Relevance of spatio-temporal heterogeneities in modelling geomicrobial reactive systems

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Zusammenfassung

Der unter der Erde liegende Bereich der kritischen Zone ist nicht nur einer der größten Kohlenstoff- und Stickstoffspeicher, sondern bietet auch 95% der weltweit geschätzten mikrobiellen Biomasse (Bakterien) einen Lebensraum. Darüber hinaus ist der Untergrund reich an mikrobieller Arten- und Funktionsvielfalt, wobei die Mikroorganismen eine Vielzahl von Möglichkeiten nutzen, um Kohlenstoff und Stickstoff aus ihrer Umgebung im Untergrund zu verstoffwechseln und aufzunehmen. Dies ist besonders herausfordernd, da der Untergrund ein oligotropher, d.h. produktionsarmer, weil energielimitierter Lebensraum ist. Insgesamt haben Mikroben ein großes Potenzial die globalen Kohlenstoff- und Stickstoffkreiläufe zu beeinflussen. Ihr Beitrag zu diesen globalen Kreisläufen ist jedoch kaum untersucht.

Die Abschätzung des mikrobiellen Beitrags an den Kohlenstoff- und Stickstoffkreisläufen im Untergrund ist nicht trivial, da der Untergrund nur begrenzte Beobachtungsmöglichkeiten bietet, räumlich heterogen ist und auf die zeitliche Dynamik der Umwelteinflüsse an der Oberfläche reagiert. Die räumliche Heterogenität des Untergrunds führt zu heterogenen Wasserflüssen, die die Verteilung der mikrobiellen Biomasse und deren Aktivität beeinflussen. Nicht nur der Wasserfluss im Untergrund, sondern auch die mikrobielle Aktivität reagiert weiterhin auf die zeitliche Dynamik der Oberflächensignale. Trotz einer Vielzahl von Studien über diese Dynamik ist die Bedeutung der räumlichen Heterogenität und der zeitlichen Dynamik für die Kohlenstoff- und Stickstoffumsatzraten noch unbekannt. Dies erschwert die Planung von Ort und Zeit der Probennahmen, die Interpretation von Felddaten und die Konzipierung geeigneter Modellierungsstudien.

Ziel dieser Arbeit ist es, die oben genannten Forschungslücken zu schließen, indem der Einfluss der räumlichen Heterogenität auf die räumliche Verteilung von

chemischen Stoffen und mikrobieller Biomasse bestimmt und so die Auswirkungen auf den Kohlenstoff- und Stickstoffumsatz im heterogenen Untergrund bewertetet werden. Die Forschungshypothesen lauten dabei wie folgt:

H1. Räumliche Heterogenität führt zu Nischen für mikrobielle Arten, die so mit anderen konkurrierenden Arten koexistieren.

H2. In räumlich heterogenen Systemen findet ein geringerer reaktiver Stoffumsatz statt als in vergleichbaren homogenen Systemen.

H3. Zeitliche Dynamik führt zu einem variierenden Nährstoffaustrag aus dem System, welcher wiederum eine Funktion der variierenden Verweilzeit im System ist.

H4. Räumliche und zeitliche Heterogenität interagieren und verstärken sich gegenseitig in ihren Auswirkungen auf das System. Eine stärkere zeitliche Dynamik in räumlich heterogenen Systemen führt zu größeren Unterschieden im Vergleich zu homogenen Systemen mit einheitlichen Bedingungen.

In meiner Arbeit habe ich numerische Modellierung verwendet, um diese Hypothesen anhand von verschiedenen Modellszenarien übergreifend zu testen. Im ersten Schritt konzipierte ich ein Reaktionsnetzwerk, das verschiedene mikrobielle Stoffumsatz- und Wachstumsstrategien sowie weitere Prozesse der Interaktion der Mikroben mit Ihrer heterogenen Umwelt abbildet. Nachfolgend entwarf ich ein Konzept für die Bestimmung der Eigenschaften der Simulationsdomäne, so dass sie je nach Bedarf repräsentativ für ungesättigte und gesättigte Zonen des Untergrunds ist. Für die porösen Medien dieser Simulationsdomäne wurde die räumliche Heterogenität mittels zweier Parameter initialisiert: Varianz und Anisotropie der räumlichen Verteilung sowohl von der log-transformierten Permeabilität (ungesättigte Zone) als auch von der log-transformierten hydraulischen Leitfähigkeit (gesättigte Zone). Anschließend setzte ich diese homogenen und heterogenen Medien einer zeitlichen Dynamik aus, bei der der Wasserfluss (durchschnittliche Wassergeschwindigkeit im System) täglich variierte. Alle Systeme wiesen im

Durchschnitt dieselben Eigenschaften auf. So war die durchschnittliche Fließgeschwindigkeit des Wassers über den gesamten instationären Simulationszeitraum in allen heterogenen und homogenen Medien sowohl in den gesättigten als auch in den ungesättigten Medien identisch. Darauf aufbauend quantifizierte ich die Auswirkungen der räumlichen Heterogenität auf die mikrobielle Aktivität und den Nährstoffkreislauf unter gesättigten und ungesättigten Bedingungen sowie die Auswirkungen der zeitlichen Dynamik in gesättigten Verhältnissen. Durch den Vergleich zu homogenen Medien und stationären Bedingungen testete ich meine Hypothesen.

Die Modellergebnisse zeigten, dass im Untergrund eine Kombination aus räumlicher Heterogenität und bestimmten Strömungsverhältnissen den Zugang der Mikroben zu Kohlenstoffsubstrat, Nährstoffen und Energiegradienten bestimmt. Abhängig von dem Transportprozess, der den konservativen Transport gelöster Stoffe bestimmte, wirkte sich die räumliche Heterogenität unterschiedlich auf das Verhalten reaktiver Stoffe aus. Meine Ergebnisse ergaben, dass Hypothese H1 in allen untersuchten reaktiven Systemen korrekt war, während Hypothesen H2 und H3 nur in ausgewählten reaktiven Systemen korrekt waren. Hypothese H4 erwies sich als falsch.

Diese Arbeit liefert Erkenntnisse darüber, wie sich mikrobielle Gemeinschaften im räumlich heterogenen Untergrund organisieren und wie sich diese Gemeinschaften unter zeitlich dynamischen Bedingungen verändern können. Um ein allgemeines, aber dennoch vorhersagbares Verhalten eines Systems zu beschreiben, war ein Kriterium unerlässlich, welches die Verweilzeit eines nichtreaktiven Tracers im System und den (durch Feldbeobachtungen) geschätzten Stoffumsatz der chemischen Spezies im System berücksichtigt. Das Verhältnis (Damköhler-Zahl, Da) dieser beiden Größen klassifiziert das System dabei als reaktions- oder strömungsdominant. Bei reaktionsdominanten Systemen wirkt sich die räumliche Heterogenität nur begrenzt auf den Umsatz von Kohlenstoff und Stickstoff aus, während bei strömungsdominanten Systemen der Einfluss deutlich stärker ist. Daher

ist es sinnvoll, die statistischen Verteilungen der Verweilzeiten in den zu untersuchenden natürlichen Systemen abzuschätzen, um das vorherrschende reaktive System zu bestimmen und dann das Ausmaß der zu erwartenden räumlich-zeitlichen Variation bei Feldbeobachtungen abzuschätzen.

Durch die Identifizierung reaktiver Systeme, die empfindlich auf eine räumlich-zeitliche Heterogenität reagieren, konnte die Unsicherheit der Modellergebnisse abgeschätzt werden. Außerdem konnten Feldbeobachtungen im Zusammenhang mit der Heterogenität der Geologie interpretiert werden. Dabei ist zu beachten, dass Feldbeobachtungen bereits von der gegebenen räumlichen Heterogenität der Geologie beeinflusst sind (und nicht wie hier aus einem Modellsystem mit angenommener hypothetisch homogener Geologie stammen). Die aus den Feldbeobachtungen abgeleiteten Parameter des Reaktionsnetzes haben daher eine entsprechende Wahrscheinlichkeitsverteilung. Diese Verteilung kann anhand der in dieser Arbeit vorgeschlagenen Beziehungen abgeleitet werden, in dem man den Verbrauch von Kohlenstoff und Stickstoff in verschiedenen räumlich heterogenen und unterschiedlich gesättigten Bereichen vergleicht. Die Übertragung von aus dem Feld abgeleiteten Reaktionsratenparametern auf andere Standorte, auf Laborbedingungen oder umgekehrt muss natürlich mit Vorsicht erfolgen. Jedoch legt diese Arbeit den Grundstein für künftige methodische Arbeiten zur Übertragung solcher Parameter zwischen verschiedenen Systemen und unterschiedlichen Skalen. Auf diese Weise hat diese Arbeit nicht nur dazu beigetragen, die Unsicherheit beim Kohlenstoff- und Stickstoffaustrag aus reaktiven unterirdischen Systemen zu quantifizieren, sondern auch die Skalierung der effektiven Ratenausprägung über verschiedene Standorte und räumliche Skalen hinweg zu unterstützen.

Der Ansatz, das reaktive System anhand von leicht abschätzbaren Indikatoren zu identifizieren, kann bei der Hochskalierung der effektiven Ratenausdrücke helfen. Modellierer müssen die Berücksichtigung oder Vernachlässigung von räumlicher Heterogenität und zeitlichen Dynamiken in jeder Studie neu begründen. Sie benötigen ebenfalls effektive Ratenausdrücke oder Ratenmodifikatoren für die

Implementierung auf größere Skalen. Diese Ratenmodifikatoren, die subskalige Heterogenitäten berücksichtigen, können dann in das Reaktionsnetzwerk integriert werden, um den reaktiven Transport auf größeren Skalen zu simulieren. So kann die Genauigkeit der Modellergebnisse beibehalten und gleichzeitig der Bedarf an Rechenressourcen verringert werden. Dies kann zu einer schnellen und genauen Vorhersage des Nährstoffaustrags in unterirdischen Systemen beitragen. Dieser Ansatz kann auch auf wirtschaftlich relevante Skalen wie die Einzugsgebietsskala übertragen werden.

Der in dieser Arbeit verwendete Ansatz ist anwendbar, übertragbar und in geeigneter Weise auf verschiedene Studien und Standorte skalierbar. Ein ganzheitliches Verständnis der mikrobiellen Gemeinschaften, ihrer Aktivität und ihres Beitrags zum Kohlenstoff- und Stickstoffkreislauf im Untergrund kann dazu beitragen, eine entscheidende Wissenslücke im globalen biogeochemischen Budget zu schließen. Darüber hinaus kann es auch dazu beitragen, das Verhalten heterogener reaktiver Systeme unter zeitlich dynamischen Bedingungen vorherzusagen, was zu einem besseren Verständnis der Ökosystemdienstleistungen führt. Dies wiederum wird dazu beitragen, den Zugang zu Wasser für die Trinkwasserversorgung, die Bewässerung und die Industrie zu sichern, was uns in der ungewissen Zukunft des Klimawandels unterstützen wird.

Summary

The Earth's Critical Zone extends from the top of the canopy till the deep subsurface. Carbon and nitrogen transform between different oxidation states and move between different ecosystems in the Critical Zone. Biogeochemical cycles describe this movement of carbon and nitrogen, and microbial species play a significant role in the natural processes that govern these biogeochemical cycles.

The subsurface compartment of the Critical Zone is not only one of the biggest stores of carbon and nitrogen, but also provides habitat for 95% of the global estimates of microbial biomass. The subsurface is also inhabited by functionally diverse microbial communities with microbial species using a variety of pathways to metabolize and take up carbon and nitrogen from their environment. They are especially creative since the subsurface is an oligotrophic, that is, energy limiting, environment. Thus, microbial species have a large potential to influence global carbon and nitrogen cycles. But their contribution to these global cycles on a global scale is not yet estimated.

The assessment of microbial contribution to carbon and nitrogen cycles in the subsurface is complicated as the subsurface provides limited observational opportunities, is spatially heterogeneous and responds to surficial temporal dynamics. The spatially heterogeneous subsurface results in heterogeneous water flux, thus influencing the distribution of microbial biomass and activity. The subsurface also exhibits temporal dynamics and responds to surface signals, with subsurficial microbial activity also varying in response to these temporally dynamic surface signals. Despite a plethora of studies on these dynamics, the relevance of sub-scale spatial heterogeneities and temporal dynamics with respect to bulk carbon and nitrogen cycling rates is not fully understood. This has strong implications for locating

and timing of sampling events, for interpreting field data, as well as for formulating suitably modelling studies.

In this work, I sought to address this gap by deriving the effect of spatial heterogeneity on the distribution of chemical species and microbial biomass, thereby assessing the impact on bulk consumption of carbona and nitrogen in the system. My research hypotheses are as follows:

H1. Spatial heterogeneity results in niches for microbial species to co-exist with other competitive species.
H2. Spatial heterogeneity results in lower consumption of reactive species in the system than expected in a homogeneous system.
H3. Temporal dynamics results in varying nutrient discharge from the domain, and this is a function of varying residence time in the domain.
H4. Spatio-temporal heterogeneities interact and result in compounding each other's effects on the system. Higher temporal dynamics in high spatially heterogeneous domains behave the most different from homogeneous domains in uniform conditions.

I used a numerical modelling approach to investigate a variety of scenarios to form a comprehensive study to explore the above-mentioned hypotheses. I conceptualized a reaction network capturing various respiration and growth strategies, and other microbial life processes, and a simulation domain representative of unsaturated and saturated zones of the subsurface. For the simulation domain, I subsequently initialized spatially heterogeneous domains using two parameters: Variance in log permeability field (in the unsaturated domain) or in log hydraulic conductivity field (in the saturated domain), and anisotropy. Next, I subjected these homogeneous and heterogeneous domains to temporal dynamics wherein the water flux (average water velocity in the system) varied on a daily basis. Since all the systems displayed the same average properties, i.e., same average water flow velocity in all the heterogeneous and

homogeneous media in both saturated and unsaturated domains, as well as same water velocity when averaged over the entire transient simulation period, I quantified the impact of spatial heterogeneity on microbial activity and nutrient cycling in the saturated and unsaturated zones and quantified the impact of temporal dynamics in the saturated zone. Thus, I tested my hypotheses with respect to homogeneous domains and uniform flow conditions.

In the subsurface, a combination of spatial heterogeneity and flow regime governed the access of microbes to carbon substrate, nutrients and energy gradients. The same domain with different dominant flow processes (diffusion v/s dispersion v/s advection) of a conservative solute responded differently to spatial heterogeneity. The results of the studies **established Hypothesis H1 to be correct in all possible reactive systems, while hypotheses H2 and H3 were correct in selected reactive systems only. Lastly, hypothesis H4 was proven to be false.**

This work provided insights into how microbial communities organize in space in the spatially heterogeneous subsurface, and how these communities may change in temporally dynamic conditions. To formulate a general yet predictive understanding of any system, a criterion that considered the residence time of a conservative tracer in the system and estimated bulk consumption of the chemical species in the system (through field observations) was essential. Their ratio (Damköhler number, Da) categorized the system to be reaction dominant or flow dominant. Reaction dominant systems exhibited limited impact of spatial heterogeneity on bulk carbon and nitrogen consumption, while flow dominant systems did. Thus, it is useful to estimate travel time distributions in the natural systems of interest to categorize the prevalent reactive system, and then to estimate the extent of spatio-temporal variation to expect in field observations.

By identifying reactive systems that were sensitive to spatio-temporal heterogeneities, the uncertainty in model outcomes could be estimated. Additionally, field observations could be interpreted in context of the heterogeneity of the

geological material. It must be noted that field observations already result from the existing spatial heterogeneity of the geological material (not from an assumed/hypothetical homogeneous geological material). Thus, any reaction network parameters that are derived from these field observations have an associated probability distribution. This distribution may be derived using the relationships proposed in this thesis comparing consumption of carbon and nitrogen in different spatially heterogeneous and variably saturated domains. While transferring field derived reaction rate parameters across sites or even to the lab or vice versa must be done with care, this thesis lays the foundation to future work on the methodology to transfer such parameters between different systems at different spatial scales. In this way, this thesis not only assisted in quantifying uncertainty in carbon and nitrogen discharge from reactive sub surficial systems, but also assisted in scaling effective rate expression across different sites and spatial scales.

The approach of identifying the reactive system of interest using easily estimable indicators can assist in upscaling effective rate expressions and modelling reactive transport at field or regional scales where the modelling community needs to carefully consider the use or rejection of sub-scale spatial heterogeneities and temporal dynamics in each study. This can assist in rapid and accurate predictions of nutrient discharge in subsurface systems. This approach can be upscaled to even policy-relevant scales such as the catchment scale.

The approach used in this thesis is applicable, transferrable, and suitably scalable across different studies and sites. A holistic understanding of microbial communities, their activity, their contribution to carbon and nitrogen cycling in the subsurface can help in filling a critical gap in the global biogeochemical budgets. Not only this, but it can also assist in forming a predictive understanding of the behavior of heterogeneous reactive systems in temporally dynamic conditions which results in an improved understanding of ecosystem services. This, in turn, will help to secure access to water for drinking, irrigation and industry, thus supporting and propelling us forward into the uncertain future of climate change.

सुखं वा यदि वा दुःखं प्रियं वा यदि वाप्रियम्.।

प्राप्तं प्राप्तमुपासीत हृदयेनापराजितः।। शान्ति १७४.३९।।

Happiness or suffering, the pleasant or the unpleasant, whatever comes, receive it with respect, and never feel defeated in your heart.

Badrinath Chaturvadi. Fate or Human Endeavour? The Question of Causality. The Mahãbhãrata: An Inquiry in the Human Condition.

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Table of Contents

Tables

Figures

Abbreviations and Symbols

µM	micromolar
µM C	micromolar Carbon
C	Concentration
D	Dimension
Da	Damköhler number
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
fg	femtogram
L	litre
m	metre
Pe	Peclet number
POC	Particulate organic carbon
Q	Discharge
TOC	Total organic carbon
ZW	zeptowatts

1 General Introduction

All the compartments of the Earth's Critical Zone (extending from the top of the vegetation canopy to aquifers in the bedrock (Giardino and Houser, 2015;Küsel et al., 2016) are connected by water flux. The movement of carbon and nitrogen, on which all life on earth depends, between different oxidation states and different ecosystems in the Earth's Critical Zone (Fig 1.1) is controlled by water flux and is captured in biogeochemical cycles. Microbial species play a significant role in the natural processes that govern these biogeochemical cycles (Falkowski et al., 2008). These microbial species are ubiquitous, having been identified in various environments, from the land surface where most of macro life exists, to extreme environments such as deep in the subsurface, oceanic sediments, and permafrost/polar regions, contributing approximately 15% to the total biomass on Earth (Bar-On et al., 2018).

The subsurface of the Critical Zone may further be categorized as shallow and deep (Fig 1.1). The shallow subsurface includes soil and the root zone, extending up till 8 meters (m) depth. The deep subsurface includes the vadose zone, or the variably saturated zone between soil and permanently saturated zone in the subsurface, and aquifers, or the permanently saturated zone in the subsurface. While 10% of the global estimates of microbial biomass inhabit the shallow subsurface, 85% of microbial biomass inhabits the deep subsurface, with the rest in marine environments (Bar-On et al., 2018). Recent inventories have also revealed the soil and deeper subsurface compartments account for almost 50% of the global carbon budget, and the subsurface is also one of the biggest storage compartments of nitrogen (McMahon and Parnell, 2014;Schlesinger and Andrews, 2000).

The presence of microbial life in the subsurface is not a recent discovery (Kotelnikova, 2002), but still astounding as the deep subsurface is predominantly oligotrophic (i.e., energy limiting) (Lever et al., 2015). With improved molecular

techniques, the subsurface has been found to be rich in microbial diversity and function (Ghiorse, 1997;Pace, 1997;Sogin et al., 2006) with microbial species using a variety of pathways to metabolize and take up carbon and nitrogen from their environment in the subsurface. Microbial species primarily require an energy gradient (LaRowe and Amend, 2015a), carbon source and other nutrients such as nitrogen and phosphorus to survive. Exploiting the energy gradient via a redox reaction using oxygen, nitrate, or any other available electron acceptor, microbial species can execute a variety of life processes. These life processes include growth, maintenance (repairing biomolecules), reactivation from dormant states etc. To allow for growth, microbial species also require a carbon source, either organic matter for heterotrophic growth or inorganic carbon for autotrophic growth. Both mechanisms of growth have been discovered in the subsurface (Anantharaman et al., 2016;Herrmann et al., 2015;Konhauser et al., 2011;Lam and Kuypers, 2011;Rivett et al., 2008;Zhang et al., 2013). Additionally, aerobic respiration, nitrate reduction, iron oxidation, ammonia oxidation, sulphate reduction account for most of the respiration pathways, and microbial species of different functional capacities interact with each other as well (Anantharaman et al., 2016). Thus, microbial species in the subsurface have a large potential to influence global carbon and nitrogen cycles but their contribution to these cycles on a global scale is not yet estimated.

The assessment of microbial contribution to carbon and nitrogen cycles in the subsurface is complicated as the subsurface provides limited observational opportunities, is spatially heterogeneous and responds to surficial temporal dynamics. The subsurface (soil, the vadose zone and aquifers) is heterogeneous at all length scales (Desbarats, 1987). Ecologically, heterogeneity refers to dissimilar or diverse constituents of a system, while statistically, it refers to the degree of similarity of two distributions (Dutilleul and Legendre, 1993). On the other hand, spatial heterogeneity refers to spatial pattern of the property of concern (Dutilleul and Legendre, 1993), and it is well-studied in geospatial analysis (Jiang, 2015).

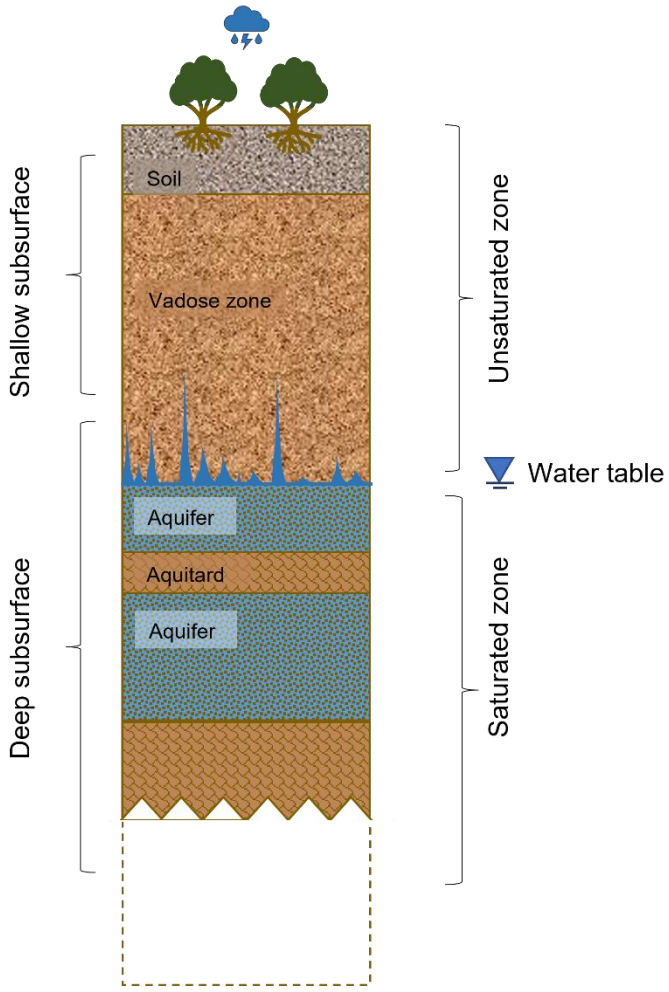

Figure 1.1 Schematic diagram of the subsurface compartment of the Earth's Critical Zone.

Spatially heterogeneous properties of the subsurface result from multiple geological factors such as geologic formation process, geologic materials, chemical composition, and physical structure. These properties include permeability, i.e., the ability of the medium to transmit fluids (Amyx et al., 1960), and porosity, i.e., the ratio of volume of void space and bulk volume of the medium, (Amyx et al., 1960), and influence the flow of water in the subsurface. Measurement of these properties in the laboratory or in-situ ignores sub-sampling scale heterogeneities (Berkowitz, 2002). These properties are then described as Gaussian process models (Gelhar and Axness, 1983;Johnson and Dreiss, 1989;Webb and Andersen, 1996), making use of a limited

parameter set: Mean of the distribution, variance in the distribution, anisotropy (degree of channelization), and spatial distance (Berkowitz, 2002;Dagan et al., 2003;Delhomme, 1979;Heße et al., 2014;Jiang, 2015;Kitanidis and Vomvoris, 1983;Rodrigo et al., 2002;Zimmerman et al., 1998). A domain of low variance in permeability and low anisotropy would be similar to a uniform layer of alluvial deposits (Davis et al., 1997;Johnson and Dreiss, 1989). In contrast, high variance and high anisotropy would be similar to a domain with clay lenses interspersed with sandy aquifers, or a domain with fractured bedrock.

This spatial heterogeneity of the subsurface results in the heterogeneous distribution of water and water flux in the subsurface, having strong implications for variability in subsurface microbial and nutrient dynamics (Cole et al., 2007;Harden et al., 1997;Holt, 2000;Küsel et al., 2016;Murphy et al., 1997;van Leeuwen, 2000).

The subsurface also responds to temporally dynamic external forcing such as weather events (Nippgen et al., 2015;Robinson et al., 2009;Yabusaki et al., 2008). Similar numerical methods also exist for describing temporal dynamics of external forcing which can take multiple forms. These forcing could be changing nutrient input from the surface or changing water flux from the surface (De Castro Ochoa and Muñoz Reinoso, 1997), or both. For example, precipitation events may be predicted using Poisson distributions (Rodriguez-Iturbe et al., 1999) using a limited set of parameters (average number of events occurring within a time interval) to describe region or hydrological regime of interest. Precipitation also varies both in form (e.g., snowfall and rainfall) and in intensity across different hydrological regimes. This results in different patterns and rates of infiltration into the shallow subsurface, further confounded by several factors including land use, land cover, slope, soil type, soil structure, root uptake, and evapotranspiration (Corradini et al., 2000;Lei et al., 2006;Lundberg et al., 2016;Okkonen et al., 2010;Pohl et al., 2006;Rascón-Ramos et al., 2021;Rasiah et al., 2007;Seyfried, 1991;Wang et al., 2008;Xue and Gavin, 2008).

Deeper in the subsurface, groundwater also exhibits temporal dynamics. Groundwater elevation (or water table in unconfined aquifers) varies on a diurnal scale since it depends on temperature and pressure. On a seasonal scale, recharge from the shallow subsurface raises the water table surface (De Castro Ochoa and Muñoz Reinoso, 1997), thus potentially increasing the hydraulic gradient in the domain.

Temporal dynamics in infiltration patterns and water flux further impact groundwater and surface water quality (Okkonen et al., 2010). Coupling temporal dynamics with spatial heterogeneity of the subsurface, a subsequent impact on microbial activity, carbon and nitrogen cycles at the lab or field scale has also been observed (Benk et al., 2019;Hofmann et al., 2020;Lohmann et al., 2020;Schwab et al., 2017;Zhou et al., 2012), giving rise to hot spots and hot moments. Hot spots and hot moments refer to points in space and in time where microbial activity is higher than the average, and higher with respect to its neighboring areas (Kuzyakov and Blagodatskaya, 2015;Parkin, 1987).

Varying groundwater head or incoming water flux results in varying water flux through the subsurface, thereby influencing chemical species distribution (Jacques et al., 2008), nutrient take-up (Gross et al., 2020), carbon turnover/discharge (Pett-Ridge et al., 2013;Pronk et al., 2020;Rezanezhad et al., 2014), subsequently impacting the physicochemical characteristics of groundwater (Zheng et al., 2019) and even the water quality of the receiving surface water bodies (Basu et al., 2010). It also results in varying microbial community structures (Zheng et al., 2019;Zhou et al., 2012) with different functional guilds dominant in recharge/high flow periods and others in recession/low flow periods (Lohmann et al., 2020;Zhou et al., 2012). Alternatively, studies suggest that microbial community structures are relatively stable (Rezanezhad et al., 2014) in regularly fluctuating redox conditions in soil as they are well-adapted to these conditions (Pett-Ridge et al., 2013).

To conclude, geomicrobial communities are complex with multiple respiration and growth strategies, even in the deep subsurface (Anantharaman et al., 2016;Christensen et al., 2001;Eilers et al., 2012;Stegen et al., 2012), and also respond to surface signals, e.g. with changing microbial activity in different seasons per prevailing flow regime, dissolved organic matter (DOM) quality (Hofmann et al., 2020;Lohmann et al., 2020;Zhou et al., 2012). Given the complexity of the response of heterogeneous natural systems to temporal dynamics, it is difficult to predict the impact of spatio-temporal heterogeneities on microbial redox dynamics in the subsurface, and carbon and nitrogen cycling thereof. In this thesis, I aim to fill this gap.

1.1 State of the art

Spatial heterogeneity in the vadose zone, and complexity of the capillary fringe influence water and solute transport (Berkowitz et al., 2004;Vogel et al., 2018). The presence and nature of DOM in soil is linked with prevailing hydrologic conditions (Bol et al., 2015;De Troyer et al., 2014;Klotzbücher et al., 2014;Van Gaelen et al., 2014), implying that water flux and associated degree of saturation controls solute transport and access to nutrients, and thereby microbial activity and nutrient export to the groundwater.

While transport processes are dependent on the degree of saturation, microbial kinetics are dependent on access to substrate and electron donor (Manzoni et al., 2016;Michaelis and Menten, 1913), which are in turn dependent on saturation/hydration profile (Ebrahimi and Or, 2015;Golparvar et al., 2021;Leinemann et al., 2016). Being a spatially heterogeneous medium, the subsurface does not allow for the water flux to be homogeneously distributed (Levy and Berkowitz, 2003;Schincariol and Schwartz, 1990). Soils and the subsurface are also dominated by preferential flow paths (Gerke, 2006;Kitanidis and Vomvoris, 1983). Microbial species are also not distributed homogeneously in the subsurface

since microbial respiration and growth is dependent on the availability of suitable carbon sources and energy gradients, access to which is governed by water flux. These spatial variations in microbial activity give rise to microbial hot spots. Similarly, temporal variations in microbial activity (and carbon and nitrogen cycling thereof) may result in hot moments.

Microbial hotspots have been hypothesized to exist in soil aggregates (Sexstone et al., 1985), and in preferential flow paths (Bundt et al., 2001). A higher rate of degradation of chemical species (20% higher than that of bulk material) by microbial species has been attributed to such preferential flow paths and macropores in soil (Pivetz and Steenhuis, 1995), while Bundt et al. (2001) found that microbial biomass in preferential flow paths can be almost twice the amount detected in bulk soil matrix. Subsequent studies have revealed coefficient of variation of microbial activity in soil cores to be higher than 20% (Raynaud and Nunan, 2014;Pallud et al., 2004;Dechesne et al., 2005;Gutiérrez Castorena et al., 2016).

A higher microbial biomass along preferential flow paths could be attributable to leveraging a variety of energy gradients and carbon substrates (Bundt et al., 2001). Alternatively, it could be a result of access to optimum conditions such as soil moisture and access to oxygenated water (Franklin et al., 2019;Or et al., 2007): Microbial activity initially increases with better access to nutrients with rise in moisture (till ~ 30% soil moisture (Barros et al., 1995), up to 50% (Schjønning et al., 2011)), and subsequently decreases due to limited gaseous diffusion at higher moisture content. Other aspects controlling microbial activity in soil include salinity, osmotic pressure, and temperature (Or et al., 2007). More recently, microbial activity has also been shown to influence the soil matrix and its structure (Li et al., 2018;Morales et al., 2010) thus providing a feedback loop between spatially heterogeneous subsurface and microbial activity. Thus, spatial heterogeneity influenced the distribution of biomass into active/dormant fractions (Couradeau et al., 2019;Grösbacher et al., 2018) with 60-80% of the soil microbial biomass estimated to be dormant (Lennon and Jones, 2011).

Data on the active/inactive fractions of microbes in the deeper subsurface and in aquifer systems is scarce. But it has been hypothesized that microbial life is hypothesized to exist in a non-growing state, or semi-active to dormant state, carrying out only the most fundamental life processes required to exist in extremely energy limiting environments such as deep below the oceanic sub-seafloor where the energy gradient available to microbial life can be as small as 10-21 zeptowatts cell^{-1}(ZW cell^{-1}) (LaRowe and Amend, 2015b). Assessing the contribution of microbial life to carbon and nitrogen cycles is complicated by these varying states of activity (active growing stage, to solely respiring, to some stage of dormancy) of microbial life in the subsurface (Stolpovsky et al., 2011;Bradley et al., 2018).

Higher microbial activity along preferential flow paths and in spatial heterogeneous subsurface is also attributable to higher microbial diversity (Horner-Devine et al., 2004) in the shallow subsurface (soil studies). But microbial diversity decreases with increasing depth (distance from the surface), potentially due to the lack of diversity of bioavailable nutrients (Du et al., 2021;Griebler et al., 2010;Humphreys, 2009;Lin et al., 2012).

Microbial and functional diversity in the subsurface have also been observed to vary in time (Blazewicz et al., 2020;Freimann et al., 2014;Hofmann et al., 2020;Schwab et al., 2017;Zhou et al., 2012). Seasonal changes such as the freeze-thaw cycle have been known to impact the physical properties of soil, thereby influencing water flux (Hayashi, 2013). Seasonal changes also result in higher microbial activity in coordination with higher substrate input from the surface (Zhou et al., 2012), changing chemical nature of DOM and translocation of younger and more labile DOM to the deeper subsurface (Benk et al., 2019). Microbial community structure also changes with season year on year, with anaerobic respiration pathways prevalent during wet periods (Lohmann et al., 2020). At the same time, extreme weather events have also been known to result in bypass-flows in variably saturated zones and reach the saturated zone depending on the geology of the site (McMillan and Srinivasan, 2015;Renck and Lehmann, 2004) perhaps resulting in no impact or reduced

microbial activity due to translocation of microbes (Yan et al., 2021). For example, increased water flux resulted in higher reduction processes in homogeneous and vertically stratified soil columns, while it resulted in higher transport in macropores (Arora and Mohanty, 2017). With weather events influencing water flux in the subsurface, there is a cascading effect on soil seepage and groundwater quality, and nutrient access of microbial species. Arora and Mohanty (2017) thus displayed that this effect is dependent on the structure of spatial heterogeneity of the matrix. At the same time, Schwab et al. (2017), Zhou et al. (2012) and Hofmann et al. (2020) linked variable diversity of microbial communities in the deep subsurface with variation of the groundwater quality in space and time, thus implying that spatio-temporal heterogeneities influenced the distribution of microbial biomass into mobile/immobile fractions (Griebler and Lueders, 2009;Grösbacher et al., 2018).

While these phenomena are of great interest and have been studied (as detailed earlier), observational opportunities in the field are limited. This constraint makes modelling studies the approach of choice to monitor and predict geomicrobial activity in the spatio-temporally heterogeneous subsurface (Molins et al., 2014). While seasonal water table and temperature fluctuations were found to be important for accurately modelling carbon discharge in a flood plain site (Arora et al., 2016), the temporally changing infiltration was found to affect this site heterogeneously (Dwivedi et al., 2018) with only naturally reduced sub-zones responding to these temporal dynamics. The model outcomes thus indicated that the geomicrobial activity responds heterogeneously to environmental disturbances such as seasonal change in water flux (Yabusaki et al., 2017) and must be further incorporated in modelling studies.

It must be noted that numerical modelling studies have historically focused on incorporating only one or two key microbial species based on site-specific geomicrobiology and hydrogeology, given the oligotrophic conditions prevalent in the subsurface (Aguilera et al., 2005;Arora et al., 2016;Herrmann et al., 2015;Schäfer et al., 1998a;Steefel et al., 2014;Thullner et al., 2007;Yabusaki et al., 2017). The

reaction networks used to describe the deep subsurface not only need to account for multiple growth and respiration strategies, as widely used already, but also dormancy and reactivation given dynamic conditions, and mobilization into the groundwater (Bradley et al., 2018). Thus, there is a need to update reaction networks that are used in reactive transport models in the deep subsurface.

Despite the extensive use of numerical modelling approaches to predict groundwater quality, parameterization of the flow field and the reaction network using lab-scale and field-scale studies remains a challenging task (Berkowitz et al., 2016). Reaction parameters may vary from the batch scale derived parameters by up to 50 times when implemented at the field scale (Rodríguez-Escales et al., 2016). Using inadequately resolved field measurements to parameterize flow and transport at the field scale may further add uncertainty to the modelling exercise. At the same time, incorporating high resolution data from lab-scale studies, e.g., pore-scale heterogeneous structure of the solid matrix, in policy-relevant catchment scale models is computationally expensive. Thus, there is a need for a methodology to evaluate the relevance of sub-scale spatio-temporal heterogeneities for long-term geomicrobial activity and carbon and nitrogen cycling thereof in natural systems of interest.

In surface water systems, methodologies such as concentration-discharge (C-Q) relationships (Creed et al., 2015;Ebeling et al., 2021;Evans and Davies, 1998;Gorski and Zimmer, 2021;Heathwaite and Bieroza, 2021;Liu et al., 2021;Saberi et al., 2021), and Damköhler number (Da, historically used in the field of chemical engineering) (Briggs et al., 2014;Pittroff et al., 2017;Oldham et al., 2013) are widely used to describe surface water systems as chemostats, and to predict the response of these systems to seasonal variations. Da is also used to describe subsurface systems (Jung and Meile, 2019) to be reaction dominant or transport dominant. Lastly, the Peclet number (Pe, also originating from the field of chemical engineering) is used to characterize the flow regime of sub surficial domains to be diffusion or dispersion or advection dominated (Field and Nash, 1997). Thus, easily estimable proxy indicators

are already extensively used to classify flow and reaction regimes in natural systems. It will be beneficial to evaluate the relevance of spatio-temporal heterogeneities for geomicrobial activity to evaluate broad system behavior such as that suggested by C-Q relationships, Da and Pe.

1.2 Research Objective

The aforementioned studies have hitherto explored microbial activity in select, somewhat simplified, scenarios (Fig. 1.2). These studies have investigated various aspects of microbial activity such as microbial biomass distribution and, microbial activity in aquifers, in hyporheic zones and in soil columns, even at the microscale with fluctuating saturation in space or in time, or both (highlighted areas in Fig. 1.2). Both lab-scale and field scale studies inform parameterization of reactive transport models, also lending uncertainty to the modelling predictions due to multiple factors such as scale-mismatch, and spatio-temporal heterogeneities in the natural systems to be simulated (Berkowitz et al., 2016). Furthermore, no functional relationship has been drawn between these different scenarios of structural heterogeneity and impact on microbial activity and nutrient cycling.

Secondly, with changing climate regime, there is large uncertainty in the future behavior of sub surficial reactive systems. Weather events are predicted to increase in intensity, and extreme events are predicted to increase in frequency as well (Trenberth, 2011), depending on the geographical region. An understanding of the impact of these changing patterns on the flow of water in the subsurface (both vadose zone and saturated aquifer) is limited. For example, weather events following intensely dry periods are expected to trigger run-off events initially with delayed infiltration into the deeper zones, but the dynamics of soil moisture, temperature and precipitation feedback are more complex (Betts et al., 1996;Schär et al., 1999). Additionally, the groundwater table is expected to continue to decline (Zektser et al., 2005) due to anthropogenic activities, impacting travel times of solutes in the

subsurface. This is expected to have a further impact on nutrient access of microbial species in the subsurface (detailed earlier). A functional relationship between different scenarios of temporal dynamics in the climate regime and resulting variability in microbial activity or nutrient cycling and discharge thereof is lacking. In my thesis, I aimed to fill these knowledge gaps.

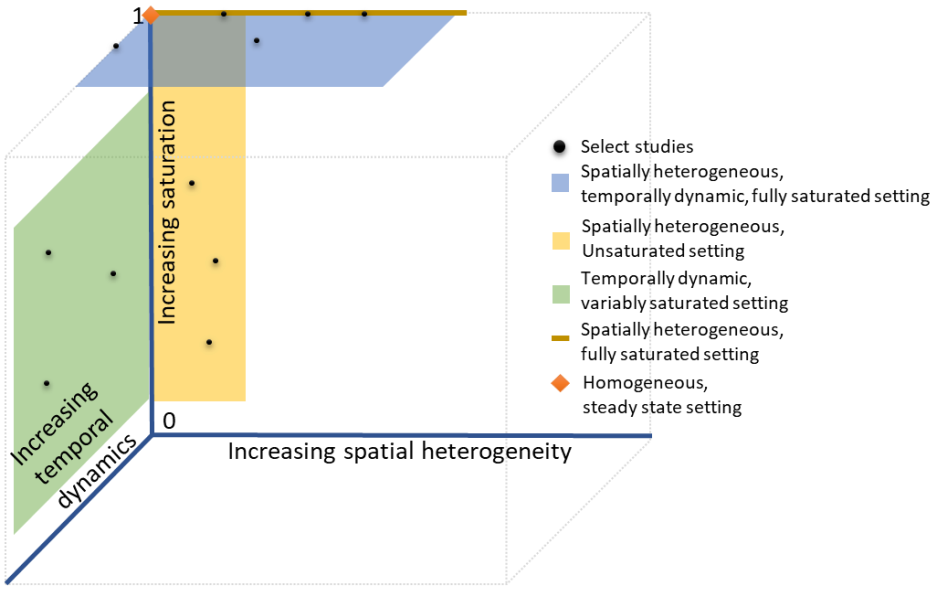

Figure 1.2 Studies incorporating spatio-temporal heterogeneities and degree of saturation to explore microbial activity in porous or fractured sub surficial media

In this thesis, I aimed to derive a functional and predictive relationship between subsurficial spatio-temporal heterogeneities and microbial redox dynamics and nutrient cycling thereof. I attempted to address the following questions:

1. How does spatial heterogeneity impact microbial nutrient cycling?
2. How do temporal dynamics impact microbial nutrient cycling?
3. How do spatio-temporal heterogeneities interact with each other and govern microbial nutrient cycling?

1.2.2 Research hypothesis

In this thesis, I aimed to address the gap of functional relationships between spatio-temporal heterogeneities and nutrient cycling in the subsurface. My research hypotheses are as follows:

H1. Spatial heterogeneity results in niches for microbial species to co-exist with other competitive species.

H2. Spatial heterogeneity results in lower consumption of reactive species in the system than expected in a homogeneous system.

H3. Temporal dynamics results in varying nutrient discharge from the domain, and this is a function of varying travel time in the domain.

H4. Spatio-temporal heterogeneities interact and result in compounding each other's effects on the system. Higher temporal dynamics in high spatially heterogeneous domains behave the most different from homogeneous domains in uniform conditions.

1.2.3 Approach

Using a numerical modelling approach, I investigated a variety of in silico scenarios to form a comprehensive study to explore the above-mentioned hypotheses. I formulated a pertinent reaction network with adequate complexity to represent microbial diversity and life processes in the subsurface. Using this, I sought to investigate the impact of spatial heterogeneity on microbial biomass distribution, activity in the subsurface, and consequent effects on biogeochemical cycles thereof in steady state conditions. I then subjected these domains at steady state in the spatially heterogeneous subsurface to temporal dynamics, with the aim to resolve the impact of temporal dynamics on microbial activity in spatially heterogeneous domains. I imposed different temporal dynamics depending on the location of the domain (i.e., in the saturated or the unsaturated zone). This assisted in setting up the problem to be as close to a physically plausible system as possible. At the same time, I used

numerical methods to ensure comparability between the different scenarios. In summary, I followed the steps below to address the research gap:

1. Formulate a reaction network capturing various respiration and growth strategies, and other microbial life processes,
2. Conceptualize simulation domain adequately representing unsaturated and saturated zones of the subsurface,
3. Formulate in silico spatially heterogeneous domains using two parameters: Variance (in log permeability field for unsaturated domains, and in log conductivity field for saturated domains) and anisotropy,
4. Quantify impact of spatial heterogeneity on microbial activity and nutrient cycling in saturated and unsaturated zones, and
5. Quantify impact of temporal dynamics in the saturated zone.

Addressing the research gap using the above stepwise approach, I am equipped to formulate recommendations when spatio-temporal heterogeneities are of concern to subsurficial microbial life in a changing climate. I used proxy indicators to express different spatio-temporal heterogeneity scenarios (such as travel time) to be able to contextualize the extent of spatio-temporal heterogeneities with respect to a base case (uniform conditions in homogeneous domains). I thus explored the use of these indicators in conjunction with existing ones (such as Da and Pe number) to build a functional relationship between spatio-temporal heterogeneities and nutrient cycling. The results of this thesis support the identification of key drivers of microbial dynamics in the Critical Zone and assist in effective upscaling these process descriptions.

1.3 Outline

The thesis consists of six (6) chapters. Chapter 1 is the current chapter, i.e., Introduction. Chapter 2 describes the approach to set up the simulation domains and

the process network used for all investigated scenarios. Chapters 3-5 describe studies investigating the above mentioned hypotheses. Parts of Chapters 2-4 have been submitted as a scientific journal article in (Khurana et al., 2021b) while Chapter 5 has been submitted as a separate scientific journal article (further information in Appendix C).

The formulation of the reaction network and conceptualization of suitable domains to investigate in the unsaturated and saturated zones of the subsurface are described in **Chapter 2**. I established the baseline system behavior that was different for domains with different saturation and flow regimes in **Chapter 3**. I then investigated the impact of spatial heterogeneity on nutrient cycling in the saturated and unsaturated zones zone in **Chapter 4**. In the vadose zone, spatial heterogeneity impacted the saturation/moisture content which further influenced travel time in the domain, as well as the concentration of dissolved oxygen in the water phase. I established that the extent of spatial heterogeneity can be expressed in terms of travel time. Consequently, I displayed that the removal of reactive species from the domain can be predicted as function of travel time in the domain. This relationship varied according to the reaction and flow regime, indicated by the Da. The work described in Chapters 2-4 enabled the evaluations of hypotheses H1 and H2. **Chapter 5** investigated the impact of temporal dynamics on fully saturated domains. I explored in detail how the impact of temporal dynamics on chemical discharge at the outlet of the domain depends on the Da. In addition, I explored how spatio-temporal heterogeneities interacted with each other in saturated domains. The work presented in Chapter 5, in combination with that presented in Chapter 4, enabled the evaluation of hypotheses H3 and H4. Finally, I discussed the results and presented the caveats of my thesis in **Chapter 6**. This assisted in forming a holistic view of how spatio-temporal heterogeneities interact in the subsurface and improving predictability of the behaviour of subsurface reactive systems with the help of limited indicators, relatively easily estimated at the field scale. Additionally, I gave an outlook into future research opportunities based on the results and shortcomings of this thesis.

2 Simulating microbial activity and nutrient cycling in the subsurface

To simulate a subsurficial reactive system of interest, a conceptual model is first needed. For this thesis, the conceptual model needs to adequately capture microbial mediated reactions transforming carbon and nitrogen in the subsurface. Incorporating microbial species explicitly allows the capture of transient conditions and associated impacts (Thullner et al., 2007). The reaction networks used for modelling soil carbon dynamics have historically employed bucket-type or pool-type systems (Thullner et al., 2007;Thullner and Regnier, 2019;Manzoni and Porporato, 2009;Yabusaki et al., 2017), while those for modelling carbon dynamics in the subsurface are seldom complex. Thus, there is a need for a microbial explicit reaction network, that represents growth conditions in the subsurface. This will assist in the evaluation of microbial mediated nutrient dynamics. Secondly, the conceptual model must also account for a simulation domain that adequately represents the flow and transport of the fluid in natural systems of interest.

In this chapter, I aimed to aimed to set up a comprehensive reaction network at the continuum scale (sub-meter scale in our case). For setting up the simulation domain and the process network, I used long-term observations from a subject site in the Hainich Critical Zone Exploratory (CZE, (Jing et al., 2018;Kohlhepp et al., 2017;Küsel et al., 2016)). This information constrained the conceptual approach and the simulated scenarios to realistic conditions, such as average hydraulic conductivity and permeability, porosity, chemical and water flux. However, since I aimed to generate a system understanding beyond the specific subject site, I considered variations from the observations thereof. These variations included considering additional scenarios for water flux in the domain (details below), extreme scenarios

for spatial heterogeneity, intensity of temporal dynamics. This enlarged the range of conditions covered by our model scenarios.

2.1 Solute transport in the simulation domain

The change in concentration of a chemical species in a reactive transport model is solved by the Advection-dispersion-reaction equation (Bauer et al., 2012):

$$\frac{\partial C}{\partial t} = \nabla(D\nabla C) - \nabla.(Cv) - R, \quad (2.1)$$

where, C is the concentration in molar [N L^{-1}], R is the rate of change in concentration of the chemical species (N L^{-1} T^{-1}), D is hydrodynamic dispersion coefficient [L^2 T^{-1}], v is the advective velocity [L T^{-1}]. The advective velocity is calculated using the Darcy equation in the saturated domain (Sun et al., 2012):

$$v = -K\frac{dh}{dl}, \quad (2.2)$$

where, v is the advective velocity, K is the hydraulic conductivity [L T^{-1}], and dh is the difference in hydraulic head [L] across a length of dl [L]. $\frac{dh}{dl}$ is referred to as the hydraulic gradient. The advective velocity in the unsaturated domain is calculated using the Richards flow (Kalbacher and Du, 2012) equation:

$$\emptyset\rho_w \frac{\partial S}{\partial p_c}\frac{\partial p_c}{\partial t} + \nabla.\left(k_{rel}K\left(\nabla p_w - \rho_w g\right)\right) = Q_w, \quad (2.3)$$

where, $\emptyset$ is the porosity, t is time [T], ρ_w is the liquid density [M L^{-3}], p_c is the capillary pressure [M L^{-1} T^{-2}] with $p_c = -p_w$, p_w is the water pressure [M L^{-1} T^{-2}], S is the water saturation [-], g is the acceleration [L T^{-2}] due to gravity, Q_w is the water discharge [L^3 T^{-1}], k_{rel} is the relative permeability [-]. Both k_{rel} and p_c are dependent on the degree of saturation in the medium. p_c and k_{rel} are given by:

$$p_c = \frac{\rho_w g}{\alpha}[S_{eff}^{-\frac{1}{m}} - 1]^{1/n}, \text{ and} \quad (2.4)$$

$$k_{rel} = S_{eff}^{1/2}[1 - 1\left(1 - S_{eff}^{1/m}\right)^{m}]^2, \quad (2.5)$$

where, m [-] and n [-] are shape defining van-Genuchten parameters, with m = 1 – (1/n), and α [-] is a van-Genuchten parameter associated with the air entry pressure. In this thesis, m = 0.3, and α = 0.5. S_{eff} is the effective Saturation given by:

$$S_{eff} = \frac{S - S_r}{S_{max} - S_r}, \quad (2.6)$$

where, S [-] is the degree of saturation, that is, ratio of pore volume occupied by water. S_{max} is the maximum saturation (0.8 in this work), and S_r (0.2 in this work) is the residual saturation of the domain.

Simulating solute transport in the subsurface is thus already a complex problem, taking into account various physical properties of the domain (permeability or conductivity, van-Genuchten parameters of unsaturated systems, porosity, viscosity of the liquid medium) and reactive processes.

2.2 Reaction component: Process Network

I conceptualized an extended biogeochemical process network to describe the turnover of carbon and nitrogen (Fig.2.1). The reaction network captured the transformation of DOC, particulate organic carbon (POC), dissolved oxygen (DO or O2), nitrate (NO3-), Sulphate (SO42-), and ammonium (NH4) by one or more of the four microbial function groups: Aerobic DOC degraders (BO2), nitrate reducers (BNO3), sulphate reducers (BSO4) and ammonia oxidizers (BNH4), adapted from the carbon dynamics and select processes described in previous studies (Manzoni and Porporato, 2009;Vogel et al., 2018).

Broadly, the network accounted for aerobic autotrophy, aerobic heterotrophy and anaerobic heterotrophy and microbial growth thereof. The transformation of the

chemical species occurs per the following processes out of which Eq. 2.7-2.10 were mediated by microbial species:

Aerobic respiration: $CH_2O + O_2 \rightarrow HCO_3^- + H^+$ (2.7)

Nitrate reduction: $CH_2O + 0.8NO_3^- + 0.8H^+ \rightarrow HCO_3^- + 0.4N_2 + 0.4H_2O + H^+$ (2.8)

Sulphate reduction: $CH_2O + 0.5SO_4^{2-} + H^+ \rightarrow HCO_3^- + 0.5HS^- + 1.5H^+$ (2.9)

Ammonia oxidation: $0.5NH_4^+ + O_2 \rightarrow 0.5NO_3^- + 0.5H_2O + H^+$ (2.10)

Hydrolysis of POC: $C_{10}H_7O_2N + 8H_2O + H^+ \rightarrow 10CH_2O + NH_4^+$ (2.11)

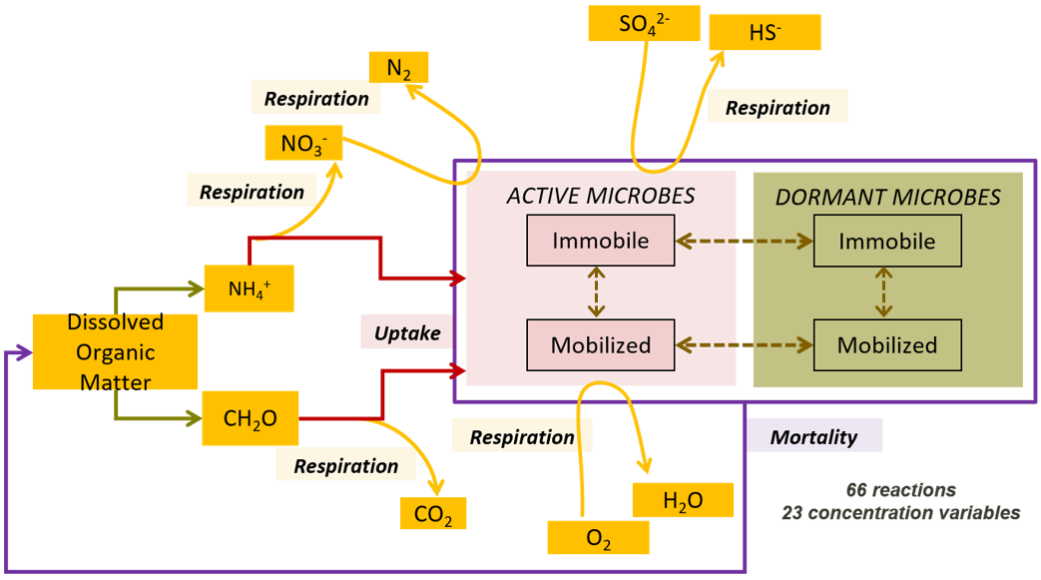

Figure 2.1: Schematic of the simulated biochemical reaction network

Each microbial functional group was further partitioned into four (4) fractions or subpopulations. While both immobile and mobile active bacteria could respire and grow and perform biogeochemical reactions, immobile and mobile inactive bacteria were able to do neither. Both active and inactive immobile bacteria were attached to the matrix of the domain, while active and inactive mobile bacteria represented

phytoplanktonic microbial species. In summary, active immobile ($X_{a,s}$), active mobile ($X_{a,w}$), inactive immobile ($X_{i,s}$) and inactive mobile ($X_{i,w}$) subpopulations for each microbial function group (X) were present in the process network.

2.2.1 Microbial respiration

Modified Michaelis-Menten type expressions to described microbial respiration (Eq. 2.12-2.15 and Table 2.1). The parameters for these processes are presented in Table A1.

1. Aerobic respiration:

$$r = \frac{1-O2min\times Kxx}{e^{\frac{Kxx}{st}}+1}\left(BO2_{a,s} + BO2_{a,w}\right) \quad (2.12)$$

2. Nitrate reduction:

$$r = \frac{1-NO3min\times Kxx}{e^{\frac{Kxx}{st}}+1}\left(BNO3_{a,s} + BNO3_{a,w}\right) \quad (2.13)$$

3. Sulphate reduction:

$$r = \frac{1-SO4min\times Kxx}{e^{\frac{Kxx}{st}}+1}\left(BSO4_{a,s} + BSO4_{a,w}\right) \quad (2.14)$$

4. Ammonia oxidation:

$$r = \frac{1-NH4min*Kxx}{e^{\frac{Kxx}{st}}+1}\left(BNH4_{a,s} + BNH4_{a,w}\right) \quad (2.15)$$

2.2.2 Microbial growth

A modified Monod type expression described microbial growth (i.e., formation of biomass carbon), further linked with microbial respiration rates using a yield coefficient, and with a dependency on the concentration and ammonium as the source of nitrogen (Eq. 2.16-2.20 and Table 2.1). The parameters for these processes are presented in Table A1.

1. Dependency on ammonium:

$$NH4limit = \frac{1}{e^{\frac{amming - NH4}{st \times amming}} + 1} \quad (2.16)$$

2. Active aerobic DOC degraders:

$$r = NH4limit \times \frac{1 - O2min \times Kxx}{e^{\frac{Kxx}{st}} + 1} Y_o \times BO2_{a,x} \quad (2.17)$$

with a = active biomass, x=s for attached and x=w for mobile bacteria

Table 2.1 Expressions controlling respiration, growth, dormancy and reactivation of microbial species

Notation	Descriptors			
	Aerobic degraders	**Nitrate reducers**	**Sulphate reducers**	**Ammonia oxidizers**
Bxx	BO2a,s and BO2a,w	BNO3a,s and BNO3a,w	BSO4a,s and BSO4a,w	BNH4a,s and BNH4a,w
Byy	BO2i,s and BO2i,w	BNO3i,s and BNO3i,w	BSO4a,s and BSO4i,w	BNH4a,s and BNH4i,w
Kxx	$1 - kmax1 \times \left(\frac{DOC}{ksodoc1 + DOC}\right) \times \left(\frac{O2}{ksox1 + O2}\right) / O2min$	$1 - kmax2 \times \left(\frac{DOC}{ksndoc + DOC}\right) \times \left(\frac{kindox}{kindox + O2}\right) \times \left(\frac{NO3}{ksno3 + NO3}\right) / NO3min$	$1 - kmax3 \times \left(\frac{DOC}{kssdoc + DOC}\right) \times \left(\frac{SO4}{ksso4 + SO4}\right) \times \left(\frac{kindox}{kindox + O2}\right) \times \left(\frac{kinno3}{kinno3 + NO3}\right) / SO4min$	$1 - kmax4 \times \left(\frac{NH4}{ksamm + NH4}\right) \times \left(\frac{O2}{ksox + O2}\right) / NH4min$

3. Active nitrate reducers:

$$r = NH4limit \times \frac{1 - NO3min \times Kxx}{e^{\frac{Kxx}{st}} + 1} Yn \times BNO3_{a,x} \quad (2.18)$$

4. Active sulphate reducers:

$$r = NH4limit \times \frac{1 - SO4min \times Kxx}{e^{\frac{Kxx}{st}} + 1} Ys \times BSO4_{a,x} \quad (2.19)$$

5. Active ammonia oxidizers:

$$r = NH4limit \times \frac{1-NH4min \times Kxx}{e^{\frac{Kxx}{st}}+1}\ Ya\ \times BNH4_{a,x} \quad (2.20)$$

2.2.3 Processes governing the location of the microbes

The process network allowed for microbial species to mobilize into the groundwater or attach to the solid matrix. Mobile bacteria are transported by the flowing water.

1. Mobilization of immobilized bacteria (Bxx) into the fluid medium (i.e. the transfer of attached bacteria into mobile bacteria) were adapted from (Rittman and McCarty, 2001) assuming additionally that high total attached biomasses led to higher detachment rates (adapted from Clement et al. (1997)):

$$r = kl\ \times\ (vq0 \times vpor0)^{0.58} \times Bxx + \frac{kdet}{e^{\frac{Bfmax-Bo2_{a,s}-BO2_{i,s}-BNO3_{a,s}-BNO3_{i,s}-BSO4_{a,s}-BSO4_{i,s}-BNH4_{a,s}-BNH4_{i,s}}{st \times Bfmax}}+1} \times Bxx \quad (2.21)$$

2. Immobilization or reattachment: Attachment rates of mobile bacteria Byy also depended on the total concentration of attached biomass:

$$r =\ katt \times Byy\ \times \left(1 - \frac{1}{e^{\frac{Bfmax-Bo2_{a,s}-BO2_{i,s}-BNO3_{a,s}-BNO3_{i,s}-BSO4_{a,s}-BSO4_{i,s}-BNH4_{a,s}-BNH4_{i,s}}{st \times Bfmax}}+1}\right) \quad (2.22)$$

2.2.4 Processes governing the activity states of microbes

The network also accounted for dormancy given inhospitable conditions for microbial growth and respiration, and reactivation. Associated parameters are presented in Table A1.

1. Deactivation/Dormancy: Deactivation rates of active bacteria (i.e., conversion of active (mobile/attached) into inactive or dormant (mobile/attached) bacteria) at unfavourable substrate conditions were expressed following Stolpovsky et al. (2011).

$$r = kdeac \times Bxx \times (1 - \frac{1}{e^{\frac{Kxx}{st}} + 1}) \quad (2.23)$$

with the term Kxx depending on the bacterial species Byy and its substrate source (see Table 2.1).

2. Reactivation: In analogy to the deactivation rates, reactivation rates were expressed as:

$$r = kreac \times Byy \times \frac{1}{e^{\frac{Kxx}{st}} + 1} \quad (2.24)$$

with the term Kxx depending on the bacterial species as described in Table 2.1.

3. Mortality: Mortality rates followed a first-order dependency on biomass concentration:

$$r = km \times fdorm \times Bxx \quad (2.25)$$

For active bacteria fdorm = 1, for inactive bacteria fdorm = 0.1. Dead bacterial biomass was added to the POM pool.

2.2.5 Miscellaneous processes

1. Hydrolysis of POC was described by first order rate kinetics: Hydrolysis of particulate organic matter added bioavailable dissolved organic carbon and ammonium back at a fixed C:N ratio (fcn) into the process network, thus completing the loop.

$$r = kpd \times POC \quad (2.26)$$

2. Background autotrophic microbial growth: Background autotrophic microbial growth adhering to Monod type kinetics again, and limited by ammonium availability was given by:

$$r = NH4limit \times kmax5 \times \left(\frac{NH4}{ksamm+NH4}\right) \quad (2.27)$$

3. Diffusion of dissolved oxygen (DO): DO diffused from the air phase to the water phase in the unsaturated domain, with a dependency on the concentration of DO in the water phase and the degree of saturation

$$r = (DO_{sat} - O2)\left(1 - \frac{1}{e^{\frac{0.4-ws}{0.4\times st}}+1}\right), \quad (2.28)$$

where, DO_{sat} is the saturation concentration of DO in groundwater (approximately 8 mg/L), ws is the degree of saturation and O2 is the concentration of DO in the liquid phase.

2.3 Simulation domain

The simulation domains set up for testing the hypotheses were two dimensional (2D, thickness defaulted to 1 m for subsequent calculations). The conceptual setup of the permanently saturated and unsaturated domains is presented in Fig. 2.2. The boundary conditions allowed for water to flow in predominantly vertically downward direction. The water flux carrying chemical and microbial species entered the domain from the top (also called inlet boundary or inlet) and it exited from the bottom (also called outlet boundary or outlet). The side boundaries of the domain were no-flow boundaries. In both saturated and unsaturated domains, I set up three (3) different flow regimes: Slow flow, medium flow and fast flow. The velocity increased by a factor of 10 between the slow and medium flow regimes, and a factor of 100 between the slow and fast flow regimes. The transport regimes accounted for advection, dispersion and diffusion. Table 2.2 summarizes these details of the

simulation domain. The chemical reaction related boundary conditions were the same in both saturated and unsaturated domains (Table 2.3).

Table 2.2 Summary of model domain setup

Parameter	Units	Saturated domain	Unsaturated domain
Domain size	m x m	0.3 x 0.5	0.3 x 0.7
Observation zone	m x m	0.3 x 0.5	0.3 x 0.5
Discretization in the observation zone (Δx x Δy)	m x m	0.01 x 0.01	0.005 x 0.005
Porosity	-	0.2	0.2
Average conductivity	$m\ d^{-1}$	$2\ 10^{-6}$	-
Average permeability	$m\ d^{-1}$	-	$2\ 10^{-8}$
Diffusion coefficient	$m^2\ d^{-1}$	$8.64\ 10^{-5}$	$8.64\ 10^{-5}$
Longitudinal dispersivity	m	0.02	0.02
Average water velocity: Slow flow	$m\ d^{-1}$	$3.8\ 10^{-4}$	$7.6\ 10^{-4}$
Average water velocity: Medium flow	$m\ d^{-1}$	$3.8\ 10^{-3}$	$7.6\ 10^{-3}$
Average water velocity: Fast flow	$m\ d^{-1}$	$3.8\ 10^{-2}$	$7.6\ 10^{-2}$

2.4 Numerical Tools

I carried out numerical simulations using OGS#BRNS (Centler et al., 2010), a tool that couples BRNS (Biochemical Reaction Network Solver (Aguilera et al., 2005;Regnier et al., 2002) with OpenGeosys (OGS, Kolditz et al. (2012)). Both BRNS and OGS are state of the art tools and have the ability to solve complex reactive transport models (Thullner et al., 2005) and groundwater flow models, respectively (Jing et al., 2018).

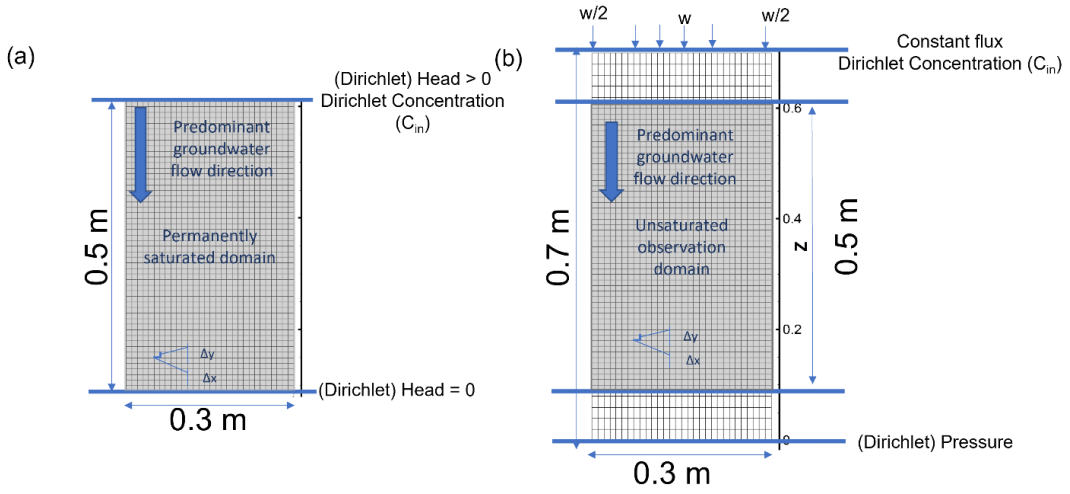

Figure 2.2 Domain set up for the (a) permanently saturated and (b) unsaturated domains, displaying dimensions, boundary conditions, discretization and predominant groundwater flow direction.

The entire workflow was realized in the Python programming language (van Rossum and Drake, 2006) (referred to as Python henceforth), taking advantage of various packages to set up the simulation scenarios, to process simulation outputs and further analysis and generation of graphical outputs. The scripts used for the Python workflow along are available in online repositories for ease of reproducibility (Khurana et al., 2021a).

Table 2.3 Dirichlet boundary condition for the reactive species at the inlet of the domain.

Chemical species	Cin (units)
POC	5 (µM C)
DOC	800 (µM C)
DO	250 (µM)
Nitrate	250 (µM)
Ammonium	60 (µM)
Sulphate	1,500 (µM)
Bx,w	2 (µM C)

As mentioned in Chapter 2.1, Richard's flow implementation solved the flow regime in the unsaturated domain. Richards flow equation being non-linear is more difficult to solve using numerical solvers. The Galerkin Finite Element Method (FEM) is used to solve the Richards flow equation (Kolditz et al., 2008) subject to enforced boundary and initial conditions in ogs5. It is known to lead to failure of water mass conservation computations (Suk et al., 2020). The errors are as high as 30% in complex problems. Having observed similar errors in the simulations carried out in the unsaturated domains, especially in extremely high spatially heterogeneous scenarios, I refined the mesh further with smaller discretization (Chapter 2.3, Fig. 2.2). This resulted in the mass balance error reducing to 3% in the homogeneous scenario, and within 5% for the in silico spatially heterogeneous scenarios. The refined mesh thus lent confidence to the simulation results of the unsaturated domain.

2.5 Data analysis

The breakthrough time is a useful metric to evaluate the matter flux in the domain. The breakthrough time of a conservative tracer was the time taken for the flux averaged concentration at the outlet of the domain to be 50% of the continuous tracer input concentration at the inlet of the domain. This enabled the evaluation of the impact of varying flow regimes with respect to the transport time scale in the simulation domains. Flux averaged concentration (C_j at cross-section j) of mobile species at each cross-section along the predominant flow direction (top to bottom) was calculated using Eq. 2.29:

$$C_j = \frac{\sum_{i=2}^{n-1}(C_{i,j}\times v_{i,j} \times \emptyset_{i,j} \times S_{i,j}) + \frac{1}{2} \times \sum_{i=1,n}(C_{i,j}\times v_{i,j} \times \emptyset_{i,j} \times S_{i,j})}{\sum_{i=2}^{n-1}(v_{i,j} \times \emptyset_{i,j} \times S_{i,j}) + \frac{1}{2} \times \sum_{i=1,n}(v_{i,j} \times \emptyset_{i,j} \times S_{i,j})}, \quad (2.29)$$

where, $C_{i,j}$ is the concentration, $v_{i,j}$ is the velocity, $\emptyset_{i,j}$ is the porosity, $S_{i,j}$ is the saturation at node [i,j] in the model domain, Δx is the discretization in the X direction, and n is the total number of nodes in the X direction. Porosity is constant across the

domain, and the saturation is 1 for the saturated domain. The unsaturated domains are effectively saturated (S_{eff} = 1) at saturation of 0.8. Since microbial species also have a mobile subpopulation, they also contain nitrogen and carbon elements. These concentrations of nitrogen and carbon were included to derive the concentration of total nitrogen and total organic carbon (TOC) at each cross-section by also considering the ratio of nitrogen and carbon present in the microbial species, given by fcn (the carbon to nitrogen ratio in biomolecules as described in Chapter 2.2). For biomass, spatially averaged concentration of all subpopulations was given by Eq. 2.30

$$C_j = \begin{cases} \frac{1}{2} \times \frac{\sum_{i=2}^{n-1}(C_{i,j}\times\emptyset_{i,j}\times S_{i,j}) + \frac{1}{2}\times\sum_{i=1,n}(C_{i,j}\times\emptyset_{i,j}\times S_{i,j})}{\sum_{i=2}^{n-1}(\emptyset_{i,j}\times S_{i,j}) + \frac{1}{2}\times\sum_{i=1,n}(\emptyset_{i,j}\times S_{i,j})}, & j = 1, yn \\ \frac{\sum_{i=2}^{n-1}(C_{i,j}\times\emptyset_{i,j}\times S_{i,j}) + \frac{1}{2}\times\sum_{i=1,n}(C_{i,j}\times\emptyset_{i,j}\times S_{i,j})}{\sum_{i=2}^{n-1}(\emptyset_{i,j}\times S_{i,j}) + \frac{1}{2}\times\sum_{i=1,n}(\emptyset_{i,j}\times S_{i,j})}, & j = 2\ to\ yn-1 \end{cases}, \quad (2.30)$$

where yn is the number of nodes in the predominant flow direction, and Δy is the discretization in the predominant flow direction.

The removal of dissolved chemical species (that is, DOC, DO, ammonium, and nitrate) from the domain was then given by Eq. 2.31.

$$Removal\ of\ chemical\ species = 1 - \frac{C_{tn}}{C_1} = 1 - \frac{C_{out}}{C_{in}}, \quad (2.31)$$

where C_{out} was the flux averaged concentration of the chemical species at the outlet (j = tn) and C_{in} was the flux averaged concentration of the chemical species at the inlet (j = 1). The Da indicated the reaction regime for each reactive species. Da is defined as the ratio of the advective transport time scale and the reaction time scale as described in Eq. 2.32.

$$Da = \frac{\tau_{transport}}{\tau_{reaction}}, \quad (2.32)$$

where, $\tau_{reaction}$ was the characteristic reaction time scale and $\tau_{transport}$ was the characteristic transport time scale given by the breakthrough time of a conservative tracer in the domain. The characteristic reaction time scale assuming 63% loss

(Pittroff et al., 2017) was thus given by Eq. 2.33 to calculate the apparent Da using values estimable in the field when $\frac{C_{out}}{C_{in}} > 5\%$.

$$Da = -\ln\frac{C_{out}}{C_{in}}, \tag{2.33}$$

If $\frac{C_{out}}{C_{in}} \leq 5\%$., the characteristic reaction time scale was then given by Eq. 2.34 and 2.35 to derive the apparent Da of the chemical species

$$\tau_{reaction} = \frac{-\ln(0.37)}{-\ln(\frac{Cy5}{Cin})} \times \tau_{y5}, \tag{2.34}$$

$$\tau_{reaction} = \frac{\tau_{y5}}{\ln(\frac{Cy5}{Cin})}, \tag{2.35}$$

where, C_{y5} was the concentration of the chemical species at the first cross-section (y = y5) when $\frac{C}{C_{in}} \leq 5\%$, and τ_{y5} was the breakthrough time for a conservative tracer at the same cross-section, i.e., y = y5. In this case, $\tau_{transport}$ case was the same as the breakthrough time of the conservative tracer in the domain (Eq. 2.36).

$$Da = \frac{breakthrough\ time}{\frac{\tau_{y5}}{\ln(\frac{Cy5}{Cin})}}, \tag{2.36}$$

The logarithm of Da to the base 10 ($\log_{10}$Da) characterized the reactive system, i.e., the domain with reaction and flow components with respect to a particular chemical species (further demonstration in Chapter 4).

2.6 Summary

In this chapter, I presented the case for the formulation of a comprehensive reaction network to simulate nutrient cycling in the subsurface using literature knowledge and geomicrobial activity identified at the subject site that adequately represented a variety of microbial life processes as well as carbon and nitrogen

transformation pathways. I conceptualized two simulation domains that described two distinct zones in the subsurface, the vadose zone and the permanently saturated deeper subsurface. The reaction network captured varying growth and respiration pathways. Its parameterization was challenging due to limited information on microbial activity in oligotrophic conditions. The presented conceptual approach and assessment scheme will be applied in Chapters 3-5 to study the effects of spatial heterogeneity and transient conditions on microbial biomass, activity and nutrient cycling.

3 Establishing baseline in saturated and unsaturated domains

The first step to assess the impact of spatio-temporal heterogeneities is to consider the baseline, or the base cases. The base cases refer to the homogeneous domain, with a uniform flow field at steady state conditions in each flow regime. Thus, there are six (6) base cases, one in each flow regime in saturated domain and also in unsaturated domain. This sets the stage for the discussion of the chemical removal and microbial biomass distribution in the spatially heterogeneous domains in Chapter 4, as well as for the same in temporally dynamic regimes in Chapter 5. To characterize the base cases, the following aspects were explored:

1. Concentration profile of chemical and microbial species along the predominant flow direction of the domain,
2. Removal of chemical species from the domain, and
3. Contribution of microbial subpopulations to the total biomass.

These results are also partially presented in Khurana et al. (2021b).

3.1 Base case in the saturated domain

Since the reaction network is complex and novel, adherence to general redox hierarchy was an important aspect to check. The residence time (or the breakthrough time) of a conservative tracer was 205 days in the slow flow regime, 24 days in the medium flow regime and 2.4 days in the fast flow regime at steady state conditions since the imposed average velocity was different in each flow regime.

3.1.1 Chemical species

The flux averaged concentration profile in the three investigated flow regimes (slow flow, medium flow, and fast flow) of chemical species in the saturated domain are presented in Fig. 3.1. The concentration at the inlet was the same for all scenarios, while it varied at the outlet. DOC concentration decreased continuously along the dominant flow direction. Initially, this reduction in DOC concentration was due to aerobic heterotrophy (henceforth referred to as activity) in all flow regimes. In slow and medium flow regimes, DOC concentrations continued to decrease due to anaerobic activity. This can be correlated with the growth and abundance of aerobic degraders (Fig. 3.3) in the same zones where DO removal and DOC reduction takes place in the upgradient parts of the domain (Fig. 3.1). In the slow flow and medium flow regimes, aerobic reducers decreased in biomass, and ammonia oxidisers and nitrate reducers emerged in suboxic (DO < 15 μM) and in anoxic conditions (where DO < 3 μM, detection limit of DO sensors (ISO, 2014)). Thus, ammonium concentration also reduced due to the growth of ammonia oxidisers and nitrate reducers.

The predominant activity in the fast flow regime, on the other hand, was aerobic since the entire domain was predominantly oxic. The DO concentration at the outlet was approximately 4 μM. There were no ideal conditions for nitrate reducers to grow and proliferate, nor for nitrate reduction to take place. Lastly, since the concentration of nitrate was still high (> 63 μM) at the outlet in all base cases, no sulphate reduction takes place. In conclusion, the reaction network adheres to overall redox hierarchy; energetically favourable aerobic degradation occurred preferentially upgradient in the domain promoted by a relatively high concentration of aerobic degraders, followed by anaerobic heterotrophy and aerobic autotrophy at lower rates.

Since the breakthrough time was the highest in the slow flow regime, the removal of reactive species, DOC (59.2%), DO (99.6%), ammonium (19.8%) and nitrate (74.7%), was also the highest there (Fig. 3.2). It follows that the rate of removal

decreased in the medium and fast flow regimes. Also, the removal of total nitrogen was the highest in the slow flow regime (57%), while the removal of TOC was the lowest there (32.6%) and highest in the medium flow regime (42.6%).

3.1.2 Microbial biomass

The total biomass concentration was the lowest in the fast flow regime (86 μM C) and the highest in the slow flow regime (122 μM C). The mobile biomass decreased with increasing flow rates, but the immobile biomass was constant between all the flow regimes. The microbial community was dominated by aerobes due to the influx of oxygenated water at the inlet.

The proportion of active aerobic degraders and ammonia oxidizers increased with increasing flow rate; it was the lowest in the slow flow regime (~5%) and it was the dominant subpopulation in the fast flow regime (~87%) (Fig. 3.4). This was primarily due to the emergence of a small oxic zone in the slow flow regime domain, which expanded further downgradient in the medium and fast flow regimes (Fig. 3.1 and Fig. 3.3), also resulting in higher aerobic activity. Consequently, the contribution of active nitrate reducers to the total biomass was lowest in the fast flow regime (~3%); they grew near the outlet of the domain (Fig. 3.3). Due to the emergence of sub-oxic zones in the medium flow regime, active nitrate reducers sustained and formed a substantial proportion of the microbial community (14% as opposed to ~4% in slow flow regime and ~3% in fast flow regime). Overall, the immobile active fraction was higher than the mobile active fraction in all flow regimes (more than 7 times in the slow flow regime, more than 4 times in the medium flow regime and more than 2 times in the fast flow regime).

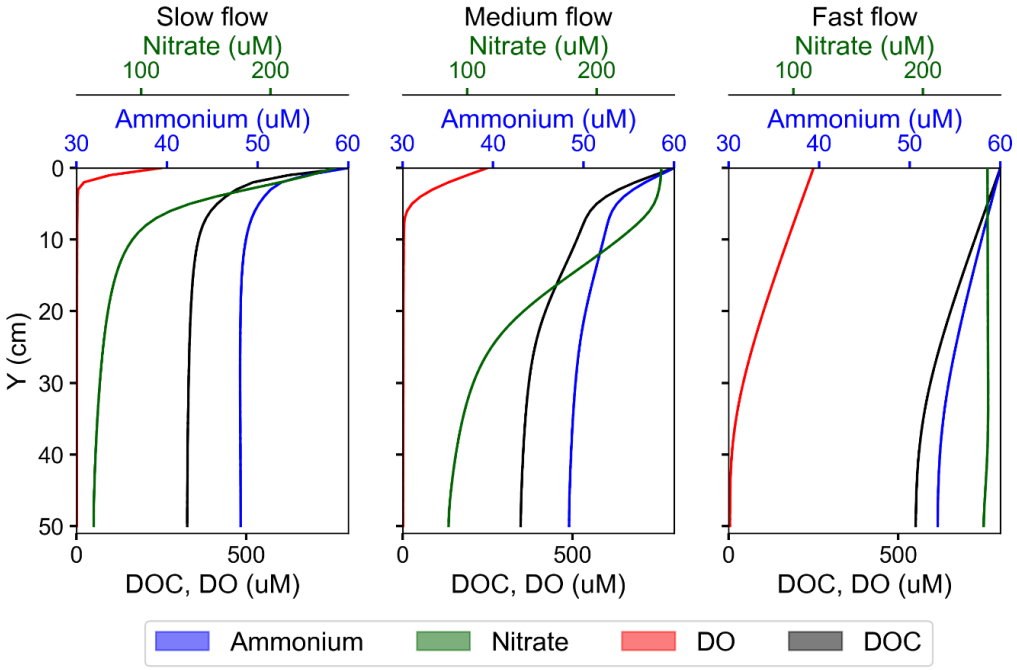

Figure 3.1 Flux averaged concentration of chemical species in the base case (homogeneous domain) in each flow regime in saturated domain.

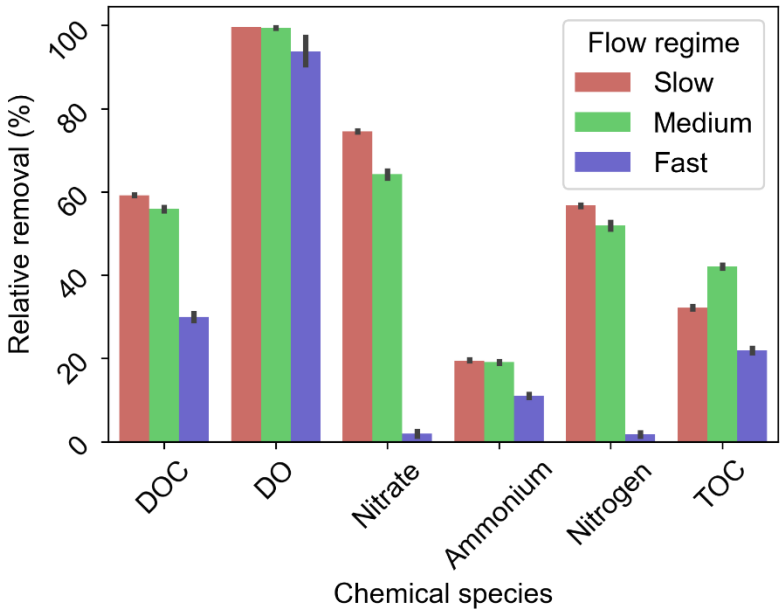

Figure 3.2 Removal of chemical species in the base case of each flow regime relative to the incoming mass flux

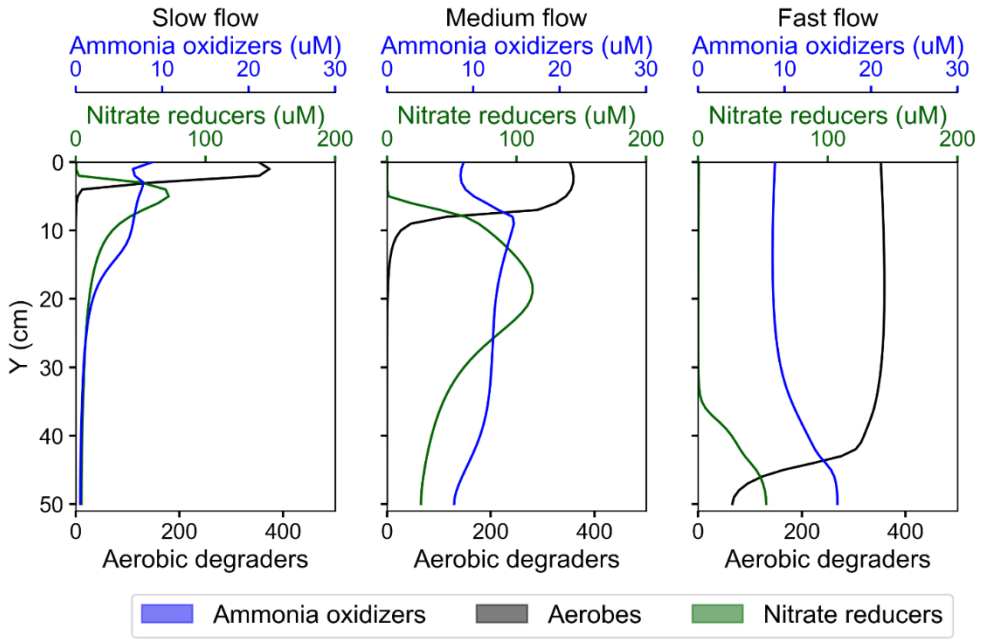

Figure 3.3 Spatially averaged concentration of chemical species in the base case (homogeneous domain) in each flow regime in the saturated domain.

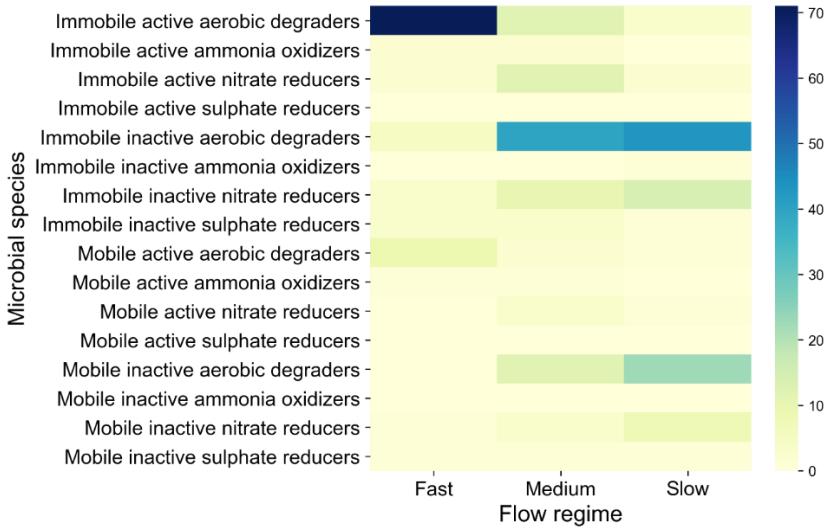

Figure 3.4 Percentage contribution of each subpopulation of each microbial functional group in the base case of each flow regime in the saturated domain.

3.2 Base case in the unsaturated domain

The average effective saturation (hereon referred to as saturation) was 0.57 in the slow flow regime, 0.65 in the medium flow regime, and 0.72 in the fast flow regime. The breakthrough time in the unsaturated domains was different from that in the saturated domain. It was 77 days at the average velocity of 7.3 10^{-4}m d^{-1} in the slow flow regime, 16 days at the average velocity of 7.3 10^{-3} m d^{-1} in the medium flow regime, and 1.6 days at the average velocity of 7.3 10^{-2} m d^{-1} in the fast flow regime.

3.2.1 Chemical species

Since the process network in the unsaturated domain allowed for diffusion of DO from the air phase to the liquid phase (Chapter 2.2.5) when the degree of saturation was low, the analysis of the simulation results of the unsaturated domain was more complex. Having said that, DO was first consumed, then ammonium in sub-oxic and/or carbon limited conditions and then nitrate in sub-oxic to anoxic conditions but carbon rich conditions, Fig 3.5). The net removal of all chemical species, except nitrate and nitrogen, from the domain decreased with increasing flow rates (Fig. 3.6).

The slow flow regime was a predominantly oxic system (in the water phase) at low degree of saturation, with DO continuously diffusing from the air phase to the water phase. This suppressed the reduction of nitrate in the downgradient region of the domain but allowed for continuous removal of ammonium via oxidation. In the medium flow regime, DO was fully consumed in the upgradient region of the system, which allowed for the reduction and removal of nitrate from the domain. The removal of ammonium via oxidation was, therefore, limited due to the limited presence of DO in the domain. The base case of the fast flow regime was predominantly oxic due to the persistent presence of DO and low aerobic heterotrophy. Thus, limited removal of nitrate and ammonium occurred in the fast flow regime.

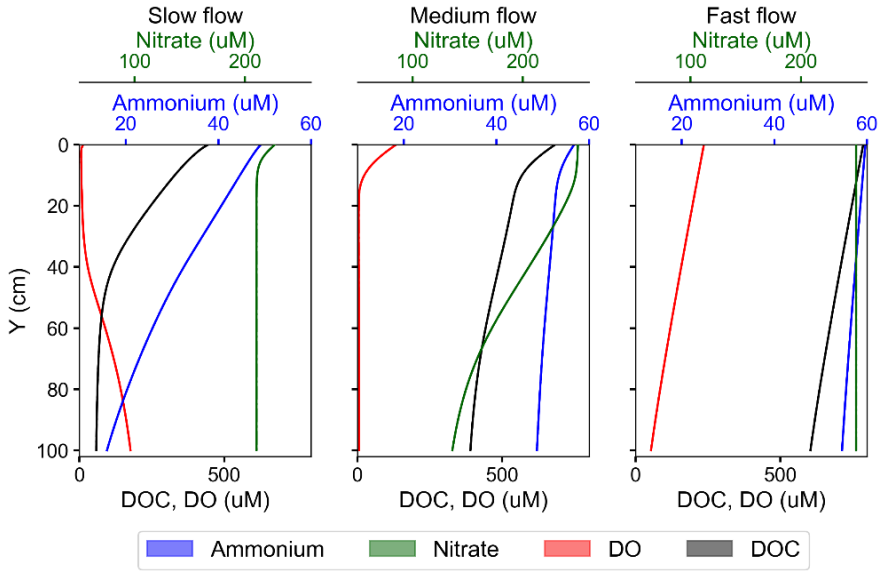

Figure 3.5 Flux averaged 1D concentration profile of chemical species in the base case (homogeneous domain) of the unsaturated domain.

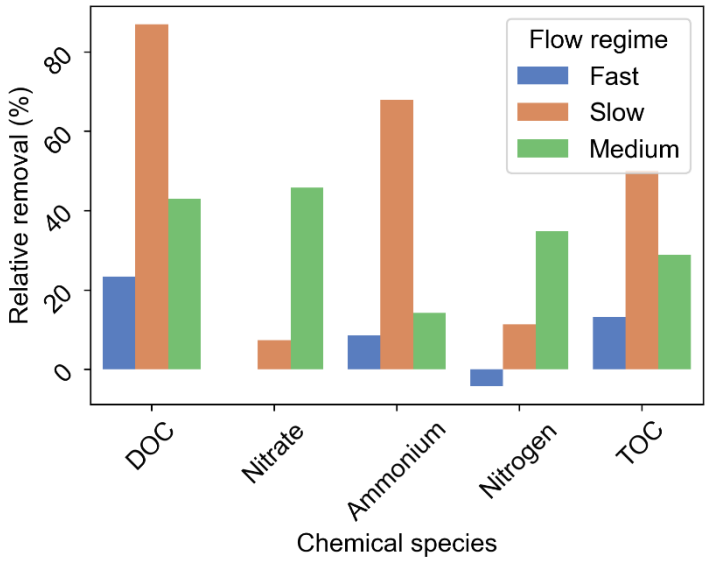

Figure 3.6 Removal of chemical species from the base case in each flow regime normalized relative to flux at inlet of the domain.

3.2.2 Microbial biomass

The total biomass in all the flow regimes was approximately the same: 265 µM C in the slow flow regime, 248 µM C in the medium flow regime and 253 µM C in the

fast flow regime. The biomass subpopulations did shift considerably among themselves, between active and inactive fractions.

In the slow flow regime, the highest contributor to microbial biomass were inactive aerobic degraders contributing 69% of total biomass in the domain (48% immobile and 21% mobile). Among active species, aerobic degraders contributed the most to the biomass in the domain (18%, 13% immobile and 5% mobile, Fig. 3.7 and Fig. 3.8). In the medium flow regime, active microbial species contributed 46% of the total microbial biomass in the domain (21% immobile and 5% mobile aerobic degraders, 14% immobile and 3% mobile nitrate reducers, and 3% immobile ammonia oxidizers). Among the inactive species, aerobic degraders were the largest contributors at 42% (33% immobile and 9% mobile). Lastly, in the fast flow regime, aerobic degraders were the largest contributors to the microbial biomass in the system (81% immobile and 7% mobile).

3.3 Discussion

The activity of geomicrobial reactive systems is dependent on a variety of factors, such as nutrient availability, access to energy gradients, pH, pore size, hydraulic conductivity, particle size distribution (Smith et al., 2018). Testing the same reaction network (with the same parameter set) in a variety of flow regimes provided a view on both reaction dominant systems and flow dominant systems. This compensated for my approach wherein I did not explore additional scenarios varying concentrations of chemical species and their influence on microbial growth and distribution. Lastly, the results discussed in this chapter are useful base cases for further studies to test the hypotheses introduced in Chapter 1.

There were several differences in microbial distribution among different functional groups as well as the abundance of each functional group within similar flow regimes between the unsaturated and saturated domains.

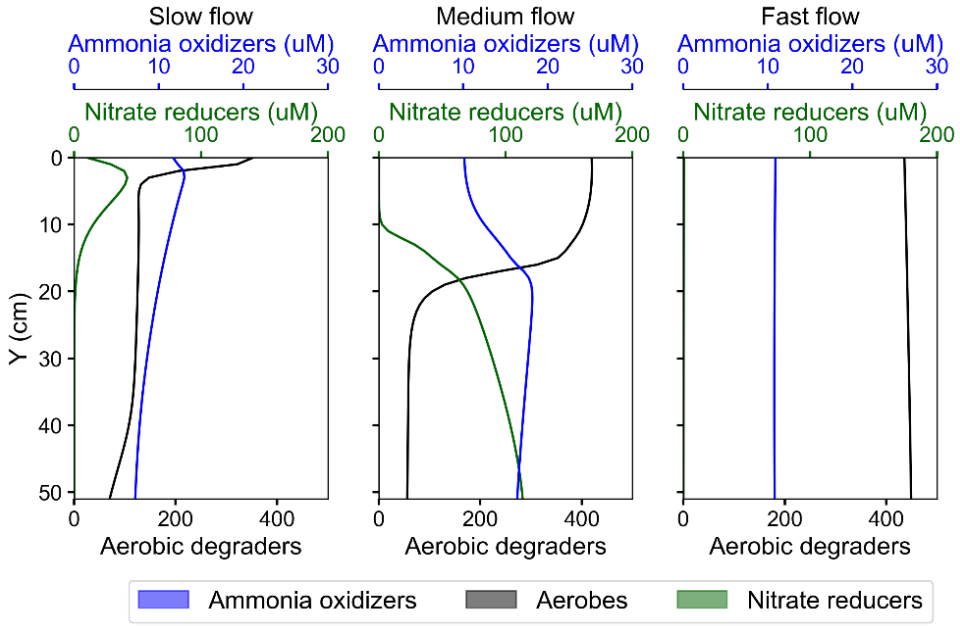

Figure 3.7 Spatially averaged immobile active biomass in the base case of the unsaturated domain.

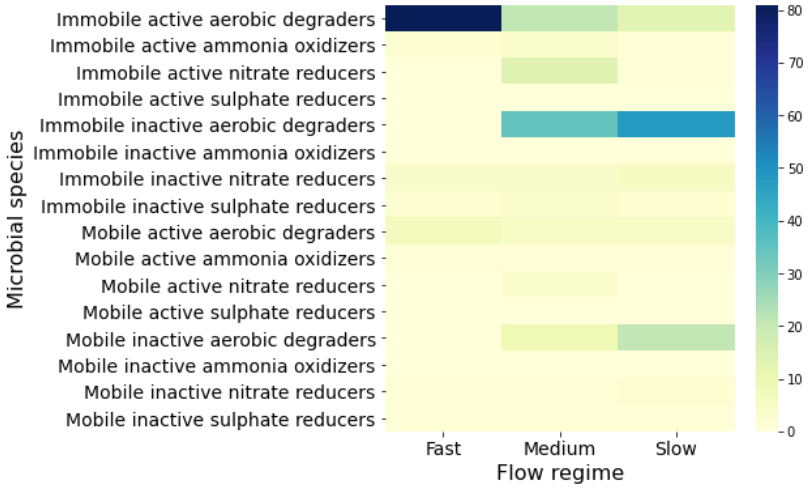

Figure 3.8 Percentage contribution of each subpopulation of each microbial functional group in the base case of each flow regime in the unsaturated domain.

Microbial abundance can be derived from carbon content in the biomass using available conversion factors varying from 5 - 39 femtogram (fg) C/cell (Fukuda et al., 1998;Vrede et al., 2002). This resulted in median values of total mobile biomass in

the saturated domain to be 10^9 to 10^{11} cells L^{-1} and in the unsaturated domain to be ~10^{14} cells L^{-1}. The biomass in the simulation results was higher than that reported in the aquifer at the subject site (Opitz et al., 2014a) while it is in the same order of magnitude of other studies (Akob and Küsel, 2011;Griebler and Lueders, 2009;Grösbacher et al., 2018;Holm et al., 1992).

Overall, the unsaturated domains contained higher biomass irrespective of the flow regime than the saturated domains. This fits with the general understanding that the shallow subsurface contains higher biomass than the deeper saturated subsurface (Magnabosco et al., 2018). Unsaturated domains provided conditions for proliferation of active aerobic degraders due to the constant diffusion of DO in low saturation sub-zones of the domain. Curiously, despite low saturation, mobile microbial species were also higher in the unsaturated domain in each flow regime. While the higher active biomass was mostly attributable to active aerobic degraders, inactive aerobic degraders were also much higher in the slow and medium flow unsaturated domains, compared to the saturated counterparts. At the same time, the biomass of the rest of the functional groups was similar in both saturated and unsaturated domains, accounting for differences in the flow regimes. For example, immobile active nitrate reducers were the highest in the medium flow regime among the unsaturated domains (at average saturation of 0.59) and they were also the highest in the medium flow regime among the saturated domains, with the nitrate reducers having a higher biomass in the lower saturation domain.

The higher active biomass in unsaturated domains resulted in carbon limited conditions in transport limited regimes (that is, slow flow regime), resulting in carbon-depleted discharge from the domain. With the exception of nitrate, the removal of dissolved chemical species was higher in the unsaturated domain. The removal of nitrate, in contrast, was lower in the unsaturated slow and medium flow regimes, while it was comparable in the medium flow regime. This points to an optimum degree of saturation that enables removal of excessive nutrients (both carbon and nitrate) at about 50-60%, slightly higher than earlier reported values by

(Barros et al., 1995) and (Schjønning et al., 2011). This is also well studied in various carbon use efficiency with respect to degree of saturation. Nitrogen uptake should also be considered by practitioners; the optimal degree of saturation for microbial activity with respect to nitrogen removal is expected to be higher than that derived from carbon use efficiency studies alone.

While the total biomass did not match the subject site observations, the relative contribution of the subpopulations of microbial species followed established findings. Interestingly, the relative contribution of the subpopulations was also the same between the unsaturated and saturated domains even though the absolute values of the biomass was different. For example, immobile microbial biomass indeed formed the majority biomass in the subsurface, with its ratio with mobile biomass changing based on nutrient availability, flow velocity and other environmental conditions (Griebler et al., 2002;Grösbacher et al., 2018), with higher ratios observed in oligotrophic conditions and lower ratios in nutrient rich conditions. This study treated oligotrophic conditions akin to transport limited systems, and nutrient rich conditions akin to transport dominant systems.

While the relative contribution of microbial species was the same in corresponding flow regimes of varying saturations, it did change between locally mixed flow regimes and advection dominated flow regime, irrespective of saturation. It is further estimated that 60%-80% of microbial biomass in soil may be inactive (Lennon and Jones, 2011), similar to the observations in the slow and medium flow regimes, but not similar to those in the fast flow regime. This points to an interplay of the flow regime and degree of saturation, both governing the access to nutrient supplies and microbial activity (Grösbacher et al., 2018;Or et al., 2007). This implies that relative abundance only gives us a partial view of the microbial activity prevalent in the domain of our interest (Hunt et al., 2013;Kim et al., 2021). To understand the extent of microbial activity, the absolute values of the active fraction of the biomass are more useful. With newer technologies equipped to better characterize activity of microbes in environmental samples (Couradeau et al., 2019), it will be easier to draw the

comparison in the future. More importantly, the immobile fraction of the active biomass must be quantified since it was invariably higher than the mobile fraction of active species (Griebler et al., 2002;Grösbacher et al., 2018).

This chapter provided preliminary insights into how varying water velocities/flow regimes and varying saturation may impact relative contribution of microbial species between inactive, active, mobile and immobile fractions in sub surficial homogeneous domains. The response of the system to chemical input may vary in spatially heterogeneous domains and/or temporally dynamic fluctuations in groundwater, further studied in Chapters 4 and 5.

3.4 Summary and Conclusions

I tested the reaction network in the homogeneous saturated domain at three different flow regimes. This is akin to testing the same in a batch culture with time being the only dimension. The process network adhered to basic redox hierarchy in that aerobic processes were energetically favourable over anaerobic processes, effectively capturing the transformation of carbon and nitrogen in the domain through a variety of pathways.

Overall, transport limited processes, flow regimes, and saturation influenced the biomass abundance, which in turn influenced the removal of the chemical species from the domain. With this chapter as the foundation, further studies may be conducted to explore the effect of spatio-temporal heterogeneities on the biogeochemical potential of subsurface systems (Chapters 4 and 5).

4 Impact of spatial heterogeneity on carbon and nitrogen cycling

In this chapter, I aimed to quantify the impact of spatially distributed properties of the sub surficial solid matrix on the in situ biogeochemical function of microorganisms using a numerical modelling approach. I focused on spatial heterogeneity alone since varying permeability and conductivity in the subsurface influences water flux, degree of saturation and thus influences the establishment of microbial hotspots (Franklin et al., 2019). To do so, I realized 12 in silico scenarios of permeability and hydraulic conductivity fields of varying heterogeneity for all simulations. I imposed boundary conditions to achieve steady state flow conditions as described in Chapter 2, keeping the average water flux the same in all scenarios belonging to a particular flow regime. I ran simulations in all the two-dimensional domains till steady state conditions were achieved. This enabled me to investigate the impact of spatially distributed matrix properties in the subsurface (permeability, van-Genuchten parameters in the unsaturated vadose zone and hydraulic conductivity of the aquifer matrix) on nutrient cycling in the subsurface with a focus on microbial activity and consumption of carbon and nitrogen thereof using reactive transport modelling.

4.1 Simulated scenarios

For each flow regime in both unsaturated and saturated domains, the homogeneous flow field was the base case scenario, described in detail in Chapter 3. I generated spatially heterogeneous domains in silico using a combination of variance in the log permeability field in the unsaturated domain and in the log hydraulic

conductivity field in the saturated domain, in addition to anisotropy to enforce highly variable flow conditions (Heße et al., 2014). The values of these parameters were based on literature and the subject site (Heath, 1983;Kohlhepp et al., 2017), aiming to represent a wide variety of geological features. See Table 4.1 for the scenario descriptions. The combination of variance and anisotropy effectively captured a variety of geological media ranging from alluvial sediments with low permeable lenses and preferential flow paths to fractured flow. Each random field was characterized by the same mean value of permeability and hydraulic conductivity in unsaturated and saturated domains, respectively.

In total, I ran 147 simulations for the three different flow regimes in unsaturated spatially heterogeneous domains, and another 147 simulations in saturated spatially heterogeneous domains. A Python package, GSTools (Müller and Schüler, 2019), generated the described spatial random fields for the spatially heterogeneous scenarios.

The van-Genuchten parameters (m and α) in the unsaturated domain varied with permeability, scaled by the ratio of permeability and mean permeability in the domain (Schlüter et al., 2013):

$$VG_{i,j}^{p} = VG_{avg}^{p} \times \frac{k_{i,j}}{k_{avg}}, \quad (4.1)$$

where, $VG_{i,j}^{p}$ is a van Genuchten parameter at node (i,j), VG_{avg}^{p} is the bulk property of the respective van Genuchten parameter for the entire domain (Chapter 2), k_{avg} is the average permeability of the domain and $k_{i,j}$ is the permeability at node (i,j). The parameters were subject to limits based on observations recorded for clayey soils (Carsel and Parrish, 1988).

Table 4.1: Summary of spatially heterogeneous scenarios investigated for each flow regime. S. No. 1 is the homogeneous base case.

S. No.	Variance in permeability	Anisotropy	Number of realizations	Category type
1	0	1	1	0:1
2	0.1	2	4	0.1:2
3	0.1	5	4	0.1:5
4	0.1	10	4	0.1:10
5	1	2	4	1:2
6	1	5	4	1:5
7	1	10	4	1:10
8	5	2	4	5:2
9	5	5	4	5:5
10	5	10	4	5:10
11	10	2	4	10:2
12	10	5	4	10:5
13	10	10	4	10:10

4.2 Data Analysis

The breakthrough time was used as a metric to characterize the spatial heterogeneity in the domain. This enabled evaluating impact of spatial heterogeneity on matter flux alone, without considering impact of reactions.

To evaluate the impact of spatial heterogeneity, I compared the mass removal of chemical species in spatially heterogeneous domains with the respective base case (homogeneous domains). I primarily used the Da (described in Chapter 2) to study the biogeochemical potential of a given domain in steady state conditions. The coefficient of variation of the concentration of these chemical species within the domain was given by Eq. 4.2.

$$cv = \frac{\sigma}{\mu}, \quad (4.2)$$

where, σ is the standard deviation of the observations and μ is the mean of the observations. A linear regression analysis of species removal vs. residence time (both in relative units to the homogeneous reference cases) for different $\log_{10}$Da ranges revealed the scalable relationship to address the impact of spatial heterogeneity on reactive species removal. For comparison with this regression analysis, the following expression predicted the impact of reducing breakthrough time alone on removal of reactive species, in case of a first order removal rate expression (Eq. 4.3):

$$C_t = C_i e^{-kt}, \quad (4.3)$$

with C_i [ML^{-3}] as initial concentration of reactive species, C_t [ML^{-3}] as concentration of reactive species at time t [T], k as first order rate constant [T^{-1}], and t as time taken for the reaction to occur. Then it follows that, normalized removal of reactive species may be described with:

$$\frac{C_i - C_t}{C_i} = 1 - e^{-kt} \quad (4.4)$$

To compare the removal of reactive species between two different time points, I adapted the above equation using Da to derive the analytical solution to the impact on removal of chemical species with respect to base case ("impact" for brevity):

$$Impact = \frac{1 - e^{-Da.tf}}{1 - e^{-Da}} \quad (4.5)$$

with tf as ratio of the time taken for the reaction to take place in the two (2) different scenarios. This was same as the ratio of breakthrough time in the heterogeneous domain and that in the base case in this work. Furthermore, the impact of reducing breakthrough time on removal of reactive species, in case of a zeroth order (i.e., constant) removal rate R_0 was given by:

$$Impact = tf\ R_0 \quad (4.6)$$

To evaluate the impact on microbial activity, I explored the contribution of active and inactive immobile and mobile fractions of the different microbial species present in the domain, and also the coefficient of variation of the concentration of microbial species and compared with their contribution in the respective base case scenarios.

4.3 Results

The characteristics of flow and transport of porous media such as conservative tracer breakthrough, microbial biomass in the domain and nutrient removal from the domain for heterogeneous domains differed from the base case in some scenarios. The base case was the homogeneous domain in all the three considered flow regimes in both unsaturated and saturated conditions (Chapter 3). I explored flux-averaged concentrations of mobile species and spatially averaged concentrations of immobile species in 1-D, along the predominant flow direction, and explored the 2-D concentration heat maps of the domain to compare the information lost when neglecting sub-sampling scale spatial heterogeneity. To aggregate results, the total microbial biomass in the domain, and nutrient removal from the domain were compared between the heterogeneous domains and respective base cases.

4.3.1 Average saturation

The average saturation was dependent on the average water flux in the system, with the fast flow regime being nearly saturated, and the slow flow regime being the driest (Chapter 3). In heterogeneous domains, the average saturation decreased monotonously with increasing variance in the log permeability field, irrespective of the flow regime (Fig 4.1) even though the average water flux in the base case and the corresponding heterogeneous domain was the same. The highest reduction in average saturation was within 20% of that in the base case (19% for slow flow regime, 17% for medium flow regime and 15% for the fast flow regime). At the same time, the coefficient of variation of saturation increased in all flow regimes with increasing

heterogeneity with the highest impact on the slow flow regime (increased to more than 0.15) and the lowest impact on the fast flow regime (increased to more than 0.13).

4.3.2 Tracer breakthrough times

For each flow regime, the tracer breakthrough time in heterogeneous domains varied from that in the base case. In both saturated and unsaturated domains, the breakthrough time in spatially heterogeneous scenarios was less than that in the corresponding base case (Fig. 4.2). This was a result of preferential flow paths that were introduced by the heterogeneous hydraulic conductivity fields. The same "category" (combination of variance and anisotropy) of heterogeneity induced varying impact depending on the flow regime, with higher average flow velocities leading to relatively stronger reductions of the breakthrough times. This difference in the impact of heterogeneity on tracer breakthrough times and thus the residence time of solutes in the domain was attributed to the dominant transport processes in the regimes. Diffusion played a stronger role in the transport processes in the slow flow regime, promoting mixing effects and reduced influence of the preferential flow paths in heterogeneous domains. This resulted in the lower deviation in breakthrough time from the base case in the slow flow regime. In contrast, in the medium and in the fast flow regime, transport was dominated by advection with little mixing between flow paths. The preferential flow paths in the heterogeneous domains therefore had a higher influence on the resulting tracer breakthrough times, and thus on the residence time of dissolved species in these regimes.

4.3.3 Distribution of chemical species

Unsaturated domains

The spatial heterogeneity resulted in varying distribution of chemical species in both saturated and unsaturated domains. In the unsaturated domain, the low flow zones were unsaturated compared to the high flow zones with preferential flowpaths,

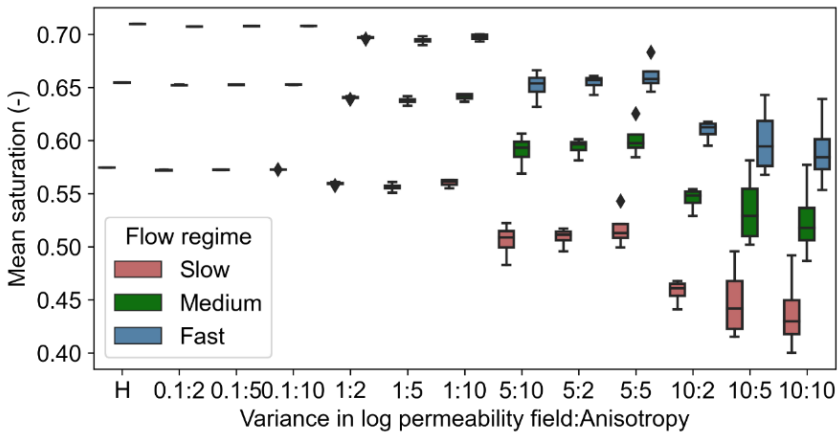

Figure 4.1 Decreasing average saturation with decreasing average water flux and increasing spatial heterogeneity

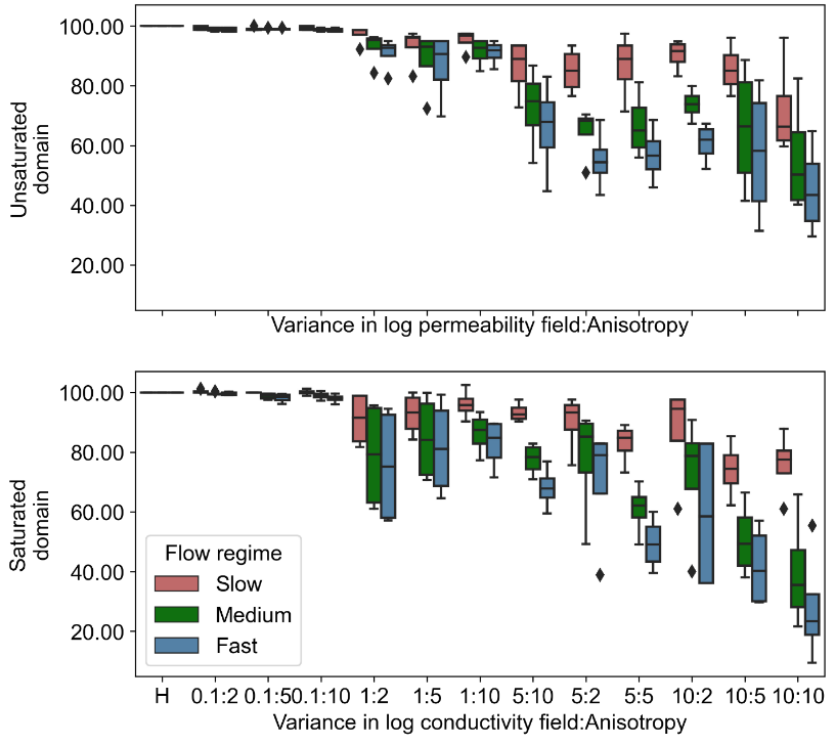

Figure 4.2 Breakthrough time in different heterogeneous scenarios normalized by that in the base case (%) in three flow regimes: Slow, medium, and fast flow in saturated and unsaturated conditions. The variance is defined as the variance in the log permeability field in unsaturated conditions, and in the log hydraulic conductivity field in saturated conditions.

resulting in persistent oxic conditions in the low flow zones and consequently, continuous diffusion of oxygen from the air phase to the water phase in these zones. However, even the spatially heterogeneous domains remained largely oxic in the slow flow regime, also evident in the low coefficient of variation of the concentration of DO (Fig. A1, Fig. A3 and Fig A4). The low flow zones thus allowed for proliferation of aerobic activity and consumption of DOC. Thus, the coefficient of variation of DOC concentration was the highest in the slow flow regime, regardless of the extent of spatial heterogeneity. At the same time, the variation in the concentration of the chemical species was not impacted by spatial heterogeneity (Fig. A3).

Saturated domains

All the saturated domains allowed for higher persistence of DO in the high flow zones. Similar to the unsaturated domains, the distribution of chemical species was not impacted by spatial heterogeneity in the slow flow regime due to high rates of microbial activity near the inlet of the domain (Fig. A2). In contrast, a larger oxic zone with aerobic activity existed in the upgradient section of the domains in medium and fast flow regimes. There, spatial heterogeneity resulted in observable shifts of the transition from oxic to sub-oxic conditions or from aerobic activity to anaerobic activity to further downgradient parts of the domain. Because of the shift of the oxic-anoxic interface, nitrate reduction took place further downgradient in the domain and at the interface of high flow and low flow zones (Fig. A2 and Fig. A5). However, the coefficient of variation of the chemical concentrations did not vary much with spatial heterogeneity despite these shifts in the oxic-anoxic interfaces (Fig. A3).

It was only in the fast flow regime, that the coefficient of variation of nitrate concentration increased with spatial heterogeneity. This is attributable to the domain being predominantly oxic. Thus, the low flow zones that allowed for marginal reduction in nitrate concentration resulted in a much higher coefficient of variation in the domains (Fig. A3).

4.3.4 Aerobic activity

Aerobic activity in the unsaturated and saturated domains was assessed separately since the diffusion of oxygen from the air phase to water phase in unsaturated domains resulted in a confounding relationship.

Unsaturated domains

In the unsaturated domains, consumption, or removal of DO was estimated by combining the rate of aerobic respiration by aerobic degraders, and that of ammonia oxidation by ammonia oxidisers. These rates represent the consumption of DO through different routes, and the impact of spatial heterogeneity on these rates with respect to the base case is presented in Fig. 4.3. The left panel of Fig. 4.3 presents this impact against changing saturation in the heterogeneous domains. The right panel presents the same data against changing breakthrough time in the domain.

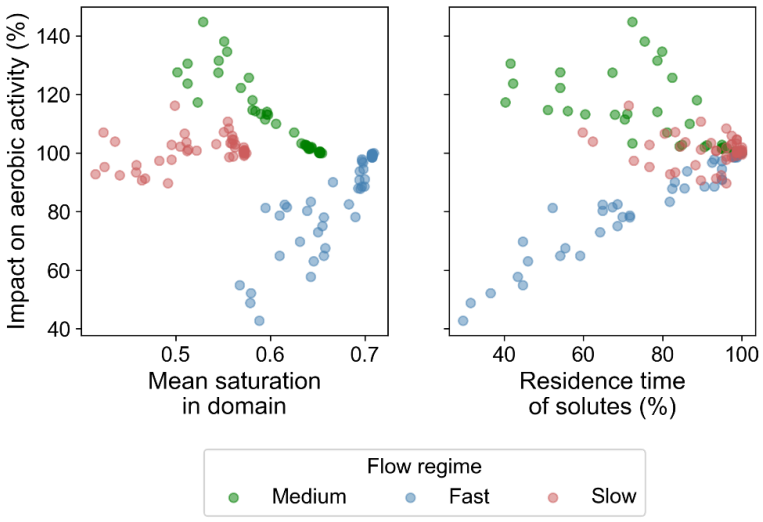

Figure 4.3 Total rate of consumption of DO normalised by that in the base case in each flow regime in unsaturated domains.

In the base case of the fast flow regime, DO was consumed at the highest rate (at ~40 μM d^{-1}) despite the fast flow regime being near saturation, while DO was consumed at the lowest rate in the slow flow regime (~0.6 μM d^{-1}) despite it being predominantly oxic. Thus, a reduction of ~15% in the aerobic activity in slow flow

regime is very little absolute variation from the base case, with the lowest aerobic activity in spatially heterogeneous domain at ~0.51 μM d^{-1}. However, in the fast flow regime, aerobic activity in heterogeneous domains decreased to ~55% of that in the base case, resulting in the DO consumption rate dropping to ~20 μM d^{-1}. In the medium flow regime, on the other hand, DO consumption increased to 140% of that in the base case with decreasing saturation and decreasing breakthrough time, from ~5 μM d^{-1} to higher than 7 μM d^{-1}.

Saturated domains

The impact of spatial heterogeneity on the consumption of DO in saturated domains was like that in the unsaturated domains for the slow and fast flow regimes (top right panel in Fig. A7). Like the unsaturated domain, there was no remarkable impact on consumption of DO in the slow flow regime, while the consumption of DO decreased monotonously with decreasing breakthrough time and increasing spatial heterogeneity in the fast flow regime (Fig. A7). In contrast to the unsaturated domain, the removal of DO was not impacted by spatial heterogeneity in the medium flow regime.

4.3.5 Removal of chemical species

With spatial heterogeneity influencing chemical species distribution, water saturation and aerobic activity, the persistence of the other chemical species in the domain was also impacted. The concentration of the reactive species leaving the spatially heterogeneous domains differed from that in the corresponding base cases. Thus, the removal of chemical species from the domain was dependent on spatial heterogeneity and the flow regime.

Unsaturated domains

In the slow flow regime, removal of chemical species was affected by spatial heterogeneity. DOC removal was marginally affected with reduction in removal by

20% in extremely high spatially heterogeneous domain, and nitrate removal decreased down to less than 5% of that in the base case. This extremely low value is due to the introduction of low saturation, oxic subzones in heterogeneous domains, leading to complete suppression of nitrate reduction, also evident in the concentration profiles (Fig A4).

In the medium flow regime, DOC removal increased with increasing heterogeneity, and nitrate removal decreased with increasing spatial heterogeneity, and the variation in the concentration of nitrate in the domain also reduced with increasing spatial heterogeneity. DOC removal, on the other hand, first increased 120% of that in the base case in select heterogeneous scenarios, and then was similar to that in the base case for highly spatially heterogeneous domains.

In the fast flow regime, DOC removal decreased linearly to ~80% of that in the base case (Fig A6). Ammonium removal was linked with DOC removal and decreased with increasing spatial heterogeneity. Nitrate removal increased by several orders of magnitude. The high scale was attributable to negligible removal of nitrate in the base case (~10^{-5} μM). Thus, even a slight increase in removal of nitrate from the domain resulted in seemingly high impact on nitrate removal, even though the absolute removal of nitrate was still low.

Saturated domains

Spatial heterogeneity did not affect removal of most chemical species in the slow flow regime. However, it impacted (decreased) the removal of carbon and nitrogen in heterogeneous domains with increasing spatial heterogeneity compared to the base cases (Fig. A7). DOC removal was less than in the base case in all the flow regimes (as low as 40% of the base case values in the fast flow regime. The removal of DO also similarly reduced in the fast flow regime while no or negligible reductions were observed for most slow and medium flow scenarios. Nitrogen removal was reduced in the slow and medium flow regimes yet reaching (as low as 70% of base case

values). One exception was nitrogen removal in the fast flow regime, which increased (up to 6 times the base values) compared to the base case.

4.3.6 Predicting impact of spatial heterogeneity on redox regimes and biomass

Da was useful to describe the above results for both saturated and unsaturated domains (Fig. 4.4-4.5) as it assisted in categorizing simulation domains with respective flow regimes and reaction regimes into 4 categories of reactive systems: Transport dominated/reaction limited where $\log_{10}Da<-1$, transport influenced where $1<\log_{10}Da<0$, reaction influenced where $0<\log_{10}Da<0.5$, and reaction dominated/transport limited where $\log_{10}Da>0.5$. Thus, the impact of spatial heterogeneity on bulk chemical removal can be predicted using estimates of Da and breakthrough time in both saturated and unsaturated domains.

4.3.7 Contribution and distribution microbial biomass

Since spatial heterogeneity affected the distribution of chemical species in the domain, the distribution of microbial biomass was likewise affected (Fig. A8 for selected heterogeneous scenarios in the unsaturated domain and Fig. A9 in the saturated domain). While aerobic immobile degraders were active and most abundant near the inlet of all the domains, and along the preferential flow paths in the downgradient zone of the domain, they were also active in low saturation and low flow zones. Ammonia oxidizers were active at the interfaces between high flow and low flow regions of all the domains as well as in carbon limited and oxygen rich unsaturated domains. They co-existed with high concentration of active aerobic degraders, but at concentrations lower by more than an order of magnitude. Since nitrate reducers also required carbon to proliferate, they only persisted in carbon-rich but oxygen depleted zones of all heterogeneous domains. They co-existed with ammonia oxidizers but typically, at higher concentrations.

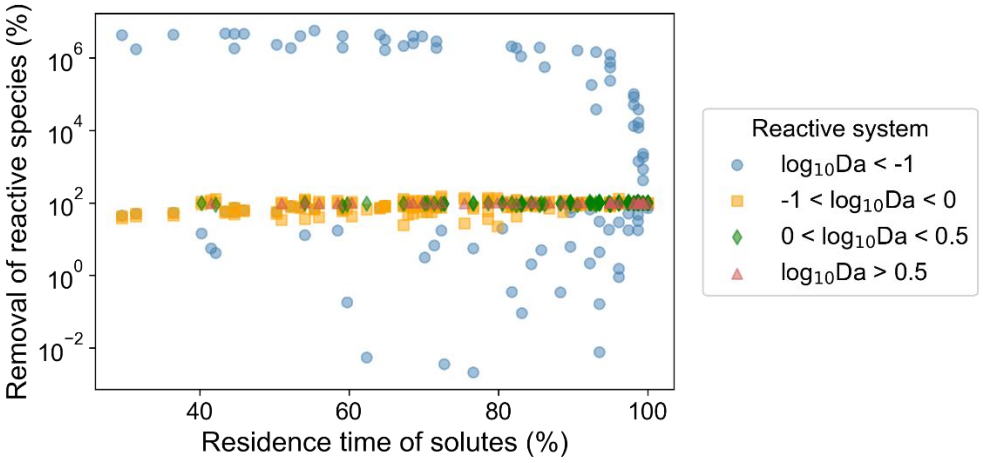

Figure 4.4 Predicting impact of spatial heterogeneity on reactive species removal in different reaction regimes indicated by $\log_{10}$Da in unsaturated domains.

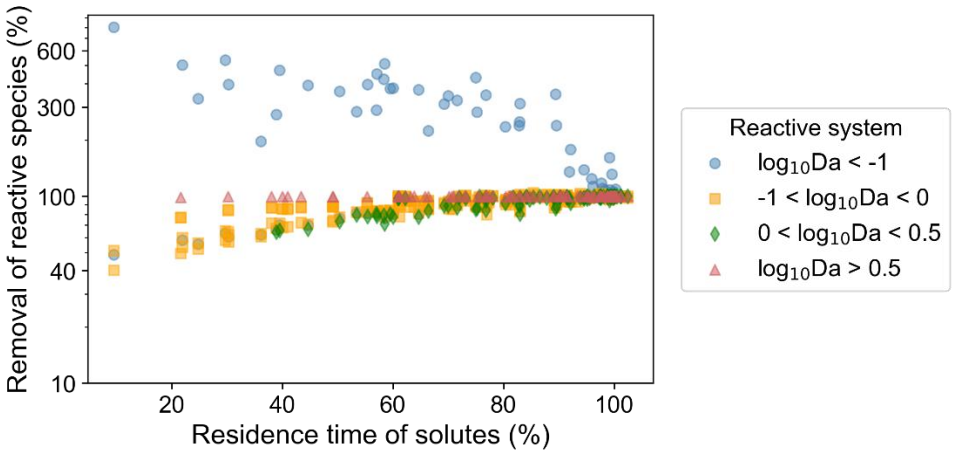

Figure 4.5 Predicting impact of spatial heterogeneity on reactive species removal in different reaction regimes indicated by $\log_{10}$Da in saturated domains.

While the distribution between inactive and active subpopulations depended on the degree of saturation and the flow regime (Fig. 4.6 and Fig. 4.7), the active immobile fraction of the biomass was much higher than active mobile fraction. Thus, the immobile fraction made a substantial contribution to nutrient cycling in all domains irrespective of the flow regime and degree of saturation. The coefficient of variation of this fraction also increased for all species with increasing spatial heterogeneity (Fig. A10) regardless of degree of saturation and flow regime.

Unsaturated domains

In the slow flow regime, the inactive fraction of microbial biomass was the highest contributor to the total biomass, also in heterogeneous domains. Whereas the immobile fraction decreased with increasing spatial heterogeneity, the mobile fraction increased. The shift in the fraction is attributable to a shift in location of aerobic degraders from immobilized on the matrix to mobile in the groundwater. The active fraction on the other hand was stable with changing spatial heterogeneity (Fig. 4.6).

In the medium flow regime, the immobile fraction of both active and inactive species decreased by up to 20% with increasing spatial heterogeneity (reduction attributable to reduction in biomass of nitrate reducers and inactive aerobic degraders), while the mobile fractions increased (Fig. A11). The inactive mobile fraction increased by up to 40%, attributable to aerobic degraders (Fig. 4.6). Except for immobile active aerobic degraders and immobile inactive nitrate reducers, the variation in the concentration of all the species increased with spatial heterogeneity.

In the fast flow regime, the active immobile fraction decreased by up to 40%, that transferred to an increase in inactive fraction (by up to 20% for each immobile and mobile). This shift in fraction is attributable to aerobic degraders, as they are the largest contributors to microbial biomass in the fast flow regime. The active mobile fraction was relatively stable with increasing spatial heterogeneity. With the exception of immobile inactive aerobic degraders and immobile active nitrate reducers, the coefficient of variation of the biomass of all microbial species increased with increasing spatial heterogeneity. Overall, active immobile biomass decreased to varying extents (depending on the flow regime) with decreasing breakthrough time and decreasing saturation (Fig. 4.6).

Saturated domains

The distribution of microbial biomass among different fractions for changing spatial heterogeneity was similar in the saturated domains (Fig. A12). As in the unsaturated domain, total active immobile biomass concentration in the domain, diverged from the base case as heterogeneity increased. The biomass of active immobile aerobic degraders decreased with increasing heterogeneity regardless of the flow regime, with lowest values reaching only 40% of the base case biomass. The biomass of immobile active ammonia oxidizers and nitrate reducers also decreased with increasing heterogeneity in slow (~75% and ~90% of base case, respectively) and medium flow regimes (30% and 85% respectively). However, the impact on the biomass of immobile active nitrate reducers was the reverse in fast flow regime (increase to 5 times the concentration in the base case). Lastly, there was no impact of spatial heterogeneity in the biomass of immobile active ammonia oxidizers in the fast flow regime.

Overall, active immobile biomass decreased with increase in spatial heterogeneity in all the flow regimes, while active mobile biomass increased marginally. Inactive immobile biomass reduced with spatial heterogeneity in slow and medium flow regimes, while it increased in the fast flow regime. Lastly, inactive mobile biomass increased with heterogeneity in all flow regimes. While the ratio of the subpopulations differed between the flow regimes, the ratio decreased linearly with respect to the base case in each flow regime (Fig. 4.7).

4.4 Discussion

In this chapter in silico scenarios representing a wide variety of geological settings to study the fate of reactive chemical species in the spatially heterogeneous subsurface revealed the influence of spatial heterogeneity on microbial redox dynamics. In total, 12 scenarios represented a variety of heterogeneous flow fields

from layered alluvial deposits to fractured bedrock, covering most physically (variance) and geometrically (anisotropy) plausible scenarios.

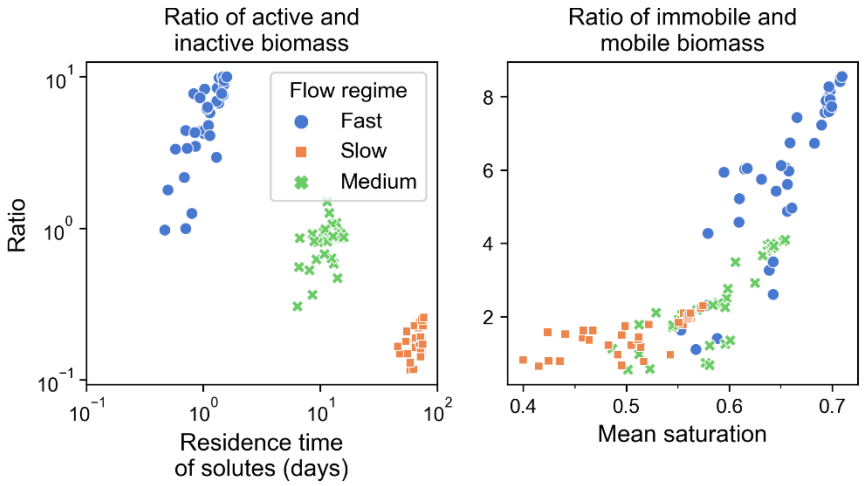

Figure 4.6 Relative contribution of subpopulations of all the microbial functional groups based on the state of activity and the location to the total biomass in spatially heterogeneous unsaturated domains.

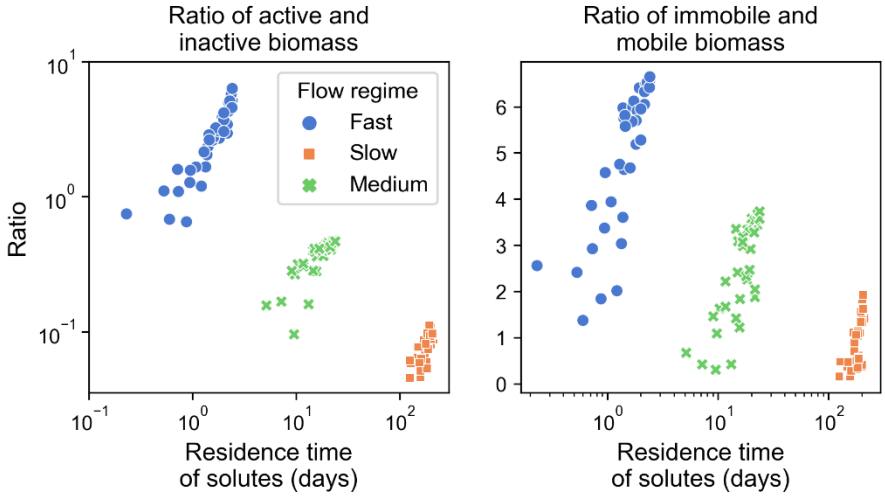

Figure 4.7 Relative contribution of subpopulations of all the microbial functional groups based on the state of activity and the location to the total biomass in spatially heterogeneous saturated domains.

As described in Chapter 2, the same comprehensive reaction network was used in all the flow regimes at varying saturation as well as in the base cases. This enabled

me to study of the impact of spatial heterogeneity on microbial activity and sub surficial nutrient dynamics.

Solute transport in the subsurface is affected by both spatial heterogeneity and the degree of saturation, displayed in the varying impact on breakthrough time with varying saturation. Lower velocity and lower saturation results in higher contribution of matrix flow for solute transport in heterogeneous domains (McIntosh et al., 1999), In contrast, preferential flow contributes more to solute transport in domains exhibiting higher degree saturation, or with higher water flux rates (Koestel et al., 2012). This resulted in varying impact on tracer breakthrough time in all heterogeneous domains at steady state conditions, with the saturated domains exhibiting a stronger reduction in breakthrough time with increasing heterogeneity. In near saturation domains, it can be as low as 40% of average flow characteristics (Koestel et al., 2013), in the same scale as that displayed in this chapter. Concurrently, the flow regimes with higher average water flux rates exhibited a stronger reduction in breakthrough time compared to the slower flow regimes.

4.4.1 Implications of spatial heterogeneity

In the subsurface, water flux influenced by spatial heterogeneity governs the distribution of microbial hotspots resulting in preferential flow paths hosting aerobic microbial activity (Franklin et al., 2019). The results presented in this chapter give important insights into how a microbial community with varying respiration strategies (both aerobic and anaerobic) may evolve in spatially heterogeneous (both saturated and unsaturated) environments.

Spatial heterogeneity results in differential distribution of saturation and velocity patterns. Drier domains (slow flow regime) exhibited higher variation in saturation compared to wetter domains (fast flow regime, (Basu et al., 2010)). Penna et al. (2009) however reported that moderately saturated soils exhibited the highest coefficient of variation. Given that these studies examined soil samples from different

locations with different topographic conditions and land use/cover (not considered in this thesis) and the simulations are also limited by enforced boundary conditions, the results are not directly comparable. However, the trend of increasing coefficient of variation with increasing spatial heterogeneity in the domain is clear.

The varying saturation in unsaturated heterogeneous domains does heavily influence gaseous diffusion. This is important to note as the rate of DO diffusion from the air phase to the water phase was higher than that of the substrate flux in the slow flow regime, making the system carbon limited (Or et al., 2007). Surprisingly, the higher variation in saturation does not result in higher variation in concentration of DO in the domain in the slow flow regime. This is because the diffusion of DO occurs throughout the domain (except for select preferential flow paths where advective transport supersedes gaseous diffusion) almost uniformly, despite the spatially heterogeneous structure. In turn, the coefficient of variation was also similar for DOC, DO and nitrate since they are consumed relatively quickly in the slow flow regime. This resulted in much lower impact of spatial heterogeneity on microbial biomass and removal of chemical species. The transport limitation of the substrate flux has thus the highest control on the distribution of microbial biomass among different subpopulations and subsequent chemical removal from the reactive system (Thullner et al., 2005).

In contrast to the slow flow regime, the domain in the fast flow regime was almost saturated, and gaseous diffusion did not influence DO concentration in the domain. The variation in DO concentration increased with increasing spatial heterogeneity and reducing breakthrough time. Further, DO concentration was higher in preferential flow paths and DO removal (and thereby DOC removal) was linearly dependent on breakthrough time alone (Sanz-Prat et al., 2016), being a reaction limited system. Given the dominant oxic system, anaerobic activity did not occur in the system at all, or to a marginal extent in the saturated domain.

The medium flow regime was advection dominated, exhibiting lower average saturation than the fast flow regime but higher than the slow flow regime. This regime, thus, did not allow for uniform gaseous diffusion along the preferential flow paths (in contrast to the slow flow regime) but allowed for higher persistence of DO in the low-flow zones of the domain (in contrast to the fast flow regime). At the same time, it allowed for higher persistence of DOC further downgradient in the domain (similar to the fast flow regime). Thus, aerobic microbial activity was limited by gaseous diffusion in these systems and enabled by advective transport processes. This interplay of gaseous diffusion and solute transport gave rise to sub-oxic and anoxic niches in downgradient areas of the domain, which allowed for the growth of nitrate reducers and higher removal of nitrate from the system, and carbon. Previous studies have also displayed higher microbial activity in higher saturation systems (Brangarí et al., 2018). In contrast, chemical species removal decreased with increasing spatial heterogeneity in the saturated medium flow regime, despite them allowing for both aerobic and anaerobic activity.

Overall, characterizing the orientation of the spatially heterogeneous soil properties, although important (Jang et al., 2017), did not accurately predict the distribution of microbial and chemical species in heterogeneous domains alone. The flow regime (and thereby the average saturation) had a critical role to play (Grösbacher et al., 2018;Gurevich et al., 2021). For the same orientation of spatially heterogeneous permeability field, DO distribution (and thereby aerobic activity) may be distributed differently in the system. Spatial heterogeneity allowed for this apparent co-occurrence of several microbial species by providing appropriate niches, similar to observations in the field (Alewell et al., 2006;Grösbacher et al., 2018;Lohmann et al., 2020;Schwab et al., 2017;Waldron et al., 2009), although the diversity of microbial communities varied in both space and time. Thus, sub-field scale geologic heterogeneity governs microbial activity (Gurevich et al., 2021), and these heterogeneities do not allow for sequential redox hierarchy to be applicable at larger scales (Alewell et al., 2006). At the same time, spatially distributed microbial

growth can also be linked to both carbon limitation (Geza et al., 2021) and nitrogen limitation (Austin et al., 2004;Schimel and Weintraub, 2003) in substrate flux limited systems, because of hydrologic controls (Kim et al., 2019;Kim et al., 2009).

While these sub-scale differences may not impact bulk microbial activity or chemical species removal (see below), they can still inform our interpretation of sampling techniques and data obtained from the subsurface: from lysimeters, monitoring wells, soil and rock cores. Since immobile microbes account for more microbial activity compared to mobile microbes (Grösbacher et al., 2018), they are necessarily the subpopulation to quantify. Estimating microbial activity based on the mobile planktonic biomass (Smith et al., 2018) present in groundwater or seepage water may thus be inaccurate. The ratio of immobile and mobile species, however, depended on the degree of saturation, the flow regime, and the breakthrough time. Thus, the results of this chapter support previous studies in that the immobile microbes are the major contributors to microbial respiration in the subsurface (Alfreider et al., 1997;Griebler et al., 2002;Grösbacher et al., 2018), by providing the link between heterogeneous structures in the domain, and also providing a scaling relationship to estimate the immobile biomass on the basis of sampled planktonic biomass. Having said that, all functional groups were present in all subpopulations, and thus, seepage water and groundwater samples are useful to evaluate microbial diversity in the reactive system of interest.

Lastly, the ratio of active and inactive biomass is predictable, given the extent of spatial heterogeneity and the prevailing flow regime in the reactive system of interest if fully saturated, or the degree of saturation if variably saturated. Since spatial heterogeneity and the flow regime combined to make nutrient limiting/nutrient scarce conditions for microorganisms in all simulated scenarios, inactive biomass reduced with both increasing spatial heterogeneity and decreasing saturation (Manzoni et al., 2014).

4.4.2 Predicting chemical removal in heterogeneous saturated reactive systems

Since spatial heterogeneity impacted microbial biomass distribution and microbial activity, it was not surprising that spatial heterogeneity also impacted carbon and nitrogen removal. In advective systems, travel time could be used as an indicator of the spatial heterogeneity of the domain (Painter, 2018), and steady state reactive transport models could be replaced by travel time models as well (Sanz-Prat et al., 2015, 2016). But for locally mixed flow regimes, this did not hold. Additionally, the validity of using the travel time approach depended on the estimated Da number of the saturated reactive system as well.

In the simulated scenarios, Da varied over 4 orders of magnitude, and the impact of spatial heterogeneity depended on the breakthrough time and $\log_{10}$Da. Since spatial heterogeneity governed the distribution of water flux and access to nutrients, it had limited impact on reaction dominated (or transport limited) systems where $\log_{10}$Da was higher than 0.5. In reaction and transport influenced systems, spatial heterogeneity impacted the mass removal in a linear relationship with the breakthrough time.

To explore the impact of decreasing breakthrough time further, I compared the trend of removal of reactive species in first order rates and zero order rates with reduced residence times with the simulation results for varying values of $\log_{10}$Da. With increasing $\log_{10}$Da (between 0 and 0.5), the root mean squared error (RMSE) between the approximated analytical solution for a first order reaction and the simulation results decreases. Additionally, the data points lie in between the solutions for first order and zero order kinetics, as it would be the case for Monod kinetics in case of reduced residence times. Consequently, the impact of spatial heterogeneity on reaction influenced systems may be described on the basis of reducing residence time alone and the results do not allow to determine if additional heterogeneity effects on removal take place. For transport influenced systems first order kinetics approximate to zero order kinetics. Additionally, the impact on mass removal of reactive species in

this domain is lower than estimated from the analytical solution. Therefore, while mass removal of reactive species reduces with reducing breakthrough times, it does not follow Monod kinetics which implies that heterogeneity has a different impact on removal than changing only the residence time. In fact, the impact of spatial heterogeneity on mass removal is lower than that predicted by reducing residence time alone.

In transport limited systems, on the other hand, the mass removal either dramatically increased in the fast flow regimes or dramatically decreased in the slow flow unsaturated domains. In the fast flow regimes, the increase is attributable to negligible removal of the corresponding nutrients in the base case (specifically, nitrogen in the fast flow regime). The heterogeneous conditions in these domains provided niches to the relevant microbial species to become active, and thus even a marginal increase in the relevant microbial activity results in a remarkably high impact on the removal of the corresponding nutrient when compared to the base case. On the same lines, in the slow flow unsaturated domains, the heterogeneous conditions and lower saturation provided for higher aerobic activity. This resulted in the complete elimination of nitrate reduction processes. The observed high impact in mass removal with heterogeneity was thus to some extent an artefact that may not represent a general trend. Overall, while heterogeneity dids have an impact on nitrogen removal in transport limited systems, the $\log_{10}$Da value remained below -1, indicating low absolute activity/removal.

4.5 Summary and Conclusions

In this chapter, I explored the impact of spatial heterogeneity on geomicrobial redox dynamics in the subsurface by exploring a wide range of unsaturated and saturated reactive systems, spatially heterogeneous domains and flow regimes, from locally mixed regimes to dominantly advective flow regimes and a complex process

network accounting for a variety of reactive species across both aerobic and anaerobic microbial processes.

A combination of variance in the log permeability (unsaturated domain) and hydraulic conductivity (saturated domain) fields, and anisotropy resulted in a variety of spatially heterogeneous domains. Reduction in solute residence time in the domain adequately represented this spatial heterogeneity. Biomass persistence, distribution, and nutrient cycling depended on the spatial heterogeneity at the sub-meter scale in the subsurface to varying extents. Flow regime played an influential role in the average behaviour of both unsaturated and saturated domains.

The total microbial biomass, as well as the subpopulations thereof (between active or dormant, mobile or immobile) depended on the flow regime and spatial heterogeneity. This had a cumulative impact on nutrient cycling in the subsurface. The activity of the microbial species in the domain was governed by the spatial heterogeneity as it influenced the distribution of nutrients and energy sources. Several microbial species that are conventionally accepted to occupy mutually exclusive niches may co-exist in the subsurface in close vicinity at varying activity states. The evolution of sub-oxic zones with anaerobic activity in predominantly oxic systems depended on both spatial heterogeneity and the flow regime, but surely impacted the overall chemical removal from the domain. Since modelers and experimentalists do not conventionally resolve these small-scale heterogeneities the accuracy of the prediction of biogeochemical cycles at the larger scale suffers. Overall, spatial heterogeneity is not of significance in transport limited reactive systems, while it is more relevant in advection dominated reactive systems.

5 Impact of temporal dynamics on carbon and nitrogen cycling in the subsurface

In this chapter, I aimed to quantify the impact of temporal dynamics on microbial activity and chemical discharge in terrestrial subsurface settings through numerical simulations since there is considerable interest in linking processes occurring in different compartments of the Earth's Critical Zone such as weather events above the surface with those in the surficial soil, root zone, the vadose zone and eventually the deep subsurface (Guo and Lin, 2016).

Linking these processes is not a trivial task as they take place along time scales from days to millions of years (Brantley et al., 2017;Guo and Lin, 2016). I used the novel and comprehensive reaction network in Chapter 2 to simulate scenarios that combined temporal fluctuations of the external forcing with different types of subsurface heterogeneities to determine the responsiveness of the subsurface reactive system. I assumed that the infiltration recharge and groundwater head fluctuated responding to weather events, diurnal, seasonal and inter-annual cycles in atmospheric conditions. The results help estimate the variability in nutrient discharge in a wide range of sub-surficial microbial reactive systems. This, in turn, contributes towards improved predictability of reactive transport models in transient conditions at field scales.

This chapter investigated the effect of temporal dynamics in forcings on microbial activity in and chemical discharge from reactive subsurficial systems. For this, I used the previously introduced model set up (Chapter 2) and used homogeneous domains in all the flow regimes in steady state conditions (Chapter 3) as the base case. Since the subsurface is spatially heterogeneous, the steady state conditions in spatially heterogeneous systems (Chapter 4) were also used as base cases. I subjected these reactive systems at steady state conditions to temporal dynamics by varying the

external forcing, that is, groundwater head at the inlet. I considered diurnal fluctuations, seasonal and inter-annual cycles in groundwater head, and thereby flow velocity, in conjunction with varying spatial heterogeneity of the aquifer.

5.1 Simulated scenarios

Based on the long-term groundwater head observations used by Jing et al. (2018), I determined that groundwater head can be described by a Gaussian process model (also known as a multivariant Gaussian distribution) with an exponential like covariance model. I, thus, generated three scenarios capturing different aspects of temporal dynamics based on this model; using three values of the variance (1,2, and 5 m^2) and two correlation time scales to incorporate annual (or seasonal with length scale as 365 days, referred to as $g_1(t)$) and super-annual cycles (with length scale as 730 days, 2 years, referred to as $g_2(t)$) in the groundwater head. Superimposing the time series $g_1(t)$ and $g_2(t)$ and centring the mean of the distribution around 1 using Eq. 5.1 resulted in the time series imposed on the inlet of the boundary (f(t)):

$$f(t) = \frac{g_1(t)+g_2(t)}{\mu}, \quad (5.1)$$

where, μ is the mean of the sum of $g_1(t)$ an $g_2(t)$. I applied the product of this time series (f(t)) with the recharge (for the unsaturated domain) and with the head (for the saturated domain) at the inlet for each scenario as the temporally varying forcing. The three imposed time series showed varying temporal characteristics (Table 5.1). The covariance of the mean-adjusted time series varied between 0.15 and 0.27. For the first time series, the memory (the time lag when autocorrelation reduces to below 0.7, see below) was 39 days, while for the third time series with the highest covariance, the traceability was 166 days. See Fig. A13 for the autocorrelation function.

Table 5.1 Summary of temporally dynamic scenarios investigated.

S. No.	Scenario name	Co-variance^	Traceability (days)
1	T1	0.15	39
2	T2	0.17	155
3	T5	0.27	166

*Super-annual (time scale of 2 years) superposed with annual cycle (time scale of 1 year, representing seasonal cycles), both having the same variance. ^The co-variance is of the resulting superposed time series

I ran transient simulations for a period of 15 years to evaluate the impact of long-term temporal dynamics. The initial conditions for these simulations were steady state conditions induced in each domain as described in Chapter 4. Even though the water flux varied in time to simulate temporal dynamics; the time averaged water flux in each domain was constant for each flow regime over the entire simulation period of 15 years for the purposes of keeping the results of the simulations comparable. In total, imposing three time series scenarios on 48 spatially heterogeneous domains and one homogeneous domain in three different flow regimes resulted in 441 simulations.

5.2 Data analysis

I recorded observations at a time interval of 5 days in all scenarios, extracting the following response variables from the simulation results:

1. Chemical/reactive species: Normalized flux averaged concentration of each species leaving the domain, and
2. Microbial biomass: Normalized total biomass of each microbial species in the domain.

The impact of temporal dynamics in the external forcing on the above response variables was characterized by cross-correlation, backward traceability, and responsiveness. Using these characteristics of response variables, I evaluated the

impact of spatio-temporal heterogeneities (base case being homogeneous domains in temporally dynamic conditions) and the impact of temporal dynamics (base case being spatially heterogeneous and homogeneous domains in steady state conditions).

5.2.1 Cross-correlation, memory and backward traceability

I used the peak value of time lagged cross-correlation (Pearson, referred to as R hereon) to investigate the strength of the link between response variables and external forcing. I adapted classification of correlation into strong, moderate and weak types from (Lehmann et al., 2021;Moore et al., 2013). Weak correlation (-0.4<R<0.4) indicated that the value did not change in a synchronized manner with the head signal at the inlet. If R>0.7, then it was strongly correlated with the forcing, indicating that the response variable is changing synchronously with the forcing.

Temporal variations in a time series signal were linearly traceable when the autocorrelation of the time series is strong even over a time lag. The time point (in days) after which the lag autocorrelation fell below 0.7 was referred to as “memory” of the time series signal.

A traceable linear relation also existed between the forcing and the response variables as long as the lag cross-correlation was strong even over a time lag. The time point (in days) after which the lag cross-correlation fell below 0.7 was referred to as "backward traceability”. In other words, backward traceability indicated the duration of the impact of the forcing on response variables of a system.

5.2.2 Responsiveness

In this chapter, the root mean squared amplitude of the variation induced in the response variables due to temporal dynamics at the inlet was referred to as “responsiveness” for brevity, calculated using Scipy package (Virtanen et al., 2020) as explained in Eq. 5.2:

$$Responsiveness = \sqrt{\max\left(Power\ spectral\ density(C_{norm}(t))\right)}, \qquad (5.2)$$

where $C_{norm}(t)$ was calculated using Eq. 5.3:

$$C_{norm}(t) = \frac{C_{out}(t)}{C_{out,base}}, \quad (5.3)$$

where $C_{out}(t)$ was the time series signal of concentration of chemical species at the outlet and $C_{out,base}$ was the concentration of the same chemical species at the outlet in steady state conditions. To assess the responsiveness of spatially heterogeneous domains specifically, I normalized it by the responsiveness (NR) of corresponding base case (homogeneous domains in temporally dynamic conditions) using Eq. 5.4:

$$NR = \frac{Responsiveness\ of\ spatially\ hetergeneous\ domain}{Responsiveness\ of\ spatially\ homogeneous\ domain} \times 100\%, \quad (5.4)$$

where, a normalized responsiveness of 100% indicated that the spatially heterogeneous domain responded the same as the homogeneous domain, given the same temporal dynamics in the forcing.

To generalize results and to move away from domain specific, flow regime specific, chemical, or microbial biomass specific discussions, I used the Da (Chapter 2) and the breakthrough time (Chapter 4) to characterize reaction regimes in steady state conditions.

5.3 Results

In the saturated domain, the temporal dynamics in the groundwater head at the inlet induced a change in the water flux in the domain. It further induced a change in the concentration of chemical species leaving the domain, concentration of microbial biomass in the domain, and also concentration of mobile biomass leaving the domain. To characterize the temporal variation, I first explored the aggregated impact on mass removal of chemical species over 15 years. I then derived the responsiveness and cross-correlation of the time series signal of variables mentioned in Chapter 5.2 in

response to the temporally dynamic forcing. I then explored the memory of the system with respect to variations induced in these variables to the forcing.

5.3.1 Homogeneous domains (base case)

Homogeneous domains provided the opportunity to consider the impact of temporal dynamics alone on microbial biomass and nutrient cycling. I considered the removal of mobile chemical species and average biomass over the entire simulation period of 15 years, and the responsiveness of the homogeneous domains to the forcing in detail.

Aggregated results

In all the flow regimes, the aggregated removal in temporally dynamic conditions and steady state conditions was approximately the same for most chemical species with the aggregated impact being marginal (within 20% of that in steady state conditions, Table B2). The notable exception was nitrate and nitrogen removal in the fast flow regime, which increased to more than twice of that in steady state conditions (240% and 211% of steady state conditions in scenario T5, respectively).

The microbial biomass also was the same in all flow regimes in temporally dynamic conditions, as that in steady state conditions. The exception was the mass of inactive ammonia oxidizers in the medium flow regime (increased by 200% in scenario T5) and active nitrate reducers in the fast flow regime (increased by more than 200%) compared to steady state conditions.

Responsiveness and correlation to forcing

The forcing induced fluctuations in the response variables in the homogeneous domains to varying degrees. The range of flux averaged concentrations of chemical species is presented in Fig. A14, and that of spatially averaged concentration of immobile active microbial biomass observed in scenario T5 in the domain is presented in Fig. A15.

The chemical species at the outlet in the slow flow regime did not respond to the forcing (Fig. A18). This was also reflected in weak to moderate correlation with the forcing (between 0 and 0.7, Table B3). Among the microbial species, the active ammonia oxidizers were the most responsive to the forcing (37% of steady state conditions in scenario T5, Fig. A19). The active biomass concentration dynamics was moderately to strongly correlated with the forcing (R >0.67, Table B5).

In the medium flow regime, DO was most responsive to the forcing (at 16% of the steady state conditions in scenario T5, Fig. A18) among the chemical species. All chemical species except ammonium and TOC were moderately to strongly correlated with the forcing (Table B3). In contrast, ammonium and TOC were weakly correlated with the forcing (<0.4). All the microbial species were responsive to the forcing, and moderately to strongly correlated (>0.77, Table B5). While inactive aerobic degraders varied the least (6%), the inactive ammonia oxidizers responded the most (62%) to the forcing.

Lastly, in the fast flow regime, the chemical species were moderately to strongly correlated with the forcing, with DO most responsive (92% in T5) and nitrogen least responsive (5.5%) to the forcing (Fig. A18). The microbial species, with exception of active mobile aerobic degraders, were also moderately to strongly correlated with the forcing, with active nitrate reducers and inactive aerobic degraders the most responsive, exceeding 70% in scenario T2.

The cross-correlation of the chemical and microbial species in the homogeneous domain to temporal dynamics are presented in Table B3 and Table B5 respectively.

5.3.2 Heterogeneous domains

Similar to homogeneous domains, I investigated the response of spatially heterogeneous domains to the forcing by first considering aggregated removal of chemical species, and then characterizing the temporal behavior of the domains in

terms of responsiveness, cross-correlation and then deriving the backward traceability.

Aggregated results

The aggregated removal of chemical species in all the flow regimes (except for nitrate in the fast flow regime) was not impacted by temporal dynamics. The time-averaged contribution of the various microbial subpopulations was also similar to that in steady state conditions. The removal of nitrate and nitrogen in the fast flow regime, however, increased to more than 200% (in scenario T5) of steady state conditions, with higher impact in low to moderate spatially heterogeneous domains.

Temporal variation in chemical discharge

The four (4) types of reactive systems identified in Chapter 4 using Da responded differently to forcings. I analysed temporally varying concentration of the chemical species leaving the domain normalized by that in steady state conditions (Fig. 5.1 and Fig. A20). For transport dominated systems, the concentration of chemical species reduced below steady state conditions (down to 40%) only in unusually low flow conditions when velocity was less than half of that in steady state conditions. But the concentration did not increase when the flow was higher than the steady state conditions. Similarly, in transport influenced systems, the concentration reduced (to less than 10%) during periods of low flow (when velocity was less than 50%) but did not increase by more than 30% even when the flow was twice of that in steady state conditions. In reaction influenced systems, the system was responsive to velocity fluctuations in both directions; the concentration reduced (to less than 10%) in low flow conditions, and the concentration increased (as high as 10 times) in high flow periods. In contrast, in reaction dominated systems, the concentration increased (as high as 12 times) in high flow conditions (velocity higher by 50%) and reduced down to 20% in low flow conditions (when velocity was less than half of steady state conditions). Fig. 5.2 presents the time series signal of the normalised velocity, and of

the median of the response variables bound by quartile ranges in the same reactive system categories. Even though the normalised concentration exceeds the steady state conditions by more than 100% in few selected scenarios when systems were characterized by $\log_{10}Da>0$ (Fig. 5.1), these extreme scenarios lie outside the third quartile (Fig. 5.2).

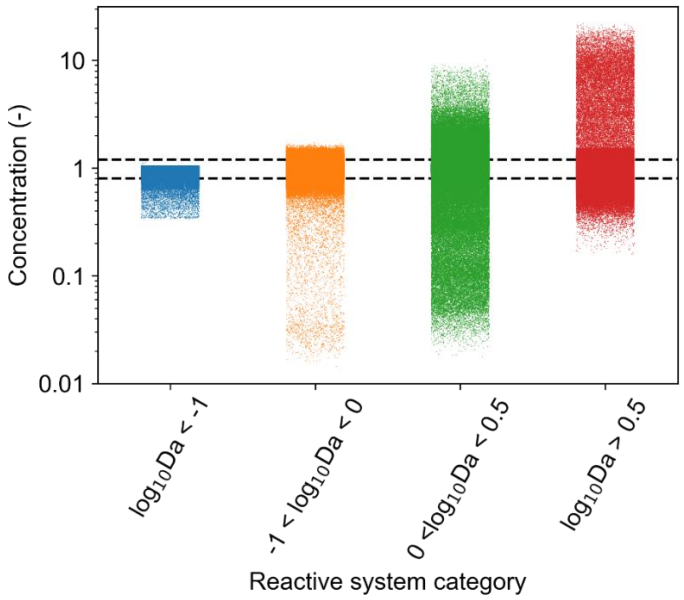

Figure 5.1 Concentration of chemical species belonging to identified categories of reaction regimes (red for reaction dominant regimes with $\log_{10}Da > 0.5$, green for regimes with $0<\log_{10}Da<0.5$, orange for regimes with $-1<\log_{10}Da<0$ and blue for reaction-limited regimes with $\log_{10}Da<-1$) in all investigated domains normalized by that in steady state conditions over the entire simulation period of 15 years for the three investigated time series (T1, T2, and T5).

Temporal variation in microbial biomass

The contribution of different fractions of microbial species also varied with temporally dynamic forcing. In all flow regimes, the ratio of active to dormant species increased with increasing velocity. The increase in the ratio was attributable to both, increasing active and decreasing dormant species with increasing velocity. The ratio of immobile and mobile species also increased with increasing velocity, but

attributable only to decreasing mobile species in the domain (Fig. 5.3). The shifting contribution of each microbial sub-population is presented in Fig. A21.

Normalized responsiveness

Since in some conditions, the outliers varied from steady state conditions by more than 10%, I explored the responsiveness of the time series signal of each reactive system. This enabled the estimation of the uncertainty band in temporally dynamic conditions with respect to homogeneous steady state domains (base cases described in Chapter 3).

Chemical species: Each chemical species responded differently to forcing based on the flow regime. The normalized responsiveness in terms of the Da of the prevailing reaction regime (estimated in corresponding steady state conditions) is presented in Fig. 5.4. Transport dominated systems with heterogeneous domains were little affected by temporal dynamics, the responsiveness of temporally dynamic spatially homogeneous domains being slightly higher. For transport influenced systems and reaction influenced systems, spatio-temporal heterogeneities induced a normalized responsiveness of 6-7 (that is, the amplitude was 500%-600% higher than in homogeneous domains). With average residence time less than ~30 days, the normalized responsiveness increased, in moderately heterogeneous domains, and then tended back towards that in homogeneous domains when average residence time reduced to approximately a day. For reaction dominated systems, the normalized responsiveness also depended on the average residence time. For average residence time of approximately one day, there was limited impact of temporal dynamics. With average residence time between one day and 30 days, the responsiveness increased with increasing spatial heterogeneity, inducing a normalized responsiveness higher than 10.

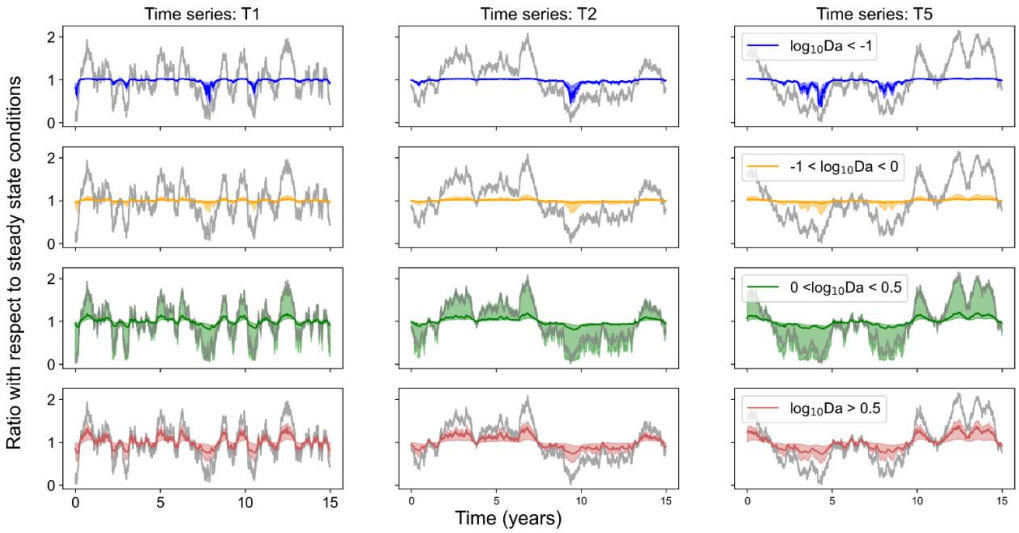

Figure 5.2 Median concentration of chemical species belonging to identified categories of reaction regimes in all investigated domains bound by 25% and 75% quartile ranges (red for reaction dominant regimes where $\log_{10}Da > 0.5$, green for regimes where, $0<\log_{10}Da<0.5$, orange for regimes with $-1<\log_{10}Da<0$ and blue for reaction-limited regimes with $\log_{10}Da<-1$) over the entire simulation period of 15 years for the three investigated time series (T1, T2, and T5).

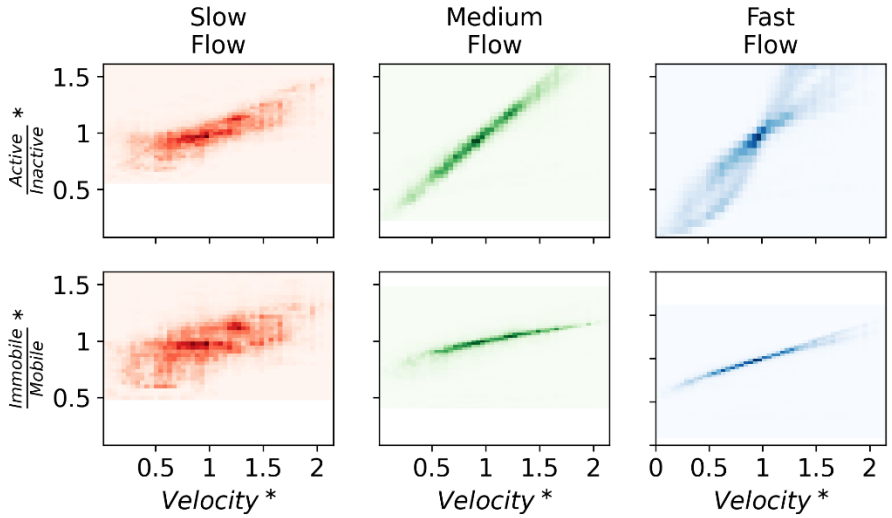

Figure 5.3 Density plot of contribution of microbial subpopulations in all investigated domains normalized by that in steady state conditions with normalized velocity in corresponding domain (velocity normalized by that in steady state conditions) over the entire simulation period of 15 years for the three investigated time series (T1, T2, and T5). Darker colours indicate higher density of points.

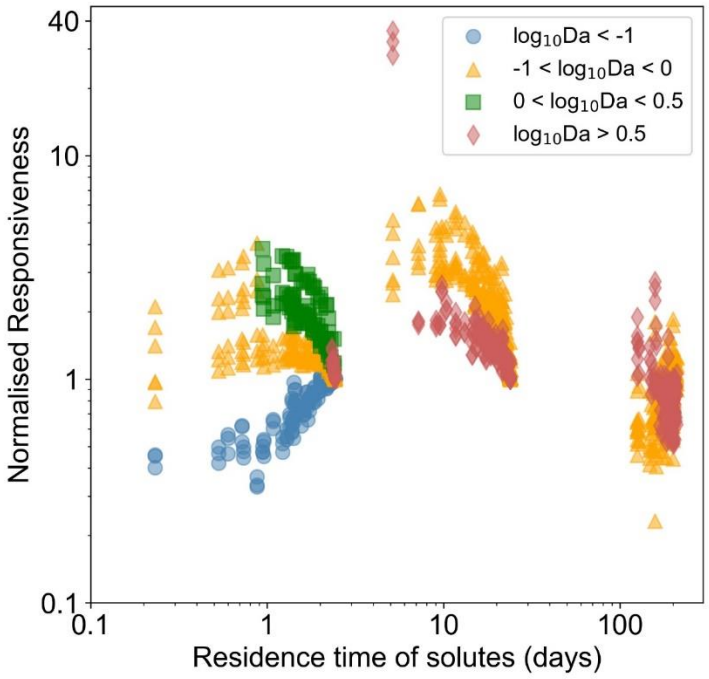

Figure 5.4 Normalised responsiveness of chemical species leaving the domain in temporally dynamic and spatially heterogeneous domains with respect to temporally dynamic homogeneous domain. The colour varies with the prevalent reaction-flow regime (red for reaction dominant regimes where log10Da > 0.5, green for reaction influenced regimes where, 0<log10Da<0.5, orange for transport influenced regimes with -1<log10Da<0 and blue for transport dominated regimes with log10Da<-1). Normalised responsiveness close to 1 indicates that the temporal dynamics induced in a spatially heterogeneous domain is similar to those induced in the corresponding homogeneous domain.

Microbial species: For all microbial species highest normalised responsiveness values were approximately 1. The normalized responsiveness of all microbial species in all flow regimes, except for aerobes in the slow and medium flow regimes, reduced with increasing spatio-temporal heterogeneity (Fig. 5.5). Refer to Fig. A26 for the normalized responsiveness of mobile active microbial species.

Cross-correlation

The chemical species were moderately to strongly correlated with the forcing in medium and fast flow regimes. In the slow flow regime, DOC and DO were weakly to

moderately positively correlated with the forcing. In contrast, TOC and nitrogen had weak to moderately negative correlation (Fig. A22).

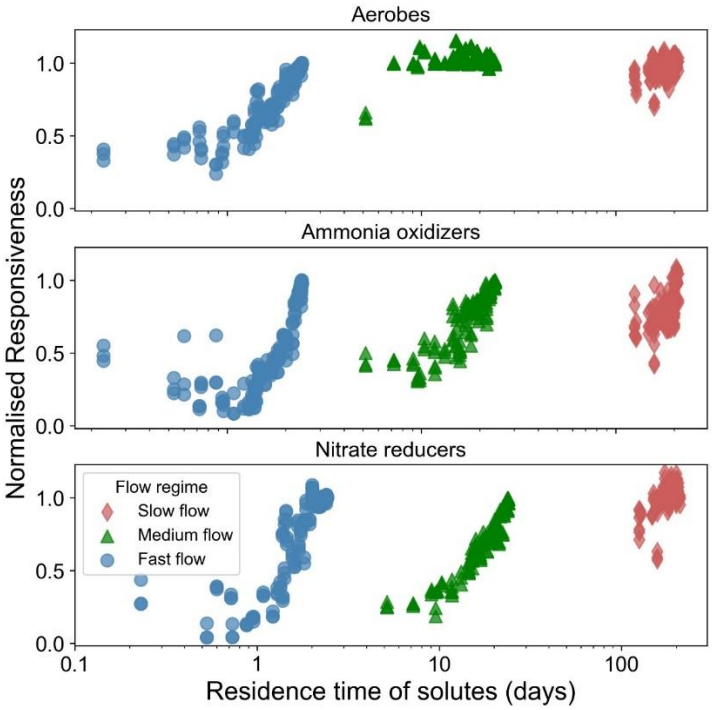

Figure 5.5 Normalised responsiveness of concentration of active fraction of biomass in the domain in response to temporal dynamics in the forcing (changing groundwater head at the inlet of the domain). The colour varies with the prevalent flow regime (red for slow flow, green for medium flow and blue for fast flow regimes). Normalised responsiveness close to 1 indicates that the temporal dynamics induced in a spatially heterogeneous domain is similar to those induced in the corresponding homogeneous domain.

The active microbial species in the medium flow regime were moderately to strongly correlated with the forcing. All the microbial species (except mobile aerobic degraders) were also similarly correlated with the forcing in the slow flow regime. Mobile aerobic degraders, on the other hand, were negatively correlated with the forcing. Lastly, all the microbial species (except aerobic degraders) were negatively correlated with the forcing in the fast flow regime. The immobile aerobic degraders were positively correlated with the forcing (Fig. A23).

Backward traceability

The variation in response variables was traced to the variation in the forcing (changes in groundwater head in this chapter). I contextualized backward traceability of the reactive systems with the memory of the temporally dynamic forcing (Table 5.1).

Chemical species: The backward traceability varied from 0 (in buffered slow flow regimes, for TOC in relatively homogeneous domains, and for nitrogen in select high response fast flow regime) to up to 245 days (nitrate in medium flow regime in T5). In the fast flow regime, the backward traceability of chemical species was less than that of the forcing in low heterogeneous domains in the fast flow regime, increasing and plateauing in domains of moderate spatial heterogeneity before decreasing again in high spatial heterogeneity scenarios for DOC, TOC and DO (Fig. A24). In the medium flow regime, the backward traceability increased with spatial heterogeneity for all chemical species except for DO, for which it remained constant. The backward traceability of chemical species was not evaluated in the slow flow regime as the correlation with the forcing was weak to moderate (Fig. A24). This meant that any fluctuation in the concentration profile of the flux averaged concentrations of mobile species at the outlet of the domain is not attributable to a change in the forcing in the slow flow regime.

Microbial species: The backward traceability for active microbial biomass varied from 0 days (ammonia oxidizers, nitrate reducers and mobile aerobes in the fast flow regime) to approximately 280 days (immobile active ammonia oxidizers in slow flow regime in T5).

The backward traceability of the microbial species biomass in the slow flow regime was the highest (active immobile aerobes, ammonia oxidizers and nitrate reducers), followed by that in the medium flow regime and the fast flow regime (Fig. A25). Except for nitrate reducers, the backward traceability remained independent of the

spatial heterogeneity in the domain. Among the nitrate reducers, the backward traceability decreased with increasing spatial heterogeneity in the medium flow regime only. The backward traceability for nitrate reducers in the slow flow and fast flow regimes also remained independent of spatial heterogeneity.

5.4 Discussion

The analysis of time series signals of chemical discharge from spatially heterogeneous saturated domains, and that of the microbial biomass subpopulations in the same domains was analogous to real-time data collected during long term environmental monitoring studies where varying groundwater head in monitoring wells is linked with varying physicochemical characteristics of groundwater. I found the variation in the response to be dependent on the flow regime and spatial heterogeneity. Here I shed light on the individual contributions of spatio-temporal heterogeneities on nutrient cycling and the consequences for modelling predictions.

5.4.1 Response of microbial biomass and nutrient cycling to temporal dynamics

The distribution of the biomass among the various types of species changed in the temporally dynamic regimes. This agrees with previous research attributing species composition to the difference in the flow regimes and the maximum carrying capacity of a reactive system (Grösbacher et al., 2018) and spatial heterogeneity of a system (Franklin et al., 2019;Khurana et al., 2021b;Or et al., 2007). Since the slow flow regime is already transport limited, the temporal variation in inactive species is not predictable. With increasing contribution of advection flow processes, the shifting microbial communities tends to be predictable (Stegen et al., 2016). However, the extent of the impact varied widely between the different heterogeneous domains and the homogeneous domains (considering the induced normalized amplitude of biomass time series signals). The biomass in high heterogeneous domains in transport dominated reactive systems was less sensitive to temporal dynamics as the

biomass in low-flow zones was not exposed to fluctuations in water flow. In contrast, locally mixed or diffusion dominated heterogeneous domains were impacted the same as homogeneous domains. Spatial heterogeneity and long transport time scales thus helped overall resilience of microbial communities to disturbances (Koenig, 2016;Koenig et al., 2017;Yabusaki et al., 2017). Thus, the relevance of spatio-temporal heterogeneities (and uncertainty thereof) for geomicrobial community structure and nutrient cycling is dependent on travel time and dominant flow processes.

As previously discussed, discharge of DO, DOC, nitrate and ammonia also varied with time. This coordinated fluctuation of microbial communities and redox conditions in line with groundwater or surface inputs was also observed in other studies. For example, King et al. (2017) demonstrated that the relative contribution of bacterial species in an in-field bioreactor changes between dry periods (oxic conditions) and wet periods (sub-oxic) conditions. Benk et al. (2019) further demonstrated that changing input of DOM between dry and wet seasons influences bacterial community evolution in a pristine oligotrophic aquifer under minimal anthropogenic impact or disturbance. Lohmann et al. (2020) also then focused on the shift from aerobic to anaerobic ammonium oxidation between summer (dry conditions) and autumn (wet conditions) at the same site. The change in relative abundance of active species in the microbial community or the change in microbial activity did not lead to an impact of the same magnitude on chemical discharge. For example, even though the contribution of active nitrate reducers decreased during low flow conditions in the medium flow regime, nitrate removal increased. This points to the low microbial activity in low-flow periods as discussed by Stegen et al. (2016). Even though nitrate reduction increased during these periods, the proliferation and activity of nitrate reducers decreased as microbial activity is limited by transport. In contrast, during high flow periods, even though the discharge of nitrate increased, the contribution of active nitrate reducers did not change from steady state conditions, while the contribution of inactive nitrate reducers decreased. This points to flushing of both microbes and organic carbon due to high flow rates

(Stegen et al., 2016). Temporal dynamics thus enable higher diversity in the subsurface microbiome by enabling mobility of microbial species higher microbial activity in high flow periods (King et al., 2017).

5.4.2 Assisting sampling decisions

The impact of temporal dynamics in forcing on the mobile species signal leaving the domain and microbial biomass within the domain can now be considered for sampling design. Low variation of mobile species at the outlet of the domain indicates that the temporal dynamics in the forcing are potentially buffered by the flow regime at the scale of the domain (Alewell et al., 2006). Alternatively, the corresponding chemical species is not affected by excessive flow rates induced in the domain, for example nitrogen in the fast flow regime. Since nitrogen removal was already marginal at steady state conditions in the fast flow regime (Khurana et al., 2021b), higher flow rates do little to reduce the consumption further. In contrast, lower flow rates induced in heterogeneous domains in the fast flow regime provide an opportunity for nitrate reducers to thrive in suboxic and anoxic microsites in heterogeneous domains, thereby resulting in increased consumption of nitrogen with both temporal dynamics and spatial heterogeneity. Lohmann et al. (2020) observed similar shifts from aerobic metabolism (ammonia oxidation during dry periods) to anaerobic metabolism (nitrate reduction and anaerobic ammonia oxidation during wet periods) in functional diversity of microbial communities in groundwater. This is a potential effect of climate change driven changing surficial processes by Stegen et al. (2016) as well. Interestingly, the low flow zones provided buffer zones to these anoxic microsites in domains with high flow rates, and therefore the amplitude of anaerobic microbial species was lower compared to temporally dynamic regimes in lower average flow rates (see discussion above).

The traceability of the signal of mobile species with temporal dynamics poses an additional challenge. The time-lag analysis provided insight into the traceability of temporal dynamics of the system to forcings (Kim et al., 2019). The lag between the

forcing of groundwater head and corresponding response in the domain and at the outlet of the domain varied as per the flow regime, as well as the microbial and chemical species of concern. Depending on the time scale of interest, the frequency of sampling and timing of sampling must be informed by occurrence of weather events as well as broader seasonal changes. While the response in dissolved chemical species signal may be immediate (shorter than observation frequency, and shorter than the traceability of the time series signal itself) in high spatio-temporal heterogeneity and high flow rate regimes, the response is induced later and lasts longer on microbial species in medium and slow flow regimes. Moreover, the traceability for microbial biomass in slow and medium flow regimes was in the same range as that of the traceability of the forcing and was largely not dependent on the residence time of the domain. Interestingly, Hofmann et al. (2020) concluded that microbial communities present in the shallow subsurface respond to changing surficial inputs over 170 days, which is in the same order of magnitude as the traceability observed in this chapter. It was also in the same order of magnitude of that observed by Zhou et al. (2012), even though their study was at the field scale as opposed to the sub-meter scale of this thesis. Thus, backward traceability of a reactive system is linked with the time scale of respective microbial growth kinetics. Microbial species with lower respiration and growth rates are slower to adapt to dynamic environmental conditions, and therefore display a large time lag (or high traceability). Microbial population distribution shifts are attributable to shifting hospitable conditions for high adaptable microbial species, and increased mobility for low adaptable microbial species(Sugiyama et al., 2018). Therefore, the time scale of temporal dynamics of environmental conditions and subsequent impact must be considered in relation to the time scale of microbial kinetics. If the time scale of changing forcing is short in comparison to the time scale of microbial kinetics, then the observable impact on microbial biomass is expected to be less. Regardless of these differences in the extent of the system response, the time series signal of dissolved chemical species and microbial biomass is linked with that of groundwater head, which is in turn linked

with surficial events. Therefore, none of these analyses may be undertaken in isolation.

5.4.3 Predicting relevance of spatio-temporal heterogeneities for chemical discharge

Since I considered a wide variety of flow regimes, a comprehensive and detailed process network, homogeneous uniform flow conditions as the base case, and stochastically generated time series of the forcing, I was in the unique position to quantify temporal variation in chemical discharge from a system relevant at the field and policy scale (Muniruzzaman and Pedretti, 2021). While there was no substantial impact of temporal dynamics on the response variables when aggregated over the entire simulation period, the response variables did fluctuate as a result of the temporally dynamic forcing. Thus, the relevance of uncertainty quantification in modelling studies must be examined with the research objective in mind.

Sub-scale temporal dynamics in chemical discharge can be characterized based on Da as in Khurana et al. (2021b). Neglecting spatial heterogeneity alone may result in errors in predicted outcomes from -60% to +500% (Khurana et al., 2021b). Transport dominated heterogeneous systems showed limited impact to temporal dynamics; most of which was attributable to the reduction of nitrate in unusually low flow conditions when velocity reduced to less than 50% of that in steady state conditions. Furthermore, the response of temporally dynamic spatially homogeneous domains was higher than that of heterogeneous domains. In other words, spatial heterogeneity dampened the impact of temporal dynamics on the reactive system. Temporal dynamics are therefore important in only unusually low flow conditions. Unusual here means that the average residence time is less than half of that in a corresponding homogeneous domain, caused by the strong heterogeneity, and at time points when the flow rate is also less than half of that in steady state conditions.

For transport or reaction influenced systems, spatio-temporal heterogeneities induce a normalised responsiveness of 6-7 (that is, moderately heterogeneous

domains respond more than homogeneous domains by 500%-600%). At the same time, high spatially heterogeneous domains respond the same as homogeneous domains. Thus, predictive uncertainty associated with temporal dynamics was adequately captured by homogeneous domains in high heterogeneity scenarios. However, in moderately heterogeneous domains, both spatial heterogeneity and temporal dynamics lead to strong deviations from the homogeneous cases and therefore heterogeneity needs to be accounted for when modelling these systems.

For reaction dominated regimes, the amplitude depended on the average residence time as well. When residence time was short (approximately a day), there was limited impact of temporal dynamics on the system. On the other hand, in medium flow systems, when residence time was higher than a fortnight, spatial heterogeneity accentuated the impact of temporal dynamics on the amplitude of the system, and the third quartile could be as high as twice the steady state conditions. Thus, spatio-temporal heterogeneities may not be neglected in these systems.

In general, the high responsiveness of the systems in the chapter was due to DO, nitrate and DOC travelling through the preferential flow paths in spatially heterogeneous domains. This behavior and extent of impact is consistent with previous studies. Gwo et al. (1996), for example, proposed that 64% of variation in fluxes is attributable to spatial heterogeneity (hydraulic conductivity and anisotropy) and temporal dynamics (rainfall intensity). Later, Van Der Hoven et al. (2005) observed even higher dynamics with a variation of DO over 2 orders of magnitude at their site, and Rein et al. (2009) observed that temporal variation in groundwater flow conditions resulted in variation of contaminant concentration (as high as the steady state/average concentration). More recently, Küsel et al. (2016) discussed variation of chemical species (such as DOC, DO, nitrate) by a factor of 3-4, both in space and in time. While a direct comparison between these previous studies and the results of this chapter is ill advised considering differences in flow regimes, conceptual model and site settings, it is worth to note that the temporal dynamics in

the response variables observed in this chapter were at similar orders of magnitude and even higher since this chapter explored a larger range of scenarios.

5.5 Summary and Conclusions

Computational costs, lack of detailed characterization of geological setting, lack of temporal resolution in field monitoring leads to modelers assuming steady state homogeneous conditions of a natural system. Field scale studies do not resolve sub-scale spatial heterogeneity, thus contributing to uncertainty with respect to both parametrization and predictive outcomes of modelling studies. Neglecting spatial heterogeneity alone results in prediction errors associated with chemical removal between 60% and 500 times of that in homogeneous domains (Khurana et al., 2021b).The uncertainty induced due to lack of characterization of spatial heterogeneity is further exacerbated by neglecting temporal dynamics. Additionally, prediction of temporal dynamics is also increasingly uncertain in a changing climate scenario. We are not yet equipped to estimate the uncertainty in microbial activity, and chemical discharge thereof from subsurface reactive systems in hitherto unforeseen scenarios. This chapter is a first attempt to capture the extent of uncertainty were spatio-temporal heterogeneities to be neglected in modelling studies. With accurate site characterization, and contextualizing temporal dynamics, this chapter can now assist in field sampling design to correlate field data with dynamics in forcing. With this work, I aimed to reduce predictive uncertainty in modelling studies.

6 Synthesis

Groundwater is a major source of freshwater supply (Oki and Kanae, 2006) and is critical to ensure water access, quality, and security to the world population. This makes variation of groundwater quality an on-going concern and indeed, numerous investigators have studied the spatio-temporal variation of groundwater quality. Various studies have described the variation of microbial abundance, distribution, diversity and associated variation in groundwater characteristics (McGuire et al., 2000;Schwab et al., 2017;Zhou et al., 2012) in both space and time, i.e., across several subject sites and also exploring seasonal variation. At the same time, computational modelers often use subject site data to parameterize their models and predict groundwater discharge as well as groundwater quality in the future. They often simplify their models limiting incorporation of spatially distributed physico-chemical properties of the system (such as hydraulic conductivity, chemical concentration, microbial biomass), or assuming steady state conditions due to paucity of field data (Berkowitz et al., 2016), or to optimize the use of computational resources. In this process, accuracy of model parameterization as well model predictions gets compromised, reflected in the mismatch of model predictions with field observations (Berkowitz et al., 2016).

I wanted to understand the repercussions of these assumptions in context of errors induced in resulting model predictions. Specifically, I wanted to identify under which conditions, the assumptions of a homogeneous domain and uniform conditions hold. In doing so, I answered the following overarching questions:

1. In which systems does spatial heterogeneity in the subsurface impact microbial activity and resulting carbon and nitrogen cycling?
2. When do temporal dynamics in the flow conditions in the subsurface affect microbial activity and the export of carbon and nitrogen from the system?

6.1 Review of hypotheses

Regarding spatial heterogeneity, I focused on the spatial distribution of matrix/material properties alone, specifically the property that governs the ability of the medium to conduct a liquid fluid (groundwater, in our case). Regarding temporal dynamics, I focused on the temporal dynamics in water flux (average water velocity in the system) alone. Thus, I was able to test my hypotheses (summarized in Table 6.1) with respect to homogeneous domains and uniform flow conditions, wherein all the systems displayed the same average properties: Same average water flow velocity in all the heterogeneous and homogeneous media in both saturated and unsaturated domains, as well as same water velocity when averaged over the entire transient simulation period. Lastly, when characterizing domains with varying flow regimes (Fast, medium and slow) and multiple reactive components (DOC, DO, nitrate, ammonium), I used the Damköhler number (Da) to qualify the reactive system as reaction or transport dominant. Thus, not only was I able to effectively test my hypotheses, but I was also able to generalize discussions to derive a broader system understanding and facilitate a transfer into practice.

I displayed that spatial heterogeneity governs the access of microbes to carbon substrate, nutrients and energy gradients (Franklin et al., 2019). However, this distribution is also impacted by the flow regime for the same orientation of spatial heterogeneity; the same domain having different dominant flow processes (diffusion v/s dispersion v/s advection) and different saturation responds differently to spatial heterogeneity (Edery et al., 2016;Nissan and Berkowitz, 2019). Spatial heterogeneity, residence time and different chemical species of concern in the system thus necessitated the use of Da.

Spatial heterogeneity did not impact or affect the spatial distribution of microbial functional groups, and the consumption of carbon and nitrogen species by microbial biomass thereof in permanently saturated, reaction dominated and with locally mixed flow regimes, where ($\log_{10}$Da > 0.5). Thus, for reaction dominant systems with locally

Table 6.1 Review of hypotheses

Hypothesis	Reaction dominant system, locally mixed flow processes	Reaction-transport mixed system, advection dominant flow processes	Advection dominant system
H1: Spatial heterogeneity results in niches for microbial species to co-exist with other competitive species.	Confirmed	Confirmed	Confirmed
H2: Spatial heterogeneity results in lower consumption of reactive species in the system than expected in a homogeneous system.	Disconfirmed	Confirmed	Disconfirmed
H3: Temporal dynamics results in varying nutrient discharge from the domain, and this is a function of varying travel time in the domain.	Confirmed	Confirmed	Confirmed
H4: Spatio-temporal heterogeneities interact and result in compounding each other's effects on the system. Higher temporal dynamics in high spatially heterogeneous domains behave the most different from homogeneous domains in uniform conditions	Confirmed	Confirmed	Disconfirmed

mixed flow regimes in permanently saturated zones, hypotheses H1 and H2 were disconfirmed. In contrast, in reaction dominant variably saturated domains, spatial heterogeneity provided for microbial niches to emerge, confirming hypothesis H1. As in the case for saturated domains, H2 was disconfirmed in reaction dominant variably saturated systems as there was no impact of spatial heterogeneity on consumption of DOC via the aerobic pathway, while there was a general reduction in anaerobic activity.

In transport influenced or reaction influenced systems where $\log_{10}Da<0.5$, spatial heterogeneity resulted in microbial niches to form regardless of degree of saturation. Thus, for systems characterized by these Da and with advection dominated flow processes, hypothesis H1 was confirmed. The bulk consumption of chemical species in these reactive systems also decreased, confirming hypothesis H2 in these systems. However, the response of the domains was noisier in variably saturated domains, pointing to a more complex interplay between the formation of microbial niches, saturation and consumption of chemical species.

In contrast, for transport dominant systems, where $\log_{10}Da<-1$, the consumption of the chemical species increased with spatial heterogeneity in saturated advection dominant flow regimes while the consumption decreased with spatial heterogeneity in locally mixed flow regimes with lower saturation. These cases were limited to the scenarios where the entire domain is predominantly oxic, and heterogeneity resulted in the emergence of microbial niches that allowed the reduction of nitrate in saturated systems and suppressed the reduction of nitrate in variably saturated systems due to the introduction of oxic subzones. Thus, for systems characterized by these Da, the hypotheses H1 was confirmed. While H2 was disconfirmed for saturated domains, it was confirmed for unsaturated domains.

By condensing the discussion of effect of spatio-temporal heterogeneities on systems cycling different chemical species, biomass distributions at different flow

rates in terms of Da, I confirmed H3, that is, residence time and reaction time scales characterized the response of the system to spatio-temporal heterogeneities.

6.2 Conceptualizing spatio-temporal heterogeneities of natural systems

Describing natural systems and dynamics therein is not a trivial task since natural systems are complex, have non-linear dynamics and are not in equilibrium (Berkowitz et al., 2016). I aimed to evaluate the relevance of spatio-temporal heterogeneities on microbial activity, and thus focused on simplifying their description to one or two easily estimable control variables. Using this approach, I was already able to draw functional relationships between the extent of spatio-temporal heterogeneities and subsequent impact on microbial biomass, distribution and biogeochemical cycling thereof.

Spatial heterogeneity in the subsurface is well-studied and well described using statistical approaches. I used Gaussian random fields to describe the permeability field (in unsaturated systems) and hydraulic conductivity field (in saturated systems) of the domain, generating spatial random fields. Since microbial activity and emergence of microbial hotspots in porous media depend on the orientation of preferential flow paths in the medium(Franklin et al., 2019), I focused on varying the permeability and anisotropy (degree of channelization) in the domain. Varying permeability and anisotropy resulted in spatially heterogeneous domains that covered most physically plausible scenarios, from inherent heterogeneity of a single formation of alluvial sediment, to a layered aquifer system such as a sandy aquifer interspersed with clay lenses, to a fractured flow system. Since I further consider the impact of these varying heterogeneous domains with respect to a homogeneous domain in different flow regimes, I am confident that a generalized discussion of results is applicable and transferable across a wide range of systems.

Like spatial heterogeneity, the conceptualization of temporal dynamics was also an important aspect to consider. Water, solute and energy are always in flux in all compartments of the Critical Zone, and measuring them provides insights into the processes of the Critical Zone (Brantley et al., 2017). Similar to geologic formations, there is also a wide variation in the nature of temporal dynamics of these fluxes across the globe. Thus, groundwater head data, being commonly observed and recorded in long-term monitoring studies, was ideal to formulate the synthetic time series data of varying external forcing in subsurface systems. I evaluated the data distribution of groundwater head in several monitoring well locations in the Nägelstadt catchment (Jing et al., 2018) and generated other randomized time series of varying groundwater head displaying the same characteristics as those observed in a real-world system. The time series of the external forcing was modelled as Gaussian process and hence could be condensed to a limited number of control variables: Variance and correlation time scale. The results of temporally dynamic scenarios were comparable since the mean of the synthetically generated time series of groundwater head was the same as the groundwater head in steady state conditions of each flow regime. This also enabled a generalized discussion of results across different climatic systems and geological materials.

Lastly, the complex reaction network accounted for different respiration strategies (both aerobic and anaerobic), growth strategies (both heterotrophic and autotrophic) and interactions between the microbial species present in different states (inactivation or reactivation, mobilization or immobilization). The parameterization of the reaction network in these numerical experiments enforced high microbial growth for some functional guilds (such as aerobic heterotrophs), and slower growth for others (such as autotrophs and anaerobic heterotrophs). Since the parameterization of these complex reaction networks is challenging (Chen et al., 2018), the focus of the thesis was not on simulating a real-world subject site as such. Instead, the focus was on adhering to established redox hierarchy in the control system, or the homogeneous domain in each flow regime. This resulted in biomass

concentrations in the modelling system (10^9 to 10^{11} cells L^{-1}) that may be higher than those observed in some sites (up to 10^8 suspended cells L^{-1} reported by Zhou et al. (2012) and Opitz et al. (2014b)), while they are in the same range of concentrations observed in other sites (Akob and Küsel, 2011;Griebler and Lueders, 2009;Grösbacher et al., 2018;Holm et al., 1992). Essentially, the parameterizations of the model scenarios covered as many physically plausible situations as possible.

In terms of incorporated processes, the reaction network does not capture abiotic processes in either water (such as chemical speciation) or on the surface (sorption), even though these processes occur abundantly in the subsurface (Maher et al., 2013;Zhang et al., 2014). Since the aim of the thesis was to study microbial activity and their role in consumption of carbon and nitrogen, abiotic reactions were not included. However, these abiotic reactions affect microbial activity (Hunter et al., 1998;Saalfield and Bostick, 2009) supporting microbial life. Therefore, it is possible that the estimates of microbial activity do not match a real-world subject site. Having said that, Edery et al. (2016)and Nissan and Berkowitz (2019) on the other hand, explored the effect of spatial heterogeneity on simple bimolecular reactions and concluded that the flow regime is a critical factor to consider, similar to the results of this thesis.

Focusing on the aspect of microbial activity alone, the reaction network also does not consider facultative organisms, that is organisms that are capable to of switching from aerobic to anaerobic respiration strategies, in the process network. Thus, in addition to a shift between active and dormant states, it is likely that microbial communities in soil shift between different respiration strategies instead (Lin et al., 2012;Pett-Ridge et al., 2013) resulting in relatively stable microbial communities during fluctuating conditions (Veach and Zeglin, 2020). While the reaction network is suitably complex to explore aspects of this thesis, it can be modified as per the requirements of future studies. In this way, this thesis provides the blueprint that can be used to answer these questions about shifting microbial communities in the subsurface under temporally fluctuating conditions.

To conclude, I am confident that my approach of using well-established statistical methods to realize different scenarios of spatio-temporal heterogeneities enables a generalized discussion of the results. It is also easily reproducible as I used open-source software and numerical solvers for generating the scenarios and for running the simulations. I am, furthermore, confident that the results will be applicable widely. The results provide insight into natural systems that are not easy to observe, enabling a predictive understanding of these systems to hitherto unforeseen external forcings.

6.3 Results and implications

With the help of this work, I add to growing knowledge of spatio-temporal variation in geomicrobiology and physico-chemical quality of water. Additionally, I provide insights into how microbial communities organize in space in the spatially heterogeneous subsurface, and how these communities may change in temporally dynamic conditions

The flow of water controls access to nutrients and energy gradients, that are essential for microbial species to survive and persist in the environment. Thus, the emergence of microbial hotspots in the subsurface, particularly oligotrophic environments, can be explained through the approach undertaken in this thesis (Franklin et al., 2019;Kuzyakov and Blagodatskaya, 2015;McClain et al., 2003;Or et al., 2007). Changing orientation of heterogeneous structures due to processes such as weathering, clogging at the pore scale leads to a patchy distribution of microbial biomass (Or et al., 2007) in the subsurface, or the microbial species at erstwhile microbial hotspot locations may switch to a dormant or inactive mode (Kuzyakov and Blagodatskaya, 2015). In addition, the microbial species may be mobilized in the groundwater or attached to the geological material. The distribution of microbial species within these states depends on numerous well-studied factors such as nutrient availability, pH, water velocity (Griebler and Lueders, 2009;Griebler et al.,

2002;Griebler et al., 2010). In summary, the ratio of attached to mobile biomass increases in nutrient scarce conditions and reduces in nutrient poor conditions (Grösbacher et al., 2018).

In this thesis, I extend the findings of previous work by proposing a method to predict the ratio of immobile and mobile as well as active and dormant cells depending on the reactive system of concern, dominant flow process, and the residence time in the system. Using the results of this thesis, the fractionation of microbial biomass into these different states is now estimable, and it can be validated by newer measurement technologies such as BONCAT-FISH (Couradeau et al., 2019).

To formulate a general yet predictive understanding of any system, I propose the use of a criterion that considers the residence time of a conservative tracer in the system and estimated bulk consumption of the chemical species in the system. Their ratio, known as the Damköhler number (Da), categorizes the system to be reaction dominant or flow dominant. While studies exploring varying nutrient discharge in surface water bodies already have a well-developed mechanism to evaluate spatio-temporally changing surface water quality (Andrea et al., 2006;Basu et al., 2010;Bieroza and Heathwaite, 2015;Bieroza et al., 2018;Herndon et al., 2015), this thesis is among the first to my knowledge to implement such an approach for subsurface systems. A similar approach was used at the continental scale by Kumar et al. (2020) focusing on nitrate leaching from soil, and a power law approach was used by Holden and Fierer (2005) to estimate the concentration of organic carbon at varying depth. The constraint for the establishment of this in subsurface systems stems from limited observational opportunities and limited long term data availability; this constraint is overcome by the numerical modelling approach.

The travel time approach simplifies reactive transport modelling in a heterogeneous medium to a simpler one-dimensional problem (Sanz-Prat et al., 2015, 2016;Waring et al., 2020). Conducting a tracer study as well as particle tracking studies to estimate travel time distributions of conservative tracers is also a well-used

and accepted approach in the field (Edmunds and Smedley, 2000;McGuire et al., 2002;Uhlenbrook et al., 2002;Valett et al., 1996). Newer technologies also take advantage of big data obtained from satellite imagery to estimate the groundwater storage, elevation, average water flux and associated variation with seasons (Henry et al., 2011;Huang et al., 2015;Li and Rodell, 2015;Pfeffer et al., 2011;Vergnes et al., 2012). This travel time approach can easily be, and already has been, scaled up to the field and regional scale (Kumar et al., 2020). This thesis adds to this state-of-the-art knowledge by clearly delineating the reactive systems where the travel time approach holds, by formulating a more realistic reaction network incorporating microbial mediated transformation of carbon and nitrogen compounds. This approach resulted in a wider variety of reactions occurring at varying time scales, that further resulted in effective Da that varied over three (3) orders of magnitude in the research. Thus, this thesis not only takes advantage of previous work, but it is also a crucial and a much more comprehensive extension of it.

In conclusion, this thesis characterizes subsurficial reactive system behavior with respect to the relevance of spatio-temporal heterogeneities on geomicrobial activity and biogeochemical cycling in the subsurface.

6.4 Outlook

By identifying reactive systems that are sensitive to spatio-temporal heterogeneities, it is now possible to estimate the error in model predictions, and to consider the field observations in context of the heterogeneity of the geological material. Field observations already result from the existing spatial heterogeneity of the geological material (not from an assumed/hypothetical homogeneous geological material). Thus, any reaction network parameters that are derived from these field observations have an associated probability distribution. This distribution may be derived using the relationships proposed in this thesis, comparing consumption of carbon and nitrogen in different spatially heterogeneous and variably saturated

domains. While transferring field derived reaction rate parameters across sites or even to the lab or vice versa requires careful thought, this thesis lays the foundation to future work on the methodology to transfer such parameters between different systems at different spatial scales. In this way, this thesis not only assists in quantifying uncertainty in carbon and nitrogen discharge from reactive sub surficial systems, but also assists in scaling effective rate expression across different sites and spatial scales.

A plethora of studies have studied microbial life processes at lab-scale (Griebler and Lueders, 2009;Griebler et al., 2002;Griebler et al., 2010;Grösbacher et al., 2018;Hofmann et al., 2020), field scale (Bouskill et al., 2019;Kumar et al., 2017;Zhou et al., 2012) and using numerical modelling (Gharasoo et al., 2012;Heße et al., 2010;Schäfer et al., 1998a, b;Stegen et al., 2016;Thullner and Regnier, 2019). For each numerical study, parameterization of the reaction network is a key feature, and modelers often spend a substantial amount of time conducting a thorough literature review or partnering with experimentalists, identifying the most suitable set of parameters, and calibrating them. This thesis supports transfer of effective rate expressions across different geological formations given the availability of travel time distributions at the sites of concern and a similar combination of dominant microbial functional groups.

A similar strategy for evaluating seasonal variation and scenarios in a changing climate can also be implemented. Climate change presents us now with hitherto unseen temporal dynamics with respect to extreme weather events (Kothavala, 1997, 1999). For example, precipitation events are now expected to occur with increasing intensity (Kothavala, 1999;Trenberth et al., 2003;Zwiers and Kharin, 1998), but reduced frequency (Gregory et al., 1997). The incidence of climate extremes such as heat waves, droughts and higher humidity in higher latitudes (Francis and Vavrus, 2012;Trenberth et al., 2003;Wetherald and Manabe, 1999) is also increasing and cold weather in mid-lower latitudes lasts longer and occurs further south than usual (Francis and Vavrus, 2012;Screen et al., 2015). It is not possible to look at the

historical data alone to be able to predict the response of subsurficial systems to these unseen temporal dynamics. Using the proposed approach to couple travel time distribution with identification of the reactive system category, however, the uncertainty associated with microbial community shifts and varying nutrient discharge from the system of concern is now predictable. This further assists in monitoring and maintaining the water quality for human consumption and sustainable habitats for other species across seasons and for several generations.

It must be noted however that the presented analysis of impact of temporal dynamics on nutrient export is from the long-term perspective; I explored aggregated impact of temporal dynamics on nutrient discharge over 15 years. The average conditions over this entire simulation period were the same as that in steady state conditions. While this maintained comparability between different scenarios, this does not really reflect real-world conditions. Groundwater storage and the water table changes with catchment, season, specific area of interest in a catchment and anthropogenic activities (Henry et al., 2011;Huang et al., 2015;Vergnes et al., 2012). Long term modelling studies suggest that groundwater discharge may increase in northern latitudes with melting permafrost (Bense et al., 2009), increase in surface water irrigated agricultural regions such as China and Thailand (Huang et al., 2015;Pholkern et al., 2018), while decrease in groundwater irrigated agricultural systems (Huang et al., 2015). This implies that the long-term mean water flux in the subsurface is not expected to be constant, but rather is expected to have either an upward or downward trend. Decreasing water flux results in longer residence times, which will shift the bulk consumption rate of chemical species in the reactive system, even shifting the system from permanently saturated to variably saturated. Increasing water flux will have an opposite effect. Thus, these dynamics are much more complex than what I explore in this thesis. Having said that, the approach established in this thesis may be followed to evaluate these complex dynamics as well. In other words, with increasing travel time in reactive systems, nitrogen consumption will first increase with increased consumption of carbon as well. But with further

increase in travel time and decreasing moisture content in the said reactive system, aerobic activity will increase resulting in higher consumption of carbon but lower consumptions of nitrate.

Last, but not the least, the presented approach can also assist in upscaling effective rate expressions. Incorporating such a complex reaction network for predicting reactive transport in larger domains is computationally expensive. Thus, the modelling community typically justifies the use or rejection of sub-scale spatial heterogeneities and temporal dynamics. Furthermore, the modelling community requires effective rate expressions or rate modifiers to model biogeochemical cycles at large scales. These rate modifiers accounting for sub-scale heterogeneities can then be incorporated into the reaction network, simulating reactive transport at larger scales, thus maintaining accuracy of model outcomes while reducing the need for computational resources. This, in turn, can assist in rapid and accurate predictions of nutrient discharge in subsurface systems. This approach can be upscaled to even policy-relevant scales such as the catchment scale. At these scales the available data observations are often lower in resolution, as they rely on satellite imagery. Thus, the presented approach can also inform relevant policymakers and ensuring water access and security at larger scales, serving larger populations.

Cumulatively, I explored the interaction of environmental variables (degree of saturation, travel time, degree of spatial heterogeneity, degree of temporal dynamics) and microbial biomass, activity and carbon and nitrogen cycling thereof in this work. Coupling this thesis with a laboratory scale experiment for validation will lend further confidence to the results. Comparison of relative contributions of immobile/mobile and dormant/active subpopulations with field observations will further help in assessing the suitability of the parameterization of the reaction network. Lastly, utilizing the methodology for a field scale subject site to predict change in nutrient discharge given a prediction of changing water flux will be a beneficial use-case for reference and scaling up.

The approach I have used is applicable, transferrable, and suitably scalable across different studies. This approach can thus be used to answer the above questions. A holistic understanding of microbial communities, their activity, their contribution to carbon and nitrogen cycling in the subsurface can help in filling a critical gap in the global biogeochemical budgets. Not only this, but it can also assist in forming a predictive understanding of the behavior of heterogeneous reactive systems in temporally dynamic conditions which results in better understanding of ecosystem services that we may be able to use for our benefit. This, in turn, will help to secure access to water for drinking, irrigation and industry, thus supporting and propelling us forward into the uncertain future of climate change.

Appendix A: Supplementary Figures

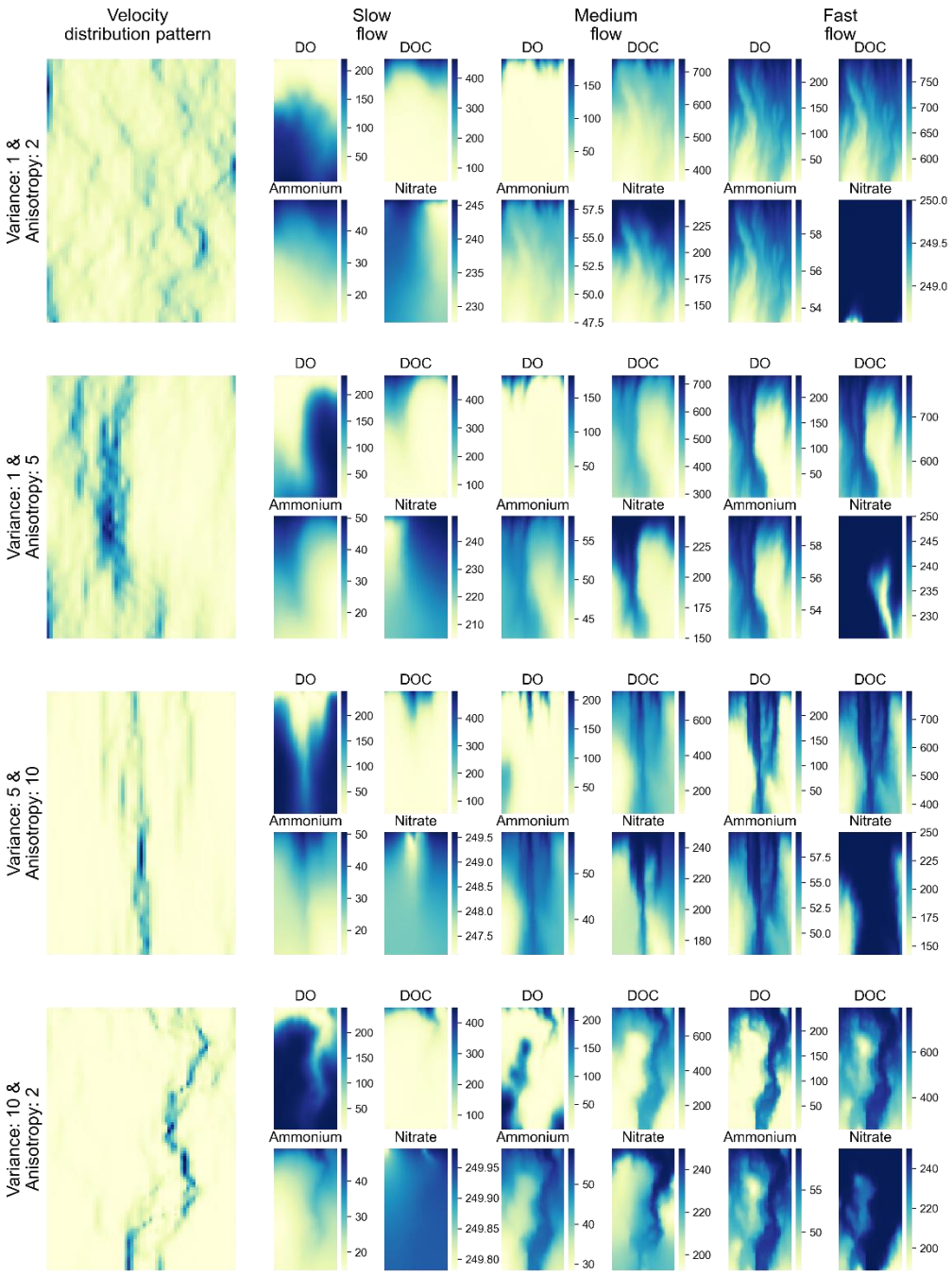

Figure A 1 2D concentration distributions of dissolved species in heterogeneous domains (μM) with the velocity distribution (in m d^{-1}) in these spatially heterogeneous unsaturated domains.

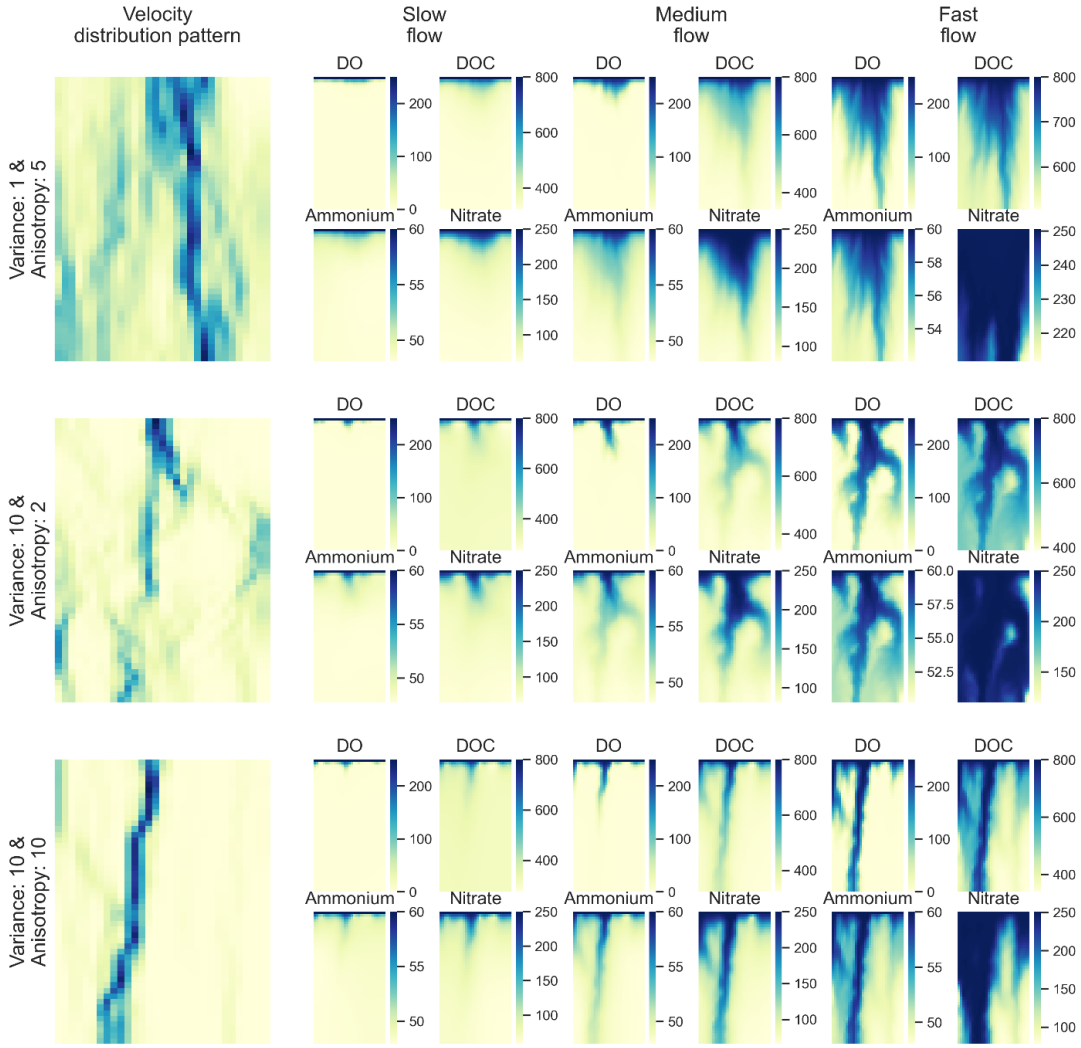

Figure A 2 2D concentration distributions of dissolved species in heterogeneous domains (μM) with the velocity distribution (in m d-1) in these spatially heterogeneous saturated domains.

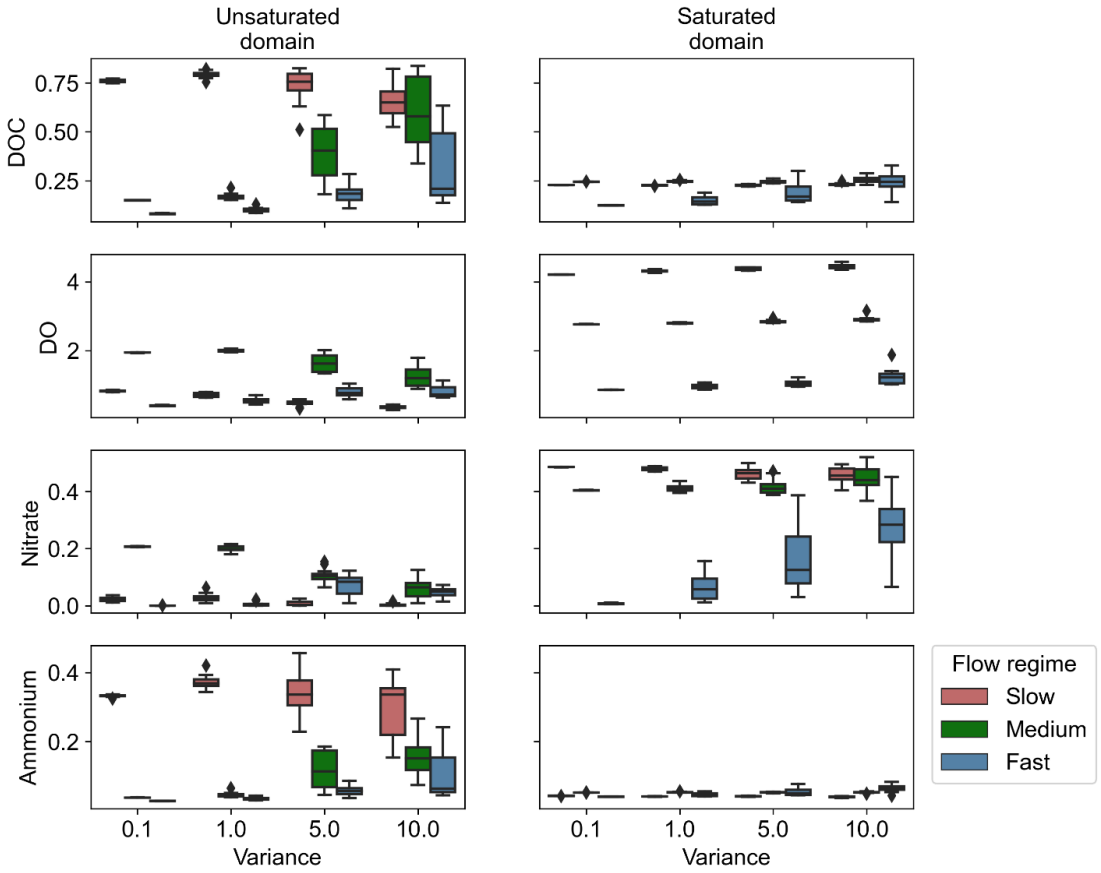

Figure A 3 Coefficient of variation of dissolved chemical species in the domain with increasing heterogeneity (variance in log permeability field in the unsaturated domain, and variance in the log hydraulic conductivity field in the saturated domain) in three different flow regimes: Slow, medium and fast flow.

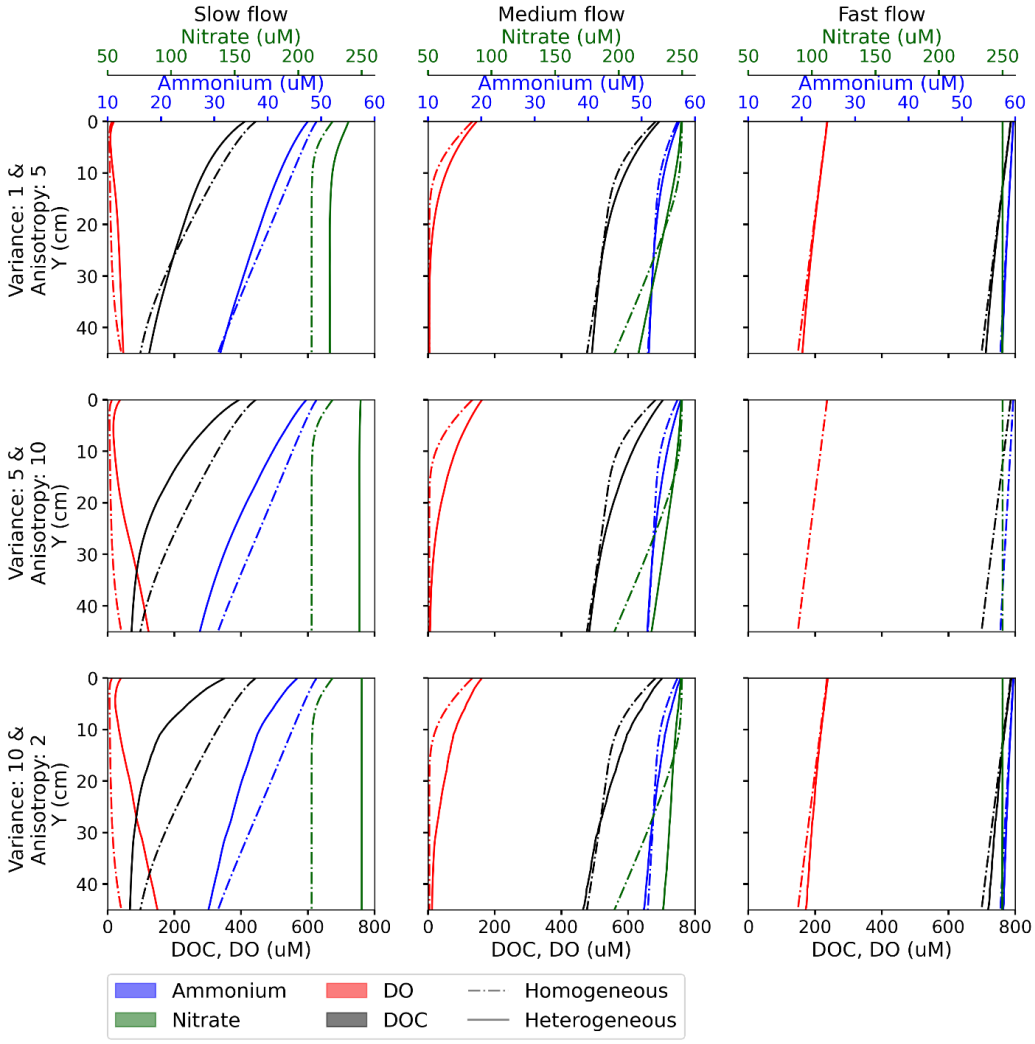

Figure A 4 Flux averaged concentrations of dissolved species in heterogeneous unsaturated domains in three types of heterogeneous scenarios (solid lines) compared to that in the homogeneous base case (dashed-dot lines) in all flow regimes

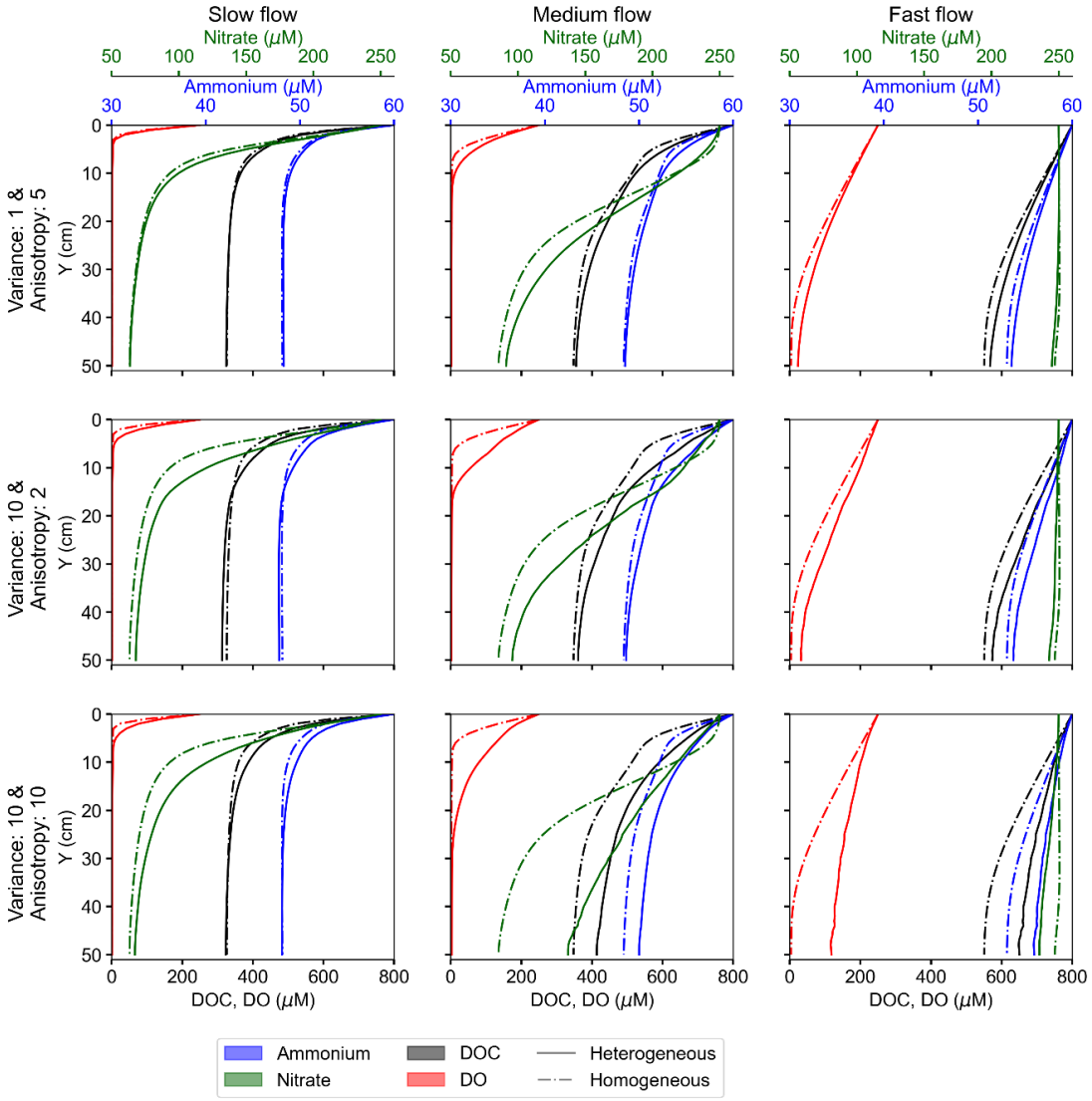

Figure A 5 Flux averaged concentrations of dissolved species in heterogeneous saturated domains in three types of heterogeneous scenarios (solid lines) compared to that in the homogeneous base case (dashed-dot lines) in all flow regimes

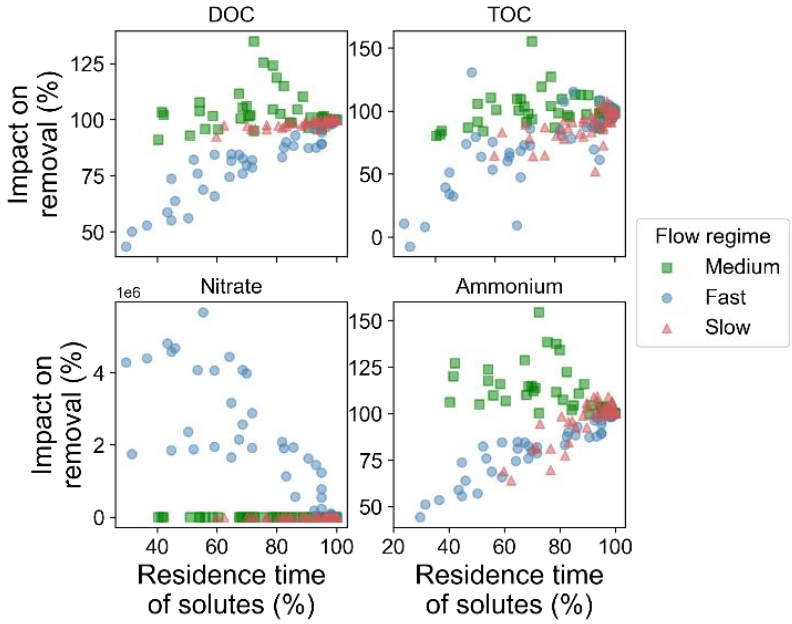

Figure A 6 Impact on removal of chemical species in heterogeneous unsaturated domains with decreasing breakthrough time and increasing spatial heterogeneity.

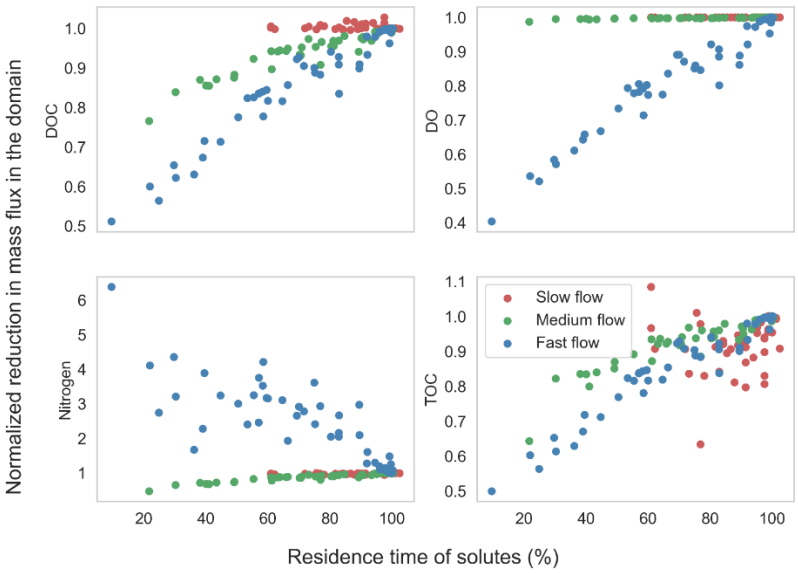

Figure A 7 Removal of chemical species in spatially heterogeneous saturated domains in different flow regimes. Values show mass flux differences between inlet and outlet of the heterogeneous domains normalized by the flux differences for the homogeneous base case of each flow regime

Supplementary Figures

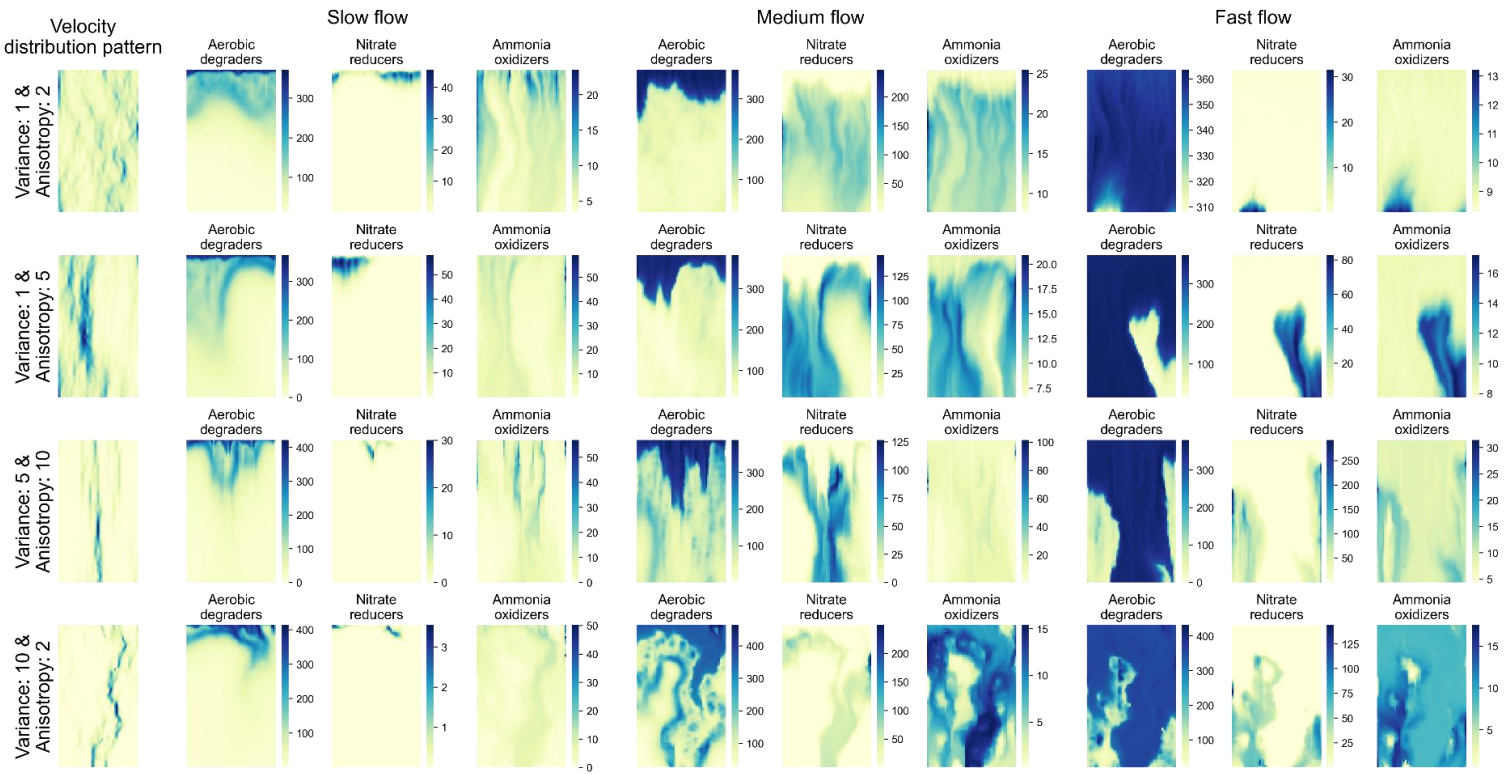

Figure A 8 2D concentration distributions of microbial species in heterogeneous unsaturated domains (µM C) with the velocity distribution (in m d^{-1}) in these domains

Supplementary Figures

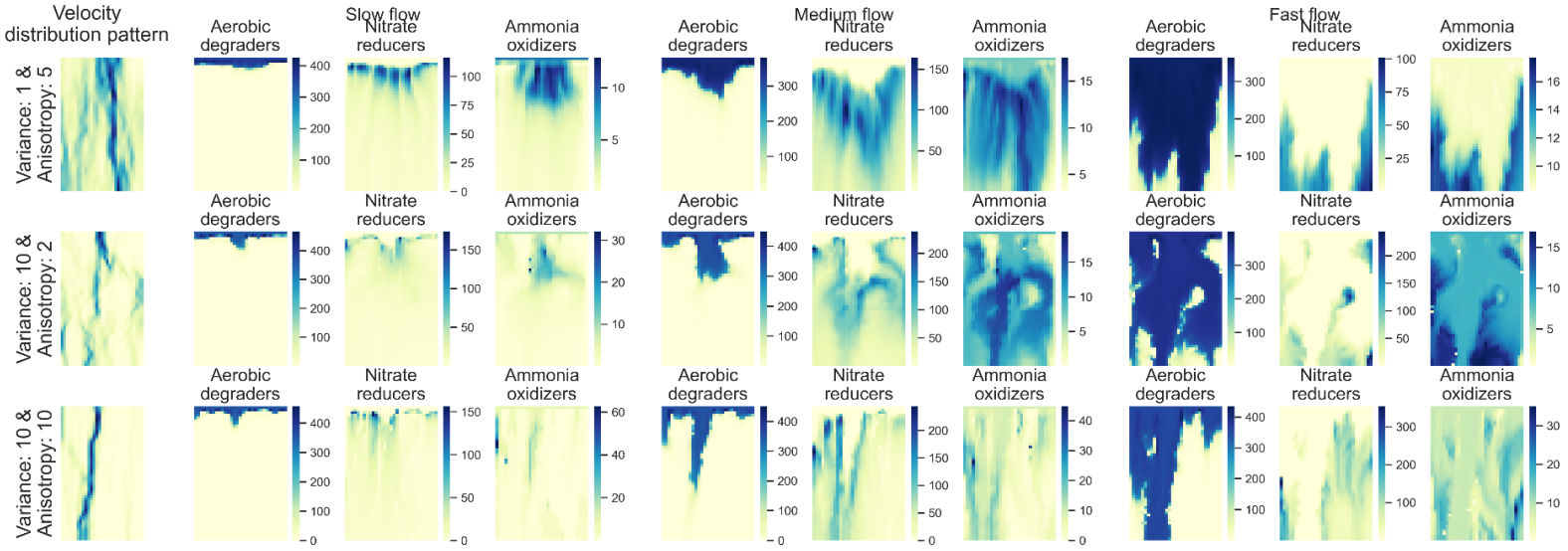

Figure A 9 2D concentration distributions of microbial species in heterogeneous saturated domains (µM C) with the velocity distribution (in m d-1) in these domains

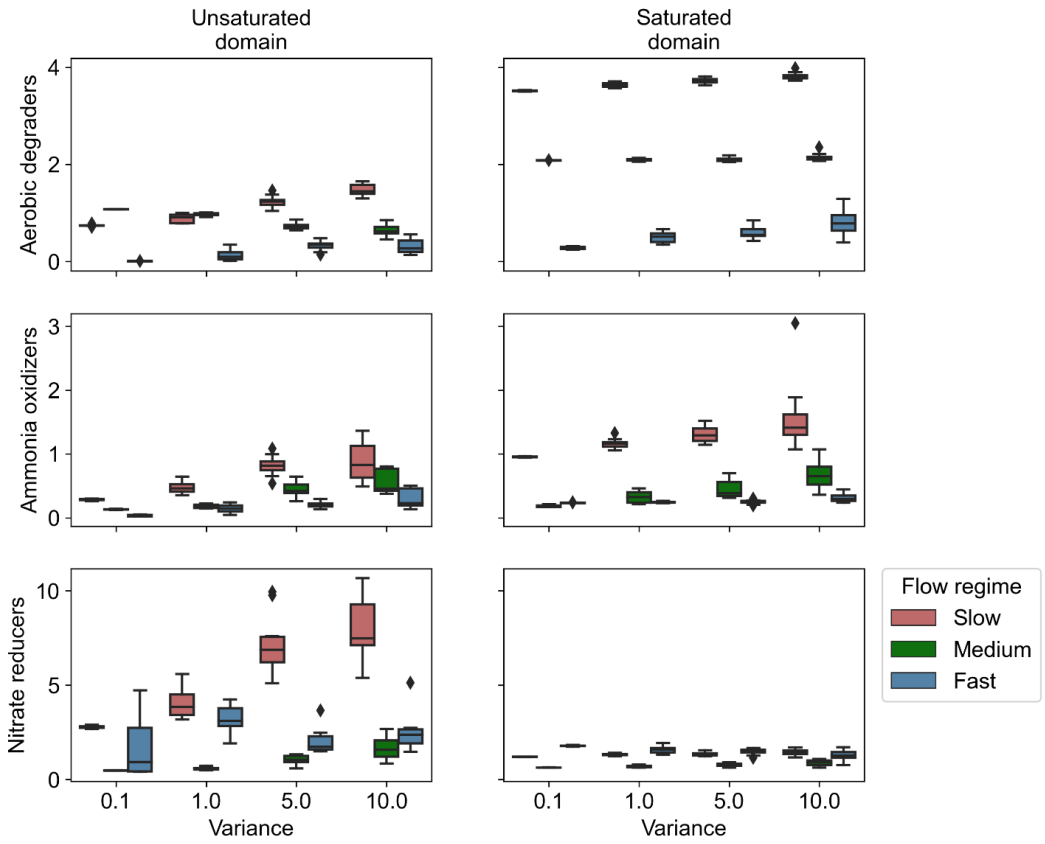

Figure A 10 Coefficient of variation of immobile active microbial species in the domain with increasing heterogeneity (variance in log permeability field in the unsaturated domain, and variance in the log hydraulic conductivity field in the saturated domain) in three different flow regimes: Slow, medium and fast flow.

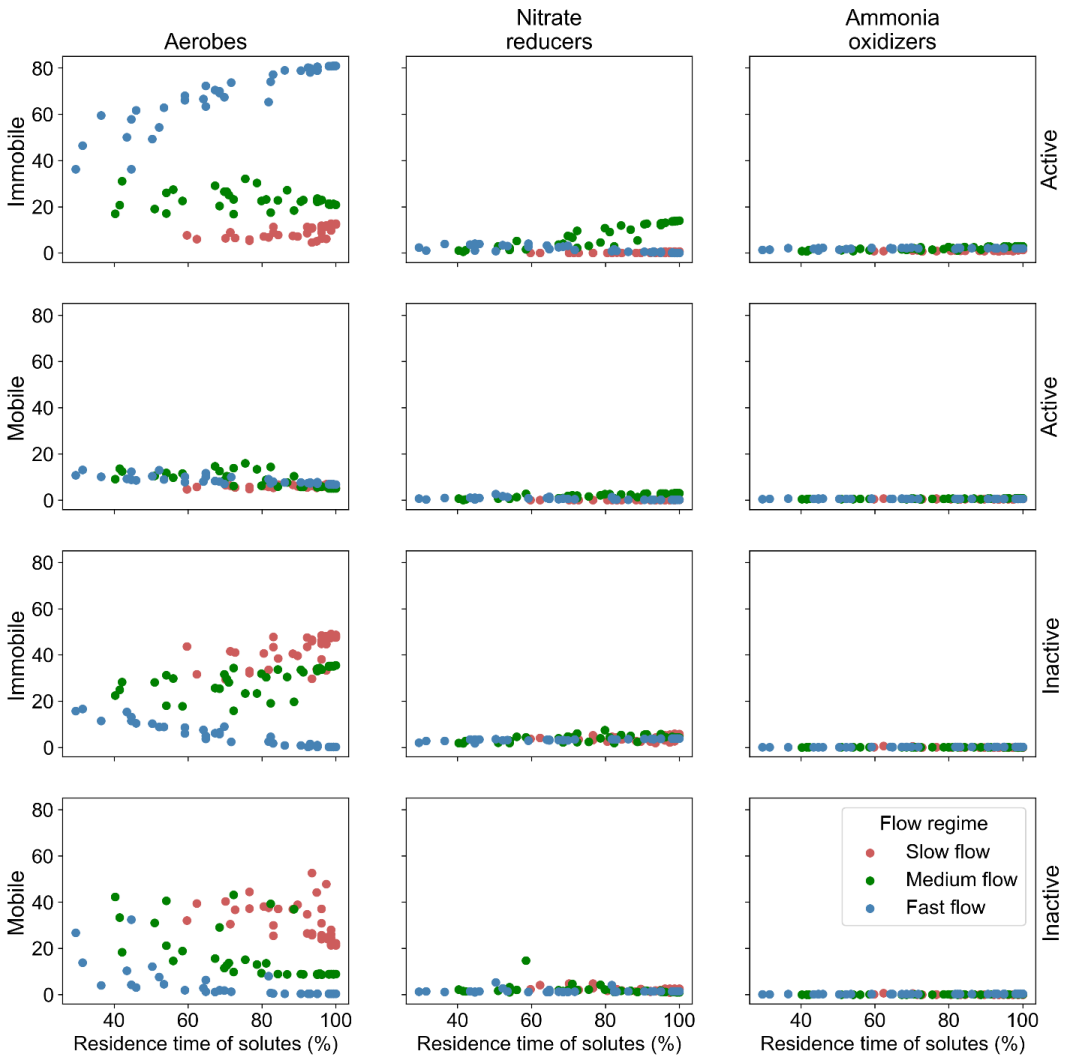

Figure A 11 Relative contribution of all subpopulations of all the microbial functional groups to the total biomass in spatially heterogeneous unsaturated domains.

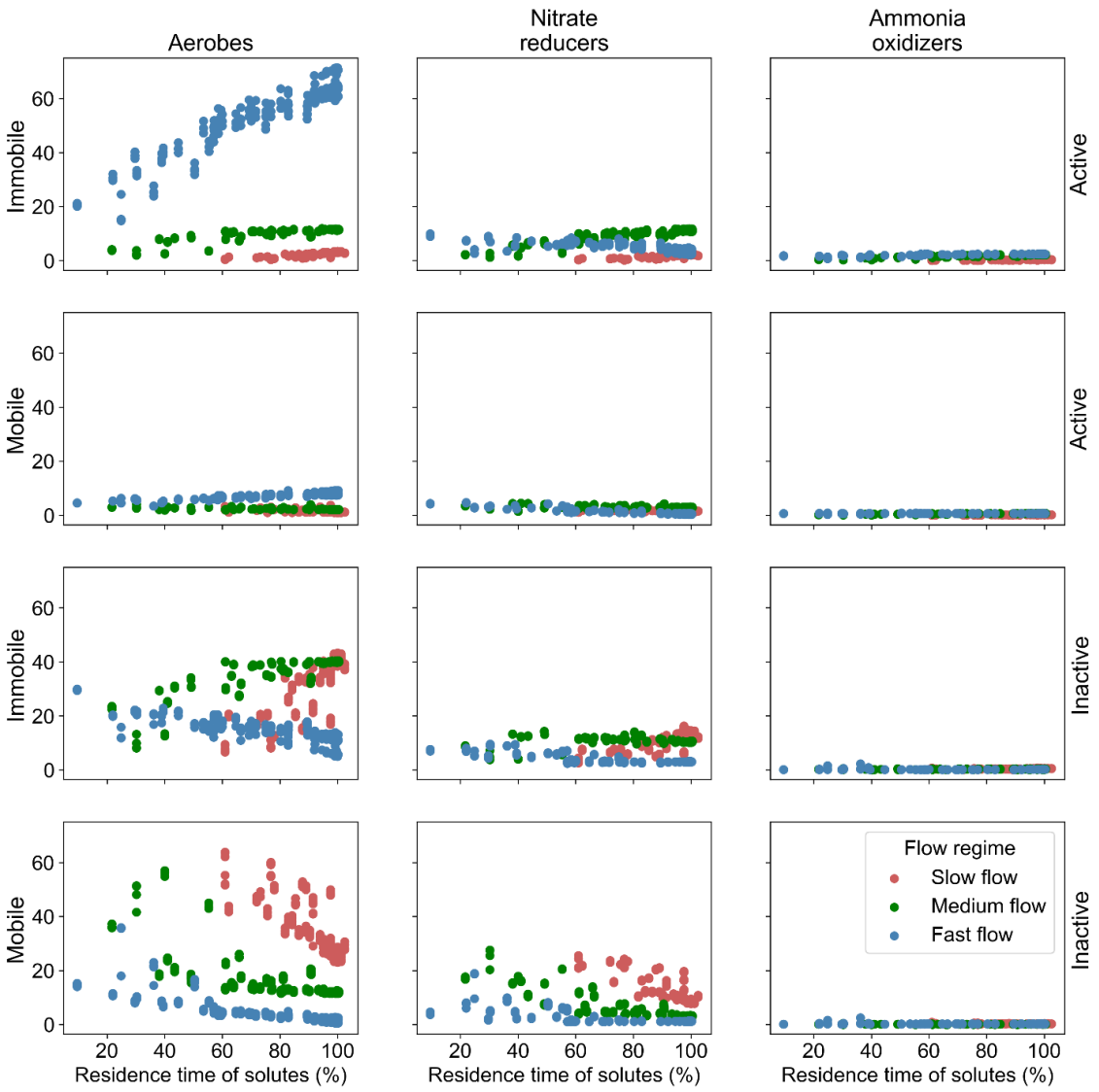

Figure A 12 Relative contribution of subpopulations of all the microbial functional groups based on the state of activity and the location to the total biomass in spatially heterogeneous saturated domains.

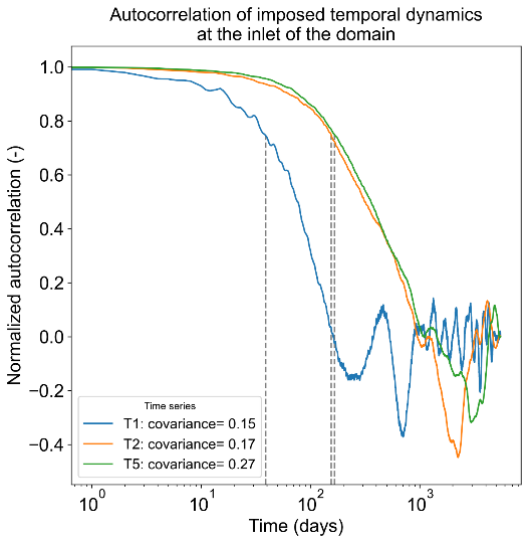

Figure A 13 Autocorrelation function of the three time series of groundwater head imposed as the boundary condition at the inlet of the domain. The dashed grey lines indicate the time point where the autocorrelation drops below 0.75 indicating the traceability of each external forcing (39 days for scenario T1, 155 days for scenario T2, 166 days for scenario T5).

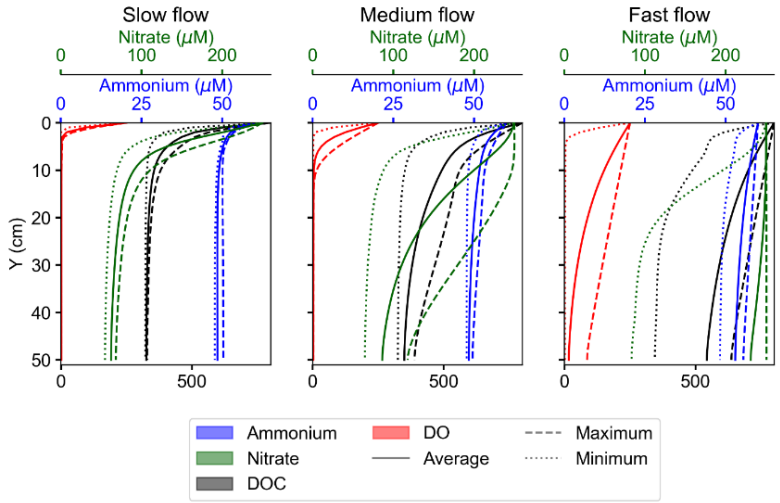

Figure A 14 Variation of flux averaged concentration of dissolved species in the homogeneous domain for the time series T5 over a simulation period of 15 years. Colour varies with the chemical species (blue for ammonium, green for nitrate, black for DOC and red for DO), and the line style varies with the value (solid for average concentration, similar to steady state conditions, dotted lines for minimum observed concentration and dashed line for maximum observed concentration).

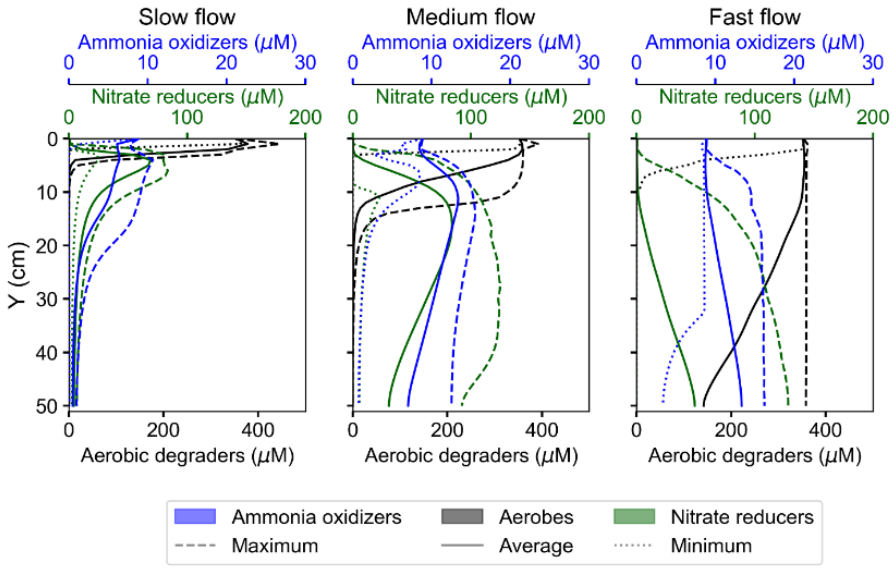

Figure A 15 Variation of spatially averaged concentration of immobile and active microbial species in the homogeneous domain for the time series T5 over a simulation period of 15 years. Colour varies with the chemical species (blue for ammonium, green for nitrate, black for DOC and red for DO), and the line style varies with the value (solid for average concentration, similar to steady state conditions, dotted lines for minimum observed concentration and dashed line for maximum observed concentration).

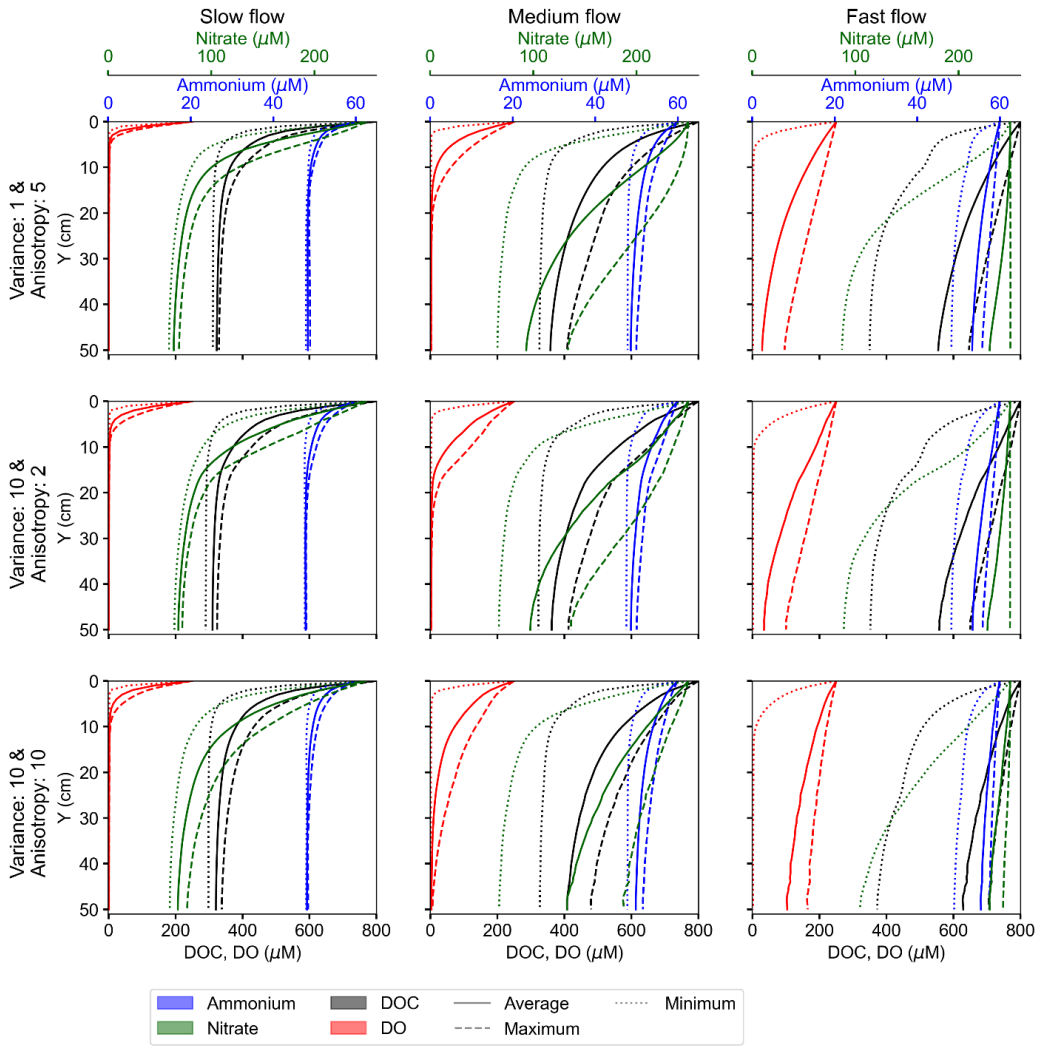

Figure A 16 Variation of flux averaged concentration of dissolved species in select heterogeneous domains (indicated by Variance and Anisotropy) for the time series T5 over a simulation period of 15 years. Colour varies with the chemical species (blue for ammonium, green for nitrate, black for DOC and red for DO), and the line style varies with the value (solid for average concentration, similar to steady state conditions, dotted lines for minimum observed concentration and dashed line for maximum observed concentration).

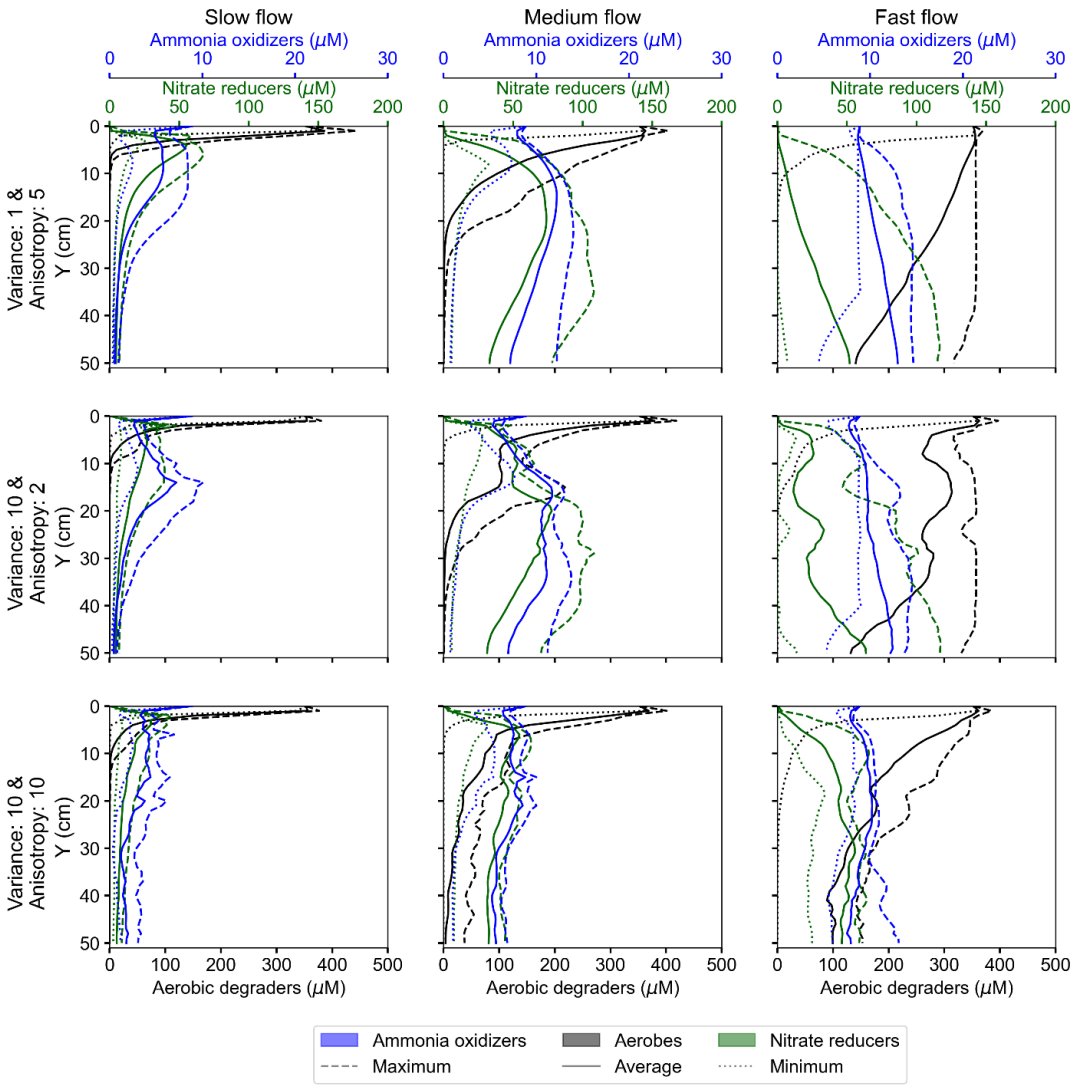

Figure A 17 Variation of spatially averaged concentration of immobile and active microbial species in select heterogeneous domains for the time series T5 over a simulation period of 15 years. Colour varies with the chemical species (blue for ammonium, green for nitrate, black for DOC and red for DO), and the line style varies with the value (solid for average concentration, like steady state conditions, dotted lines for minimum observed concentration and dashed line for maximum observed concentration).

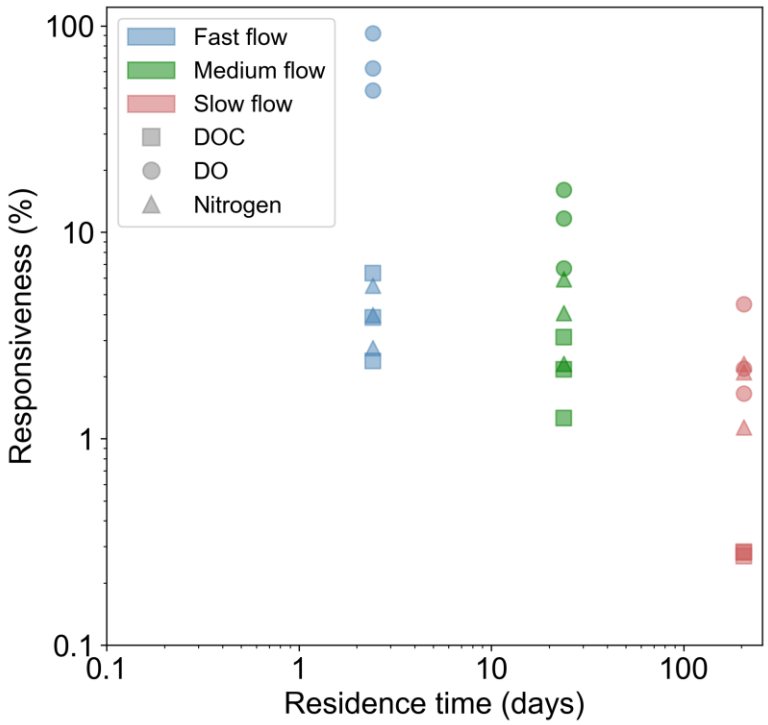

Figure A 18 Responsiveness (%) of flux averaged concentrations leaving the homogeneous domain in temporally dynamic conditions with respect to steady state conditions. The colour varies with the prevalent flow regime (red for slow flow, green for medium flow and blue for fast flow regimes) and symbol with the chemical species (square for DOC, circle or DO and triangle for nitrogen). The intensity of temporal dynamics is indicated by the temporal dynamics factor (ratio of residence time in steady state conditions and the traceability of the external forcing). The lower the value, the higher the temporal dynamics.

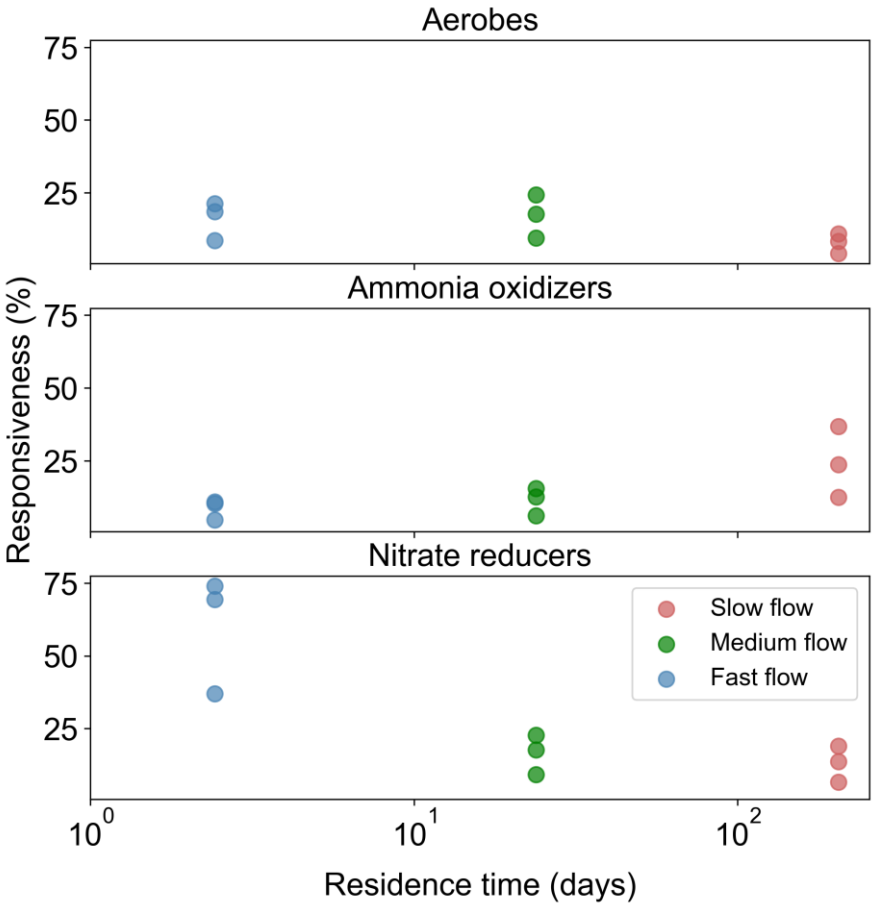

Figure A 19 Responsiveness (%) of active immobile biomass in the homogeneous domain in temporally dynamic conditions with respect to steady state conditions. The colour varies with the prevalent flow regime (red for slow flow, green for medium flow and blue for fast flow regimes).

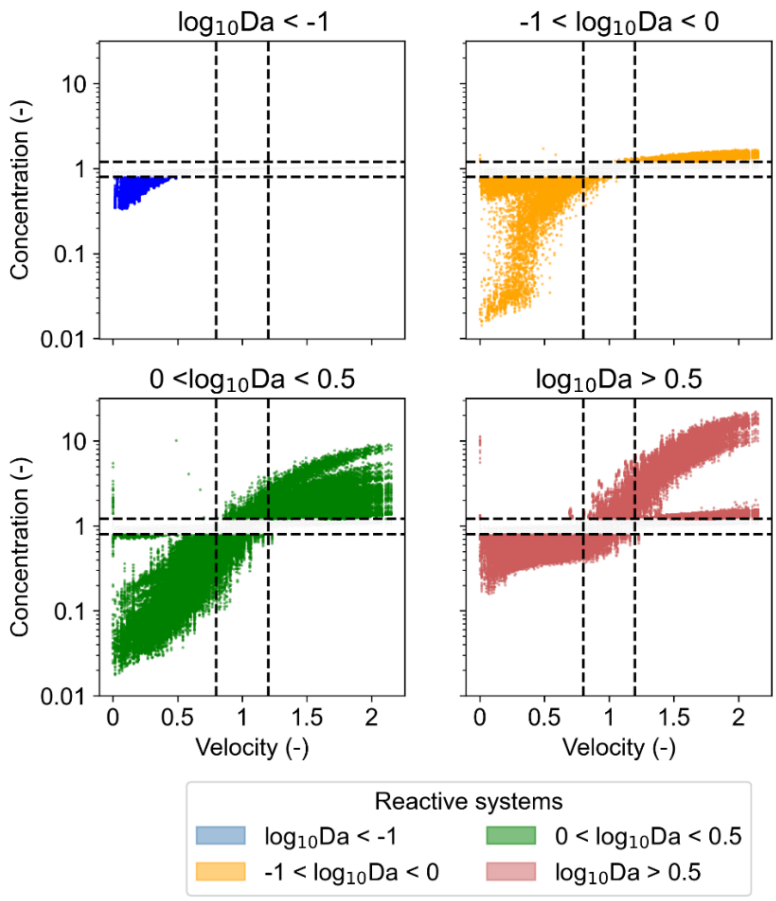

Figure A 20 Concentration of chemical species belonging to identified categories of reaction regimes (red for reaction dominant regimes with $\log_{10}Da > 0.5$, green for regimes with $0<\log_{10}Da<0.5$, orange for regimes with $-1<\log_{10}Da<0$ and blue for reaction-limited regimes with $\log_{10}Da<-1$) in all investigated domains normalised by that in steady state conditions with changing normalised velocity in corresponding domain (velocity normalised by that in steady state conditions) over the entire simulation period of 15 years for the three investigated time series (T1, T2, and T5). Points are coloured when the change in concentration is 20% or higher, otherwise points are grey. Dashed black lines mark a change of 20% in normalised concentration and in normalised velocity.

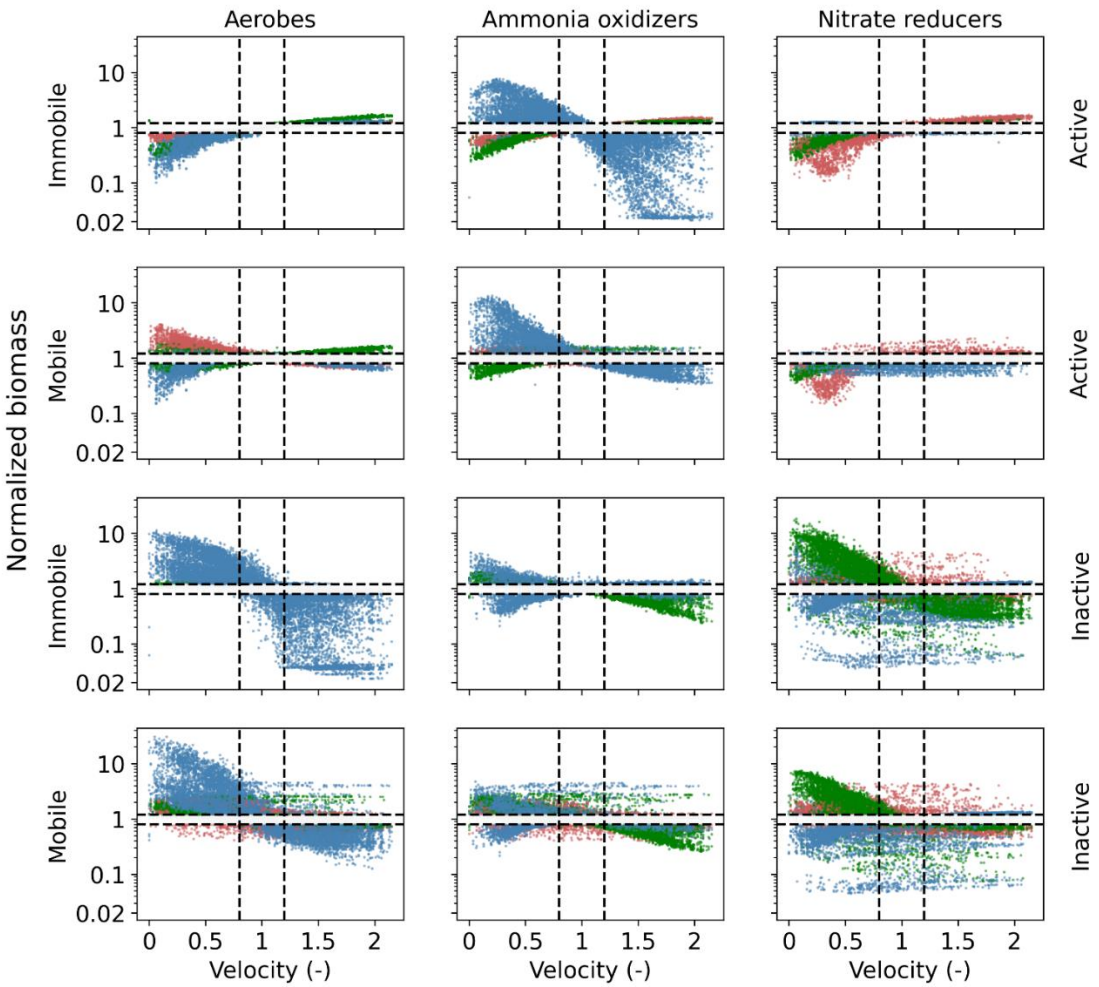

Figure A 21 Contribution of microbial biomass in all investigated domains normalised by that in steady state conditions with changing normalised velocity in corresponding domain (velocity normalised by that in steady state conditions) over the entire simulation period of 15 years for the three investigated time series (T1, T2, and T5). Points are coloured when the change in concentration is 20% or higher, otherwise points are grey. Dashed black lines mark a change of 20% in normalised concentration and in normalised velocity.

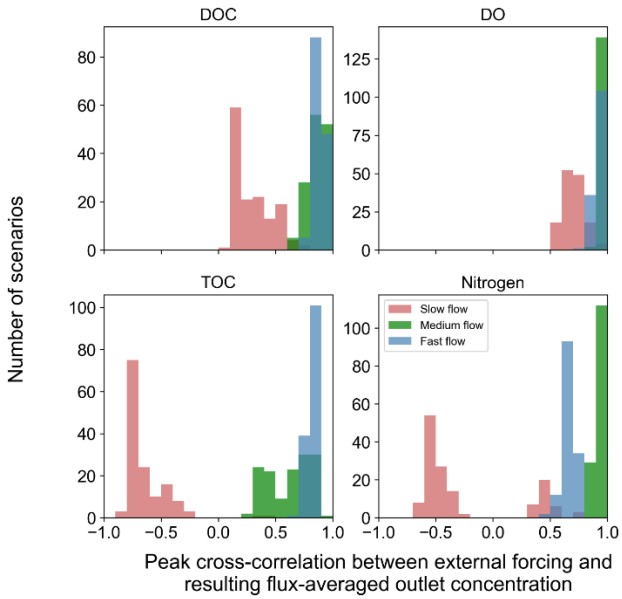

Figure A 22 Distribution of peak cross-correlation of concentration of chemical species leaving the domain in response to temporally dynamic externally forcing at the inlet of the domain.

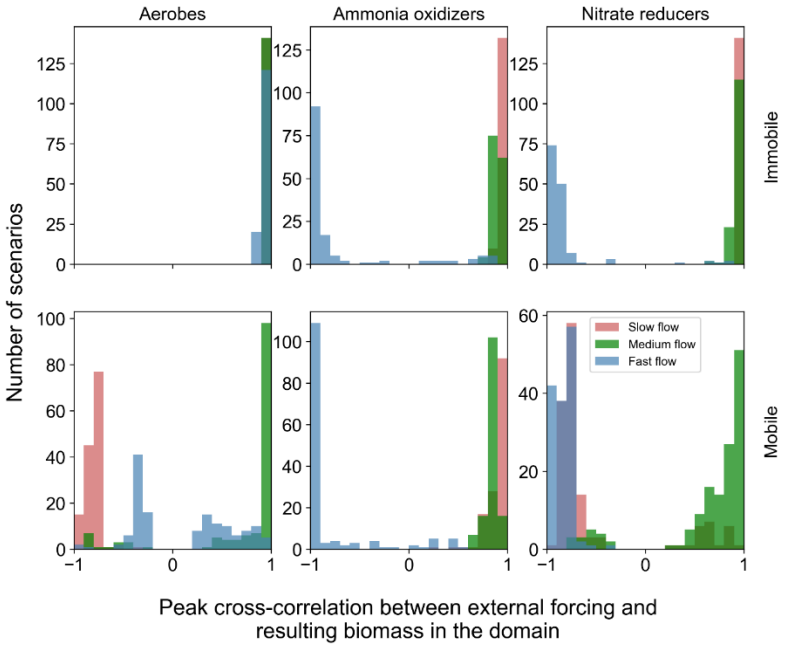

Figure A 23 Distribution of peak cross-correlation of concentration of active fraction of microbial species in the domain in response to temporally dynamic externally forcing at the inlet of the domain.

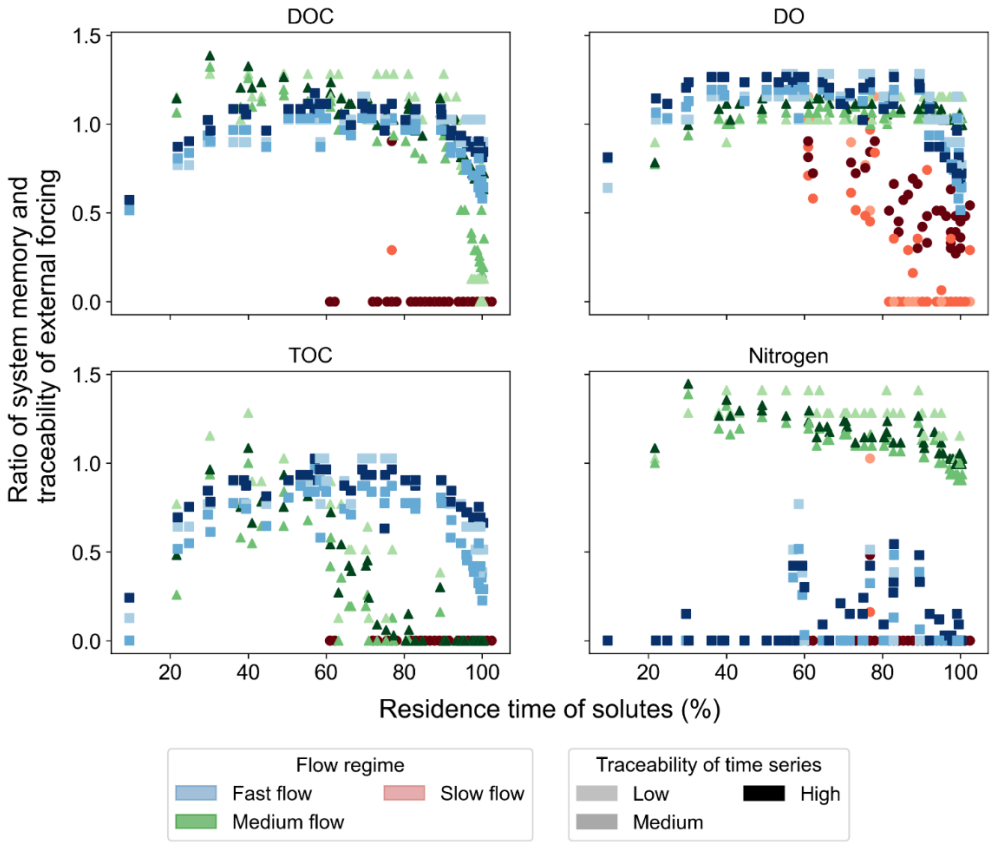

Figure A 24 Ratio of traceability of time series signal of chemical species (DOC, DO, TOC, nitrogen) and traceability of the forcing (changing groundwater head at the inlet of the domain). The colour varies with the prevalent flow regime (red for slow flow, green for medium flow and blue for fast flow regimes) and the intensity of the colour varies with the traceability of the external forcing (lighter colours represent scenario T1, and dark colours represent scenario T5). The spatially heterogeneous domains are indicated by the reducing residence time of solutes derived in steady state conditions, where a value of 100% indicates the base case or the homogeneous domain. Lower residence time indicates a higher spatially heterogeneous domain.

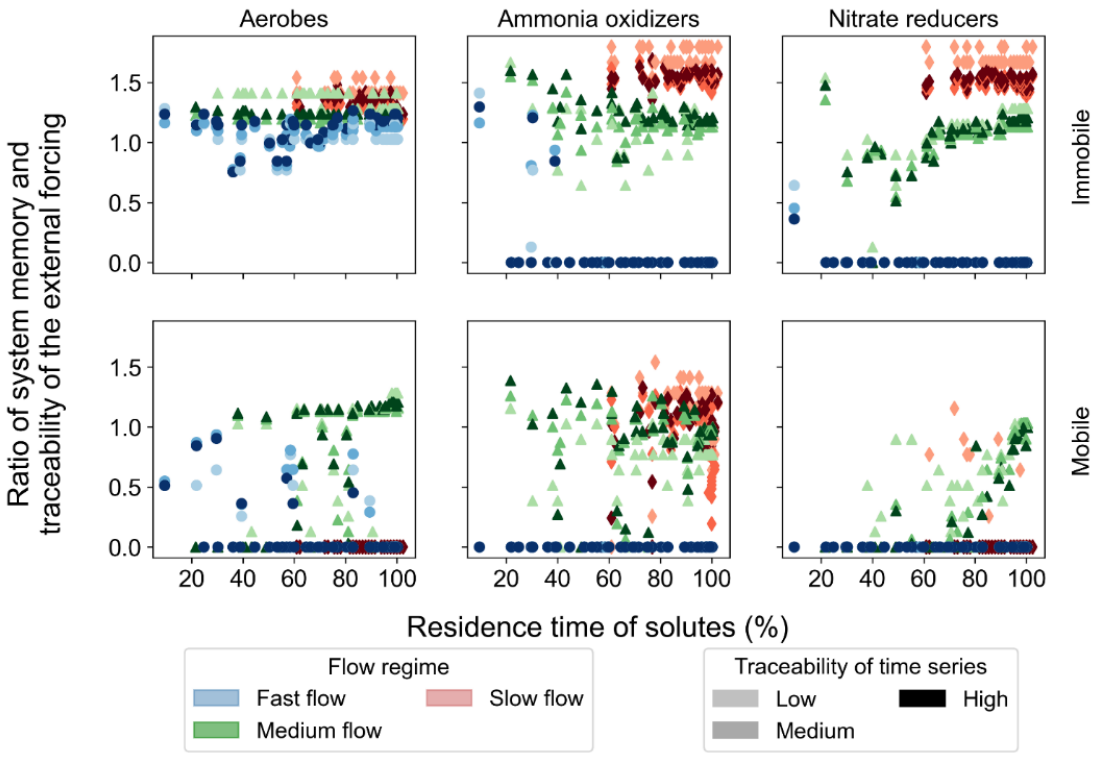

Figure A 25 Ratio of system memory of the domain and traceability of the external forcing (changing groundwater head at the inlet of the domain) for different active microbial species (aerobic degraders, ammonia oxidisers and nitrate reducers). The colour varies with the prevalent flow regime (red for slow flow, green for medium flow and blue for fast flow regimes) and the intensity of the colour varies with the traceability of the external forcing (lighter colours represent scenario T1, and dark colours represent scenario T5). The spatially heterogeneous domains are indicated by the reducing residence time of solutes derived in steady state conditions, where a value of 100% indicates the base case or the homogeneous domain. Lower residence time indicates a higher spatially heterogeneous domain. The system memory of mobile microbes in most domains is 0 in temporally dynamic heterogeneous domains, leading to overlapping data points in the figure.

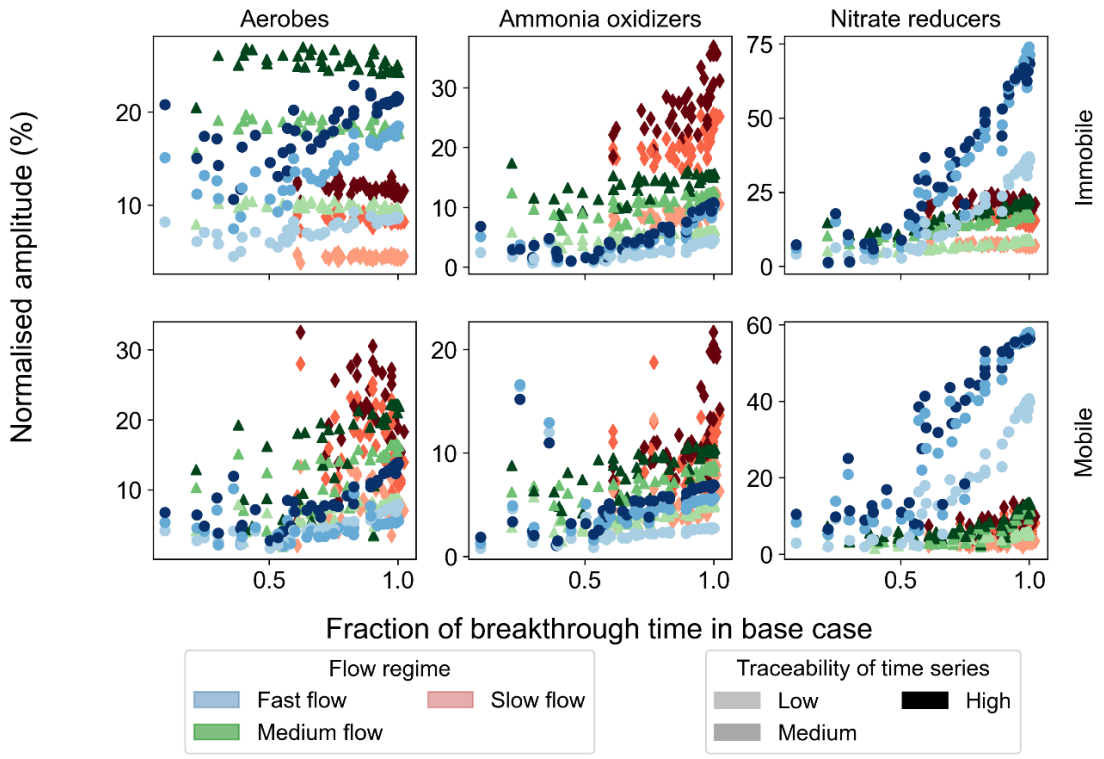

Figure A 26 Normalized amplitude (%) of active immobile biomass in the homogeneous domain in temporally dynamic conditions with respect to steady state conditions.

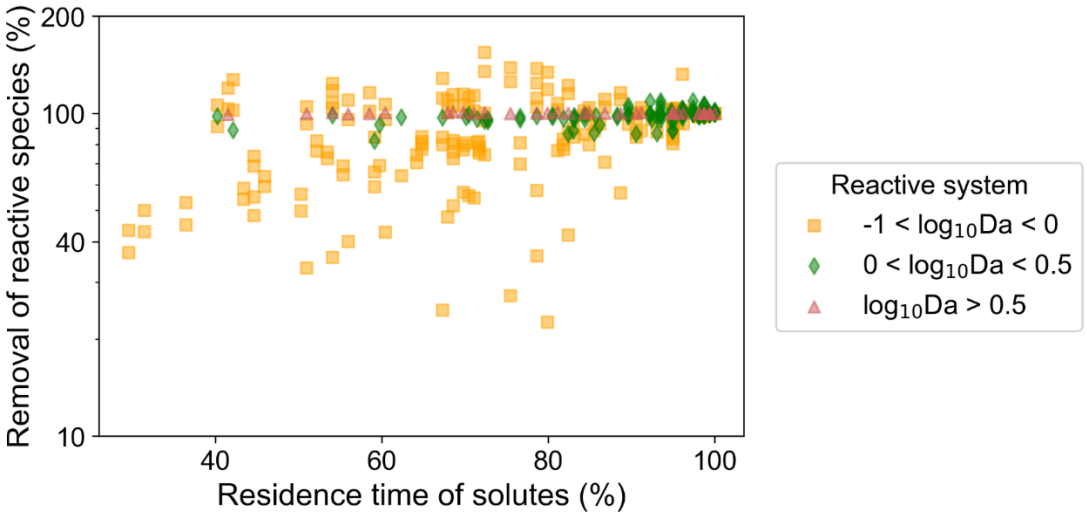

Figure A 27 Predicting impact of spatial heterogeneity on reactive species removal in different reaction regimes indicated by $\log_{10}$Da in saturated domains.

Appendix B: Supplementary Tables

Table B 1 Parameterization of microbial respiration and growth processes

S. No.	Description	Notation	Value	Units	Source
1	Rate constant for aerobic reduction of DOC	kmax1	1	d^{-1}	Calibrated
2	Minimum biomass normalized rate value for aerobic respiration to be favourable	o2min	0.06	d^{-1}	Calibrated
3	Yield coefficient for growth of aerobic degraders of DOC	Yo	0.25	-	calibrated, based on Thullner et al. (2005)
4	Half-velocity DOC concentration	ksodoc	1,000	µM C	calibrated, based on concentrations observed in the field
5	Half velocity oxygen concentration	ksox	2-	µM	Thullner et al. (2005), Wang and Van Cappellen (1996)
6	Rate constant for nitrate reduction	kmax2	0.9	d^{-1}	calibrated, based on Schäfer et al. (1998b)
7	Minimum biomass normalized rate value for respiration to be favourable	no3min	0.1	d^{-1}	Calibrated
8	Yield coefficient for growth of nitrate reducers	Yn	0.17	-	calibrated, based on Clement et al. (1997) and Thullner et al. (2005)
9	Half-velocity DOC concentration	ksndoc	1,000	µM C	calibrated, based on concentrations observed in the field
10	Half velocity nitrate concentration	ksno3	100	µM	calibrated, based on Clément et al. (1997) and André et al. (2011)
11	Inhibition constant for presence of oxygen	kindox	1	µM	calibrated, based on detection limits of sensors defining anaerobic conditions
12	Rate constant for sulphate reduction	kmax3	0.03	d^{-1}	Calibrated
13	Minimum biomass normalized rate value for respiration to be favourable	so4min	0.0039	d^{-1}	Calibrated
14	Yield coefficient for growth of sulphate reducers	Ys	0.02	-	calibrated, based on Thullner et al. (2005)

S. No.	Description	Notation	Value	Units	Source
15	Half-velocity DOC concentration	kssdoc	1,000	µM C	calibrated, based on concentrations observed in the field
16	Half velocity sulphate concentration	ksso4	1,000	µM	calibrated, based on Pallud and Van Cappellen (2006), Thullner et al. (2005) and Boudreau and Westrich (1984)
17	Inhibition constant for presence of oxygen	kindox	1	µM	calibrated, based on detection limits of sensors defining anaerobic conditions
18	Inhibition constant for presence of nitrate	kinno3	50	µM	Calibrated
19	Rate constant for ammonia oxidation	kmax4	0.1	d^{-1}	Calibrated
20	Minimum biomass normalized rate value for respiration to be favourable	ammin	0.004	d^{-1}	Calibrated
21	Yield coefficient for growth of ammonia oxidizers	Ya	0.0038		Calibrated
22	Half-velocity Ammonia concentration	ksamm	20	µM	calibrated based on conditions observed in the field
23	Half velocity oxygen concentration	ksox	20	µM	De Brabandere et al. (2014), Kalvelage et al. (2013), Seitzinger et al. (2006)
24	Sigmoidal function slope parameter	st	0.1	-	Stolpovsky et al. (2011)
25	Minimum concentration of Ammonium for growth to remain favourable	amming	10	µM	calibrated based on review by Jin et al. (2013)
26	Maximum/Carrying capacity at a node	Bfmax	500	µM C	calibrated, based on Fukuda et al. (1998), Vrede et al. (2002) and Grösbacher et al. (2018).
27	Mobilisation rate constant due to exceedance of carrying capacity	kdet	1	µM C d^{-1}	calibrated, based on Clément et al. (1997)

S. No.	Description	Notation	Value	Units	Source
28	Immobilisation rate constant	katt	0.3	µM C d^{-1}	calibrated, based on kdet
29	Deactivation/dormancy rate constant	kdeac	1	d^{-1}	calibrated, based on Stolpovsky et al. (2016)
30	Reactivation rate constant	kreac	0.3	d^{-1}	calibrated, based on Stolpovsky et al (2016).
31	Mortality rate constant	km	0.01	d^{-1}	calibrated, based on Clément et al. (1997)
32	Hydrolysis constant	kpd	0.03	d^{-1}	calibrated
33	Carbon Nitrogen ratio for hydrolysis of particulate organic matter	fcn	10:1	-	calibrated, based on Wang and van Cappellen (1996)
34	Desorption constant	kl	0.00544	-	Rittmann and McCarty (2001)
35	Rate constant for background activity	kmax5	0.00038	d^{-1}	calibrated

Table B 2 Relative removal of dissolved species (%) leaving the domain in three (3) flow regimes - slow flow, medium flow and fast flow and three (3) temporally dynamic scenarios in the homogeneous domain aggregated over 15 years of simulation period.

Chemical species/Removal in ->	Slow flow				Medium flow				Fast flow			
	T0	T1	T2	T5	T0	T1	T2	T5	T0	T1	T2	T5
Ammonium	19.8	19.4	19.4	19.4	19.4	19.1	19.0	18.9	11.5	11.1	11.0	10.7
DO	99.6	99.6	99.6	99.6	99.4	99.4	99.4	99.3	98.3	93.8	93.1	89.5
DOC	59.2	59.2	59.2	59.2	56.5	56.0	55.8	55.4	31.1	29.9	29.9	29.0
Nitrate	74.7	74.6	74.5	74.4	65.8	64.4	64.1	62.9	1.14	1.74	2.15	2.74
Nitrogen	57.0	56.8	56.7	56.5	53.1	52.0	51.7	50.8	1.14	1.62	1.95	2.41
TOC	32.6	32.4	32.2	31.7	42.6	42.1	42.0	41.6	22.7	21.8	21.8	21.2

Table B 3 Peak correlation of concentration of chemical species at the outlet of the domain in response to temporal dynamics at the inlet of the domain in three flow regimes in the homogeneous domain

Chemical Species	Slow flow			Medium flow			Fast flow		
	T1	T2	T5	T1	T2	T5	T1	T2	T5
Ammonium	0.1	0.2	0.3	0.3	0.3	0.3	0.5	0.5	0.6
DO	0.6	0.6	0.7	0.9	1.0	1.0	0.8	0.8	0.9
DOC	0.2	0.1	0.2	0.7	0.7	0.8	0.8	0.8	0.9
Nitrate	0.6	0.8	0.8	0.9	1.0	1.0	0.7	0.7	0.7
Nitrogen	0.4	0.6	0.5	0.9	0.9	0.9	0.6	0.6	0.7
TOC	0.6	0.8	0.7	0.4	0.3	0.4	0.7	0.7	0.8

Table B 4 Biomass (B in µM C) in the homogeneous domain of each microbial species in three (3) flow regimes and in three (3) temporally dynamic scenarios averaged over 15 years of simulation period.

Microbial species	Slow flow				Medium flow				Fast flow			
	T0	T1	T2	T5	T0	T1	T2	T5	T0	T1	T2	T5
Total biomass (µM C)	124	126	129	133	96.6	97.1	97.3	97.8	89.3	89.3	89.3	89.4
Fraction of each species: Active immobile(%)												
Aerobes	3.45	3.38	3.28	3.17	11.5	11.5	11.4	11.5	71.5	65.0	64.1	61.2
Ammonia oxidizers	0.45	0.41	0.40	0.37	2.21	2.20	2.11	2.21	2.34	2.38	2.39	2.41
Nitrate reducers	2.42	2.38	2.30	2.22	11.4	11.3	11.0	11.4	2.26	2.98	3.53	4.34
Fraction of each species: Active mobile(%)												
Aerobes	1.03	1.08	1.05	1.09	2.11	2.10	2.10	2.11	9.23	8.05	7.93	7.45
Ammonia oxidizers	0.23	0.21	0.21	0.19	0.67	0.66	0.64	0.67	0.64	0.65	0.65	0.65
Nitrate reducers	1.22	1.24	1.23	1.25	2.89	2.87	2.77	2.89	0.26	0.45	0.51	0.66
Fraction of each species: Inactive immobile(%)												
Aerobes	43.3	42.7	41.9	41.0	40.4	40.3	40.4	40.4	5.04	10.5	10.9	12.9
Ammonia oxidizers	0.54	0.58	0.55	0.54	0.29	0.28	0.32	0.29	0.17	0.16	0.16	0.16

Nitrate reducers	14.5	14.5	14.1	13.8	10.1	10.3	10.4	10.1	3.11	3.14	3.04	2.96
Microbial species	**Slow flow**				**Medium flow**				**Fast flow**			
	T0	**T1**	**T2**	T5	**T0**	**T1**	**T2**	T5	**T0**	**T1**	**T2**	T5
Fraction of each species: Inactive mobile(%)												
Aerobes	23.1	23.5	24.7	25.8	11.9	11.9	12.1	11.9	0.47	1.75	1.84	2.40
Ammonia oxidizers	0.29	0.33	0.32	0.34	0.12	0.12	0.14	0.12	0.26	0.24	0.24	0.24
Nitrate reducers	7.72	7.91	8.22	8.52	2.95	3.03	3.17	2.95	1.33	1.25	1.24	1.20

Table B 5 Peak correlation of biomass (B in μM C) in the homogeneous domain of each microbial species in three (3) flow regimes and in three (3) temporally dynamic scenarios

Microbial species	Slow flow			Medium flow			Fast flow		
	T1	T2	T5	T1	T2	T5	T1	T2	T5
Active fixed Aerobes	0.97	0.98	0.98	0.99	1.00	1.00	0.91	0.93	0.90
Active fixed ammonia oxidizers	0.87	0.93	0.94	0.90	0.92	0.90	0.94	0.96	0.94
Active fixed nitrate reducers	0.95	0.98	0.99	0.97	0.98	0.98	0.88	0.90	0.88
Active mobile aerobes	0.76	0.73	0.74	0.99	1.00	1.00	0.21	0.32	0.32
Active mobile ammonia oxidizers	0.76	0.73	0.81	0.84	0.87	0.84	0.96	0.97	0.96
Active mobile nitrate reducers	0.70	0.72	0.70	0.95	0.95	0.92	0.74	0.71	0.74
Inactive fixed aerobes	0.68	0.89	0.91	0.97	0.96	0.96	0.89	0.92	0.89
Inactive fixed ammonia oxidizers	0.78	0.66	0.46	0.86	0.87	0.85	0.91	0.90	0.56
Inactive fixed nitrate reducers	0.89	0.85	0.86	0.97	0.99	0.99	0.83	0.87	0.67
Inactive mobile aerobes	0.73	0.88	0.86	0.93	0.91	0.91	0.78	0.81	0.78
Inactive mobile ammonia oxidizers	0.73	0.72	0.79	0.80	0.79	0.77	0.96	0.97	0.95
Inactive mobile nitrate reducers	0.73	0.87	0.85	0.96	0.97	0.96	0.79	0.83	0.65

Appendix C: Publications from this thesis

Chapters 2,3 and 4/ Khurana et al., 2021

The description of the model set up in the saturated domain, the reaction network, and all results pertaining to the numerical experiments in the saturated domain are based on the following publication, currently in review:

Khurana, S., Heße, F., Hildebrandt, A., and Thullner, M.: Predicting the impact of spatial heterogeneity on microbial redox dynamics and nutrient cycling in the subsurface, Biogeosciences Discussions [preprint], in review, https://doi.org/10.5194/bg-2021-72, 2021.

Chapter 5/ Khurana et al., 2021

The results of numerical experiments in transient conditions in the saturated domain are based on the following publication, currently submitted for review:

Khurana, S., Heße, F., Hildebrandt, A., and Thullner, M.: Should we worry about surficial dynamics when assessing nutrient cycling in the groundwater? Front. Water - Water and Hydrocomplexity Manuscript ID: 780297, submitted, 2021.

Selbständigkeitserklärung

Ich erkläre, dass ich die vorliegende Arbeit selbständig und unter Verwendung der angegebenen Hilfsmittel, persönlichen Mitteilungen und Quellen angerfetigt habe.

Ort, Datum Unterschrift der Verfasserin

References

Aguilera, D. R., Jourabchi, P., Spiteri, C., and Regnier, P.: A knowledge-based reactive transport approach for the simulation of biogeochemical dynamics in Earth systems, Geochemistry, Geophysics, Geosystems, 6, n/a-n/a, 10.1029/2004gc000899, 2005.

Akob, D. M., and Küsel, K.: Where microorganisms meet rocks in the Earth's Critical Zone, Biogeosciences, 8, 3531-3543, 10.5194/bg-8-3531-2011, 2011.

Alewell, C., Paul, S., Lischeid, G., Küsel, K., and Gehre, M.: Characterizing the redox status in three different forested wetlands with geochemical data, Environmental Science and Technology, 40, 7609-7615, 10.1021/es061018y, 2006.

Alfreider, A., Krossbacher, M., and Psenner, R.: Groundwater samples do not reflect bacterial densities and activity in subsurface systems, Water Research, 31, 832-840, 1997.

Amyx, J. W., Daniel M. Bass, J., and Whiting, R. L.: Petroleum Reservoir Engineering, United States of America, 1960.

Anantharaman, K., Brown, C. T., Hug, L. A., Sharon, I., Castelle, C. J., Probst, A. J., Thomas, B. C., Singh, A., Wilkins, M. J., Karaoz, U., Brodie, E. L., Williams, K. H., Hubbard, S. S., and Banfield, J. F.: Thousands of microbial genomes shed light on interconnected biogeochemical processes in an aquifer system, Nat Commun, 7, 13219, 10.1038/ncomms13219, 2016.

André, L., Pauwels, H., Dictor, M. C., Parmentier, M., and Azaroual, M.: Experiments and numerical modelling of microbially-catalysed denitrification reactions, Chemical Geology, 287, 171-181, 10.1016/j.chemgeo.2011.06.008, 2011.

Andrea, B., Francesc, G., Jérôme, L., Eusebi, V., and Francesc, S.: Cross-site comparison of variability of DOC and nitrate c-q hysteresis during the autumn-winter period in three Mediterranean headwater streams: A synthetic approach, Biogeochemistry, 77, 327-349, 10.1007/s10533-005-0711-7, 2006.

Arora, B., Spycher, N. F., Steefel, C. I., Molins, S., Bill, M., Conrad, M. E., Dong, W., Faybishenko, B., Tokunaga, T. K., Wan, J., Williams, K. H., and Yabusaki, S. B.: Influence of hydrological, biogeochemical and temperature transients on subsurface carbon fluxes in a flood plain environment, Biogeochemistry, 127, 367-396, 10.1007/s10533-016-0186-8, 2016.

Arora, B., and Mohanty, B. P.: Influence of Spatial Heterogeneity and Hydrological Perturbations on Redox Dynamics: A Column Study, Procedia Earth and Planetary Science, 17, 869-872, https://doi.org/10.1016/j.proeps.2017.01.046, 2017.

Austin, A. T., Yahdjian, L., Stark, J. M., Belnap, J., Porporato, A., Norton, U., Ravetta, D. A., and Schaeffer, S. M.: Water pulses and biogeochemical cycles in arid and semiarid ecosystems, Oecologia, 141, 221-235, 10.1007/s00442-004-1519-1, 2004.

Bar-On, Y. M., Phillips, R., and Milo, R.: The biomass distribution on Earth, Proceedings of the National Academy of Sciences of the United States of America, 115, 6506-6511, 10.1073/pnas.1711842115, 2018.

Barros, N., Gomez-Orellana, I., Feijóo, S., and Balsa, R.: The effect of soil moisture on soil microbial activity studied by microcalorimetry, Thermochimica Acta, 249, 161-168, 10.1016/0040-6031(95)90686-X, 1995.

Basu, N. B., Destouni, G., Jawitz, J. W., Thompson, S. E., Loukinova, N. V., Darracq, A., Zanardo, S., Yaeger, M., Sivapalan, M., Rinaldo, A., and Rao, P. S. C.: Nutrient loads exported from managed catchments reveal emergent biogeochemical stationarity, Geophysical Research Letters, 37, 10.1029/2010GL045168, 2010.

Bauer, S., Beyer, C., McDermott, C., Kosakowski, G., Krug, S., Park, C.-H., Pichot, G., Shao, H., Sun, Y., and Taron, J.: Mass Transport, in: Thermo-Hydro-Mechanical-Chemical Processes in Porous Media: Benchmarks and Examples, edited by: Kolditz, O., Görke, U.-J., Shao, H., and Wang, W., Springer Berlin Heidelberg, Berlin, Heidelberg, 201-231, 2012.

Benk, S. A., Yan, L., Lehmann, R., Roth, V.-N., Schwab, V. F., Totsche, K. U., Küsel, K., and Gleixner, G.: Fueling Diversity in the Subsurface: Composition and Age of Dissolved Organic Matter in the Critical Zone, Frontiers in Earth Science, 7, 10.3389/feart.2019.00296, 2019.

Bense, V. F., Ferguson, G., and Kooi, H.: Evolution of shallow groundwater flow systems in areas of degrading permafrost, Geophysical Research Letters, 36, 10.1029/2009GL039225, 2009.

Berkowitz, B.: Characterizing flow and transport in fractured geological media: A review, Advances in Water Resources, 25, 861-884, 10.1016/S0309-1708(02)00042-8, 2002.

Berkowitz, B., Silliman, S. E., and Dunn, A. M.: Impact of the Capillary Fringe on Local Flow, Chemical Migration, and Microbiology, Vadose Zone Journal, 3, 534-548, 2004.

Berkowitz, B., Dror, I., Hansen, S. K., and Scher, H.: Measurements and models of reactive transport in geological media, Reviews of Geophysics, 54, 930-986, https://doi.org/10.1002/2016RG000524, 2016.

Betts, A. K., Ball, J. H., Beljaars, A. C. M., Miller, M. J., and Viterbo, P. A.: The land surface-atmosphere interaction: A review based on observational and global modeling perspectives, Journal of Geophysical Research Atmospheres, 101, 7209-7225, 10.1029/95JD02135, 1996.

Bieroza, M. Z., and Heathwaite, A. L.: Seasonal variation in phosphorus concentration-discharge hysteresis inferred from high-frequency in situ monitoring, Journal of Hydrology, 524, 333-347, 10.1016/j.jhydrol.2015.02.036, 2015.

Bieroza, M. Z., Heathwaite, A. L., Bechmann, M., Kyllmar, K., and Jordan, P.: The concentration-discharge slope as a tool for water quality management, Science of the Total Environment, 630, 738-749, 10.1016/j.scitotenv.2018.02.256, 2018.

Blazewicz, S. J., Hungate, B. A., Koch, B. J., Nuccio, E. E., Morrissey, E., Brodie, E. L., Schwartz, E., Pett-Ridge, J., and Firestone, M. K.: Taxon-specific microbial growth and mortality patterns reveal distinct temporal population responses to rewetting in a California grassland soil, ISME Journal, 14, 1520-1532, 10.1038/s41396-020-0617-3, 2020.

Bol, R., Lücke, A., Tappe, W., Kummer, S., Krause, M., Weigand, S., Pütz, T., and Vereecken, H.: Spatio-temporal variations of dissolved organic matter in a german forested mountainous headwater catchment, Vadose Zone Journal, 14, 10.2136/vzj2015.01.0005, 2015.

Boudreau, B. P., and Westrich, J. T.: The dependence of bacterial sulfate reduction on sulfate concentration in marine sediments, Geochimica et Cosmochimica Acta, 48, 2503-2516, 10.1016/0016-7037(84)90301-6, 1984.

Bouskill, N. J., Conrad, M. E., Bill, M., Brodie, E. L., Cheng, Y., Hobson, C., Forbes, M., Casciotti, K. L., and Williams, K. H.: Evidence for Microbial Mediated NO3− Cycling Within Floodplain Sediments During Groundwater Fluctuations, Frontiers in Earth Science, 7, 10.3389/feart.2019.00189, 2019.

Bradley, J. A., Amend, J. P., and LaRowe, D. E.: Bioenergetic Controls on Microbial Ecophysiology in Marine Sediments, Front Microbiol, 9, 180, 10.3389/fmicb.2018.00180, 2018.

Brangarí, A. C., Fernàndez-Garcia, D., Sanchez-Vila, X., and Manzoni, S.: Ecological and soil hydraulic implications of microbial responses to stress – A modeling analysis, Advances in Water Resources, 116, 178-194, 10.1016/j.advwatres.2017.11.005, 2018.

Brantley, S. L., McDowell, W. H., Dietrich, W. E., White, T. S., Kumar, P., Anderson, S. P., Chorover, J., Lohse, K. A., Bales, R. C., Richter, D. D., Grant, G., and Gaillardet, J.: Designing a network of critical zone observatories to explore the living skin of the terrestrial Earth, Earth Surface Dynamics, 5, 841-860, 10.5194/esurf-5-841-2017, 2017.

Briggs, M. A., Lautz, L. K., and Hare, D. K.: Residence time control on hot moments of net nitrate production and uptake in the hyporheic zone, Hydrological Processes, 28, 3741-3751, 10.1002/hyp.9921, 2014.

Bundt, M., Widmer, F., Pesaro, M., Zeyer, J., and Blaser, P.: Preferential flow paths: biological 'hot spots' in soils, Soil Biology and Biochemistry, 33, 729-738, 2001.

Carsel, R. F., and Parrish, R. S.: Developing Joint Probability Distributions of Soil Water Retention Characteristics, Water Resources Research, 24, 755-769, 1988.

Centler, F., Shao, H., De Biase, C., Park, C.-H., Regnier, P., Kolditz, O., and Thullner, M.: GeoSysBRNS—A flexible multidimensional reactive transport model for simulating biogeochemical subsurface processes, Computers & Geosciences, 36, 397-405, 10.1016/j.cageo.2009.06.009, 2010.

Chen, Z., Shi, L., Ye, M., Zhu, Y., and Yang, J.: Global sensitivity analysis for identifying important parameters of nitrogen nitrification and denitrification under model uncertainty and scenario uncertainty, Journal of Hydrology, 561, 884-895, 10.1016/j.jhydrol.2018.04.031, 2018.

Christensen, T. H., Kjeldsen, P., Bjerg, P. L., Jensen, D. L., Christensen, J. B., Baun, A., Albrechtsen, H. J., and Heron, G.: Biogeochemistry of landfill leachate plumes, Applied Geochemistry, 16, 659-718, 10.1016/S0883-2927(00)00082-2, 2001.

Clement, T. P., Peyton, B. M., Skeen, R. S., Jennings, D. A., and Petersen, J. N.: Microbial growth and transport in porous media under denitrification conditions: experiments and simulations, Journal of Contaminant Hydrology, 24, 269-285, 1997.

Cole, J. J., Prairie, Y. T., Caraco, N. F., McDowell, W. H., Tranvik, L. J., Striegl, R. G., Duarte, C. M., Kortelainen, P., Downing, J. A., Middelburg, J. J., and Melack, J.: Plumbing the Global Carbon Cycle: Integrating Inland Waters into the Terrestrial Carbon Budget, Ecosystems, 10, 172-185, 10.1007/s10021-006-9013-8, 2007.

Corradini, C., Melone, F., and Smith, R. E.: Modeling local infiltration for a two-layered soil under complex rainfall patterns, Journal of Hydrology, 237, 58-73, 10.1016/S0022-1694(00)00298-5, 2000.

Couradeau, E., Sasse, J., Goudeau, D., Nath, N., Hazen, T. C., Bowen, B. P., Chakraborty, R., Malmstrom, R. R., and Northen, T. R.: Probing the active fraction of soil microbiomes using BONCAT-FACS, Nature Communications, 10, 10.1038/s41467-019-10542-0, 2019.

Creed, I. F., McKnight, D. M., Pellerin, B. A., Green, M. B., Bergamaschi, B. A., Aiken, G. R., Burns, D. A., Findlay, S. E. G., Shanley, J. B., Striegl, R. G., Aulenbach, B. T., Clow, D. W., Laudon, H., McGlynn, B. L., McGuire, K. J., Smith, R. A., and Stackpoole, S. M.: The river as a chemostat: Fresh perspectives on dissolved organic matter flowing down the river continuum, Canadian Journal of Fisheries and Aquatic Sciences, 72, 1272-1285, 10.1139/cjfas-2014-0400, 2015.

Dagan, G., Fiori, A., and Janković, I.: Flow and transport in highly heterogeneous formations: 1. Conceptual framework and validity of first-order approximations, Water Resources Research, 39, SBH141-SBH1412, 10.1029/2002WR001717, 2003.

Davis, J. M., Wilson, J. L., Phillips, F. M., and Gotkowitz, M. B.: Relationship between fluvial bounding surfaces and the permeability correlation structure, Water Resources Research, 33, 1843-1854, 10.1029/97wr01003, 1997.

De Castro Ochoa, F., and Muñoz Reinoso, J. C.: Model of long-term water-table dynamics at Donana National park, Water Research, 31, 2586-2596, 10.1016/S0043-1354(97)00098-5, 1997.

De Troyer, I., Merckx, R., Amery, F., and Smolders, E.: Factors controlling the dissolved organic matter concentration in pore waters of agricultural soils, Vadose Zone Journal, 13, 10.2136/vzj2013.09.0167, 2014.

Dechesne, A., Pallud, C., Bertolla, F., and Grundmann, G. L.: Impact of the microscale distribution of a Pseudomonas strain introduced into soil on potential contacts with indigenous bacteria, Applied and Environmental Microbiology, 71, 8123-8131, 10.1128/AEM.71.12.8123-8131.2005, 2005.

Delhomme, J. P.: Spatial variability and uncertainty in groundwater flow parameters: A geostatistical approach, Water Resources Research, 15, 269-280, 10.1029/WR015i002p00269, 1979.

Desbarats, A. J.: Numerical estimation of effective permeability in sand-shale formations, Water Resources Research, 23, 273-286, 10.1029/WR023i002p00273, 1987.

Du, X., Deng, Y., Li, S., Escalas, A., Feng, K., He, Q., Wang, Z., Wu, Y., Wang, D., Peng, X., and Wang, S.: Steeper spatial scaling patterns of subsoil microbiota are shaped by deterministic assembly process, Molecular Ecology, 30, 1072-1085, 10.1111/mec.15777, 2021.

Dutilleul, P., and Legendre, P.: Spatial Heterogeneity against Heteroscedasticity: An Ecological Paradigm versus a Statistical Concept, Oikos, 66, 152-171, 1993.

Dwivedi, D., Arora, B., Steefel, C. I., Dafflon, B., and Versteeg, R.: Hot Spots and Hot Moments of Nitrogen in a Riparian Corridor, Water Resources Research, 54, 205-222, 10.1002/2017wr022346, 2018.

Ebeling, P., Kumar, R., Weber, M., Knoll, L., Fleckenstein, J. H., and Musolff, A.: Archetypes and Controls of Riverine Nutrient Export Across German Catchments, Water Resources Research, 57, 10.1029/2020WR028134, 2021.

Ebrahimi, A., and Or, D.: Hydration and diffusion processes shape microbial community organization and function in model soil aggregates, Water Resources Research, 51, 9804-9827, 10.1002/2015wr017565, 2015.

Edery, Y., Porta, G. M., Guadagnini, A., Scher, H., and Berkowitz, B.: Characterization of Bimolecular Reactive Transport in Heterogeneous Porous Media, Transport in Porous Media, 115, 291-310, 10.1007/s11242-016-0684-0, 2016.

Edmunds, W. M., and Smedley, P. L.: Residence time indicators in groundwater: The East Midlands Triassic sandstone aquifer, Applied Geochemistry, 15, 737-752, 10.1016/S0883-2927(99)00079-7, 2000.

Eilers, K. G., Debenport, S., Anderson, S., and Fierer, N.: Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil, Soil Biology and Biochemistry, 50, 58-65, 10.1016/j.soilbio.2012.03.011, 2012.

Evans, C., and Davies, T. D.: Causes of concentration/discharge hysteresis and its potential as a tool for analysis of episode hydrochemistry, Water Resources Research, 34, 129-137, 10.1029/97WR01881, 1998.

Falkowski, P. G., Fenchel, T., and Delong, E. F.: The Microbial Engines That Drive Earth's Biogeochemical Cycles, Science, 320, 1034-1039, 10.1126/science.1153213, 2008.

Field, M. S., and Nash, S. G.: Risk assessment methodology for karst aquifers: (1) Estimating karst conduit-flow parameters', Environmental Monitoring and Assessment, 47, 1-21, 10.1023/A:1005753919403, 1997.

Francis, J. A., and Vavrus, S. J.: Evidence linking Arctic amplification to extreme weather in mid-latitudes, Geophysical Research Letters, 39, 10.1029/2012GL051000, 2012.

Franklin, S., Vasilas, B., and Jin, Y.: More than Meets the Dye: Evaluating Preferential Flow Paths as Microbial Hotspots, Vadose Zone Journal, 18, 190024, https://doi.org/10.2136/vzj2019.03.0024, 2019.

Freimann, R., Bürgmann, H., Findlay, S. E. G., and Robinson, C. T.: Spatio-temporal patterns of major bacterial groups in alpine waters, PLoS ONE, 9, 10.1371/journal.pone.0113524, 2014.

Fukuda, R., Ogawa, H., Nagata, T., and Koike, I.: Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments, Applied and Environmental Microbiology, 64, 3352-3358, 10.1128/aem.64.9.3352-3358.1998, 1998.

Gelhar, L. W., and Axness, C. L.: Three-dimensional stochastic analysis of macrodispersion in aquifers, Water Resources Research, 19, 161-180, 10.1029/WR019i001p00161, 1983.

Gerke, H. H.: Preferential flow descriptions for structured soils, Journal of Plant Nutrition and Soil Science, 169, 382-400, 10.1002/jpln.200521955, 2006.

Geza, M., Deb, S. K., Leinauer, B., Stanek, S., Sevostianova, E., and Serena, M.: Modeling NO3–N leaching during establishment of turfgrasses irrigated with tailored reclaimed water, Vadose Zone Journal, 20, e20112, https://doi.org/10.1002/vzj2.20112, 2021.

Gharasoo, M., Centler, F., Regnier, P., Harms, H., and Thullner, M.: A reactive transport modeling approach to simulate biogeochemical processes in pore structures with pore-scale heterogeneities, Environmental Modelling & Software, 30, 102-114, 10.1016/j.envsoft.2011.10.010, 2012.

Ghiorse, W. C.: Subterranean Life, Science, 275, 789-790, 1997.

Giardino, J. R., and Houser, C.: A Summary and Future Direction of the Principles and Dynamics of the Critical Zone. In: Developments in Earth Surface Processes, 2015.

Golparvar, A., Kästner, M., and Thullner, M.: Pore-scale modeling of microbial activity: What we have and what we need, Vadose Zone Journal, 20, 10.1002/vzj2.20087, 2021.

Gorski, G., and Zimmer, M. A.: Hydrologic regimes drive nitrate export behavior in human-impacted watersheds, Hydrology and Earth System Sciences, 25, 1333-1345, 10.5194/hess-25-1333-2021, 2021.

Gregory, J. M., Mitchell, J. F. B., and Brady, A. J.: Summer drought in Northern Midlatitudes in a time-dependent CO2 climate experiment, Journal of Climate, 10, 662-686, 10.1175/1520-0442(1997)010<0662:SDINMI>2.0.CO;2, 1997.

Griebler, C., Mindl, B., Slezak, D., and Geiger-Kaiser, M.: Distribution patterns of attached and suspended bacteria in pristine and contaminated shallow aquifers studied with an in situ sediment exposure microcosm, Aquatic Microbial Ecology, 28, 117-129, 2002.

Griebler, C., and Lueders, T.: Microbial biodiversity in groundwater ecosystems, Freshwater Biology, 54, 649-677, 10.1111/j.1365-2427.2008.02013.x, 2009.

Griebler, C., Stein, H., Kellermann, C., Berkhoff, S., Brielmann, H., Schmidt, S., Selesi, D., Steube, C., Fuchs, A., and Hahn, H. J.: Ecological assessment of groundwater ecosystems - Vision or illusion?, Ecological Engineering, 36, 1174-1190, 10.1016/j.ecoleng.2010.01.010, 2010.

Grösbacher, M., Eckert, D., Cirpka, O. A., and Griebler, C.: Contaminant concentration versus flow velocity: drivers of biodegradation and microbial growth in groundwater model systems, Biodegradation, 29, 211-232, 10.1007/s10532-018-9824-2, 2018.

Gross, A., Lin, Y., Weber, P. K., Pett-Ridge, J., and Silver, W. L.: The role of soil redox conditions in microbial phosphorus cycling in humid tropical forests, Ecology, 101, 10.1002/ecy.2928, 2020.

Guo, L., and Lin, H.: Critical Zone Research and Observatories: Current Status and Future Perspectives, Vadose Zone Journal, 15, 10.2136/vzj2016.06.0050, 2016.

Gurevich, H., Baram, S., and Harter, T.: Measuring nitrate leaching across the critical zone at the field to farm scale, Vadose Zone Journal, 20, e20094, https://doi.org/10.1002/vzj2.20094, 2021.

Gutiérrez Castorena, E. V., Gutiérrez-Castorena, M. D. C., González Vargas, T., Cajuste Bontemps, L., Delgadillo Martínez, J., Suástegui Méndez, E., and Ortiz Solorio, C. A.: Micromapping of microbial hotspots and biofilms from different crops using digital image mosaics of soil thin sections, Geoderma, 279, 11-21, 10.1016/j.geoderma.2016.05.017, 2016.

Gwo, J. P., Toran, L. E., Morris, M. D., and Wilson, G. V.: Subsurface Stormflow Modeling with Sensitivity Analysis Using a Latin-Hypercube Sampling Technique, Ground Water, 34, 811-818, 10.1111/j.1745-6584.1996.tb02075.x, 1996.

Harden, J. W., O'Neill, K. P., Trumbore, S. E., Veldhuis, H., and Stocks, B. J.: Moss and soil contributions to the annual net carbon flux of a maturing boreal forest, Journal of Geophysical Research Atmospheres, 102, 28805-28816, 1997.

Hayashi, M.: The cold vadose zone: Hydrological and ecological significance of frozen-soil processes, Vadose Zone Journal, 12, 2013.

Heath, R. C.: Basic groundwater-hydrology, U.S. Geological Survey, U.S. Geological Survey Water-Supply Paper, 81, 1983.

Heathwaite, A. L., and Bieroza, M.: Fingerprinting hydrological and biogeochemical drivers of freshwater quality, Hydrological Processes, 35, 10.1002/hyp.13973, 2021.

Henry, C. M., Allen, D. M., and Huang, J.: Groundwater storage variability and annual recharge using well-hydrograph and GRACE satellite data, Hydrogeology Journal, 19, 741-755, 10.1007/s10040-011-0724-3, 2011.

Herndon, E. M., Dere, A. L., Sullivan, P. L., Norris, D., Reynolds, B., and Brantley, S. L.: Landscape heterogeneity drives contrasting concentration-discharge relationships in shale headwater catchments, Hydrology and Earth System Sciences, 19, 3333-3347, 10.5194/hess-19-3333-2015, 2015.

Herrmann, M., Rusznyak, A., Akob, D. M., Schulze, I., Opitz, S., Totsche, K. U., and Kusel, K.: Large fractions of CO2-fixing microorganisms in pristine limestone aquifers appear to be involved in the oxidation of reduced sulfur and nitrogen compounds, Appl Environ Microbiol, 81, 2384-2394, 10.1128/AEM.03269-14, 2015.

Heße, F., Harms, H., Attinger, S., and Thullner, M.: Linear exchange model for the description of mass transfer limited bioavailability at the pore scale, Environmental Science & Technology, 44, 2064-2071, 2010.

Heße, F., Prykhodko, V., Schlüter, S., and Attinger, S.: Generating random fields with a truncated power-law variogram: A comparison of several numerical methods, Environmental Modelling & Software, 55, 32-48, 10.1016/j.envsoft.2014.01.013, 2014.

Hofmann, R., Uhl, J., Hertkorn, N., and Griebler, C.: Linkage Between Dissolved Organic Matter Transformation, Bacterial Carbon Production, and Diversity in a Shallow Oligotrophic Aquifer: Results From Flow-Through Sediment Microcosm Experiments, Frontiers in Microbiology, 11, 10.3389/fmicb.2020.543567, 2020.

Holden, P. A., and Fierer, N.: Microbial processes in the vadose zone, Vadose Zone Journal, 4, 1-21, 2005.

Holm, P. E., Nielsen, P. H., Albrechtsen, H. J., and Christensen, T. H.: Importance of unattached bacteria and bacteria attached to sediment in determining potentials for degradation of xenobiotic organic contaminants in an aerobic aquifer, Applied and Environmental Microbiology, 58, 3020-3026, 10.1128/aem.58.9.3020-3026.1992, 1992.

Holt, M. S.: Sources of chemical contaminants and routes into the freshwater environment, Food and Chemical Toxicology, 38, S21-S27, https://doi.org/10.1016/S0278-6915(99)00136-2, 2000.

Horner-Devine, M. C., Carney, K. M., and Bohannan, B. J. M.: An ecological perspective on bacterial biodiversity, Proceedings of the Royal Society B: Biological Sciences, 271, 113-122, 10.1098/rspb.2003.2549, 2004.

Huang, Y., Salama, M. S., Krol, M. S., Su, Z., Hoekstra, A. Y., Zeng, Y., and Zhou, Y.: Estimation of human-induced changes in terrestrial water storage through integration of GRACE satellite detection and hydrological modeling: A case study of the Yangtze River basin, Water Resources Research, 51, 8494-8516, 10.1002/2015WR016923, 2015.

Humphreys, W. F.: Hydrogeology and groundwater ecology: Does each inform the other?, Hydrogeology Journal, 17, 5-21, 10.1007/s10040-008-0349-3, 2009.

Hunt, D. E., Lin, Y., Church, M. J., Karl, D. M., Tringe, S. G., Izzo, L. K., and Johnson, Z. I.: Relationship between abundance and specific activity of bacterioplankton in open ocean surface waters, Applied and Environmental Microbiology, 79, 177-184, 10.1128/AEM.02155-12, 2013.

Hunter, K. S., Wang, Y., and Van Cappellen, P.: Kinetic modeling of microbially-driven redox chemistry of subsurface environments: coupling transport, microbial metabolism and geochemistry, Journal of Hydrology, 209, 53-80, 1998.

ISO, I. O. f. S.: ISO: 17289:2014-Water quality - Determination of dissolved oxygen - Optical sensor method. 2014.

Jacques, D., Šimůnek, J., Mallants, D., and van Genuchten, M. T.: Modelling coupled water flow, solute transport and geochemical reactions affecting heavy metal migration in a podzol soil, Geoderma, 145, 449-461, 10.1016/j.geoderma.2008.01.009, 2008.

Jang, E., He, W., Savoy, H., Dietrich, P., Kolditz, O., Rubin, Y., Schüth, C., and Kalbacher, T.: Identifying the influential aquifer heterogeneity factor on nitrate reduction processes by numerical simulation, Advances in Water Resources, 99, 38-52, 10.1016/j.advwatres.2016.11.007, 2017.

Jiang, B.: Geospatial analysis requires a different way of thinking: the problem of spatial heterogeneity, GeoJournal, 80, 1-13, 10.1007/sl0708-014-9537-y, 2015.

Jing, M., Heße, F., Kumar, R., Wang, W., Fischer, T., Walther, M., Zink, M., Zech, A., Samaniego, L., Kolditz, O., and Attinger, S.: Improved regional-scale groundwater representation by the coupling of the mesoscale Hydrologic Model (mHM v5.7) to the groundwater model OpenGeoSys (OGS), Geosci. Model Dev., 11, 1989-2007, 10.5194/gmd-11-1989-2018, 2018.

Johnson, N. M., and Dreiss, S. J.: Hydrostratigraphic interpretation using indicator geostatistics, Water Resources Research, 25, 2501-2510, 10.1029/WR025i012p02501, 1989.

Jung, H., and Meile, C.: Upscaling of microbially driven first-order reactions in heterogeneous porous media, J Contam Hydrol, 224, 103483, 10.1016/j.jconhyd.2019.04.006, 2019.

Kalbacher, T., and Du, Y.: Richards Flow, in: Thermo-Hydro-Mechanical-Chemical Processes in Porous Media: Benchmarks and Examples, edited by: Kolditz, O., Görke, U.-J., Shao, H., and Wang, W., Springer Berlin Heidelberg, Berlin, Heidelberg, 125-142, 2012.

Khurana, S., Heße, F., Hildebrandt, A., and Thullner, M.: Spatial heterogeneity impact on microbial redox dynamics (Version v1.0.0). Zenodo, 2021a.

Khurana, S., Heße, F., Hildebrandt, A., and Thullner, M.: Predicting the impact of spatial heterogeneity on microbial redox dynamics and nutrient cycling in the subsurface, Biogeosciences Discussions [preprint], in review, https://doi.org/10.5194/bg-2021-72, 2021b.

Kim, K. H., Yun, S. T., Choi, B. Y., Chae, G. T., Joo, Y., Kim, K., and Kim, H. S.: Hydrochemical and multivariate statistical interpretations of spatial controls of nitrate concentrations in a shallow alluvial aquifer around oxbow lakes (Osong area, central Korea), J Contam Hydrol, 107, 114-127, 10.1016/j.jconhyd.2009.04.007, 2009.

Kim, K. H., Michael, H. A., Field, E. K., and Ullman, W. J.: Hydrologic Shifts Create Complex Transient Distributions of Particulate Organic Carbon and Biogeochemical Responses in Beach Aquifers, Journal of Geophysical Research: Biogeosciences, 124, 3024-3038, 10.1029/2019jg005114, 2019.

Kim, T., Hite, M., Rogacki, L., Sealock, A. W., Sprouse, G., Novak, P. J., and LaPara, T. M.: Dissolved oxygen concentrations affect the function but not the relative abundance of nitrifying bacterial populations in full-scale municipal wastewater treatment bioreactors during cold weather, Science of the Total Environment, 781, 10.1016/j.scitotenv.2021.146719, 2021.

King, A. J., Preheim, S. P., Bailey, K. L., Robeson, M. S., Roy Chowdhury, T., Crable, B. R., Hurt, R. A., Mehlhorn, T., Lowe, K. A., Phelps, T. J., Palumbo, A. V., Brandt, C. C., Brown, S. D., Podar, M., Zhang, P., Lancaster, W. A., Poole, F., Watson, D. B., Fields, M., Chandonia, J. M., Alm, E. J., Zhou, J., Adams, M. W. W., Hazen, T. C., Arkin, A. P., and Elias, D. A.: Temporal Dynamics of In-Field Bioreactor Populations Reflect the Groundwater System and Respond Predictably to Perturbation, Environmental Science and Technology, 51, 2879-2889, 10.1021/acs.est.6b04751, 2017.

Kitanidis, P. K., and Vomvoris, E. G.: A geostatistical approach to the inverse problem in groundwater modeling (steady state) and one-dimensional simulations, Water Resources Research, 19, 677-690, 10.1029/WR019i003p00677, 1983.

Klotzbücher, T., Kaiser, K., and Kalbitz, K.: Response of dissolved organic matter in the forest floor of a temperate spruce stand to increasing throughfall, Vadose Zone Journal, 13, 10.2136/vzj2013.10.0180, 2014.

Koenig, S.: Modelling Spatiotemporal Dynamics of Biodegradation under Disturbances, 2016.

Koenig, S., Worrich, A., Banitz, T., Harms, H., Kaestner, M. E., Miltner, A., Wick, L. Y., Frank, K., Thullner, M., and Centler, F.: Dynamic response of a virtual microbial ecosystem to recurrent disturbances - Mechanistic insights into functional and structural resistance, Frontiers in Microbiology, 2017.

Koestel, J. K., Moeys, J., and Jarvis, N. J.: Meta-analysis of the effects of soil properties, site factors and experimental conditions on solute transport, Hydrology and Earth System Sciences, 16, 1647-1665, 10.5194/hess-16-1647-2012, 2012.

Koestel, J. K., Norgaard, T., Luong, N. M., Vendelboe, A. L., Moldrup, P., Jarvis, N. J., Lamandé, M., Iversen, B. V., and Wollesen De Jonge, L.: Links between soil properties and steady-state solute transport through cultivated topsoil at the field scale, Water Resources Research, 49, 790-807, 10.1002/wrcr.20079, 2013.

Kohlhepp, B., Lehmann, R., Seeber, P., Küsel, K., Trumbore, S. E., and Totsche, K. U.: Aquifer configuration and geostructural links control the groundwater quality in thin-bedded carbonate–siliciclastic alternations of the Hainich CZE, central Germany, Hydrology and Earth System Sciences, 21, 6091-6116, 10.5194/hess-21-6091-2017, 2017.

Kolditz, O., Delfs, J. O., Bürger, C., Beinhorn, M., and Park, C. H.: Numerical analysis of coupled hydrosystems based on an object-oriented compartment approach, Journal of Hydroinformatics, 10, 227-244, 10.2166/hydro.2008.003, 2008.

Kolditz, O., Bauer, S., Bilke, L., Böttcher, N., Delfs, J. O., Fischer, T., Görke, U. J., Kalbacher, T., Kosakowski, G., McDermott, C. I., Park, C. H., Radu, F., Rink, K., Shao, H., Shao, H. B., Sun, F., Sun, Y. Y., Singh, A. K., Taron, J., Walther, M., Wang, W., Watanabe, N., Wu, Y., Xie, M., Xu, W., and Zehner, B.: OpenGeoSys: an open-source initiative for numerical simulation of thermo-hydro-mechanical/chemical (THM/C) processes in porous media, Environmental Earth Sciences, 67, 589-599, 10.1007/s12665-012-1546-x, 2012.

Konhauser, K. O., Kappler, A., and Roden, E. E.: Iron in microbial metabolisms, Elements, 7, 89-93, 10.2113/gselements.7.2.89, 2011.

Kotelnikova, S.: Kotelnikova S.. Microbial production and oxidation of methane in deep subsurface. Earth-Sci Rev 58: 367-395, Earth-Science Reviews, 58, 367-395, 10.1016/S0012-8252(01)00082-4, 2002.

Kothavala, Z.: Extreme precipitation events and the applicability of global climate models to the study of floods and droughts, Mathematics and Computers in Simulation, 43, 261-268, 10.1016/s0378-4754(97)00008-6, 1997.

Kothavala, Z.: The duration and severity of drought over eastern Australia simulated by a coupled ocean-atmosphere GCM with a transient increase in CO2, Environmental Modelling and Software, 14, 243-252, 10.1016/S1364-8152(98)00076-0, 1999.

Kumar, R., Heße, F., Rao, P. S. C., Musolff, A., Jawitz, J. W., Sarrazin, F., Samaniego, L., Fleckenstein, J. H., Rakovec, O., Thober, S., and Attinger, S.: Strong hydroclimatic controls on vulnerability to subsurface nitrate contamination across Europe, Nature Communications, 11, 10.1038/s41467-020-19955-8, 2020.

Kumar, S., Herrmann, M., Thamdrup, B., Schwab, V. F., Geesink, P., Trumbore, S. E., Totsche, K.-U., and Küsel, K.: Nitrogen Loss from Pristine Carbonate-Rock Aquifers of the Hainich Critical Zone Exploratory (Germany) Is Primarily Driven by Chemolithoautotrophic Anammox Processes, Frontiers in Microbiology, 8, 10.3389/fmicb.2017.01951, 2017.

Küsel, K., Totsche, K. U., Trumbore, S. E., Lehmann, R., Steinhäuser, C., and Herrmann, M.: How Deep Can Surface Signals Be Traced in the Critical Zone? Merging Biodiversity with Biogeochemistry Research in a Central German Muschelkalk Landscape, Frontiers in Earth Science, 4, 10.3389/feart.2016.00032, 2016.

Kuzyakov, Y., and Blagodatskaya, E.: Microbial hotspots and hot moments in soil: Concept & review, Soil Biology and Biochemistry, 83, 184-199, 10.1016/j.soilbio.2015.01.025, 2015.

Lam, P., and Kuypers, M. M. M.: Microbial nitrogen cycling processes in oxygen minimum zones, Annual Review of Marine Science, 3, 317-345, 10.1146/annurev-marine-120709-142814, 2011.

LaRowe, D. E., and Amend, J. P.: Catabolic rates, population sizes and doubling/replacement times of microorganisms in natural settings, American Journal of Science, 315, 167-203, 10.2475/03.2015.01, 2015a.

LaRowe, D. E., and Amend, J. P.: Power limits for microbial life, Front Microbiol, 6, 718, 10.3389/fmicb.2015.00718, 2015b.

Lehmann, K., Lehmann, R., and Totsche, K. U.: Event-driven dynamics of the total mobile inventory in undisturbed soil account for significant fluxes of particulate organic carbon, Science of the Total Environment, 756, 10.1016/j.scitotenv.2020.143774, 2021.

Lei, T., Pan, Y., Liu, H., Zhan, W., and Yuan, J.: A run off-on-ponding method and models for the transient infiltration capability process of sloped soil surface under rainfall and erosion impacts, Journal of Hydrology, 319, 216-226, 10.1016/j.jhydrol.2005.06.029, 2006.

Leinemann, T., Mikutta, R., Kalbitz, K., Schaarschmidt, F., and Guggenberger, G.: Small scale variability of vertical water and dissolved organic matter fluxes in sandy Cambisol subsoils as revealed by segmented suction plates, Biogeochemistry, 131, 1-15, 10.1007/s10533-016-0259-8, 2016.

Lennon, J. T., and Jones, S. E.: Microbial seed banks: the ecological and evolutionary implications of dormancy, Nat Rev Microbiol, 9, 119-130, 10.1038/nrmicro2504, 2011.

Lever, M. A., Rogers, K. L., Lloyd, K. G., Overmann, J., Schink, B., Thauer, R. K., Hoehler, T. M., and Jorgensen, B. B.: Life under extreme energy limitation: a synthesis of laboratory- and field-based investigations, FEMS Microbiol Rev, 39, 688-728, 10.1093/femsre/fuv020, 2015.

Levy, M., and Berkowitz, B.: Measurement and analysis of non-Fickian dispersion in heterogeneous porous media, Journal of Contaminant Hydrology, 64, 203-226, 10.1016/S0169-7722(02)00204-8, 2003.

Li, B., and Rodell, M.: Evaluation of a model-based groundwater drought indicator in the conterminous U.S, Journal of Hydrology, 526, 78-88, 10.1016/j.jhydrol.2014.09.027, 2015.

Li, B., Pales, A. R., Clifford, H. M., Kupis, S., Hennessy, S., Liang, W. Z., Moysey, S., Powell, B., Finneran, K. T., and Darnault, C. J. G.: Preferential flow in the vadose zone and interface dynamics: Impact of microbial exudates, Journal of Hydrology, 558, 72-89, 2018.

Lin, X., McKinley, J., Resch, C. T., Kaluzny, R., Lauber, C. L., Fredrickson, J., Knight, R., and Konopka, A.: Spatial and temporal dynamics of the microbial community in the Hanford unconfined aquifer, ISME Journal, 6, 1665-1676, 10.1038/ismej.2012.26, 2012.

Liu, W., Birgand, F., Tian, S., and Chen, C.: Event-scale hysteresis metrics to reveal processes and mechanisms controlling constituent export from watersheds: A review☆, Water Research, 200, 10.1016/j.watres.2021.117254, 2021.

Lohmann, P., Benk, S., Gleixner, G., Potthast, K., Michalzik, B., Jehmlich, N., and Bergen, M. V.: Seasonal Patterns of Dominant Microbes Involved in Central Nutrient Cycles in the Subsurface, Microorganisms, 8, 10.3390/microorganisms8111694, 2020.

Lundberg, A., Ala-Aho, P., Eklo, O., Klöve, B., Kværner, J., and Stumpp, C.: Snow and frost: Implications for spatiotemporal infiltration patterns - a review, Hydrological Processes, 30, 1230-1250, 10.1002/hyp.10703, 2016.

Magnabosco, C., Lin, L. H., Dong, H., Bomberg, M., Ghiorse, W., Stan-Lotter, H., Pedersen, K., Kieft, T. L., van Heerden, E., and Onstott, T. C.: The biomass and biodiversity of the continental subsurface, Nature Geoscience, 11, 707-717, 10.1038/s41561-018-0221-6, 2018.

Maher, K., Bargar, J. R., and Brown Jr, G. E.: Environmental speciation of actinides, Inorganic Chemistry, 52, 3510-3532, 10.1021/ic301686d, 2013.

Manzoni, S., and Porporato, A.: Soil carbon and nitrogen mineralization: Theory and models across scales, Soil Biology and Biochemistry, 41, 1355-1379, 10.1016/j.soilbio.2009.02.031, 2009.

Manzoni, S., Schaeffer, S. M., Katul, G., Porporato, A., and Schimel, J. P.: A theoretical analysis of microbial eco-physiological and diffusion limitations to carbon cycling in drying soils, Soil Biology and Biochemistry, 73, 69-83, 10.1016/j.soilbio.2014.02.008, 2014.

Manzoni, S., Moyano, F., Kätterer, T., and Schimel, J.: Modeling coupled enzymatic and solute transport controls on decomposition in drying soils, Soil Biology and Biochemistry, 95, 275-287, 10.1016/j.soilbio.2016.01.006, 2016.

McClain, M. E., Boyer, E. W., Dent, C. L., Gergel, S. E., Grimm, N. B., Groffman, P. M., Hart, S. C., Harvey, J. W., Johnston, C. A., Mayorga, E., McDowell, W. H., and Pinay, G.: Biogeochemical Hot Spots and Hot Moments at the Interface of Terrestrial and Aquatic Ecosystems, Ecosystems, 6, 301-312, 10.1007/s10021-003-0161-9, 2003.

McGuire, J. T., Smith, E. W., Long, D. T., Hyndman, D. W., Haack, S. K., Klug, M. J., and Velbel, M. A.: Temporal variations in parameters reflecting terminal-electron-accepting processes in an aquifer contaminated with waste fuel and chlorinated solvents, Chemical Geology, 169, 471-485, 10.1016/S0009-2541(00)00223-0, 2000.

McGuire, K. J., DeWalle, D. R., and Gburek, W. J.: Evaluation of mean residence time in subsurface waters using oxygen-18 fluctuations during drought conditions in the mid-Appalachians, Journal of Hydrology, 261, 132-149, 10.1016/S0022-1694(02)00006-9, 2002.

McIntosh, J., McDonnell, J. J., and Peters, N. E.: Tracer and hydrometric study of preferential flow in large undisturbed soil cores from the Georgia Piedmont, USA, Hydrological Processes, 13, 139-155, 10.1002/(SICI)1099-1085(19990215)13:2<139::AID-HYP703>3.0.CO;2-E, 1999.

McMahon, S., and Parnell, J.: Weighing the deep continental biosphere, FEMS Microbiol Ecol, 87, 113-120, 10.1111/1574-6941.12196, 2014.

McMillan, H. K., and Srinivasan, M. S.: Characteristics and controls of variability in soil moisture and groundwater in a headwater catchment, Hydrology and Earth System Sciences, 19, 1767-1786, 10.5194/hess-19-1767-2015, 2015.

Michaelis, L., and Menten, M. L.: Die kinetik der invertinwirkung, Biochem. z, 49, 352, 1913.

Molins, S., Trebotich, D., Yang, L., Ajo-Franklin, J. B., Ligocki, T. J., Shen, C., and Steefel, C. I.: Pore-scale controls on calcite dissolution rates from flow-through laboratory and numerical experiments, Environ Sci Technol, 48, 7453-7460, 10.1021/es5013438, 2014.

Moore, D. S., Notz., W. I., and Flinger, M. A.: The basic practice of statistics (6th ed.), W. H. Freeman and Company, New York, NY, 2013.

Morales, V. L., Parlange, J. Y., and Steenhuis, T. S.: Are preferential flow paths perpetuated by microbial activity in the soil matrix? A review, Journal of Hydrology, 393, 29-36, 10.1016/j.jhydrol.2009.12.048, 2010.

Müller, S., and Schüler, L.: GeoStat-Framework/GSTools: Bouncy Blue (Version v1.0.1). Zenodo, http://doi.org/10.5281/zenodo.2543658, 2019.

Müller, S.: GeoStat-Framework/ogs5py: v1.1.1 (Version v1.1.1). Zenodo, 2020.

Muniruzzaman, M., and Pedretti, D.: Mechanistic models supporting uncertainty quantification of water quality predictions in heterogeneous mining waste rocks: a review, Stochastic Environmental Research and Risk Assessment, 35, 985-1001, 10.1007/s00477-020-01884-z, 2021.

Murphy, E. M., Ginn, T. R., Chilakapati, A., Resch, C. T., Phillips, J. L., Wietsma, T. W., and Spadoni, C. M.: The influence of physical heterogeneity on microbial degradation and distribution in porous media, Water Resources Research, 33, 1087-1103, 10.1029/96wr03851, 1997.

Nippgen, F., McGlynn, B. L., and Emanuel, R. E.: The spatial and temporal evolution of contributing areas, Water Resources Research, 51, 4550-4573, 10.1002/2014WR016719, 2015.

Nissan, A., and Berkowitz, B.: Reactive Transport in Heterogeneous Porous Media Under Different Péclet Numbers, Water Resources Research, 55, 10119-10129, 10.1029/2019WR025585, 2019.

Oki, T., and Kanae, S.: Global hydrological cycles and world water resources, Science, 313, 1068-1072, 10.1126/science.1128845, 2006.

Okkonen, J., Jyrkama, M., and Kløve, B.: A conceptual approach for assessing the impact of climate change on groundwater and related surface waters in cold regions (Finland), Hydrogeology Journal, 18, 429-439, 10.1007/s10040-009-0529-9, 2010.

Oldham, C. E., Farrow, D. E., and Peiffer, S.: A generalized Damköhler number for classifying material processing in hydrological systems, Hydrology and Earth System Sciences, 17, 1133-1148, 10.5194/hess-17-1133-2013, 2013.

Opitz, S., Kusel, K., Spott, O., Totsche, K. U., and Herrmann, M.: Oxygen availability and distance to surface environments determine community composition and abundance of ammonia-oxidizing prokaroytes in two superimposed pristine limestone aquifers in the Hainich region, Germany, FEMS Microbiol Ecol, 90, 39-53, 10.1111/1574-6941.12370, 2014a.

Opitz, S., Küsel, K., Spott, O., Totsche, K. U., and Herrmann, M.: Oxygen availability and distance to surface environments determine community composition and abundance of ammonia-oxidizing prokaroytes in two superimposed pristine limestone aquifers in the Hainich region, Germany, FEMS Microbiology Ecology, 90, 39-53, 10.1111/1574-6941.12370, 2014b.

Or, D., Smets, B. F., Wraith, J. M., Dechesne, A., and Friedman, S. P.: Physical constraints affecting bacterial habitats and activity in unsaturated porous media - a review, Advances in Water Resources, 30, 1505-1527, 10.1016/j.advwatres.2006.05.025, 2007.

Pace, N. R.: A molecular view of microbial diversity and the biosphere, Science, 276, 734-740, 10.1126/science.276.5313.734, 1997.

Painter, S. L.: Multiscale Framework for Modeling Multicomponent Reactive Transport in Stream Corridors, Water Resources Research, 54, 7216-7230, 10.1029/2018WR022831, 2018.

Pallud, C., Dechesne, A., Gaudet, J. P., Debouzie, D., and Grundmann, G. L.: Modification of spatial distribution of 2,4-dichlorophenoxyacetic acid degrader microhabitats during growth in soil columns, Applied and Environmental Microbiology, 70, 2709-2716, 10.1128/AEM.70.5.2709-2716.2004, 2004.

Pallud, C., and Van Cappellen, P.: Kinetics of microbial sulfate reduction in estuarine sediments, Geochimica et Cosmochimica Acta, 70, 1148-1162, 10.1016/j.gca.2005.11.002, 2006.

Parkin, T. B.: SOIL MICROSITES AS A SOURCE OF DENITRIFICATION VARIABILITY, Soil Science Society of America Journal, 51, 1194-1199, 10.2136/sssaj1987.03615995005100050019x, 1987.

Penna, D., Borga, M., Norbiato, D., and Dalla Fontana, G.: Hillslope scale soil moisture variability in a steep alpine terrain, Journal of Hydrology, 364, 311-327, 10.1016/j.jhydrol.2008.11.009, 2009.

Pett-Ridge, J., Petersen, D. G., Nuccio, E., and Firestone, M. K.: Influence of oxic/anoxic fluctuations on ammonia oxidizers and nitrification potential in a wet tropical soil, FEMS Microbiology Ecology, 85, 179-194, 10.1111/1574-6941.12111, 2013.

Pfeffer, J., Boucher, M., Hinderer, J., Favreau, G., Boy, J. P., De Linage, C., Cappelaere, B., Luck, B., Oi, M., and Le Moigne, N.: Local and global hydrological contributions to time-variable gravity in Southwest Niger, Geophysical Journal International, 184, 661-672, 10.1111/j.1365-246X.2010.04894.x, 2011.

Pholkern, K., Saraphirom, P., and Srisuk, K.: Potential impact of climate change on groundwater resources in the Central Huai Luang Basin, Northeast Thailand, Science of the Total Environment, 633, 1518-1535, 10.1016/j.scitotenv.2018.03.300, 2018.

Pittroff, M., Frei, S., and Gilfedder, B. S.: Quantifying nitrate and oxygen reduction rates in the hyporheic zone using 222Rn to upscale biogeochemical turnover in rivers, Water Resources Research, 53, 563-579, https://doi.org/10.1002/2016WR018917, 2017.

Pivetz, B. E., and Steenhuis, T. S.: Soil Matrix and Macropore Biodegradation of 2,4-D, Journal of Environmental Quality, 24, 564-570, https://doi.org/10.2134/jeq1995.00472425002400040002x, 1995.

Pohl, S., Marsh, P., and Liston, G. E.: Spatial-temporal variability in turbulent fluxes during spring snowmelt, Arctic, Antarctic, and Alpine Research, 38, 136-146, 10.1657/1523-0430(2006)038[0136:SVITFD]2.0.CO;2, 2006.

Pronk, G. J., Mellage, A., Milojevic, T., Smeaton, C. M., Engel, K., Neufeld, J. D., Rezanezhad, F., and Van Cappellen, P.: Carbon turnover and microbial activity in an artificial soil under imposed cyclic drainage and imbibition, Vadose Zone Journal, 19, 10.1002/vzj2.20021, 2020.

Rascón-Ramos, A. E., Martínez-Salvador, M., Sosa-Pérez, G., Villarreal-Guerrero, F., Pinedo-Alvarez, A., Santellano-Estrada, E., and Corrales-Lerma, R.: Soil moisture dynamics in response to precipitation and thinning in a semi-dry forest in northern Mexico, Water (Switzerland), 13, 10.3390/w13010105, 2021.

Rasiah, V., Armour, J. D., and Cogle, A. L.: Statistical characterization of impact of system variables on temporal dynamics of groundwater in highly weathered regoliths, Hydrological Processes, 21, 2435-2446, 10.1002/hyp.6404, 2007.

Raynaud, X., and Nunan, N.: Spatial ecology of bacteria at the microscale in soil, PLoS ONE, 9, 10.1371/journal.pone.0087217, 2014.

Regnier, P., O'Kane, J. P., Steefel, C. I., and vanderborght, J. P.: Modeling complex multi-component reactive-transport systems: towards a simulation environment based on the concept of a Knowledge Base, Applied Mathematical Modelling, 26, 913-927, 2002.

Rein, A., Bauer, S., Dietrich, P., and Beyer, C.: Influence of temporally variable groundwater flow conditions on point measurements and contaminant mass flux estimations, J Contam Hydrol, 108, 118-133, 10.1016/j.jconhyd.2009.06.005, 2009.

Renck, A., and Lehmann, J.: Rapid water flow and transport of inorganic and organic nitrogen in a highly aggregated tropical soil, Soil Science, 169, 330-341, 10.1097/01.ss.0000128016.00021.3d, 2004.

Rezanezhad, F., Couture, R. M., Kovac, R., O'Connell, D., and Van Cappellen, P.: Water table fluctuations and soil biogeochemistry: An experimental approach using an automated soil column system, Journal of Hydrology, 509, 245-256, 10.1016/j.jhydrol.2013.11.036, 2014.

Rivett, M. O., Buss, S. R., Morgan, P., Smith, J. W. N., and Bemment, C. D.: Nitrate attenuation in groundwater: A review of biogeochemical controlling processes, Water Research, 42, 4215-4232, 10.1016/j.watres.2008.07.020, 2008.

Robinson, D. A., Lebron, I., Kocar, B., Phan, K., Sampson, M., Crook, N., and Fendorf, S.: Time-lapse geophysical imaging of soil moisture dynamics in tropical deltaic soils: An aid to interpreting hydrological and geochemical processes, Water Resources Research, 46, 10.1029/2008WR006984, 2009.

Rodrigo, J., Capilla, J. E., and Gómez-Hernández, J. J.: The impact of random function model assumption for the identification fo hydraulic conductivity fields in fractured media: Gaussian and non-Gaussian approaches, Acta Universitatis Carolinae, Geologica, 46, 540-543, 2002.

Rodríguez-Escales, P., Folch, A., van Breukelen, B. M., Vidal-Gavilan, G., and Sanchez-Vila, X.: Modeling long term Enhanced in situ Biodenitrification and induced heterogeneity in column experiments under different feeding strategies, Journal of Hydrology, 538, 127-137, 10.1016/j.jhydrol.2016.04.012, 2016.

Rodriguez-Iturbe, I., Porporato, A., Rldolfi, L., Isham, V., and Cox, D. R.: Probabilistic modelling of water balance at a point: The role of climate, soil and vegetation, Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences, 455, 3789-3805, 10.1098/rspa.1999.0477, 1999.

Saalfield, S. L., and Bostick, B. C.: Changes in iron, sulfur, and arsenic speciation associated with bacterial sulfate reduction in ferrihydrite-rich systems, Environmental Science and Technology, 43, 8787-8793, 10.1021/es901651k, 2009.

Saberi, L., Crystal Ng, G. H., Nelson, L., Zhi, W., Li, L., La Frenierre, J., and Johnstone, M.: Spatiotemporal Drivers of Hydrochemical Variability in a Tropical Glacierized Watershed in the Andes, Water Resources Research, 57, 10.1029/2020WR028722, 2021.

Sanz-Prat, A., Lu, C., Finkel, M., and Cirpka, O. A.: On the validity of travel-time based nonlinear bioreactive transport models in steady-state flow, J Contam Hydrol, 175-176, 26-43, 10.1016/j.jconhyd.2015.02.003, 2015.

Sanz-Prat, A., Lu, C., Finkel, M., and Cirpka, O. A.: Using travel times to simulate multi-dimensional bioreactive transport in time-periodic flows, J Contam Hydrol, 187, 1-17, 10.1016/j.jconhyd.2016.01.005, 2016.

Schäfer, D., Schäfer, W., and Kinzelbach, W.: Simulation of reactive processes related to biodegradation in aquifers. 1. Structure of the three-dimensional reactive transport model, Journal of Contaminant Hydrology, 31, 167-186, 10.1016/S0169-7722(97)00060-0, 1998a.

Schäfer, D., Schäfer, W., and Kinzelbach, W.: Simulation of reactive processes related to biodegradation in aquifers. 2. Model application to a column study on organic carbon degradation, Journal of Contaminant Hydrology, 31, 187-209, 10.1016/S0169-7722(97)00061-2, 1998b.

Schär, C., Lüthi, D., Beyerle, U., and Heise, E.: The soil-precipitation feedback: A process study with a regional climate model, Journal of Climate, 12, 722-741, 10.1175/1520-0442(1999)012<0722:tspfap>2.0.co;2, 1999.

Schimel, J. P., and Weintraub, M. N.: The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: A theoretical model, Soil Biology and Biochemistry, 35, 549-563, 10.1016/S0038-0717(03)00015-4, 2003.

Schincariol, R. A., and Schwartz, F. W.: An experimental investigation of variable density flow and mixing in homogeneous and heterogeneous media, Water Resources Research, 26, 2317-2329, 10.1029/WR026i010p02317, 1990.

Schjønning, P., Thomsen, I. K., Petersen, S. O., Kristensen, K., and Christensen, B. T.: Relating soil microbial activity to water content and tillage-induced differences in soil structure, Geoderma, 163, 256-264, 10.1016/j.geoderma.2011.04.022, 2011.

Schlesinger, W. H., and Andrews, J. A.: Soil respiration and the global carbon cycle, Biogeochemistry, 48, 7-20, 2000.

Schlüter, S., Vogel, H.-J., Ippisch, O., and Vanderborght, J.: Combined Impact of Soil Heterogeneity and Vegetation Type on the Annual Water Balance at the Field Scale, Vadose Zone Journal, 12, 10.2136/vzj2013.03.0053, 2013.

Schwab, V. F., Herrmann, M., Roth, V. N., Gleixner, G., Lehmann, R., Pohnert, G., Trumbore, S., Küsel, K., and Totsche, K. U.: Functional diversity of microbial communities in pristine aquifers inferred by PLFA- and sequencing-based approaches, Biogeosciences, 14, 2697-2714, 10.5194/bg-14-2697-2017, 2017.

Screen, J. A., Deser, C., and Sun, L.: Projected changes in regional climate extremes arising from Arctic sea ice loss, Environ.Res.Lett., 10, 10.1088/1748-9326/10/8/084006, 2015.

Sexstone, A. J., Revsbech, N. P., Parkin, T. B., and Tiedje, J. M.: DIRECT MEASUREMENT OF OXYGEN PROFILES AND DENITRIFICATION RATES IN SOIL AGGREGATES, Soil Science Society of America Journal, 49, 645-651, 10.2136/sssaj1985.03615995004900030024x, 1985.

Seyfried, M. S.: Infiltration patterns from simulated rainfall on a semiarid rangeland soil, Soil Science Society of America Journal, 55, 1726-1734, 10.2136/sssaj1991.03615995005500060037x, 1991.

Smith, H. J., Zelaya, A. J., De León, K. B., Chakraborty, R., Elias, D. A., Hazen, T. C., Arkin, A. P., Cunningham, A. B., and Fields, M. W.: Impact of hydrologic boundaries on microbial planktonic and biofilm communities in shallow terrestrial subsurface environments, FEMS microbiology ecology, 94, 10.1093/femsec/fiy191, 2018.

Sogin, M. L., Morrison, H. G., Huber, J. A., Welch, D. M., Huse, S. M., Neal, P. R., Arrieta, J. M., and Herndl, G. J.: Microbial diversity in the deep sea and the underexplored "rare biosphere", Proceedings of the National Academy of Sciences of the United States of America, 103, 12115-12120, 10.1073/pnas.0605127103, 2006.

Steefel, C. I., Appelo, C. A. J., Arora, B., Jacques, D., Kalbacher, T., Kolditz, O., Lagneau, V., Lichtner, P. C., Mayer, K. U., Meeussen, J. C. L., Molins, S., Moulton, D., Shao, H., Šimůnek, J., Spycher, N., Yabusaki, S. B., and Yeh, G. T.: Reactive transport codes for subsurface environmental simulation, Computational Geosciences, 19, 445-478, 10.1007/s10596-014-9443-x, 2014.

Stegen, J. C., Lin, X., Konopka, A. E., and Fredrickson, J. K.: Stochastic and deterministic assembly processes in subsurface microbial communities, ISME Journal, 6, 1653-1664, 10.1038/ismej.2012.22, 2012.

Stegen, J. C., Fredrickson, J. K., Wilkins, M. J., Konopka, A. E., Nelson, W. C., Arntzen, E. V., Chrisler, W. B., Chu, R. K., Danczak, R. E., Fansler, S. J., Kennedy, D. W., Resch, C. T., and Tfaily, M.: Groundwater-surface water mixing shifts ecological assembly processes and stimulates organic carbon turnover, Nature Communications, 7, 10.1038/ncomms11237, 2016.

Stolpovsky, K., Martinez-Lavanchy, P., Heipieper, H. J., Van Cappellen, P., and Thullner, M.: Incorporating dormancy in dynamic microbial community models, Ecological Modelling, 222, 3092-3102, 10.1016/j.ecolmodel.2011.07.006, 2011.

Sugiyama, A., Masuda, S., Nagaosa, K., Tsujimura, M., and Kato, K.: Tracking the direct impact of rainfall on groundwater at Mt. Fuji by multiple analyses including microbial DNA, Biogeosciences, 15, 721-732, 10.5194/bg-15-721-2018, 2018.

Suk, H., Chen, J.-S., Park, E., and Kihm, Y. H.: Practical Application of the Galerkin Finite Element Method with a Mass Conservation Scheme under Dirichlet Boundary Conditions to Solve Groundwater Problems, Sustainability, 12, 10.3390/su12145627, 2020.

Sun, F., Watanabe, N., and Delfs, J.-O.: Groundwater Flow, in: Thermo-Hydro-Mechanical-Chemical Processes in Porous Media: Benchmarks and Examples, edited by: Kolditz, O., Görke, U.-J., Shao, H., and Wang, W., Springer Berlin Heidelberg, Berlin, Heidelberg, 107-123, 2012.

Thullner, M., Van Cappellen, P., and Regnier, P.: Modeling the impact of microbial activity on redox dynamics in porous media, Geochimica et Cosmochimica Acta, 69, 5005-5019, 10.1016/j.gca.2005.04.026, 2005.

Thullner, M., Regnier, P., and Van Cappellen, P.: Modeling Microbially Induced Carbon Degradation in Redox-Stratified Subsurface Environments: Concepts and Open Questions, Geomicrobiology Journal, 24, 139-155, 10.1080/01490450701459275, 2007.

Thullner, M., and Regnier, P.: Microbial Controls on the Biogeochemical Dynamics in the Subsurface, Reviews in Mineralogy and Geochemistry, 85, 265-302, 10.2138/rmg.2019.85.9, 2019.

Trenberth, K. E., Dai, A., Rasmussen, R. M., and Parsons, D. B.: The changing character of precipitation, Bulletin of the American Meteorological Society, 84, 1205-1217+1161, 10.1175/BAMS-84-9-1205, 2003.

Trenberth, K. E.: Changes in precipitation with climate change, Climate Research, 47, 123-138, 10.3354/cr00953, 2011.

Uhlenbrook, S., Frey, M., Leibundgut, C., and Maloszewski, P.: Hydrograph separations in a mesoscale mountainous basin at event and seasonal timescales, Water Resources Research, 38, 31-31-31-14, 10.1029/2001wr000938, 2002.

Valett, H. M., Morrice, J. A., Dahm, C. N., and Campana, M. E.: Parent lithology, surface-groundwater exchange, and nitrate retention in headwater streams, Limnology and Oceanography, 41, 333-345, 10.4319/lo.1996.41.2.0333, 1996.

Van Der Hoven, S. J., Solomon, D. K., and Moline, G. R.: Natural spatial and temporal variations in groundwater chemistry in fractured, sedimentary rocks: Scale and implications for solute transport, Applied Geochemistry, 20, 861-873, 10.1016/j.apgeochem.2004.11.013, 2005.

Van Gaelen, N., Verheyen, D., Ronchi, B., Struyf, E., Govers, G., Vanderborght, J., and Diels, J.: Identifying the transport pathways of dissolved organic carbon in contrasting catchments, Vadose Zone Journal, 13, 10.2136/vzj2013.11.0199, 2014.

van Leeuwen, F. X. R.: Safe Drinking Water: the Toxicologist's Approach, Food and Chemical Toxicology, 38, S51-S58, https://doi.org/10.1016/S0278-6915(99)00140-4, 2000.

van Rossum, G., and Drake, F. L., Jr.: The Python Language Reference Manual (version 3.2), edited by: Drake, F. L., Jr., Network Theory Ltd, 120 pp., 2006.

Veach, A. M., and Zeglin, L. H.: Historical Drought Affects Microbial Population Dynamics and Activity During Soil Drying and Re-Wet, Microbial Ecology, 79, 662-674, 10.1007/s00248-019-01432-5, 2020.

Vergnes, J. P., Decharme, B., Alkama, R., Martin, E., Habets, F., and Douville, H.: A simple groundwater scheme for hydrological and climate applications: Description and offline evaluation over France, Journal of Hydrometeorology, 13, 1149-1171, 10.1175/JHM-D-11-0149.1, 2012.

Virtanen, P., Gommers, R., Oliphant, T. E., Haberland, M., Reddy, T., Cournapeau, D., Burovski, E., Peterson, P., Weckesser, W., Bright, J., van der Walt, S. J., Brett, M., Wilson, J., Millman, K. J., Mayorov, N., Nelson, A. R. J., Jones, E., Kern, R., Larson, E., Carey, C. J., Polat, İ., Feng, Y., Moore, E. W., VanderPlas, J., Laxalde, D., Perktold, J., Cimrman, R., Henriksen, I., Quintero, E. A., Harris, C. R., Archibald, A. M., Ribeiro, A. H., Pedregosa, F., van Mulbregt, P., and Contributors, S.: SciPy 1.0: Fundamental Algorithms for Scientific Computing in Python, Nature Methods, 17, 261-272, 10.1038/s41592-019-0686-2, 2020.

Vogel, L. E., Pot, V., Makowski, D., Garnier, P., and Baveye, P. C.: To what extent do uncertainty and sensitivity analyses help unravel the influence of microscale physical and biological drivers in soil carbon dynamics models?, Ecological Modelling, 383, 10-22, 10.1016/j.ecolmodel.2018.05.007, 2018.

Vrede, K., Heldal, M., Norland, S., and Bratbak, G.: Elemental composition (C, N, P) and cell volume of exponentially growing and nutrient-limited bacterioplankton, Applied and Environmental Microbiology, 68, 2965-2971, 10.1128/AEM.68.6.2965-2971.2002, 2002.

Waldron, P. J., Wu, L., Van Nostrand, J. D., Schadt, C. W., He, Z., Watson, D. B., Jardine, P. M., Palumbo, A. V., Hazen, T. C., and Zhou, J.: Functional gene array-based analysis of microbial community structure in groundwaters with a gradient of contaminant levels, Environmental Science and Technology, 43, 3529-3534, 10.1021/es803423p, 2009.

Wang, X. P., Cui, Y., Pan, Y. X., Li, X. R., Yu, Z., and Young, M. H.: Effects of rainfall characteristics on infiltration and redistribution patterns in revegetation-stabilized desert ecosystems, Journal of Hydrology, 358, 134-143, 10.1016/j.jhydrol.2008.06.002, 2008.

Wang, Y., and Van Cappellen, P.: A multicomponent reactive transport model of early diagenesis: Application to redox cycling in coastal marine sediments, Geochemica et Cosmochimica Acta, 60, 2993-3014, 1996.

Waring, B. G., Sulman, B. N., Reed, S., Smith, A. P., Averill, C., Creamer, C. A., Cusack, D. F., Hall, S. J., Jastrow, J. D., Jilling, A., Kemner, K. M., Kleber, M., Liu, X. J. A., Pett-Ridge, J., and Schulz, M.: From pools to flow: The PROMISE framework for new insights on soil carbon cycling in a changing world, Global Change Biology, 26, 6631-6643, 10.1111/gcb.15365, 2020.

Webb, E. K., and Andersen, M. P.: Simulation of preferential flow in three-dimensional, heterogeneous conductivity fields with realistic internal architecture, Water Resources Research, 32, 533-545, 10.1029/95WR03399, 1996.

Wetherald, R. T., and Manabe, S.: Detectability of summer dryness caused by greenhouse warming, Climatic Change, 43, 495-511, 10.1023/A:1005499220385, 1999.

Xue, J., and Gavin, K.: Effect of rainfall intensity on infiltration into partly saturated slopes, Geotechnical and Geological Engineering, 26, 199-209, 10.1007/s10706-007-9157-0, 2008.

Yabusaki, S. B., Fang, Y., and Waichler, S. R.: Building conceptual models of field-scale uranium reactive transport in a dynamic vadose zone-aquifer-river system, Water Resources Research, 44, 10.1029/2007WR006617, 2008.

Yabusaki, S. B., Wilkins, M. J., Fang, Y., Williams, K. H., Arora, B., Bargar, J., Beller, H. R., Bouskill, N. J., Brodie, E. L., Christensen, J. N., Conrad, M. E., Danczak, R. E., King, E., Soltanian, M., R., Spycher, N. F., Steefel, C. I., Tokunaga, T. K., Versteeg, R., Waichler, S.

T., and Wainwright, H. M.: Water Table Dynamics and Biogeochemical Cycling in a Shallow, Variably-Saturated Floodplain, Environmental Science & Technology, 51, 3307-3317, 10.1021/acs.est.6b04873, 2017.

Yan, L., Hermans, S. M., Totsche, K. U., Lehmann, R., Herrmann, M., and Küsel, K.: Groundwater bacterial communities evolve over time in response to recharge, Water Research, 201, 10.1016/j.watres.2021.117290, 2021.

Zektser, S., Loáiciga, H. A., and Wolf, J. T.: Environmental impacts of groundwater overdraft: Selected case studies in the southwestern United States, Environmental Geology, 47, 396-404, 10.1007/s00254-004-1164-3, 2005.

Zhang, Q., Lei, H. M., and Yang, D. W.: Seasonal variations in soil respiration, heterotrophic respiration and autotrophic respiration of a wheat and maize rotation cropland in the North China Plain, Agricultural and Forest Meteorology, 180, 34-43, 10.1016/j.agrformet.2013.04.028, 2013.

Zhang, S., Ho, Y. F., Creeley, D., Roberts, K. A., Xu, C., Li, H. P., Schwehr, K. A., Kaplan, D. I., Yeager, C. M., and Santschi, P. H.: Temporal variation of iodine concentration and speciation (127I and 129I) in wetland groundwater from the Savannah River Site, USA, Environmental Science and Technology, 48, 11218-11226, 10.1021/es502003q, 2014.

Zheng, T., Deng, Y., Wang, Y., Jiang, H., O'Loughlin, E. J., Flynn, T. M., Gan, Y., and Ma, T.: Seasonal microbial variation accounts for arsenic dynamics in shallow alluvial aquifer systems, Journal of Hazardous Materials, 367, 109-119, 10.1016/j.jhazmat.2018.12.087, 2019.

Zhou, Y., Kellermann, C., and Griebler, C.: Spatio-temporal patterns of microbial communities in a hydrologically dynamic pristine aquifer, FEMS Microbiology Ecology, 81, 230-242, 10.1111/j.1574-6941.2012.01371.x, 2012.

Zimmerman, D. A., De Marsily, G., Gotway, C. A., Marietta, M. G., Axness, C. L., Beauheim, R. L., Bras, R. L., Carrera, J., Dagan, G., Davies, P. B., Gallegos, D. P., Galli, A., Gómez-Hernández, J., Grindrod, P., Gutjahr, A. L., Kitanidis, P. K., Lavenue, A. M., McLaughlin, D., Neuman, S. P., RamaRao, B. S., Ravenne, C., and Rubin, Y.: A comparison of seven geostatistically based inverse approaches to estimate transmissivities for modeling advective transport by groundwater flow, Water Resources Research, 34, 1373-1413, 10.1029/98WR00003, 1998.

Zwiers, F. W., and Kharin, V. V.: Changes in the extremes of the climate simulated by CGC GCM2 under CO 2 doubling, Journal of Climate, 11, 2200-2222, 10.1175/1520-0442(1998)011<2200:CITEOT>2.0.CO;2, 1998.