- , NO_2^- , and NH_4^+) wastewaters. CWs are easy to operate and cost-effective, with generally low capital and operational expenses (Phipps & Crumpton, 1994; Wu et al., 2014). As agricultural/municipal/industrial wastewater is directed to and flows through CWs, N is transformed through processes such as plant uptake, nitrification, denitrification, dissimilatory nitrate reduction to ammonia (DNRA), and/or anaerobic ammonium oxidation (anammox) (Figure 1) (Picardal, 2012; Sebilo et al., 2006). While denitrification and anammox remove N from wastewater as gaseous products, nitrification and DNRA only change the N form (Figure 1). The impact of seasonal temperature, N loading capacity, dissolved oxygen (O_2) transfer, organic matter source and quantity, microbial species, wetland age, and hydraulic retention time play critical roles in N attenuation efficiency (Carrey et al., 2014; Grau-Martínez et al., 2017; Valhondo et al., 2020).

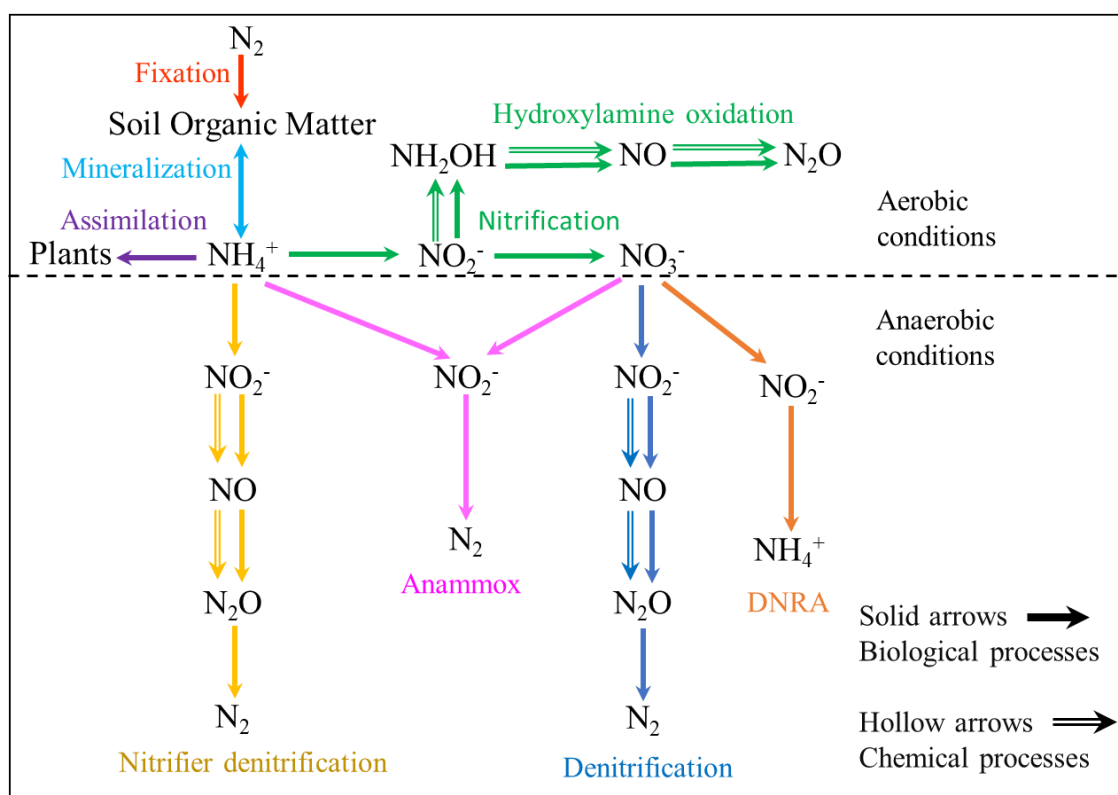
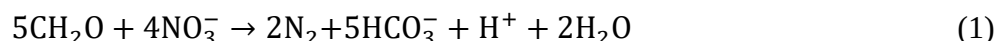


Figure 1. A. scheme showing biological and chemical N attenuation pathways in groundwater in aerobic and anaerobic conditions (modified after Abu et al., (2022)).

Even though all the aforementioned N cycling pathways can take place in CWs, denitrification is considered the main N removal process (Saeed & Sun, 2017), which reduces NO_3^- to $\text{N}_{2(g)}$ through intermediate steps driven by denitrifying bacteria (Figure 1). Bacteria responsible for N species transformations are abundant in surface and subsurface waters (Beauchamp et al., 1989). NO_3^- reductase bacteria are primarily facultative anaerobic denitrifying heterotrophs that oxidize organic compounds to generate energy for growth and cell synthesis (Reaction 1).

Anaerobic biological denitrification occurs with oxygen concentration below $2 \text{ mg}\cdot\text{L}^{-1}$ (Singleton et al., 2007).



Denitrification efficiency in many CWs is less than 50% due to the limitation of organic matter (Li et al., 2014; Torrijos et al., 2016). To improve the removal of NO_3^- in CWs, the biologically enhanced denitrification (BED) approach is adopted to provide external organic matter as a source of electron donors.

In long-term nitrate remediation treatments, using pure organic carbon sources such as glucose, acetate, ethanol and methanol could be costly (Margalef-Martí et al., 2019a). Therefore, the concept of the circular economy offers an optimal solution by transforming waste products into valuable resources. Industrial residues provide an alternative cheap and easily accessible electron donor source aligning with the principles of the circular economy. Various organic matter sources, including whey (Margalef-Martí et al., 2019b), plant-based compost and woodchips (Grau-Martínez et al., 2017; Valhondo et al., 2020c), and glucose (Calderer et al., 2010), have been investigated in micro and meso-scale studies to enhance NO_3^- removal.

Nitrogen microbial-mediated reactions tend to alter the distribution of the light and heavy isotopes of N and O in the unreacted substrate, and the product pools according to the isotopic fractionation factor, which quantifies the relative segregation between the light and heavy isotopes. The dissolved nitrate molecule's N and O heavy isotopes are progressively enriched in the residual NO_3^- during NO_3^- reduction. The isotopologues of NO_3^- provide valuable information about nitrogen cycling and environmental management. They play a pivotal role in tracing nitrate sources and identifying their origins, which is crucial for understanding nutrient inputs. Also, they allow for evaluating natural and/or induced denitrification efficiency. Contrary to biological denitrification, dilution or mixing does not modify its isotopic composition, although it may decrease NO_3^- concentration (Mariotti et al., 1981). This is especially useful in field studies since rainfall could affect NO_3^- concentration but not the isotopic composition to allow better evaluation of the extent of nitrate reduction. Multi-isotopic studies coupled with chemical data have proven valuable tools for studying biological denitrification in water resources (Otero et al., Otero). Numerical modeling tools are another powerful technique for research and investigations because they allow synthesis and interpretation of data, evaluation of complex processes, testing of scenarios and prediction of long-term reactions beyond the range of laboratory experiments (Zhu, 2012). Numerical models have been developed to characterize the fate and transport of NO_3^- in groundwater resources, which have been applied in laboratory experiments (André et al., 2011; Rodríguez-Escales et al., 2014) and in the field (Kildsgaard & Engesgaard, 2001; Kinzelbach et al., 1991). Although NO_3^- isotopologues have been successfully employed to study and characterize groundwater N transformation processes (Carrey et al., 2013; Margalef-Martí et al., 2019b), limitations in quantifying multiple reactions have been observed (Abu et al., 2022). Therefore, coupling chemical and isotopic tools to numerical modeling could provide a better understanding of nitrate attenuation processes.

An example of a N-polluted basin is the Lerma basin, a small catchment area (7.33 km^2) in northeastern Spain (Zaragoza), located on the left bank of the Ebro River. Over the past two decades, the Lerma basin has been subjected to anthropogenic activities, primarily irrigation agriculture (Abraham et al., 2013) and fertilization (Merchán et al., 2015). Agricultural run-off in the Lerma basin has significantly increased N exports to the surrounding water bodies and has

caused the principal recipient water body (Arba River) to be polluted with NO_3^- (Merchán et al., 2015). In 2015, a CW was built in the Lerma basin to remove N before discharge into the environment. Details on the construction and operation of the Lerma Basin CW can be found in (Margalef-Martí et al., 2019a; Merchán et al., 2015).

Biologically enhanced denitrification through CW application was previously tested on the Lerma basin run-off water into the CW at a laboratory scale using corn stubble, wheat hay, and animal compost to determine their potential to remove NO_3^- , and a pilot test using corn stubble was tested at field scale (Margalef-Martí et al., 2019a). However, results showed that denitrification was not sustained at high flow rates for long time periods. For example, the application of corn stubble at the field-scale resulted in at least 60% NO_3^- removal using a flow rate of $16 \text{ L} \cdot \text{s}^{-1}$, but this efficiency was limited to a maximum of three months due to the vertical diffusion of O_2 . Therefore, there is a need to characterize other possible organic matter sources and the best treatment strategy to improve their sustainability and increase nitrate removal efficiency.

Industrial wine waste holds considerable promise as a valuable carbon source for advancing research in biological denitrification. Wine residues are cost-effective and align with the circular economy concept by reusing discarded materials. This approach supports the United Nations Sustainable Development Goals (SDGs 6 & 7) related to clean water, waste reduction, and resource efficiency (United Nations, 2022), which are increasingly important in today's research and industrial landscape. The use of wine waste reduces the environmental impact associated with carbon dioxide (CO_2) and methane (CH_4) gas emissions during wine waste disposal (Niculescu & Ionete, 2023). Therefore, efficiently processing wine waste as electron donors in denitrification can contribute to diminishing these greenhouse gas emissions (Ioannou et al., 2015). Liquid organic waste from wine residues contains a higher concentration of nutrients, including organic carbon, making it readily available for denitrification processes and potentially accelerating these reactions compared to solid organic waste.

In this context, the aim of this study is to determine the potential of two different industrial wine residues to stimulate biological denitrification at the laboratory scale as they may provide an economically feasible solution to nitrate remediation while providing waste recycling (circular economy) and could be easily applied to field studies. Batch and column laboratory experiments were performed with water from the Lerma basin using chemical and multi-isotopic tools to quantify the treatment efficiency. Geochemical models describing denitrification processes have received relatively limited attention in scientific research. Therefore, this study also aimed at simulating the evolution of NO_3^- , NO_2^- , non-purgeable dissolved organic carbon (NPDOC) concentrations, $\delta^{15}\text{N}$ of NO_3^- and NO_2^- using numerical models in order to have a verified tool for future field applications and other case studies in similar experimental conditions.

2 Materials and Methods

2.1 Laboratory experiments

Batch assays consisted of 250 mL crystal Pyrex[®] reactors containing 150 mL of field water (FW) and 75 g of gravel material obtained from the CW of the Lerma Basin. Some parts of the CW are covered with gravelly material, providing subsurface flow to limit the vertical diffusion of O_2 , and the gravel used in the laboratory experiments is used to mimic the field conditions. Field water, in our context, refers to water from the Lerma basin entering the CW that was sampled in 25 L gallons and stored in the fridge at 4 °C until use.

The industrial wine wastes were obtained from Destileria San Valero Sociedad Cooperativa Limitada, Cariñena (Spain). The company produces liquid by-products from lees and pomace from 45 partner wineries. The liquid wastes produced during the first and second fermentation in the winemaking process are called "*lías*" and "*vínico*", respectively. *Lías* and *vínico* were used as electron donor sources to enhance biological denitrification in the experiments. The Total Organic Carbon (TOC) concentration was 20177.9 and 33531.1 mg·L⁻¹ in *lías* and *vínico*, respectively.

Two sets of batch experiments (13 samples each) were carried out to test the potential of these two liquid wastes to induce biological denitrification (Figure 2a and 2b). The first set of batch experiments was biostimulated with *lías* and shall be referred to as B-L hereafter. The second set was biostimulated with *vínico* and shall be called B-V hereafter. The reactors were covered with aluminium crimp caps with septa (Figure 2a and 2b) and purged with Ar to remove O₂(g) and create an anaerobic environment. Both series of experiments included a control reactor with field water and gravel but with no added waste (Control).

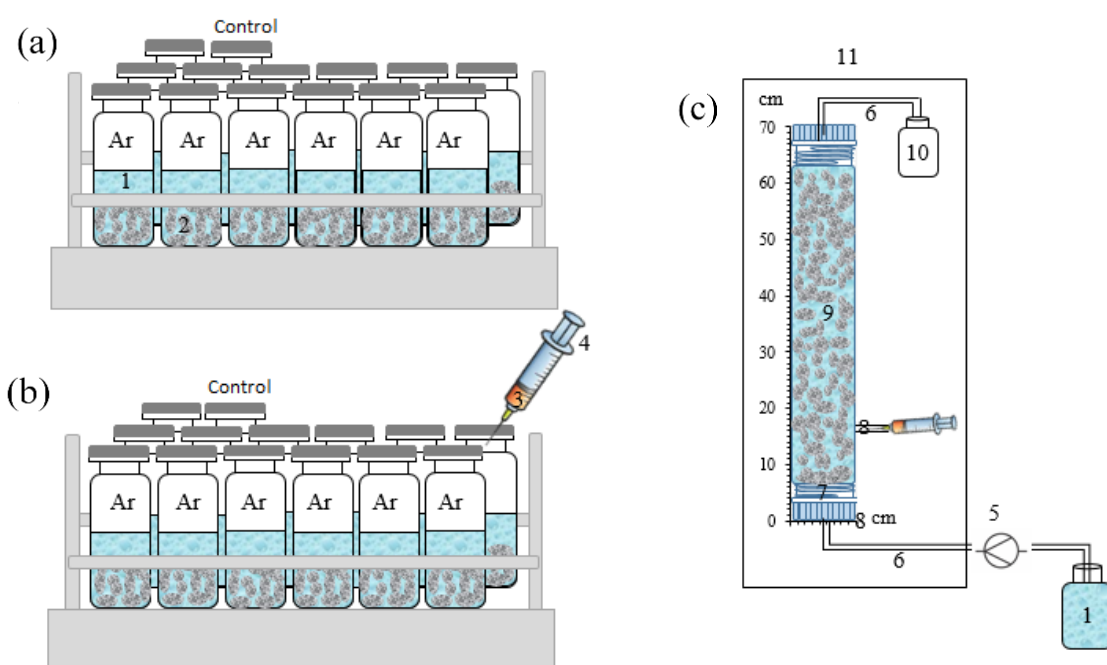


Figure 2. A scheme of the experimental set-up: (a) B-L; (b) B-V; (c) C-L with components: 1, field water; 2, gravel material; 3, wine residue; 4, syringe; 5, peristaltic pump; 6, Tygon tubes; 7, silica balls; 8, injection point; 9, glass column; 10, outflow water reservoir; 11, refrigerating chamber. For a more detailed description, see Margalef-Martí et al. (2019b).

A column experiment was performed to mimic field conditions. The column experiment was biostimulated with *lías*, which shall be called C-L hereafter. In section 3.1, it is explained why the column experiment was not biostimulated with *vínico*. Field water was pumped from the inflow reservoir through a 70 cm long glass column (8 cm diameter) and discharged into an outflow reservoir by a peristaltic pump (Micropump Reglo digital four channels, ISMATEC) (Figure 2c). The outflow reservoir affixed to the glass column by a Tygon tube was installed in a refrigerating chamber. Similar Tygon tubes connected the glass column to the peristaltic pump and the inflow reservoir (Figure 2c). The top and bottom 2.5 cm of the glass column were filled with 5 mm diameter silica balls to provide homogenous flow at these parts of the glass column, and the

remaining glass column was filled with gravel from the CW (volume = 1.6 L) to simulate field conditions by providing a heterogeneous porous media. A constant flow rate of $0.5 \text{ mL} \cdot \text{min}^{-1}$ was maintained throughout the experiment. The organic matter source was injected at 16 cm from the bottom of the column daily for four days. Afterwards, the injection was stopped, allowing NO_3^- to recover to its initial concentration. Sampling was carried out only at the outflow reservoir. Before the injection of organic carbon into the system, a conservative Cl^- tracer test was carried out to assess the system performance and determine the hydraulic and transport parameters of the column. Considering this study's scope and objectives, we did not conduct experimental measurements of biomass concentrations or assess the community dynamics of denitrifying bacteria in batch and column experiments.

2.2 Analytical methods

pH, Eh and electrical conductivity were measured using pH, Eh and conductivity meters immediately after sampling. Samples for chemical and isotopic analyses were prepared at the laboratory of the MAiMA-UB research group and analyzed at the Centres Científics i Tecnològics of the Universitat de Barcelona (CCiT-UB). The biostimulated reactors were sequentially sacrificed at specific time intervals based on the denitrification dynamics, while the control reactors were sacrificed at the end of the experiment (the time for sample sacrifice can be found in Tables S2 & S3 of Supporting Information SI). Sampled volumes for NPDOC, cations and anions analyses were filtered through a $0.22 \mu\text{m}$ Millipore® filter, acidified with HCl (for NPDOC analysis) and HNO_3 (for analysis of cations) and stored at $+4^\circ\text{C}$. Dissolved inorganic carbon (DIC) was measured using the titration method (METROHM 702 SM Titrino). TOC and NPDOC were analyzed with the organic matter combustion method (TOC 500 SHIMADZU). Major cations (Ca, Na, K and Mg) were analyzed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Perkin–Elmer Optima 8300) and trace elements (Fe, Mn, Si, B, Zn, Co, Cu, Pb, Cd and Mo) by Inductively Coupled Plasma Mass Spectrometry, ICP-MS, Perkin Elmer NexIon 350D. The concentration of anions NO_3^- , NO_2^- , SO_4^{2-} and Cl^- was analyzed by ion chromatography with UV detectors (UV detection limit 214 nm). Samples for $\delta^{15}\text{N}$ - NO_3^- and $\delta^{18}\text{O}$ - NO_3^- analyses were kept frozen until analyzed. The nitrogen and oxygen isotopic abundance of dissolved nitrate ($\delta^{15}\text{N}$ - NO_3^- and $\delta^{18}\text{O}$ - NO_3^-) were analyzed using the cadmium (Cd) and sodium azide (NaN_3) reduction methods (McIlvin & Altabet, 2005; Ryabenko et al., 2009). Notations were expressed as δ relative to the international standards (V-SMOW for $\delta^{18}\text{O}$ and Atmospheric N_2 for $\delta^{15}\text{N}$). The international standards USGS 32, USGS 34 and USGS 35 and the internal laboratory standards, CCiT-IWS- NO_3 and CCiT-IWS- NO_2 , were used to correct the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- and NO_2^- , respectively. The wide range of patterns ensures that the values of the analyzed samples fall within the range of pattern values in accordance with the recommendations of the IAEA (Coplen, 2011), thus ensuring the accurate correction of the analytical data. The reference values of these standards and the reproducibilities are presented in Table S1 of Supporting Information SI.

2.2 Modeling

Geochemical calculations and modeling have been carried out with the GibbsStudio software (Nardi & de Vries, 2017) based on PHREEQC version 3 code (Parkhurst & Appelo, 2013) and the iso.dat database.

PHREEQC is a computer program used worldwide for geochemical calculations. PHREEQC can quantify various physical and chemical processes such as aqueous, mineral, gas,

solid-solution, surface-complexation, ion-exchange equilibria, kinetically controlled reactions, and one-dimensional transport. The iso.dat database provided with the code used in this study contains data for the simulation of isotopic fractionation of carbon, hydrogen, nitrogen, oxygen, and sulfur (Parkhurst & Appelo, 2013). Equations considered in the models are presented in Supporting Information SI and in the conceptual model section.

GibbsStudio helps to manage water chemistry sample data and run PHREEQC models in a unified productive graphical environment (Nardi & de Vries, 2017). The model-data calibration was performed using CeresStudy within the GibbsStudio environment. This process employed the least-squares curve fitting technique, using the Levenberg–Marquardt algorithm, often referred to as LMA or simply LM, for nonlinear parameter estimation. The selection of the Levenberg–Marquardt algorithm in this study was driven by its robustness, rapid convergence, and versatility (Nardi & de Vries, 2017). Nitrate and nitrite concentrations of the batch experiments were used for the calibration process. In our analysis, the Least Square Error (LSE) is utilized to determine the best-fit line that minimizes the sum of squared differences between observed data points and predicted values.

3 Experimental results and discussion

3.1 Batch results and discussion

Chemical data of the field water used in the experiments are summarized in Table S2 of Supporting Information SI. Results of the batch experiments with *lías* and *vínico* are summarized in Tables S2 and S3, respectively, of Supporting Information SI. In batch experiments, the measured conductivities and pH were constant over time. Conductivities averaged 2.37 ± 0.05 mS·cm⁻¹ in B-L and 2.52 ± 0.01 mS·cm⁻¹ in B-V, and pH's averaged 7.85 ± 0.12 in B-L and 7.58 ± 0.08 in B-V experiments. Geochemical calculations with the Phreeqc software revealed a constant ionic strength over time.

The concentrations of NO₃⁻, NO₂⁻, and NPDOC for B-L and B-V are shown in Figure 3a and 3b, respectively. In the early stages of batch experiments, wine waste dissolution raised nitrate levels above the inflow water concentration after injecting the organic residue into the batch reactors. Results revealed an essential NO₃⁻ concentration increase from 1.7 mM to 2.8 mM in B-L and from 1.7 mM to 2.6 mM in B-V in 12 hours. In the control reactor, changes in NO₃⁻ concentration were not observed over time, indicating that the NO₃⁻ increase was linked to additional NO₃⁻ provided by the wine residues. In the biological denitrification studies with whey, Margalef-Martí et al. (2019b) and Oliveira et al. (2002) pointed out that industrial liquid wastes could be a potential source of NO₃⁻, denitrifying bacteria and cations, so wine residues could account for the increased NO₃⁻ concentration in B-L and B-V.

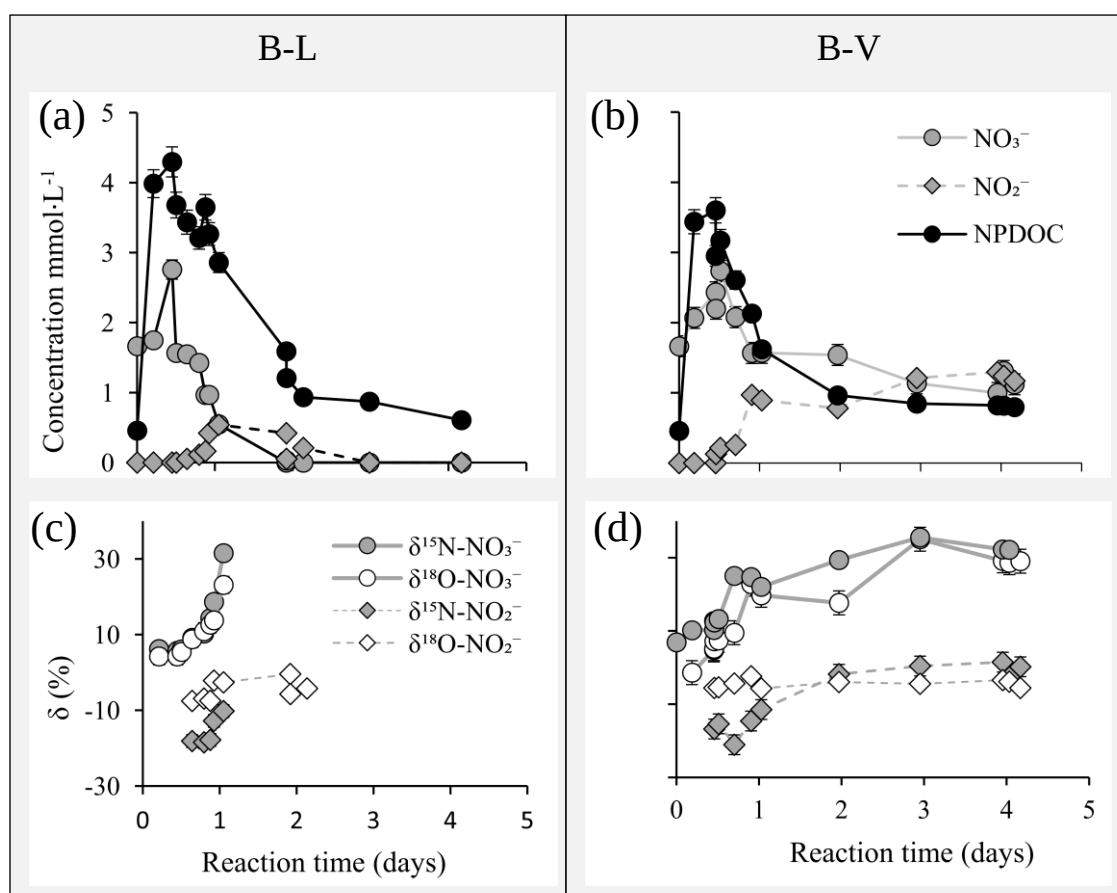


Figure 3. Chemical and isotopic results in B-L and B-V: evolution of NO_3^- , NO_2^- and NPDOC concentration in (a) B-L and (b) B-V experiments; evolution of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- and NO_2^- in (c) B-L and (d) B-V experiments. Error bars show a 5% error associated with experimental data; non-visible error bars are within the symbols.

NO_3^- attenuation in the batch reactors was linked to external organic carbon supplied by the wine wastes. In B-L, NO_3^- attenuation was quick, and NO_3^- was completely removed in two days (Figure 3a), demonstrating an efficient N removal capacity. The rapid NO_3^- attenuation could be explained by the capacity of *lías* to supply organic carbon to the latent denitrifying bacteria of the field water and gravel, which will quickly acclimate upon injection of the wine waste. In B-V, NO_3^- slightly decreased from peak NO_3^- concentration (2.6 mM to 1.1 mM) at the end of the experiment, representing 35% NO_3^- removal. Different N attenuation efficiencies were observed in the batch experiments, attributed to the composition of the wine residues used. *Lías* contained highly labile organic carbon content, resulting in high N attenuation in B-L. *Vínico*, on the other hand, mainly contains humic and fulvic acids with less labile organic carbon and, hence, lower efficiency in removing NO_3^- (Akunna et al., 1993).

NO_3^- reduction in B-L was coupled with a slight NO_2^- accumulation (up to 0.5 mM) on day one. NO_2^- decreased afterwards and was below detection ($<2\cdot 10^{-4}$ mM) after day 3 (Figure 3a), while NO_3^- reduction in B-V was associated with a significant NO_2^- accumulation during the entire experiment (up to 3 mM) (Figure 3b), attributed to the inhibition of NO_2^- reductase production due to incomplete NO_3^- attenuation (Kraft et al., 2011). This NO_2^- accumulation could be controlled by the organic carbon concentration, pH and bacterial community (Glass & Silverstein, 1998; Gómez

et al., 2000). The pH identified in our batch experiments (7.6-7.9) was within the favourable pH range for NO_2^- reduction (Glass & Silverstein, 1998). Hence, bacterial growth was the main factor controlling NO_2^- accumulation; therefore, it could only be expected at the beginning of the treatment.

In batch experiments, NPDOC rapidly increased from 0.4 mM (NPDOC concentration field water) to peak concentrations of 4.3 mM in B-L and 3.6 mM in B-V after injecting the wine wastes. NPDOC concentration gradually decreased afterwards to 0.6 mM in B-L and 0.8 mM in B-V (Figure 3a and 3b). The oxidation of organic matter increased bicarbonate concentration in the outflow water (from 4.8 mM to 7.2 mM in B-L and from 5.2 mM to 6.2 mM in B-V). High labile organic carbon in B-L suggests high organic carbon consumption, producing more bicarbonate, which explains the low residual NPDOC and high bicarbonate concentration observed in B-L (Table S2 of Supporting Information SI).

Geochemical calculations have shown that calcite was oversaturated in the outflow water ($\text{SI}=0.7$ in B-L and 0.6 in B-V) (Table S2 and S3 of Supporting Information SI). The equilibrium with amorphous calcite may explain the buffering of pH in the batch experiments, keeping the pH constant over time. Calcite precipitation has also been observed in similar biological denitrification experiments (Mastrocicco et al., 2011; Rodríguez-Escales et al., 2014).

Figure 3c and 3d show $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- and NO_2^- versus time for B-L and B-V, respectively. During both experiments, the residual NO_3^- became enriched in the heavy isotopes over time, correlating with a decrease in NO_3^- concentration. $\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$ increased from $+6.2\text{‰}$ and $+4.2\text{‰}$ to $+31.5\text{‰}$ and $+23.2\text{‰}$, respectively, in B-L (Figure 3c) and increased from -1.4‰ and $+6.9\text{‰}$ to $+29.1\text{‰}$ and $+32.2\text{‰}$ respectively in B-V (Figure 3d). The enrichment of the heavy isotopes indicates that NO_3^- attenuation in these experiments was caused by denitrification (Carrey et al., 2013; Kendall et al., 2007). Because of the low C/N ratio in our study, we discarded the possibility of DNRA, which has been found to be important in high C and low NO_3^- conditions at C/N ratios greater than 5 (Burgin & Hamilton, 2007). The NO_2^- produced progressively became enriched in the heavy isotopes with $\delta^{15}\text{N}-\text{NO}_2^-$ increasing from -18.5‰ to -10.2‰ in B-L (Figure 3c) and from -21.1‰ to -1.5‰ in B-V (Figure 3d). Since during denitrification, the $\delta^{18}\text{O}-\text{NO}_2^-$ may equilibrate with $\delta^{18}\text{O}-\text{H}_2\text{O}$ (Kool et al., 2007), a progressive increase of $\delta^{18}\text{O}-\text{NO}_2^-$ was not observed in the batch experiments (Figure 3c and 3d).

NO_3^- attenuation is often characterized by isotope fractionation, controlled by the kinetic isotope effect (Mariotti et al., 1981). In a closed system, the magnitude of fractionation is characterized using the Rayleigh distillation Equation 2, where ε is the kinetic isotope fractionation, C_0 and C_t are the initial and residual concentration at time zero and t , respectively, and R_0 and R_t are the isotopic ratios at time zero and t , respectively.

$$\ln \left(\frac{R_t}{R_0} \right) = \varepsilon \cdot \ln \left(\frac{C_t}{C_0} \right) \quad (2)$$

The values of R are calculated according to Equation 3, where ε is the isotopic composition (‰).

$$R = \frac{\delta}{1000} + 1 \quad (3)$$

The natural logarithm of the remaining NO_3^- fraction (C_t/C_0) was linearly correlated with the Ln of the isotope ratios (R_t/R_0) with the slope representing ϵ . $\epsilon^{15}\text{N}_{\text{NO}_3}$ and $\epsilon^{18}\text{O}_{\text{NO}_3}$ were -16.5 ± 2.7 ‰ and -12.1 ± 1.8 ‰ in B-L, and -32.0 ± 4.8 ‰ and -27.6 ± 3.3 ‰ in B-V respectively (Figure 4a). The calculated $\epsilon^{15}\text{N}_{\text{NO}_2}$ fractionation was -3.5 ± 0.9 ‰ ($R^2=0.81$) and -7.8 ± 2.2 ‰ ($R^2=0.62$) for B-L and B-V, respectively.

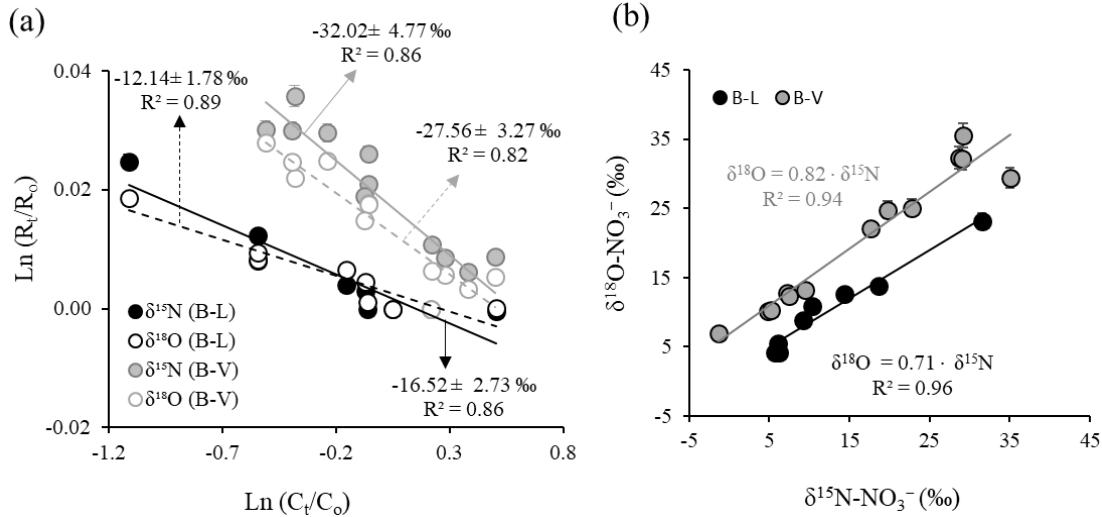


Figure 4. Isotopic data analyses in B-L and B-V: (a) evolution of the natural logarithm of the substrate remaining fraction (C_t/C_0) versus the isotope ratios (R_t/R_0) with slopes corresponding to the isotopic fractionation values in B-L and B-V. Solid continuous lines show the regression line for N with the slope indicated by the solid arrow lines, and dashed lines show the regression line for O with the slopes indicated by dashed arrow lines. (b) a plot of $\delta^{15}\text{N}$ against $\delta^{18}\text{O}$ showing a positive linear correlation in B-L and B-V and the regression line showing the slope ($\epsilon\text{O}/\epsilon\text{N}$). Error bars show a 5% error associated with experimental data; non-visible error bars are within the symbols.

Limited NO_3^- removal in the case of B-V might show larger fractionation (i.e., more negative $^{15}\epsilon$) values consistent with Mariotti et al. (1988), who proposed that larger fractionation is expected for a lower denitrification rate. The $\epsilon^{15}\text{N}_{\text{NO}_3}$ and $\epsilon^{18}\text{O}_{\text{NO}_3}$ obtained in our work are consistent with values reported in the literature for denitrification, ranging from $+6.5$ ‰ to -39.0 ‰ and -1.0 ‰ to -30.4 ‰, respectively (Grau-Martínez et al., 2017; Margalef-Martí et al., 2019a) for freshwater denitrification studies, depending on the environmental conditions, organic matter source employed and biomass community. $\delta^{15}\text{N}-\text{NO}_3^-$ versus $\delta^{18}\text{O}-\text{NO}_3^-$ showed a positive linear correlation with a slope of 0.71 in B-L and 0.82 in B-V (Figure 4b), indicating a high enrichment effect on $\epsilon^{15}\text{N}_{\text{NO}_3}$. Although these values agree with literature ratios for biological denitrification (1 to 0.5), they are not consistent regarding many laboratory studies where the ratio is close to 1 (Carrey et al., 2014; Kendall et al., 2007). The deviation could be explained by NO_2^- reoxidation to NO_3^- , the type of bacteria involved in NO_3^- reduction or the reduction rate of NO_3^- (Buchwald & Casciotti, 2010; Casciotti, 2009; Granger & Wankel, 2016; Wunderlich et al., 2013). $\epsilon^{18}\text{O}_{\text{NO}_3}/\epsilon^{15}\text{N}_{\text{NO}_3}$ ratio at the field scale can be influenced by atmospheric nitrate deposition, which can complicate the denitrification influence on nitrate isotope composition assessment (Elliott et al., 2019). Denitrification has often been shown to exhibit a greater enrichment effect on $\delta^{15}\text{N}_{\text{NO}_3}$

than $\delta^{15}\text{O}_{\text{NO}_3}$, which is seldom affected by denitrification in field-scale studies ($\epsilon^{18}\text{O}_{\text{NO}_3}/\epsilon^{15}\text{N}_{\text{NO}_3}$ close to 0.5) (Critchley et al., 2014; Otero et al., 2009). $\epsilon^{18}\text{O}_{\text{NO}_3}$ to $\epsilon^{15}\text{N}_{\text{NO}_3}$ ratios serve as an indicator of denitrification efficiency and enable the distinction between bacterial activity and other processes that lead to a reduction in nitrate concentration without affecting the isotopic composition (Buyanjargal et al., 2023; Kendall et al., 2007). The batch degradation results have shown that *lías* has the potential to remove NO_3^- from the water through denitrification at a C/N ratio of 1.25, and NO_3^- attenuation is coupled with quick removal of NO_2^- from water. Under similar experimental conditions, *vínico* could only remove about 35% of NO_3^- , not enough to obtain NO_3^- concentrations below the required limit ($0.81 \text{ mmol}\cdot\text{L}^{-1}$), and NO_2^- accumulated in water, exceeding the maximum concentration allowed (0.11 mM NO_2^-). Because of this different behaviour, *lías* was chosen for the experimental column study, while *vínico* was discarded. The goal of the column experiment was to determine the efficacy of continuous treatment and to model the results for a better understanding of NO_3^- attenuation dynamics prior to a field upscaling.

3.2 Column results and discussion

Results of the column experiment with *lías* are summarized in Table S4 of Supporting Information SI. *Lías* was injected daily into the column until day 4. During biostimulation, conductivity and pH averaged $2.2\pm 0.2 \text{ mS}\cdot\text{cm}^{-1}$ and 8.2 ± 0.1 , respectively, with no significant fluctuations in time. Eh slightly increased from 0.19 to 0.23 V at the beginning of the experiment and decreased afterwards to 0.14 V. Eh evolution demonstrated that the injection of *lías* created reducing conditions in the column, which were necessary for NO_3^- reduction.

Unlike the batch experiments, no initial increase in NO_3^- concentration was identified in the outflow water of the column experiment (Figure 5a); therefore, NO_3^- injected with *lías* was either not transported or consumed in the column.

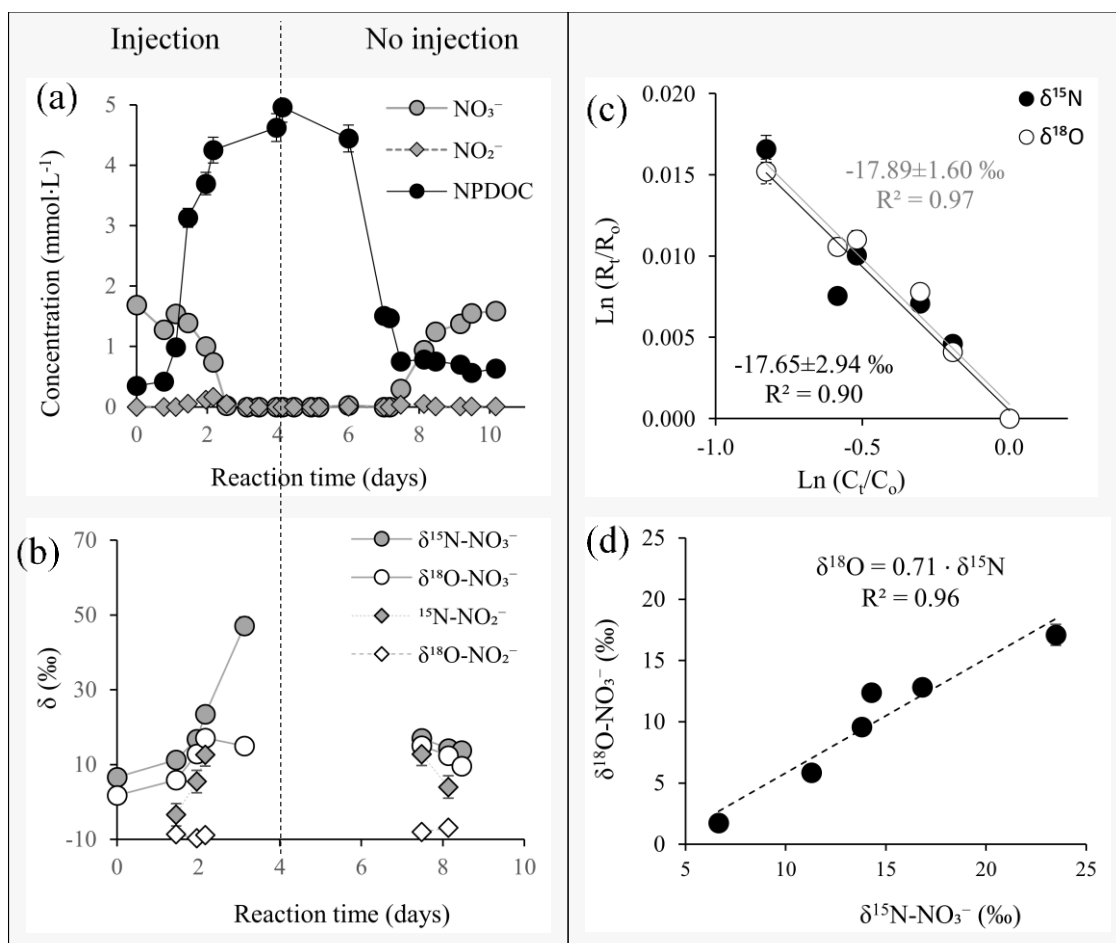


Figure 5. Chemical and isotopic data analyses: (a) NO₃⁻, NO₂⁻ and NPDOC evolution in C-L. (b) evolution of δ¹⁵N and δ¹⁸O of NO₃⁻ and NO₂⁻ over time in C-L. The dashed vertical line separates the injection period from the no-injection period (a and b). (c) A plot of the natural logarithm of the substrate remaining fraction against the isotope ratios showing a negative linear correlation with slopes depicting the isotopic fractionation values in C-L. (d) A plot δ¹⁵N against δ¹⁸O showing a positive linear correlation in C-L. Error bars show a 5% error associated with experimental data with non-visible error bars within the symbols.

NO₃⁻ attenuation began quickly after the first injection, and NO₃⁻ was removed entirely from the outflow water in three days (Figure 5a). NO₃⁻ remained undetected in the outflow until day 7. NO₃⁻ recovery began after day seven and fully recovered to the initial concentration on day 10 (Figure 5a). NO₂⁻ did not significantly accumulate in the outflow water during the experiment. NPDOC concentration progressively increased from 0.4 mM to about 5.0 mM at the end of the injections (day 4 to day 6). The increased NPDOC in the outflow water is attributed to external C supplied by *lías*. NPDOC quickly decreased to 0.8 mM on day seven (Figure 5a) and remained the same throughout the experiment. Similar to the results observed in the batch experiments, the δ¹⁵N and δ¹⁸O of NO₃⁻ increased over time (Figure 5b). δ¹⁵N-NO₃⁻ and δ¹⁸O-NO₃⁻ increased from +6.6 ‰ and +1.7 ‰ to +23.5 ‰ and +17.1 ‰, respectively (Figure 5b). Similar to B-L, a positive linear trend was observed for δ¹⁵N-NO₃⁻ versus δ¹⁸O-NO₃⁻ bi-isotopic plot with a slope of 0.71 (Figure 5b), confirming denitrification as the NO₃⁻ attenuation pathway, as in the batch experiment. Typical of denitrification, the natural logarithm of the substrate's remaining fraction was correlated to the

isotope ratios by a negative linear relationship (Figure 5c). The calculated $\varepsilon^{15}\text{N}_{\text{NO}_3}$ and $\varepsilon^{18}\text{O}_{\text{NO}_3}$ were -17.7 ± 2.9 ‰ and -17.9 ± 1.6 ‰, respectively (Figure 5c), and the $\varepsilon^{18}\text{O}_{\text{NO}_3}/\varepsilon^{15}\text{N}_{\text{NO}_3}$ obtained in C-L is 1.0, characteristic of laboratory biological denitrification studies (Carrey et al., 2013; Grau-Martínez et al., 2017). $\delta^{15}\text{N}\text{-NO}_3^-$ versus $\delta^{18}\text{O}\text{-NO}_3^-$ showed a positive linear correlation with a slope of 0.71 in B-C (Figure 5d). Similar to the batch experiments, $\delta^{15}\text{N}$ of the generated NO_2^- increased from -3.4 ‰ to $+12.8$ ‰ and $\delta^{18}\text{O}\text{-NO}_2^-$ equilibrated with $\delta^{18}\text{O}\text{-H}_2\text{O}$.

The isotopic data of the column experiment implies that any additional nitrate supplied by the wine waste underwent degradation within the column and did not reach the outflow reservoir. As a result, we did not observe the mixing of the heavy isotopes of the residual nitrate with fresh nitrate provided by the wine waste during the injection period. Consequently, the Rayleigh equation successfully reproduced the experimental data, treating it as a closed system. The similar ε values observed in batch and column *lías* experiments suggest that the same processes were degrading NO_3^- in both experiments, attributed to the same organic matter source (*lías*) used. It also demonstrates that various factors such as nitrate supply, variations in biomass, mixing, flow velocity, and the potential presence of preferential flow paths in the column did not significantly impact the denitrification process within the column experiment. This conclusion is further supported by the fact that complete nitrate degradation was achieved in similar timeframes. The calculated laboratory isotope fractionation values are valuable for estimating denitrification efficiency for environmental applications. Laboratory-determined fractionation values are more reliable for quantifying denitrification efficiency than field fractionation values, as field values can overestimate denitrification rates (Böttcher et al., 1990; Mariotti et al., 1988). Therefore, controlled laboratory-scale fractionation values help to mitigate potential interferences from other attenuation processes under field conditions, establishing a more reliable basis for quantifying denitrification efficiency. However, applying these values to field-scale studies is challenging due to the natural variability influenced by factors like soil composition, microbial communities, and substrate availability (Maggi & Riley, 2010, 2009). Field-specific dynamics like transport and mixing (Green et al., 2010) and environmental changes such as temperature fluctuations, pH shifts and O_2 levels (Druhan et al., 2014) make it challenging to apply laboratory-derived fractionation values to field denitrification accurately. In order to ensure greater accuracy and precision in the laboratory findings and numerical models for field-scale applications, it is necessary to perform robust field measurements and establish long-term monitoring protocols to accommodate the intricate and constantly evolving dynamics of natural systems.

The batch and column experiments have shown that *lías* is a viable electron donor capable of inducing denitrification in nitrate-contaminated water. *Lías* does not seem to pose a pollution risk, as the concentration of heavy metals remained unchanged before and after the injection of *lías*, suggesting that *lías* does not contain heavy metal concentration high enough to significantly increase the levels in the field water, making it a safe option for remediating nitrate-polluted water bodies. According to the results obtained in this study, the electron donor feeding strategy is considered appropriate for future field application at the CW to improve denitrification efficiency.

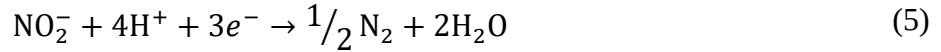
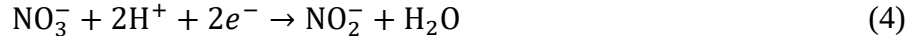
4 Geochemical modeling

4.1 Conceptual Model

The model describes the coupled reaction between NO_3^- reduction and organic matter oxidation, linked to denitrifying bacterial growth and decay and the evolution of NO_3^- and NO_2^-

isotopic composition in a fully saturated system at a constant temperature of 25 °C. The same conceptual has been used to model the batch and column experiments.

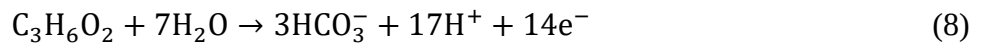
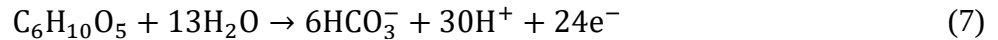
NO_3^- reduction is assumed to occur in a two-step process. In the first step, nitrate is reduced to nitrite according to Reaction 4; in the second step, nitrite is reduced to dinitrogen gas according to Reaction 5.



Reactions 4 and 5 are assumed to be kinetically driven and are quantified using the dual Monod kinetics, the most used approach in biological denitrification models (Calderer et al., 2010; Chen & MacQuarrie, 2004). Monod kinetics assumes that the degradation rates are limited by both the electron donor and the electron acceptor concentration. In our model, Reactions 4 and 5 are quantified using Equation 6, where R_{EA} is the NO_3^- (for Reaction 4) or NO_2^- (for Reaction 5) degradation rate ($\text{mol L}^{-1}\cdot\text{s}^{-1}$), $[\text{EA}]$ is the electron acceptor concentration (NO_3^- or NO_2^- depending on the reaction) ($\text{mol}\cdot\text{L}^{-1}$), $[\text{ED}]$ is *lías/vínico* concentration ($\text{mol}\cdot\text{L}^{-1}$), K_{EA} and K_{ED} are the electron acceptor and electron donor half-saturation constants respectively ($\text{mol}\cdot\text{L}^{-1}$), k_{max} is the maximum rate of the electron donor consumption (s^{-1}), and $[\text{X}_{\text{EA}}]$ is biomass concentration ($\text{mol}\cdot\text{L}^{-1}$), which is different for each Reaction (4 and 5). k_{max} indicates the maximum rate at which the biological process can occur when substrate concentration is not limiting, while the half-saturation constants reflect the microorganism's affinity for a specific nutrient, representing the limiting nutrient concentration at which it can grow at half its maximum rate.

$$R_{\text{EA}} = -k_{\text{max}} \cdot [\text{X}_{\text{EA}}] \cdot \left(\frac{[\text{EA}]}{[\text{EA}] + K_{\text{EA}}} \right) \cdot \left(\frac{[\text{ED}]}{[\text{ED}] + K_{\text{ED}}} \right) \quad (6)$$

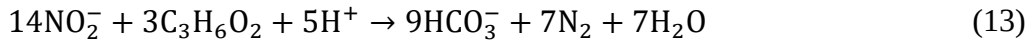
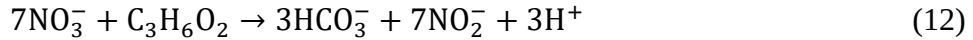
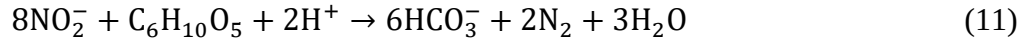
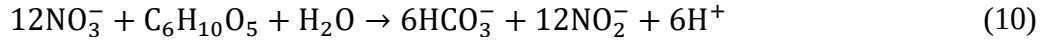
Reactions 4 and 5 are linked to the oxidation of *lías* and *vínico*. In our study, we did not conduct a specific analysis to determine the organic matter composition of the *lías* stimulant employed in our research. Previous investigations have extensively examined polysaccharides in the context of biological denitrification studies (Kim et al., 2002; Liu et al., 2013). Considering the observed fractionation values compared to existing literature, we infer that the labile organic carbon source contributing to the enhancement of denitrification in our study likely exhibits similarity with those previously characterized for polysaccharides. For the sake of simplicity, the simplest polysaccharide, $\text{C}_6\text{H}_{10}\text{O}_5$, is considered representative of *lías*, and the simplest fatty acid, $\text{C}_3\text{H}_5\text{O}_2$, is considered to represent *vínico*. The oxidation of these compounds occurs according to Reactions 7 and 8.



The rate of Reactions 9 and 8 is given by Equation 9, where k is the specific rate of organic matter degradation (dimensionless), $[\text{ED}]$ is the concentration of $\text{C}_6\text{H}_{10}\text{O}_5$ for Reaction 7 or the concentration of $\text{C}_3\text{H}_5\text{O}_2$ for Reaction 8 ($\text{mol}\cdot\text{L}^{-1}$), and $R_{\text{NO}_3^-}$ and $R_{\text{NO}_2^-}$ are the degradation rates of nitrate and nitrite, respectively, calculated with Equation 6.

$$R_{\text{OM}} = k \cdot [\text{ED}] \cdot [k_1 \cdot R_{\text{NO}_3^-} + k_2 \cdot R_{\text{NO}_2^-}] \quad (9)$$

k_1 and k_2 are the ratios of the stoichiometric coefficient between the organic carbon and the electron acceptor (C/N) in a balanced chemical reaction. In the case of *lías*, k_1 and k_2 were 0.5 and 0.75, respectively, according to Reactions 10 and 11. For *vínico*, k_1 and k_2 took values of 0.43 and 0.64, according to Reactions 12 and 13. k_1 and k_2 relate nitrate/nitrite reduction to organic matter consumption, which directly affects the rate and efficiency of denitrification processes.



The model considers that bacterial communities degrading nitrate and nitrite are different, as well as those degrading *lías* and *vínico*. The value of $[\text{X}_{\text{EA}}]$ in Equation 6 will depend on each specific case (the organic matter used and the reduction step). The growth and decay of biomass concentration of each bacterial community ($[\text{X}_{\text{EA}}]$, $\text{mol} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$) are calculated according to Equation 14, where b and Y are the biomass decay constant and biomass growth yield coefficient, respectively (dimensionless and different for each bacterial community), and R_{EA} is the degradation rate of nitrate or nitrite, calculated according to Equation 6. The bacterial growth yield represents the amount of biomass generated for each unit of organic matter consumed, while the decay constant indicates the rate at which bacteria die in the absence of sufficient organic matter.

$$R_{\text{X}_{\text{EA}}} = -Y \cdot R_{\text{EA}} - b \cdot [\text{X}_{\text{EA}}] \quad (14)$$

The change in the isotopic composition of N in nitrate and nitrite ($\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{15}\text{N}-\text{NO}_2^-$) during denitrification has been modeled according to the first-order degradation kinetics described in Van Breukelen & Prommer (2008) and shown in Equation 15, where ε is the isotope fractionation (that is different for nitrate and nitrite), $[\delta^{\text{H}}\text{EA}]/([\delta^{\text{H}}\text{EA}] + [\delta^{\text{L}}\text{EA}])$ is N isotope ratio of NO_3^- and NO_2^- , and R_{EA} is the degradation rate of nitrate or nitrite, calculated from Equation 6.

$$R_{\delta^{\text{H}}\text{EA}} = R_{\text{EA}} \cdot \frac{[\delta^{\text{H}}\text{EA}]}{[\delta^{\text{H}}\text{EA}] + [\delta^{\text{L}}\text{EA}]} \cdot \varepsilon \quad (15)$$

Aqueous speciation is assumed to occur in equilibrium, and concentrations are calculated with the mass action law, which has been considered for all the aqueous species listed in Table S5 of SI. To be consistent with the experimental data, a constant CO_2 partial pressure of $10^{-2.6}$ atm has been considered, and calcite precipitation in equilibrium is allowed, following the principles of the mass action law using the iso.dat database. Instead of using amorphous calcite, for which thermodynamic data was unavailable in the database, crystalline calcite has been used but is only left to precipitate for saturation indices higher than 0.7.

A homogeneous porous substrate media is considered for the batch reactions and transport models. The column transport model has considered one-dimensional transport due to advection and diffusion coupled with chemical reactions under a constant flow.

4.2 Numerical model

The initial pore water composition for the batch and column models is the field water composition (FW) in Table S2 of the Supporting Information (SI). An initial nitrate concentration of 2.8 mM with initial $\delta^{15}\text{N-NO}_3^- = +5.7\text{‰}$ and $\delta^{18}\text{O-NO}_3^- = +4.3\text{‰}$ for B-L and 2.6 mM with initial $\delta^{15}\text{N-NO}_3^- = +4.8\text{‰}$ and $\delta^{18}\text{O-NO}_3^- = +10.3\text{‰}$ for B-V were considered for the batch models after peak nitrate increase due the wine waste injection and dissolution in 12 hours. In the C-L, nitrate measurements at the top of the column did not reveal a concentration rise from wine waste injection and an initial nitrate concentration of 1.7 mM with initial $\delta^{15}\text{N-NO}_3^- = +6.6\text{‰}$ and $\delta^{18}\text{O-NO}_3^- = +1.7\text{‰}$ was chosen. In both the batch and column models, NO_2^- and N_2 initial concentrations were set at 0 mmol L^{-1} . After some preliminary calculations, an initial biomass concentration of 0.005 mol $\cdot \text{L}^{-1}$ and 0.003 mol $\cdot \text{L}^{-1}$ were chosen for the *lías* and *vínico* experiments, respectively, and an initial concentration of organic matter of 0.4 mM obtained from the experimental results was chosen in both cases. In our models, the average isotopic fractionation values based on our experimental results, $\epsilon^{15}\text{N}_{\text{NO}_3} = -18\text{‰}$ and $\epsilon^{15}\text{N}_{\text{NO}_2} = -4\text{‰}$ for B-L/C-L and $\epsilon^{15}\text{N}_{\text{NO}_3} = -29\text{‰}$ for B-V have been chosen for Equation 15. The initial moles of calcite were set to zero in phase assemblage, allowing calcite to precipitate at saturation indices > 0.7 . The values of the reaction rate parameters (k_{max} , K_{ED} , K_{EA} , Y , k , and b) for equations 2, 3, and 4 were initially those from Rodríguez-Escales et al. (2014), who conducted batch experiments under similar experimental conditions (Table 1).

The column experiment was modeled considering a one-dimensional system of 70 cm length discretized in 70 cells of 1 cm length. A constant temperature of 25°C was chosen in the batch and transport simulations. No flow and transport boundary conditions were considered for the batch models. However, for the column model, we employed forward flow and flux boundary conditions for the first and last cells. A velocity of $3.5 \cdot 10^{-6} \text{ m s}^{-1}$, a dispersivity of 0.005 m and a diffusion coefficient of $3.0 \cdot 10^{-10} \text{ m}^2 \text{ s}^{-1}$, estimated from a conservative tracer test (Figure S1 and Table S6 of Supporting Information SI), were used in the transport modeling.

The batch experiments models were run for four and a half days in 415 time steps, while the column model was run for 10 days in 2857 time steps. To ensure the numerical stability of the column model, the grid spacing and the step length fulfilled the Péclet number (< 2) and the Courant number (< 1).

4.3 Modeling results

The initially estimated reaction rate parameters were calibrated using nitrate/nitrite concentration of our batch experiments with CeresStudy in GibbsStudio (Nardi and de Vries, 2017) to obtain the best fit. For comparison purposes, Table 1 presents our initial guesses, the range of values reported in the existing literature, and the best-fit parameters obtained through our calibration process. The associated uncertainties, calculated as the root mean square errors resulting from the Levenberg–Marquardt fitting algorithm, are detailed in Table S6 of the Supporting Information (SI).

Table 1

Fitted Reaction Rate Parameters for Equations Used in our Model Estimated From GibbsStudio (Batch and Column Experiments) and Reported Values in the Literature.

Model Parameter	Range of values	Initial guess	Fitted values	
			B-L/C-L	B-V
K_{EA,NO_3^-} (mol·L ⁻¹)	$1.0 \cdot 10^{-6}$ - $1.7 \cdot 10^{-4}$ ^{a,b,c,d,e}	$1.4 \cdot 10^{-5}$	$5.0 \cdot 10^{-4}$	$5.0 \cdot 10^{-4}$
K_{EA,NO_2^-} (mol·L ⁻¹)	$1.0 \cdot 10^{-6}$ - $1.7 \cdot 10^{-4}$ ^{a,b,c,d,e}	$1.4 \cdot 10^{-5}$	$6.0 \cdot 10^{-5}$	$6.0 \cdot 10^{-5}$
K_{ED,NO_3^-} (mol·L ⁻¹)	$8.3 \cdot 10^{-6}$ - $1.0 \cdot 10^{-1}$ ^{a,b,c,d,e}	$2.2 \cdot 10^{-5}$	$2.2 \cdot 10^{-5}$	$2.2 \cdot 10^{-5}$
K_{ED,NO_2^-} (mol·L ⁻¹)	$8.3 \cdot 10^{-6}$ - $1.0 \cdot 10^{-1}$ ^{a,b,c,d,e}	$2.2 \cdot 10^{-5}$	$1.0 \cdot 10^{-6}$	$1.0 \cdot 10^{-6}$
k_{max,NO_3^-} (s ⁻¹)	$1.0 \cdot 10^{-3}$ - $3.9 \cdot 10^{-7}$ ^{a,b,c,d,e}	$3.9 \cdot 10^{-7}$	$5.0 \cdot 10^{-7}$	$5.0 \cdot 10^{-7}$
k_{max,NO_2^-} (s ⁻¹)	$5.2 \cdot 10^{-4}$ - $7.0 \cdot 10^{-8}$ ^{a,b,c,d,e}	$4.4 \cdot 10^{-7}$	$5.0 \cdot 10^{-7}$	$5.0 \cdot 10^{-7}$
Y, NO_3^- (moles C-cells/moles C-OC)	$0.05 - 0.73$ ^{a,b,c,d,e,f}	0.57	0.53	0.53
Y, NO_2^- (moles C-cells/moles C-OC)	$0.05 - 0.73$ ^{a,b,c,d,e,f}	0.57	0.53	0.53
b, NO_3^- (s ⁻¹)	$0 - 5.8 \cdot 10^{-7}$ ^{a,b,c,d,e}	$1.2 \cdot 10^{-7}$	$3.0 \cdot 10^{-6}$	$3.2 \cdot 10^{-5}$
b, NO_2^- (s ⁻¹)	$0 - 5.8 \cdot 10^{-7}$ ^{a,b,c,d,e}	$1.2 \cdot 10^{-7}$	$3.0 \cdot 10^{-6}$	$5.0 \cdot 10^{-5}$
k		210	950	1400

^aChen & MacQuarrie (2004). ^bKillingstad et al. (2002). ^cKinzelbach et al. (1991). ^dMastrocicco et al. (2011). ^eRodríguez-Escales et al. (2014). ^fParkhurst & Appelo, (2013).

The batch model does not consider the increase of NO_3^- concentration due to the dissolution of wine waste, and the initial time corresponds to the maximum NO_3^- concentration reached in 12 hours. The simulated pH well reproduced the experimental results in both the batch and column experiments (Figure S2 of Supporting Information SI). Eh quickly reduced from +0.28 V to - 0.19 V in B-L and B-V models and remained constant throughout the simulation.

In addition to nitrate, nitrite, dissolved organic carbon and biomass concentrations that several authors have modeled in biological denitrification (André et al., 2011; Green et al., 1995), our model incorporates nitrogen isotope composition of nitrate and nitrite, which has not gained much attention to a significant extent. Our model simulates the evolution of NO_3^- , NO_2^- , $\delta^{15}N-NO_3^-$, $\delta^{15}N-NO_2^-$ in B-L and B-V (Figure 6a and 6c respectively). The model also reproduces NPDOC concentration in B-L and B-V, shown in Figure 6b and 6d, respectively. Table S7 in the Supporting Information (SI) reveals a remarkably high level of accuracy achieved by your predictive model.

Similarly to the experimental results, NO_3^- and NO_2^- were quickly removed from the solution in B-L, while in B-V, NO_3^- was not completely removed and was coupled to a high NO_2^- accumulation (Figure 6a and 6c). NO_3^- reduction in the model was related to the increase of $\delta^{15}N-NO_3^-$ in the remaining NO_3^- fraction and the generated NO_2^- . An advantage of the geochemical

isotope model is its ability to simulate $\delta^{15}\text{N}$ of NO_3^- and NO_2^- at low NO_3^- and NO_2^- concentrations below the detection limit by laboratory measurements ($0.2 \text{ mg}\cdot\text{L}^{-1}$ for NO_3^- and $0.01 \text{ mg}\cdot\text{L}^{-1}$ for NO_2^-). However, extremely low levels of NO_3^- and NO_2^- can result in very high isotopic values. When simulating $\delta^{15}\text{N}$ - NO_2^- of the low NO_2^- experiment (B-L), the model fitted the experimental $\delta^{15}\text{N}$ - NO_2^- using the experimentally determined $\varepsilon^{15}\text{N}_{\text{NO}_2}$ equal to -4 ‰ but a higher $\varepsilon^{15}\text{N}_{\text{NO}_2}$ of -18 ‰ instead of -8 ‰ was imposed in B-V to accurately reproduce the experimental results. Similar $\delta^{15}\text{N}$ - NO_2^- results were obtained by Sebilo et al. (2019) for a high NO_2^- system, where the model could simulate the experimental results by using a higher $\varepsilon^{15}\text{N}_{\text{NO}_2}$ of -10 ‰ than the value obtained in batch experiments (-4.2 ‰). The behavior of $\varepsilon^{15}\text{N}_{\text{NO}_2}$ in our models within a high-nitrite system may be associated with nitrite's potential reoxidation to nitrate, which was not previously considered. This is a crucial factor that should be incorporated into future models in the transferability of the laboratory results to field-scale studies.

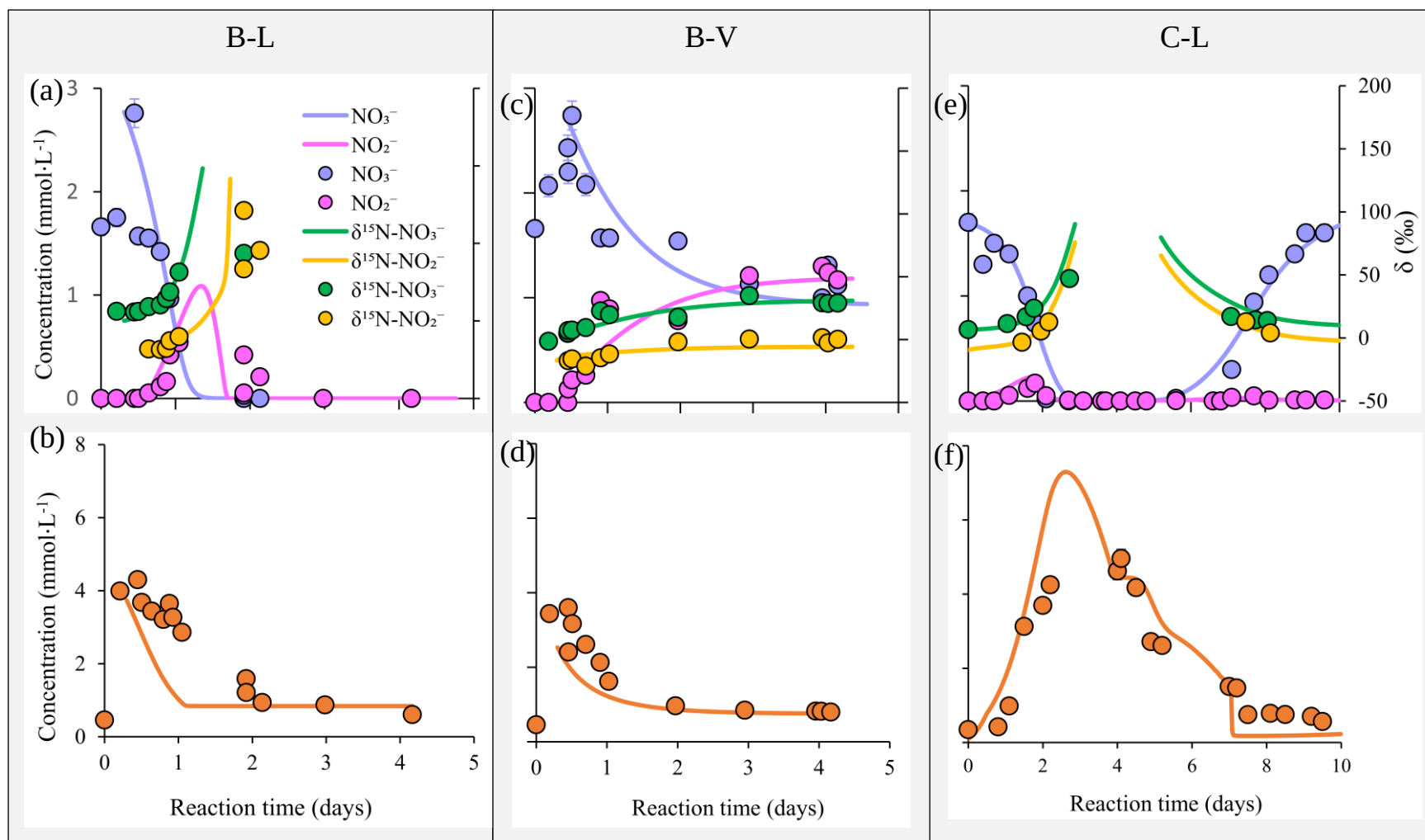


Figure 6. Modeling results (lines) versus experimental data (dots) for NO_3^- , NO_2^- and NPDOC concentrations and $\delta^{15}\text{N}$ of NO_3^- and NO_2^- of the batch and column experiments. Error bars indicate a 5% margin of error with experimental data (non-visible error bars within circles).

By applying the same reaction rate parameters utilized in the B-L model, the C-L model demonstrates the capability to replicate the concentrations of NO_3^- , NO_2^- and NPDOC, as well as $\delta^{15}\text{N}$ of NO_3^- and NO_2^- accurately (Figure 6c and 6f). Because the peak NO_3^- concentration occurs at the start of the column experiment, the initial time of the column model coincides with the initial time of the experiment. Eh in the C-L model showed more pronounced reducing conditions up to day 4 (-0.26 V), probably due to the injection of *lías* in the column. Subsequently, Eh rapidly increased to 0.75 V and remained constant through day 10. Like the B-L model, the C-L model achieves complete removal of nitrogen species from the solution and simulates $\delta^{15}\text{N}$ of NO_3^- and NO_2^- at low nitrate and nitrite concentrations (Figure 6c and 6f).

The same reaction rate parameters (k_{max} , K_{ED} , K_{EA} , Y , k and b) used in B-L and C-L might have contributed to the slight discrepancy between experimental and model results, particularly NPDOC (Figure 6b and 6d). Our model assumed constant transport parameters throughout the column and over time. Most biomass often colonizes around the injection point, thus, modifying the dispersivity and other associated transport parameters in the column, which has been reported in similar experiments (Kildsgaard & Engesgaard, 2001; Rodríguez-Escales et al., 2016). Other studies have shown that the biofilm colonization and calcite precipitation over time in biological remediation studies lasting more than two weeks tends to modify the porosity and hydraulic conductivity (Arnon et al., 2005; Hill & Sleep, 2002; Seifert & Engesgaard, 2007).

The biomass dynamics suggest that endogenous respiration did not play a significant role in denitrification in our model. At complete NO_3^- exhaustion with no external carbon source, the biomass population remained steady with no substantial decay to provide additional carbon, which may explain the slight delay before the onset of NO_3^- recovery. Rodríguez-Escales et al. (2014), in batch experiments using glucose and ethanol as electron donors, found that endogenous respiration did not contribute significantly to nitrate reduction (less than 10%).

5 Conclusions

This work evaluates the denitrification potential of two industrial wine residues (*lías* and *vínico*), accomplished by characterizing batch and column experiments and using chemical and isotopic techniques. The efficiency of nitrate removal depended on the type of wine residue used. The batch results showed that *lías* had the capacity to promote complete nitrate attenuation at a C/N ratio of 1.25 with no hazardous intermediate products. Under similar experimental conditions, *vínico* could only attenuate 35% nitrate and was linked to a high NO_2^- accumulation. *Lías* was selected for the column experiment due to its high nitrate removal capacity. At the same C/N ratio, complete denitrification was achieved when *lías* was injected daily into the column for four days. The column experimental results indicate the viability of the controlled injection for utilizing wine waste in wetland applications to enhance denitrification rates during specific time intervals when denitrification processes are most active. The controlled injection method offers flexibility in adjusting injection parameters to achieve high denitrification rates. However, it emphasizes the importance of tailoring wine waste application to the characteristics and limitations of the specific wetland site.

The calculated isotope fractionation ($\epsilon^{15}\text{N}_{\text{NO}_3}$ and $\epsilon^{18}\text{O}_{\text{NO}_3}$) in the batch experiments with *vínico* were $-32 \pm 5 \text{ ‰}$ and $-28 \pm 3 \text{ ‰}$, respectively, while with *lías*, $\epsilon^{15}\text{N}_{\text{NO}_3}$ and $\epsilon^{18}\text{O}_{\text{NO}_3}$ were -17 ± 3

‰ and -12 ± 2 ‰, respectively, in batch experiments and -18 ± 3 ‰ and -18 ± 2 ‰, respectively, in column experiments. Laboratory-derived isotope fractionation values are valuable for quantifying denitrification efficiency in environmental applications, providing greater reliability than field-based values. However, applying these laboratory values to field-scale studies is challenging due to natural variability and environmental dynamics. Robust field measurements and long-term monitoring protocols are essential to ensure more accurate results in applying controlled laboratory results in numerical models and field settings.

The developed numerical model described the transformation of nitrogen species in the batch and column experiments by combining nitrate and nitrite respiration, nitrate and nitrite reductase bacterial growth and decay, organic matter oxidation, calcite precipitation reactions and nitrate and nitrite isotopic fractionation. Using experimental and model data allows a comprehensive understanding of the processes occurring during nitrate attenuation and could be useful in field studies.

Acknowledgements

This publication is part of the grant PACE-ISOTEC (CGL2017-87216-C4-1-R) funded by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe", the grant REMEDIATE (TED2021-131005B-C31) funded by MCIN/AEI/10.13039/501100011033 and by "European Union NextGenerationEU/PRTR", the grant NPP-SOL (PCI2023-143359) funded by MCIN/AEI/10.13039/501100011033 and cofunded by the European Union under the "PRIMA2022 Programme-Section2" and the project AgroSOS (PID2019-108057RB-I00) funded by the Ministry of Science and Innovation – Feder EU Funds. It has also been funded by the Consolidated Research Group MAG (2021-SGR-00308). The authors are thankful to the CCI of the University of Barcelona for the analytical support. Abu Alex would also like to thank the Agency for Management of University and Research Grants of the Generalitat de Catalunya and Fundació Bosch i Gimpera de la Universitat de Barcelona for the PhD grant (2019 FI_B 01059) and the L'Institut de Recerca de l'Aigua (IdRA)-Universitat de Barcelona for their support.

Open Research

Supplementary Information (SI) and the experimental data used in this study are freely available on Zenodo (Abu et al., 2023). The Phreeqc version three software used in this work is available online at <https://water.usgs.gov/water-resources/software/PHREEQC/index.html>, and the GibbsStudio version three software is also available at <https://gibbsstudio.io/>.

References

- Abrahao, R., Causapé, J., Moreno-Mateos, D., & Comín, F. (2013). Nitrate and Salt Water Contamination Associated with the Transition of an Agrarian Basin into an Irrigated Area. *Water Environment Research*, 85(2), 105–112. <https://doi.org/10.2175/106143012x13560205144254>
- Abu, A., Carrey, R., Valhondo, C., Domènech, C., Otero, N., Soler, A., Diaz-Cruz, S. (2022). Pathways and Efficiency of Nitrogen Attenuation in Wastewater Effluent Through Soil Aquifer Treatment. *Journal of Environmental Management*, 321(May).

- <https://doi.org/10.2139/ssrn.4127243>
- Abu, A., Carrey, R., Navarro-Ciurana, D., Margalef-Martí, R., Albert, S., Neus, O., Domènech, C. (2023). Chemical and isotopic data of laboratory batch and column experiments using industrial wine wastes to biostimulate denitrification [Dataset]. *Zenodo*. <https://doi.org/10.5281/zenodo.8027789>
- Akunna, J. C., Bizeau, C., & Moletta, R. (1993). Nitrate and nitrite reductions with anaerobic sludge using various carbon sources: Glucose, glycerol, acetic acid, lactic acid and methanol. *Water Research*, 27(8), 1303–1312. [https://doi.org/10.1016/0043-1354\(93\)90217-6](https://doi.org/10.1016/0043-1354(93)90217-6)
- André, L., Pauwels, H., Dictor, M. C., Parmentier, M., & Azaroual, M. (2011). Experiments and numerical modelling of microbially-catalyzed denitrification reactions. *Chemical Geology*, 287(3–4), 171–181. <https://doi.org/10.1016/j.chemgeo.2011.06.008>
- Arnon, S., Adar, E., & Ronen, Z. (2005). *Impact of microbial activity on the hydraulic properties of fractured chalk*. 76, 315–336. <https://doi.org/10.1016/j.jconhyd.2004.11.004>
- Beauchamp, E. G., Trevors, J. T., & Paul, J. W. (1989). Carbon Sources for Bacterial Denitrification. In *Advances in soil science* (Vol. 10, pp. 113–142). https://doi.org/10.1007/978-1-4613-8847-0_3
- Böttcher, J., Strebel, O., Voerkelius, S., Schmidt, H.L., 1990. Using isotope fractionation of nitrate-nitrogen and nitrate-oxygen for evaluation of microbial denitrification in a sandy aquifer. *J. Hydrol.* 114, 413–424. [https://doi.org/10.1016/0022-1694\(90\)90068-9](https://doi.org/10.1016/0022-1694(90)90068-9)
- Buchwald, C., & Casciotti, K. L. (2010). Oxygen isotopic fractionation and exchange during bacterial nitrite oxidation. *Limnology and Oceanography*, 55(3), 1064–1074. <https://doi.org/10.4319/lo.2010.55.3.1064>
- Burgin, A. J., & Hamilton, S. K. (2007). Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. *Frontiers in Ecology and the Environment*, 5(2), 89–96. [https://doi.org/10.1890/1540-9295\(2007\)5\[89:HWOTRO\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2007)5[89:HWOTRO]2.0.CO;2)
- Buyanjargal, A., Kang, J., Lee, J. H., & Jeon, S. W. (2023). Nitrate removal rates, isotopic fractionation, and denitrifying bacteria in a woodchip-based permeable reactive barrier system: a long-term column experiment. *Environmental Science and Pollution Research*, 30(13), 36364–36376. <https://doi.org/10.1007/s11356-022-24826-4>
- Calderer, M., Gibert, O., Martí, V., Rovira, M., De Pablo, J., Jordana, S., Bruno, J. (2010). Denitrification in presence of acetate and glucose for bioremediation of nitrate-contaminated groundwater. *Environmental Technology*, 31(7), 799–814. <https://doi.org/10.1080/09593331003667741>
- Carrey, R., Otero, N., Soler, A., Gómez-Alday, J. J., & Ayora, C. (2013). The role of Lower Cretaceous sediments in groundwater nitrate attenuation in central Spain: Column experiments. *Applied Geochemistry*, 32, 142–152. <https://doi.org/10.1016/j.apgeochem.2012.10.009>
- Carrey, R., Otero, N., Vidal-Gavilan, G., Ayora, C., Soler, A., & Gómez-Alday, J. J. (2014). Induced nitrate attenuation by glucose in groundwater: Flow-through experiment. *Chemical Geology*, 370(2), 19–28. <https://doi.org/10.1016/j.chemgeo.2014.01.016>
- Casciotti, K.L., 2009. Inverse kinetic isotope fractionation during bacterial nitrite oxidation. *Geochim. Cosmochim. Acta* 73, 2061–2076. <https://doi.org/10.1016/j.gca.2008.12.022>
- Chen, D. J. Z., & MacQuarrie, K. T. B. (2004). Numerical simulation of organic carbon, nitrate, and nitrogen isotope behavior during denitrification in a riparian zone. *Journal of Hydrology*, 293(1–4), 235–254. <https://doi.org/10.1016/j.jhydrol.2004.02.002>

- Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry*, 25(17), 2538–2560. <https://doi.org/10.1002/rcm.5129>
- Critchley, K., Rudolph, D. L., Devlin, J. F., & Schillig, P. C. (2014). Stimulating in situ denitrification in an aerobic, highly permeable municipal drinking water aquifer. *Journal of Contaminant Hydrology*, 171, 66–80. <https://doi.org/10.1016/j.jconhyd.2014.10.008>
- Druhan, J. L., Steefel, C. I., Conrad, M. E., & DePaolo, D. J. (2014). A large column analog experiment of stable isotope variations during reactive transport: I. A comprehensive model of sulfur cycling and $\delta^{34}\text{S}$ fractionation. *Geochimica et Cosmochimica Acta*, 124, 366–393. <https://doi.org/10.1016/j.gca.2013.08.037>
- Elliott, E. M., Yu, Z., Cole, A. S., & Coughlin, J. G. (2019). Isotopic advances in understanding reactive nitrogen deposition and atmospheric processing. *Science of the Total Environment*, 662, 393–403. <https://doi.org/10.1016/j.scitotenv.2018.12.177>
- Glass, C., & Silverstein, J. (1998). Denitrification kinetics of high nitrate concentration water. *Water Research*, 32(3), 831–839. [https://doi.org/10.1016/S0043-1354\(97\)00260-1](https://doi.org/10.1016/S0043-1354(97)00260-1)
- Gómez, M. A., González-López, J., & Hontoria-García, E. (2000). Influence of carbon source on nitrate removal of contaminated groundwater in a denitrifying submerged filter. *Journal of Hazardous Materials*, 80(1–3), 69–80. [https://doi.org/10.1016/S0304-3894\(00\)00282-X](https://doi.org/10.1016/S0304-3894(00)00282-X)
- Granger, J., & Wankel, S. D. (2016). Isotopic overprinting of nitrification on denitrification as a ubiquitous and unifying feature of environmental nitrogen cycling. *Proceedings of the National Academy of Sciences*, 113(42), E6391–E6400. <https://doi.org/10.1073/pnas.1601383113>
- Grau-Martínez, A., Torrentó, C., Carrey, R., Rodríguez-Escales, P., Domènech, C., Ghiglieri, G., Otero, N. (2017). Feasibility of two low-cost organic substrates for inducing denitrification in artificial recharge ponds: Batch and flow-through experiments. *Journal of Contaminant Hydrology*, 198(2), 48–58. <https://doi.org/10.1016/j.jconhyd.2017.01.001>
- Green, C. T., Böhlke, J. K., Bekins, B. A., & Phillips, S. P. (2010). Mixing effects on apparent reaction rates and isotope fractionation during denitrification in a heterogeneous aquifer. *Water resources research*, 46(8). <https://doi.org/10.1029/2009WR008903>
- Green, M., Schnitzer, M., & Tarre, S. (1995). Kinetics of a fluidized-bed reactor for groundwater denitrification. *Applied Microbiology and Biotechnology*, 43(1), 188–193. <https://doi.org/10.1007/BF00170642>
- Hill, D. D., & Sleep, B. E. (2002). Effects of biofilm growth on flow and transport through a glass parallel plate fracture. 56, 227–246. [https://doi.org/10.1016/S0169-7722\(01\)00210-8](https://doi.org/10.1016/S0169-7722(01)00210-8)
- Ioannou, L. A., Puma, G. L., & Fatta-Kassinos, D. (2015). Treatment of winery wastewater by physicochemical, biological and advanced processes: A review. *Journal of Hazardous Materials*, 286, 343–368. <https://doi.org/10.1016/j.jhazmat.2014.12.043>
- Kendall, C., Elliott, E. M., & Wankel, S. D. (2007). Tracing anthropogenic inputs of nitrogen to ecosystems. In *Stable Isotopes in Ecology and Environmental Science* (2nd ed., pp. 375–449).
- Kildsgaard, J., & Engesgaard, P. (2001). Numerical analysis of biological clogging in two-dimensional sandbox experiments. *Journal of Contaminant Hydrology*, 50(3–4), 261–285. [https://doi.org/10.1016/S0169-7722\(01\)00109-7](https://doi.org/10.1016/S0169-7722(01)00109-7)
- Killingstad, M. W., Widdowson, M. A., & Smith, R. L. (2002). Modeling Enhanced In Situ Denitrification in Groundwater. *Journal of Environmental Engineering*, 128(6), 491–504. [https://doi.org/10.1061/\(asce\)0733-9372\(2002\)128:6\(491\)](https://doi.org/10.1061/(asce)0733-9372(2002)128:6(491))

- Kim, Y. S., Nakano, K., Lee, T. J., Kanchanatawee, S., & Matsumura, M. (2002). On-site nitrate removal of groundwater by an immobilized psychrophilic denitrifier using soluble starch as a carbon source. *Journal of Bioscience and Bioengineering*, 93(3), 303–308. [https://doi.org/10.1016/S1389-1723\(02\)80032-9](https://doi.org/10.1016/S1389-1723(02)80032-9)
- Kinzelbach, W., Schäfer, W., & Herzer, J. (1991). Numerical modeling of natural and enhanced denitrification processes in aquifers. *Water Resources Research*, 27(6), 1123–1135. <https://doi.org/10.1029/91WR00474>
- Kool, D. M., Wrage, N., Oenema, O., Dolfing, J., & Van Groenigen, J. W. (2007). Oxygen exchange between (de) nitrification intermediates and H₂O and its implications for source determination of NO₃ and N₂O: a review. *Rapid Communications in Mass Spectrometry: RCM*, 21, 3569–3578. <https://doi.org/10.1002/rcm>
- Kraft, B., Strous, M., & Tegetmeyer, H. E. (2011). Microbial nitrate respiration - Genes, enzymes and environmental distribution. *Journal of Biotechnology*, 155(1), 104–117. <https://doi.org/10.1016/j.jbiotec.2010.12.025>
- Li, Y., Zhu, G., Ng, W. J., & Tan, S. K. (2014). A review on removing pharmaceutical contaminants from wastewater by constructed wetlands: Design, performance and mechanism. *Science of the Total Environment*, 468–469, 908–932. <https://doi.org/10.1016/j.scitotenv.2013.09.018>
- Liu, F., Huang, G., Fallowfield, H., Guan, H., Zhu, L., & Hu, H. (2013). *Study on heterotrophic-autotrophic denitrification permeable reactive barriers (HAD PRBs) for in situ groundwater remediation*. Springer Science & Business Media.
- Lu, S., Zhang, P., Jin, X., Xiang, C., Gui, M., Zhang, J., & Li, F. (2009). Nitrogen removal from agricultural run-off by full-scale constructed wetland in China. *Hydrobiologia*, 621(1), 115–126. <https://doi.org/10.1007/s10750-008-9636-1>
- Maggi, F., & Riley, W. J. (2010). Mathematical treatment of isotopologue and isotopomer speciation and fractionation in biochemical kinetics. *Geochimica et Cosmochimica Acta*, 74(6), 1823–1835. <https://doi.org/10.1016/j.gca.2009.12.021>
- Maggi, F., & Riley, W. J. (2009). Transient competitive complexation in biological kinetic isotope fractionation explains nonsteady isotopic effects: Theory and application to denitrification in soils. *Journal of Geophysical Research: Biogeosciences*, 114(G4). <https://doi.org/10.1029/2008JG000878>
- Margalef-Martí, R., Carrey, R., Merchán, D., Soler, A., Causapé, J., & Otero, N. (2019a). Feasibility of using rural waste products to increase the denitrification efficiency in a surface flow constructed wetland. *Journal of Hydrology*, 578(April). <https://doi.org/10.1016/j.jhydrol.2019.124035>
- Margalef-Martí, R., Carrey, R., Soler, A., & Otero, N. (2019b). Evaluating the potential use of a dairy industry residue to induce denitrification in polluted water bodies: A flow-through experiment. *Journal of Environmental Management*, 245(March), 86–94. <https://doi.org/10.1016/j.jenvman.2019.03.086>
- Mariotti, A., Germon, J. C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., & Tardieux, P. (1981). Experimental determination of nitrogen kinetic isotope fractionation: Some principles; illustration for the denitrification and nitrification processes. *Plant and Soil*, 62(3), 413–430. <https://doi.org/10.1007/BF02374138>
- Mariotti, André, Landreau, A., & Simon, B. (1988). ¹⁵N isotope biogeochemistry and natural denitrification process in groundwater: Application to the chalk aquifer of northern France. *Geochimica et Cosmochimica Acta*, 52(7), 1869–1878. [https://doi.org/10.1016/0016-7037\(88\)90010-5](https://doi.org/10.1016/0016-7037(88)90010-5)

- Mastrocicco, M., Colombani, N., Salemi, E., & Castaldelli, G. (2011). Reactive modeling of denitrification in soils with natural and depleted organic matter. *Water, Air, and Soil Pollution*, 222(1–4), 205–215. <https://doi.org/10.1007/s11270-011-0817-6>
- McIlvin, M. R., & Altabet, M. A. (2005). Chemical conversion of nitrate and nitrite to nitrous oxide for nitrogen and oxygen isotopic analysis in freshwater and seawater. *Analytical Chemistry*, 77(17), 5589–5595. <https://doi.org/10.1021/ac050528s>
- Merchán, D., Causapé, J., Abrahão, R., & García-Garizábal, I. (2015). Assessment of a newly implemented irrigated area (Lerma Basin, Spain) over a 10-year period. I: Water balances and irrigation performance. *Agricultural Water Management*, 158, 277–287. <https://doi.org/10.1016/j.agwat.2015.04.016>
- Nardi, A., & de Vries, L. M. (2017). *GibbsStudio, Barcelona Science Technologies SL, Barcelona, Spain*. Retrieved from <https://gibbsstudio.io/>
- Niculescu, V. C., & Ionete, R. E. (2023). An Overview on Management and Valorisation of Winery Wastes. *Applied Sciences*, 13(8), 5063. <https://doi.org/10.3390/app13085063>
- Oliveira, C. P., Beatriz A. Gloria, M., F. Barbour, J., & A. Scanlan, R. (2002). Nitrate, Nitrite, and Volatile Nitrosamines in Whey-Containing Food Products. *Journal of Agricultural and Food Chemistry*, 43(4), 967–969. <https://doi.org/10.1021/jf00052a023>
- Otero, N., Torrentó, C., Soler, A., Menció, A., & Mas-Pla, J. (2009). Monitoring groundwater nitrate attenuation in a regional system coupling hydrogeology with multi-isotopic methods: The case of Plana de Vic (Osona, Spain). *Agriculture, Ecosystems and Environment*, 133(1–2), 103–113. <https://doi.org/10.1016/j.agee.2009.05.007>
- Parkhurst, D. L., & Appelo, C. A. J. (2013). Description of Input and Examples for PHREEQC Version 3 — A Computer Program for Speciation, Batch-Reaction, One-Dimensional Transport, and Inverse Geochemical Calculations. *U.S. Geological Survey Techniques and Methods, Book 6, Chapter A43*, 6–43A. [https://doi.org/10.1016/0029-6554\(94\)90020-5](https://doi.org/10.1016/0029-6554(94)90020-5)
- Phipps, R. G., & Crumpton, W. G. (1994). Factors affecting nitrogen loss in experimental wetlands with different hydrologic loads. *Ecological Engineering*, 3(4), 399–408. [https://doi.org/10.1016/0925-8574\(94\)00009-3](https://doi.org/10.1016/0925-8574(94)00009-3)
- Picardal, F. (2012). Abiotic and microbial interactions during anaerobic transformations of Fe(II) and NO_x-. *Frontiers in Microbiology*, 3(MAR), 1–7. <https://doi.org/10.3389/fmicb.2012.00112>
- Rodríguez-Escales, P., Breukelen, B. M. Van, Vidal-gavilan, G., Soler, A., & Folch, A. (2014). Integrated modeling of biogeochemical reactions and associated isotope fractionations at batch scale : A tool to monitor enhanced biodenitrification applications. *Chemical Geology*, 365, 20–29. <https://doi.org/10.1016/j.chemgeo.2013.12.003>
- Rodríguez-Escales, P., Folch, A., van Breukelen, B. M., Vidal-Gavilan, G., & Sanchez-Vila, X. (2016). Modeling long term Enhanced in situ Biodenitrification and induced heterogeneity in column experiments under different feeding strategies. *Journal of Hydrology*, 538, 127–137. <https://doi.org/10.1016/j.jhydrol.2016.04.012>
- Ryabenko, E., Altabet, M. A., & Wallace, D. W. R. (2009). Effect of chloride on the chemical conversion of nitrate to nitrous oxide for $\delta^{15}\text{N}$ analysis. *Limnology and Oceanography: Methods*, 7(7), 545–552. <https://doi.org/10.4319/lom.2009.7.545>
- Saeed, T., & Sun, G. (2017). A comprehensive review on nutrients and organics removal from different wastewaters employing subsurface flow constructed wetlands. *Critical Reviews in Environmental Science and Technology*, 47(4), 203–288. <https://doi.org/10.1080/10643389.2017.1318615>

- Sebilo, M., Aloisi, G., Mayer, B., Perrin, E., Vaury, V., Mothet, A., & Laverman, A. M. (2019). Controls on the Isotopic Composition of Nitrite ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) during Denitrification in Freshwater Sediments. *Scientific Reports*, 9(1), 1–14. <https://doi.org/10.1038/s41598-019-54014-3>
- Sebilo, M., Billen, G., Mayer, B., Billiou, D., Grably, M., Garnier, J., & Mariotti, A. (2006). Assessing nitrification and denitrification in the seine river and estuary using chemical and isotopic techniques. *Ecosystems*, 9(4), 564–577. <https://doi.org/10.1007/s10021-006-0151-9>
- Seifert, D., & Engesgaard, P. (2007). *Use of tracer tests to investigate changes in flow and transport properties due to bioclogging of porous media*. 93, 58–71. <https://doi.org/10.1016/j.jconhyd.2007.01.014>
- Singleton, M. J., Esser, B. K., Moran, J. E., Hudson, G. B., McNab, W. W., & Harter, T. (2007). Saturated zone denitrification: Potential for natural attenuation of nitrate contamination in shallow groundwater under dairy operations. *Environmental Science and Technology*, 41(3), 759–765. <https://doi.org/10.1021/es061253g>
- Torrijos, V., Gonzalo, O. G., Trueba-Santiso, A., Ruiz, I., & Soto, M. (2016). Effect of by-pass and effluent recirculation on nitrogen removal in hybrid constructed wetlands for domestic and industrial wastewater treatment. *Water Research*, 103, 92–100. <https://doi.org/10.1016/j.watres.2016.07.028>
- United Nations, 2022. The Sustainable Development Goals Report 2022, United Nations publication issued by the Department of Economic and Social Affairs. <https://unstats.un.org/sdgs/report/2022/>
- Valhondo, C., Martínez-Landa, L., Carrera, J., Díaz-Cruz, S. M., Amalfitano, S., & Levantesi, C. (2020). Six artificial recharge pilot replicates to gain insight into water quality enhancement processes. *Chemosphere*, 240. <https://doi.org/10.1016/j.chemosphere.2019.124826>
- Van Breukelen, B. M., & Prommer, H. (2008). Beyond the Rayleigh equation: Reactive transport modeling of isotope fractionation effects to improve quantification of biodegradation. *Environmental Science and Technology*, 42(7), 2457–2463. <https://doi.org/10.1021/es071981j>
- WHO. (2017). Guidelines for Drinking-Water Quality: Fourth Edition Incorporating the First Addendum. *Guidelines for Drinking-Water Quality: Fourth Edition Incorporating the First Addendum*, 541. Retrieved from <https://apps.who.int/iris/bitstream/handle/10665/254637/9789241549950-eng.pdf;jsessionid=C9942C5DBCC2BDC53A892D79B3844EE8?sequence=1%0Ahttp://www.ncbi.nlm.nih.gov/pubmed/28759192>
- Wu, S., Kusch, P., Brix, H., Vymazal, J., & Dong, R. (2014). Development of constructed wetlands in performance intensifications for wastewater treatment: A nitrogen and organic matter targeted review. *Water Research*, 57, 40–55. <https://doi.org/10.1016/j.watres.2014.03.020>
- Wunderlich, A., Meckenstock, R.U., Einsiedl, F., 2013. A mixture of nitrite-oxidizing and denitrifying microorganisms affects the $\delta^{18}\text{O}$ of dissolved nitrate during anaerobic microbial denitrification depending on the $\delta^{18}\text{O}$ of ambient water. *Geochim. Cosmochim. Acta* 119, 31–45. <https://doi.org/10.1016/j.gca.2013.05.028>
- Zhu, C. (2012). Geochemical Modeling in Environmental and Geological Studies. In *Encyclopedia of Sustainability Science and Technology* (pp. 4094–4104). <https://doi.org/10.1007/978-1-4419-0851-3>

Water Resources Research

Supporting Information for PAPER 1

Enhancing Nitrate Removal with Industrial Wine Residue: Insights from Laboratory Batch and Column Experiments using Chemical, Isotopic, and Numerical Modeling Tools.

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Water Resources Research

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Introduction

The supporting Information (SI) comprises experimental data sets from two batch experiments and a column experiment. The data sets have been categorized according to the experiment type. Geochemical calculations with the Phreeqc software have also been included in the SI as this helps better understand the experimental results. The results that our analytical techniques could not analyze in the data sets, such as low chemical concentrations beyond the detectable range of our analytical techniques and the subsequent isotopic composition, have been indicated.

Text S1.

Our biological denitrification models were carried out with the Phreeqc software version three code (Parkhurst and Appelo, 2013), which can be found online at <https://water.usgs.gov/water-resources/software/PHREEQC/index.html>. GibbsStudio version three software was used to produce our models in a unified, productive graphical environment and is also available at <https://gibbsstudio.io/>.

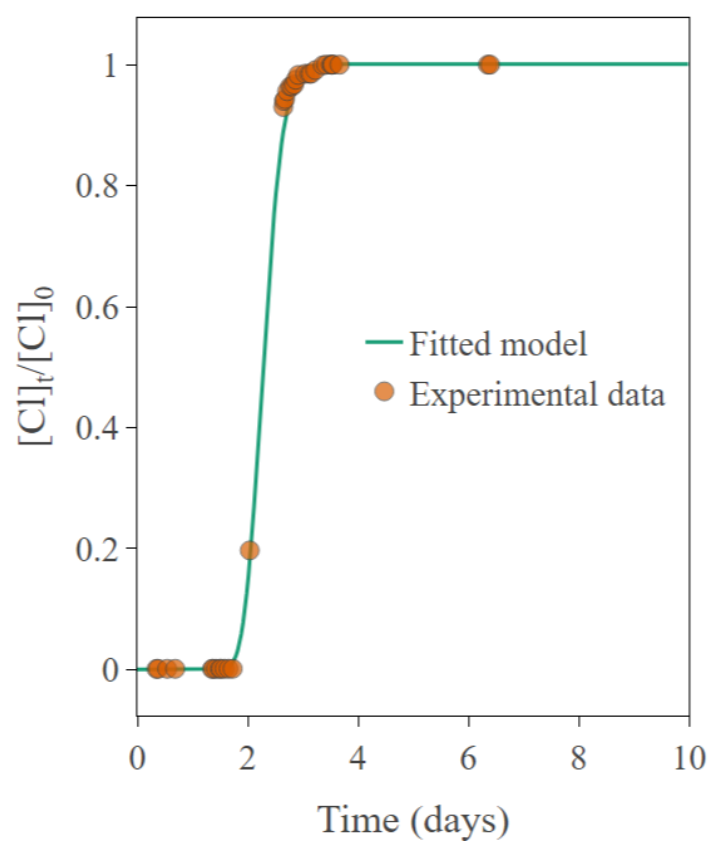


Figure S1. Cl^- conservative tracer test. The dots represent experimental data, and the solid line represents the fitted model.

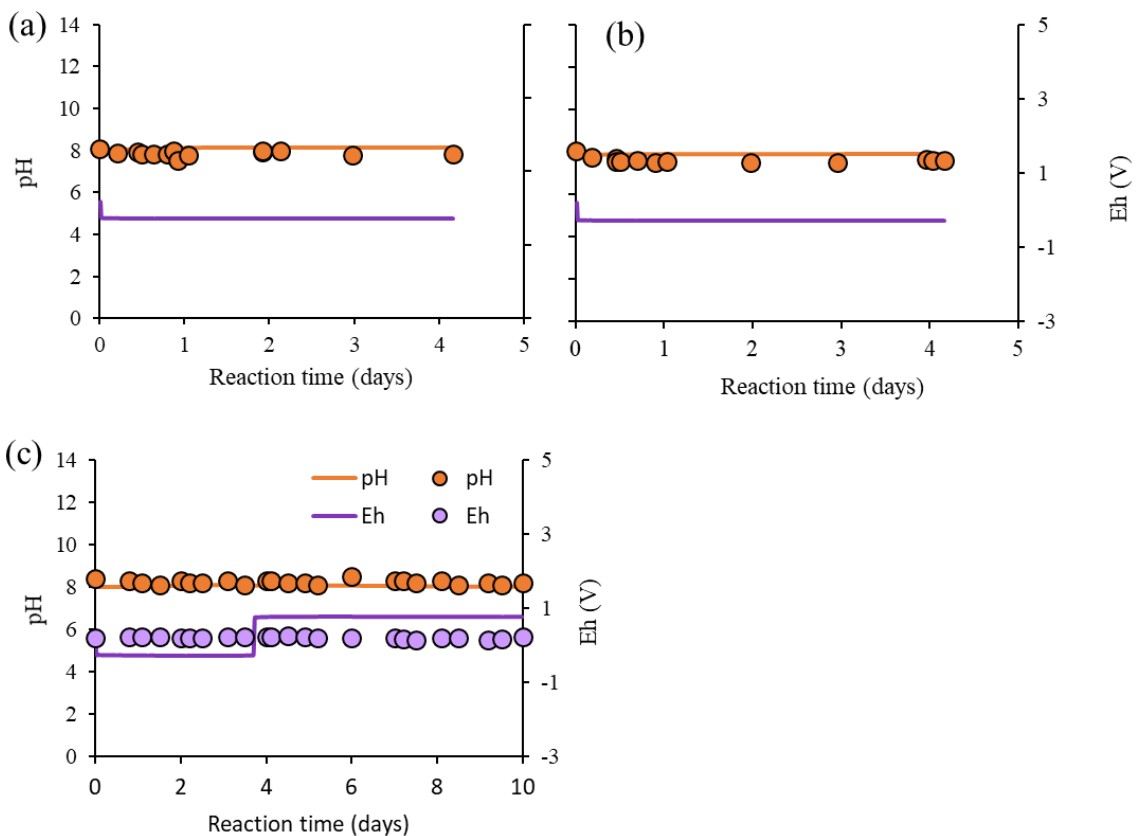


Figure S2. Evolution of pH and Eh in (a) B-L (b) B-V and (c) C-L. The dot represents experimental results, and solid lines represent the fitted model.

Table S1*International and Laboratory (CCiT-UB) Standards and Reproducibility for the Isotopic Analyses.*

Standard	Reference value [‰]		
	$\delta^{15}\text{N-NO}_3$	$\delta^{18}\text{O-NO}_3$	$\delta^{15}\text{N-NO}_2$
USGS-32	+180.0	+25.3	
USGS-34	-1.8	-27.9	
USGS-35	+2.7	+57.3	
CCiT-IWS-NO3	+16.9	+28.5	
CCiT-IWS-NO2			-28.5
Reproducibility (1σ)	$\pm 1.0 \text{ ‰}$	$\pm 1.5 \text{ ‰}$	$\pm 1.0 \text{ ‰}$

Table S2

Chemical Composition (mmol·L⁻¹) and $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ (‰) of NO_3^- and NO_2^- in B-L During Biostimulation. Ionic Strength and Saturation Indices of Calcite and Gypsum Calculated With the Phreeqc Software and Phreeqc Code (Parkhurst and Appelo, 2013). NA Means Not Analyzed, FW is Field Water, and Control is the Control Reactor.

Reactor	FW	1	2	3	4	5	6	7	8	9	10	11	12	13	Control
Days	0	0.21	0.45	0.50	0.64	0.80	0.88	0.93	1.05	1.92	1.92	2.13	2.98	4.17	5.00
pH	8.1	7.9	7.9	7.8	7.8	7.8	8.0	7.5	7.8	7.9	8.0	7.96	7.8	7.8	NA
T (°C)	28.4	27.5	28	28	28.4	28.4	28.4	27.5	27.5	28.4	28.4	28.4	28.4	28.4	NA
Conductivity (mS·cm ⁻¹)	2.36	2.38	2.39	2.52	2.38	2.38	2.41	2.38	2.39	2.33	2.32	2.34	2.32	2.33	NA
Ca	2.16	2.28	2.14	2	2.02	2.2	2.27	2.26	2.17	2.17	2.13	2.2	2.22	2.27	NA
Mg	2.94	3.24	2.99	2.76	2.86	3.02	3.01	3.2	2.95	3.02	3.03	3.1	3.12	2.99	NA
Na	11.4	12	11.3	10.4	10.8	11.4	11.2	12	10.9	11.6	11.6	11.9	11.9	11.6	NA
K	0.13	0.21	0.19	0.17	0.19	0.21	0.19	0.2	0.2	0.21	0.22	0.32	0.2	0.19	NA
Si	0.39	0.38	0.38	0.35	0.35	0.36	0.37	0.38	0.37	0.37	0.37	0.38	0.38	0.38	NA
S	3.19	3.43	3.12	2.88	2.88	3.12	3.14	3.35	3.04	3.2	3.26	3.34	3.34	3.3	NA
B	0.012	0.027	0.024	0.021	0.021	0.024	0.024	0.026	0.024	0.024	0.025	0.025	0.025	0.025	NA
P	0.001	0.006	0.002	0.002	0.002	0.001	0.046	0.004	0.053	0.004	0.000	0.001	0.000	0.001	NA
Cl ⁻	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	NA
HCO ₃ ⁻	0.46	3.99	4.3	3.68	3.44	3.21	3.65	3.27	2.86	1.59	1.21	0.94	0.87	0.61	NA

Annex 1: Paper 1

Reactor	FW	1	2	3	4	5	6	7	8	9	10	11	12	13	Control
NO ₃ ⁻	1.66	1.75	2.76	1.57	1.55	1.42	0.97	0.97	0.55	<3E-03	0.03	0.03	<3E-03	<3E-03	1.66
NO ₂ ⁻	<2E-04	<2E-04	<2E-04	<2E-04	0.05	0.11	0.16	0.42	0.54	0.42	0.05	0.21	<2E-04	<2E-04	<2E-04
$\delta^{15}\text{N-NO}_3^-$	NA	6.2	5.7	6.1	9.2	10.3	14.4	18.7	31.5	NA	NA	NA	NA	NA	NA
$\delta^{18}\text{O-NO}_3^-$	+NA	4.2	4.2	5.4	8.8	10.9	12.6	13.8	23.2	NA	NA	NA	NA	NA	NA
$\delta^{15}\text{N-NO}_2^-$	NA	NA	NA	NA	-18.1	-18.5	-17.8	-12.8	-10.2	NA	NA	NA	NA	NA	NA
$\delta^{18}\text{O-NO}_2^-$	NA	NA	NA	NA	-7.5	-6.9	-7.5	-2.2	-2.6	-0.4	-5.7	-4.2	NA	NA	NA
Ionic strength (mol·L ⁻¹)	0.029	0.026	0.027	0.028	0.028	0.027	0.028	0.028	0.027	0.027	0.028	0.028	0.028	0.028	NA
SI_Calcite	0.65	0.6	0.83	0.72	0.64	0.63	0.83	0.33	0.63	0.81	0.84	0.84	0.71	0.76	NA
SI_Gypsum	-1.25	-1.35	-1.29	-1.31	-1.3	-1.35	-1.28	-1.26	-1.31	-1.29	-1.3	-1.28	-1.27	-1.27	NA

Table S3

Chemical Composition (mmol·L⁻¹) and $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ (‰) of NO_3^- and NO_2^- in B-V During Biostimulation. Ionic Strength and Saturation Indices of Calcite and Gypsum Calculated With the Phreeqc Software and Phreeqc Code (Parkhurst and Appelo, 2013). NA Means Not Analyzed, FW is Field Water, and Control is the Control Reactor.

Reactor	FW	1	2	3	4	5	6	7	8	9	10	11	12	13	Control
Days	0	0.19	0.45	0.45	0.46	0.51	0.7	0.9	1.03	1.97	2.95	3.95	4.03	4.17	5
pH	8.1	7.7	7.7	7.6	7.6	7.5	7.6	7.5	7.5	7.5	7.5	7.7	7.6	7.6	NA
T (°C)	28.4	27.5	28	28	28	28	28.4	27.5	27.5	28.4	28.4	28.4	28.4	28.4	NA
Conductivity (mS·cm ⁻¹)	2.36	2.51	2.53	2.51	2.52	2.54	2.53	2.52	2.54	2.52	2.5	2.53	2.54	2.52	NA
Ca	2.16	2.49	2.32	2.34	2.45	2.35	2.44	2.58	2.3	2.48	2.68	2.75	2.62	2.55	NA
Mg	2.94	3.36	2.94	3.05	3.22	2.98	3.14	3.37	2.93	3	3.12	3.21	3.04	3.08	NA
Na	11.4	12.2	11	11.3	12	11	11.6	12.4	10.8	11.3	11.8	12.2	11.5	11.7	NA
K	0.13	0.74	0.71	0.7	0.77	0.75	0.82	0.8	0.81	0.73	0.6	0.74	0.74	0.66	NA
Si	0.39	0.41	0.36	0.38	0.4	0.37	0.38	0.42	0.36	0.38	0.41	0.42	0.4	0.4	NA
S	3.19	3.48	3.02	3.13	3.25	3.04	3.17	3.51	3.02	3.12	3.36	3.39	3.25	3.24	NA
B	0.012	0.030	0.026	0.026	0.026	0.026	0.027	0.029	0.026	0.027	0.028	0.029	0.028	0.027	NA
P	0.001	0.042	0.020	0.022	0.024	0.025	0.017	0.034	0.025	0.022	0.019	0.024	0.029	0.022	NA
Cl ⁻	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	9.7	NA
HCO ₃ ⁻	0.46	3.44	3.6	2.95	2.41	3.17	2.61	2.13	1.62	0.96	0.85	0.82	0.82	0.8	NA
NO ₃ ⁻	1.66	2.07	2.43	1.5	2.2	2.74	2.08	1.57	1.57	1.54	1.13	1	1.31	1.12	1.66
NO ₂ ⁻	<2E-04	<2E-04	<2E-04	<2E-04	0.13	0.21	0.26	0.97	0.89	0.78	1.21	1.3	1.24	1.17	<2E-04
$\delta^{15}\text{N}$ -NO ₃ ⁻	NA	-1.4	4.8	5.2	7.2	7.5	9.5	22.8	19.7	17.7	35	29.2	28.6	29	NA

Annex 1: Paper 1

Reactor	FW	1	2	3	4	5	6	7	8	9	10	11	12	13	Control
$\delta^{18}\text{O-NO}_3^-$	NA	6.9	10.2	10.3	12.7	12.3	13.3	25	24.7	22.1	29.4	35.5	32.3	32.2	NA
$\delta^{15}\text{N-NO}_2^-$	NA	NA	NA	NA	-16.8	-15.4	-21.1	-14.6	-11.5	-1.8	0.4	1.5	-2.6	0.2	NA
$\delta^{18}\text{O-NO}_2^-$	NA	NA	NA	NA	-5.6	-5.4	-4.4	-2.4	-5.7	-4	-4.4	-3.4	-3.8	-5.6	NA
Ionic strength (mol·L ⁻¹)	0.029	0.03	0.028	0.028	0.028	0.03	0.029	0.031	0.028	0.029	0.031	0.03	0.03	0.03	NA
SI_Calcite	0.65	0.55	0.41	0.52	0.35	0.41	0.42	0.39	0.37	0.37	0.61	0.4	0.5	0.5	NA
SI_Gypsum	-1.25	-1.22	-1.27	-1.29	-1.28	-1.25	-1.26	-1.21	-1.29	-1.25	-1.19	-1.2	-1.23	-1.22	NA

Table S4

Chemical Composition (mmol·L⁻¹) and $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ (‰) of NO_3^- and NO_2^- in C-L During Biostimulation. Ionic Strength and Saturation Indices of Calcite and Gypsum Calculated With the Phreeqc Software and Phreeqc Code (Parkhurst and Appelo, 2013). NA Means Not Analyzed.

Days	0	0.8	1.1	1.5	2	2.2	2.5	3.5	4	4.5	5.2	6	7.5	8.1	8.5	9.5	10.2
pH	8.4	8.3	8.2	8.1	8.3	8.2	8.2	8.1	8.3	8.2	8.1	8.5	8.2	8.3	8.1	8.1	8.2
Eh (V)	0.199	0.225	0.224	0.225	0.202	0.219	0.221	0.241	0.236	0.254	0.220	0.198	0.161	0.198	0.212	0.144	NA
Conductivity (mS·cm ⁻¹)	1.5	2.0	2.3	2.3	2.3	2.3	2.2	2.2	2.3	2.3	2.2	2.4	2.3	2.3	2.3	2.4	2.3
Ca	0.77	1.61	2.2	2.31	2.34	2.23	2.13	2.08	2.13	2.11	2.01	2.15	1.98	2.08	2.14	2.18	2.16
Mg	0.34	1.21	2.11	2.34	2.47	2.43	2.59	2.7	2.91	2.96	3.02	3.13	3.11	3.16	3.22	3.3	3.32
Na	10.93	12.68	13.22	13.41	13.67	13	12.8	12.64	12.7	12.52	12.24	13.19	12.12	12.09	12.26	12.2	12.22
K	1.3	0.24	0.75	0.39	1.51	0.82	1.15	0.32	1.18	0.21	0.69	0.6	0.53	0.79	0.33	0.27	0.29
Si	1.22	2.59	3.47	3.63	3.67	3.56	3.6	3.64	3.74	3.74	3.74	3.95	3.62	3.62	3.69	3.74	3.78
S	1.21	2.51	3.26	3.35	3.41	3.39	3.58	3.47	3.47	3.47	3.72	3.92	3.49	3.51	3.76	3.73	3.62
B	0.017	0.022	0.025	0.029	0.033	0.032	0.029	0.027	0.026	0.027	0.026	0.028	0.027	0.027	0.027	0.027	0.028
P	0.0009	0.0020	0.0005	0.0001	0.0001	0.0006	0.0018	0.0015	0.0017	0.0001	0.0023	0.0003	0.0024	0.002	0.0014	0.002	0.002
Cl ⁻	9.65	9.25	9.03	9.74	10.53	9.82	10.54	9.4	10.18	9.15	10.2	10.6	9.91	10.12	9.87	9.93	9.68
HCO ₃ ⁻	2.33	NA	4.38	NA	5.33	5.6	NA	NA	NA	NA	6.11	NA	6	NA	NA	NA	NA
NO ₃ ⁻	1.69	1.28	1.54	1.39	1.00	0.74	0.02	<3E-03	<3E-03	<3E-03	<3E-03	0.03	0.3	0.94	1.25	1.55	1.59
NO ₂ ⁻	<2E-04	<2E-04	<2E-04	0.05	0.12	0.17	0.05	<2E-04	<2E-04	<2E-04	<2E-04	0.003	0.034	0.048	0.008	0.009	0.009

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Days	0	0.8	1.1	1.5	2	2.2	2.5	3.5	4	4.5	5.2	6	7.5	8.1	8.5	9.5	10.2
$\delta^{15}\text{N} - \text{NO}_3^-$	+6.6	NA	NA	+11.3	+16.8	+23.5	NA	NA	NA	NA	NA	NA	+17	+14.3	+13.8	NA	NA
$\delta^{18}\text{O} - \text{NO}_3^-$	+1.7	NA	NA	+5.9	+12.8	+17.1	NA	NA	NA	NA	NA	NA	+15.1	+12.4	+9.6	NA	NA
$\delta^{15}\text{N} - \text{NO}_2^-$	NA	NA	NA	-3.4	+5.5	+12.6	NA	NA	NA	NA	NA	NA	+12.8	+4	NA	NA	NA
$\delta^{18}\text{O} - \text{NO}_2^-$	NA	NA	NA	-8.6	-9.7	-8.9	NA	NA	NA	NA	NA	NA	-8	-6.9	NA	NA	NA
Ionic strength (mol·L ⁻¹)	0.017	0.023	0.028	0.027	0.03	0.028	0.028	0.028	0.029	0.028	0.03	0.029	0.029	0.029	0.029	0.03	0.032
SI_Calcite	0.47	0.82	0.85	0.9	1.09	1	1	0.9	1.1	1.01	1.29	1.01	1.1	1.07	0.92	1.02	1.03
SI_Gypsum	-1.97	-1.43	-1.21	-1.24	-1.22	-1.24	-1.26	-1.26	-1.25	-1.25	-1.24	-1.25	-1.28	-1.3	-1.26	-1.25	-1.22

Table S5

Elements, Solution Species and the Phases Considered in the Geochemical Systems Using the Iso.Dat Database.

ELEMENTS				
C	Ca	Mg	Na	K
N	Si	S	P	Cl
O	B	e ⁻		
SOLUTION SPECIES				
H ₃ O ⁺	H ₂ O	H ₂	Ca ⁺²	CaCO ₃
CaHCO ₃ ⁺	CaSO ₄	CaHSO ₄ ⁺	CaPO ₄ ⁻	CaHPO ₄
CaH ₂ PO ₄ ⁺	CaOH ⁺	Mg ⁺²	MgSO ₄	MgHCO ₃ ⁺
MgCO ₃	MgHPO ₄	MgPO ₄ ⁻	MgH ₂ PO ₄ ⁺	MgOH ⁺
K ⁺	KOH	KHPO ₄ ⁻	KSO ₄ ⁻	Na ⁺
NaSO ₄ ⁻	NaHCO ₃	NaCO ₃ ⁻	NaHPO ₄ ⁻	NaOH
H ₄ SiO ₄	H ₃ SiO ₄ ⁻	H ₂ SiO ₄ ⁻²	O ₂	Cl ⁻
NO ₃ ⁻	NO ₂ ⁻	N ₂	HCO ₃ ⁻	CO ₃ ⁻²
CO ₂	PO ₄ ⁻³	HPO ₄ ⁻²	H ₂ PO ₄ ⁻	HS ⁻
H ₂ S	S ⁻²	SO ₄ ⁻²	HSO ₄ ⁻	
PHASES				
Calcite	CO ₂ (g)			

Table S6

Transport Parameters Obtained From Cl⁻ Conservative Tracer Test and the Fitted Parameters.

	Experimental	Fitted parameters
Velocity	3.8·10 ⁻⁶ m·s ⁻¹	3.5·10 ⁻⁶ m·s ⁻¹
Porosity	0.46	
Dispersivity		0.005 m
Diffusion coefficient		3.0·10 ⁻¹⁰ m ² ·s ⁻¹

Table S7

Least Square Errors of Reaction Rate Parameters for the Calibration of the Batch Experiments Using The Levenberg–Marquardt Algorithm (Nardi and de Vries, 2017)

Parameter	Least Square Error	
	B-V	B-L
K _{EA,NO₃}	0.006	0.006
K _{EA,NO₂}	0.080	0.080
K _{ED,NO₃}	0.006	0.006
K _{ED,NO₂}	0.080	0.080
k _{max,NO₃}	0.007	0.007
k _{max,NO₂}	0.080	0.080
b _{NO₃}	0.006	0.05
b _{NO₂}	0.080	0.05
k	0.012	0.012

Supplementary equations

More information on the equations employed can be found in the Phreeqc manual (Parkhurst & Appelo 2013).

In the PHREEQC code, equilibrium among aqueous species is calculated according to the mass action law (S1). K_{eq} is a temperature-dependent equilibrium constant (mol kgw^{-1})^{*n*} where *n* depends on the stoichiometric coefficients of the reactants and products of any chemical reaction. The log of the equilibrium constant (K_{eq}) and its temperature dependence for each reaction are defined in the iso.dat database. S_i is the stoichiometric coefficient of master species *m* in species *i* defined in the iso.dat database (dimensionless), and a_i is the activity of the species *i* (mol kgw^{-1}).

$$K_{eq} = \prod a_i^{S_i} \quad (\text{S1})$$

The activity of species *i* is related to the molality of species *i* (mol kgw^{-1}), m_i by equation S2, where γ_i is the activity coefficient of species *i* (dimensionless), which is calculated according to the Davies equation (S3), where *A* is a constant at a given temperature ($\text{\AA}^{-1}$), μ is the ionic strength (mol kgw^{-1}), and Z_i is the ionic charge of species *i* (dimensionless). The activity coefficient parameters are defined in the iso.dat database.

$$a_i = \gamma_i \cdot m_i \quad (\text{S2})$$

$$\log \gamma_i = -AZ_i^2 \left(\frac{\sqrt{\mu}}{1 + \sqrt{\mu}} - 0.3\mu \right) \quad (\text{S3})$$

The ionic strength of the aqueous solution is calculated according to equation S4, where N_{aq} is the number of aqueous species (dimensionless).

$$\mu = \frac{1}{2} \sum_i^{N_{aq}} Z_i^2 m_i \quad (\text{S4})$$

Pure phase equilibria are implemented using the mass-action law following equation S5, where K_{sp} is the equilibrium constant for the pure phase defined in the iso.dat database, S_p is the stoichiometric coefficient of master species *m* in the dissolution reaction (dimensionless), and a_i is the activity of the species *i* (mol kgw^{-1}).

$$K_{sp} = \prod a_i^{S_p} \quad (\text{S5})$$

The mineral's saturation index (SI_p) is defined following equation S6, where *IAP* is the ion activity product, and K_{sp} is the solubility product of the pure phase defined in the iso.dat database.

$$SI = \log \left(\frac{IAP}{K_{sp}} \right) \quad (\text{S6})$$

The one-dimensional reactive transport is simulated in PHREEQC using the Advection-Reaction-Dispersion (ARD) equation according to S7. $\partial c / \partial t$ is the temporal change in concentration ($\text{mol kgw}^{-1} \text{s}^{-1}$), where *C* is the concentration (mol kgw^{-1}) and *t* is time (s). $-v \partial C / \partial x$ is the advective transport, representing the spatial transport of the substance with the fluid flow ($\text{mol kgw}^{-1} \text{s}^{-1}$), where *v* is the pore water flow velocity (m s^{-1}) and *x* is the distance (m). $D_L (\partial^2 C) / (\partial x^2)$ is the dispersive transport, describing the spreading or diffusion of the substance, where D_L is the hydrodynamic dispersion coefficient ($\text{m}^2 \text{s}^{-1}$, $D_L = D_e + \alpha_L v$, with D_e the effective diffusion coefficient, and α_L the dispersivity (m)). $\partial q / \partial t$ is the chemical interaction term, describing the

temporal change in concentration in the solid phase due to reactions, where q is the solid phase concentration in mol kgw^{-1} .

$$\left(\frac{\partial C}{\partial t}\right) = -v\left(\frac{\partial C}{\partial x}\right) + D_L\left(\frac{\partial^2 C}{\partial x^2}\right) - \left(\frac{\partial q}{\partial t}\right) \quad (\text{S7})$$

The ARD equation is solved with an explicit finite difference scheme forward in time, central in space for dispersion and upward for advective transport. The chemical interactive term for each element is calculated separately from the transport part for each time step and is the sum of all the equilibrium and non-equilibrium reaction rates.

ANNEX 2: PAPER 2

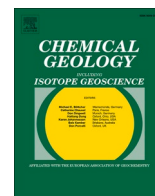
ABIOTIC NITRITE REDUCTION WITH FERROUS IRON

Isotopic and geochemical modeling approach to evaluate abiotic nitrite reduction by ferrous iron

Published on 17 January 2024

Abu, A., Carrey, R., Navarro-Ciurana, D., Margalef-Marti, R., Soler, A., Otero, N., Domènech, C., 2024. Isotopic and geochemical modeling approach to evaluate abiotic nitrite reduction by ferrous iron. Chem. Geol., 647, 121942.

<https://doi.org/10.1016/j.chemgeo.2024.121942>



Isotopic and geochemical modeling approach to evaluate abiotic nitrite reduction by ferrous iron

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ARTICLE INFO

Editor: Michael E. Boettcher

Keywords:

Chemodenitrification

Isotope fractionation

N₂O site preference

Numerical modeling

ABSTRACT

Nitrite reduction has often been treated as a biotic process in water treatment systems, but it also occurs abiotically, and it is difficult to distinguish both reactions as they can co-occur at field-scale. The potential reduction of NO₂⁻ was tested in 3 anaerobic experimental scenarios using a NO₂⁻ bearing solution amended with: i) siderite (FeCO₃) (Sid experiment); ii) Fe(II) solution from FeCl₂·4H₂O(s) (DFe experiment); and iii) siderite mixed with Fe(II) solution (Sid+DFe experiment). Non-sterilized batch experiments were carried out in anaerobic conditions with an initial ratio of nitrogen to dissolved iron of 5 in DFe and Sid+DFe (Sid had no initially dissolved Fe(II)) and 1000 mg L⁻¹ of siderite in Sid and Sid+DFe experiments. At the end of the experiments, the NO₂⁻ removed was 3% for Sid, 54% for DFe and 84% for Sid+DFe. The NO₂⁻ concentration decrease over time was characterized by an enrichment in the δ¹⁵N_{NO2} of the unreacted NO₂⁻, increasing from -26.9 ‰ to -26.4 ‰ (Sid), -18.0 ‰ (DFe) and -15.9 ‰ (Sid+DFe). The calculated ε¹⁵N_{NO2} for Sid was -11.8 ‰, whereas for DFe was -12.0 ‰ and Sid+DFe was -13.0 ‰, suggesting a common NO₂⁻ degradation mechanism in all experiments. The Rayleigh distillation equation showed that the generated N₂O was the final product of the abiotic nitrite reduction reaction, and the calculated N₂O site preference (SP) was 22.5 ± 0.7 ‰ for DFe and 23.5 ± 0.5 ‰ for Sid+DFe. The continuous N₂O measurement showed that only 25.3 ± 5.1% in DFe and 31.0 ± 6.3% in Sid+DFe of the generated N₂O in water was recovered in the headspace vials, suggesting that a large portion of the produced N₂O(aq) in solution did not diffuse from water. The coupled NO₂⁻ reduction and Fe(II) oxidation followed a second-order kinetic reaction with a rate equal to (9.39 ± 0.36)·10⁻⁴·[NO₂⁻]·[Fe(II)] (mol L⁻¹ s⁻¹) in all experiments. The experimental conditions supported by the Rayleigh distillation equation using the experimentally calculated ε¹⁵N values, coupled with NO₂⁻ isotopic data and N₂O SP values, showed that biological denitrification had a negligible influence on nitrite reduction and that chemodenitrification was the main NO₂⁻ attenuation pathway. A geochemical model coupling the kinetic chemodenitrification, isotope fractionation, aqueous speciation in equilibrium and precipitation and dissolution of calcite has been implemented and reproduced the experimental results. The geochemical model developed in our study can be applied to similar experimental studies and to field-scale studies to predict the efficiency of abiotic nitrite reduction treatments using Fe(II).

1. Introduction

Groundwater nitrate (NO₃⁻) pollution, which is attributed principally to agricultural activities, is one of the modern global societal challenges

as it causes major environmental and public health problems in many parts of the world (Arauzo, 2017). NO₃⁻ impairs water quality, and ingesting water containing high NO₃⁻ or NO₂⁻ concentrations endangers human life with health hazards, including methemoglobinemia and

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<https://doi.org/10.1016/j.chemgeo.2024.121942>

Received 5 July 2023; Received in revised form 15 January 2024; Accepted 16 January 2024

Available online 17 January 2024

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gastric and stomach cancer (Fan and Steinberg, 1996; WHO, 2017). Groundwater NO_3^- concentration in many European areas exceeds the safe drinking water threshold of 50 mg L^{-1} of NO_3^- and requires the application of induced remediation methods (EU parliament, 2020). Groundwater NO_3^- attenuation occurs mainly through natural or induced biological denitrification, where NO_3^- is reduced to gaseous products, generating nitrite (NO_2^-) as an intermediate product (Fig. 1). Other biotic processes, such as dissimilatory nitrate reduction to ammonium (DNRA), anaerobic ammonium oxidation (anammox), nitrifier denitrification and nitrification, also contribute to groundwater nitrite transformation processes (Fig. 1).

Nitrite reduction was often reported to be microbially catalyzed, but recent findings have proven that NO_2^- is chemically reactive and can also be abiotically reduced to N_2O by Fe(II) oxidation in anaerobic conditions (Chen et al., 2020a; Liu et al., 2019; Wankel et al., 2017). Iron is a common redox-active metal in the environment (Kappler and Straub, 2005), which occurs as dissolved, sorbed or structural Fe(II). Fe and N redox cycles are frequently interrelated, affecting N compound transformations in groundwater (Emerson et al., 2010). The oxidation of Fe (II) to Fe(III), mediated by nitrate-dependant bacteria, uses NO_3^- as an electron acceptor (Bryce et al., 2018), producing nitrite as an intermediate product. Sørensen and Thorling (1991) and Picardal (2012) reported simultaneous biotic and abiotic NO_2^- reduction during biological denitrification in batch cultures. Therefore, in Fe-rich environments, the biotic-abiotic processes might co-occur, and the former process may further reduce the N_2O produced by the latter process to N_2 . Quantifying the biotic and abiotic components of NO_2^- reduction is significant to understand the geochemical coupling of N and Fe redox cycles and to evaluate the potential efficiency of nitrate remediation strategies using Fe(II) (Kopf et al., 2013; Liu et al., 2019).

Stable isotopes of N ($\delta^{15}\text{N}$) and O ($\delta^{18}\text{O}$) have proven to be an effective tool for characterizing and quantifying environmental transformations of N compounds (Kendall et al., 2007; Margalef-Martí et al., 2020). Dual isotope systematics of NO_2^- transformation has been characterized during biological denitrification (Grau-Martínez et al., 2019; Martin and Casciotti, 2016), nitrification (Granger and Wankel, 2016; Sebilo et al., 2006), nitrifier denitrification (Ostrom and Ostrom, 2012; Wrage et al., 2001), anammox (Casciotti et al., 2010) and NO_2^- re-oxidation to NO_3^- (Buchwald and Casciotti, 2010). The kinetic isotope effect of N and O linked with chemodenitrification, which represents an essential control on NO_2^- isotopic composition in Fe-rich environments,

has not been the object of research until recently and less information is available compared to biological processes (Buchwald et al., 2016; Jones et al., 2015; Martin and Casciotti, 2016; Sebilo et al., 2019). During nitrite reduction (biotic and abiotic), the light N and O isotopes preferentially react faster, leading to the accumulation of the heavy isotopes of the unreacted nitrite ($^{15}\text{N}_{\text{NO}_2}$ and $^{18}\text{O}_{\text{NO}_2}$), which differentiates chemodenitrification and denitrification from processes that do not alter changes in the nitrite isotopic composition, such as mixing or dilution (Kopf et al., 2013). Consequently, the heavy isotopes of the generated N_2O ($^{15}\text{N}_{\text{N}_2\text{O}}$ and $^{18}\text{O}_{\text{N}_2\text{O}}$) progressively increase, approaching the initial NO_2^- isotopic composition in the case of the chemodenitrification, as it is the final product of the reaction. However, it is not still clear if it is possible to distinguish the biotic from the abiotic reactions as both processes show similar patterns of isotopic fractionation. The site preference (SP) of the generated N_2O and geochemical numerical modeling, including isotopic data, are promising tools that could be helpful to distinguish biotic and abiotic processes after coupling with all other data. Coupling the isotopic characterization of NO_2^- and N_2O could, therefore, be useful in identifying the nitrous oxide production pathway.

The two N atoms of N_2O differ in the nature of their covalent bonds and tend to acquire distinct isotopic compositions. The difference in $\delta^{15}\text{N}$ between the central N atom (N_α) and outer N atom (N_β) of N_2O ($\text{N}_\beta\text{-N}_\alpha\text{-O}$) provides site-specific isotopic information. SP does not depend on the isotopic composition of the substrate (NO_2^- in the case of chemodenitrification) and does not exhibit fractionation during its production, making SP a powerful indicator of the N_2O production pathway (Heil et al., 2014; Jones et al., 2015). N_2O SP values have successfully been used as indicators of nitrifier denitrification (-10 to 0 ‰), hydroxylamine oxidation and fungal denitrification (33 to 37 ‰), heterotrophic denitrification (-5 to 5 ‰) as sources of N_2O (Li et al., 2022; Ostrom and Ostrom, 2012).

Numerical simulations provide an essential tool for research in contaminant degradation processes, which allows the quantitative evaluation of complex processes. They also serve to predict the extent and consequences of geochemical reactions, interpret experimental data and test scenarios beyond laboratory experiment ranges, and for field-scale application (Zhu, 2012). While Monod kinetic equations are often used in modeling biotic NO_2^- reduction (Betlach and Tiedje, 1981), the kinetic model approach has been developed to characterize abiotic nitrite reduction and include pseudo-first-order, second-order nitrite degradation rate equations and others (Benaiges-Fernandez et al., 2020;

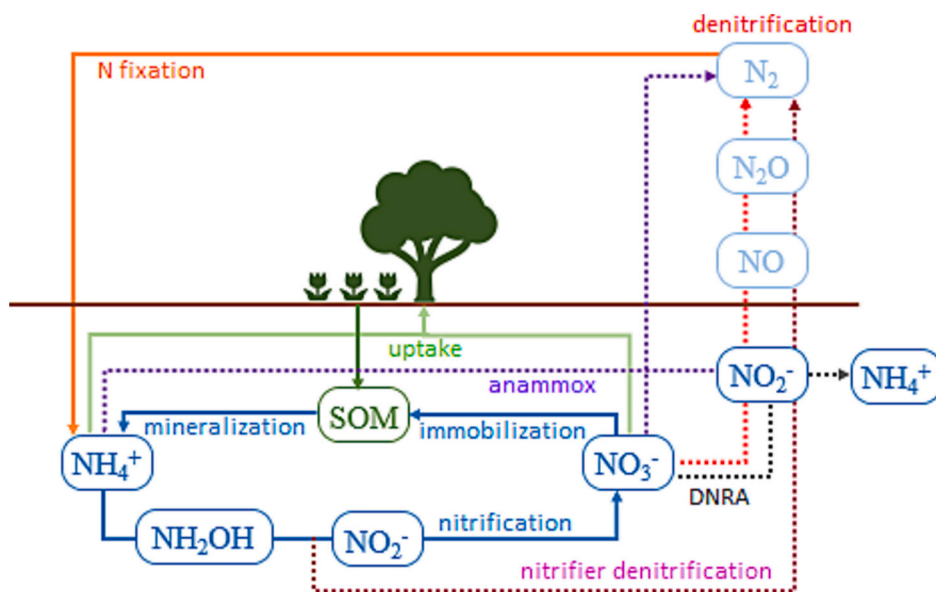


Fig. 1. Schematic model of N transformation pathways in aerobic (continuous lines) and anaerobic (dotted lines) conditions. SOM is soil organic matter (Modified after Margalef-Martí et al., 2021).

Liu et al., 2019). Despite several studies applied isotopic tools to identify and quantify the degradation pathways of NO_2^- , only a few studies focused on quantifying the isotope fractionation of NO_2^- during abiotic NO_2^- reduction by Fe(II) (Chen et al., 2021; Kopf et al., 2013). Therefore, improving the abiotic NO_2^- reduction numerical model is essential to understand the geochemical processes controlling N transformations.

Siderite, the most common iron carbonate mineral, can be used as a larger reservoir of Fe(II) with high dissolution rate to catalyze natural and induced denitrification in polluted water (Dunlop and Özdemir, 2015; Nowak et al., 2023). Siderite was consequently chosen in the present work to investigate its effect on abiotic batch experiments with particular emphasis on nitrite reduction and nitrous oxide production dynamics. Dissolved synthetic Fe(II) was also added in some of the experiments to assess its potential to increase the reactivity. Three scenarios (only siderite (Sid), only dissolved iron II (DFe) and dissolved iron + siderite (Sid+DFe)) were tested. Isotopic tools were employed to characterize nitrite attenuation and compare the calculated fractionation values to the range of values reported in the literature for different nitrite reduction processes. The fractionation values were then applied to the numerical modeling. Based on our knowledge, combining laboratory experiments with the dual NO_2^- and N_2O isotope modeling has never been applied so far in chemodenitrification. In this context, we also aim to develop a conceptual and numerical geochemical model that can reproduce our experimental results in order to upscale it to future field-scale studies.

2. Materials and methods

2.1. Experimental setup

Three parallel sets of anaerobic batch experiments, named Sid, DFe, and Sid+DFe, were performed under non-sterile conditions using 25 mL headspace (HS) vials. Based on preliminary batch experiments tests, 13 vials were chosen for the DFe and Sid+DFe experiments, while 4 vials were chosen for Sid experiments (negligible or extremely low reactivity was observed in the absence of DFe). Each vial for the Sid experiment was filled with 10 mg of micro-sized siderite and mixed with 10 mL of synthetic groundwater (SW, Table 1) prepared to simulate field conditions. Vials for DFe experiments were filled with 10 mL of SW and amended with 10 mg of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}(\text{s})$, and vials for Sid+DFe experiments were filled with 10 mg of siderite, mixed with 10 mL of the SW and amended with 10 mg of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}(\text{s})$. An initial ratio between N and dissolved Fe(II) of 5 was kept in DFe and Sid+DFe experiments, while no dissolved Fe was added in the Sid experiments. Each vial was immediately crimped with a butyl rubber stopper and purged with Argon gas during the first two minutes to create anaerobic conditions. All the batch experiments were carried out at ambient temperatures (18 °C to 25 °C) and kept in darkness under continuous shaking to avoid photodegradation processes. They lasted 8 days based on the preliminary batch experiments, and duplicate vials for each treatment set

Table 1

Composition of the synthetic water (SW) and the mass concentration of reagents used to prepare it. * only in DFe and Sid+DFe experiments.

Chemical	mol L ⁻¹	Reagent	mol L ⁻¹
pH	7.60	NaHCO_3 (Sigma-aldrich 99.5%)	$3.60 \cdot 10^{-3}$
pe	-3.00	KH_2PO_4 (Alfa aesar 98–100.5%)	$0.04 \cdot 10^{-3}$
Ca	$8.77 \cdot 10^{-4}$	$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (Sigma-aldrich 99–102%)	$1.24 \cdot 10^{-3}$
Mg	$1.24 \cdot 10^{-3}$	KCl (Sigma-aldrich 99%)	$1.50 \cdot 10^{-3}$
K	$2.55 \cdot 10^{-3}$	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Pancreac 74–80%)	$8.77 \cdot 10^{-3}$
Na	$6.44 \cdot 10^{-3}$	Na_2SO_4 (Alfa aesar 99%)	$1.42 \cdot 10^{-3}$
S	$1.42 \cdot 10^{-3}$	KNO_3 (Alfa aesar >96%)	$1.01 \cdot 10^{-3}$
C	$3.60 \cdot 10^{-3}$	$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (Pancreac 98%)	$5.03 \cdot 10^{-3*}$
P	$3.67 \cdot 10^{-5}$		
Cl^-	$5.75 \cdot 10^{-3}$		
NO_2^-	$1.2 \cdot 10^{-3}$		

were sacrificed in turns at different times.

2.2. Analytical methods

Samples obtained from conducted experiments were prepared for chemical and isotopic analyses at the laboratory of the MAIMA-UB research group and analyzed at the Centres Científics i Tecnològics of the Universitat de Barcelona (CCiT-UB). The headspace of each vial was first extracted using a gas syringe and stocked in exetainer flasks for subsequent N_2O concentration and isotopic analyses. After obtaining the gaseous samples, the headspace vials were decapsulated, and the liquid fraction was obtained and filtered using the 0.22 μm Millipore® PTFE hydrophilic syringe filters. One aliquot of the filtered liquid fraction was immediately used to convert NO_2^- to N_2O using the azide reduction method for NO_2^- concentration and isotope analysis (McIlvin and Altabet, 2005; Ryabenko et al., 2009). Nitrite concentration standards were then analyzed to calculate the samples' nitrite and nitrous oxide concentrations from the samples' peak amplitudes determined by isotope ratio mass spectroscopy (IRMS), as illustrated in Fig. S1 and summarized in Table S1. We employed this method to avoid issues associated with the oxidation of Fe(II) to Fe(III) and the subsequent precipitation of Fe (III) oxy(hydr)oxides in water and to prevent any possible reduction reactions of nitrite that might persist after the vials were sacrificed. Two vials were sacrificed at each time point, and each vial sample was analyzed by duplicate. The results at each time correspond to the average of these four measurements.

The isotopic compositions of N ($\delta^{15}\text{N}$) and O ($\delta^{18}\text{O}$) of dissolved NO_2^- were analyzed following the sodium azide reduction method, which converts NO_2^- to N_2O (McIlvin and Altabet, 2005; Ryabenko et al., 2009), immediately after sample collection to avoid further reduction of NO_2^- or other chemical reactions. The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ composition of the generated N_2O with the sodium azide ($\delta^{15}\text{N}_{\text{NO}_2}$, $\delta^{18}\text{O}_{\text{NO}_2}$) and of the N_2O collected from the HS of the batch experiments were analyzed using a Pre-Con (Thermo Scientific) coupled to an IRMS (Finnigan MAT 253, Thermo Scientific). The bulk and positional isotope of N_2O ($\delta^{15}\text{N}_{\text{N}_2\text{O}}^{\text{bulk}}$, $\delta^{15}\text{N}_{\text{N}_2\text{O}}^{\alpha}$, $\delta^{18}\text{O}_{\text{N}_2\text{O}}$, $\delta^{15}\text{N}_{\text{N}_2\text{O}}^{\beta}$) were measured using the arrays of mass/charge ratios of 46, 45 and 44 for the N_2O^+ molecular ion and 31 and 30 for the NO^+ fragment of the N_2O molecule. $\delta^{15}\text{N}_{\text{N}_2\text{O}}^{\text{bulk}}$ is the bulk $\delta^{15}\text{N}$ of N_2O , $\delta^{15}\text{N}_{\text{N}_2\text{O}}^{\alpha}$ is the isotopic composition of the central N of N_2O , and $\delta^{15}\text{N}_{\text{N}_2\text{O}}^{\beta}$ is the isotopic composition of the outer N ($\text{N}^{\beta}\text{-N}^{\alpha}\text{-O}$). Therefore, the $\delta^{15}\text{N}$ of N_2O^+ provides the $\delta^{15}\text{N}_{\text{N}_2\text{O}}^{\text{bulk}}$, the $\delta^{15}\text{N}$ of NO^+ provides the $\delta^{15}\text{N}_{\text{N}_2\text{O}}^{\alpha}$, and the $\delta^{15}\text{N}_{\text{N}_2\text{O}}^{\beta}$ is derived from Eq. (1). The site preference (SP) of $\text{N}_2\text{O}(\text{g})$ can be then calculated from $\delta^{15}\text{N}_{\text{N}_2\text{O}}^{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{N}_2\text{O}}^{\alpha}$ according to Eq. (2). Isotopic notation is expressed in terms of δ (‰) relative to the international standard: atmospheric N_2 (AIR) for $\delta^{15}\text{N}$ and Vienna Standard Mean Oceanic Water (V-SMOW) for $\delta^{18}\text{O}$. Hence, $\delta = (R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}$, where R is the isotope ratio ($\delta^{15}\text{N}/\delta^{14}\text{N}$ and $\delta^{18}\text{O}/\delta^{16}\text{O}$). NO_2^- isotopic composition was calibrated using the UB internal working standard CCiT-IWS ($\delta^{15}\text{N} = +28.5$ ‰, $1\sigma = \pm 0.1$ for $\delta^{15}\text{N}_{\text{NO}_2}$), which is analyzed at every set of 5 to 6 samples by duplicate. N_2O was calibrated using the international standard USGS-51 ($\delta^{15}\text{N} = +1.3$ ‰, $\delta^{18}\text{O} = +41.2$ ‰, $1\sigma = \pm 0.5$ for $\delta^{18}\text{O}_{\text{N}_2\text{O}}$ and ± 0.1 for $\delta^{15}\text{N}_{\text{N}_2\text{O}}$).

$$\delta^{15}\text{N}_{\text{N}_2\text{O}(\text{g})}^{\text{bulk}} (\text{‰}) = (\delta^{15}\text{N}_{\text{N}_2\text{O}(\text{g})}^{\alpha} + \delta^{15}\text{N}_{\text{N}_2\text{O}(\text{g})}^{\beta}) / 2 \quad (1)$$

$$\text{SP} (\text{‰}) = (\delta^{15}\text{N}_{\text{N}_2\text{O}(\text{g})}^{\alpha} - \delta^{15}\text{N}_{\text{N}_2\text{O}(\text{g})}^{\beta}) \quad (2)$$

The kinetic isotope fractionation (ϵ) associated with nitrite reduction was quantified by the Rayleigh distillation equation (Eq. (3)), where C_{initial} and C_{residual} are the initial and residual nitrite concentration (mol L⁻¹), respectively, and R_{initial} and R_{residual} are the isotope ratios of the initial and residual nitrite.

$$\text{Ln} \left(\frac{R_{\text{residual}}}{R_{\text{initial}}} \right) = \epsilon \cdot \text{Ln} \left(\frac{C_{\text{residual}}}{C_{\text{initial}}} \right) \quad (3)$$

2.3. Numerical method

Geochemical calculations and modeling have been carried out with the GibbsStudio software (Nardi and de Vries, 2017) based on PHREEQC version 3 code (Parkhurst and Appelo, 2013) and the iso.dat and wateq4f.dat databases supplied with the PHREEQC code. GibbsStudio helped to manage water chemistry sample data and run PHREEQC models in a unified productive graphical environment.

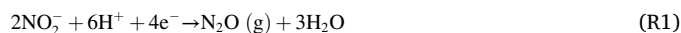
3. Results and discussion

3.1. NO_2^- attenuation dynamics

Low nitrite reduction was identified in the batch experiments containing only siderite, as only 3% of NO_2^- was removed (Fig. 2A), which is similar to experimental results conducted with other similar Fe(II) mineral compounds (siderite, magnetite, olivine) by Margalef-Marti et al. (2020) and Rakshit et al. (2008). However, in the conducted experiments involving DFe and Sid+DFe treatments, higher rates of NO_2^- reduction were observed. 54% and 84% of NO_2^- were removed in DFe and Sid+DFe, respectively, as NO_2^- levels decreased from an initial concentration of 1.10 mM to 0.51 mmol L^{-1} in DFe, and to 0.17 mmol L^{-1} in Sid+DFe (Fig. 2A).

Nitrite reduction in the batch experiments led to generation of gaseous nitrous oxide in the vial headspaces. $\text{N}_2\text{O}(\text{g})$ rarely accumulated in the Sid experiment but increased to 0.187 mmol $\text{N}_2\text{O}(\text{g})/\text{L H}_2\text{O}$ and 0.194 mmol $\text{N}_2\text{O}(\text{g})/\text{L H}_2\text{O}$ in DFe and Sid+DFe, respectively, on day 7 (Fig. 2B).

In our experiments, we did not use sediments or add microorganisms, and the absence of these biological components substantially restricts the potential for biological denitrification. Additionally, the relatively short duration of our experiments limits biological processes. Microbial denitrification, in the absence of sediments or added microorganisms, necessitates an extended period for microbial populations to establish and promote substantial nitrite reduction. A study by Visser et al. (2020) and Margalef-Marti et al. (2020) demonstrated that biological denitrification, without sediments or microbial inoculation, progresses slowly, with less than a 3% nitrite reduction rate observed over approximately 200 days. Fe(II) is recognized for inducing slower rates of biological denitrification compared to organic carbon sources (Rahman et al., 2019), which diminishes the likelihood of significant biological denitrification occurring within our experimental conditions. Consequently, we have considered that our experimental conditions are abiotic. Considering abiotic nitrite reduction, N- NO_2^- attenuation and N- N_2O production are expected in a 1:1 stoichiometry according to reaction (R1).



However, the nitrous oxide recovered in the vial headspaces in our experiments did not match the expected except for the first half of the experiments in Sid, which partially accounted for the NO_2^- removed from the vials (Fig. 3A-C).

The non-stoichiometry may be attributed to the formation of the intermediary compound NO, which may bind to mineral surfaces and prevent further reduction (Collman et al., 2008; Dhakal et al., 2013) or the further reduction of N_2O to N_2 . The absence of biological components in our experiments restricts the potential N_2O to N_2 reduction. Few authors have measured other gases than N_2O , and when measured, only minimal to no NO or N_2 nitrogen mass recoveries from these other gases were identified (Buchwald et al., 2016; Dhakal et al., 2021; Kampschreur et al., 2011; Rakshit et al., 2016; Wankel et al., 2017). Another process that might have contributed to the non-stoichiometry of the expected and measured N_2O is the leakage of N_2O through the butyl rubber stoppers or the low diffusion of aqueous N_2O into the HS. The reduced recoveries in our current study may be linked to the low diffusion of N_2O into the vial's headspace, potentially influenced by metal chlorides in the prepared water. Previous studies have shown that halides, including metal chlorides, can impact N_2O recoveries (Ryabenko et al., 2009). Several authors have observed a non-stoichiometry between N- NO_2^- removed and generated N- $\text{N}_2\text{O}(\text{g})$ during NO_2^- reduction, as a wide range of gaseous N_2O recoveries has been reported. In fact, <50% of N- NO_2^- removed during nitrite reduction is usually recovered as N- $\text{N}_2\text{O}(\text{g})$ in abiotic conditions (Chen et al., 2020a; Dhakal et al., 2021; Jones et al., 2015; Rakshit et al., 2016). In the present experiments, the N_2O recovery decreased from 36% to 19% in Sid+DFe, and significant fluctuations have been observed in DFe, with a maximum recovery rate (46%) at 36 h and a recovery rate of 25% on day 7 (Fig. 3D). The formation of iron oxide minerals may sorb NO_2^- intermediate reduction product (NO) or Fe (II), influencing $\text{N}_2\text{O}(\text{g})$ production and transformation (Chen et al., 2020a; Jones et al., 2014, 2015), which may explain changes in the recovery rate over time. N- N_2O in the liquid phase (undiffused N- N_2O) in the experiments of Sid+DFe accounted for 50–96% of the transformed N in the water and 68 to 98% in DFe experiments (Table S2). N- N_2O in the gas and liquid phases and the residual N- NO_2^- always equal the initial NO_2^- concentration in all the experiments. This indicates that N_2O leakages through the butyl rubber stoppers, the formation of intermediary NO, and the reduction of N_2O to $\text{N}_2(\text{g})$ did not occur in our experiments. Consequently, we can conclude that the main reason for not observing 100% N_2O recovery in the headspace vials is the solubility of N_2O in water, which retained a larger portion of the N_2O . The percentage of total N- N_2O compared to the total N varied from 2.3 to 60.0% in Sid+DFe and 0.2 to 61.3% in DFe experiments (Table S2).

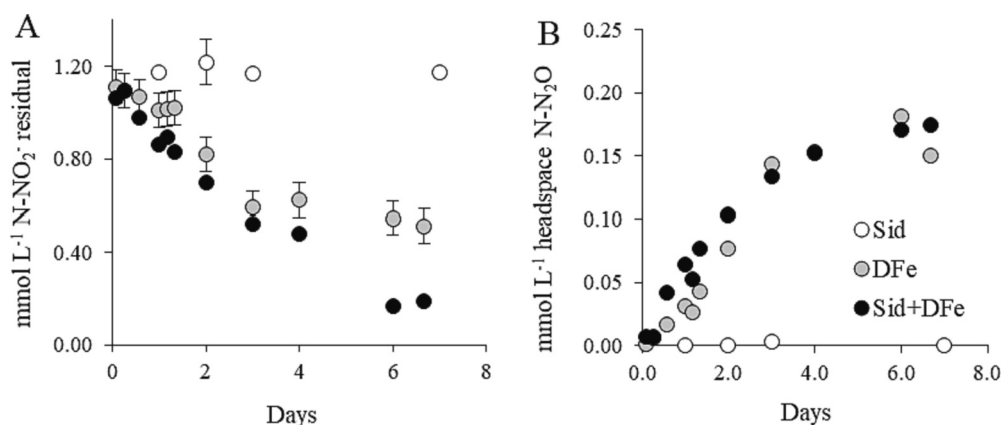


Fig. 2. A) Evolution of residual NO_2^- in solution. B) Evolution of $\text{N}_2\text{O}(\text{g})$ recovered in vial headspaces. The error bars represent the standard error (SE) of duplicate samples, and non-visible error bars are small and embedded within the data points.

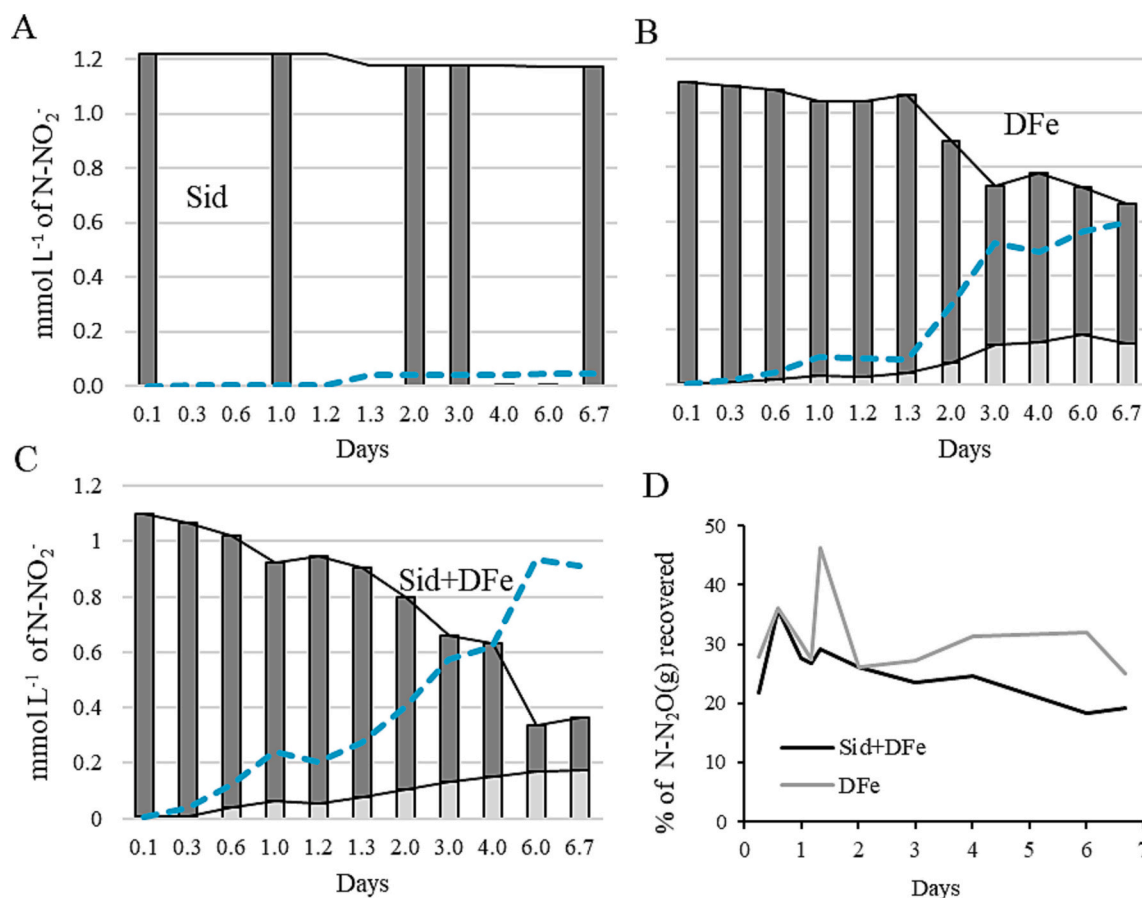


Fig. 3. Bar graphs showing the distribution of the residual NO_2^- (dark grey bars), the recovered $\text{N}_2\text{O(g)}$ (light grey bars), and the N_2O expected from the degradation of NO_2^- (transformed N-NO_2^-) (dashed lines) in the experiments of Sid (A), DFe (B) and Sid+DFe (C). D) The percentage distribution of recovered N_2O over time.

NO_3^- was monitored with paper test stripes and was consistently below the detection limit. However, NH_4^+ was not measured. The initial prepared water did not contain NH_4^+ , and we did not consider the possibility of DNRA since NO_3^- was not present, discarding NH_4^+ formation in our experiments through DNRA or through nitrite abiotic reduction. Furthermore, in previous nitrite reduction experiments, NH_4^+ has often not been detected (Margalef-Martí et al., 2020; Rakshit et al., 2016).

Nitrite reduction in this study was linked with a $\delta^{15}\text{N}_{\text{NO}_2}$ enrichment over time. A significant $\delta^{15}\text{N}_{\text{NO}_2}$ increase was observed in DFe and Sid+DFe experiments for the first two and a half days, corresponding to the period marked by high nitrite degradation, and remained in a steady state afterwards (Fig. 4A). $\delta^{15}\text{N}_{\text{NO}_2}$ values increased from -26.9 to -26.4 ‰ (Sid), -18.0 ‰ (DFe) and -15.9 ‰ (Sid+DFe) (Table S3). Due to $\delta^{18}\text{O}_{\text{NO}_2}$ equilibration with $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ during NO_2^- reduction, the obtained $\delta^{18}\text{O}_{\text{NO}_2}$ values in this study were not correlated with the residual NO_2^- concentration (Supplementary Information). For this reason, no further discussion is provided on the $\delta^{18}\text{O}_{\text{NO}_2}$ or $\delta^{18}\text{O}_{\text{N}_2\text{O}}$ results. The $\delta^{15}\text{N}_{\text{bulk}}$ values of the generated $\text{N}_2\text{O(g)}$ increased over time, with the most significant increment identified during the first two and a half days: $\delta^{15}\text{N}_{\text{bulk}}$ composition increased from -52.6 to -36.5 ‰ in DFe and from -57.1 to -38.6 ‰ in Sid+DFe (Fig. 4B).

The transformation of nitrogen oxides is often characterized by kinetic isotope fractionation (Mariotti et al., 1981), which follows the Rayleigh distillation Eq. (2) in a closed system. The calculated $\epsilon^{15}\text{N}_{\text{NO}_2}$ values were -11.8 ± 11.0 ‰ (Sid), -12.0 ± 1.4 ‰ (DFe) and -13.0 ± 1.1 ‰ (Sid+DFe) (Fig. 4C). The preliminary batch experiments had shown that nitrite reduction in the Sid experiment was very slow, and duplicate samples were analyzed at only 4 time points, resulting in a large error with the ϵ value. Even though its coefficient of determination

(R^2) is very low, the ϵ value is considered to be correct as it is similar to the other two. The similar ϵ values obtained suggest that the same process degraded nitrite in these experiments. The ϵ values obtained in this study are consistent with ϵ values reported in the literature for laboratory abiotic nitrite reduction studies, ranging from -19.7 to $+16.5$ ‰ for nitrite reduction by dissolved Fe(II) plus Fe(II) mineral (Benaiges-Fernandez et al., 2020; Chen et al., 2021; Grabb et al., 2017; Margalef-Martí et al., 2020), -8.6 to $+12.9$ ‰ for only dissolved Fe(II) (Benaiges-Fernandez et al., 2020; Buchwald et al., 2016; Grabb et al., 2017), and -9.4 to $+16.4$ ‰ for only Fe(II)-containing minerals (Grabb et al., 2017). In contrast, biotic nitrite reduction processes have yielded isotope fractionation values of -23.5 to -1.0 ‰ ($\epsilon^{15}\text{N-NO}_2^-$) and -9.8 to -1.0 ‰ ($\epsilon^{18}\text{O-NO}_2^-$) (Brunner et al., 2013; Bryan et al., 1983; Martin and Casciotti, 2016). The Rayleigh distillation equation for NO_2^- and N_2O fractions and the changes in its $\delta^{15}\text{N}$ results (Fig. 4D) show that $\text{N}_2\text{O(g)}$ is the final product formed, and fractionation did not affect $\text{N}_2\text{O(aq)}$ diffusion to $\text{N}_2\text{O(g)}$ recovered in the vial headspaces. This is evidenced as the $\delta^{15}\text{N}_{\text{bulk}}$ composition of the generated N_2O approached the initial $\delta^{15}\text{N-NO}_2^-$ value (Fig. 4D), which also explains the incomplete 100% N_2O recovery in the headspace vials due to the high solubility of N_2O in water. The natural logarithm of the generated $\text{N}_2\text{O(g)}$ was positive and linearly correlated with the $\delta^{15}\text{N}_{\text{bulk}}$ with slopes of 3.2 ± 0.2 ‰ in DFe and 4.7 ± 0.6 ‰ in Sid+DFe (Fig. S2). The calculated SP values, 22.5 ± 0.5 ‰ (DFe) and 23.5 ± 0.7 ‰ (Sid+DFe) are consistent with previous findings for chemodenitrification (0 to 30.2 ‰) (Grabb et al., 2017; Li et al., 2022). The SP values determined in this study differ from previously reported SP values associated with heterotrophic denitrification (-5.8 to 8.9 ‰: Haslun et al., 2018; Li et al., 2022; Rohe et al., 2017; Sutka et al., 2006; Toyoda et al., 2017; Winther et al., 2018; Yamazaki

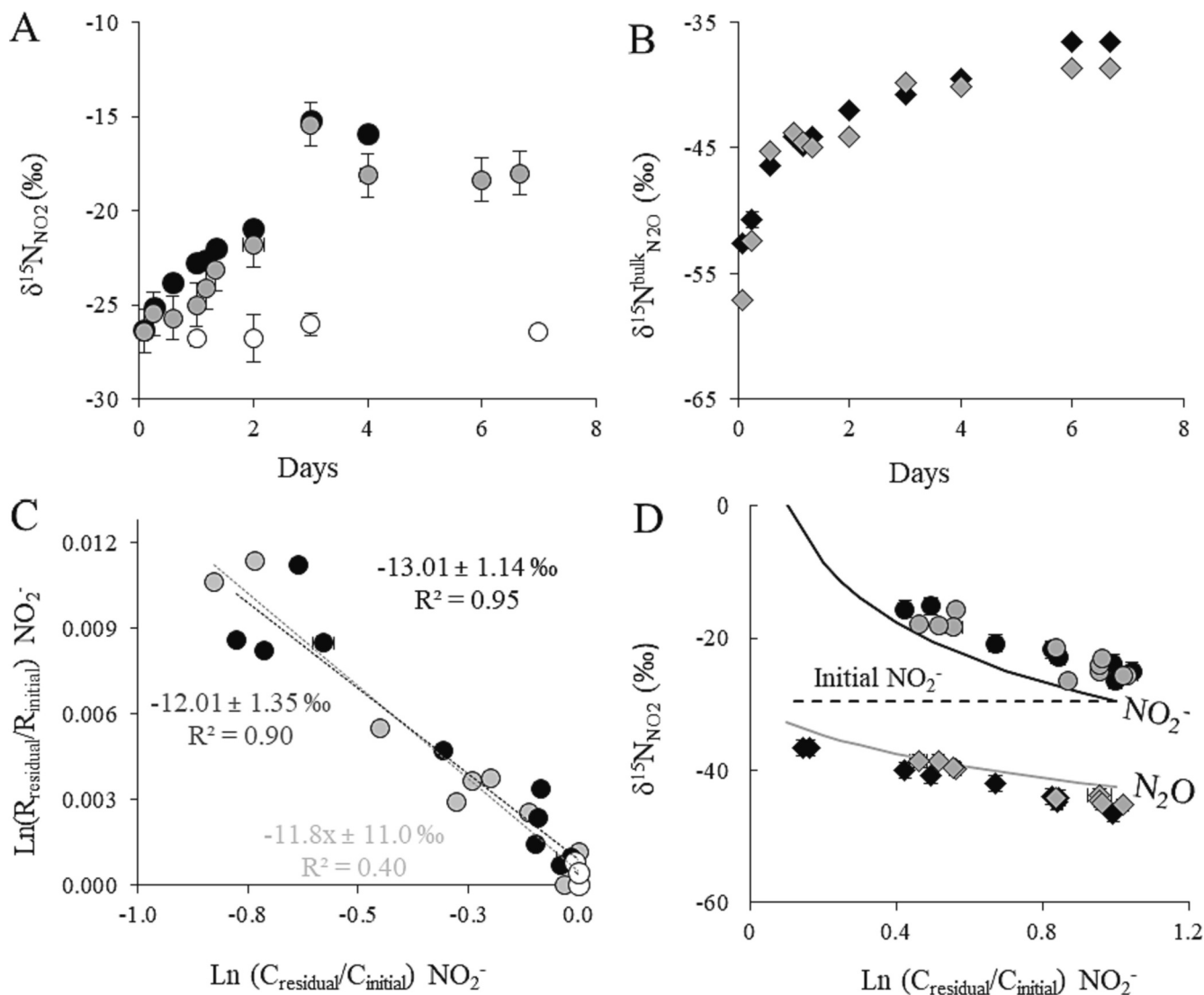


Fig. 4. Evolution of $\delta^{15}\text{N}_{\text{NO}_2}$ (A) and $\delta^{15}\text{N}_{\text{N}_2\text{O}}$ (B) during abiotic nitrite reduction. C) A plot of the natural logarithm of unreacted nitrite fraction against the isotope ratio with slopes indicating the isotope fractionation. D) The Rayleigh distillation model using the average ϵ value (12 ‰) and the experimentally analyzed $\delta^{15}\text{N}$ of the unreacted NO_2^- and generated N_2O . The error bars represent the standard error (SE) of duplicate samples, and non-visible error bars are small and embedded within the data points. Black, grey and white colors indicate Sid+DFe, DFe and Sid experiments, respectively. Circles and diamonds represent experimental results, and solid lines represent the Rayleigh model.

et al., 2014) and nitrifier denitrification (−10 to 0 ‰: Ostrom and Ostrom, 2012), although, they exhibit a similarity to the SP values associated with fungal denitrification (21.9 to 37 ‰: Ostrom and Ostrom, 2012; Rohe et al., 2014), and hydroxylamine oxidation (29 to 33 ‰: Ostrom and Ostrom, 2012; Tischer et al., 2022), but these biological processes are not occurring in our studied systems. Considering the factors mentioned above, we can infer that chemodenitrification is the main process for nitrite reduction in our study, and the isotopic data shows characteristic patterns associated with chemodenitrification process.

Although siderite reactivity with nitrite was almost negligible, it was observed that when it was mixed with dissolved Fe(II), the nitrite removal rate was increased up to 84%, while nitrite removal induced by dissolved Fe(II) alone was 54%. The increased nitrite reduction suggested that siderite might have contributed to the increased chemodenitrification rate, which is consistent with previously conducted studies reporting increased reactivity of Fe(II) in the presence of minerals surfaces (Dhakal et al., 2021; Jones et al., 2014; Rakshit et al., 2016, 2008; Visser et al., 2020). During chemodenitrification, not all the Fe(II) dissolved in water is usually involved in the direct nitrite

reduction: some portion may bind to the siderite surface to form reactive surfaces, which acts as a surface catalyst, increasing the Fe(II) electron density improving its potential as a reductant (Jones et al., 2015; Rakshit et al., 2016). Similar to Benaiges-Fernandez et al. (2020) and Chen et al. (2020b), the reduction of nitrite only by dissolved Fe(II) in the DFe batch experiment occurred without a mineral catalyst, which is contrary to earlier reports by Cooper et al. (2003) and Rakshit et al. (2008) that postulated that chemodenitrification required a mineral catalyst. The chemical reduction of nitrite by only Fe(II) involves the electron transfer process. Nitrite, in this process, is bound to Fe(II) to form Fe(II)- NO_2^- complex, where electrons are transferred to nitrite, producing nitrous oxide and Fe(III) oxyhydroxides (Chen et al., 2021; Rakshit et al., 2016).

3.2. Kinetic analyses during abiotic nitrite reduction

Nitrite concentration evolution with time revealed that nitrite degradation followed a pseudo-first order kinetics with respect to nitrite concentration according to Eq. (4), where $d[\text{NO}_2^-]/dt$ is the nitrite degradation rate ($\text{mol L}^{-1} \text{ s}^{-1}$), and k is the pseudo-first-order rate constant (s^{-1}). Once integrated, Eq. (4) becomes a linear equation if $\ln$

$[\text{NO}_2^-]$ is expressed as a function of time (Eq. (5)), and then, the rate constant and the initial nitrite concentration can be obtained after a linear regression of the experimental values (Fig. 5A) (Table 2).

$$\frac{d[\text{NO}_2^-]}{dt} = -k \cdot [\text{NO}_2^-] \quad (4)$$

$$\text{Ln}[\text{NO}_2^-] = \text{Ln}[\text{NO}_2^-]_0 - kt \quad (5)$$

The estimated initial nitrite concentrations were $1.21 \pm 0.02 \text{ mmol L}^{-1}$, $1.11 \pm 0.05 \text{ mmol L}^{-1}$ and $1.19 \pm 0.07 \text{ mmol L}^{-1}$ in Sid, DFe and Sid+DFe experiments, respectively (Table 2). These values are very close and consistent with the initial NO_2^- concentration used in the experiments (Table 1). However, k values are $(4.94 \pm 4.85) \cdot 10^{-8} \text{ s}^{-1}$, $(1.48 \pm 0.17) \cdot 10^{-6} \text{ s}^{-1}$, and $(3.31 \pm 0.20) \cdot 10^{-6} \text{ s}^{-1}$ for Sid, DFe and Sid+DFe, significantly different between conducted batch experiments (Table 2).

In order to ascertain the dependence of rate constant k with Fe(II) aqueous concentration, we have performed preliminary geochemical calculations to estimate Fe(II) aqueous concentration since it was not measured. In the geochemical calculations, the experimental procedure has been reproduced. First, SW (Table 1) was equilibrated with atmospheric $\text{CO}_2(\text{g})$ ($p_{\text{CO}_2(\text{g})} = 10^{-3.5} \text{ atm}$) and with calcite (in case of oversaturation) as SW was not prepared in a controlled atmosphere. Total aqueous Fe(II) concentration, $[\text{Fe(II)}]_{\text{T}}$, was calculated after equilibrating this CO_2 equilibrated SW (from now on, CESW) with Fe-minerals before nitrite reduction starts. The amount of initial siderite considered in the calculation was $8.63 \cdot 10^{-3} \text{ mmol L}^{-1}$ in Sid and DFe+Sid experiments, while no siderite was considered to be initially present in the DFe experiment. Moreover, 5 mmol L^{-1} of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ was added to the solution in DFe and Sid+DFe experiments. In all cases, amorphous Fe(III) hydroxide ($\text{Fe(OH)}_3(\text{am})$) and calcite were left to precipitate if oversaturated. The composition of the CESW before and after equilibration with Fe-minerals, values of $[\text{Fe(II)}]_{\text{T}}$, the remaining amount of siderite and the precipitated $\text{Fe(OH)}_3(\text{am})$ obtained in these calculations are summarized in Table 3.

The SW pH (7.6) dropped to 6.52, 3.12 and 5.48 in Sid, DFe and Sid+DFe, respectively, after the preliminary calculations. Similarly, the Eh's reduced from 0.17 to -0.13 V in Sid, from 0.69 to 0.08 V in DFe and from 0.25 to -0.08 V Sid+DFe (Fig. S3), showing reducing conditions. These initial conditions are favorable for nitrite reduction as acidic

Table 2

Regression analysis for nitrite reduction over time, shown in Fig. 5.

Experiment	Equation of regression line	R^2
Sid	$\text{Ln}[\text{NO}_2^-] = (-4.9 \pm 4.9 \cdot 10^{-8}) \cdot t - (6.7 \pm 0.1)$	0.3
DFe	$\text{Ln}[\text{NO}_2^-] = (-1.48 \pm 0.17 \cdot 10^{-6}) \cdot t - (6.80 \pm 0.05)$	0.96
Sid+DFe	$\text{Ln}[\text{NO}_2^-] = (-3.31 \pm 0.20 \cdot 10^{-6}) \cdot t - (6.70 \pm 0.06)$	0.97

conditions ($\text{pH} < 6$) have often been found to promote nitrite reduction in experimental laboratory studies (Dhakal et al., 2013; Kampschreur et al., 2011).

A plot of the pseudo-first-order constants, k (s^{-1}), versus these calculated $[\text{Fe(II)}]_{\text{T}}$ (mol L^{-1}) shows a positive linear relationship (Fig. 5B), from which the k dependence on Fe concentration can be obtained. Therefore, Eq. (4) can be further developed to Eq. (6), where nitrite degradation rate ($\text{mol L}^{-1} \text{ s}^{-1}$) is a second-order kinetic rate with a constant value of $(9.39 \pm 0.36) \cdot 10^{-4} \text{ L mol}^{-1} \text{ s}^{-1}$.

$$\frac{d[\text{NO}_2^-]}{dt} = (9.39 \pm 0.36) \cdot 10^{-4} \cdot [\text{NO}_2^-] \cdot [\text{Fe(II)}] \quad (6)$$

The k values obtained in the current study ($4.94 \cdot 10^{-8}$ – $3.31 \cdot 10^{-6} \text{ s}^{-1}$), according to Eq. (4), were in agreement with the range of values reported in the literature ($3.89 \cdot 10^{-8}$ – $2.78 \cdot 10^{-5} \text{ s}^{-1}$; Buchwald et al., 2016; Jones et al., 2014; Rakshit et al., 2008). Additionally, the overall second-order nitrite reduction rate constant (k') calculated for the current batch experiments ($9.39 \cdot 10^{-4} \text{ mol L}^{-1} \text{ s}^{-1}$) according to Eq. (6), was also in concordance with the range of values ($1.53 \cdot 10^{-4}$ – $9.38 \cdot 10^{-4} \text{ mol L}^{-1} \text{ s}^{-1}$) obtained for similar chemodenitrification experiments (Benaiges-Fernandez et al., 2020; Rakshit et al., 2008; Robinson et al., 2021).

3.3. Conceptual and numerical model

The model presented in the current study describes the coupled reaction between chemical nitrite reduction and Fe(II) oxidation, linked to the evolution of NO_2^- and N_2O isotopic composition in a fully saturated batch system and at a constant temperature of 25°C . The linked nitrite reduction and Fe(II) oxidation were considered a single-step process expressed in reaction (R2) and quantified by Eq. (6). This is an overall process simplification, as surface catalytic effects by direct and indirect

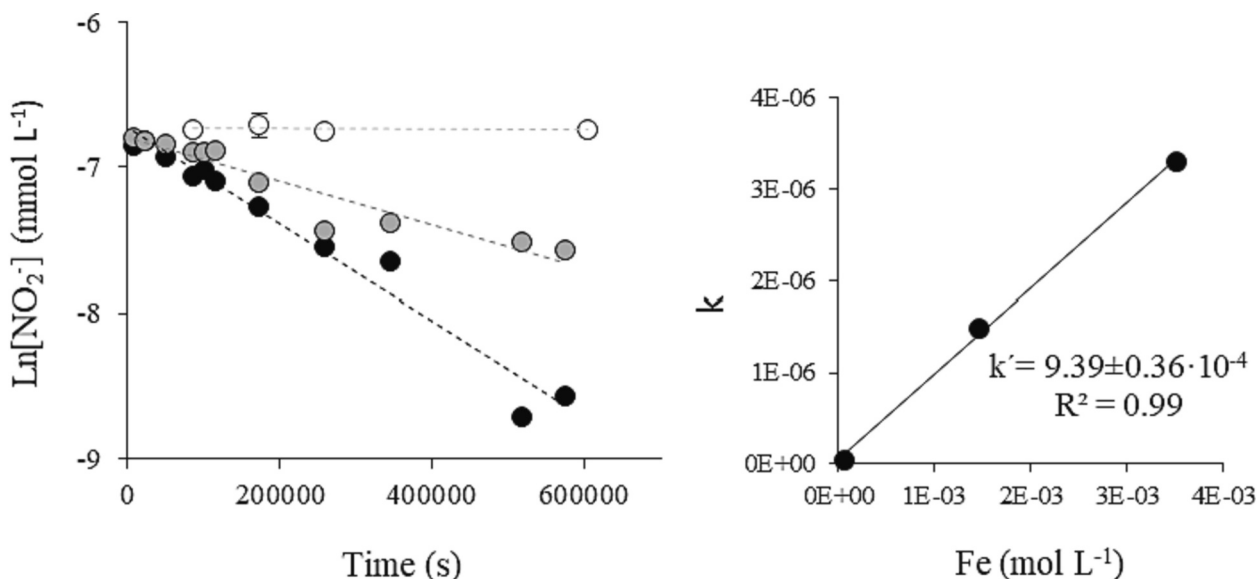


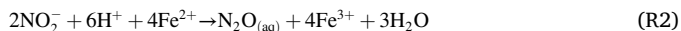
Fig. 5. A) Natural logarithm of NO_2^- concentration versus time. B) The pseudo-first-order rate constants versus the total aqueous Fe(II) concentration before NO_2^- reduction. The white, grey and black colors in A represent Sid, DFe and Sid+DFe, respectively. The error bars represent the standard error (SE) of duplicate samples, and non-visible error bars are small and embedded within the data points. The analysis of the regression lines can be seen in Table 2.

Table 3

CESW composition before and after equilibrating with Fe-minerals and mineral composition after equilibration for Sid, DFe and Sid+DFe cases.

Chemicals	pH	Eh V	Ca mM	Mg mM	K mM	Na mM	Cl mM	S mM	P mM	C mM	Fe(II) _T mM	Siderite mM	Fe(OH) ₃ mM
CESW	8.62	0.60	0.16	1.24	2.55	6.44	5.75	1.42	0.04	2.36	–	–	–
CESW-Sid	6.53	0.17	0.16	1.24	2.55	6.44	5.75	1.42	0.04	5.95	0.05	5.04	3.54
CESW-DFe	3.11	0.69	0.16	1.24	2.55	6.44	15.75	1.42	0.04	2.36	1.46	–	2.42
CESW-Sid+DFe	5.48	0.25	0.16	1.24	2.55	6.44	15.75	1.42	0.04	4.40	3.51	6.58	3.54

sorption onto siderite are not considered in our model. The model does not consider the formation of $\text{N}_2\text{O(g)}$ neither.



The change in the $\delta^{15}\text{N}$ of NO_2^- during nitrite reduction has been modeled according to first-order degradation kinetics described in Van Breukelen and Prommer (2008) (Eq. (7)), where ϵ is the experimental kinetic isotope fractionation, $[\delta^{15}\text{N}_{\text{NO}_2^-}]/([\delta^{15}\text{N}_{\text{NO}_2^-}] + [\delta^{14}\text{N}_{\text{NO}_2^-}])$ is N isotopic ratio of NO_2^- , and $d[\text{NO}_2^-]/dt$ is the NO_2^- degradation rate, calculated from Eq. (6).

$$R_{\delta \text{ HEA}} = \frac{d[\text{NO}_2^-]}{dt} \cdot \frac{[\delta^{15}\text{N}_{\text{NO}_2^-}]}{[\delta^{15}\text{N}_{\text{NO}_2^-}] + [\delta^{14}\text{N}_{\text{NO}_2^-}]} \cdot \epsilon \quad (7)$$

The prepared water composition obtained after the initial geochemical calculations (Table 3) with an initial nitrite concentration of 1.18 mol L^{-1} and the amounts of siderite and amorphous Fe(III) hydroxide indicated in Table 3 have been considered as initial conditions for the different batch experiments. In all cases, siderite, calcite and amorphous Fe(III) hydroxide are left to precipitate or dissolve in equilibrium. The average isotopic fractionation value from the experimental data (ϵ of -12.0 ‰) was considered for Eq. (7). According to the experimental data, all the models were run for 7 days in 70 equal steps. Sensitivity cases have been run to account for k uncertainties.

The models well reproduced nitrite concentration experimental results in Sid, DFe and Sid+DFe. The same initial nitrite concentration, which was taken as the average of three experimental sets, was slightly lower compared to the initial nitrite concentration of the Sid experiments, which accounts for the high nitrite experimental results. Similar to the experimental data, 5–6%, 61–63%, and 84–86% of nitrite is removed in Sid, DFe and Sid+DFe, respectively, and was paralleled with

an increase of $\delta^{15}\text{N}_{\text{NO}_2^-}$ value from -26.9 to $-26.1 \pm 0.3 \text{ ‰}$ (Sid), $-15.3 \pm 0.5 \text{ ‰}$ (DFe) and $-4.2 \pm 0.8 \text{ ‰}$ (Sid+DFe) in the remaining nitrite (Fig. 6A). The isotope model could simulate $\delta^{15}\text{N}_{\text{NO}_2^-}$ at low NO_2^- concentrations, below analytical capabilities.

As the models simulate the total aqueous N_2O generated in the solution, modeled results overestimate measured $\text{N}_2\text{O(g)}$, as experimental results measure the N_2O diffused from the solution into the HS, which only occurs in a small percentage (Fig. 6B). However, considering the total N_2O calculated using Henry's Law, comprising both the gas and liquid phases N_2O , the results align closely with the model results for DFe and Sid, but slightly lower in the Sid+DFe (Fig. 6B).

Geochemical calculation with the GibbsStudio software indicated that amorphous Fe-oxyhydroxide was formed in all the batch experiments. $\text{Fe}(\text{OH})_3(\text{am})$ slightly increased from 3.5 to 3.7 mmol L^{-1} in Sid and significantly increased from 2.2 to 2.9 mmol L^{-1} in DFe and 3.5 to 5.5 mmol L^{-1} in Sid+DFe. 0.1 and 1.5 mmol L^{-1} of siderite were dissolved in Sid and Sid+DI, respectively, and calcite did not form in any of the models due to the low concentration of Ca ions in SW (Table 2). In DFe and Sid+DFe models, more Fe(II) was oxidized, resulting in a higher rate of nitrite reduction, forming more Fe-oxyhydroxides, which is consistent with the experimental data as brown precipitates were observed in DFe and Sid+DFe experiments. The small amount of siderite dissolved in Sid explains the slow nitrite reduction, as siderite dissolution is the only Fe(II) source. On the other hand, the large amount of dissolved siderite in Sid+DFe may also contribute to improving nitrite reduction, which is often observed in iron-rich environments (Buchwald et al., 2016; Jones et al., 2015).

The numerical model could predict concentrations below detection by our analytical techniques. While nitrous oxide was not detected in the headspace of vials in the Sid experiments, the model successfully

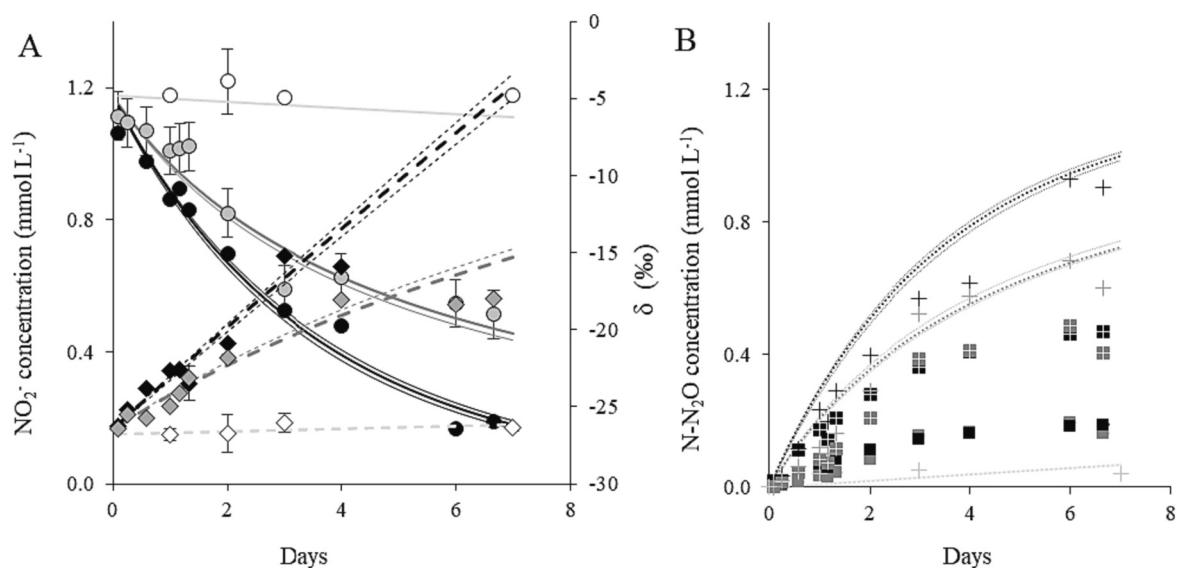


Fig. 6. A) NO_2^- concentration and $\delta^{15}\text{N}_{\text{NO}_2^-}$ evolution over time for the three experiments. White, grey and black colors represent Sid, DFe and Sid+DFe, whereas circles and diamonds indicate experimental NO_2^- and $\delta^{15}\text{N}_{\text{NO}_2^-}$, respectively, and lines are model results. B) Recovered $\text{N-N}_2\text{O}$ in gas phase (squares), calculated $\text{N-N}_2\text{O}$ in liquid phase (plus inside squares), transformed N (plus) and simulated total $\text{N-N}_2\text{O}$ (lines) evolution over time. The thin outer lines represent the uncertainty due to constant rate error. The error bars represent the standard error (SE) of duplicate samples, and non-visible error bars are small and embedded within the data points.

predicted small amount of aqueous nitrous oxide concentration. Consequently, it would be intriguing to consider monitoring the aqueous nitrous oxide concentration in future denitrification and chemodenitrification experiments. At the laboratory scale, it will require a long time to study nitrite reactivity with only siderite due to the low reactivity as in our case, but our numerical model provides an essential advantage of its ability to predict the reactivity of nitrite and only siderite over extended periods beyond the laboratory range. Our model could also be used as a tool for similar experimental studies and field applications.

4. Conclusions

This study investigated the reaction of NO_2^- by Fe(II) oxidation using chemical, isotopic data and numerical modeling. Results showed that the reactivity of siderite alone was low, removing only 3% of nitrite from the water. Dissolved Fe(II) induced nitrite reduction without a mineral catalyst, achieving 54% of nitrite removal. The NO_2^- reduction rate was enhanced when dissolved Fe(II) was mixed with siderite, removing 84% of nitrite from the solution. The calculated nitrogen of nitrous oxide in the gas and liquid phases was equal to the nitrite nitrogen transformed in water, showing that N_2O was the final product generated. This also suggested N_2O leakage through the butyl rubber stoppers, and the formation of intermediary NO did not occur in our experiments. Henry's Law showed that 50 to 98% of the total N_2O generated in water was dissolved, and low N_2O recovery in the headspace of the vials was attributed mainly to low diffusion of aqueous N_2O . The isotopic fractionation (ϵ) and site preference (SP) values calculated were -12.0 ± 1.4 ‰ and 22.5 ± 0.7 ‰ for DFe, respectively, whereas for Sid+DFe were -13.0 ± 1.1 ‰ (ϵ) and 23.5 ± 0.5 ‰ (SP). The isotopic data in this study showed that the main process for nitrite reduction in the experiments was chemodenitrification.

The present study developed a geochemical model describing the oxidation-reduction reaction between Fe(II) and nitrite and nitrous oxide production according to a second-order kinetics that could simulate the experimental results. The evolution of $\delta^{15}\text{N}$ of NO_2^- was reproduced following the first-order kinetics. The experimental conditions of our study, coupled with nitrite and nitrous oxide isotopic data, the Rayleigh distillation equation and the geochemical model complementary tools, showed that chemodenitrification was the main nitrite attenuation process and that the influence of biological processes was limited. This study underscores the applicability of abiotic nitrite reduction concentration and isotope models across diverse experimental and field scenarios to understand nitrogen species reaction abiotic pathways.

CRedit authorship contribution statement

Alex Abu: Data curation, Formal analysis, Writing – original draft. **Raúl Carrey:** Conceptualization, Data curation, Formal analysis, Supervision, Writing – review & editing. **Dídac Navarro-Ciurana:** Supervision, Writing – review & editing. **Rosanna Margalef-Marti:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. **Albert Soler:** Funding acquisition, Writing – review & editing. **Neus Otero:** Funding acquisition, Writing – review & editing. **Cristina Domènech:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work is part of the research project ISOREM (REMEDATE) (TED2021-131005B-C31) funded by MCIN/AEI/10.13039/501100011033 and by “European Union NextGenerationEU/PRTR” and the grant PACE-ISOTEC (CGL2017-87216-C4-1-R) funded by MCIN/AEI/10.13039/501100011033 and by “ERDF A way of making Europe”. Support for the research was also received through the project 2021-SGR-00308 (Consolidate Research Group MAGH) from the Catalan Government. The authors are grateful for the analytical support of the Centres Científics i Tecnològics de la Universitat de Barcelona (CCiT-UB). Abu Alex would also like to thank the Agència de Gestió d'Ajuts Universitaris i de Recerca de la Generalitat de Catalunya (2019 FI_B 01059) for the PhD grant and L'Institut de Recerca de l'Aigua (IdRA)-Universitat de Barcelona for their support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemgeo.2024.121942>.

References

- Arauzo, M., 2017. Vulnerability of groundwater resources to nitrate pollution: a simple and effective procedure for delimiting Nitrate Vulnerable zones. *Sci. Total Environ.* 575, 799–812. <https://doi.org/10.1016/j.scitotenv.2016.09.139>.
- Benaiges-Fernandez, R., Offeddu, F.G., Margalef-Marti, R., Palau, J., Urmeneta, J., Carrey, R., Otero, N., Cama, J., 2020. Geochemical and isotopic study of abiotic nitrite reduction coupled to biologically produced Fe(II) oxidation in marine environments. *Chemosphere* 260, 127554. <https://doi.org/10.1016/j.chemosphere.2020.127554>.
- Betlach, M.R., Tiedje, J.M., 1981. Kinetic Explanation for Accumulation of Nitrite, Nitric Oxide, and Nitrous Oxide during Bacterial Denitrification. *Appl. Environ. Microbiol.* 42, 1074–1084. <https://doi.org/10.1128/aem.42.6.1074-1084.1981>.
- Brunner, B., Contreras, S., Lehmann, M.F., Matantseva, O., Rollog, M., Kalvelage, T., Klockgether, G., Lavik, G., Jetten, M.S.M., Kartal, B., Kuypers, M.M.M., 2013. Nitrogen isotope effects induced by anammox bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 110, 18994–18999. <https://doi.org/10.1073/pnas.1310488110>.
- Bryan, B.A., Shearer, G., Skeeters, J.L., Kohl, D.H., 1983. Variable expression of the nitrogen isotope effect associated with denitrification of nitrite. *J. Biol. Chem.* 258, 8613–8617. [https://doi.org/10.1016/s0021-9258\(18\)32100-8](https://doi.org/10.1016/s0021-9258(18)32100-8).
- Bryce, C., Blackwell, N., Schmidt, C., Otte, J., Huang, Y.M., Kleindienst, S., Tomaszewski, E., Schad, M., Warter, V., Peng, C., Byrne, J.M., Kappler, A., 2018. Microbial anaerobic Fe(II) oxidation – Ecology, mechanisms and environmental implications. *Environ. Microbiol.* 20, 3462–3483. <https://doi.org/10.1111/1462-2920.14328>.
- Buchwald, C., Casciotti, K.L., 2010. Oxygen isotopic fractionation and exchange during bacterial nitrite oxidation. *Limnol. Oceanogr.* 55, 1064–1074. <https://doi.org/10.4319/lo.2010.55.3.1064>.
- Buchwald, C., Grabb, K., Hansel, C.M., Wankel, S.D., 2016. Constraining the role of iron in environmental nitrogen transformations: dual stable isotope systematics of abiotic NO_2^- reduction by Fe(II) and its production of N_2O . *Geochim. Cosmochim. Acta* 186, 1–12. <https://doi.org/10.1016/j.gca.2016.04.041>.
- Casciotti, K.L., McIlvin, M., Buchwald, C., 2010. Oxygen isotopic exchange and fractionation during bacterial ammonia oxidation. *Limnol. Oceanogr.* 55, 1805. <https://doi.org/10.4319/lo.2010.55.4.1805>.
- Chen, G., Chen, D., Li, F., Liu, T., Zhao, Z., Cao, F., 2020a. Dual nitrogen-oxygen isotopic analysis and kinetic model for enzymatic nitrate reduction coupled with Fe(II) oxidation by *Pseudogulbenkiania* sp. strain 2002. *Chem. Geol.* 534, 119456. <https://doi.org/10.1016/j.chemgeo.2019.119456>.
- Chen, D., Yuan, X., Zhao, W., Luo, X., Li, F., Liu, T., 2020b. Chemodenitrification by Fe (II) and nitrite: pH effect, mineralization and kinetic modeling. *Chem. Geol.* 541. <https://doi.org/10.1016/j.chemgeo.2020.119586>.
- Chen, G., Zhao, W., Yang, Y., Chen, D., Wang, Y., Li, F., Zhao, Z., Cao, F., Liu, T., 2021. Chemodenitrification by Fe(II) and nitrite: Effects of temperature and dual N[and]O isotope fractionation. *Chem. Geol.* 575, 120258. <https://doi.org/10.1016/j.chemgeo.2021.120258>.
- Collman, J.P., Dey, A., Yang, Y., Decréau, R.A., Ohta, T., Solomon, E.I., 2008. Intermediates involved in the two electron reduction of NO to N₂O by a functional synthetic model of heme containing bacterial NO reductase. *J. Am. Chem. Soc.* 130, 16498–16499. <https://doi.org/10.1021/ja807700n>.
- Cooper, D.C., Picardal, F.W., Schimmelmann, A., Coby, A.J., 2003. Chemical and Biological Interactions during Nitrate and Goethite Reduction by *Shewanella*

- putrefaciens 200. *Appl. Environ. Microbiol.* 69, 3517–3525. <https://doi.org/10.1128/AEM.69.6.3517>.
- Dhakal, P., Matocha, C.J., Huggins, F.E., Vandiviere, M.M., 2013. Nitrite reactivity with magnetite. *Environ. Sci. Technol.* 47, 6206–6213. <https://doi.org/10.1021/es304011w>.
- Dhakal, P., Coyne, M.S., McNear, D.H., Wendroth, O.O., Vandiviere, M.M., D'Angelo, E. M., Matocha, C.J., 2021. Reactions of nitrite with goethite and surface Fe(II)-goethite complexes. *Sci. Total Environ.* 782, 146406 <https://doi.org/10.1016/j.scitotenv.2021.146406>.
- Dunlop, D.J., Özdemir, Ö., 2015. Magnetizations in rocks and minerals. In: *Treatise on Geophysics: Second Edition*, pp. 255–308. <https://doi.org/10.1016/B978-0-444-53802-4.00102-0>.
- Emerson, D., Fleming, E.J., McBeth, J.M., 2010. Iron-oxidizing bacteria: an environmental and genomic perspective. *Annu. Rev. Microbiol.* 64, 561–583. <https://doi.org/10.1146/annurev.micro.112408.134208>.
- EU parliament, 2020. Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption. *Off. J. Eur. Union* 2019, 1–62.
- Fan, A.M., Steinberg, V.E., 1996. Health implications of nitrate and nitrite in drinking water: an update on methemoglobinemia occurrence and reproductive and developmental toxicity. *Regul. Toxicol. Pharmacol.* 23, 35–43. <https://doi.org/10.1006/rtp.1996.0006>.
- Grabb, K.C., Buchwald, C., Hansel, C.M., Wankel, S.D., 2017. A dual nitrite isotopic investigation of chemodenitrification by mineral-associated Fe(II) and its production of nitrous oxide. *Geochim. Cosmochim. Acta* 196, 388–402. <https://doi.org/10.1016/j.gca.2016.10.026>.
- Granger, J., Wankel, S.D., 2016. Isotopic overprinting of nitrification on denitrification as a ubiquitous and unifying feature of environmental nitrogen cycling. *Proc. Natl. Acad. Sci. U. S. A.* 113, E6391–E6400. <https://doi.org/10.1073/pnas.1601383113>.
- Grau-Martínez, A., Torrentó, C., Carrey, R., Soler, A., Otero, N., 2019. Isotopic evidence of nitrate degradation by a zero-valent iron permeable reactive barrier: batch experiments and a field scale study. *J. Hydrol.* 570, 69–79. <https://doi.org/10.1016/j.jhydrol.2018.12.049>.
- Haslun, J.A., Ostrom, N.E., Hegg, E.L., Ostrom, P.H., 2018. Estimation of isotope variation of N₂O during denitrification by *Pseudomonas aureofaciens* and *Pseudomonas chlororaphis*: Implications for N₂O source apportionment. *Biogeosciences* 15, 3873–3882. <https://doi.org/10.5194/bg-15-3873-2018>.
- Heil, J., Wolf, B., Bru, N., Emmenegger, L., Vereecken, H., Mohn, J., 2014. Site-specific N isotopic signatures of abiotically produced N₂O, 139, pp. 72–82. <https://doi.org/10.1016/j.gca.2014.04.037>.
- Jones, A.M., Griffin, P.J., Collins, R.N., Waite, T.D., 2014. Ferrous iron oxidation under acidic conditions - the effect of ferric oxide surfaces. *Geochim. Cosmochim. Acta* 145, 1–12. <https://doi.org/10.1016/j.gca.2014.09.020>.
- Jones, L.C., Peters, B., Lezama Pacheco, J.S., Casciotti, K.L., Fendorf, S., 2015. Stable isotopes and iron oxide mineral products as markers of chemodenitrification. *Environ. Sci. Technol.* 49, 3444–3452. <https://doi.org/10.1021/es504862x>.
- Kampschreur, M.J., Kleerebezem, R., de Vet, W.W.J.M., Van Loosdrecht, M.C.M., 2011. Reduced iron induced nitric oxide and nitrous oxide emission. *Water Res.* 45, 5945–5952. <https://doi.org/10.1016/j.watres.2011.08.056>.
- Kappler, A., Straub, K.L., 2005. Geomicrobiological cycling of iron. *Mol. Geomicrobiol.* 59, 85–108. <https://doi.org/10.2138/rmg.2005.59.5>.
- Kendall, C., Elliott, E.M., Wankel, S.D., 2007. Tracing anthropogenic inputs of nitrogen to ecosystems. In: *Stable Isotopes in Ecology and Environmental Science*, pp. 375–449. <https://doi.org/10.1002/9780470691854.ch12>.
- Kopf, S.H., Henny, C., Newman, D.K., 2013. Ligand-enhanced abiotic iron oxidation and the effects of chemical versus biological iron cycling in anoxic environments. *Environ. Sci. Technol.* 47, 2602–2611. <https://doi.org/10.1021/es3049459>.
- Li, S., Wang, S., Ji, G., 2022. Influences of carbon sources on N₂O production during denitrification in freshwaters: activity, isotopes and functional microbes. *Water Res.* 226, 119315 <https://doi.org/10.1016/j.watres.2022.119315>.
- Liu, T., Chen, D., Luo, X., Li, X., Li, F., 2019. Microbially mediated nitrate-reducing Fe(II) oxidation: Quantification of chemodenitrification and biological reactions. *Geochim. Cosmochim. Acta* 256, 97–115. <https://doi.org/10.1016/j.gca.2018.06.040>.
- Margalef-Martí, R., Carrey, R., Benito, J.A., Martí, V., Soler, A., Otero, N., 2020. Nitrate and nitrite reduction by ferrous iron minerals in polluted groundwater: Isotopic characterization of batch experiments. *Chem. Geol.* 548, 119691 <https://doi.org/10.1016/j.chemgeo.2020.119691>.
- Margalef-Martí, R., Llovet, A., Carrey, R., Ribas, A., Domene, X., Mattana, S., Chin-Pampillo, J., Mondini, C., Alcañiz, J.M., Soler, A., Otero, N., 2021. Impact of fertilization with pig slurry on the isotopic composition of nitrate retained in soil and leached to groundwater in agricultural areas. *Appl. Geochem.* 125 <https://doi.org/10.1016/j.apgeochem.2020.104832>.
- Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., Tardieux, P., 1981. Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. *Plant Soil* 62, 413–430. <https://doi.org/10.1007/BF02374138>.
- Martin, T.S., Casciotti, K.L., 2016. Nitrogen and oxygen isotopic fractionation during microbial nitrite reduction. *Limnol. Oceanogr.* 61, 1134–1143. <https://doi.org/10.1002/lno.10278>.
- McIlvin, M.R., Altabet, M.A., 2005. Chemical conversion of nitrate and nitrite to nitrous oxide for nitrogen and oxygen isotopic analysis in freshwater and seawater. *Anal. Chem.* 77, 5589–5595. <https://doi.org/10.1021/ac050528s>.
- Nardi, A., de Vries, L.M., 2017. *GibbsStudio*. Barcelona Science Technologies SL, Barcelona, Spain.
- Nowak, J., Szubka, M., Klimontko, J., Mariola, K., 2023. Thermal decomposition of siderite and characterization of the decomposition products under O₂ and CO₂ atmospheres. *Minerals* 13, 1066. <https://doi.org/10.3390/min13081066>.
- Ostrom, N.E., Ostrom, P.H., 2012. The isotopomers of nitrous oxide: analytical considerations and application to resolution of microbial production pathways. *Adv. Isotope Geochim.* 453–476. https://doi.org/10.1007/978-3-642-10637-8_23.
- Parkhurst, D.L., Appelo, C.A.J., 2013. Description of Input and Examples for PHREEQC Version 3: A Computer Program for Speciation, Batch-Reaction, One-Dimensional Transport, and Inverse Geochemical Calculations. U.S. Geological Survey. <https://doi.org/10.1097/00000446-195210000-00005>.
- Picardal, F., 2012. Abiotic and microbial interactions during anaerobic transformations of Fe(II) and NO_x. *Front. Microbiol.* 3, 1–7. <https://doi.org/10.3389/fmicb.2012.00112>.
- Rahman, M.M., Roberts, K.L., Grace, M.R., Kessler, A.J., Cook, P.L.M., 2019. Role of organic carbon, nitrate and ferrous iron on the partitioning between denitrification and DNRA in constructed stormwater urban wetlands. *Sci. Total Environ.* 666, 608–617. <https://doi.org/10.1016/j.scitotenv.2019.02.225>.
- Rakshit, S., Matocha, C.J., Coyne, M.S., 2008. Nitrite reduction by siderite. *Soil Sci. Soc. Am. J.* 72, 1070–1077. <https://doi.org/10.2136/sssaj2007.0296>.
- Rakshit, S., Matocha, C.J., Coyne, M.S., Sarkar, D., 2016. Nitrite reduction by Fe(II) associated with kaolinite. *Int. J. Environ. Sci. Technol.* 13, 1329–1334. <https://doi.org/10.1007/s13762-016-0971-x>.
- Robinson, T., Latta, D.E., Notini, L., Schilling, K.E., Scherer, M.M., 2021. Abiotic reduction of nitrite by Fe(II): a comparison of rates and N₂O production Thomas. *Environ. Sci.* <https://doi.org/10.1039/d1em00222h>.
- Rohe, L., Anderson, T.H., Braker, G., Flessa, H., Giesemann, A., Lewicka-Szczebak, D., Wrage-Mönnig, N., Well, R., 2014. Dual isotope and isotopomer signatures of nitrous oxide from fungal denitrification - a pure culture study. *Rapid Commun. Mass Spectrom.* 28, 1893–1903. <https://doi.org/10.1002/rcm.6975>.
- Rohe, L., Well, R., Lewicka-Szczebak, D., 2017. Use of oxygen isotopes to differentiate between nitrous oxide produced by fungi or bacteria during denitrification. *Rapid Commun. Mass Spectrom.* 31, 1297–1312. <https://doi.org/10.1002/rcm.7909>.
- Ryabenko, E., Altabet, M.A., Wallace, D.W.R., 2009. Effect of chloride on the chemical conversion of nitrate to nitrous oxide for $\delta^{15}\text{N}$ analysis. *Limnol. Oceanogr. Methods* 7, 545–552. <https://doi.org/10.4319/lom.2009.7.545>.
- Sebilo, M., Billen, G., Mayer, B., Billioud, D., Grably, M., Garnier, J., Mariotti, A., 2006. Assessing nitrification and denitrification in the seine river and estuary using chemical and isotopic techniques. *Ecosystems* 9, 564–577. <https://doi.org/10.1007/s10021-006-0151-9>.
- Sebilo, M., Aloisi, G., Mayer, B., Perrin, E., Vauri, V., Mothet, A., Laverman, A.M., 2019. Controls on the isotopic composition of nitrite ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) during denitrification in freshwater sediments. *Sci. Rep.* 9, 1–14. <https://doi.org/10.1038/s41598-019-54014-3>.
- Sørensen, J., Thorling, L., 1991. Stimulation by lepidocrocite (7-FeOOH) of Fe(II)-dependent nitrite reduction. *Geochim. Cosmochim. Acta* 55, 1289–1294. [https://doi.org/10.1016/0016-7037\(91\)90307-Q](https://doi.org/10.1016/0016-7037(91)90307-Q).
- Sutka, R.L., Ostrom, N.E., Ostrom, P.H., Breznak, J.A., Gandhi, H., Pitt, A.J., Li, F., 2006. Distinguishing nitrous oxide production from nitrification and denitrification on the basis of isotopomer abundances. *Appl. Environ. Microbiol.* 72, 638–644. <https://doi.org/10.1128/AEM.72.1.638-644.2006>.
- Tischer, J., Zopfi, J., Frey, C., Magyar, P.M., Brand, A., Oswald, K., Jegge, C., Frame, C. H., Miracle, M.R., Soria-Perpinya, B., Vicente, E., Lehmann, M.F., 2022. Isotopic signatures of biotic and abiotic N₂O production and consumption in the water column of meromictic, ferruginous Lake La Cruz (Spain). *Limnol. Oceanogr.* 67, 1760–1775. <https://doi.org/10.1002/lno.12165>.
- Toyoda, S., Yoshida, N., Koba, K., 2017. Isotopocule analysis of biologically produced nitrous oxide in various environments. *Mass Spectrom. Rev.* 36 (2), 32, 135–160. <https://doi.org/10.1002/mas>.
- Van Breukelen, B.M., Prommer, H., 2008. Beyond the Rayleigh equation: reactive transport modeling of isotope fractionation effects to improve quantification of biodegradation. *Environ. Sci. Technol.* 42, 2457–2463. <https://doi.org/10.1021/es071981j>.
- Visser, A.N., Wankel, S.D., Niklaus, P.A., Byrne, J.M., Kappler, A.A., Lehmann, M.F., 2020. Impact of reactive surfaces on the abiotic reaction between nitrite and ferrous iron and associated nitrogen and oxygen isotope dynamics. *Biogeosciences* 17, 4355–4374. <https://doi.org/10.5194/bg-17-4355-2020>.
- Wankel, S.D., Ziebis, W., Buchwald, C., Charoenpong, C., Beer, D., Dentinger, J., Xu, Z., Zengler, K., 2017. Evidence for fungal and chemodenitrification based N₂O flux from nitrogen impacted coastal sediments. *Nat. Commun.* 8, 1–11. <https://doi.org/10.1038/ncomms15595>.
- WHO, 2017. *Guidelines for Drinking-Water Quality: Fourth Edition Incorporating the First Addendum*. Guidel. Qual. Fourth Ed. Inc. First Add, p. 541.
- Winther, M., Balslev-Harder, D., Christensen, S., Priemé, A., Elberling, B., Crosson, E., Blunier, T., 2018. Continuous measurements of nitrous oxide isotopomers during incubation experiments. *Biogeosciences* 15, 767–780. <https://doi.org/10.5194/bg-15-767-2018>.
- Wrage, N., Velthof, G.L., Van Beusichem, M.L., Oenema, O., 2001. Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biol. Biochem.* 33, 1723–1732. [https://doi.org/10.1016/S0038-0717\(01\)00096-7](https://doi.org/10.1016/S0038-0717(01)00096-7).
- Yamazaki, T., Hozuki, T., Arai, K., Toyoda, S., Koba, K., Fujiwara, T., Yoshida, N., 2014. Isotopomeric characterization of nitrous oxide produced by reaction of enzymes extracted from nitrifying and denitrifying bacteria. *Biogeosciences* 11, 2679–2689. <https://doi.org/10.5194/bg-11-2679-2014>.
- Zhu, C., 2012. Geochemical modeling in environmental and geological studies. In: *Encyclopedia of Sustainability Science and Technology*, pp. 4094–4104. <https://doi.org/10.1007/978-1-4419-0851-3>.

Chemical Geology

Supporting Information for PAPER 2

Isotopic and geochemical modeling approach to evaluate abiotic nitrite reduction by ferrous iron

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Submitted to Chemical Geology

Table S11. The internal working standard (IWS-NO₂) used for the calculation of nitrite and nitrous oxide concentration measurement

IWS NO ₂ (nmol)	Peak Amplitude (mV)	Peak Area (V ² S)	$\delta^{18}\text{O-NO}_2^-$ (‰)	$\delta^{15}\text{N-NO}_2^-$ (‰)
75	3631	9.228	30.392	-18.526
75	3689	9.269	30.424	-18.639
150	7202	19.118	30.567	-18.627
150	6937	18.326	30.527	-18.625
225	10693	30.914	30.757	-18.423
225	10545	30.133	30.818	-18.397

Table S2. The distribution of the liquid phase N₂O and gas phase N₂O during N transformation in abiotic nitrite reduction

	Days	Residual NO ₂ ⁻ mM	Transformed N-NO ₂ ⁻ mM	Gas phase N-N ₂ O mM	Liquid phase N-N ₂ O mM
Sid+DFe	0.1	1.0612	0.0000	0.0071	0.0179
	0.3	1.0937	0.0325	0.0076	0.0192
	0.6	0.9774	0.1163	0.0449	0.1125
	1.0	0.8600	0.2338	0.0694	0.1739
	1.2	0.8953	0.1985	0.0571	0.1432
	1.3	0.8285	0.2653	0.0832	0.2086
	2.0	0.6962	0.3975	0.1113	0.2792
	3.0	0.5249	0.5688	0.1436	0.3602
	4.0	0.4780	0.6157	0.1633	0.4095
	6.0	0.1652	0.9286	0.1837	0.4606
	6.7	0.1894	0.9043	0.1870	0.4690
DFe	0.1	1.1131	0.0000	0.0006	0.0015
	0.3	1.0935	0.0196	0.0059	0.0147
	0.6	1.0685	0.0446	0.0174	0.0435
	1.0	1.0089	0.1042	0.0338	0.0848
	1.2	1.0162	0.0969	0.0287	0.0719
	1.3	1.0211	0.0920	0.0458	0.1147
	2.0	0.8203	0.2928	0.0825	0.2069
	3.0	0.5895	0.5236	0.1538	0.3857
	4.0	0.6237	0.4894	0.1646	0.4127
	6.0	0.5459	0.5672	0.1945	0.4877
	6.7	0.5125	0.6006	0.1617	0.4056
Sid	1.0	1.2181	0.0000	0.0000	0.0000
	2.0	1.1766	0.0414	0.0000	0.0000
	3.0	1.1766	0.0500	0.0057	0.0074
	7.0	1.1681	0.0414	0.0008	0.0010

Table S3. Concentration of NO_2^- and $\text{N}_2\text{O}(\text{g})$ (mM), $\delta^{15}\text{N}$ of NO_2^- and $\text{N}_2\text{O}(\text{g})$ and SP (‰) analyzed in Sid, DFe and Sid+DFe. NA means not analyzed, and NC means not calculated

	Days	Residual NO_2^- mM	Recovered N_2O mM	$\delta^{15}\text{N}_{\text{NO}_2}$ ‰	$\delta^{18}\text{N}_{\text{NO}_2}$ ‰	$\delta^{15}\text{N}^{\text{bulk}}_{\text{N}_2\text{O}}$ ‰	SP ‰
Sid+DFe	0.1	1.0612	0.0071	-26.2994	-17.9	-52.5940	NC
	0.3	1.0937	0.0076	-25.1544	-17.7	NA	NC
	0.6	0.9774	0.0449	-23.8294	-16.6	-46.5060	24.1380
	1.0	0.8600	0.0694	-22.7324	-17.8	-43.9640	NC
	1.2	0.8953	0.0571	-22.6404	-19.3	-44.8650	NC
	1.3	0.8285	0.0832	-23.4644	-18.7	-44.0740	NC
	2.0	0.6962	0.1113	-20.9474	-20.4	-42.0240	NC
	3.0	0.5249	0.1436	-15.1744	-22.9	-40.7510	23.3820
	4.0	0.4780	0.1633	-15.8854	-24.5	NA	NC
	6.0	0.1652	0.1837	-33.4464	-24.7	-36.5570	NC
	6.7	0.1894	0.1870	-29.4954	-26.8	-36.5460	23.0820
DFe	0.1	1.1131	0.0006	-26.3984	-17.5	-57.1110	NC
	0.3	1.0935	0.0059	-25.4614	-17.4	-52.3840	NC
	0.6	1.0685	0.0174	-25.7134	-19.6	-45.2720	21.6880
	1.0	1.0089	0.0338	-24.9944	-20.8	-44.0955	NC
	1.2	1.0162	0.0287	-24.1044	-20.7	-44.5430	NC
	1.3	1.0211	0.0458	-23.1254	-20.2	-44.9320	NC
	2.0	0.8203	0.0825	-21.8054	-18.4	-44.1310	NC
	3.0	0.5895	0.1538	-15.4304	-21.7	-39.8350	22.9580
	4.0	0.6237	0.1646	-18.1064	-23.4	NA	NC
	6.0	0.5459	0.1945	-18.3634	-24.3	-38.6350	NC
	6.7	0.5125	0.1617	-18.0064	-24.1	-38.6380	22.8120
Sid	1.0	1.2181	0.0000	-26.7954	-18.3	NA	NC
	2.0	1.1766	0.0000	-26.7434	-17.6	NA	NC
	3.0	1.1766	0.0057	-26.0254	-18.8	-39.8460	NC
	7.0	1.1681	0.0008	-26.3874	-16.5	-40.9140	NC

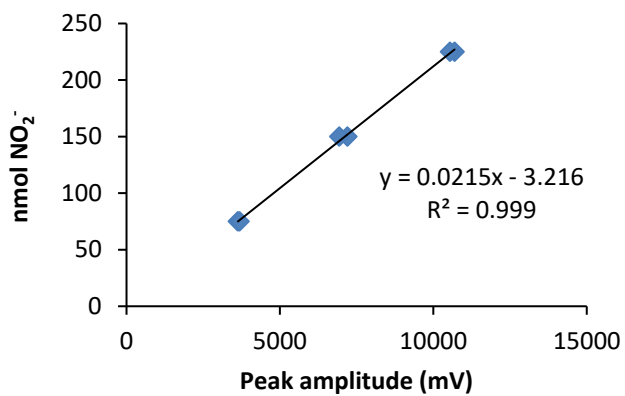


Fig. S1. A plot of the amount of nitrite and the peak amplitude of the IRMS for the determination of nitrite and nitrous oxide concentration

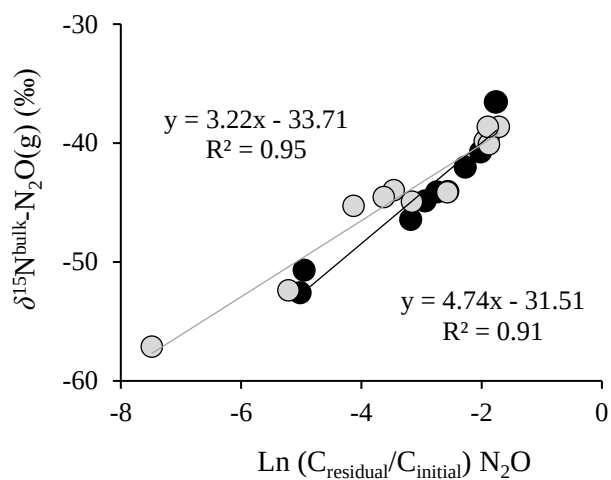


Fig. S2. A plot of the natural logarithm of generated gas phase nitrous oxide fraction against $\delta^{15}\text{N}^{\text{bulk}}_{\text{N}_2\text{O}(\text{g})}$.

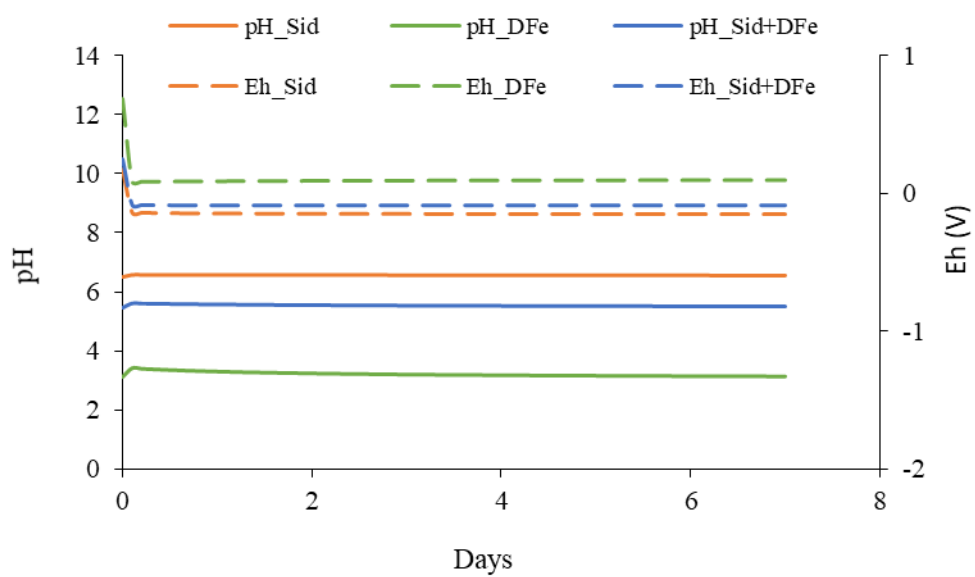


Fig. S3. Evolution of pH and Eh in Sid, DFe and Sid+DFe.

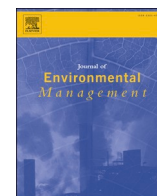
ANNEX 3: PAPER 3

PILOT SOIL AQUIFER TREATMENT USING PRBS

Pathways and efficiency of nitrogen attenuation in wastewater effluent through Soil Aquifer Treatment.

Published on 19th August 2022

Abu, A., Carrey, R., Valhondo, C., Domènech, C., Otero, N., Soler, A., Martínez-Landa, L., Carrera, J., Diaz-Cruz, S., 2022. Pathways and Efficiency of Nitrogen Attenuation in Wastewater Effluent Through Soil Aquifer Treatment. J. Environ. Manage., 321, 115927. <https://doi.org/10.2139/ssrn.4127243>



Research article

Pathways and efficiency of nitrogen attenuation in wastewater effluent through soil aquifer treatment

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ARTICLE INFO

Keywords:

Isotopic fractionation
Enhanced biological denitrification
Permeable reactive barrier
Soil aquifer treatment
Nitrification
Denitrification

ABSTRACT

Soil Aquifer Treatment (SAT) is used to increase groundwater resources and enhance the water quality of wastewater treatment plant (WWTP) effluents. The resulting water quality needs to be assessed. In this study, we investigate attenuation pathways of nitrogen (N) compounds (predominantly NH_4^+) from a secondary treatment effluent in pilot SAT systems: both a conventional one (SAT-Control system) and one operating with a permeable reactive barrier (PRB) to provide extra dissolved organic carbon to the recharged water. The goal is to evaluate the effectiveness of the two systems regarding N compounds by means of chemical and isotopic tools. Water chemistry (NO_3^- , NH_4^+ , Non-Purgeable Dissolved Organic Carbon (NPDOC), and O_2) and isotopic composition of NO_3^- ($\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$) and NH_4^+ ($\delta^{15}\text{N}-\text{NH}_4^+$) were monitored in the inflow and at three different sections and depths along the aquifer flow path. Chemical and isotopic results suggest that coupled nitrification-denitrification were the principal mechanisms responsible for the migration and distribution of inorganic N in the systems and that nitrification rate decreased with depth. At the end of the study period, 66% of the total N in the solution was removed in the SAT-PRB system and 69% in the SAT-Control system, measured at the outlet of the systems. The residual N in solution in the SAT-PRB system had an approximately equal proportion of $\text{N}-\text{NH}_4^+$ and $\text{N}-\text{NO}_3^-$ while in the SAT-Control system, the residual N in solution was primarily $\text{N}-\text{NO}_3^-$. Isotopic data also confirmed complete NO_3^- degradation in the systems from July to September with the possibility of mixing newly generated NO_3^- with the residual NO_3^- in the substrate pool.

1. Introduction

Nitrogen (N) pollution has become an important threat to water resources (Ren et al., 2014; Shi et al., 2019). Anthropogenic activities involving intensive fertilizer application and animal waste disposal represent the main global sources of N contamination (Arauzo, 2017; Zirkle et al., 2016). Furthermore, domestic, and industrial wastewater and septic-system effluents also serve as a significant source of N and

contaminants of emerging concern (CECs). In manure and wastewater, N is mainly found as ammonium (NH_4^+) which, under aerobic conditions, is oxidized to nitrate (NO_3^-) (nitrification) as



Nitrate contamination impairs its quality (Bourke et al., 2019). This fact has gained global attention in recent years as NO_3^- concentrations have been broadly reported above the 50 mgNO_3^-/L drinking water

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<https://doi.org/10.1016/j.jenvman.2022.115927>

Received 12 May 2022; Received in revised form 19 July 2022; Accepted 31 July 2022

Available online 19 August 2022

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threshold value (European Union, 2006; 1991). The ingestion of NO_3^- contaminated water is linked to health hazards such as methemoglobinemia in infants and young children (WHO, 2004) and stomach cancer (Volkmer et al., 2005). Furthermore, nitrate is usually the limiting factor for algal growth, so increasing its concentration leads to eutrophication of groundwater dependent water bodies (Withers et al., 2014).

The main natural process to remove NO_3^- in aquifers is the reduction of NO_3^- to dinitrogen gas ($\text{N}_{2(g)}$) through a sequence of microbial reduction reactions (denitrification) (Korom, 1992; Matchett et al., 2019) (Fig. 1).



Natural attenuation of NO_3^- can occur in anaerobic conditions in groundwater with oxygen levels below 1–2 mg/L (Singleton et al., 2007). Denitrifying bacteria responsible for these transformations are ubiquitous in surface and subsurface waters (Beauchamp et al., 1989) and they are predominantly facultative anaerobic denitrifying heterotrophs generating energy for growth and cell synthesis via the oxidation of organic compounds



Autotrophic denitrifiers in groundwater use reduced inorganic compounds such as pyrite, sulfide, sulfite, S^0 , HS^- , Fe(II) , Mn(II) and H_2 as electron donors. Nonetheless, numerous studies have shown that the availability of electron donors is the main limiting factor for denitrification under natural aquifer conditions (King et al., 2012; Pabich et al., 2001). Other nitrogen attenuation pathways include (1) ammonium (NH_4^+) oxidation to nitrogen gas ($\text{N}_{2(g)}$) using nitrite (NO_2^-) as the electron acceptor in anaerobic conditions (anaerobic ammonium oxidation, or Anammox) (2) anaerobic respiration by microorganisms using nitrate as electron acceptor (dissimilatory nitrate reduction to ammonium (DNRA)) and (3) plant uptake (assimilation). The main reactions involving the N transformation pathways are summarized in Fig. 1.

Microbial-mediated reactions tend to alter the distribution of the isotopes between the substrate and product pools. For this reason, stable isotopes of N in compounds such as NO_3^- and NH_4^+ ($\delta^{15}\text{N}-\text{NO}_3^-$, $\delta^{18}\text{O}-\text{NO}_3^-$ and $\delta^{15}\text{N}-\text{NH}_4^+$) have been successfully used to study and characterize natural and induced denitrification processes (Adebawale et al., 2019; Carrey et al., 2013; Margalef-Martí et al., 2019) as well as nitrification processes (Castellano-Hinojosa et al., 2020).

Biologically enhanced denitrification (BED), which involves the biological stimulation of heterotrophic denitrification, has become a strategy to create optimized conditions by providing an external electron donor source in the aquifer (Critchley et al., 2014; Gibert et al., 2019; Margalef-Martí et al., 2019). This technology has evolved over the past decade in an attempt to clean up NO_3^- in groundwater to regulatory limits at a low cost. BED has been evaluated in several contexts and

approaches like ex-situ groundwater remediation techniques (Baú and Mayer, 2008) and in situ bioremediation techniques such as in (I) artificial recharge ponds (Grau-Martínez et al., 2018), (II) permeable reactive barriers (PRB), reactive media installed perpendicular to flow path (Gibert et al., 2019; Valhondo et al., 2014) and (III) carbon injection systems (Critchley et al., 2014; Margalef-Martí et al., 2019).

Soil Aquifer Treatment (SAT) is a technique often employed to augment groundwater resources and improve water quality in water-stressed zones by promoting the infiltration of effluents from wastewater treatment plants (WWTPs) (Drewes, 2009; Maeng et al., 2010). Conventional wastewater treatment removes a large fraction of the biochemical oxygen demand and suspended solids from wastewater (Jokela et al., 2017; Naidoo and Olaniran, 2013) but is deficient in other water quality indicators such as N and organic compounds, such as CECs (Sonune and Ghate, 2004; Valhondo et al., 2014). N species and CECs are frequent in WWTP effluents (Díaz-Cruz and Barceló, 2008; Kuster et al., 2010; Maeng et al., 2010). The implementation of a PRB based on organic substrates at the bottom of infiltration basins has proven to increase the removal of NO_3^- and CECs from the recharged water SAT-PRB system using plant-based compost as reactive barriers has proven to successfully promote BED in artificial recharge ponds (Grau-Martínez et al., 2018). The barrier operates by releasing dissolved organic carbon, which acts as the electron donor to stimulate the consumption of diverse electron acceptors, including NO_3^- and favouring the CECs' biotransformation, improving the recharged water quality, and reducing NO_3^- leakage to the aquifer (Valhondo et al., 2015). The nitrogen cycle and the attenuation of organic contaminants across PRBs must be evaluated during the application SAT-PRB to assess its effectiveness. The rate of denitrification in SAT systems applying PRBs has shown to vary over time and is a function of the NO_3^- concentration reaching the reactive media, the residence time of NO_3^- within the reactive media and temperature (Robertson et al., 2008). Furthermore, wastewater often contains N as NH_4^+ rather than NO_3^- , affecting the efficiency of the SAT-PRB.

This work evaluates two pilot SAT systems that receive secondary treatment effluent from a WWTP located in the North of Catalonia, Spain. One system has a PRB consisting of plant compost blended with sand and a small fraction of clay (SAT-PRB system, T4) and the other has no PRB (SAT-Control system, T2) Fig. 2. The biochemical processes controlling the migration and speciation of the N compounds in the wastewater effluent are investigated to:

- (1) understand the nitrogen cycle during SAT operations, so as to
- (2) understand the prevailing processes affecting N in SAT, and to
- (3) evaluate the potential of SAT and SAT-PRB to remove N

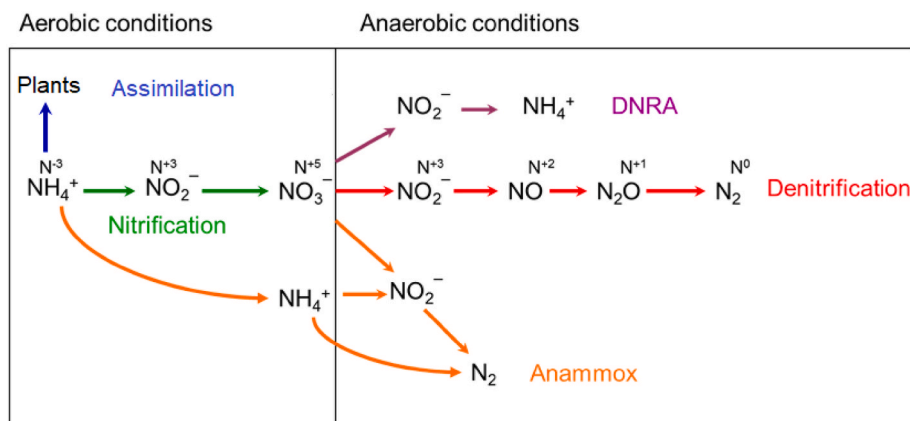


Fig. 1. Biogeochemical processes controlling the fate of N compounds in groundwater. Each coloured arrow indicates a specific N transformation pathway.

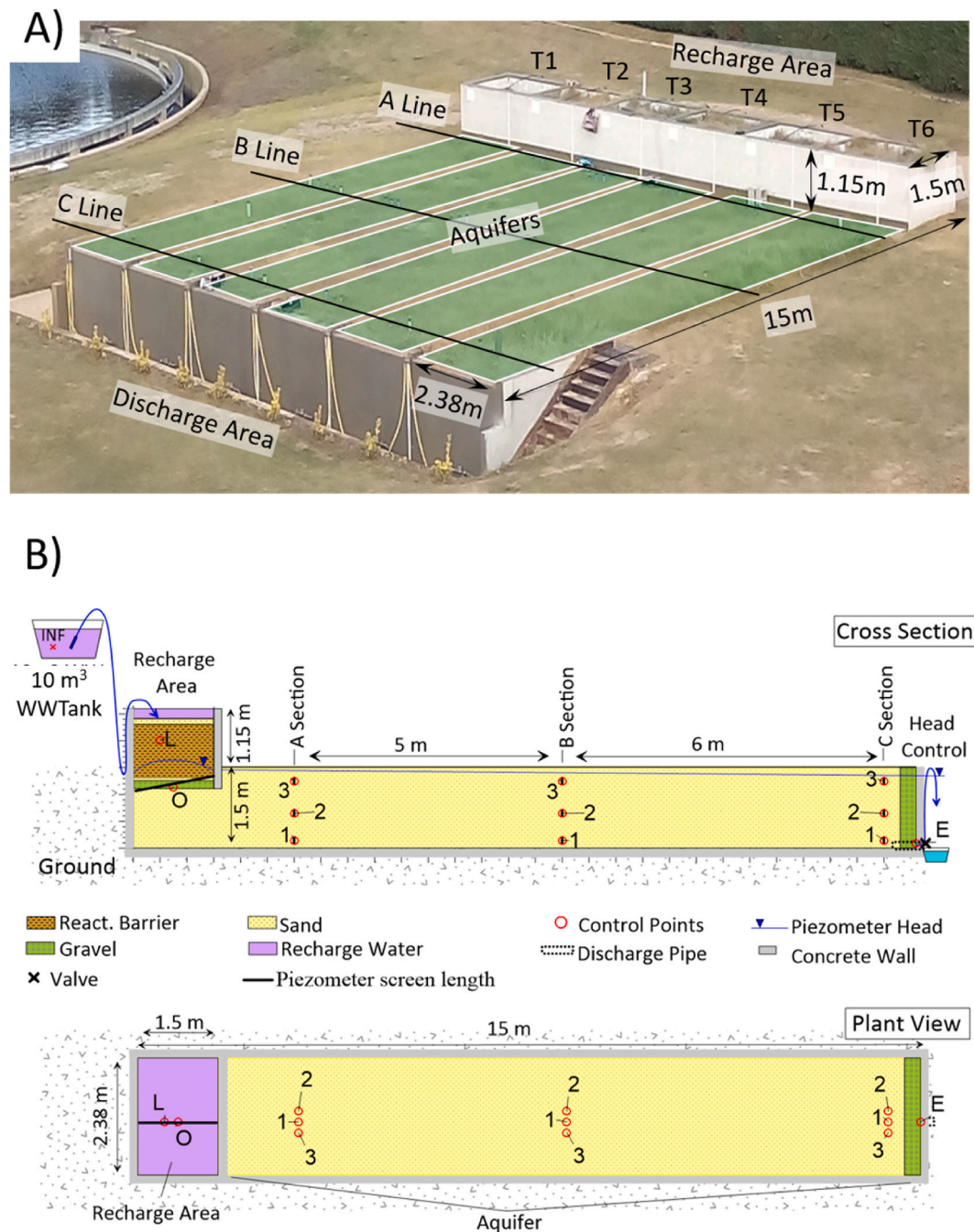


Fig. 2. A) General view of the experimental site, including recharge and discharge areas, piezometer sections and replicates T1 to T6; B) Cross-section and plan view of each system and monitoring points: O, A, B and C are the sampling sections along the flow path, INF = inflow water and E = outflow water; 1, 2 and 3 are the three sampling piezometers at each section (Deep, Middle and Shallow, respectively). A detailed description can be found in (Valhondo et al., 2020a,b).

2. Materials and methods

2.1. Site description

Six mesoscale SAT systems, named from T1 to T6, were built in the facility of a WWTP (Fig. 2A and B). Each system consists of a $15 \times 2.38 \times 1.5$ m (length $\times$ width $\times$ height) sand canal, emulating the aquifer, coupled to a $1.5 \times 2.38 \times 1.15$ m box emulating the infiltration basin. The infiltration box was filled with fine sand ($\phi = 0.1\text{--}0.2$ mm) in the SAT-Control system (T2) and with a 1 m thick PRB in the remaining tanks. Plants were left to grow naturally at the surface of the infiltration areas and sand canals. Different plants, primarily grama grass, grew immediately after construction. The bottom 40 cm of each system was

filled with coarse sand to favour the vertical flow to the discharge pipe (Fig. 2B). The systems were fed, during recharge episodes, with the effluent of the secondary treatment of the WWTP using PRIUS dosing pumps (EMEC, Rieti, Italy). The elevation of the discharge pipe, which collected the water from the bottom of the system, controlled the head in the outlet.

Ten piezometers were installed in each system for sampling and monitoring three distances from the infiltration area and at three depths. Piezometer "O" is a completely screened crosswise piezometer located just below the infiltration basin (Fig. 2B). Three 10-cm screened piezometers (1 at 1.3–1.4 m, 2 at 0.8–0.9 m, and 3 at 0.3–0.4 m depth) were placed in three sections (A, B, and C, at 1.5, 6.5, and 12.5 m from the recharge area, respectively) (Fig. 2B). Additionally, the inlet and outlet

of each system were sampled and monitored frequently. A detailed description of the systems can be found in (Valhondo et al., 2020b).

2.2. System performance and sampling collection

Four recharge episodes were performed during 2018. The first one lasted 27 days (from January, 5th through February, 1st) and aimed at conditioning the systems. The second, third and fourth recharge episodes (RE-2, RE-3 and RE-4) lasting 126 days (February to June), 65 days (July to September), and 74 days (October to January 2019), respectively, aimed at studying the performance of the systems.

An average flow rate of 0.4 m/d fed each system in all four recharge episodes. During these episodes, water samples were collected from the inlet, the monitoring points and the outlets of the systems for chemical and isotopic analyses. Field parameters (O_2 , pH, Eh, Electrical Conductivity, and temperature) were measured during sampling using a multiparameter probe (YSI, Yellow Spring, OH, USA).

2.3. Analytical methods

Samples for Non-Purgeable Dissolved Organic Carbon (NPDOC) and chemical analyses were collected in muffled glass bottles, filtered through 0.22 μ m membrane filters, acidified with HCl (for NPDOC analysis) or HNO_3 (for cations determination) and stored at 4 °C. NPDOC was measured with a TOC-VCSH analyser Shimadzu (Kyoto, Japan). Cations were analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES) using an iCAP 6500 instrument (Thermo Fisher Scientific, Massachusetts, USA). NH_4^+ was analysed using an ORION Ion-Selective Electrode (ISE, Thermo Fisher Scientific, Massachusetts, USA). The main anions were measured by ion chromatography (IC) using a Dionex AQUION (Dionex, Sunnyvale, CA, USA) with an Ionpack AS9 2 $\times$ 250 mm column and Na_2CO_3 9 mM solution as eluent. Samples for NH_4^+ , $\delta^{15}N-NH_4^+$, $\delta^{15}N-NO_3^-$, and $\delta^{18}O-NO_3^-$ analysis were kept frozen until analysed.

2.4. Isotopic methods

The nitrogen and oxygen isotopic abundance in nitrate ($\delta^{15}N-NO_3^-$ and $\delta^{18}O-NO_3^-$) were analysed using the cadmium (Cd) and sodium azide (NaN_3) reduction methods (McIlvin and Altabet, 2005; Ryabenko et al., 2009). The nitrogen isotopic abundance of NH_4^+ ($\delta^{15}N-NH_4^+$) was measured using the sodium hypobromite ($NaBrO$) and sodium azide reduction methods (Ryabenko et al., 2009; Zhang et al., 2007).

The standards used were the Vienna Standard Mean Oceanic Water (V-SMOW) for $\delta^{18}O-NO_3^-$ and $\delta^{18}O-NO_2^-$ analyses and AIR (Atmospheric N_2) for $\delta^{15}N-NO_3^-$ analyses. To correct the $\delta^{15}N-NO_3^-$ and $\delta^{18}O-NO_3^-$ values, three international standards (USGS 32, 34 and 35) and one internal laboratory standard (CCIT-IWS-NO3) were used. To correct $\delta^{15}N-NH_4^+$, two international standards (USGS-25 and IAEA-N2) and two internal laboratory standards (CCIT-IWS-NO2 and CCIT-IWS-NH4)

Table 1

Standards and reproducibility for isotopic analysis. International and laboratory (CCIT) standards used for the normalization of the results.

Analysis	Standard	Reference value [‰]	Reproducibility (1 σ)
$\delta^{15}N-NO_3^-$	USGS-32,	+180.0	$\pm 1.0\%$
	USGS-34,	-1.8	
	USGS-35	+2.7	
	CCIT-IWS	+16.9	
$\delta^{18}O-NO_3^-$	USGS-32,	+25.3	$\pm 1.5\%$
	USGS-34,	-27.9	
	USGS-35	+57.3	
	CCIT-IWS	+28.5	
$\delta^{15}N-NH_4^+$	USGS-25	-30.4	$\pm 1.0\%$
	IAEA-N2	+20.3	
	CCIT-IWS-NO2	-28.5	
	CCIT-IWS-NH4	-0.8	

were employed (Table 1). The samples for chemical and isotopic analyses were prepared at the laboratory of the MAiMA-UB research group and analysed at the Centres Científics i Tecnològics of the Universitat de Barcelona (CCiT-UB).

3. Results

Results of NH_4^+ , NO_3^- , NPDOC and O_2 concentration and isotopic data are available as Supplementary information (Table S1).

3.1. Chemical and isotopic results of inflow water

The average NH_4^+ concentration of the inflow water during RE-2, RE-3 and RE-4 varied significantly ranging from 0.93 to 5.54 mM. The measured NH_4^+ in the inflow water was higher during RE-3 (average of 4.2 mM, SD = 0.7, n = 20) while RE-2 and RE-4 measured similar NH_4^+ concentrations (Fig. 3). NO_3^- in the wastewater was below the detection limit (<0.01 mM) during all recharge episodes. The average O_2 measured was 0.27 mM (SD = 0.14, n = 18), 0.12 mM (SD = 0.09, n = 7) and 0.20 mM (SD = 0.03, n = 2) during RE-2, RE-3 and RE-4 respectively. RE-2 measured maximum and minimum NPDOC concentrations of 2.11 mM and 1.08 mM, respectively with an average of 1.41 mM and RE-3 measured maximum and minimum concentrations of 1.48 mM and 1.10 mM, respectively with an average concentration of 1.31 mM and RE-4 measured an average NPDOC concentration of 0.94 mM.

The $\delta^{15}N-NH_4^+$ of the inflow water showed considerable constant values with an average of +9.6‰ (n = 8 and SD = 0.80).

3.2. Chemical and isotopic results along the flow path of the systems

The average concentration of NH_4^+ , NO_3^- , NPDOC and O_2 measured at the sampling points are shown in Fig. 3. RE-2 and RE-4 had similar NH_4^+ concentrations whereas slightly higher values were observed in RE-3 (Fig. 3) in both systems. For the 3 recharge episodes studied in both systems, NH_4^+ concentration progressively decreased from O to C and slightly increased from C to the outlet (E) for all recharge episodes. RE-2 showed a slight increase of NH_4^+ from O to A (Fig. 3). The NO_3^- concentrations displayed similar behaviour in both systems; measured NO_3^- concentrations were very low at O, A and B and significantly increased from C to the outlet (E) during all the recharge episodes (Fig. 3). NPDOC concentration showed, overall, a decrease from the inflow water, which showed no significant differences between recharge episodes, to the outlet (Fig. 3). The NPDOC concentration in the SAT-PRB increases at O compared to the inflow due to the DOC release from the RB, especially during RE-2 (2.065 ± 0.12 mM) and RE-3 (1.965 ± 0.46 mM) (Fig. 3). NPDOC concentration in the SAT-PRB system decreased progressively from O to E due to the oxidation of DOC. The concentration of O_2 decreased from the inlet (INF) to O in both systems during the three recharge episodes (Fig. 3). The O_2 concentration remained practically the same from the O to C and showed a slight increase from C to E except in RE-4 in both systems (Fig. 3).

3.3. Chemical and isotopic results with depth in the systems

Detailed profiles of NH_4^+ , NO_3^- , NPDOC and O_2 with depth at 1, 2 and 3 points of A, B and C sections are presented in the supporting information (Table S2) and summarized in Fig. 4, where boxplots represent samples belonging to all the different depths at each section for each recharge episode. The concentration of NH_4^+ displayed an increasing profile with depth in monitoring points of section C of both systems and section A of the SAT-Control system, with an increase and a slight decrease in section B of both systems. NH_4^+ in the SAT-PRB system at section A which was high at the top showed decreasing and an increasing profile with depth (Fig. 4A). NO_3^- concentration showed no important change with depth at all sections in both systems (Fig. 4B). The NPDOC decreased from the top to the bottom at all sections of the

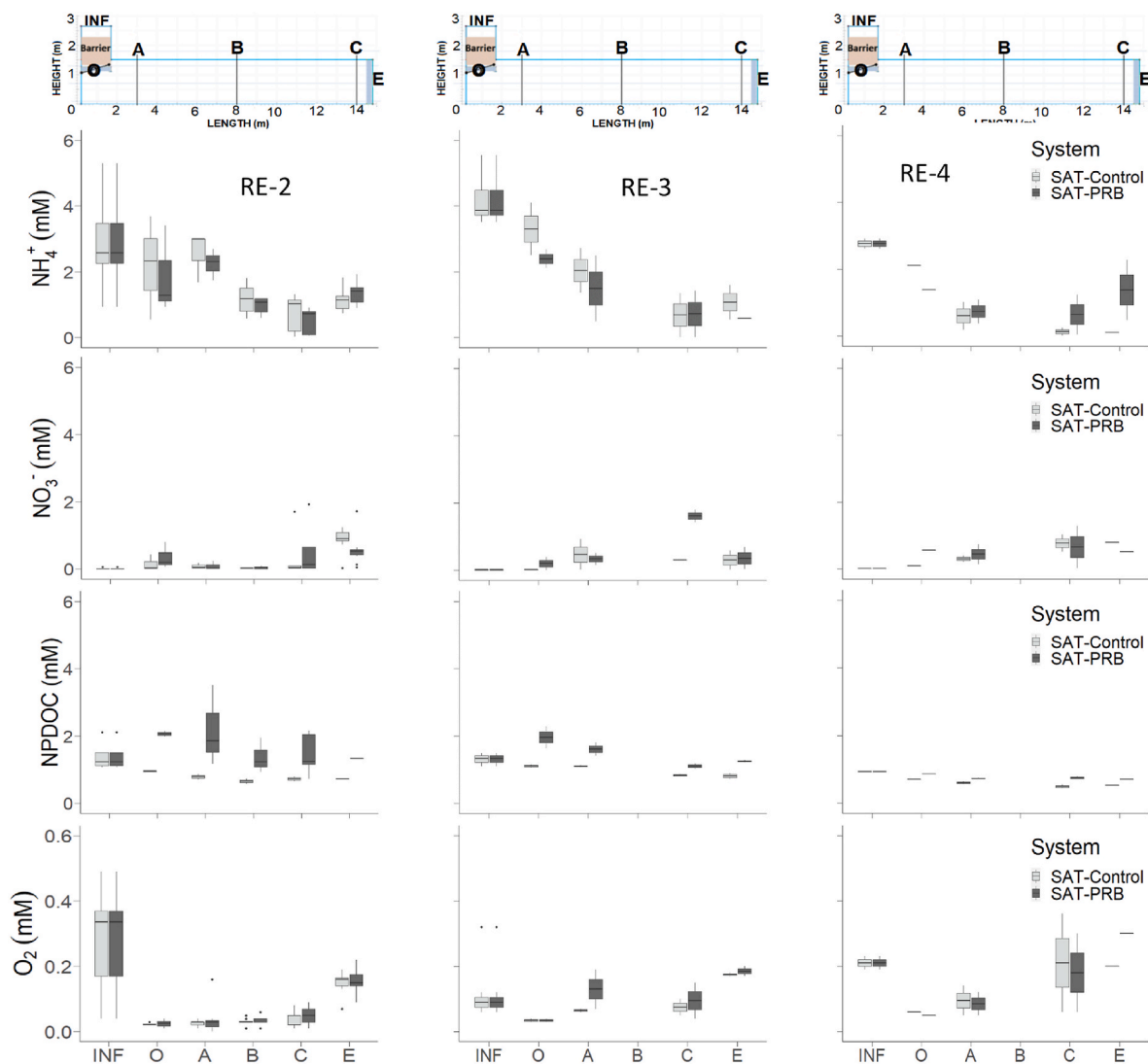


Fig. 3. Boxplots of NH_4^+ , NPDOC, O_2 and NO_3^- aqueous concentration for all recharge episodes along the flow path. A scheme showing sections along the systems is also provided. Concentrations are indicated by the box plots and the outliers are represented by dots. Values in A, B, and C were calculated with the samples of the different depths at each section. INF = inflow water concentration and E is the outflow water concentration. First boxplot at each section (light grey) = SAT-Control system and the second boxplot (dark grey) = SAT-PRB system.

SAT-PRB system while exhibiting no significant decrease at all the sections of the SAT-Control system. The NPDOC in SAT-PRB systems was significantly higher due to the release of DOC from the RB (Fig. 4C). O_2 also slightly decreased from the top to the bottom of the systems at all the sampling points (Fig. 4D). The isotopic composition of NH_4^+ ($\delta^{15}\text{N}-\text{NH}_4^+$) in the SAT-Control system ranged from +8.4‰ to +29.4‰ where the lowest values were found in sample points O (just below the PRB) and the highest values were observed in sample points B and slight decrease afterwards at C and the outlet (Table S1). $\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$ ranged from +4.6‰ to +27‰ and from +9.2‰ to +41.9‰, respectively, where the highest values were observed in sample point O and decreased along the flow path with the lowest values observed in the outlet (Table S1). The isotopic composition of $\delta^{15}\text{N}-\text{NH}_4^+$ in the SAT-PRB system ranged from +9.6‰ to +34.7‰ where lower values were found in sample points O and increased along the flow path with the highest values observed in sample points C and slightly decreased at the outlet (Table S1). $\delta^{15}\text{N}-\text{NO}_3^-$ and $\delta^{18}\text{O}-\text{NO}_3^-$ ranged from +5.6‰ to +22.9‰ and from +10.5‰ to +38.2‰, respectively, where the highest values were observed in sample points A and decreased along the flow path with the lowest values observed in the outlet (Table S1).

4. Discussion

4.1. Ammonium migration and transformations: spatial variability

The inflow water employed in the systems showed NH_4^+ concentrations ranging from 0.93 to 5.54 mM, with $\delta^{15}\text{N}-\text{NH}_4^+$ ranging from +8.2‰ to +10.4‰. These values are in agreement with an organic pollution source (Smith and Miller, 2006) as expected since the inflow water is urban wastewater and a wastewater discharge from nearby hospitals. NH_4^+ concentration in the inflow water showed an annual maximum in summer attaining a peak concentration in September (Fig. 6) which could be attributed to the population increase that doubles during summer months, linked to tourism, which implies a higher sewage input of organic N.

Microbial oxidation of NH_4^+ to NO_3^- (nitrification) appears to be the main transformation mechanism of the inorganic N (NH_4^+ + NO_3^-) species in the systems. During nitrification, NH_4^+ concentration decreases coupled with an O_2 decrease and an increase of NO_3^- concentration along the flow path. Considering nitrification as the only reaction that produces the decrease of NH_4^+ in the systems, an increase of NO_3^- is expected following the stoichiometry of the reaction (Eq. (1) and (1) mol of NO_3^-).

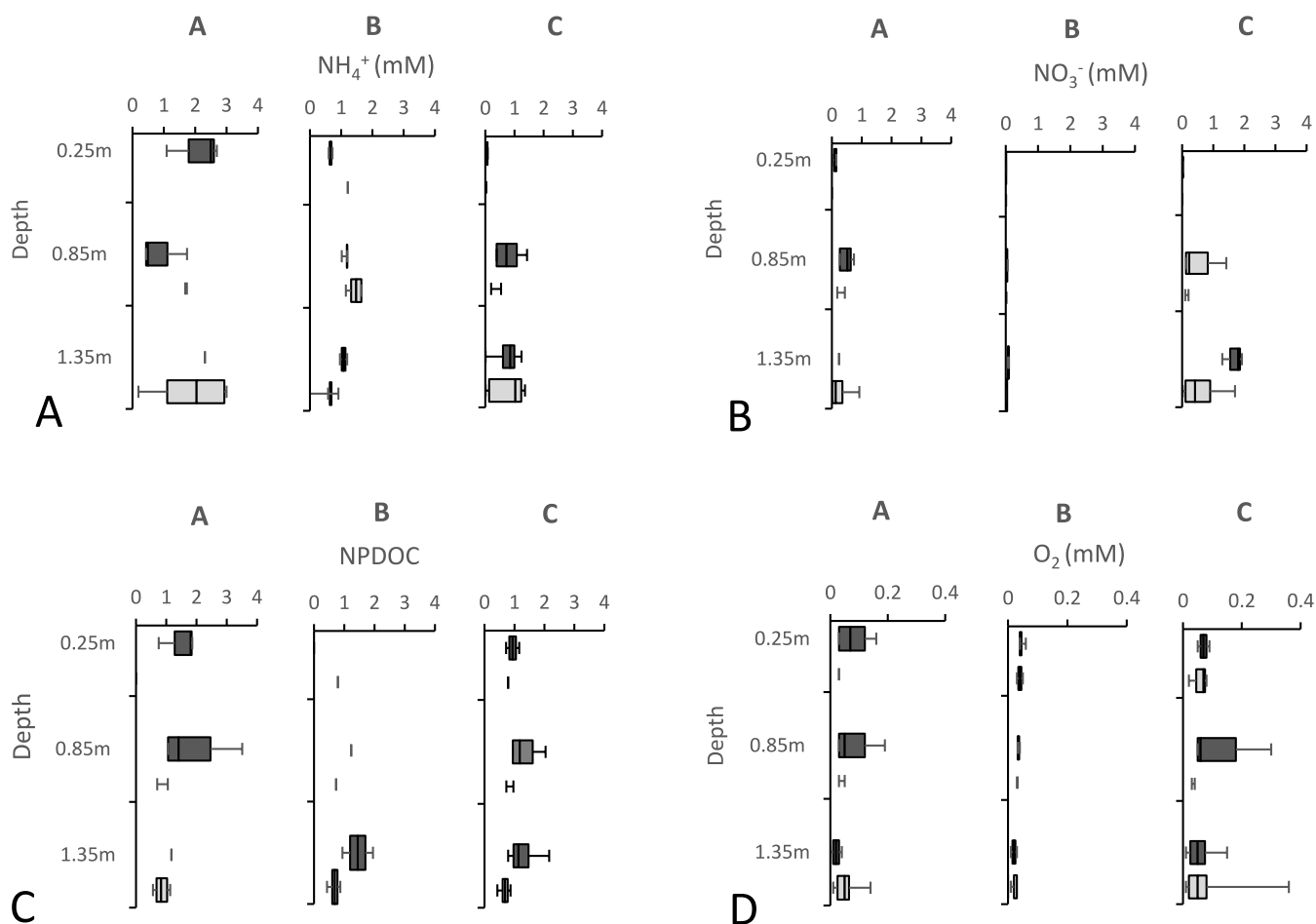


Fig. 4. The evolution of NH_4^+ (A figure), NO_3^- (B figure), NPDOC (C figure) and O_2 (D figure) aqueous concentration with depth at A, B and C sections in the systems (Concentrations are indicated by the box plots and the outliers represented by the dots plotted using all temporal series. First boxplot at each depth (light grey) = SAT-Control system and the second boxplot (dark grey) = SAT-PRB system).

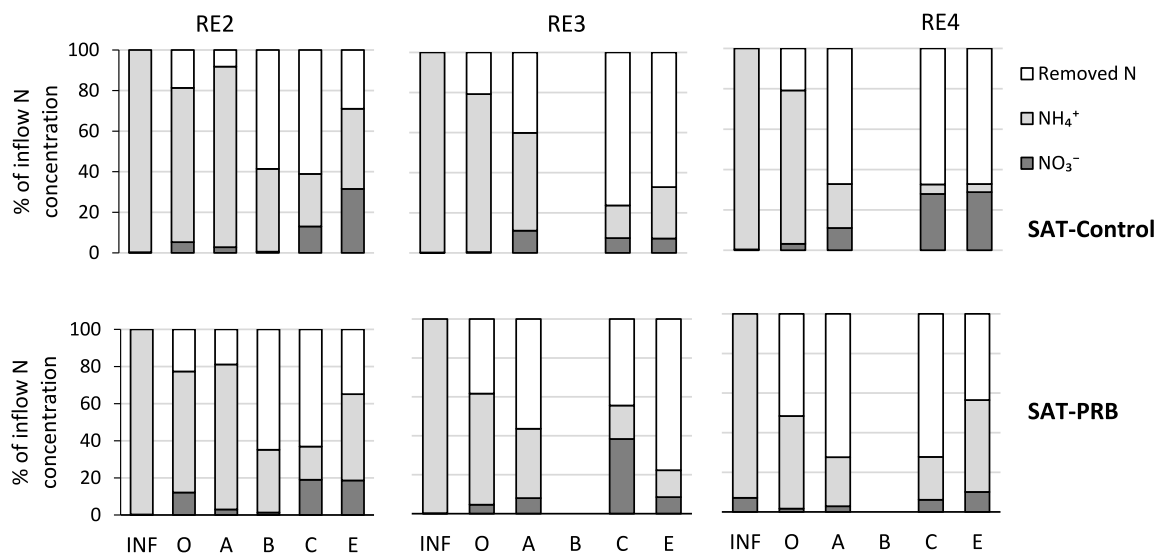


Fig. 5. Comparison of the % distribution of inflow NH_4^+ , residual NO_3^- , and nitrogen removed along the flow path in the systems for all depth profiles during each recharge episode for SAT-Control (top) and SAT-PRB (bottom) systems.

However, the measured NO_3^- concentration in the field does not match this stoichiometry. Total N measured in water is below the expected value, suggesting that other processes may be responsible for N removal. The deficit in N (N_{deficit}) along the flow path in the systems (Fig. 5) can be calculated as the difference between the total N in the inflow water and the residual N dissolved in solution (Eq. 4).

$$N_{\text{deficit}} = N\text{-NH}_4^+ \text{INF} - (N\text{-NH}_4^+ \text{res} + N\text{-NO}_3^- \text{res}) \quad (4)$$

where $N\text{-NH}_4^+ \text{INF}$ is the NH_4^+ concentration in the inflow water (nitrate was negligible in the inflow, Table S1), $N\text{-NH}_4^+ \text{res}$ and $N\text{-NO}_3^- \text{res}$ are the residual concentration of NH_4^+ and NO_3^- in each sample point in the system. The observed N deficit can be explained either by NH_4^+ consumption or by another process such as sorption of NH_4^+ (Alshameri et al., 2018; Fidel et al., 2018; Wang et al., 2015) or plant assimilation (Desimone and Howes, 1998; Lusby et al., 1998) or due to contemporaneous production and consumption of nitrates (coupled nitrification-denitrification reaction) (Kim et al., 1997; Lusby et al., 1998; Smith et al., 2006) or Anammox reactions (Burgin and Hamilton, 2007; Castro-barros et al., 2017). In fact, genetic analyses of the microbial communities present in the barriers demonstrate a potential for all these microbially mediated processes to occur (Hellman et al., 2022). Clay is a component of the PRB and it could provide sorption sites for cation exchange. Since no isotopic fractionation is expected during sorption, comparing the $\epsilon^{15}\text{N}\text{-NH}_4^+$ for the different recharge periods cannot confirm or discard its occurrence. Still, sorption will not affect dramatically long-term N removal processes because its main effect is to buffer inflow fluctuations, which is confirmed by the fact that similar $\epsilon^{15}\text{N}\text{-NH}_4^+$ was observed in the SAT-PRB system in all recharge episodes

(Table S1) and explained further in the section dealing with isotopic fractionation.

Plant assimilation could be relevant in the recharge area, especially during plant growth at the beginning of the study. Nevertheless, plant assimilation is reduced once plants are fully grown, and plant decay can also contribute as a source of organic N in the systems. Denitrification can also be important, especially after infiltration, when O_2 concentration is low. The latter hypothesis is confirmed using the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- since the highest values for these parameters were observed at O and A in both the SAT-Control and SAT-PRB systems. This suggests that part of the NO_3^- produced by nitrification is quickly reduced due to the low O_2 and high NPDOC. A plot of ^{15}N vs. ^{18}O confirms that denitrification is taking place in the systems (Fig. 7 C).

During the RE-2 and RE-3, NH_4^+ concentration removed along the SAT-PRB system was significantly higher than in the SAT-Control system (Fig. 3A). More efficient nitrification was expected in the SAT-Control system, than in the SAT-PRB system where O_2 is used to degrade its higher NPDOC, thus inhibiting nitrification as reported in previous groundwater and nitrifying biofilm studies (Jie et al., 2009; Ling and Chen, 2005). The unexpected higher removal rate of NH_4^+ may be linked to sorption but the higher $\delta^{15}\text{N}\text{-NH}_4^+$ values measured in the SAT-PRB system support that nitrification was higher in this system. We attribute this paradox to the NO_3^- produced from nitrification of NH_4^+ is then denitrified upon reaching the PRB due to reducing conditions created at the reactive barrier base (O) as the NPDOC consumes O_2 in the inflow water.

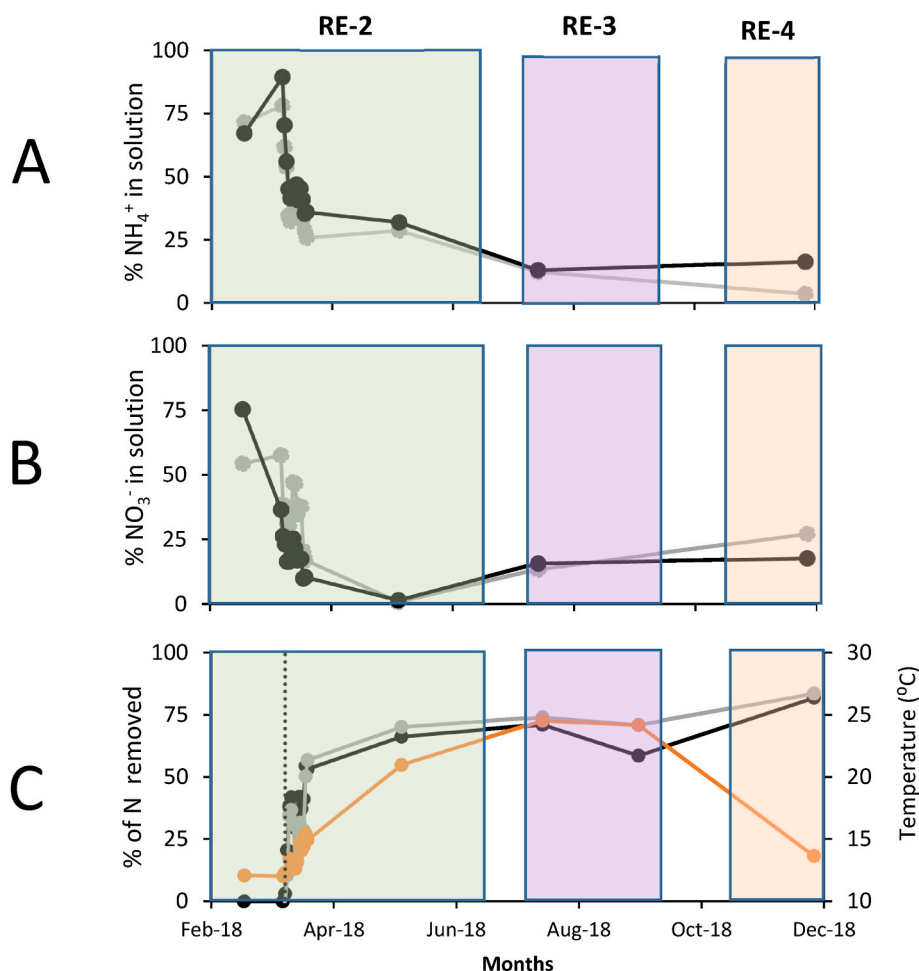


Fig. 6. (A) % distribution of residual NH_4^+ in solution at E; (B) % distribution of residual NO_3^- in solution at E; (C) the % of N ($\text{NH}_4^+ + \text{NO}_3^-$) removed from the solution and the average temperature in both systems. Grey circles represent the SAT-Control system, black circles represent the SAT-PRB system, and orange circles represent the average temperature in both systems. The dotted line represents the point below which we have zero N removal from the systems. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

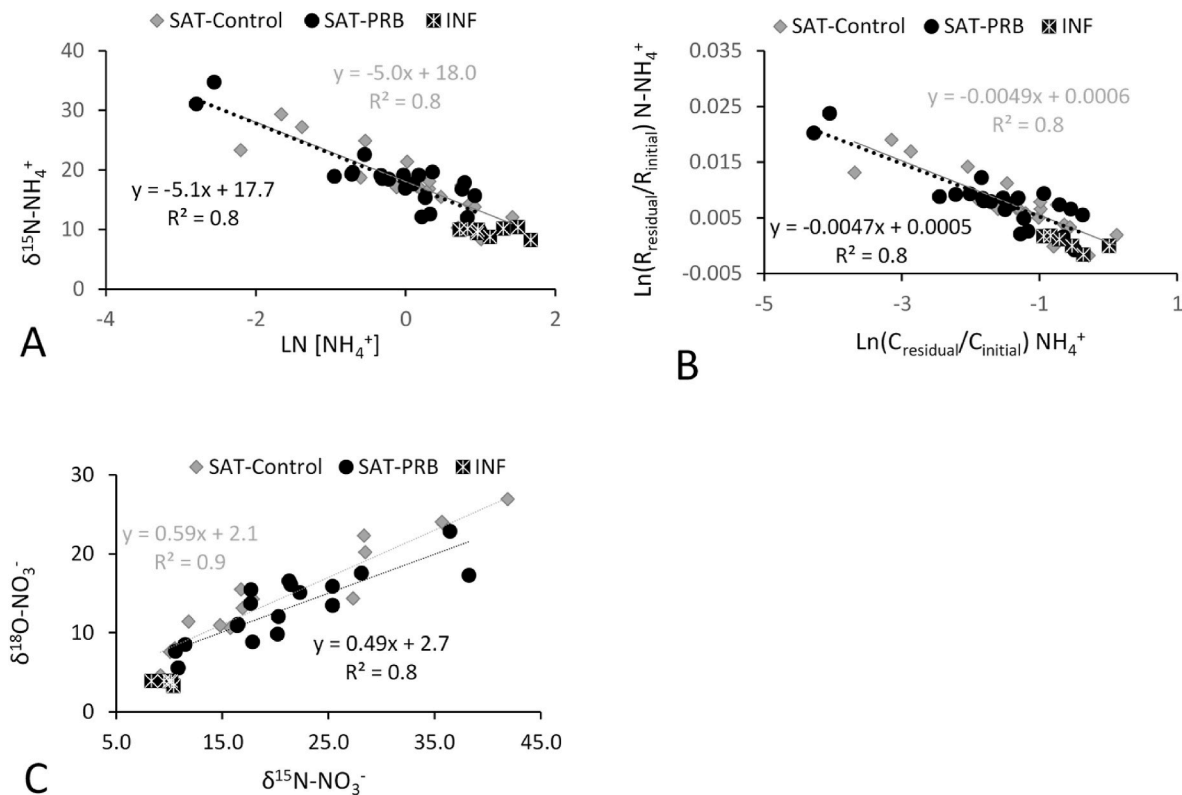


Fig. 7. Evolution of $\delta^{15}\text{N-NH}_4^+$ versus $[\text{NH}_4^+]$ in SAT-Control and SAT-PRB systems (A and B). Relationship between $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ during NO_3^- degradation in systems, (C).

4.2. Nitrate production and reduction: spatial variability

No significant change in NO_3^- concentration along the flow path from O to C was observed but a significant increase at the outlet was detected in both systems. The increase in NO_3^- at the outlets (E) could be explained by an increased nitrification rate favored by flux of O_2 , which we attribute to the grama grass prevalent in the canals. The isotopic evolution of NO_3^- along the flow path showed typical denitrification trends suggesting that this reaction could be taking place. Both nitrification and organic carbon oxidation favour anoxic conditions in the systems; the observed NO_3^- reduction could, therefore, be linked to NPDOC consumption providing energy for denitrifying bacteria. Nonetheless, dissimilatory NO_3^- reduction to NH_4^+ (DNRA) might have contributed to NO_3^- removal as well. However, the N_{deficit} (Eq. (4)) observed cannot be explained by DNRA suggesting that denitrification was the principal NO_3^- removal process.

At each particular section, maximum NH_4^+ accumulation was observed at the bottom of the systems (Fig. 4) suggesting that the rate of nitrification decreased with depth. More aerobic conditions prevailing in the shallower part of the systems favored nitrification resulting in higher nitrification occurring at the top of the systems which is consistent with studies on nitrification-denitrification rates reported in the literature (Kim et al., 1997; Smith et al., 2006). The upper points of our systems are in contact with the atmosphere so oxygen diffusion can account for these higher values at the top. Maximum NO_3^- was not observed at the top of our systems due to the contemporaneous production and consumption of NO_3^- . The higher rate of nitrification at the top of the systems produced a higher consumption of O_2 creating anoxic conditions for denitrification to occur. This assumption is consistent with the high concentration of NH_4^+ and low O_2 observed at the bottom of the systems (Fig. 4).

In the SAT-Control system, NPDOC decreases with depth but the variability of SAT-PRB is due to the release of DOC from the barrier and

the different travel times of the water at the bottom of the systems. The water flow in the bottom of the systems is slower than in the middle and the top of the systems where flow is faster (based on the tracer tests performed data not shown). Therefore, the water flowing through the bottom of the systems has more time for the DOC to degrade.

4.3. Temporal variability of NO_3^- and NH_4^+ production and reduction rates at the outlet

It was observed that the processes responsible for N attenuation in the systems were more effective during RE-3 (July to September) as NH_4^+ concentration decreased from 4.19 mM in the inflow to 0.69 mM at the C section in the SAT-Control system and 0.58 mM in the outlet of the SAT-PRB system (Fig. 3). Low residual NO_3^- concentration was measured suggesting a high denitrification rate during RE-3. For example, 31.5% (RE-2), 7.2% (RE-3) and 28.9% (RE-4) of the total N were measured in the SAT-Control system and 18.7% (RE-2), 8.5% (RE-3) and 10.8% (RE-4) of the total N was measured in the SAT-PRB system at the outlet. Temperatures measured at the infiltration water increased from 12.0 °C to 24.2 °C (March to May) during RE-2, from 26.1 °C to 29.3 °C (July to September) during RE-3 and decreased to 15.4 °C from October to December during RE-4. High N attenuation during RE-3 could be attributed to the high temperature and the rapid acclimatization of the latent denitrifying bacteria at the beginning of RE-3 (Margalef-Marti et al., 2019). The dry periods between the recharge episodes did not significantly influence N attenuation in the systems. At the start of RE-2, RE-3 and RE-4, the latent biomass community might have quickly adapted when recharge resumed.

During 3 days in March 2018, residual N at the outlet (E) of the systems was higher than the total inorganic N ($\text{NH}_4^+ + \text{NO}_3^-$) in the inflow water (INF) (Fig. 6C). This reflects the sudden drop in inflow concentration due to a storm (a unitary sewage system feeds the WWTP so that wastewater is diluted during rain events). It is therefore assumed

zero N removal in the systems during this period (February 16, 2018 to March 27, 2021) as depicted in Fig. 6C.

Residual inorganic N progressively decreased in the outflow of the systems beyond the March event (dotted line in Fig. 6C). Biomass growth and adaptation in the systems coupled with the increasing temperatures during the warm period may have increased the N removal rates, thereby, decreasing the residual inorganic N along time. The average N removal rate increased from 31% of the total discharged N (RE-2) to 72% (RE-3) and from 38% (RE-2) to 65% (RE-3) in the SAT-Control and the SAT-PRB systems respectively (Fig. 6C). In both cases, the % increase of the N removal rate from RE-2 to RE-3 suggests that temperature played a significant role in the N removal from the systems (Fig. 6C). Still, while acknowledging the impact of temperature, the fact that N removal continued to increase during RE-4 when temperatures dropped, suggests that microbial community growth and adaptation also played a significant role. The NH_4^+ progressively decreased from 72% to 4% of total N inflow in the SAT-Control system and 67%–16% in the SAT-PRB system while NO_3^- decreased from 54% to 27% (0.80 mM) of total N inflow in the SAT-Control system and from 75% to 18% in the SAT-PRB system measured at the outlets at the end of the study period (Fig. 6B). The significant fluctuations observed may be attributed to the composition of the recharge water during the recharge episodes, fluctuations in the inflow concentrations (population seasonality and rainfall), and variations in biomass during non-recharge periods, which affect the prevailing reactions. The residual N measured at the outlet of both systems are very similar. However, differences were observed in the dominant N species measured at the outlet of the two systems and the recharge periods. For the SAT-Control system, during RE-2, NH_4^+ was the main N form (1% NO_3^- and 29% NH_4^+), during RE-3 an approximately equal proportion of NO_3^- and NH_4^+ was observed (14% NO_3^- and 13% NH_4^+) and finally, during RE-4 NO_3^- was the dominant species (27% NO_3^- and 4% NH_4^+) (Fig. 6A and B). For the SAT-PRB system, NH_4^+ was also the dominant species (2% NO_3^- and 32% NH_4^+) in RE-2, and during both RE-3 and RE-4 an approximately equal proportion of NO_3^- and NH_4^+ was observed (16–18% NO_3^- and 13–16% NH_4^+) (Fig. 6A and B).

4.4. Isotopic fractionation

NH_4^+ and NO_3^- transformations are often characterized by isotopic fractionation (Casciotti et al., 2003; Mariotti et al., 1981), which reflects that reaction rates depend on the isotopes (Casciotti et al., 2011). The degree of fractionation is affected by the extent of substrate consumption, and processes that compete for the substrate (Wankel et al., 2007). The magnitude of fractionation is characterized using the Rayleigh distillation equation, which expresses a power law relationship between the evolution of the isotopic ratio ($R = \delta + 1$) and that of the species concentration, or more conveniently.

$$\text{Ln} \left(\frac{R_{\text{residual}}}{R_{\text{initial}}} \right) = \epsilon \text{Ln} \left(\frac{C_{\text{residual}}}{C_{\text{initial}}} \right) \quad (5)$$

where ϵ is the isotope enrichment factor. This equation can be linearized as.

$\delta_{\text{residual}} = \epsilon (1 + \delta_{\text{initial}}) \text{Ln}(C_{\text{residual}}/C_{\text{initial}})$. Indeed, a linear correlation was observed in both systems between $\delta^{15}\text{N-NH}_4^+$ and the natural logarithm (Ln) of the residual NH_4^+ (Fig. 7A). The resulting enrichment factor ($\epsilon^{15}\text{N-NH}_4^+$) in the SAT-Control and the SAT-PRB systems was -5‰ ($R^2 = 0.8$). That is, the transformation of NH_4^+ to NO_3^- resulted in an increased $\delta^{15}\text{N-NH}_4^+$ in the residual NH_4^+ pool. The lighter isotopes ($\delta^{14}\text{N-NH}_4^+$) are preferentially incorporated into the generated NO_3^- resulting in $\delta^{15}\text{N-NH}_4^+$ enriched residual NH_4^+ and low $\delta^{15}\text{N-NO}_3^-$ in the generated NO_3^- . This is consistent with similar studies of NH_4^+ migration and reaction in contaminated groundwater where a decrease in NH_4^+ concentration yielded an increase in $\delta^{15}\text{N-NH}_4^+$ (Smith and Miller, 2006; Venkiteswaran et al., 2019). $\epsilon^{15}\text{N-NH}_4^+$ values reported in the literature for nitrification range from -10‰ to -28‰ in laboratory culture and

field-scale studies (Sebilo et al., 2006). Lower $\epsilon^{15}\text{N-NH}_4^+$ (in absolute values) could be explained by some potential contribution of plant assimilation in the systems. Hellman et al. (2022) suggested that the mixed vegetation at the infiltration area contributed to the NH_4^+ attenuation in the SAT-PRB system at the beginning of the experiment. The assimilation of N-NH_4^+ by different plant species reported by Emmerton et al. (2001), estimated an increase in $\delta^{15}\text{N-NH}_4^+$ of 2‰. The study on the uptake of ionic NH_4^+ by two plant species by Yoneyama et al. (2001) showed that the magnitude of the uptake of NH_4^+ depended on the concentrations of NH_4^+ and the plant species with $\epsilon^{15}\text{N-NH}_4^+$ ranging from -2 to -8.1‰ and -3.9 and -24.1‰ for low concentrations of NH_4^+ (0.04–0.2 mM) and -9.4 to -18‰ and -13.4 to -28.9‰ at high concentrations (0.5–4 mM). Consequently, it is somewhat difficult to distinguish between nitrification and plant assimilation isotopically. In the systems, evaluated plant assimilation must be mainly located in the infiltration area and limited to the shallowest portions of the canals, where vegetation is limited and surficial. The vertical transport of atmospheric O_2 into the systems resulted in a high rate of nitrification between sections A, B and C. The diffused O_2 was consumed during nitrification keeping its content similar to the O_2 in the infiltrated water. Note, however, that the conditions (relatively high concentrations of both electron donors and acceptors) favour a highly dynamic biological activity, with relatively fast growth of biofilms and parallel changes in redox conditions (Carrera et al., 2022). Results of $\delta^{15}\text{N-NH}_4^+$ in our systems showed constant isotopic fractionation from O to the outlets (Fig. 6B). This can be explained by two hypotheses. The first is that assimilation and nitrification have similar fractionation. The second hypothesis is that fractionation related to assimilation is limited and the main process affecting the isotopic composition of $\delta^{15}\text{N-NH}_4^+$ would be nitrification.

The low $\delta^{15}\text{N-NO}_3^-$ in the generated nitrate compared to $\delta^{15}\text{N-NH}_4^+$ can be explained by the preferential incorporation of the lighter isotopes to the nitrate substrate produced by nitrification. The increase in $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ in the systems was associated with a corresponding decrease in the residual nitrate concentration linked to denitrification. This was evidenced as the NO_3^- concentration decreased from 1.24 mM to 0.8 mM in the SAT-Control system and from 1.72 mM to 0.52 mM in the SAT-PRB system measured at the outlets (end of the monitoring period). A linear correlation between $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ evolution in the systems is observed with slopes of 0.6 and 0.5 in the SAT-Control and the SAT-PRB systems, respectively (Fig. 7C). The deviation of $\delta^{15}\text{N-NO}_3^-$: $\delta^{18}\text{O-NO}_3^-$ from the 1:1 linear relationship can be explained by (I) the variance of the $\delta^{18}\text{O}$ of the O atom donors (O_2 : $\text{H}_2\text{O} = 1:2$) during the oxidation of NH_4^+ to hydroxylamine NH_2OH to NO_2^- and NO_2^- to NO_3^- (Kumar et al., 1983) (II) O isotope exchange and fractionation (Martin and Casciotti, 2016).

Several authors have reported diverse $\epsilon^{15}\text{N-NO}_3^-$ and $\epsilon^{18}\text{O-NO}_3^-$ for groundwater studies ranging from -8.6‰ to -22.9‰ and -5.5‰ to -18.3‰ for $\epsilon^{15}\text{N-NO}_3^-$ and $\epsilon^{18}\text{O-NO}_3^-$ respectively (Carrey et al., 2013; Fukada et al., 2003). However, in the present experiment, the linear relationship typical for microbial nitrate reduction between $\delta^{15}\text{N-NO}_3^-$, $\delta^{18}\text{O-NO}_3^-$ and the natural logarithm of the residual NO_3^- was not observed (Fig. S3). As there is NO_3^- production and reduction at the same time $\epsilon^{15}\text{N-NO}_3^-$ and $\epsilon^{18}\text{O-NO}_3^-$ could not be modelled as a closed system using the Rayleigh model. This limits the use of isotopes to quantify NO_3^- degradation in coupled nitrification-denitrification systems. Fig. 8 shows $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ and the substrate remaining fraction from the present study using literature $\epsilon^{15}\text{N-NO}_3^-$ and $\epsilon^{18}\text{O-NO}_3^-$ produced by compost ($\epsilon^{15}\text{N-NO}_3^- = -10.4\text{‰}$, $\epsilon^{18}\text{O-NO}_3^- = -9\text{‰}$) (Grau-Martínez et al., 2017) and low $\epsilon^{15}\text{N-NO}_3^-$ and $\epsilon^{18}\text{O-NO}_3^-$ of -3‰ . Some samples follow the trend expected for denitrification as NO_3^- fraction decreases. However, some samples of a very low fraction of residual NO_3^- showed lower $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ close to initial isotopic composition. This can be explained considering that in some areas of the systems, denitrification was able to completely remove NO_3^- and, after that, nitrification continues producing new NO_3^- that would have lower

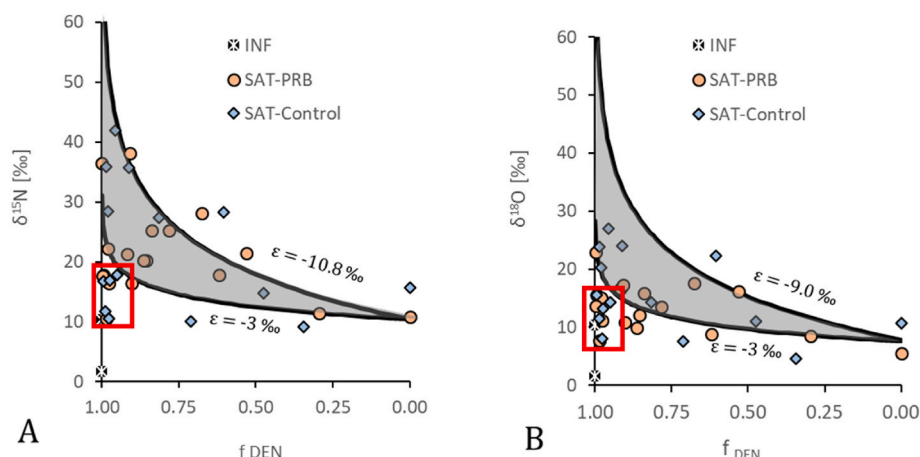


Fig. 8. Isotopic fractionation for $\epsilon^{15}\text{N-NO}_3 = -10.8\text{‰}$ (solid line) and -3.0‰ (dashed line) (A) and $\epsilon^{18}\text{O-NO}_3 = -9\text{‰}$ (solid line) and 3.0‰ (dashed line) (B) using the Rayleigh equation. The shaded region shows the evolution of $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ between $\epsilon = -10.8\text{‰}$ and -3.0‰ (A) and -9‰ and -3.0‰ (B) normally observed in denitrification studies in similar conditions (Grau-Martínez et al., 2017). Dots and square boxes are $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ experimental data in MAR-PRB and SAT-Control systems. The asterisks show $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ in the inflow (INF) and the box highlights the $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ values that do not fall between the fractionation (extreme deviation).

$\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$. The linearity of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ and the natural logarithm of the residual NO_3^- during reduction can, therefore, be affected by the mixing of newly generated NO_3^- with the residual NO_3^- in the substrate pool for nitrification-denitrification processes in the present study.

5. Conclusion

We studied the removal of dissolved N from N-contaminated inflow water in two SAT mesocosm systems. Conventional SAT and SAT-PRB systems were compared. Results showed that both systems are efficient in removing N since the net N removed reached up to 69% in the conventional SAT and 66% in the SAT-PRB, and this removal seems to grow with time and be temperature-dependent. Contemporaneous production and consumption of NO_3^- (nitrification-denitrification) were the principal processes active in the systems. The NH_4^+ and NO_3^- isotopic characterization confirmed that nitrification and denitrification were accompanied by the enrichment of the residual substrate concentration in heavy isotopes. The isotopic fractionation associated with NO_3^- degradation could not be obtained in this study due to constant production and reduction of NO_3^- .

The plant compost in the SAT-PRB system installed to favour CECs removal did not introduce a significant change in the global N removal. The isotopic fractionation calculated for $\delta^{15}\text{N-NH}_4^+$ was -5‰ in both systems. The residual inorganic N remained in the SAT-Control system predominantly as NO_3^- and in the SAT-PRB as equal proportions of NH_4^+ and NO_3^- , suggesting that the plant compost in the PRB increased the electron donor source and contributed to the higher rate of denitrification in the SAT-PRB system.

The concentration of NO_3^- in inflow water was below the EU nitrate threshold limit (50 mg/L - NO_3^-) throughout the whole experiment in the SAT-PRB system except at the beginning of the first recharge episode (average = 33.2 mg/L). In the SAT-Control system, the concentration of NO_3^- in the inflow water was above the EU nitrate threshold limit during the entire experiment except during the warm periods when the temperature was high (average = 51.4).

The concentration of NH_4^+ in the inflow water in both systems was above the maximum EU threshold value for NH_4^+ (5 mg/L - NH_4^+) throughout the entire experiment (average = 55.0 mg/L in the SAT-PRB system, and 54.5 mg/L in the SAT-Control system). The presence of PRB favored the removal of NO_3^- with time. The SAT-PRB had NO_3^- below safe water drinking limit, but NH_4^+ above safe water drinking limit and the SAT-Control system had both NO_3^- and NH_4^+ above safe water drinking limit, rendering water from both systems inappropriate for direct human consumption.

Funding

This work has been financed by the following projects: EU project ACWAPUR (Water JPI -WaterWorks 2014 code PCIN-2015-239), PACE-ISOTEC (CGL2017-87216-C4-1-R) financed by the Spanish Government and AEI/FEDER from the UE, the Water JPI (MARadentro-PCI2019-103603), the project RESTORA (CA210/18/00040) from the Catalan Water Agency, and the Consolidated Research Group MAG (2017-SGR-1733), financed by the Catalan Government. IDAEA-CSIC is a Center of Excellence Severo Ochoa (Spanish Ministry of Science and Innovation, Project CEX2018-000794-S).

The EU Water JPI project MARADENTRO (PCI2019-103603-WW2017), the Catalan Research Project RESTORA (ACA210/18/00040) and AGAUR (AQU - 2017 SGR 1485).

Authorship statement

All authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any publication before its appearance in the *Journal of Environmental Management*.

Conception and design of study: C. Valhondo, L. Martínez-Landa, R. Carrey, J. Carrer, N. Otero; acquisition of data: R. Carrey, C. Valhondo, L. Martínez-Landa; analysis and/or interpretation of data: A. Abu, R. Carrey, C. Valhondo, L. Martínez-Landa; Drafting the manuscript: A. Abu, R. Carrey; revising the manuscript critically for important intellectual content: C. Valhondo, C. Domènech, A. Soler, S. Diaz-Cruz, L. Martínez-Landa, R. Carrey, J. Carrera, N. Otero; Approval of the version of the manuscript to be published (the names of all authors must be listed): C. Domènech, A. Soler, S. Diaz-Cruz, A. Abu, L. Martínez-Landa, C. Valhondo, R. Carrey, J. Carrera, N. Otero.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors are grateful to Consorci de la Costa Brava Girona (CCBGi), the staff of the Palamós WWTP for their unconditional help, and the AGAUR-SGR2017-1485 research group. Abu Alex would like to thank the The Agency for Management of University and Research Grants of the Generalitat de Catalunya for the Ph.D. grant (2019 FI_B 01059) and the CCIT of University of Barcelona for the analytical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2022.115927>.

References

- Adebawale, T., Surapaneni, A., Faulkner, D., McCance, W., Wang, S., Currell, M., 2019. Science of the Total Environment Delineation of contaminant sources and denitrification using isotopes of nitrate near a wastewater treatment plant in peri-urban settings. *Sci. Total Environ.* 651, 2701–2711. <https://doi.org/10.1016/j.scitotenv.2018.10.146>.
- Alshameri, A., He, H., Zhu, J., Xi, Y., Zhu, R., Ma, L., Tao, Q., 2018. Applied Clay Science Adsorption of ammonium by different natural clay minerals : Characterization. kinetics and adsorption isotherms 159, 83–93. <https://doi.org/10.1016/j.clay.2017.11.007>.
- Arauzo, M., 2017. Vulnerability of groundwater resources to nitrate pollution: a simple and effective procedure for delimiting Nitrate Vulnerable Zones. *Sci. Total Environ.* 575, 799–812. <https://doi.org/10.1016/j.scitotenv.2016.09.139>.
- Baú, D.A., Mayer, A.S., 2008. Optimal design of pump-and-treat systems under uncertain hydraulic conductivity and plume distribution. *J. Contam. Hydrol.* 100, 30–46. <https://doi.org/10.1016/j.jconhyd.2008.05.002>.
- Beauchamp, E.G., Trevors, J.T., Paul, J.W., 1989. Carbon Sources for Bacterial Denitrification 10, 113–142. https://doi.org/10.1007/978-1-4613-8847-0_3.
- Bourke, S.A., Iwanyszyn, M., Kohn, J., Jim Hendry, M., 2019. Sources and fate of nitrate in groundwater at agricultural operations overlying glacial sediments. *Hydrol. Earth Syst. Sci.* 23, 1355–1373. <https://doi.org/10.5194/hess-23-1355-2019>.
- Burgin, A.J., Hamilton, S.K., 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. *Front. Ecol. Environ.* 5, 89–96. [https://doi.org/10.1890/1540-9295.2007.5\[89:HWOTRO\]2.0.CO;2](https://doi.org/10.1890/1540-9295.2007.5[89:HWOTRO]2.0.CO;2).
- Carrera, J., Saaltink, M.W., Soler-Sagarra, J., Wang, J., Valhondo, C., 2022. Reactive transport: a review of basic concepts with emphasis on biochemical processes. *Energies* 15 (3), 925. <https://doi.org/10.3390/en15030925>.
- Carrey, R., Otero, N., Soler, A., Gómez-Alday, J.J., Ayora, C., 2013. The role of Lower Cretaceous sediments in groundwater nitrate attenuation in central Spain: column experiments. *Appl. Geochem.* 32, 142–152. <https://doi.org/10.1016/j.apgeochem.2012.10.009>.
- Casciotti, K.L., Sigman, D.M., Ward, B.B., 2003. Linking diversity and stable isotope fractionation in ammonia-oxidizing bacteria. *Geomicrobiol. J.* 20, 335–353. <https://doi.org/10.1080/01490450303895>.
- Casciotti, K.L., Buchwald, C., Santoro, A.E., Frame, C., 2011. Assessment of nitrogen and oxygen isotopic fractionation during nitrification and its expression in the marine environment. In: *Methods in Enzymology*, first ed. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-381294-0.00011-0>.
- Castellano-Hinojosa, A., Charteris, A.F., Müller, C., Jansen-Willems, A., González-López, J., Bedmar, E.J., Carrillo, P., Cárdenas, L.M., 2020. Occurrence and 15N-quantification of simultaneous nitrification and denitrification in N-fertilised soils incubated under oxygen-limiting conditions. *Soil Biol. Biochem.* 143 <https://doi.org/10.1016/j.soilbio.2020.107757>.
- Castro-barros, C.M., Jia, M., Loosdrecht, M.C.M., Van, Volcke, E.I.P., Winkler, M.K.H., 2017. Bioresource Technology Evaluating the potential for dissimilatory nitrate reduction by anammox bacteria for municipal wastewater treatment. *Bioresour. Technol.* 233, 363–372. <https://doi.org/10.1016/j.biortech.2017.02.063>.
- Critchley, K., Rudolph, D.L., Devlin, J.F., Schilling, P.C., 2014. Stimulating in situ denitrification in an aerobic, highly permeable municipal drinking water aquifer. *J. Contam. Hydrol.* 171, 66–80. <https://doi.org/10.1016/j.jconhyd.2014.10.008>.
- Desimone, L.A., Howes, L., 1998. Nitrogen transport and transformations in a shallow aquifer receiving wastewater discharge: a mass balance approach. *Water Resour. Res.* 34 (2), 271–285. <https://doi.org/10.1029/97WR03040>.
- Díaz-Cruz, M.S., Barceló, D., 2008. Trace organic chemicals contamination in ground water recharge. *Chemosphere* 72, 333–342. <https://doi.org/10.1016/j.chemosphere.2008.02.031>.
- Drewes, J.E., 2009. Ground water replenishment with recycled water - water quality improvements during managed aquifer recharge. *Ground Water* 47, 502–505. <https://doi.org/10.1111/j.1745-6584.2009.00587.5.x>.
- Emmerton, K.S., Callaghan, T.V., Jones, H.E., Leake, J.R., Michelsen, A., Read, D.J., 2001. Assimilation and isotopic fractionation of nitrogen by mycorrhizal and nonmycorrhizal subarctic plants. *New Phytol.* 151, 513–524. <https://doi.org/10.1046/j.1469-8137.2001.00179.x>.
- Fidel, R.B., Laird, D.A., Spokas, K.A., 2018. Sorption of ammonium and nitrate to biochars is electrostatic and pH-dependent. *Sci. Rep.* 1–10. <https://doi.org/10.1038/s41598-018-35534-w>.
- Fukada, T., Hiscock, K.M., Dennis, P.F., Grischek, T., 2003. A dual isotope approach to identify denitrification in groundwater at a river-bank infiltration site, 37, pp. 3070–3078. [https://doi.org/10.1016/S0043-1354\(03\)00176-3](https://doi.org/10.1016/S0043-1354(03)00176-3).
- Gibert, O., Assal, A., Devlin, H., Elliot, T., Kalin, R.M., 2019. Performance of a field-scale biological permeable reactive barrier for in-situ remediation of nitrate-contaminated groundwater. *Sci. Total Environ.* 659, 211–220. <https://doi.org/10.1016/j.scitotenv.2018.12.340>.
- Grau-Martínez, A., Torrentó, C., Carrey, R., Rodríguez-Escales, P., Domènech, C., Ghiglieri, G., Soler, A., Otero, N., 2017. Feasibility of two low-cost organic substrates for inducing denitrification in artificial recharge ponds: batch and flow-through experiments. *J. Contam. Hydrol.* 198, 48–58. <https://doi.org/10.1016/j.jconhyd.2017.01.001>.
- Grau-Martínez, A., Folch, A., Torrentó, C., Valhondo, C., Barba, C., Domènech, C., Soler, A., Otero, N., 2018. Monitoring induced denitrification during managed aquifer recharge in an infiltration pond. *J. Hydrol.* 561, 123–135. <https://doi.org/10.1016/j.jhydrol.2018.03.044>.
- Hellman, M., Valhondo, C., Martínez-Landa, L., Carrera, J., Juhanson, J., Hallin, S., 2022. Nitrogen removal capacity in microbial communities developing in compost- and woodchip-based multipurpose reactive barriers for aquifer recharge with wastewater. *Front. Microbiol.* 13 <https://doi.org/10.3389/fmicb.2022.877990>.
- Jie, H.U., Daping, L.L., Qiang, L.I.U., Yong, T.A.O., Xiaohong, H.E., Xiaomei, W., Xudong, L.L., Ping, G.A.O., 2009. Effect of organic carbon on nitrification efficiency and community composition of nitrifying biofilms, 21, pp. 387–394. [https://doi.org/10.1016/S1001-0742\(08\)62281-0](https://doi.org/10.1016/S1001-0742(08)62281-0).
- Jokela, P., Eskola, T., Heinonen, T., Tanttu, U., Tyrväinen, J., Artimo, A., 2017. Raw water quality and pretreatment in managed aquifer recharge for drinking water production in Finland. *Water (Switzerland)* 9. <https://doi.org/10.3390/w9020138>.
- Kim, D.H., Matsuda, O., Yamamoto, T., 1997. Nitrification, denitrification and nitrate reduction rates in the sediment of Hiroshima Bay, Japan. *J. Oceanogr.* 53, 317–324. [https://doi.org/10.1016/S0967-0653\(98\)80283-4](https://doi.org/10.1016/S0967-0653(98)80283-4).
- King, A., Jensen, V., Fogg, G.E., Harter, T., 2012. Groundwater remediation and management for nitrate. Addressing nitrate California's drink. *Water with a focus tulare lake basin salinas val. Groundw* 51.
- Korom, S.F., 1992. Natural denitrification in the saturated zone: a review. *Water Resour. Res.* 28 (6), 1657–1668. <https://doi.org/10.1029/92WR00252>.
- Kumar, S., Nicholas, D.J.D., Williams, E.H., 1983. Definitive 15N NMR evidence that water serves as a source of "O" during nitrite oxidation by *Nitrobacter agilis*. *FEBS Lett.* 152, 71–74. [https://doi.org/10.1016/0014-5793\(83\)80484-0](https://doi.org/10.1016/0014-5793(83)80484-0).
- Kuster, M., Díaz-Cruz, S., Rosell, M., López de Alda, M., Barceló, D., 2010. Fate of selected pesticides, estrogens, progestogens and volatile organic compounds during artificial aquifer recharge using surface waters. *Chemosphere* 79, 880–886. <https://doi.org/10.1016/j.chemosphere.2010.02.026>.
- Ling, J., Chen, S., 2005. Impact of organic carbon on nitrification performance of different biofilters. *Aquacult. Eng.* 33 (2), 150–162. <https://doi.org/10.1016/j.aquaeng.2004.12.002>.
- Lusby, F.E., Gibbs, M.M., Cooper, A.B., Thompson, K., 1998. The fate of groundwater ammonium in a lake edge wetland. *American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America* 27 (No. 2), 459–466. <https://doi.org/10.2134/jeq1998.00472425002700020029x>.
- Maeng, S.K., Ameda, E., Sharma, S.K., Grützmacher, G., Amy, G.L., 2010. Organic micropollutant removal from wastewater effluent-impacted drinking water sources during bank filtration and artificial recharge. *Water Res.* 44, 4003–4014. <https://doi.org/10.1016/j.watres.2010.03.035>.
- Margalef-Martí, R., Carrey, R., Soler, A., Otero, N., 2019. Evaluating the potential use of a dairy industry residue to induce denitrification in polluted water bodies: a flow-through experiment. *J. Environ. Manag.* 245, 86–94. <https://doi.org/10.1016/j.jenvman.2019.03.086>.
- Mariotti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., Tardieux, P., 1981. Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes. *Plant Soil* 62, 413–430. <https://doi.org/10.1007/BF02374138>.
- Martin, T.S., Casciotti, K.L., 2016. Nitrogen and oxygen isotopic fractionation during microbial nitrite reduction. *Limnol. Oceanogr.* 61, 1134–1143. <https://doi.org/10.1002/lno.10278>.
- Matchett, L.S., Goulding, K.W.T., Webster, C.P., Haycock, N.E., 2019. Denitrification in Riparian Bu Er Zones: the Role of Floodplain Hydrology, vol. 13, pp. 10–11. [https://doi.org/10.1002/\(SICI\)1099-1085\(199907\)13:10%3C1451::AID-HYP822%3E3.0.CO;2](https://doi.org/10.1002/(SICI)1099-1085(199907)13:10%3C1451::AID-HYP822%3E3.0.CO;2).
- McIlvin, M.R., Altabet, M.A., 2005. Chemical conversion of nitrate and nitrite to nitrous oxide for nitrogen and oxygen isotopic analysis in freshwater and seawater. *Anal. Chem.* 77, 5589–5595. <https://doi.org/10.1021/ac050528s>.
- Naidoo, S., Olaniran, A.O., 2013. Treated wastewater effluent as a source of microbial pollution of surface water resources. *Int. J. Environ. Res. Publ. Health* 11, 249–270. <https://doi.org/10.3390/ijerph110100249>.
- Pabich, W.J., Valiela, I., Hemond, H.F., 2001. Relationship between DOC concentration and vadose zone thickness and depth below water table in groundwater of Cape Cod. *U.S.A. Biogeochemistry* 55, 247–268. <https://doi.org/10.1023/A:1011842918260>.
- Ren, Y., Xu, Z., Zhang, X., Wang, X., Sun, X., Ballantine, D.J., Wang, S., 2014. Nitrogen pollution and source identification of urban ecosystem surface water in Beijing. *Front. Environ. Sci. Eng.* 8, 106–116. <https://doi.org/10.1007/s11783-012-0474-z>.
- Robertson, W.D., Vogan, J.L., Lombardo, P.S., 2008. Nitrate removal rates in a 15-year-old permeable reactive barrier treating septic system nitrate. *Ground Water Monit. Remed.* 28, 65–72. <https://doi.org/10.1111/j.1745-6592.2008.00205.x>.

- Ryabenko, E., Altabet, M.A., Wallace, D.W.R., 2009. Effect of chloride on the chemical conversion of nitrate to nitrous oxide for $\delta^{15}\text{N}$ analysis. *Limnol Oceanogr. Methods* 7, 545–552. <https://doi.org/10.4319/lom.2009.7.545>.
- Sebilo, M., Billen, G., Mayer, B., Billiou, D., Grably, M., Garnier, J., Mariotti, A., 2006. Assessing nitrification and denitrification in the Seine river and estuary using chemical and isotopic techniques. *Ecosystems* 9, 564–577. <https://doi.org/10.1007/s10021-006-0151-9>.
- Shi, P., Zhang, Y., Song, J., Li, P., Wang, Y., Zhang, Xiaoming, Li, Z., Bi, Z., Zhang, Xin, Qin, Y., Zhu, T., 2019. Response of nitrogen pollution in surface water to land use and social-economic factors in the Weihe River watershed, northwest China. *Sustain. Cities Soc.* 50, 101658. <https://doi.org/10.1016/j.scs.2019.101658>.
- Singleton, M.J., Esser, B.K., Moran, J.E., Hudson, G.B., McNab, W.W., Harter, T., 2007. Saturated zone denitrification: potential for natural attenuation of nitrate contamination in shallow groundwater under dairy operations. *Environ. Sci. Technol.* 41, 759–765. <https://doi.org/10.1021/es061253g>.
- Smith, R.L., Miller, D.N., 2006. Ammonium Transport and Reaction in Contaminated Groundwater: Application of Isotope Tracers and Isotope Fractionation Studies, vol. 42, pp. 1–19. <https://doi.org/10.1029/2005WR004349>.
- Smith, R.L., Baumgartner, L.K., Miller, D.N., Repert, D.A., Böhlke, J.K., 2006. Assessment of nitrification potential in ground water using short-term, single-well injection experiments. *Microb. Ecol.* 51, 22–35. <https://doi.org/10.1007/s00248-004-0159-7>.
- Sonune, A., Ghate, R., 2004. Developments in wastewater treatment methods. *Desalination* 167, 55–63. <https://doi.org/10.1016/j.desal.2004.06.113>.
- Union, European, 1991. Council directive of 12 december 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources. *Directive 91/676/EEC. Off. J. Eur. Union L* 375, 1–13.
- Union, European, 2006. Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration. *Off. J. Eur. Union L* 372, 19–31.
- Valhondo, C., Carrera, J., Ayora, C., Barbieri, M., Nödler, K., Licha, T., Huerta, M., 2014. Behavior of nine selected emerging trace organic contaminants in an artificial recharge system supplemented with a reactive barrier. *Environ. Sci. Pollut. Res.* 21, 11832–11843. <https://doi.org/10.1007/s11356-014-2834-7>.
- Valhondo, C., Carrera, J., Ayora, C., Tubau, I., Martínez-Landa, L., Nödler, K., Licha, T., 2015. Characterizing redox conditions and monitoring attenuation of selected pharmaceuticals during artificial recharge through a reactive layer. *Sci. Total Environ.* 512 (513), 240–250. <https://doi.org/10.1016/j.scitotenv.2015.01.030>.
- Valhondo, C., Carrera, J., Martínez-Landa, L., Wang, J., Amalfitano, S., Levantesi, C., Díaz-Cruz, M.S., 2020a. Reactive barriers for renaturalization of reclaimed water during soil aquifer treatment. *Water* 12 (4), 1012. <https://doi.org/10.3390/w12041012>.
- Valhondo, C., Martínez-Landa, L., Carrera, J., Díaz-Cruz, S.M., Amalfitano, S., Levantesi, C., 2020b. Six artificial recharge pilot replicates to gain insight into water quality enhancement processes. *Chemosphere* 240. <https://doi.org/10.1016/j.chemosphere.2019.124826>.
- Venkiteswaran, J.J., Schiff, S.L., Ingalls, B.P., 2019. Quantifying the fate of wastewater nitrogen discharged to a Canadian river. *Facets* 4, 315–335. <https://doi.org/10.1139/facets-2018-0028>.
- Volkmer, B.G., Ernst, B., Simon, J., Kuefer, R., Bartsch, G., Bach, D., Gschwend, J.E., 2005. Influence of nitrate levels in drinking water on urological malignancies: a community-based cohort study. *BJU Int.* 95, 972–976. <https://doi.org/10.1111/j.1464-410X.2005.05450.x>.
- Wang, B., Lehmann, J., Hanley, K., Hestrin, R., Enders, A., 2015. Chemosphere Adsorption and desorption of ammonium by maple wood biochar as a function of oxidation and pH. *Chemosphere* 138, 120–126. <https://doi.org/10.1016/j.chemosphere.2015.05.062>.
- Wankel, S.D., Kendall, C., Pennington, J.T., Chavez, F.P., Paytan, A., 2007. Nitrification in the euphotic zone as evidenced by nitrate dual isotopic composition: observations from Monterey Bay, California. *Global Biogeochem. Cycles* 21, 1–13. <https://doi.org/10.1029/2006GB002723>.
- WHO, 2004. Guidelines for Drinking-Water Quality, third ed., vol. 1. WHO Libr. Cat. 1 <https://apps.who.int/iris/handle/10665/42852>.
- Withers, P.J., Neal, C., Jarvie, H.P., Doody, D.G., 2014. Agriculture and eutrophication: where do we go from here? *Sustainability* 6 (9), 5853–5875. <https://doi.org/10.3390/su6095853>.
- Yoneyama, T., Matsumaru, T., Usui, K., Engelaar, W.M.H.G., 2001. Discrimination of nitrogen isotopes, during absorption of ammonium and nitrate at different nitrogen concentrations by rice (*Oryza sativa* L.) plants. *Plant Cell Environ.* 24, 133–139. <https://doi.org/10.1046/j.1365-3040.2001.00663.x>.
- Zhang, L., Altabet, M.A., Wu, T., Hadas, O., 2007. Sensitive measurement of $\text{NH}_4^+ + 15\text{N}$ ($\delta^{15}\text{NH}_4^+$) at natural abundance levels in fresh and saltwaters. *Anal. Chem.* 79, 5297–5303. <https://doi.org/10.1021/ac070106d>.
- Zirkle, K.W., Nolan, B.T., Jones, R.R., Weyer, P.J., Ward, M.H., Wheeler, D.C., 2016. Assessing the relationship between groundwater nitrate and animal feeding operations in Iowa (USA). *Sci. Total Environ.* 566–567, 1062–1068. <https://doi.org/10.1016/j.scitotenv.2016.05.130>.

Supporting Information PAPER 3

Pathways and efficiency of nitrogen attenuation in wastewater effluent through Soil Aquifer Treatment.

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Supplementary Table S1. NH_4^+ , NO_3^- , NPDOC and O_2 concentration (mM) and the isotopic data measured in systems. (BDL means below detection limit and NA means data is not available or measured).

Reference	System	Section	Temp (°C)	NH_4^+ (mM)	NO_3^- (mM)	NPDOC (mM)	O_2 (mM)	$\delta^{15}\text{N-NH}_4^+$	$\delta^{18}\text{O-NO}_3^-$	$\delta^{15}\text{N-NO}_3^-$
INF180306	Inflow	INF	-	2.59	0.01	2.11	NA	9.4	NA	NA
INF180307	Inflow	INF	15.40	2.27	0.01	NA	0.23	10.1	NA	NA
INF180323	Inflow	INF	-	3.96	0.01	NA	NA	NA	NA	NA
INF180325	Inflow	INF	12.00	0.93	0.06	NA	0.27	NA	NA	NA
INF180326	Inflow	INF	13.20	1.50	0.01	NA	0.28	NA	NA	NA
INF180327	Inflow	INF	13.40	2.06	BDL	NA	0.15	10.0	NA	NA
INF180328	Inflow	INF	15.1	2.46	BDL	NA	0.08	NA	NA	NA
INF180329	Inflow	INF	15.6	3.29	BDL	NA	0.06	NA	NA	NA
INF180330	Inflow	INF	15.6	3.51	BDL	NA	0.35	NA	NA	NA
INF180331	Inflow	INF	14.8	3.46	BDL	NA	0.35	NA	NA	NA
INF180401	Inflow	INF	15.1	2.46	0.01	NA	0.34	NA	NA	NA
INF180402	Inflow	INF	15.6	2.33	BDL	NA	0.49	NA	NA	NA
INF180403	Inflow	INF	17.9	2.57	BDL	NA	0.37	NA	NA	NA
INF180404	Inflow	INF	16.7	2.23	0.01	NA	0.33	NA	NA	NA
INF180405	Inflow	INF	16.8	2.19	BDL	NA	0.40	NA	NA	NA
INF180406	Inflow	INF	17.8	4.34	0.01	NA	0.40	NA	NA	NA
INF180407	Inflow	INF	18.8	4.24	0.01	NA	0.37	NA	NA	NA
INF180511	Inflow	INF	22.4	5.29	BDL	1.08	0.37	8.2	NA	NA
INF180523	Inflow	INF	22	3.07	BDL	1.14	0.05	8.8	NA	NA
INF180525	Inflow	INF	24.2	2.63	0.01	1.31	0.04	NA	NA	NA
INF180720	Inflow	INF	27.2	3.51	0.01	NA	0.08	NA	NA	NA
INF180724	Inflow	INF	27.4	4.57	BDL	NA	0.09	NA	NA	NA
INF180725	Inflow	INF		3.86	0.01	NA	NA	NA	NA	NA
INF180727	Inflow	INF	27.5	3.77	0.01	NA	0.09	NA	NA	NA
INF180730	Inflow	INF	27.3	3.67	0.01	1.10	0.06	10.1	NA	NA

Supplementary Table S1. NH_4^+ , NO_3^- , NPDOC and O_2 concentration (mM) and the isotopic data measured in systems. (BDL means below detection limit and NA means data is not available or measured).

Reference	System	Section	Temp (°C)	NH_4^+ (mM)	NO_3^- (mM)	NPDOC (mM)	O_2 (mM)	$\delta^{15}\text{N-NH}_4^+$	$\delta^{18}\text{O-NO}_3^-$	$\delta^{15}\text{N-NO}_3^-$
INF180731	Inflow	INF	29.3	4.39	BDL	1.41	0.07	NA	NA	NA
INF 180829	Inflow	INF	26.8	NA	NA	NA	NA	10.4	NA	NA
INF180905	Inflow	INF	26.1	5.54	0.01	1.48	0.32	NA	NA	NA
INF181210	Inflow	INF	14.60	2.61	0.01	0.92	0.19	NA	NA	NA
INF181211	Inflow	INF	15.4	2.92	0.01	0.95	0.23	NA	NA	NA
T2O180327	Control	P-O	11	0.55	0.43	NA	0.03	NA	7.6	10.0
T2O180405	Control	P-O	16.1	NA	NA	NA	0.02	NA	NA	NA
T2O180511	Control	P-O	18.8	3.69	0.01	0.94	0.02	NA	15.5	16.7
T2O180525	Control	P-O	21	2.32	0.02	0.97	0.02	14.3	13.1	16.9
T2O180731	Control	P-O	27	4.10	0.02	1.15	0.03	12.1	23.8	35.8
T2O180905	Control	P-O	24.1	2.51	0.01	1.06	0.04	13.8	NA	NA
T2O181211	Control	P-O	13.40	2.11	0.09	0.70	0.06	NA	NA	NA
T2A1180327	Control	P-A1	11.9	NA	BDL	NA	0.01	NA	NA	NA
T2A1180511	Control	P-A1	17.8	1.68	0.18	0.70	0.03	NA	14.3	17.8
T2A1180731	Control	P-A1	27	1.37	0.92	1.08	0.07	16.8	22.3	28.3
T2A1181210	Control	P-A1	15.00	0.19	0.40	0.56	0.05	NA	NA	NA
T2A1180405	Control	P-A1		NA	NA	NA	0.01	NA	NA	NA
T2A2180511	Control	P-A2	18.4	3.00	0.01	0.87	0.03	NA	NA	NA
T2A3180327	Control	P-A3	10.6	NA	NA	NA	0.03	NA	NA	NA
T2A3180405	Control	P-A3		NA	NA	NA	0.03	NA	NA	NA
T2A3180511	Control	P-A3	18.2	3.00	0.05	0.79	0.04	NA	20.2	28.4
T2A3180731	Control	P-A3	26.5	2.72	0.01	1.13	0.06	8.4	11.4	11.8
T2A3181210	Control	P-A3	13.40	1.02	0.21	0.64	0.14	NA	NA	NA
T2B1180327	Control	P-B1	12.5	NA	NA	NA	0.01	NA	NA	NA
T2B1180516	Control	P-B1	16.6	0.69	0.04	NA	0.03	NA	NA	NA

Supplementary Table S1. NH_4^+ , NO_3^- , NPDOC and O_2 concentration (mM) and the isotopic data measured in systems. (BDL means below detection limit and NA means data is not available or measured).

Reference	System	Section	Temp (°C)	NH_4^+ (mM)	NO_3^- (mM)	NPDOC (mM)	O_2 (mM)	$\delta^{15}\text{N-NH}_4^+$	$\delta^{18}\text{O-NO}_3^-$	$\delta^{15}\text{N-NO}_3^-$
T2B1180525	Control	P-B1	17.1	0.58	0.00	0.58	0.03	NA	NA	NA
T2B1 180523	Control	P-B1	26.8	NA	NA	NA	NA	24.9	NA	NA
T2B2180516	Control	P-B2	16.6	1.60	0.04	NA	0.03	NA	NA	NA
T2B2 180523	Control	P-B2		NA	NA	NA	NA	17.3	NA	NA
T2B2180525	Control	P-B2	17.8	1.15	0.01	0.73	0.03	NA	NA	NA
T2B3180327	Control	P-B3		NA	NA	NA	0.03	NA	NA	NA
T2B3180516	Control	P-B3	16	1.81	0.01	NA	0.05	NA	NA	NA
T2B3 180523	Control	P-B3		NA	NA	NA	NA	17.0	NA	NA
T2B3180525	Control	P-B3	19.4	1.21	0.01	0.66	0.04	NA	NA	NA
T2C1180307	Control	P-C1	11.50	1.14	1.70	0.76	0.02	NA	4.6	9.2
T2C1180327	Control	P-C1		NA	NA	NA	0.01	NA	NA	NA
T2C1180523	Control	P-C1	16.7	1.03	0.02	0.66	0.02	18.4	NA	NA
T2C1180731	Control	P-C1	23.4	1.36	0.31	0.79	0.05	18.1	24.1	35.6
T2C1181210	Control	P-C1	15.00	0.25	1.03	0.43	0.06	NA	NA	NA
T2C2180523	Control	P-C2	17.4	1.32	0.04	0.69	0.03	16.4	8.1	10.5
T2C3180306	Control	P-C3	10.40	0.20	0.10	0.73	0.02	NA	27.0	41.9
T2C3180327	Control	P-C3		NA	NA	NA	0.07	NA	NA	NA
T2C3180523	Control	P-C3	18.9	0.03	0.01	0.79	0.08	NA	NA	NA
T2C3180731	Control	P-C3	24.3	0.01	NA	0.87	0.10	NA	NA	NA
T2C3181210	Control	P-C3	12.50	0.01	0.52	0.55	0.36	NA	NA	NA
T2Ex180307	Control	Outlet	11.70	1.63	1.24	NA	0.07	NA	10.6	15.7
T2Ex180326	Control	Outlet	12.20	1.18	0.87	NA	0.16	NA	NA	NA
T2Ex180327	Control	Outlet	12.70	1.28	0.79	NA	NA	NA	11.0	14.8
T2Ex180328	Control	Outlet	12.5	1.32	0.88	NA	0.17	NA	NA	NA
T2Ex180329	Control	Outlet	13.5	1.14	1.02	NA	0.14	NA	NA	NA

Supplementary Table S1. NH_4^+ , NO_3^- , NPDOC and O_2 concentration (mM) and the isotopic data measured in systems. (BDL means below detection limit and NA means data is not available or measured).

Reference	System	Section	Temp ($^{\circ}\text{C}$)	NH_4^+ (mM)	NO_3^- (mM)	NPDOC (mM)	O_2 (mM)	$\delta^{15}\text{N-NH}_4^+$	$\delta^{18}\text{O-NO}_3^-$	$\delta^{15}\text{N-NO}_3^-$
T2Ex180330	Control	Outlet	13.3	1.14	1.08	NA	0.17	NA	NA	NA
T2Ex180331	Control	Outlet	12.9	1.23	1.17	NA	0.17	NA	NA	NA
T2Ex180401	Control	Outlet	12.7	0.92	1.17	NA	0.19	NA	NA	NA
T2Ex180402	Control	Outlet	13.2	0.87	1.09	NA	0.16	NA	NA	NA
T2Ex180403	Control	Outlet	15.1	0.86	0.90	NA	0.14	NA	NA	NA
T2Ex180404	Control	Outlet	14.2	0.79	0.85	NA	0.16	NA	NA	NA
T2Ex180405	Control	Outlet	14.5	0.74	0.83	NA	0.16	NA	NA	NA
T2Ex180406	Control	Outlet	15.9	1.26	0.90	NA	0.14	NA	NA	NA
T2Ex180407	Control	Outlet	14.9	1.10	0.73	NA	0.13	NA	NA	NA
T2Ex180523	Control	Outlet	22	0.89	0.03	0.73	0.14	17.1	NA	NA
T2Ex180622	Control	Outlet	20.3	1.82	NA	NA	0.07	NA	NA	NA
T2Ex180731	Control	Outlet	24.7	0.55	0.59	0.72	0.17	18.7	14.4	27.3
T2Ex180916	Control	Outlet	24.4	1.60	0.01	0.89	0.18	15.5	NA	NA
T2Ex181211	Control	Outlet	13.70	0.11	0.80	0.53	0.20	NA	NA	NA
T4O180327	MAR-PRB	P-O	11.3	0.93	0.79	NA	0.03	NA	8.5	11.5
T4O180511	MAR-PRB	P-O	18.72	3.41	0.06	1.98	0.04	NA	NA	NA
T4O180525	MAR-PRB	P-O	20.4	1.29	0.19	2.15	0.02	15.4	12.1	20.3
T4O180731	MAR-PRB	P-O	27.2	2.11	0.39	2.29	0.04	16.8	9.9	20.2
T4O180905	MAR-PRB	P-O	24.1	2.68	0.00	1.64	0.03	9.6	NA	NA
T4O180405	MAR-PRB	P-O	15.7	NA	NA	NA	0.01	NA	NA	NA
T4O181211	MAR-PRB	P-O	13.70	1.39	0.56	0.86	0.05	NA	NA	NA
T4A1180327	MAR-PRB	P-A1	11.4	NA	NA	NA	0.01	NA	NA	NA
T4A1180405	MAR-PRB	P-A1	14.2	NA	NA	NA	0.00	NA	NA	NA
T4A1180511	MAR-PRB	P-A1	17.5	2.31	0.24	1.17	0.02	NA	16.6	21.3
T4A1180731	MAR-PRB	P-A1	26.3	0.49	0.52	1.41	0.19	19.6	15.9	25.3

Supplementary Table S1. NH_4^+ , NO_3^- , NPDOC and O_2 concentration (mM) and the isotopic data measured in systems. (BDL means below detection limit and NA means data is not available or measured).

Reference	System	Section	Temp (°C)	NH_4^+ (mM)	NO_3^- (mM)	NPDOC (mM)	O_2 (mM)	$\delta^{15}\text{N-NH}_4^+$	$\delta^{18}\text{O-NO}_3^-$	$\delta^{15}\text{N-NO}_3^-$
T4A1181210	MAR-PRB	P-A1	15.20	0.38	0.74	0.71	0.05	NA	NA	NA
T4A2180511	MAR-PRB	P-A2	18.42	1.74	0.01	3.51	0.03	NA	NA	NA
T4A3180327	MAR-PRB	P-A3		NA	NA	NA	0.04	NA	NA	NA
T4A3180405	MAR-PRB	P-A3	13.2	NA	NA	NA	0.03	NA	NA	NA
T4A3180511	MAR-PRB	P-A3	18.1	2.69	0.01	1.86	0.16	NA	15.5	17.7
T4A3180731	MAR-PRB	P-A3	26.2	2.50	0.15	1.81	0.07	15.7	17.3	38.2
T4A3181210	MAR-PRB	P-A3	13.30	1.09	0.14	0.75	0.12	NA	NA	NA
T4B1180327	MAR-PRB	P-B1	12	NA	NA	NA	0.01	NA	NA	NA
T4B1180516	MAR-PRB	P-B1	16.6	1.19	0.10	NA	0.03	19.6	22.9	36.5
T4B1180525	MAR-PRB	P-B1	17.1	0.96	0.06	0.94	0.03	19.2	NA	NA
T4B2180516	MAR-PRB	P-B2	16.6	1.18	0.06	NA	0.04	21.8	7.7	10.5
T4B2180525	MAR-PRB	P-B2	17.9	1.19	0.00	1.95	0.04	19.2	NA	NA
T4B3180327	MAR-PRB	P-B3		NA	NA	NA	0.04	NA	NA	NA
T4B3180516	MAR-PRB	P-B3	17.1	0.60	0.00	NA	0.06	NA	NA	NA
T4B3180525	MAR-PRB	P-B3		0.72	0.01	1.23	0.04	19.1	NA	NA
T4C1180307	MAR-PRB	P-C1	11.70	0.91	1.92	2.16	0.02	NA	16.1	21.4
T4C1180327	MAR-PRB	P-C1		NA	NA	NA	0.01	NA	NA	NA
T4C1180523	MAR-PRB	P-C1	17.1	0.80	0.03	1.24	0.04	18.5	13.7	17.6
T4C1180731	MAR-PRB	P-C1	23	1.43	1.42	1.18	0.04	19.7	8.9	17.8
T4C1181210	MAR-PRB	P-C1	15.20	1.24	1.29	0.79	0.06	NA	NA	NA
T4C2180523	MAR-PRB	P-C2	17.8	0.73	0.23	2.04	0.06	18.6	10.9	16.4
T4C3180306	MAR-PRB	P-C3	10.40	0.06	NA	0.72	0.09	NA	15.1	22.3
T4C3180327	MAR-PRB	P-C3		NA	NA	NA	0.05	NA	NA	NA
T4C3180523	MAR-PRB	P-C3	19.9	0.08	0.01	1.16	0.08	34.7	NA	NA
T4C3180731	MAR-PRB	P-C3	23.9	0.01	1.80	1.03	0.15	NA	17.6	28.1

Supplementary Table S1. NH_4^+ , NO_3^- , NPDOC and O_2 concentration (mM) and the isotopic data measured in systems. (BDL means below detection limit and NA means data is not available or measured).

Reference	System	Section	Temp (°C)	NH_4^+ (mM)	NO_3^- (mM)	NPDOC (mM)	O_2 (mM)	$\delta^{15}\text{N-NH}_4^+$	$\delta^{18}\text{O-NO}_3^-$	$\delta^{15}\text{N-NO}_3^-$
T4C3181210	MAR-PRB	P-C3	12.60	0.05	0.02	0.71	0.30	NA	NA	NA
T4Ex180307	MAR-PRB	Outlet	12.50	1.53	1.72	NA	0.22	NA	5.6	10.8
T4Ex180326	MAR-PRB	Outlet	11.9	1.35	0.55	NA	0.19	NA	NA	NA
T4Ex180327	MAR-PRB	Outlet	11.90	1.45	0.54	NA	0.21	NA	NA	NA
T4Ex180328	MAR-PRB	Outlet	12.30	1.38	0.57	NA	0.17	NA	NA	NA
T4Ex180329	MAR-PRB	Outlet	13.4	1.49	0.55	NA	0.16	NA	NA	NA
T4Ex180330	MAR-PRB	Outlet	13.2	1.46	0.58	NA	0.14	NA	NA	NA
T4Ex180331	MAR-PRB	Outlet	12.8	1.56	0.64	NA	0.19	NA	NA	NA
T4Ex180401	MAR-PRB	Outlet	12.6	1.11	0.63	NA	0.17	NA	NA	NA
T4Ex180402	MAR-PRB	Outlet	13.3	1.10	0.51	NA	0.15	NA	NA	NA
T4Ex180403	MAR-PRB	Outlet	14.3	1.05	0.44	NA	0.15	NA	NA	NA
T4Ex180404	MAR-PRB	Outlet	14	1.02	0.39	NA	0.14	NA	NA	NA
T4Ex180405	MAR-PRB	Outlet	14.3	0.90	0.39	NA	0.13	NA	NA	NA
T4Ex180406	MAR-PRB	Outlet	15	1.54	0.43	NA	0.15	NA	NA	NA
T4Ex180407	MAR-PRB	Outlet	15	1.54	0.45	NA	0.15	NA	NA	NA
T4Ex180523	MAR-PRB	Outlet	20	0.99	0.05	1.33	0.09	NA	11.1	16.5
T4Ex180622	MAR-PRB	Outlet	19.9	1.92	0.13	NA	0.09	NA	NA	NA
T4Ex180731	MAR-PRB	Outlet	24.4	0.58	0.69	1.28	0.17	22.6	13.5	25.3
T4Ex180916	MAR-PRB	Outlet	24	2.28	0.02	1.22	0.20	NA	NA	NA
T4Ex181211	MAR-PRB	Outlet	13.60	0.48	0.52	0.70	0.30	NA	NA	NA

Supplementary Table S2. NH_4^+ , NO_3^- , NPDOC and O_2 aqueous concentration (mM) measured at the 3 sampling piezometer depths (NA means data is not available or measured).

Reference	Treatment	Sampling point	Depth	NH_4^+	NO_3^- (mM)	DOC (mM)	O_2 (mM)
T2A3180327	Control	P-A3	0.3-0.4 m	NA	NA	NA	0.03
T2A3180405	Control	P-A3	0.3-0.4 m	NA	NA	NA	0.03
T2A3180511	Control	P-A3	0.3-0.4 m	3.00	0.05	0.79	0.04
T2A3180731	Control	P-A3	0.3-0.4 m	2.72	0.01	1.13	0.06
T2A3181210	Control	P-A3	0.3-0.4 m	1.02	0.21	0.64	0.14
T2B3180327	Control	P-B3	0.3-0.4 m	NA	NA	NA	0.03
T2B3180516	Control	P-B3	0.3-0.4 m	1.81	0.01	NA	0.05
T2B3180525	Control	P-B3	0.3-0.4 m	1.21	0.01	0.66	0.04
T2C3180306	Control	P-C3	0.3-0.4 m	0.20	0.10	0.73	0.02
T2C3180327	Control	P-C3	0.3-0.4 m	NA	NA	NA	0.07
T2C3180523	Control	P-C3	0.3-0.4 m	0.03	0.01	0.79	0.08
T2C3180731	Control	P-C3	0.3-0.4 m	0.01	NA	0.87	0.10
T2C3181210	Control	P-C3	0.3-0.4 m	0.01	0.52	0.55	0.36
T2A2180511	Control	P-A2	0.8-0.9 m	3.00	0.01	0.87	0.03
T2B2180516	Control	P-B2	0.8-0.9 m	1.60	0.04	NA	0.03
T2B2180525	Control	P-B2	0.8-0.9 m	1.15	0.01	0.73	0.03
T2C2180523	Control	P-C2	0.8-0.9 m	1.32	0.04	0.69	0.03
T2A1180511	Control	P-A1	1.3-1.4 m	1.68	0.18	0.70	0.03
T2A1180731	Control	P-A1	1.3-1.4 m	1.37	0.92	1.08	0.07
T2A1181210	Control	P-A1	1.3-1.4 m	0.19	0.40	0.56	0.05
T2A1180405	Control	P-A1	1.3-1.4 m	NA	NA	NA	0.01
T2B1180327	Control	P-B1	1.3-1.4 m	NA	NA	NA	0.01
T2B1180516	Control	P-B1	1.3-1.4 m	0.69	0.04	NA	0.03
T2B1180525	Control	P-B1	1.3-1.4 m	0.58	0.00	0.58	0.03
T2C1180307	Control	P-C1	1.3-1.4 m	1.14	1.70	0.76	0.02
T2C1180327	Control	P-C1	1.3-1.4 m	NA	NA	NA	0.01
T2C1180523	Control	P-C1	1.3-1.4 m	1.03	0.02	0.66	0.02
T2C1180731	Control	P-C1	1.3-1.4 m	1.36	0.31	0.79	0.05
T2C1181210	Control	P-C1	1.3-1.4 m	0.25	1.03	0.43	0.06
T4A3180327	MAR-PRB	P-A3	0.3-0.4 m	NA	NA	NA	0.04
T4A3180405	MAR-PRB	P-A3	0.3-0.4 m	NA	NA	NA	0.03
T4A3180511	MAR-PRB	P-A3	0.3-0.4 m	2.69	0.01	1.86	0.16
T4B3180327	MAR-PRB	P-B3	0.3-0.4 m	NA	NA	NA	0.04
T4B3180516	MAR-PRB	P-B3	0.3-0.4 m	0.60	0.00	NA	0.06
T4B3180525	MAR-PRB	P-B3	0.3-0.4 m	0.72	0.01	1.23	0.04
T4C3180306	MAR-PRB	P-C3	0.3-0.4 m	0.06	NA	0.72	0.09
T4C3180327	MAR-PRB	P-C3	0.3-0.4 m	NA	NA	NA	0.05

Supplementary Table S2. NH_4^+ , NO_3^- , NPDOC and O_2 aqueous concentration (mM) measured at the 3 sampling piezometer depths (NA means data is not available or measured).

Reference	Treatment	Sampling point	Depth	NH_4^+	NO_3^- (mM)	DOC (mM)	O_2 (mM)
T4C3180523	MAR-PRB	P-C3	0.3-0.4 m	0.08	0.01	1.16	0.08
T4C3180731	MAR-PRB	P-C3	0.3-0.4 m	0.01	1.80	1.03	0.15
T4C3181210	MAR-PRB	P-C3	0.3-0.4 m	0.05	0.02	0.71	0.30
T4A3180731	MAR-PRB	P-A3	0.3-0.4 m	2.50	0.15	1.81	0.07
T4A3181210	MAR-PRB	P-A3	0.3-0.4 m	1.09	0.14	0.75	0.12
T4A2180511	MAR-PRB	P-A2	0.8-0.9 m	1.74	0.01	3.51	0.03
T4B2180516	MAR-PRB	P-B2	0.8-0.9 m	1.18	0.06	NA	0.04
T4B2180525	MAR-PRB	P-B2	0.8-0.9 m	1.19	0.00	1.95	0.04
T4C2180523	MAR-PRB	P-C2	0.8-0.9 m	0.73	0.23	2.04	0.06
T4B1180327	MAR-PRB	P-B1	1.3-1.4 m	NA	NA	NA	0.01
T4B1180516	MAR-PRB	P-B1	1.3-1.4 m	1.19	0.10	NA	0.03
T4B1180525	MAR-PRB	P-B1	1.3-1.4 m	0.96	0.06	0.94	0.03
T4C1180307	MAR-PRB	P-C1	1.3-1.4 m	0.91	1.92	2.16	0.02
T4C1180327	MAR-PRB	P-C1	1.3-1.4 m	NA	NA	NA	0.01
T4C1180523	MAR-PRB	P-C1	1.3-1.4 m	0.80	0.03	1.24	0.04
T4C1180731	MAR-PRB	P-C1	1.3-1.4 m	1.43	1.42	1.18	0.04
T4C1181210	MAR-PRB	P-C1	1.3-1.4 m	1.24	1.29	0.79	0.06

Supplementary Table S3. Isotopic fractionation of ammonium ($\varepsilon^{15}\text{N-NH}_4^+$) during recharge episode.

Recharge episode	$\varepsilon^{15}\text{N-NH}_4^+$ [‰] (R^2)	
	SAT-PRB	Control
RE-2	-5 ‰ (0.9)	-7 ‰ (0.8)
RE-3	-5 ‰ (0.5)	-5 ‰ (0.5)
RE-4	-5 ‰ (0.7)	-5 ‰ (1.0)

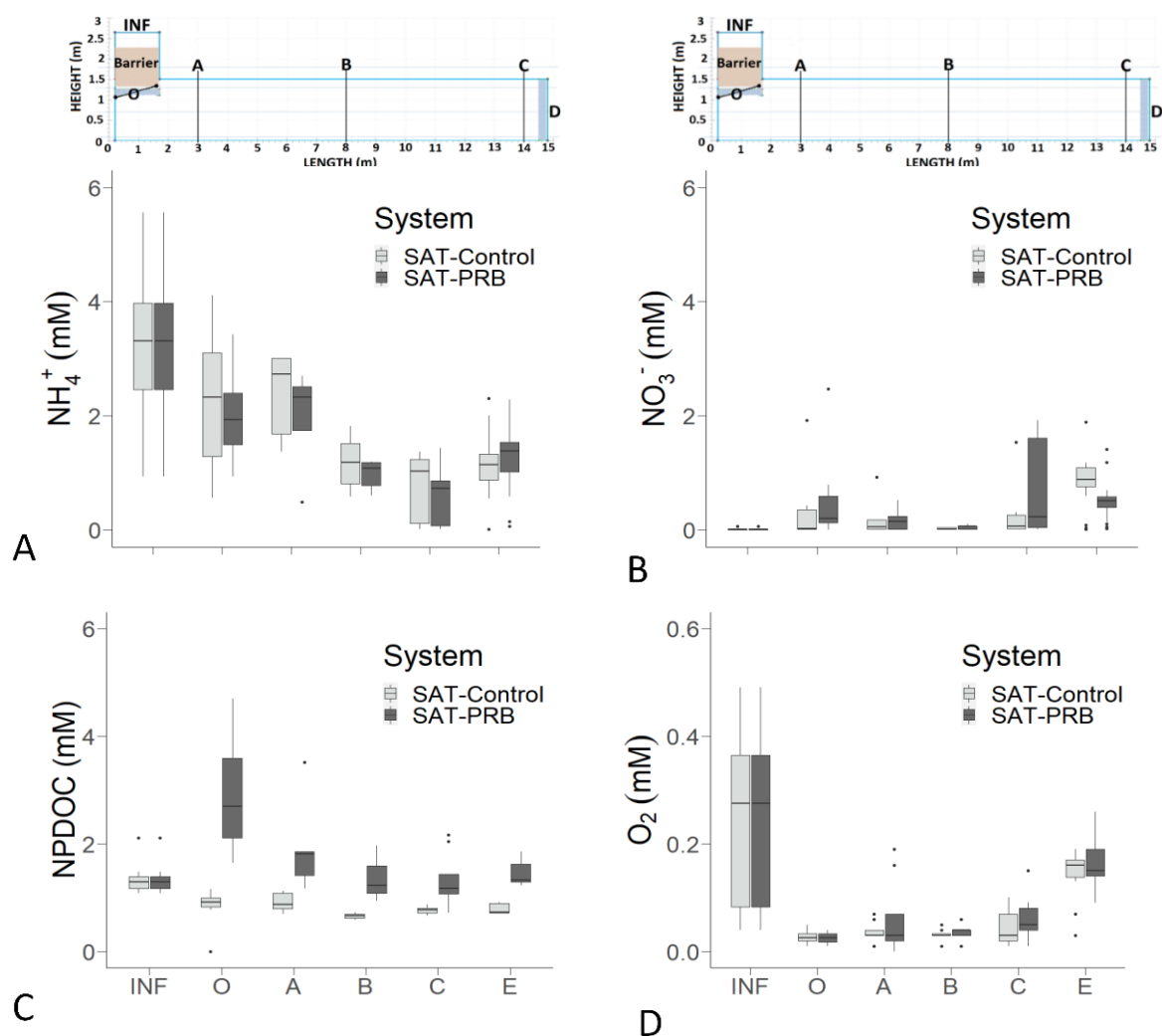


Fig. S1. NH_4^+ , NPDOC, O_2 and NO_3^- aqueous concentration along the flow path in systems (all recharge episodes) and a scheme showing sections along the systems (Concentrations are indicated by the box plots and the outliers represented by the dots, values in A, B, and C, have been calculated using the samples belonging to all the different depths at each section. INF = inflow concentration and D is the outflow concentration).

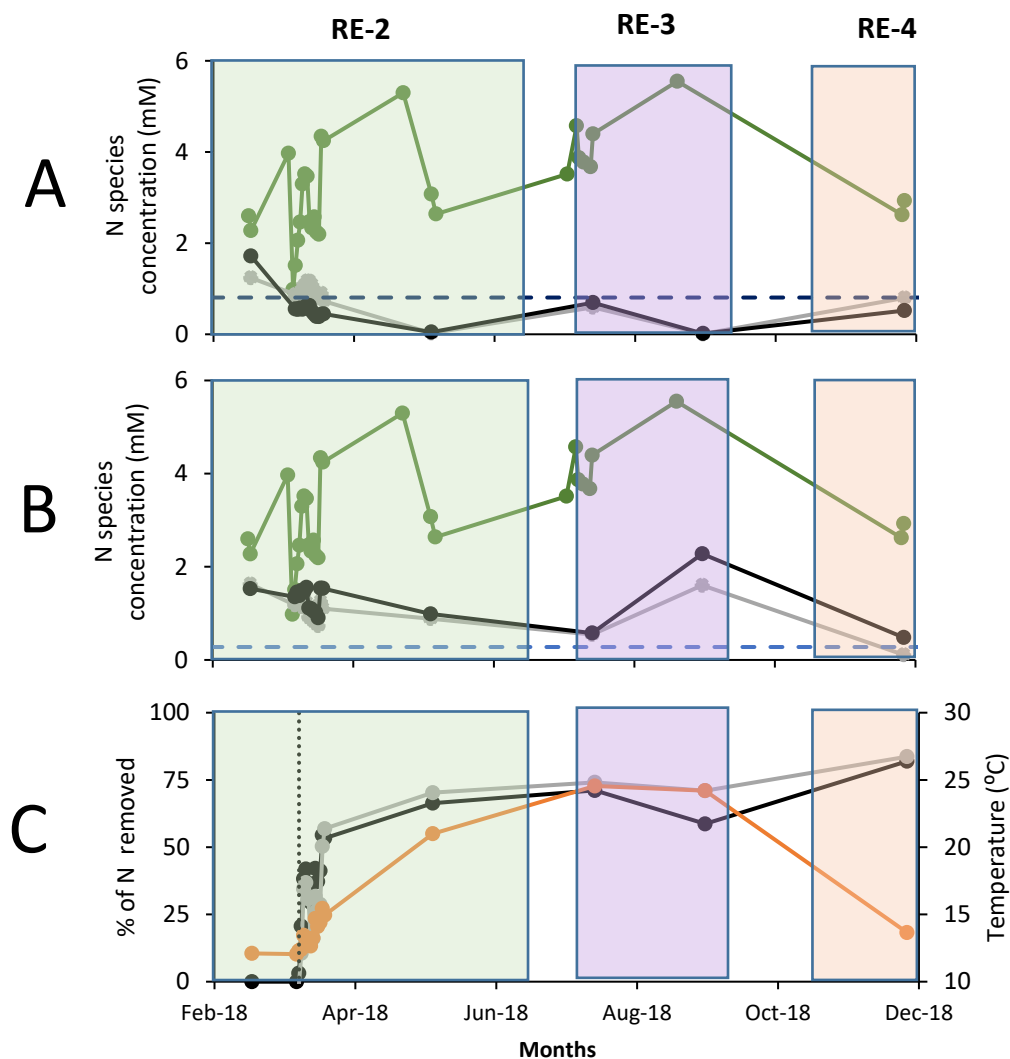


Fig. S2. (A) Distribution of INF NH_4^+ (total N) and residual NO_3^- concentration at E; (B) distribution of INF NH_4^+ and residual NH_4^+ concentration at E; (C) the % of N ($\text{NH}_4^+ + \text{NO}_3^-$) removed from solution and the average temperature in both systems. Grey circles represent the Control system, black circles represent SAT-PRB system, green circles represent INF NH_4^+ and orange circles represent the average temperature in both systems. The dashed lines represent NO_3^- threshold value (figure A) and NH_4^+ threshold value (figure B) and the dotted line represents the point below which we have zero N removal from the systems (figure C).

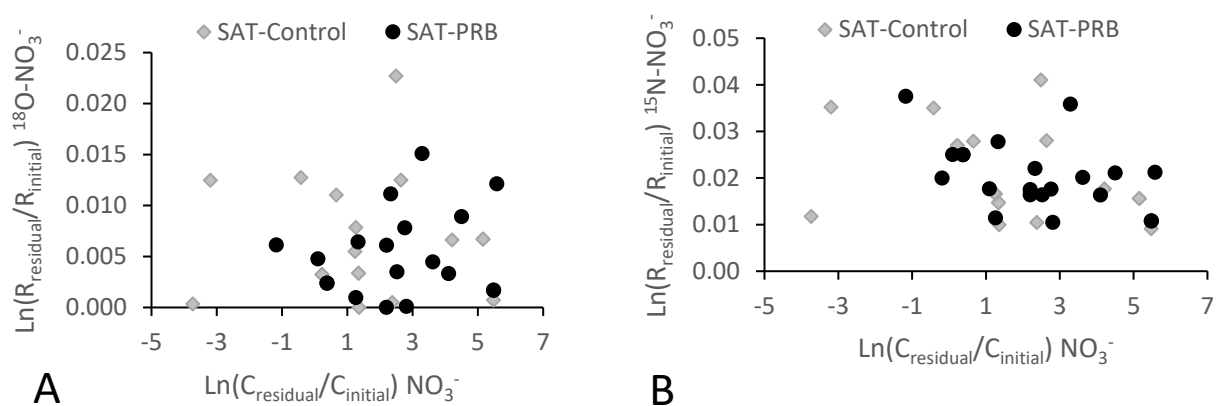


Fig. S3. Evolution $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ versus $[\text{NO}_3^-]$ in SAT-Control and SAT-PRB systems (A and B).

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PHD THESIS

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MAY 2024



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