UNIVERSITÉ DU QUÉBEC À MONTRÉAL

# LA SUCCESSION PRIMAIRE DES COMMUNAUTÉS MICROBIENNES SESSILES ET PLANCTONIQUES DANS UN AQUIFÈRE DE LA FORMATION DU COVEY HILL AU QUÉBEC

MÉMOIRE

PRÉSENTÉ

COMME EXIGENCE PARTIELLE

AU PROGRAMME DE MAITRISE EN BIOLOGIE

PAR

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Avertissement

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**TABLE DES MATIÈRES**

**LISTE DES FIGURES**

**LISTE DES TABLEAUX**

**LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES**

| | |
|---|---|
| ADN | Acide désoxyribonucléique |
| ARN | Acide ribonucléique |
| ANOVA | Analysis of variance |
| ASV | Amplicon Sequence Variant |
| B1, B2, B3 | Bioréacteur 1, 2 et 3 |
| CERMO – FC | Centre d'Excellence en Recherche sur les Maladies Orphelines – Fondation Courtois |
| DIC | Dissolved Inorganic Carbon |
| DOC | Dissolved Organic Carbon |
| E1, E2 | Expérience 1, Expérience 2 |
| GRIL | Groupe de recherche interuniversitaire en Limnologie |
| NCBI | National Center for Biotechnology Information |
| NMDS | Non-Metric Multidimensional Scaling |
| PCR | Polymerase Chain Reaction |
| VarPart | Variance Partitioning |

**Résumé**

La subsurface et les aquifères qui y sont présents jouent plusieurs rôles essentiels dans nos vies. Que ce soit comme source d'eau potable ou d'hydrocarbures ou pour éventuellement pouvoir y entreposer du carbone pour lutter contre les changements climatiques, notre société ne peut s'en passer. Il est aussi maintenant reconnu qu'une multitude de bactéries, d'archées et d'eucaryotes interagissant avec presque tout ce qui compose la subsurface colonisent cet environnement. Suite à une perturbation permettant la colonisation d'un milieu dénué de vie, la séquence dans laquelle ces microorganismes se succéderont et l'influence que le carbone organique et inorganique dissouts auront sur cette colonisation sont toutefois encore très peu connus, particulièrement dans les premiers jours suivant cette perturbation. Nous avons donc reproduit un aquifère de la formation géologique du Covey Hill en laboratoire et avons étudié les 24 premiers jours de la succession d'un environnement stérile chez les bactéries et les eucaryotes. Nous avons observé une succession en deux phases chez toutes les communautés observées (bactéries et eucaryotes sessiles et planctoniques). La première phase, durant quelques jours, a vu une grande diversité de microorganismes s'installant dans le nouvel environnement, particulièrement dans les communautés planctoniques. Suite à cette première phase, il y a probablement l'établissement d'un biofilm dans la communauté bactérienne sessile et les changements deviennent soudainement beaucoup plus lents. La croissance des microorganismes semble généralement avoir été limitée par divers nutriments tel que l'azote, mais l'effet du carbone organique sur la succession, qui a seulement pu être étudié pour les communautés sessiles, a été beaucoup plus faible que prévu. Le carbone organique semble avoir été présent en quantité suffisamment grandes pour que, peu importe les fluctuations, il y en avait toujours une quantité suffisante pour les besoins de la communauté. Il n'a donc pas eu d'effet significatif. En ce qui a trait au carbone inorganique, il a eu comme unique effet, à grandes concentrations, d'augmenter la diversité alpha des bactéries planctoniques. Ce résultat inattendu est probablement expliqué par le fait que le carbone inorganique a une grande influence sur le pH d'un milieu. Ce pH plus bas aurait donc nuit aux taxa dominants et permis à une plus grande diversité de microorganismes de se développer. De plus, les communautés eucaryotes étaient majoritairement composées de protiste vraisemblablement mixotrophes. Ces microorganismes occupaient une place beaucoup plus importante dans les communautés que les hétérotrophes et ce, malgré l'abondance de carbone organique. Finalement, mis-à-part de la communauté eucaryote sessile, les communautés semblaient très bien isolées les unes des autres après les quelques premiers jours chaotiques; la communauté des jours précédent expliquait presqu'entièrement la communauté d'un jour donné.

Mots clés : Subsurface continentale, communautés microbiennes, microbes sessiles, succession microbienne, génomique

**Abstract**

The subsurface and the aquifers contained within play multiple crucial roles in our lives. Whether it be for drinking water, hydrocarbon extraction or, eventually, for carbon storage to fight climate change, our society could not live without these resources. It also is now widely accepted that a multitude of bacteria, eucaryotes and archaea interacting with almost everything that comprises the subsurface colonize this environment. These communities' behavior in the first few days following a perturbation eliminating all life of an environment and the influence organic and inorganic carbon will have on that succession are, however, still severely understudied. To help further our knowledge of this behavior, we reproduced an aquifer of the Covey Hill geological formation in a laboratory setting and studied the first 24 days of colonization of a sterile environment for bacteria and eukaryotes. We observed a succession taking place in 2 phases in all studied communities (sessile and planktonic bacteria and eucaryotes). The first phase, lasting only a few days, saw important changes as a great diversity of microorganisms installed themselves in the pristine environment. The diversity was especially high in the planktonic communities. Following this initial colonization and, most likely, the creation of a biofilm in the sessile communities, the changes suddenly became much slower. The changes in the communities seems to generally have been limited by nutrients such as nitrogen, but the effect of organic carbon, that could only be studied on sessile communities, was much weaker than expected; it likely always was present in high enough concentration that, no matter the fluctuations, there always was enough of it to meet the communities' needs so there were no significant effects on the communities. Regarding inorganic carbon, it increased the alpha diversity of the planktonic bacteria when in high concentration. We believe this is explained by inorganic carbon lowering an environment's pH. This lower pH would therefore have inhibited the growth of the dominant taxa and allowed greater diversity to develop. Regarding the eukaryotes, they were mostly likely mixotrophic protists that occupied a much more important place in the community than the heterotrophs despite the abundance of organic carbon. Finally, apart from the sessile eucaryotes, the communities seemed to be mostly insulated from one another following the first few chaotic days; the communities from the previous days explained almost entirely the composition of a specific day.

Keywords: continental subsurface, microbial communities, sessile microbes, microbial succession, genomic

# 0. Introduction

## 0.1 Les habitats de la subsurface

La subsurface est un milieu complexe situé sous la surface du sol et des océans et est composée, entre autres choses, de sédiments, de matière organique et inorganique, de gaz dissous et d'eau souterraine (Smith *et al.*, 2018). L'eau présente dans cet environnement provient, entre autres, de l'infiltration d'eau de surface lors d'évènements de recharge tel que la fonte des neiges ou des précipitations (Yan *et al.*, 2020). Une fois rendue dans la subsurface, elle peut circuler dans les pores des roches, les failles, ou entre les particules sédimentaires pour s'accumuler et former des étendues d'eau, c'est-à-dire des aquifères (Falkenmark *et al.*, 2003). L'eau souterraine qui circule dans ces aquifères représente une grande proportion de l'eau potable accessible sur terre (Kundzewicz et Döli, 2009) et est également une source importante d'eau pour l'irrigation, l'agriculture et l'industrie (Falkenmark *et al.*, 2003; Smith *et al.*, 2018). Ces aquifères hébergent aussi une multitude de microorganismes vivant sous une de deux formes. Ils sont soit fixés sur des surfaces (roches ou particules), c'est-à-dire sessiles, ou flottants librement dans l'eau souterraine, c'est-à-dire planctoniques (Marshall, 2013). Les dynamiques de ces communautés sont, à ce jour, peu connues (Sharma *et al.*, 2024). Cet amalgame complexe de microorganismes et de divers facteurs géochimiques entre fréquemment en interaction avec diverses activités anthropiques souterraines telles que l'entreposage de déchets nucléaires (Fredrickson et Balkwill, 2006) ou l'exploration et l'exploitation des hydrocarbures (Hull *et al.*, 2018). Par exemple, dans le cas de la fracturation hydraulique, soit l'injection sous pression de liquide de fracturation dans la subsurface pour créer ou élargir des fissures permettant de libérer des gisements gaziers, le développement de communautés microbiennes introduites ou déplacées par ce liquide peut entraîner divers problèmes, comme la transformation des hydrocarbures (Vick *et al.*, 2019), leur dégradation durant l'extraction, l'accélération de la corrosion de l'équipement utilisé pour l'extraction, ou encore l'obstruction de divers conduits de cet équipement (Hull *et al.*, 2018).

Les aquifères sont également étudiés dans le but de les utiliser pour la séquestration du carbone (Emerson *et al.*, 2016) et de l'hydrogène (Jin et Sengupta, 2024) dans le contexte de la lutte contre les changements climatiques. Ils sont finalement étudiés dans le but plus global de mieux comprendre les cycles biogéochimiques régissant, entre autres, les cycles du carbone, de l'azote, du soufre, de divers composés métalliques, et de composés organiques ou de contaminants (Thullner et Regnier, 2019). Il est donc crucial de chercher à mieux comprendre comment ces communautés microbiennes influencent ces

divers cycles et les substances présentes dans la subsurface, leur fonctionnement écologique et comment leur composition et leurs fonctions peuvent évoluer au fil du temps.

### 0.2 La diversité et les dynamiques d'assemblage des communautés microbiennes dans les habitats souterrains

Les trois domaines de la vie, c'est-à-dire les bactéries, les archées (les deux procaryotes) et les eucaryotes sont présents dans la subsurface dans les communautés sessile et planctonique (Griebler et Lueders, 2009; Groult *et al.*, 2022). Le mode de vie sessile serait particulièrement important dans les milieux pauvres en carbone et en nutriments. Cela est probablement dû aux surfaces rocheuses offrant une plus grande diversité minérale et de niches que l'environnement dissout (Griebler et Lueders, 2009). L'utilisation du style de vie planctonique serait donc principalement une stratégie de déplacement pour des microorganismes sessiles (Marshall, 2013). Cela pourrait expliquer pourquoi l'abondance de la communauté sessile est de plusieurs ordres de grandeur supérieurs à celle de la communauté planctonique (Griebler et Lueders, 2009; Sharma *et al.*, 2024). Certains microorganismes semblent toutefois préférer le mode de vie planctonique, notamment certaines archées méthanogènes (Lehman *et al.*, 2001).

Certains microorganismes sessiles sont également capables de former des micro-colonies, c'est-à-dire des agrégats multicellulaires pouvant former un biofilm, une substance polymérique extracellulaire conférant une résistance accrue aux stress externes tels que les métaux lourds et la dessiccation (Smith *et al.*, 2018). La formation de ce biofilm est toutefois dépendante de la densité et de l'organisation de la communauté microbienne et, dépendamment des genres microbiens présents dans la communauté, ces besoins préalables à la formation du biofilm peuvent varier de façon complexe (Zhou *et al.*, 2020). Ces biofilms jouent un rôle essentiel dans la formation des communautés sessiles de la subsurface puisqu'en plus de conférer un avantage aux genres bactériens capables d'en former un, les eucaryotes peuvent coexister dans ces biofilms bactériens (Zirnstein *et al.*, 2012) et une substance similaire peut être produite par les archées (van Wolferen *et al.*, 2018). Ces biofilms devraient donc fortement influencer les communautés sessiles de la subsurface et pourraient également expliquer pourquoi la proportion de cellules viables (McMahon et Parnell, 2014) et l'abondance absolue (Dong *et al.*, 2021; Sharma *et al.*, 2024) sont plus élevées chez les populations sessiles que chez les populations planctoniques. Les archées semblent toutefois avoir une tendance inverse aux deux autres domaines puisqu'elles ont une plus grande abondance et une plus grande diversité sous forme planctonique que sessile (Hug *et al.*, 2015; Patel *et al.*, 2024).

L'ordre dans lequel les espèces ou les communautés de microorganismes s'installent (leur succession) et la fréquence et l'intensité du changement de ces communautés sont toujours très mal comprises. Particulièrement les changements à court terme, et ce, tout autant dans un environnement artificiel, comme le liquide de forage (Hull *et al.*, 2018), que dans un environnement plus naturel, comme un aquifère (Sharma *et al.*, 2024).

La succession primaire des microorganismes correspond à la colonisation d'un environnement dénué de vie. Les études sur la succession des communautés microbiennes dans les aquifères sont importantes dans des environnements sains dans le but de comprendre la dynamique de l'assemblage de ces communautés et de futures études pourraient permettre de comprendre la réaction des communautés aux perturbations et aux contaminations pour pouvoir essayer de prédire l'impact des diverses perturbations qui affecteront ces environnements essentiels à nos vies. Des obstacles évidents se posent toutefois à cette expansion de notre compréhension telle que la difficulté d'accès au milieu d'étude. Cela signifie donc que divers aspects de cet environnement sont peu étudiés et peu compris.

**0.3 Influence des facteurs abiotiques sur les communautés sessiles et planctoniques**

Le type de surface sur laquelle les microorganismes sessiles se développent de même que la composition de cette surface contrôleraient de 70 à 90% de la variance phylogénétique (Jones, A. A. et Bennett, 2017), et ce, peu importe les autres pressions environnementales. Certaines tendances dans la composition de la communauté sont effectivement observables à différentes étapes de la succession et en fonction de la chimie du milieu telles que la présence de réducteurs ou d'oxydants du soufre, de Gram positives ou d'acidophiles (Jones, A. A. et Bennett, 2017). La composition chimique du milieu exercerait aussi une influence plus générale sur les communautés. Par exemple, le carbone organique est corrélé positivement avec l'accumulation de biofilm et avec la diversité alpha (Jones, A. A. et Bennett, 2017). Les microorganismes semblent également capables de coloniser sélectivement des surfaces, préférant les surfaces offrant des nutriments bénéfiques, mais évitant les éléments potentiellement toxiques (Casar *et al.*, 2020; Roberts, 2004). Il a aussi été observé que, dans les milieux oligotrophes, de nombreux microorganismes de la subsurface seraient chimiolithotrophes, c'est-à-dire qu'ils dépendraient de minéraux et de carbone inorganique pour soutenir divers processus biogéochimiques (Jones, S. E. et Lennon, 2010). Dans un contexte de colonisation d'un environnement sessile (succession primaire), les microorganismes provenant de la communauté planctonique et colonisant une surface vierge auraient donc un niveau d'activité très limité dans la communauté planctonique, mais, une fois qu'ils s'installent sur des surfaces rocheuses qui leur sont favorables, ils augmenteraient leur activité métabolique et

deviendraient des membres actifs de la communauté sessile (Anantharaman *et al.*, 2016). D'ailleurs, plusieurs de ces organismes présentaient une plasticité phénotypique leur permettant d'utiliser divers donneurs et receveurs d'électrons à différents moments, ce qui leur permettrait d'occuper diverses niches à travers le temps ou en fonction de l'environnement dans lequel ils se retrouvent (Anantharaman *et al.*, 2016; Fierer *et al.*, 2010).

Un autre aspect important à considérer est un principe fondamental de l'écologie microbienne postulant que «tout est partout, mais l'environnement sélectionne» (Baas Becking et Beijerinck, 1934, cités et traduits par De Wit et Bouvier, 2006). Une énorme diversité de microorganismes en dormance ou faiblement actifs métaboliquement serait présente en tout temps dans un écosystème, mais seulement une faible proportion de ces microorganismes se développerait et deviendrait active (De Wit et Bouvier, 2006). En supposant une sensibilité suffisante des appareils de détection utilisés pour analyser ladite communauté, des espèces s'activant seulement très tard dans la succession pourraient donc être détectées tout le long du processus de succession, mais leur abondance ne commencera à varier de façon importante qu'à un moment précis. Cela requiert toutefois des microorganismes d'être capable d'alterner entre trois différents niveaux d'activité distincte en fonctions des conditions environnantes. Tout d'abord, ils peuvent avoir un niveau d'activité permettant la croissance et un développement normal. Ce niveau d'activité est uniquement présent en condition optimale; il est très rarement rencontré en nature et entraîne, entre autres, une utilisation des ressources disponibles, une augmentation de la population et la production de métabolites. Ces métabolites sont potentiellement toxiques ou, dans d'autres situations, peuvent permettre ou influencer la vie d'autres genres microbiens. Lorsque les conditions ne sont plus optimales, les microorganismes peuvent transitionner à un deuxième niveau d'activité. Ce niveau ne permet pas la croissance, mais permet de maintenir à un niveau normal les activités autres que la croissance. Il entraînera également une modification des stratégies de survie. Par exemple, les microorganismes commenceront à produire des protéines permettant de mieux résister aux stress, réduiront leur taille ou produiront des spores. Finalement, lorsque les conditions sont plus difficiles, ce qui est très fréquent dans les milieux tels que la subsurface, les procaryotes peuvent entrer dans un état d'activité extrêmement limité où leur activité métabolique consiste presque uniquement à réparer les dommages causés à leur ADN et où ils sont largement dormants (Price et Sowers, 2004). Ces stratégies, plus fréquentes chez les microorganismes dans les écosystèmes ayant des ressources limitées, peuvent être fréquemment observées chez les taxons procaryotes communs, mais elles peuvent également être observées chez les eucaryotes. Elles permettent aux microorganismes de résister à différents stress tels que la température ou la dessiccation (Jones, S. E. et Lennon, 2010). Cette capacité

qu'ont certains microorganismes d'entrer dans un état de dormance pourrait expliquer diverses tendances écologiques microbiennes telles que les successions saisonnières répétées, la présence d'une biosphère rare et la résistance aux perturbations (Jones, S. E. et Lennon, 2010). Les microorganismes rares sont également plus souvent actifs; ils ont le potentiel d'augmenter rapidement en nombre lorsque les conditions sont propices (Jones, S. E. et Lennon, 2010). Ils constituent souvent une grande partie de la diversité d'une communauté, particulièrement dans les environnements pauvres en ressources, et peuvent être responsables de divers processus métaboliques essentiels à la communauté (Jones, S. E. et Lennon, 2010).

La formation de spores provenant de la communauté sessile et les individus en dormance provenant d'une source, comme les zones de recharge des aquifères (Jones, S. E. et Lennon, 2010) ou d'un autre endroit de l'aquifère contribueraient également à la diversité de la communauté planctonique (Marshall, 2013). Des individus sessiles morts retourneraient également augmenter artificiellement l'abondance de la communauté planctonique (Hazen *et al.*, 1991).

**0.4 Les facteurs influençant la succession des microorganismes de la subsurface**

Il a également longtemps été supposé qu'il y aurait peu de variation temporelle dans les communautés sessiles et planctoniques de la subsurface puisque les fluctuations environnementales seraient peu intenses (peu ou pas de lumière dans la subsurface, peu de variation de température et, dépendamment de la profondeur, peu de carbone organique facilement utilisable issu de la surface) (Yan *et al.*, 2020). Or, il existe diverses variations dans ou entre les communautés telles que des variations temporelles à court terme et saisonnières associées à des changements dans la composition chimique de l'eau et aux évènements de recharge (Yan *et al.*, 2020). La connectivité d'un aquifère avec la surface, le type de roche le composant et sa porosité peuvent également influencer les communautés occupant l'aquifère (Yan *et al.*, 2020). Vu ces facteurs influençant la sortie de leur latence, les microorganismes dominants feraient preuve d'une biogéographie spatiale et temporelle malgré le fait qu'ils soient normalement détectables en très petites quantités dans une grande diversité d'environnements (Hug *et al.*, 2015). Ces facteurs influenceraient donc à la fois la diversité alpha et bêta des écosystèmes (Dong *et al.*, 2021) et dépendraient fortement du degré d'isolation par rapport à la surface (Hubalek *et al.*, 2016).

La source d'énergie principalement utilisée par une communauté influencerait également sa succession. Dépendamment de la chimie du milieu, de la proximité avec la surface et des interactions du milieu étudié avec la surface, trois stratégies d'utilisations de sources de carbone sont effectivement possibles (Fierer *et al.*, 2010). La première possibilité est l'utilisation de lumière ou l'oxydation de

composés inorganique pour produire de l'énergie. Cette stratégie autotrophe serait principalement utilisée lorsque le carbone organique est rare ou inexistant, entraînant des changements de communauté relativement lents. La deuxième option, ayant lieu si le substrat contient suffisamment de carbone organique, est une colonisation initiale se faisant par des microorganismes hétérotrophes endogènes utilisant le carbone organique issu du substrat (les roches composant l'environnement dans lequel les microorganismes se développent) pour faire de la fermentation ou de la respiration. Dans cette situation, la disponibilité du carbone et des divers nutriments utilisés par la communauté variera à travers le temps dû à la présence de microorganismes les utilisant. Finalement, le troisième type de succession peut avoir lieu lorsque les microorganismes ont la possibilité d'utiliser du carbone issu de sources externes au milieu, c'est-à-dire du carbone exogène. Ces microorganismes utiliseraient donc, comme pour le deuxième type de succession, la fermentation ou la respiration comme source principale d'énergie, mais le carbone organique utilisé proviendrait de sources externes plutôt que du substrat. Cela signifierait donc que le substrat ne devrait pas être influencé par la croissance de la communauté et que le développement initial peut être rapide. L'apport de carbone et donc, le développement de la communauté, peut toutefois varier grandement à travers le temps en fonction de facteurs externes à l'habitat tel que les changements des saisons, la fonte des neiges ou les grandes pluies (Fierer *et al.*, 2010).

En plus du type de carbone utilisé, le taxon microbien dominant influence également la succession prenant place dans une communauté (Fierer *et al.*, 2010). En effet, même si les ressources restent les mêmes, si, par exemple, le taxon dominant change d'un taxon bactérien à fongique, les besoins des deux taxons seront différents, ce qui modifiera les nutriments disponibles pour les autres membres de la communauté, influençant le développement de l'ensemble de la communauté (Fierer *et al.*, 2010).

La succession des communautés planctoniques et sessiles semble également différer, tant au niveau de la diversité alpha que bêta même si les deux environnements sont physiquement extrêmement proches l'un de l'autre (Dong *et al.*, 2021).

**0.5 Divergence des résultats observés dans les études menées**

Finalement, il est important de noter les nombreuses différences entre les diverses études de la succession recensées. Effectivement, la méthodologie, la sensibilité, la fréquence et le lieu d'échantillonnage sont tous très variables. Il peut donc être difficile de départager les comportements

spécifiques à un environnement ou une méthodologie et ceux qui sont généralisables à l'ensemble des communautés microbiennes de la subsurface.

Nous n'avons pas non plus pu trouver d'études analysant la succession quotidienne. Or, il a été démontré que des changements importants par rapport à la biomasse et la diversité ont lieu dans les premiers jours (Hull *et al.*, 2018; Sharma *et al.*, 2024). Nous nous attendons donc à observer de grands changements, mais ne savons pas précisément en quoi ils consisteront.

**0.6 Objectifs et hypothèses**

Les objectifs de ce projet de recherche sont donc de caractériser les processus temporels (Objectif 1) impliqués dans et les facteurs géochimiques (Objectif 2) influençant la succession primaire des communautés microbiennes lors de la colonisation de la surface des roches (communautés sessiles) et de l'eau (communautés planctoniques) dans un écosystème d'aquifère au Québec.

**Hypothèse 1**: Étant donné que l'aquifère étudié a précédemment été observé avoir une concentration élevée en carbone organique dissout (Patel *et al.*, 2024), la diversité alpha de la communauté sessile devrait être très élevée initialement alors que les microorganismes colonisent un environnement vierge sans compétition avant de diminuer abruptement alors que la compétition augmente et qu'il y a un début de formation du biofilm. La diversité devrait ensuite augmenter à nouveau alors que le biofilm devient plus hétérogène (Fillinger *et al.*, 2019). La diversité de la communauté planctonique, quant à elle, devrait être plus élevée que la communauté sessile (Sharma *et al.*, 2024), mais moins varier avec le temps, puisque la majorité des microorganismes la composant devrait être en dormance ou faiblement actifs (De Wit et Bouvier, 2006). Similairement, la diversité bêta des différentes communautés sessiles ou planctoniques devrait varier à travers le temps et converger vers une diversité commune (Vick *et al.*, 2019).

**Hypothèse 2**: En ce qui a trait à l'effet du carbone organique et inorganique dissout, puisque, comme mentionné précédemment, la concentration en carbone organique devrait être élevée (Patel *et al.*, 2024), les microorganismes hétérotrophes devraient rapidement prendre le dessus sur les autotrophes et nous devrions assister à une succession de microorganismes hétérotrophes dans les deux communautés. Le carbone organique devrait donc avoir un effet beaucoup plus grand que le carbone inorganique et cet effet devrait être plus grand dans la communauté planctonique puisque les paramètres physico-chimiques semblent avoir une plus grande influence sur celle-ci. La communauté sessile, quant à elle, devrait être plus influencée par l'environnement biotique (Fillinger *et al.*, 2019). Le carbone organique

devrait aussi exercer une plus grande influence sur la diversité alpha de la communauté sessile (Jones, A. A. et Bennett, 2017).

Les résultats de ce projet de recherche seront publiés dans le journal Frontiers in Microbiology et ont été revus par les pairs.

BEAUREGARD-TOUSIGNANT, S & LAZAR, CS. In press. Primary succession of microbial communities in an aquifer from the Covey Hill formation in Quebec, Canada. *Front. Microbiol.*

# 1. Primary succession of communities in an aquifer from the Covey Hill formation, Quebec, Canada

## 1.1 Abstract

Aquifers in the continental subsurface have long been exploited for their resources. However, given the technical difficulties in accessing recurring subsurface samples, their community diversity and temporal dynamics remain not well understood. Here, we investigated the effects of time and organic and inorganic carbon concentration variation on primary succession of microbial communities belonging to the Bacteria and Eukaryota domains colonizing rock surfaces and groundwater from a shallow fractured sandstone aquifer with a very high concentration of organic carbon and low concentration of nitrogen compounds. We attempted to recreate its physicochemical environment in a triplicate bioreactor setup and let the communities grow for 24 days. The sessile and planktonic communities were sampled daily in independent experiments and identified based on their 16S (Bacteria) or 18S (Eukaryote) rRNA genes. Time was the parameter with the strongest correlation both with alpha and beta diversity. The primary succession of all communities seems to have been divided into two temporal phases: in the first phase, approximately the two first days, the variations in community composition and diversity were high. In the second phase, the variation is more progressive and lasted until the end of the experiment. As expected in an aquifer rich in organic carbon, bacteria were mostly heterotrophs, except in the first few days where there were some chemolithotrophs, and eukaryotes were heterotrophs and likely mixotrophs. Unexpectedly, the alpha diversity of the sessile and planktonic communities varied following similar patterns, but the planktonic ones varied with a wider amplitude. Regarding carbon's effect, organic and inorganic carbon concentration variation explained a much smaller proportion of the variation in alpha and beta diversity than expected. We believe this is due to its high concentration throughout the incubation and to the strong limiting effect of other factors such as nitrogen concentration and pH. The communities of both Bacteria and Eukaryotes were more active than expected and their temporal dynamics and interactions should be further investigated in varying carbon, nitrogen and other nutrient concentrations to better understand how different perturbations can affect subsurface communities and, subsequently, us.

## 1.2 Introduction

The continental subsurface provides us with countless services and resources, ranging from hydrocarbons and drinking water (Pearce et al., 2023, Smith et al., 2018) to potentially storing $CO_2$ (Emerson et al., 2016) or $H_2$ (Jin and Sengupta, 2024) to help mitigate climate change. Most if not all of these underground resources are influenced by subsurface microorganisms able to use and transform them (Jewell et al., 2016, Jin and Sengupta, 2024, Pearce et al., 2023). These communities also are essential to multiple biogeochemical cycles (Jewell et al., 2016, Katayama et al., 2023, Kumar et al., 2017) and remove nitrates from contaminated groundwater (Jakus et al., 2021). The impact of subsurface microbial activity on the biogeochemical cycles taking place in the subsurface and how the communities may be affected and react to perturbations taking place there is important, but still poorly understood.

These communities are comprised of all three domains of life (Bacteria, Archaea and, Eukaryote) (Rajala and Bomberg, 2023) and most often originate from surface water leaching into the subsurface in aquifer ecosystems (Yan et al., 2021). Once in the subsurface, these microorganisms can adopt one of two lifestyles: they can either be attached to a surface, in which case they are considered as sessile, or they can be free floating in (ground)water, in which case they are planktonic (Marshall, 2013). These sessile and planktonic communities, likely taxonomically distinct (Flynn et al., 2013), interact with each other (Rajala and Bomberg, 2023) and vary differently through time (Dong et al., 2021, Fillinger et al., 2019, Rajala and Bomberg, 2023) both in their metabolic activities and taxonomic composition (Dong et al., 2021). Predation and competition for resources between eukaryotes and bacteria are also known to take place (Herrmann et al., 2020) but are still poorly understood. While the sessile communities have been shown to be more active and faster growing than their planktonic counterparts (Griebler and Lueders, 2009, Sharma et al., 2024), the planktonic microbes seem to be more diverse (Patel et al., 2024, Sharma et al., 2024, Yan et al., 2020).

A few studies have observed the changes of subsurface community composition throughout the colonization of a sterile environment, also known as primary succession, in pristine aquifers (Sharma et al., 2024) or in flowback water from hydraulic fracturing wells (Hull et al., 2018). However, these studies sampled infrequently, looked at succession on a longer time scale and observed great changes occurring at every sampling point (Sharma et al. sampled after 0, 2, 4, 8 days and later; and Hull et al. sampled after 1, 4, 7 days and later). Some other studies took interest into the influence that outside events such as groundwater recharge had on the succession (Fillinger et al., 2021, Fillinger et al., 2019), but the effect of the time and of the perturbations often ends up being confounded. Most of these studies focused on bacteria and seldom studied the eukaryotic communities despite strong interactions such as predation and competition for resources taking place (Herrmann et al., 2020). In summary, the temporal dynamics of both bacteria and eukaryotes and the dynamics unique to the eukaryotes and the interactions between these two domains still are poorly understood despite being important.

The high variation observed in the early days of colonization may be explained by a multitude of factors including environmental ones. Indeed, the resources made available or used by microorganisms colonizing the environment (Fierer et al., 2010) and the competitive exclusion that may come from the activity of other microorganisms (Lee et al., 2023, Wetherington et al., 2022) could enable and favor different microorganisms to thrive through time. For example, some sessile prokaryotes are able to create biofilms, an extracellular polymer substance increasing the microorganisms' resistance to external

stresses such as desiccation and nutrient deficiencies (Smith et al., 2018). This provides a strong competitive advantage to the microorganisms able to produce and live in it. The biofilm, however, is dependent on the microbial cell density; the community must reach a certain threshold density for the taxa to start forming a biofilm and that threshold varies between different taxa (Zhou et al., 2020). A wide variety of eukaryotes also thrive in the biofilm despite not being able to form one themselves, thus influencing strongly biofilm dynamics (Zirnstein et al., 2012).

While it is generally agreed that the development of all subsurface communities is partly stochastic (Fillinger et al., 2019), some factors relating to the community composition (Sharma et al., 2024) and the water geochemistry seem to play an important role (Fillinger et al., 2019) with parameters such as organic and inorganic carbon concentration and composition (Schwab et al., 2019), organic and inorganic electron donors (Herrmann et al., 2017), nitrogen compounds (Schwab et al., 2016), dissolved oxygen and the distance from the surface (Fillinger et al., 2019) playing a major role in the shaping of the community; potentially exercising more influence than time itself (Yan et al., 2020). The sessile community also seems to be strongly influenced by selection and carbon availability is expected to play an important role in determining the community composition (Rajala and Bomberg, 2023).

If the concentration of organic carbon in an environment is low or nonexistent, it is expected that autotrophs will be the first colonizers. Some chemolithotrophs, can mineralize $CO_2$ and play essential roles in nitrate reduction in oxic and anoxic environments (Kumar et al., 2017, Schwab et al., 2019, Visser et al., 2024). These slow growing early colonizers subsequently are preyed upon by metazoan top predators (Herrmann et al., 2020). Meanwhile, in an environment containing a high concentration of organic carbon, heterotrophs should play a bigger role in the early stages of succession (Fierer et al., 2010) and there is a negative correlation between fungi and chemolithotrophs (Herrmann et al., 2020). The carbon sources of a community and the interactions between prokaryotes and eukaryotes thus plays a crucial role in shaping subsurface communities.

In this study, we assessed the influence of time and dissolved organic and inorganic carbon concentration variation during the primary succession of sessile and planktonic microorganisms in a pristine fractured sandstone aquifer with a high carbon concentration and very low nitrogen concentration. Using amplicon sequencing targeting bacteria and eukaryotes, and a bioreactor setup, we followed succession of sessile microbial communities during the colonization of rock surfaces every 24 hours for 24 days, as well as daily succession of the planktonic microbial communities in the water. We hypothesized that the sessile communities would vary markedly more than the planktonic ones. The

planktonic communities also were expected to be very weakly active and their alpha and beta diversity were not expected to vary much except if sessile microorganisms were to be inserted into the planktonic community. Since the carbon concentration was expected to be high, heterotrophs were expected to dominate the whole incubation, and the initial community growth was expected to be high. This study furthers our still limited understanding of the short-term community dynamics, the effect of different nutrient limitation and the interactions between these communities. When combined with future studies regarding the interactions of the different communities and the influence of varying nutrient concentration and of different contaminants on subsurface communities, it should enable us to better understand how aquifers may influence and respond to perturbations in the subsurface.

### 1.3 Material and methods

#### 1.3.1 Experimental set-up

Given the near-impossible task of collecting subsurface rock samples daily to study microbial succession in an aquifer ecosystem, the *in situ* environment was recreated as closely as possible in a triplicate bioreactor setup (bioreactor 1, B1; bioreactor 2, B2; and bioreactor 3, B3).

Two different experiments were performed. One to examine sessile microbial colonization of rock surfaces from planktonic microbes and a second to investigate planktonic microbial colonization of groundwater from colonized rocks. In the first experiment (*E1*), the collected groundwater was circulated through the bioreactors and into collection bottles in an open system similar to an aquifer habitat where water only flows in one direction. The output water and a rock pellet were collected daily from each triplicate bioreactor for genomic analysis. Since the water was only collected once it had flowed through the bioreactor, the water collection did not disturb the incubation. The second experiment (*E2*) occurred in two phases. The first phase was conducted the same way as for the study of sessile succession, but for 21 days instead of 24 and without collecting any rock pellets to avoid disturbing the sessile community. The second phase then took place where first, the bioreactors were emptied of their water, only leaving the colonized rock pellets in the system. Then, autoclaved, freshly sampled groundwater was circulated for 24 days in the system to observe how the microorganisms that previously colonized the rock pellets would detach and settle in the groundwater. Three pellets from each bioreactor were collected twice: once when the bioreactors were emptied between that first and second phase and once on the last day of the second phase to assess how the community on the rocks had changed over time. It was decided to not collect rock pellets more frequently to avoid disturbing the water succession process as much as possible. A summary of the sampling schedule is presented in Table 1.

**Table 1.1**. Summary of the samples collected and processed for bacterial 16S and eukaryote 18S rRNA gene sequencing, and analyses. B, bioreactor; E, experiment.

| Experiment | Rate of sampling | Bioreactor 1 | Bioreactor 2 | Bioreactor 3 |
|---|---|---|---|---|
| **E1** | Rock and water samples collected each day for 24 days | - 24 rock | - 24 rock | - 24 rock |
| | | - 24 water | - 24 water | - 24 water |
| **E2 Phase 1** | Water emptied from bioreactors after 21 days Rock pellets are left in place | | | |
| **E2 Phase 2** | Water samples collected each day for 24 days | - 24 water | - 24 water | - 24 water |
| | Rock samples (3) collected at Day 1 and Day 24 | - 6 rock | - 6 rock | - 6 rock |

### 1.3.1 Study site and sampling

Groundwater was sampled during the summer of 2022 from a 1.5 m deep well using a capillary pump (Waterra D-25 Foot Valve, Canada) near Covey Hill, in the province of Quebec, Canada (45°00'27.6"N 73°49'10.5"W). The well was first purged to get rid of stagnant water by pumping until the water's physico-chemical parameters (temperature, percentage of dissolved $O_2$, pH and conductivity), measured using a multi-parameter probe (YSI 650 MDS, USA), were stabilized (approximately 30L of purged water). The geochemical and physical properties were measured *in situ* (Table 2). The aquifer is composed of fractured sandstone and has an estimated flow rate of $2 \times 10^{-5}$ to $4 \times 10^{-5}$ m/s (Nastev et al., 2008). Despite limited study, further characterization of the aquifer can be obtained from the literature (Levison et al., 2014, Nastev et al., 2008).

**Table 1.2**. Raw geochemical measurements for all field samples.

| Sample type | Temperature (℃) | pH | Dissolved $O_2$ (%) | DIC (mg/L) | DOC (mg/L) | Conductivity (µS/cm) | NO2 (mg/L) | NO3 (mg/L) | NHx (mg/L) |
|---|---|---|---|---|---|---|---|---|---|
| Peatbog *E1* | 19.04 | 4.04 | 91.2 | | | 22 | | | |
| Groundwater *E1* | 13.55 | 4.73 | 80.9 | 13.41 | 29.21 | 24 | 0.01 | 0.0015 | 0.08 |
| Peatbog *E2* | 20.41 | 3.83 | 61.4 | 11.8 | 39.4 | 17 | | | |
| Groundwater *E2* | 18.34 | 4.42 | 61.6 | 15.6 | 25.85 | 28 | 0.02 | 0.0005 | 0.08 |

Following the purge, 1L and 10L of groundwater were collected in alternance until four 1L sterile polypropylene bottles (Nalgene, Sigma-Aldrich, St. Louis, MO, USA) and three 10L opaque, sterile PYREX low-actinic glass bottles were filled. The 1L bottles enabled us to establish the initial *in situ* groundwater microbial community diversity and composition and the 10L bottles were used for the bioreactor incubations. Four 1L bottles were samples to attest whether community composition of the initial groundwater changed during sampling and would be similar in all three starting bioreactors. Since the geological composition at 1.5 meters is the same for the surface rocks (Girard et al., 2015), we sampled rocks from the surface to be used in the bioreactors to recreate the mineral environment of the aquifer. Because the aquifer is recharged from a nearby peat bog (Levison et al., 2014), 1L of water was also collected from its surface to assess the influence of that recharge site on the aquifer communities. A visual summary of the experimental set-up can be found in Supplementary Material Figure 1.

### 1.3.2 Water geochemical analyses

To determine the dissolved inorganic and organic carbon concentration (DIC and DOC) of the water in the field and during the experiments, samples were taken and filtered on 0.45 µm filters (Sarstedt®, Numbrecht, Germany) into gas-free glass bottles. These bottles were stored at 4°C and analyzed with an OI Analytical Aurora 1030W TOC Analyzer (https://www.oico.com/1030W) using a persulfate oxidation method at the GRIL-Université du Québec à Montréal (UQAM) analytical laboratory.
Regarding nitrogen compound, to determine ammonium and ammonia ($NH_x$) concentrations, water samples were collected in plastic scintillation vials after filtration on a 0.2 µm polyether sulfone filter (Sarstedt®, Numbrecht, Germany). Samples were analyzed on a Flow Solution 3100 autosampler using a chloramine reaction with salicylate to form indophenol blue dye (EPA Method 350.1). For measurement of nitrates ($NO_3$) and nitrites ($NO_2$), water samples were collected in plastic scintillation vials after filtration on a 0.45 µm polyethersulfone filter (Sarstedt®, Numbrecht, Germany). Samples were analyzed with a continuous flow analyzer (OI Analytical Flow Solution 3100©, OI Analytical, College Station, TX,

USA) using an alkaline persulfate digestion method, coupled with a cadmium reactor, following a standard protocol (Patton et Kryskalla, 2003) at the GRIL-Université du Québec à Montréal (UQAM) analytical laboratory.

### 1.3.3 Rock pellets preparation

The rock slab used to prepare the rock pellets was collected in the vicinity of the sampling well and was well embedded in the ground to minimize the likelihood of it having been deposited there by human activity. In order to ensure consistency between pellets and bioreactors, we selected a rock slab that was big enough to allow us to make all the pellets required for the experiment from that single slab. To create the pellets, the slab was first cleaned with a brush, soap and water to remove any dirt and vegetation. Core plugs were then drilled into the slab using a 5/8 inches diamond hole saw (Milwaukee, 49-56-0513, Taiwan) fixed on a drill press (18'' nova voyager dvr, King Industrial, Canada). These plugs were then cut approx. 0.3 cm thick and ground to fit into the bioreactor's slots using a rotary tool (DREMEL 3000, DREMEL, Mount Prospect, IL, USA). The pellets obtained were further cleaned by vortexing them in milli-Q water and detergent, cleaning them and finally, sonicating (Sonifier Cell Disruptor 185, Branson, Rungis, France) them for one minute. These pellets were then air dried and autoclaved before being installed into the bioreactor. The whole bioreactor setup with the tubing and the pellets was autoclaved prior to incubation experiments.

### 1.3.4 Bioreactor set-up

The bioreactors (CBR 90 Standard CDC Biofilm Reactor, BioSurface Technologies,Bozeman, MT, USA) were run in triplicate and continuously agitated with a magnetic stirrer. They also were wrapped in aluminum foil and placed in a dark incubation chamber in a windowless basement to minimize as much as possible light exposure. Using a peristaltic pump (IPC, Ismatec, Malente, Germany), groundwater (directly from the well for *E1* and *E2* phase one, autoclaved for *E2* phase 2) was pumped through these bioreactors at a rate of 0.289 ml/min. The bioreactors contained 8 columns of 3 pellets each for a total of 24 pellets per replicate. The bioreactors were supplied with 200 sccm of a varying mix of gasses ($N_2$, $O_2$ and $CO_2$) using a gas mixer MCQ GB100 (Monkey Industrial Supply, USA). This mix was calculated based on dissolved oxygen and pH measured in the groundwater on the day of sampling and the temperature of the growth chambers was set at the temperature measured in the field (Table 2). The dissolved $O_2$ and pH in the water were measured daily by collecting 50 ml of water from each bioreactor and using a probe (accumet XL600, Fisher Scientific, USA). These readings were then used to adjust the gas flow to the

bioreactors to keep the abiotic conditions as close as possible to the ones measured in the groundwater extracted from the aquifer on the day of sampling.

A blank run was performed for 21 days using the same set-up previously described but using sterile milli-Q water and rock pellets prepared as the previous ones but also dipped 3 times in HCl 10% to remove DNA traces and then rinsed using de-ionized water before being autoclaved. DNA was extracted from one of the rock pellets obtained from each bioreactor, to be considered as the contamination caused by the rock pellet handling and removed from further analysis.

The water that flowed out of the bioreactors was filtered on sterile polyether sulfone membrane filters with 0.2 µm diameter pores (Sartorius, Göttingen, Germany) using a vacuum pump (Welch 2019B-01, Welch, USA). They were then kept at -80°C until extraction. Similarly, the pellets collected daily were put into sterile tubes and stored at -20°C until extraction. A visual summary of the experimental set-up can be found in Supplementary Material Figure 1.

#### 1.3.5 Groundwater sterilization for the second phase of the second experiment

We first tried sterilizing the groundwater in 1L clear PYREX bottles using a UV-clave (Benchmark, U.S.A), but it failed to reduce DNA concentrations below the detection threshold of the Qubit™. It then was decided to autoclave the groundwater, and this successfully reduced DNA concentration below detection thresholds. It was, however, observed that flakes formed in the water following the autoclave. Blanks of the sterilized water were also sequenced and removed from the subsequent dataset.

#### 1.3.6 DNA extraction and sequencing

DNA was extracted from the water filters using the DNeasy PowerWater kit (QIAGEN, Germany). The DNA from the sessile community (the rock pellets) was extracted using the protocol detailed in Patel et al. (2024). First, a 2x AE elution buffer was prepared. The rock chip was then placed in a 50 mL Falcon tube with 1 g of autoclaved 0.1 mm zircon/silica beads and 2 mL of 2x AE buffer, and vortexed for 30 s. Then, 500 µL of 20% SDS was added, and the solution was vortexed for 1 min at maximum speed. The solution was then centrifuged for 5 min at 8000× g, and the rock chip was put into a 15 mL Falcon tube. 2 mL of phenol:chloroform:isoamyl alcohol (25:24:1) was added, the solution was vortexed at maximum speed for 1 min and then centrifuged for 5 min at 8000× g. The obtained aqueous phase was transferred to a new 50 mL tube, and 2 mL of chloroform was added. The new tube was then vortexed for 1 min and centrifuged for 10 min at 8000× g. Afterwards, the supernatant was transferred into a new 50 mL tube. To precipitate the DNA present in the sample, 2 µL of glycogen (20 mg/mL), 200 µL of sodium acetate

(3M), and 4 mL of 100% ethanol were added to the aqueous phase. The tube then was inverted and incubated overnight at 4 ◦C. The next day, the tube was centrifuged for 30 min at 8000× g. The obtained pellet was dried out and then rinsed with 0.5 mL of 70% ethanol. The tube was then centrifuged for 10 min at 8000× g, and the ethanol was completely removed. The pellet was finally re-suspended in 100 µL of 1x Tris EDTA (TE) buffer and stored at -20°C until further processing. Blank kit extractions were performed, and the sequences obtained from these runs were removed from analysis. The amplification of the community of B1, day 21 of the first experiment did not work so this sample has been removed from further analyses.

Despite our best efforts, the archaeal domain could not be reliably amplified and thus, was not analysed. Similar observations were made in Patel et al. (2024). This may be due to their low relative abundance when compared to bacteria and lower number of gene copies per cell (Hoshino, T. and Inagaki, F., 2019, Opitz et al., 2014) potentially putting them below our detection threshold. There also may have been a mismatch of primers making the PCR amplification less effective and the co-extraction of humic acids may have hindered the PCR reactions (Hoshino, T. and Inagaki, F., 2019). These four factors combined pose some great challenges to PCR amplification and, especially, to archaeal amplification. The effect of these factors may be particularly strong during the early stages of primary succession where the absolute abundance of microorganisms must have been extremely low.

The primer pairs B341F–B785R (5’-CCTACGGGAGGCAGCAG-3’ and 5’-GACTACHVGGGTATCTAATCC-3’) was used for the Bacteria domain, E960F–E1438R (5’-GGCTTAATTTGACTCAACRCG-3’ and 5’-GGGCATCACAGACCTGTTAT-3’) for the Eukaryote, and A340F-A915R (5’-CCC TAH GGG GYG CAS CA-3’ and 5’-GTG CTC CCC CGC CAA TTC CT-3’) for the Archaea (the amplification conditions can be found in the Supplementary Material Table 1). Samples were sent to the CERMO-FC (Center of Excellence in Research on Orphan Diseases – Fondation Courtois) at UQAM to be sequenced using Miseq Illumina and a Miseq Reagent v3 600-cycle kit (Illumina, San Diego, CA, USA) with a paired reading of 300 bp. Before sequencing, Phix control library (Illumina) was spiked into the amplicon pool to improve the unbalanced base composition. Negative PCR controls and the control samples for water and rock samples were sequenced for the two domains. The raw reads were deposited into the National Center for Biotechnology Information (NCBI) under the BioProject ID (PRJNA1159903).

**1.3.7 Bioinformatics and statistical analyses**

For all analysis, the R software (v4.2.2; R Core Team 2022) was used. The DADA2 package (v1.26.0; Callahan et al. 2016) was used to process the raw reads and produce amplicon sequence variant (ASV)

tables. Sequences present in the controls were considered contaminants and were removed from the datasets using the decontam package (v1.24.0; Davis et al. 2018). For both domains (Bacteria and Eukaryote), rarefaction was carried out using the median sequencing depth method. Taxonomy was assigned using the Silva database v.138.1 for the prokaryotes (Glöckner et al., 2017; Quast et al., 2013; Yilmaz et al., 2013), and the PR2 database for the eukaryotes (Del Campo et al., 2018; Guillou et al., 2013; Vaulot et al., 2021; Vaulot et al., 2022). The ggplot2 package (v3.5.1; Wickham 2016) was used for data visualization. A significance threshold of 0.05 was used for all experiments. The environmental parameters (DIC and DOC concentrations, pH, percentage of dissolved oxygen, time and bioreactors) were tested for autocorrelation using the ggpairs function with a Pearson correlation coefficient. If the correlation exceeded the 0.7 threshold, only one of the parameters was conserved. The pH and the percentage of dissolved oxygen had high correlations with multiple parameters of both experiments and thus, were excluded and, since DOC and time were strongly colinear for the second experiment *E2*, DOC was excluded from that analysis.

The community was first visualized by stacked bar chart. The proportion of shared ASVs between the sessile and planktonic communities on each day for *E1*, and the unique share of ASV for each lifestyle (planktonic or sessile) was further established to determine the temporal behavior of each community.

Since the Shannon index can be influenced either by richness or evenness of the community, alpha diversity, richness and evenness indices were computed using the estimate_richness function of the Phyloseq package (v1.48.0; McMurdie and Holmes 2012). The alpha diversity index used were the Shannon index (using the natural logarithm), the observed richness and Pielou's index for evenness. The richness was the observed richness, selected since it is a component of the Shannon index. The evenness was Pielou's index, calculated by dividing the Shannon index by the natural logarithm of the observed richness. Thus, by comparing the variations of richness and evenness, we could have a direct explanation of the variations of Shannon index. The variations in all these measures were analyzed using the vegan package (v2.6-6.1; Dixon 2003). Finally, a time series analysis of the variation of Shannon index through time, the variations of DIC and DOC concentrations and the variations between bioreactors was performed. To do so, hierarchical generalized additive models (HGAM) were done by using the mgcv package (v1.9-1; Wood 2010) as the data was not expected to be normal and we also wanted to factor in the influence of parameters that were expected to have an unknown, but most likely non-linear influence on the variable of interest. We also desired to include hierarchical effects to account for the fact that each bioreactor inevitably had minute differences (e.g., pellet composition, differences in the

initial community) that could influence the outcome of the experiment, especially since a part of the community variation is expected to be stochastic. DIC and DOC concentration variations were suspected of having a great influence on alpha diversity. Since the relationship and linearity of their influence was unknown, we decided to use an anisotropic tensor function including DIC and DOC concentration and their interaction for the modelling of the sessile community. However, since DOC concentration and time were highly correlated in the second experiment, it was removed from the modelling of *E2*. Time and the individual bioreactors were also suspected of having a strong influence on Shannon indices, so time was included as a main effect and the variation of each bioreactor from the main effect was included to enable us to determine a general trend and the unique behavior of each bioreactor. A random effect for the bioreactors was also included to accommodate the varying initial alpha diversity of each bioreactor. The Gamma family with an identity link function was used as the data is strictly positive and not necessarily normal. A k-check was performed and the qq-plot, residuals vs fitted, and histogram of residuals were visually inspected to determine the validity of the model and the potential biases. We set the hyperparameter m = 1 for the group trends (Pedersen et al., 2019). The models were constructed on this framework:

Shannon ~ te(dic, doc) + s(time) + s(time, by = bioreactor, m = 1) + s(bioreactor, bs = "re")

For beta diversity analyses, visualization of the data was computed using NMDS ordination and clustering (20 iterations), with the ordinate function of the Phyloseq package. A db-RDA (capscale function, vegan package) and a subsequent one degree of freedom sequential contrast ANOVA (999 permutations, anova.cca function, vegan package) were performed on both domains to determine if the lifestyle, the experiment and the bioreactors were significantly different from one another. We then carried out a db-RDA (capscale function, vegan package), a one degree of freedom sequential contrast ANOVA (999 permutations, anova.cca function, vegan package) and variance partitioning (varpart function, vegan package) to determine which proportion of the variance in the beta diversity could be attributed to the different geochemical parameters and if the bioreactors behaved in similar ways through time. Given the nature of contrast tests, this test only allowed us to compare B2 and B3 to B1. In the case where both B2 and B3 were not significantly different from B1, a post-hoc test would have been performed to compare B2 and B3 to determine if all three bioreactors were not significantly different. It however never was the case.

Finally, the origin (or contribution from previous time point communities) of each community was determined using the FEAST algorithm for microbial source tracking of the FEAST package (v0.1.0;

Shenhav et al. 2019). The community of the same lifestyle from the previous days, the community from the other lifestyle, the groundwater sampled in the field and the peat bog water from the sampling day were used to determine how much of the community of a single day could be explained by each of these different sources.

During the first incubation (*E1*), the measures of the physico-chemical parameters of the second day were used for the first day as well for the alpha and beta diversity variation analysis in relation to geochemical parameters since, during the incubation, we were not able to retrieve enough water to measure them on the first day.

**1.4 Results**

**1.4.1 Water physico- and geo-chemical features**

The bioreactor set-up was not able to accurately reproduce the *in situ* pH and dissolved oxygen percentage despite all of the pumped gas being either $CO_2$ (to decrease pH) or $O_2$ (to increase the percentage in dissolved oxygen). The recreated conditions however were all well within normal variation observed in the aquifer during the summer of the sampling (2022). The measurements taken *in* situ reveal that the aquifer was oxic (% dissolved $O_2$ *E1*: 80.9, *E2*: 61.6), acid (pH *E1*: 4.73, *E2*: 4.42) and had high concentrations in DIC (*E1*: 13.41 mg/L, *E2*: 15.6 mg/L) and DOC (*E1*: 29.21 mg/L, *E2*: 25.85 mg/L). Its conductivity was low (*E1*: 24 µS/cm, *E2*: 28 µS/cm) and its nitrogen content was very low (*E1:* $NO_2$: 0.01 mg/L, $NO_3$: 0.0015 mg/L, $NH_x$: 0.08 mg/L, E2: $NO_2$: 0.02 mg/L, $NO_3$: 0.0005 mg/L, $NH_x$: 0.08 mg/L). In the first experiment, pH remained mostly below 6 (Mean: 5.03) whereas in *E2*, pH remained below 5 (Mean: 4.41). Both experiments were oxic with a mean percentage of dissolved $O_2$ of 55.34% for *E1* and of 59.70% for *E2* (Supplementary Material Table 2). DIC concentration (Mean *E1*: 41.78 mg/L, Mean *E2*: 48.93) (Supplementary Material Table 2) had generally higher values in B1 than B2 and B3 in *E1* (Supplementary Material Figure 2). DOC concentrations remained rather similar for all three bioreactors for both experiments (Supplementary Material Figure 2) and had a mean concentration of 29.17 mg/L for *E1* and of 19.16 mg/L for *E2* (Supplementary Material Table 2).

Physico- and geo-chemical parameters remained largely constant in-between bioreactors except for DIC concentration in *E1* (daily primary succession of sessile microbial communities on rock surfaces) (Supplementary Material Figure 2). During *E1*, pH was correlated with DIC concentration and time (Pearson correlation coefficient: 0.613 and 0.542) and the percentage of dissolved oxygen was correlated with DIC and DOC concentration, time and pH (0.361, 0.328, 0.657 and 0.679). During *E2* (primary

succession of planktonic communities in groundwater), DOC concentration was strongly correlated with time, and pH was correlated with DIC concentration (0.895 and 0.807) (Supplementary Material Figure 2). Given their high collinearity with multiple parameters, pH and dissolved oxygen concentration were removed from the analysis and DOC concentration was removed from the analysis of the second experiment to avoid collinearity problems with time.

### 1.4.2 Community taxonomic composition

Overall, throughout the incubations, 13,067 bacterial ASVs and 3,467 eukaryotic ASVs were observed. For experiment *E1*, for the Bacteria domain, the initial *in situ* groundwater had a high diversity, and no genera occupied more than 10% of these communities (Figure 1A). Unclassified (unc.) *Acetobacteraceae* and unc. *Thermodesulfovibrionia* were the most abundant genera for the first liter recovered before the first 10 L were pumped (00.1) and the second liter collected after the first 10L was pumped (00.2). The third pumped liter (after the second 10L) saw a gradual change in the *in situ* groundwater bacterial community (00.3) where unc. *Rhodocyclaceae*, *Rhodoblastus*, unc. *Thermodesulfovibrionia* and unc. *Hydrogenophilaceae* were most abundant, while the last liter collected after all the groundwater for the incubation experiments was recovered (00.4) saw the introduction of unc. *Xanthobacteraceae* in the main genera. For the eukaryotes, the initial *in situ* groundwater sample 00.1 contained unc. *Eukaryota*, unc. *Pezizomycotina* fungi, and unc. *Arachnida*, while sample 00.2 contained unc. *Pezizomycotina*, unc. *Eukaryota* and *Paramicrosporidium*; and samples 00.3 and 00.4 had unc. *Pezizomycotina*, unc. *Eukaryota* and the fungi, *dipodascopsis* and *Suillus* (Figure 1B).

Regarding the Bacteria domain of *E1* for the sessile community, the first 2 days showed the most variation and differences compared to the following days within each bioreactor, but also between them (Figure 1A). The first day for B1 was dominated by *Sulfurimonas*, *Pseudomonas*, and Candidatus (cand.) *Nitrotoga* while B2 was dominated by cand. *Solibacter*, *Rheinheimera*, and unc. UBA12409 (*Nitrospirota* phylum) and B3 by *Sulfurimonas*, unc. *Magnetospirillaceae*, and *Sideroxydans*. On the second day all three bioreactors were dominated by *Sulfurimonas*, *Pseudomonas*, and *Collimonas*, and for all bioreactors, *Pseudomonas* dominated the following days. For bioreactor B1, the same 3 genera dominated until day 5, where *Undibacterium* was more abundant than *Sulfurimonas*. On day 9, *Polaromonas* was more abundant than *Collimonas*, and slowly increased until the end of the incubations. For bioreactors 2 and 3, *Collimonas* was another major genus for days 4 and 5. After day 5, alongside *Pseudomonas*, unc. *Oxalobacteraceae*, *Undibacterium*, and *Rhodoferax* dominated.

In the planktonic bacterial community of *E1*, the community coming from the groundwater directly, a succession of heterotrophs can be observed, but there also is the presence of a chemolithotroph, *Sulfurimonas*, all throughout the incubation. After the first day where it had a high relative abundance, it stayed at a constant abundance of less than 10% for the remainder of the incubation. There also was the presence of a photosynthetic bacteria, *Rhodoblastus* (Spring et al., 2013), from day 8 onwards (Supplementary Material Figure 3A).

For *E1*, for the sessile community of the Eukaryote domain, the first few days (2 to 4 depending on the bioreactor) had a very high diversity and changed rapidly (Figure 1B). The first day for B1, was dominated by unc. TSAR, unc. *Dothideomycetes*, and *Sphaeroeca*, while days 2 and 3 contained many unc. *Embryophyceae*. The first day for B2 and B3 was dominated by unc. *Embryophyceae* and unc. *Dothideomycetes*. After 5 days of incubation, for all bioreactors, unc. *Synuracea* and unc. *Chrysophyceae* became the most important genera. *Sandonidae*_X became more abundant around day 10 followed by *Apoikiospumella* around day 16. The samples collected in the field differed from the samples from the first days for both domains, containing a much broader diversity that did not appear in a similar way in the incubation afterwards.

In *E1*, regarding the planktonic community used to colonize the sessile community, accurate identification could not be achieved for most ASV, but there was the presence of potentially mixotrophic algae (*Apoikiospumella*, unc. *Chrysophyceae* and unc. *Synuraceae*) as well fungi (*Rozellomycota*_XXX and *Pezizomycotina*) and *Goussia1*, a parasite (Jowers et al., 2023) (Supplementary Material Figure 3B).

For the Bacteria domain of *E2*, the main genera present in the sessile communities colonizing the sterile water were *Rhodoferax*, unc. *Acetobacteraceae*, unc. *Oxalobacteraceae* and *Undibacterium* (Supplementary Material Figure 4A). Regarding the aquifer, no genera occupied more than 10% of the initial communities, but the main ones were unc. *Acetobacteraceae*, unc. *Thermodesulfovibrionia*, unc. *Rhodocyclaceae*, *Sulfurimonas*, and *Desulfobacca* for the first liter recovered before the first 10 L were pumped (00.1); *Sulfurimonas*, unc. *Thermodesulfovibrionia*, unc. *Rhodocyclaceae*, unc. *Desulfocapsaceae* and unc. *Hydrogenophilaceae* for 00.2; unc. *Thermodesulfovibrionia*, *Sulfurimonas*, unc. *Rhodocyclaceae*, and *Clostridium* for 00.3; and unc. *Thermodesulfovibrionia*, *Clostridium*, *Sulfurimonas*, and *Desulfobacca* for 00.4 (Figure 1C).

For the planktonic community of the Bacteria domain for *E2*, the first 2 days showed the most variation and differences compared to the following days within each bioreactor (Figure 1C). The first day of

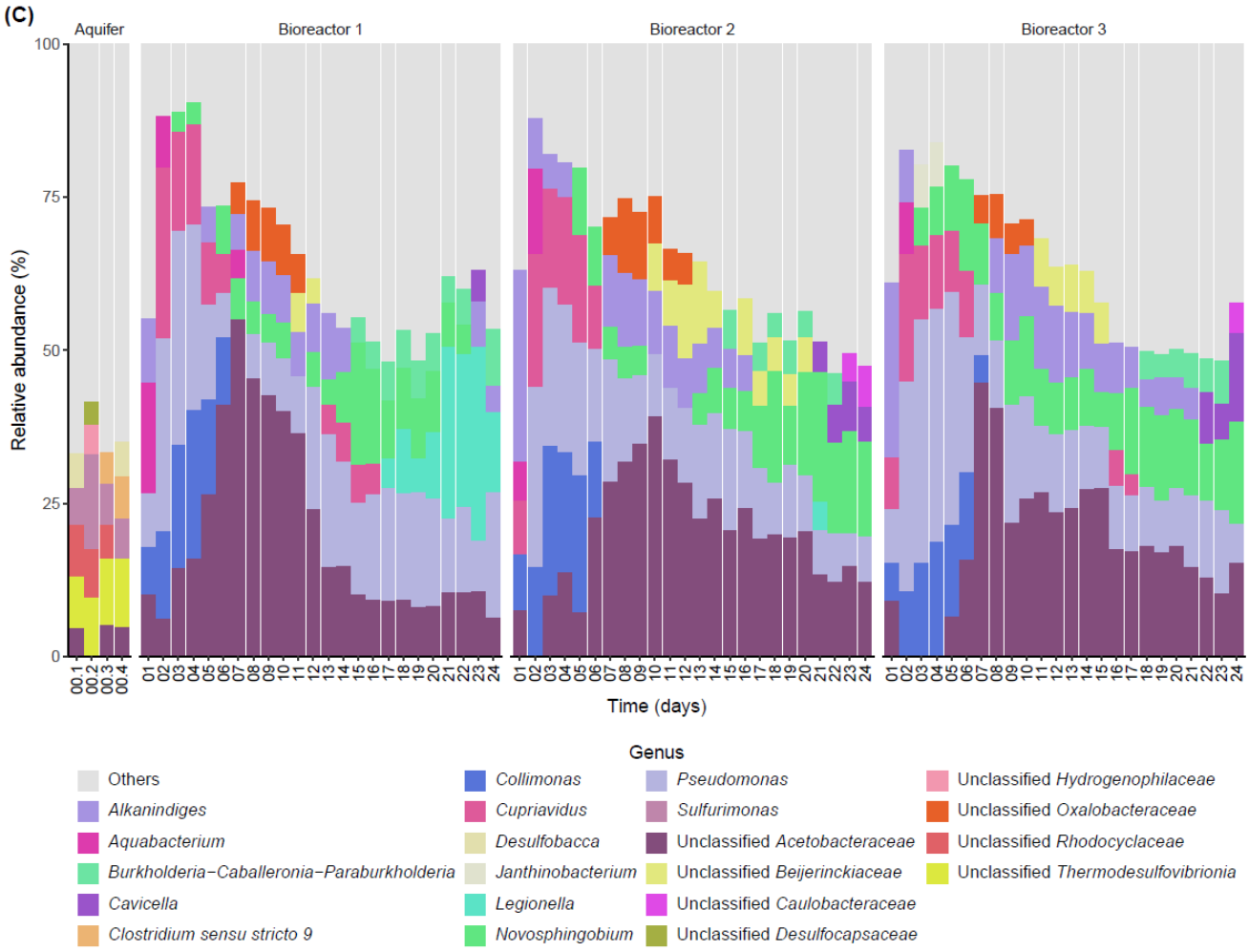
incubation was dominated by *Aquabacterium*, *Alkanindiges*, unc. *Acetobacteraceae*, *Pseudomonas*, *Collimonas*, and *Cupriavidus*. The second day of incubation, all three bioreactors were dominated by *Pseudomonas*, *Cupriavidus*, *Collimonas*, *Alkanindiges*, and *Aquabacterium*. *Pseudomonas*, *Collimonas*, and *Cupriavidus* also dominated the bioreactors for days 4 and 5 (B1 and B3) and days 4, 5 and 6 for B2. Then, unc. *Acetobacteraceae* took a predominant role and increased until days 7 to 10 and then varied in relative abundance until the end of the incubations. *Novosphingobium* and *Pseudomonas*, together with unc. *Acetobacteraceae* were then the most important genera until the end.

For the eukaryotes of *E2*, the most abundant genera of the initial *in situ* groundwater were unc. *Pezizomycotina* fungi, unc. *Eukaryota* and unc. *Opisthokonta* for the first liter recovered before the first 10 L were pumped (00.1) and the last (00.4) and for 00.2 and 00.3, unc. *Pezizomycotina*, unc. *Eukaryota* and *Stenostomum* were the most abundant, still not having more than ca. 10% of relative abundance (Figure 1D). The rock pellets of *E2* were colonized at more than 75% by *Apoikiospumella* algae, but this colonization markedly decreased on the 24th day (Supplementary Material Figure 4B).

For the planktonic eukaryotic community of *E2*, *Apoikiospumella* was the most abundant genera during the first days of the incubations (Figure 1D). Then *Spumella* dominated until the days 15-16. Afterwards, *Apoikiospumella* increased again in relative abundance. *Sandonidae*_Clade-N/F-A were important during the first 7-8 days for B1 and B2. Unc. *Chrysophyceae* were abundant during the middle of the incubations for B1 and the unc. *Synuraceae* for B3. Finally, *Vorticella* increased at the end of the incubations after days 13 for B1 and 20 for B2 and B3.


(A)
Aquifer
Bioreactor 1
Bioreactor 2
Bioreactor 3
Relative abundance (%)
100
75
50
25
0
00.1
00.2
00.3
00.4
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 22 23 24
Time (days)
Genus
Others
Acidocella
Candidatus Nitrotoga
Candidatus Solibacter
Clostridium sensu stricto 9
Collimonas
Desulfobacca
Gallionella
Glaciimonas
Herminiimonas
Legionella
Massilia
Mycobacterium
Polaromonas
Pseudomonas
Rheinheimera
Rhodoblastus
Rhodoferax
Sideroxydans
Sulfurimonas
Undibacterium
Unclassified 4−29−1
Unclassified Acetobacteraceae
Unclassified Acidimicrobiia
Unclassified Desulfocapsaceae
Unclassified Hydrogenophilaceae
Unclassified Magnetospirillaceae
Unclassified Oxalobacteraceae
Unclassified Rhodocyclaceae
Unclassified Thermodesulfovibrionia
Unclassified UBA12409
Unclassified Xanthobacteraceae


(B)
Aquifer
Bioreactor 1
Bioreactor 2
Bioreactor 3
Relative abundance (%)
100
75
50
25
0
00.1
00.2
00.3
00.4
01
02
03
04
05
06
07
08
09
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
Time (days)
Genus
Others
Apatococcus
Apoikiospumella
CONTH_2_XXX
Cryptococcus
Cryptomonas
Dinobryon
Dipodascopsis
Gonapodya
Goussia1
Monocystis
Mortierella
Naegleria
Paramicrosporidium
Phaeosphaeria
Phyllocoptes
Rhynchoscolex
Rozellomycota_XXX
Sandonidae_Clade−N/F−A
Sandonidae_X
Sphaeroeca
Spumella
Suillus
Tetrahymena
Unclassified Actinocephalidae
Unclassified Amphifilaceae
Unclassified Arachnida
Unclassified Chrysophyceae
Unclassified Dothideomycetes
Unclassified Embryophyceae_XX
Unclassified Eukaryota
Unclassified Eurotiomycetes
Unclassified Hartmannellidae
Unclassified Opisthokonta
Unclassified Pezizomycotina
Unclassified Stephanoascales
Unclassified Synuraceae
Unclassified TSAR


(C)
Aquifer
Bioreactor 1
Bioreactor 2
Bioreactor 3
Relative abundance (%)
100
75
50
25
0
Time (days)
Genus
Others
Alkanindiges
Aquabacterium
Burkholderia−Caballeronia−Paraburkholderia
Cavicella
Clostridium sensu stricto 9
Collimonas
Cupriavidus
Desulfobacca
Janthinobacterium
Legionella
Novosphingobium
Pseudomonas
Sulfurimonas
Unclassified Acetobacteraceae
Unclassified Beijerinckiaceae
Unclassified Caulobacteraceae
Unclassified Desulfocapsaceae
Unclassified Hydrogenophilaceae
Unclassified Oxalobacteraceae
Unclassified Rhodocyclaceae
Unclassified Thermodesulfovibrionia

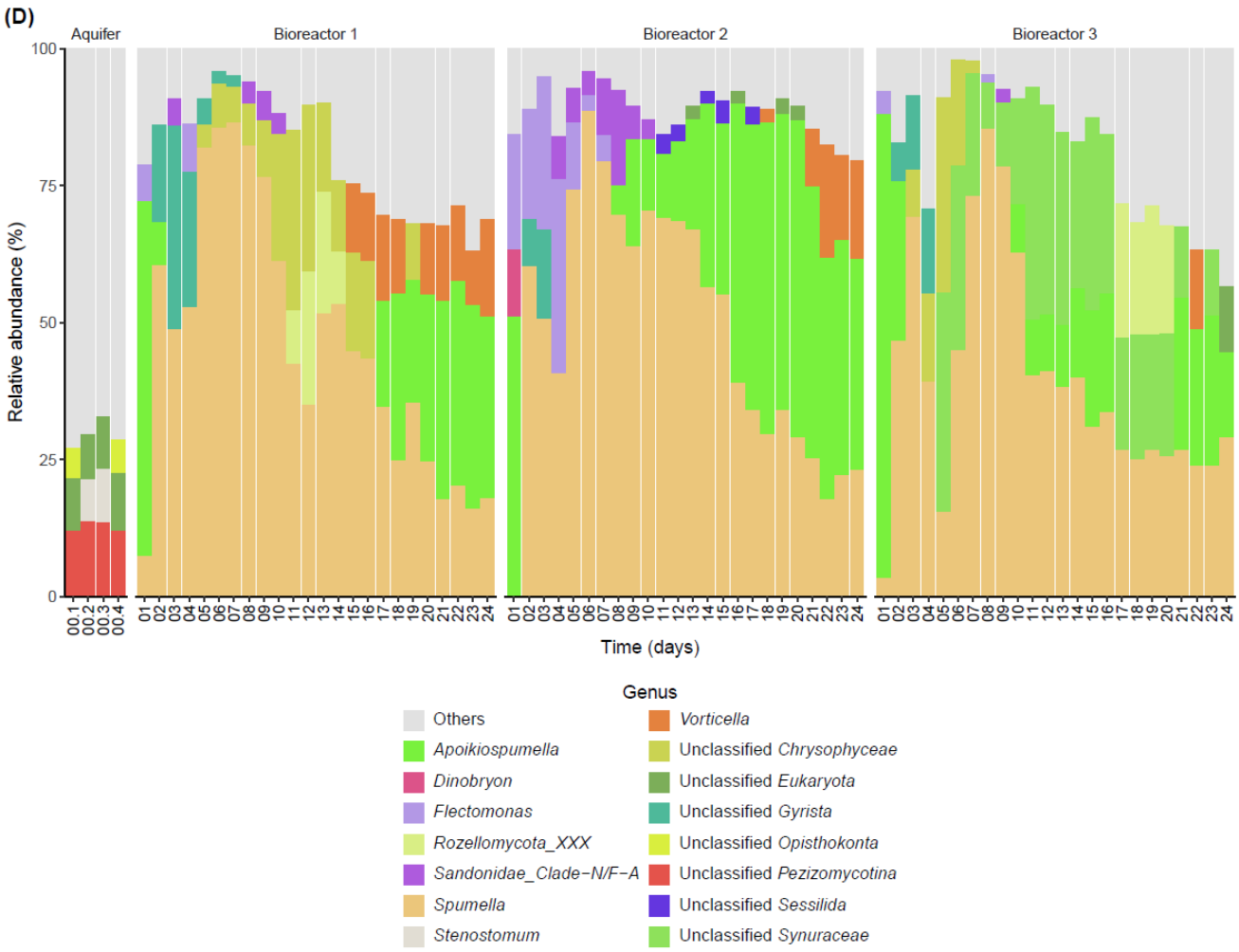
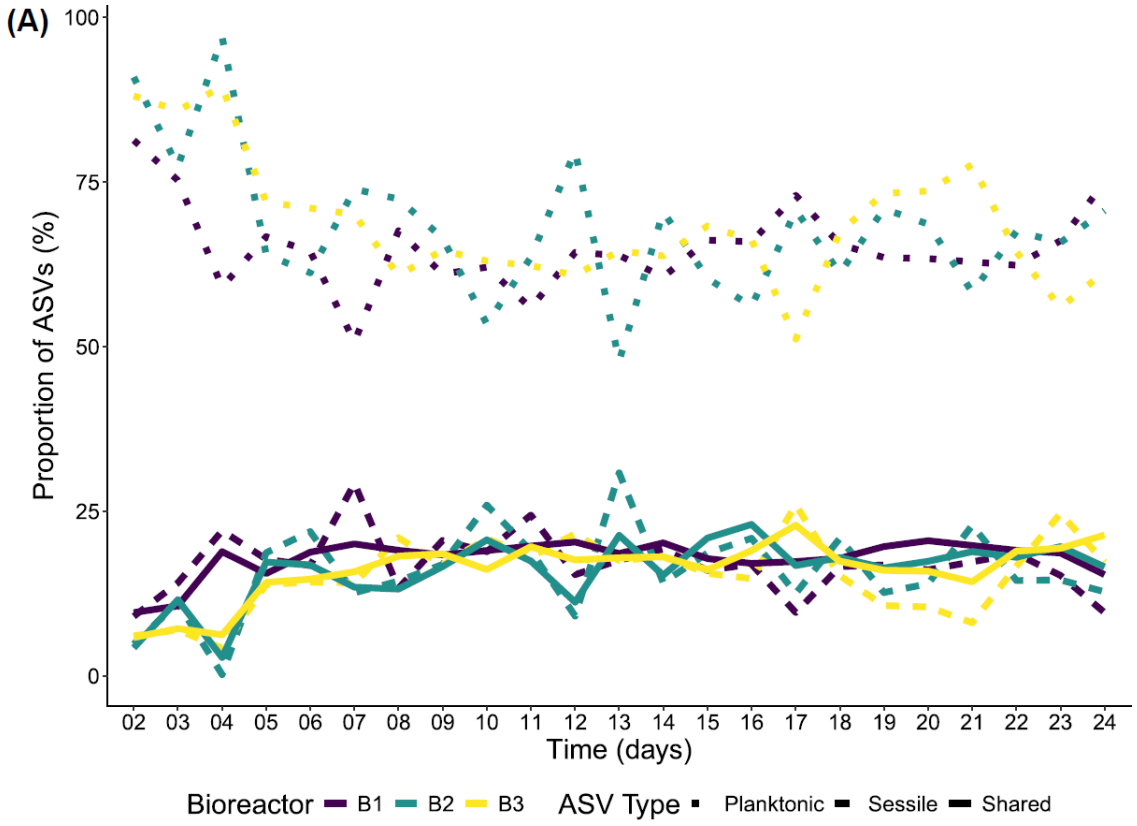



**Figure 1.1**. Taxonomic identification of each community based on 16S/18S rRNA gene sequencing: (A) sessile Bacteria of *E1*; (B) sessile Eukaryotes of *E1*; (C) planktonic Bacteria of *E2*; and (D) planktonic Eukaryotes of *E2*.

### 1.4.3 Shared ASVs between sessile and planktonic communities

For the Bacteria domain of *E1*, the percentage of shared ASVs between the sessile and planktonic communities on the second day of incubations started between 5 and 12% and increased to reach a plateau at 20 to 25% after 5 days of incubation (Figure 2A). For the Eukaryote domain, the percentage of shared ASVs started at 2.5% and increased to reach a peak of 15% after 19 days of incubation and slightly decreased afterwards until the end of the incubations to end around 10% on day 24 except for B1 that, on day 24, had more than 50% of its ASVs unique to the sessile community (Figure 2B). Most of the unique ASVs were found in the planktonic communities of both domains, close to 90% on the first day of the incubations and decreased to reach a plateau of 60 to 75% on day 6 for the Bacteria and day 15 for

the eukaryotes (except for B1 which behaved differently on day 24). The unique ASVs present in the sessile communities were close to zero on the first day and remained at 25% or less for the rest of the incubation days for the Bacteria, and at 15% or less for the eukaryotes. Since the sessile communities used to colonize planktonic communities were only sampled on the first and last days of *E2*, the information is limited to only 2 timepoints and will not be discussed further.



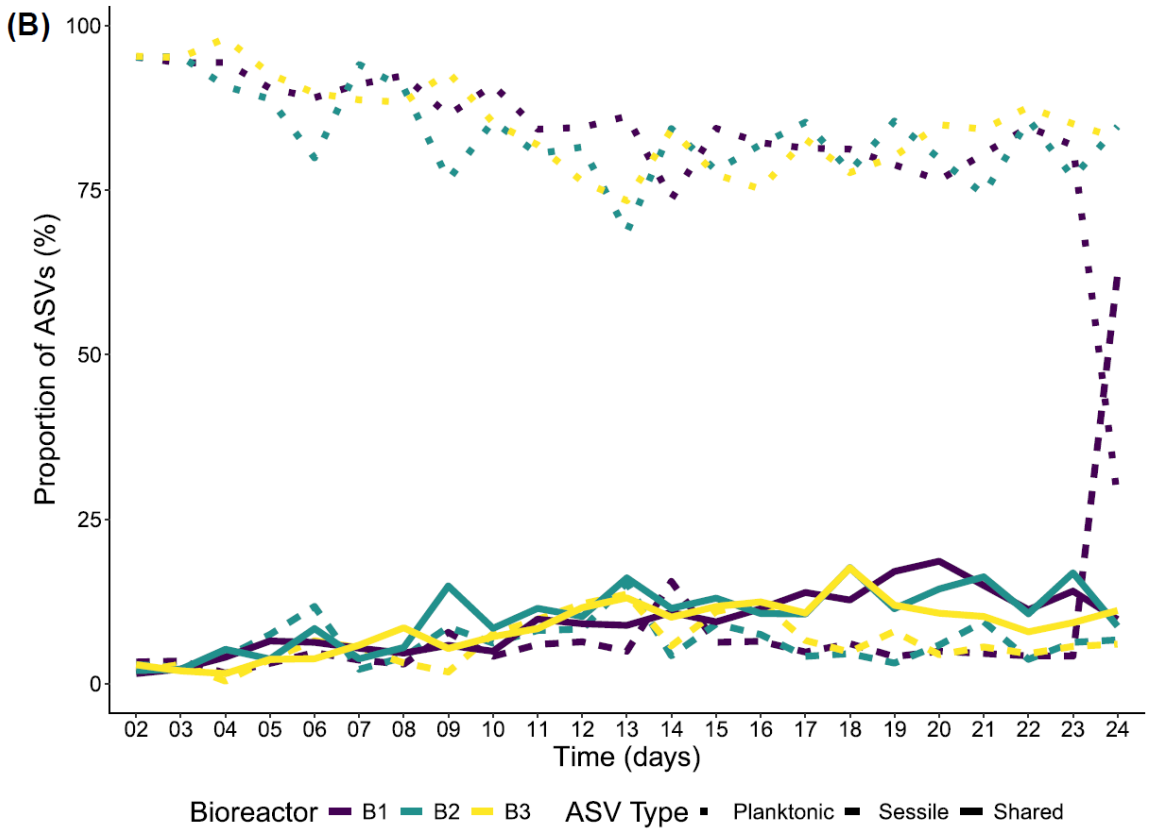



**Figure 1.2**. Shared and unique ASV percentage through time between the sessile and planktonic communities of *E1*. (A) Bacteria; and (B) Eukaryotes.

### 1.4.4 Alpha-diversity variation and modeling

The alpha-diversity values (Shannon index) ranged, for the incubation, from 2.302 to 4.370 for the sessile bacteria of *E1*, from 2.766 to 4.514 for the planktonic bacteria of *E2*, from 1.189 to 4.252 for the sessile eukaryotes of *E1* and from 0.9521 to 3.0671 for the planktonic eukaryotes of *E2*. (Supplementary Material Figure 5). The Shannon index of the sessile bacterial communities of *E1* started fairly low (less than 3, except for B3 at around 4), increased on day 2 to more than 4 and then decreased sharply on day 3 before stabilizing and slowly increasing for the remainder of the experiment. The planktonic bacteria of *E2* followed a rather similar trend, but the highest index of the first few days, close to 4, was on day one, followed by a sharp drop on day 2 and a subsequent increase for the remainder of the experiment with a plateau of around 4.25 starting around day 17. Regarding the eukaryotes, the sessile communities of *E1* started high as well (between 2.5 and 4) but decreased until close to day 7 to be around 1.5 before increasing slowly to be around 2 on the last day. The planktonic eukaryotes of *E2* were around 2 on the

first day (except B3). A subsequent decrease to 1 followed until ca. day 7 followed by a strong increase to end between 2 and 3.

The Shannon index of planktonic and sessile communities was relatively high, and the initial evenness of the sessile communities was high (Supplementary Material Figure 6) whereas the initial richness of the planktonic communities was high (Supplementary Material Figure 7). Following these first few days of high variation, both richness and evenness followed broadly similar trends to the Shannon index.

The richness and the Shannon index also were generally higher in the *in situ* samples than during the incubation period. The eukaryotes, except for the sessile community of *E1*, had a much broader variation between the four samples collected *in situ* than the bacteria (Supplementary Material Figure 5 and 7). The evenness of both domains for the sessile experiments of *E1* was close to the evenness of the early days of the incubation, but the evenness of the *in situ* samples of *E2* were generally higher than what was observed during the incubation (Supplementary Material Figure 6).

Overall, the models aiming to attribute the source of variations of Shannon index for each community and each domain were able to explain much more of the variation in alpha diversity for the primary succession of the planktonic communities (*E2*) than the sessile one (*E1*) (Supplementary Material Table 3). The models for both lifestyles of the bacterial communities, however, were unable to accommodate the much higher diversity measured during the first 2 days for the sessile community (especially the second day) and during the first day for the planktonic community (Figures 3A and 3B). The Shannon index for these days were ca. 4, but the models fitted an index closer to three. These divergences, along with day 24 of B1 of the sessile eukaryotes of *E1* (Figure 3C) were the only major divergences between the data and the model since the modeling of the planktonic eukaryotes of *E2* was fairly successful (Figure 3D).

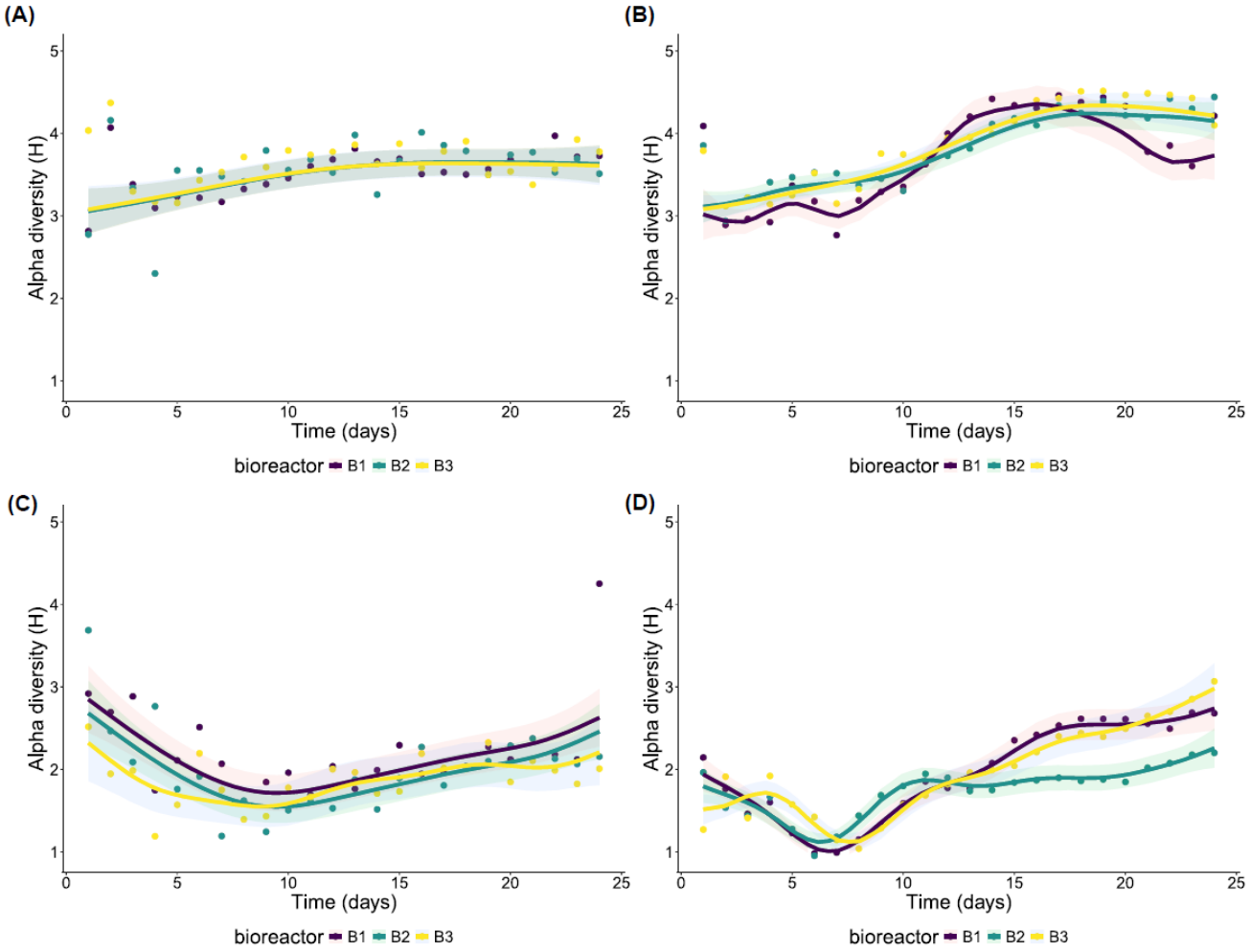



**Figure 1.3**. Combined effect of the shared trend of time and the individual effect of each bioreactor on the alpha diversity. (A) sessile bacteria of *E1*; (B) planktonic bacteria of *E2*; (C) sessile eukaryotes of *E1*; and (D) planktonic eukaryotes of *E2*.

The shared trend of time was the only parameter of said models that was always significantly correlated to the alpha diversity (Supplementary Material Table 3). The influence of time on both bacterial sessile and planktonic communities' alpha-diversity indices increased until the 16th day of incubation and stabilized or slightly decreased afterwards (Figures 4A and 4B). On the other hand, the influence of time on both eukaryotic communities' indices decreased up to day 7 for the planktonic community and day 9 for the sessile community and increased afterwards until the end of the experiment (Figures 4C and 4D). For both domains, the variations between the minimal and maximal influence of the shared trend of time are greater in the planktonic community than in the sessile one (Supplementary Material Table 3). Based on this metric, time seems to have been correlated with a higher variation of the alpha diversity in

the planktonic community than in the sessile one. The planktonic lifestyle thus may provide a wider ranging availability of niches through time or a varying amount of competition depending on time whereas the sessile community is more stable in the diversity of available niches it provides, supported by the fact that it had a significantly higher evenness (Supplementary Material Table 4).

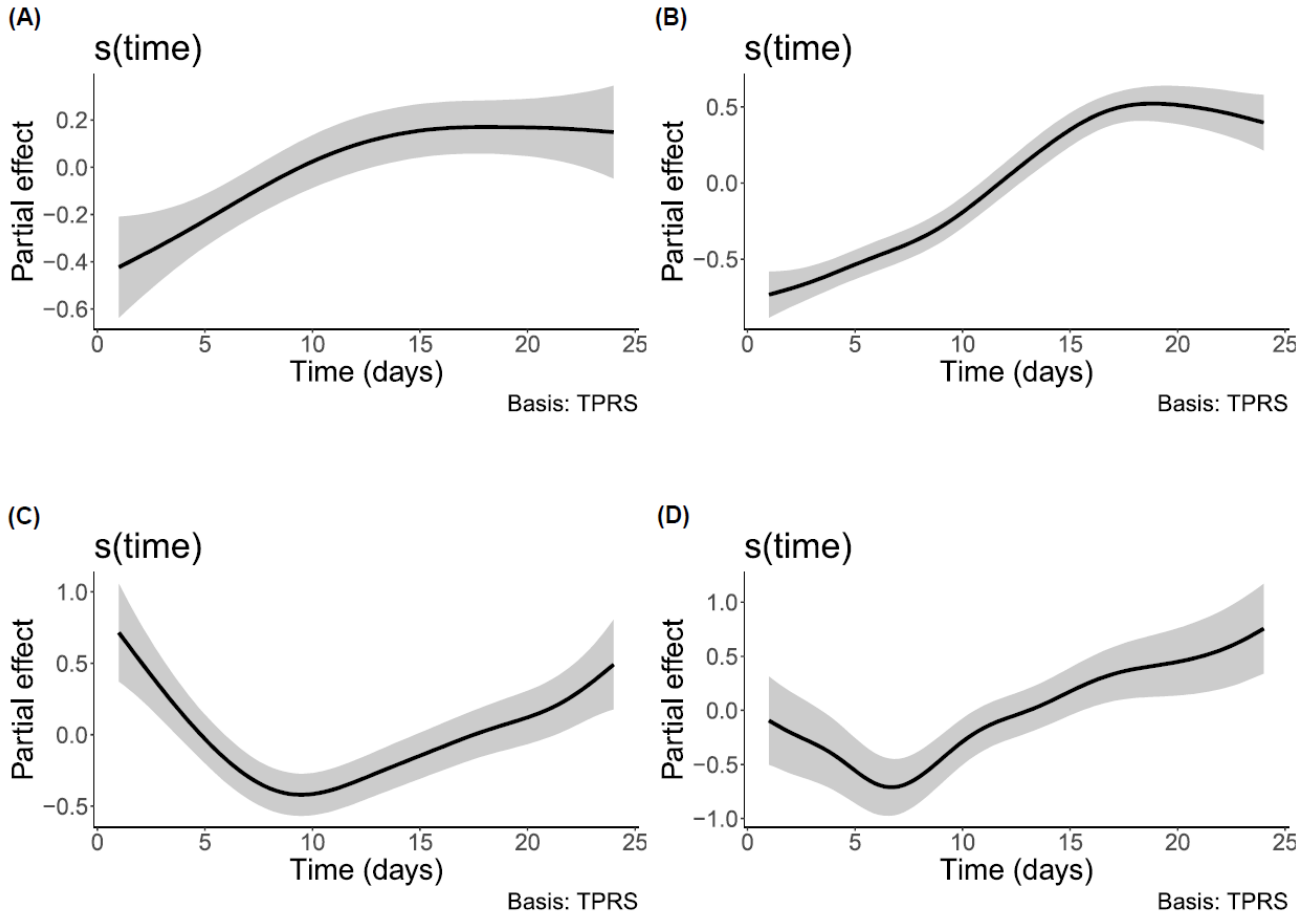



**Figure 1.4**. Shared trend of the effect of time on alpha diversity. The shaded area indicates the 0.95 credible interval. (A) sessile bacteria of *E1*; (B) planktonic bacteria of *E2*; (C) sessile eukaryotes of *E1*; and (D) planktonic eukaryotes of *E2*.

It should however be noted that the individual variations of each bioreactor of the planktonic community are more important than in the sessile one. When the unique variations of each bioreactor from the shared trend were significant in the sessile communities, they were following similar trends to the other bioreactors, but with slightly varying amplitudes or added smaller variations whereas one of the three bioreactors diverged in an important way from the two others in both planktonic domains.

The modelled effect of inorganic carbon concentration variation on alpha diversity was significant for the bacterial planktonic community of *E2* where DIC concentration had a rather stable effect until it reached approximately 55 mg/L when the effect then increased until reaching the maximum measured concentration of 73.83 mg/L (Supplementary Material Figure 8). For the other communities, both organic and inorganic carbon did not have a significant effect on alpha diversity.

### 1.4.5 NMDS ordination of beta-diversity and sample clustering

All the stresses for the sessile and planktonic communities were around 0.1 except for *E1* for the eukaryotes that was of 0.17 (Figure 5). All the communities' variations therefore seem to be reasonably accurately represented by the NMDS graphs. For *E1*, for both domains, the NMDS ordination shows that the early days of incubation led to distinct sessile communities, compared to the later days which clustered closely together (Figures 5A and 5B). These observations were confirmed by the cluster dendrograms, highlighting a very distinct sessile community for the first and often the second day of incubation for both domains (Supplementary Material Figure 9). For the bacteria, the dendrograms also show that the second cluster regrouping samples from days 3 or 4 to 24 were subdivided further into 2 clusters and more. However, succession as assessed by these subclusters seems to have occurred differently in each bioreactor.

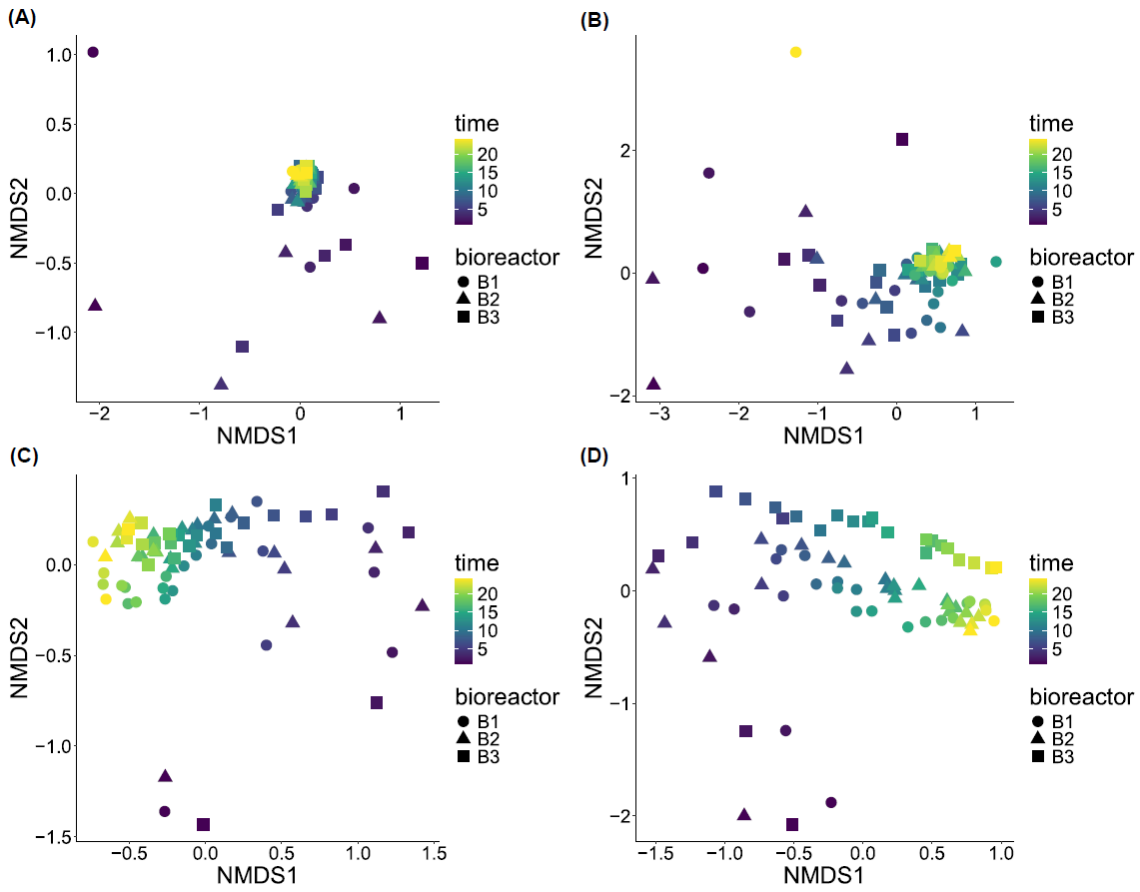



**Figure 1.5**. NMDS of the beta diversity of all communities based on Bray-Curtis dissimilarities. (A) sessile bacteria of *E1*; (B) sessile eukaryotes of *E1*; (C) planktonic bacteria of *E2*; and (D) planktonic eukaryotes of *E2*.

For *E2*, for both domains, succession of planktonic communities appears to be more continuous over time, since the early day samples don't cluster as separately from the later day samples, compared to *E1* (Figures 5C and 5D). For the Bacteria domain, the cluster dendrograms show indeed one cluster containing the first 4 to 6 days of incubation, and do not show the first day standing out as much as for *E1* (Supplementary Material Figure 9).

With the aim of performing a sensitivity analysis, NMDS analyses using Hellinger transformed data (Supplementary Material Figure 10) and Bray-Curtis dissimilarity index (Figure 5) were performed. While the Hellinger transformation made the sessile eukaryotes' plot unreadable since most points were stacked on top of each other, it made the similarity between later and earlier samples of both planktonic communities disappear. It therefore seems likely that the similarity between earlier and later samples of the planktonic communities is not ecologically meaningful and solely an artifact of the ordination,

namely a horseshoe effect. This effect is caused by the samples from the early and later days having a high number of the same ASVs that are missing from both samples. The model then interprets this similar lack in a lot of ASV as a similarity while, biologically, the early and later days environments most likely excluded these ASVs for very different biological reasons (Borcard et al., 2011).

### 1.4.6 Db-RDA, ANOVA and variance partitioning

All of the samples of both domains were compared using a db-RDA and a one degree of freedom sequential contrast ANOVA (999 permutations) on the db-RDA results. The different lifestyles, experiments and bioreactors were all significantly different (Supplementary Material Table 5).

The db-RDA followed by the ANOVA and the variance partitioning performed on all the communities indicated that the time was the main driver of variation (Supplementary Material Table 6). Time explained more of the variation in the planktonic communities than in the sessile ones and it was always a significant explanatory variant in the ANOVA. Based on vector orientation in the db-RDA, time also was positively correlated to varying degrees with organic and inorganic carbon (Supplementary Material Figure 11). Regarding the individual effect of each bioreactors, they did not influence the community in similar ways (Supplementary Material Figure 11 and Supplementary Material Table 6).

Regarding the effect of the variation of organic and inorganic carbon concentration on beta diversity, it played a small, but statistically significant role in all communities. The interaction of DIC and DOC concentrations for both domains, however, was not significant (Supplementary Material Table 6).

### 1.4.7 Microbial source tracking

The first few days of all communities were explained by communities of multiple lifestyle and origins. Then, in the following days, the origin of each communities mostly were the microorganisms of the previous days of the same community (Supplementary Material Figure 12). There however were occasional days, different for each community, where a community from another lifestyle explained an important part of a given community's origin on a given day. For example, days 12 and 22 for the bacterial sessile community of *E1*, days 21 to 23 for the planktonic bacterial community of *E2* and days 5, 9, 12 to 14 and 18 to 20 for the planktonic eukaryotes. The major exception to this trend is the sessile eukaryotes. The sessile community of the previous days and the planktonic community alternated to explain a major part of the community and the 'unknown' source tended to decrease through time. The planktonic source also generally tended to explain a higher proportion of the community before day eight. After this day, despite great variations, the sessile community from the previous days explained a

higher proportion of the sessile community. The peat bogs will not be discussed further since they explained 0% of the variation for all communities (Supplementary Material Figure 12).

### 1.5 Discussion

Time was shown to be the parameter most correlated with both alpha and beta diversity variations in all communities and the primary succession seems to have been separated into two phases. The first one, where high variation and high diversity were observed, had a duration of approximately 2 days and the second one, where change was much more progressive, lasted for the remainder of the experiment. Based on the taxonomic identification of each community, sessile bacteria of *E1* underwent succession from chemolithotrophs to heterotrophs and planktonic bacteria mostly underwent a succession of heterotrophs. Regarding eukaryotes, both sessile and planktonic communities saw a succession of likely mixotrophic microorganisms and heterotrophs. The alpha diversity of planktonic and sessile communities of both domains followed similar patterns, but the planktonic communities varied with a broader amplitude. This is opposite to what was expected. Even though they were expected to be weakly active, not multiply that much and originate from the sessile communities, the planktonic communities were correlated with larger variations both in alpha and beta diversity both in the Bacteria and Eukaryote domains. Following the first few chaotic days, both domains were well insulated from the sessile communities, which also comes into contradiction with our expectation of the planktonic communities being mostly formed by dormant or weakly active microorganisms. The effect of the variation in concentration of DIC and DOC also was much weaker than expected both on alpha and beta diversity. This suggests that in a geochemically stable environment rich in organic carbon and low in nitrogen compounds and electrolytes, planktonic microorganisms are more active than expected in the primary succession and that pH or other nutrients than carbon such as N, P, S and Fe restrict the variation of these communities. Further investigation on these communities by following a broader array of nutrients throughout the incubations would help shed light on the unique community dynamics and the influence each nutrient has.

It also highlights the need to use multi-omics approaches to distinguish between sessile and planktonic microorganisms since the current filtering methods used in this study and in most of the literature to analyse planktonic communities do not allow for distinction between planktonic microorganisms and free-floating ones on small sediments.

### 1.5.1 Temporal primary succession of the sessile community

In *E1*, the sessile communities had a high evenness in the first few days which led to a high alpha diversity index which then decreased as evenness decreased sharply after day 4. The alpha diversity then increased more slowly from day five onwards as richness increased. This may represent the early stages of solid surface colonization (Griebler and Lueders, 2009) where there was less competition between available niches in the beginning of the primary succession in the sessile environment. The early colonizers could therefore multiply at a faster rate. Once the available space on the pellets began filling up, the communities convened toward a similar beta diversity, like what has been observed in a coal seam (Vick et al., 2019). The evenness decreased until day five and stabilized until the end of the experiment. These changes also coincided with the establishment of a few dominant genera around day 4 and the end of the transition from chemolithotrophs, mainly *Sulfurimonas*, but also *Sideroxydans*, to heterotrophs such as *Pseudomonas*, unc. *Acetobacteraceae*, unc. *Oxalobacteraceae*, *Undibacterium*, *Rhodoferax*, *Polaromonas* and *Collimonas*. While we could not reliably identify the ASVs to the species level, all these genera contain species capable of forming biofilms (Besemer et al., 2012, Song et al., 2015, Thi et al., 2020). Thus, biofilm formation may have occurred as soon as the cell density was sufficient.

Regarding the sessile eukaryotes of *E1*, the first few days (2 to 4 depending on the bioreactor) of the succession in the communities saw rapid taxonomic changes, going from being comprised of fungi, protists and many other heterotrophs to being mostly composed of the potentially photosynthetic autotrophs unc. *Synuracea* and unc. *Chrysophyceae*. It should however be noted that some of these microorganisms may have mixotrophic capabilities (Cavalier-Smith and Chao, 2006, Siver, 2003). *Sandonidae*_X also increased in relative abundance followed by the bacterivorous *Apoikiospumella*. Photosynthetic or mixotrophic protists always occupied a predominant place in the community, occupying above 50% of the relative abundance for most of the days. This predominance of potentially photosynthetic or mixotrophic microorganisms is rather surprising since there was a high concentration of available carbon to be consumed by heterotrophs and the incubations took place in a dark growth chamber, the bioreactors used for the incubation were wrapped in aluminum foil and the bottles used to feed water into the system were also obscured. The tubes connecting the bioreactors to the water collection bottles and the collection bottles themselves were, however, not obscured and may have let some light into the system whenever the growth chamber's doors were opened daily for sampling. The fact that we did not observe similar metabolic taxa in the Bacteria domain despite the presence of

cyanobacteria, *Rhodoblastus* and other photosynthetic genera in the initial *in situ* groundwater would however make the mixotrophic explanation more likely.

After ca. 6 days, the bacterial sessile communities originated mostly from the sessile communities of the previous days. The critical days determining the future characteristics of a community would therefore be the first few days with the community of the subsequent days being fairly well insulated from the other ones and the internal dynamics of the community determining its changes. The groundwater during the incubations and the groundwater collected on the sampling day seem to exceptionally have had an important influence on days 12 and 22. As long as the geochemical environment is kept stable, the community thus seems to be fairly well insulated from the influence of other communities except on a few specific days. The sessile eukaryotes of *E1* are the exception to this insulation. They did not show any particular patterns in all three bioreactors, alternating between being mostly explained by the sessile community of the previous days and the planktonic community. The decreasing influence of the planktonic community after day 8 may however indicate that this community, if given enough time, would stabilize itself as the others did. It also should be noted that the interactions between the heterotrophic and mixotrophic eukaryotes and the various prokaryotes and dissolved organic carbon species available as carbon sources most likely played a role in shaping the sessile communities (Herrmann et al., 2020) and should be investigated further. Especially given the presence, for example, of fungal taxa competing for dissolved organic carbon with heterotrophic bacteria and of *Apoikiospumella*, a bacterivorous taxon (Grossmann et al., 2016).

### 1.5.2 Primary succession of the planktonic community

In the planktonic bacterial community of *E2*, the alpha diversity variation through time followed similar patterns as in the sessile bacterial community of *E1* albeit with a broader amplitude. There also doesn't seem to have been a first period of the succession where autotrophic microorganisms played an important role in the community, there only was a succession of heterotrophs in the dominant genera. There thus seems to have been a different response between the sessile and planktonic communities regarding metabolic diversity during the community succession. Also, as previously observed, the observed richness of the planktonic communities was much higher than the one of the sessile communities (Patel et al., 2024, Sharma et al., 2024, Yan et al., 2020), especially in the first few days. This high richness led to a higher Shannon index and, following the strong decrease in richness during the first or second day, the evenness increased, but more slowly. This also led to an increase in the Shannon index. In summary, the planktonic communities were composed of a wide range of genera with highly

varying abundances in the early days, but the number of genera quickly decreased, and the fewer remaining genera had a progressively more equal distribution as the experiment progressed.

Regarding the planktonic eukaryotes of *E2*, likely mixotrophic microorganisms also dominated most of the incubation despite the presence of heterotrophs and of high organic carbon concentrations. *Spumella*, *Vorticella* and unc. *Synuraceae* represented the vast majority of the community for most of the experiment and *Apoikiospumella* increased strongly in abundance in the latter half of the experiment. This highlights once again the importance of better understanding the interaction dynamics between heterotrophs and mixotrophs to better understand these environments.

The planktonic eukaryotes of *E2* displayed a behavior similar to both sessile and planktonic bacterial communities where, following the first few chaotic days, the community of a given sample is explained almost entirely by the same community of the previous days. This suggests that most communities in stable biogeochemical conditions are well insulated from outside biotic influence. The community of the sessile eukaryotes of *E1*, however, were frequently influenced by the planktonic community colonizing them. It thus seems that planktonic eukaryotes would frequently but briefly interact with the sessile communities in *E1*, but the sessile microorganisms would seldom go into the planktonic community in *E2*. Repeated experiments are needed to determine if the eukaryotic sessile communities are repeatedly much less constant in the origin of their communities or if the observed trend was an anomaly. Given that such erratic behavior was observed in all three bioreactors, this tendency however seems rather generalized. We also only have two time point for the sessile and planktonic interactions in *E2* so further investigation is warranted.

The planktonic communities of *E1* were extracted directly from the aquifer and thus had an unknown age whereas the planktonic communities of *E2* were undergoing primary succession starting on the first day of the second phase. It therefore is not surprising for both of them to have had a markedly different behavior throughout the 24 days of the incubation. In addition to being taxonomically distinct, the planktonic communities extracted from the aquifer used to colonize the rock pellets of *E1* displayed a more stable behavior with less fluctuation in genera than in the planktonic communities of *E2*. These older communities had likely reached an equilibrium that could not be reached within 24 days of the beginning of primary succession for the planktonic community of *E2* (Fillinger et al., 2019).

### 1.5.3 Sessile and planktonic communities: how do they differ and how are they related?

Since the sessile communities were expected to be more active than the planktonic ones and increase in abundance faster (Griebler and Lueders, 2009, Sharma et al., 2024), it was expected for time to play a more important role in the sessile communities than in the planktonic ones. However, we observed the opposite trend: while time correlated in a small but significant way with the beta diversity of all communities, it explained a bigger part of the variation of the planktonic communities than of the sessile ones. The planktonic communities also, as expected, had a higher richness (Patel et al., 2024, Sharma et al., 2024, Yan et al., 2020), and a higher share of unique ASVs (Sharma et al., 2024). This may indicate that the planktonic lifestyle provided a wide-ranging availability of niches depending on time whereas the higher evenness of the sessile community indicates a higher stability in the provided niches.

The high proportion of ASVs present in the colonized community, but not in the colonizing community also highlights the challenges associated with detecting scarce ASVs. For example, the ASVs detected in the planktonic communities of *E2* most likely fell below the detection threshold in the colonizing sessile communities but increased in abundance once in the planktonic communities. The protocol used to extract DNA from the planktonic community may also have been more effective or extracted fewer mineral impurities which would likely have influenced the effectiveness of the PCR and the sequencing

The fact that similar behavior has been observed in all three bioreactors, however, seems to indicate that this is to be expected. It may have been caused by eukaryotes interacting with a bacterial biofilm but being unable to form one themselves (Zirnstein et al., 2012) and thus, being more or less well insulated from the planktonic community. That would explain why the planktonic community was the source of the sessile community in the early days and, following the establishment of the biofilm, the microorganisms that positively interacted with the biofilm were generally better insulated from outside influence following day 8 despite important planktonic influences still occurring on some days. This general trend of communities changing through time and being explained almost entirely by their own communities of the previous days also comes in stark contradiction to the literature that expects the planktonic community to be populated by weakly active microorganisms (Griebler and Lueders, 2009, Sharma et al., 2024, Wilhartitz et al., 2009) that, in the context of primary succession, would come from the sessile community. Flow cytometry may be helpful to get the absolute abundance of microorganisms and distinguish between living and dead microorganisms once sufficient cell concentration has been reached (Zacharias et al., 2015).

As observed for the alpha diversity of the planktonic communities where time was correlated with a stronger variation of alpha diversity, the beta diversity of the planktonic communities had a stronger correlation with time than the sessile ones. This is opposite to our initial hypothesis as it was expected that the sessile community would be more active and faster growing and changing. Such expectations were, however, based on studies led in a different, limestone-rich environments (Sharma et al., 2024, Wilhartitz et al., 2009). This difference in the geological environment may explain why the microorganism's behavior differed from what we observed. The general trend nonetheless was for a stronger variation of planktonic communities.

### 1.5.4 Influence of environmental parameters on microbial communities

Since *in situ* and incubation conditions differed between both sampling times, it unsurprisingly led to different communities. All communities were nonetheless incubated in an oxic environment rich in dissolved organic and inorganic carbon, but poor in nitrogen compounds and their temporal dynamics and taxonomic composition were similar.

The DIC and DOC concentrations were high, and their variations correlated with the alpha and beta diversity variations in a much weaker way than expected. Both organic and inorganic carbon concentration variations explained a higher proportion of the variation of the beta diversity of the eukaryotes than of the bacteria. This is opposite to the influence of inorganic carbon concentration variation on alpha diversity that explained a bigger proportion of the bacteria than the eukaryotes. This may therefore mean that the variation in DIC concentration correlated to stark changes in the composition of the community (alpha diversity) of the planktonic bacterial community, but these changes were weaker between the different days sampled (beta diversity).

It also was expected for the variation in concentration of organic carbon to have an influence on the composition of the communities and especially on the shift from autotrophs to heterotrophs. While its high concentration most likely caused heterotrophs to quickly dominate the chemolithotrophs (Fierer et al., 2010), its variation does not seem to have had an important influence. This may be because, with a minimum DOC concentration for the first experiment of 24 mg/L and of 16 mg/L for the second, these concentrations were much higher than the rough approximation of maximum 4 mg/L of DOC that is usually found in groundwater (Regan et al., 2017). While there is no known contamination source for the aquifer we sampled (J. Chabot-Grégoire, personal communication, 2024), these much higher than normal concentrations usually are indicative of contamination (Regan et al., 2017). The nitrogen levels measured in the aquifer, however, were low, as usually observed in pristine aquifers (Kumar et al., 2017,

Morgenstern and Daughney, 2012) and may have restricted community growth (Schwab et al., 2016). The analysis of nitrogen concentration throughout the incubation would allow us to observe more precisely its variations in concentration and its subsequent influence on the community as it likely was an important factor restricting community growth. The low conductivity levels measured *in situ* also indicate low concentration of electrolytes in the water (Gray, 2004) so other electrolytes such as Fe (Jakus et al., 2021), P (Rogers et al., 1998) and S (Labrenz et al., 2013) compounds may be in low concentration and restricting growth.

Regarding the planktonic bacterial community, the correlation between its variation and the variation in concentration of inorganic carbon may be explained by the fact that, in an acidic environment such as the groundwater we used for our experiment, most of the inorganic carbon present in solution is carbonic acid (dissolved $CO_2$) (Cole and Prairie, 2024). Since the heterotrophs composing most of the community do not use inorganic carbon as a carbon source, this correlation between DIC concentration variation and the planktonic bacterial community of *E2* may be due to the effect the variations in $CO_2$ concentration had on the variation in pH and subsequently on the growing conditions of the microorganisms of the community. The measured pH of the second experiment (pH: min = 4.17, median = 4.41, max = 4.89) indeed remained much below the optimal pH of the most abundant prokaryotic taxa (Belmok et al., 2023, Hommel and Ahnert, 1999). While the optima cited in the literature generally are for specific species and the microorganisms could not reliably be identified to the species level, it supports the possibility that the fluctuations in pH measured in the community generally may have limited and influenced the growth of the communities whereas the fluctuations of DOC concentration did not since DOC's absolute concentration always was fairly high. It also would explain why only the higher concentrations of DIC affected the alpha diversity of the community; the pH had to be below a certain threshold to influence in an important way the microorganisms during the incubation. Similarly, the eukaryotic dominant genera may have been limited by pH (Moser and Weisse, 2011, Sudo and Aiba, 1973), low nitrogen concentrations and the low concentration of other nutrients (Poikane et al., 2022). The overbearing influence of these factors would explain the weak correlations between carbon concentration variation and diversity as these other parameters determined the changes occurring in the community. The proportion to which mixotrophy was an important strategy in the community also has yet to be established and likely played a crucial role in shaping it.

### 1.5.5 Methodological limitations

All of these distinctions noted between sessile and planktonic communities and contradictions between them and the literature may be explained by the fact that we kept the geochemical parameters as stable as possible throughout the experiment, studied both prokaryotes and eukaryotes and studied the short term primary succession while studies generally analysed communities over longer periods and in changing environments (Dong et al., 2021, Fillinger et al., 2021, Fillinger et al., 2019). The trends observed in these studies may therefore not directly translate to ours since the geochemical variations tends to exert a strong influence on these communities. The studies with closer methodology to ours are extremely rare (Patel et al., 2024, Sharma et al., 2024) and took a more limited interest into the planktonic and eukaryotic communities and so, comparison is more difficult.

It also should be noted that we classified the planktonic communities as the ones extracted from the filters and the sessile communities as the ones that were attached to the rock pellets, as is usually the case (Fillinger et al., 2021, Gios et al., 2023, Patel et al. 2024, Sharma et al., 2024, Yan et al., 2020). This means that we could not differentiate between the free-floating microorganisms and the ones living a sessile lifestyle on small free-floating sediments. It therefore cannot be ruled out that the sessile microorganisms living on flowing sediments were the most active sub-community that was studied from the groundwater and the planktonic community per se only was weakly active, as has been observed in the literature. There also likely was a community of ultra-small bacteria that could not be studied since they were smaller than the 0.2 µm pores of our filters and thus, were not detected by our methods (Herrmann et al., 2019). Multi-omics methods enable to better understand the distinction between these varying communities (Atencio et al., 2025, Smith et al., 2018), but, given the challenging DNA extraction and purification of the microorganisms living in these environments, the frequent co-extraction of PCR inhibitors and the small knowledge we have of these unique taxa, such methods are seldom used.

## 1.6 Conclusions

To summarize, time was consistently correlated to the variations in the diversity of the communities. Its influence varied based on the lifestyle and the domain, but it always was the parameter with the strongest correlation with diversity variation. The alpha and beta diversity of the planktonic communities varied much more through time than expected and, contrary to what was expected, they seemed to be active independently from the sessile one. The variation in the studied concentrations of inorganic carbon displayed a much weaker correlation than expected with alpha and beta diversity and was mostly

relevant to explain the variation in the alpha diversity of the prokaryotic planktonic communities. When analyzed, the variation in organic carbon concentration failed to show an important influence on the communities, but its high concentration likely played an important role in determining the composition and temporal dynamics of the different communities. This much weaker than expected influence of carbon concentration variation is likely due to the low pH of the environment or the very low concentration of other nutrients such as nitrogen restricting the development of these communities.

This study is the first we could find that analysed the daily succession and interactions of subsurface bacteria and eukaryotes in sessile and planktonic communities. It enabled a higher temporal resolution of the subsurface communities' dynamics and reduced the influence of variation in concentration of geo-chemical parameter. The high concentration of organic carbon and low concentration of nitrogen, however, likely exerted a strong influence on the composition of the communities and their dynamics. We were able to observe the clear demarcation, especially in the bacteria, of the primary succession into two distinct temporal phases. The first was of a few days where high variability prevailed. The second phase, of which we could not observe the end, was characterized by slower changes. Regarding community composition, the first phase contained bacterial chemolithotrophs whereas the second phase was almost exclusively bacterial heterotrophs. The eukaryotes did not show such a stark contrast in the variation of community composition between the two phases, displaying likely mixotrophic taxa throughout the incubation. These communities' temporal dynamics and the interactions between prokaryotes and eukaryotes still are little understood and warrant further investigation in varying nutrient concentrations and, if possible, using multi-omics, to better understand how different perturbations may affect these subsurface communities and how they may subsequently respond.

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## 2. Discussion

L'objectif de cette étude était à la fois d'observer la succession primaire quotidienne des communautés microbiennes dans un aquifère simulé et l'influence du carbone organique et inorganique sur ces communautés. Il s'agit d'une première étape dans le but de mieux comprendre la succession quotidienne des bactéries et des eucaryotes et leur interaction, chose qui n'a jamais été étudiée selon notre revue de littérature. Ce que nous avons pu observer c'est qu'à la fois dans les communautés sessiles et planctoniques bactériennes et eucaryotes, la succession ayant lieu dans les 24 premiers jours semble se diviser en deux phases. Une première ayant lieu dans les quelques premiers jours, représentant probablement l'assemblage initial de la communauté (Fillinger *et al.*, 2019) et une seconde phase débutant juste après et dont nous n'avons pas pu observer la fin. Plus concrètement, la phase initiale se caractérise par des diversités alpha et bêta très élevées et variant rapidement et de façon importante alors que la seconde phase se caractérise généralement par une variation plus progressive des diversités alpha et bêta.

Il est important de noter que l'indice de Shannon est composé de deux éléments : la richesse, démontrant le nombre d'ASV présent dans une communauté et l'équité, démontrant si les ASV sont présents dans une abondance relative similaire ou si certains sont prépondérants sur les autres (Whittaker, 1972). La tendance observée de diminution rapide dans la diversité alpha semble être expliquée par une différente composante de la diversité dépendamment du mode de vie de la communauté. Effectivement, la richesse des premiers jours de la communauté planctonique était particulièrement élevée et a diminué très rapidement alors que l'équité des communautés sessiles était beaucoup plus élevée que celle des communautés planctoniques et a diminué rapidement suivant les premiers jours. Cette tendance est observable dans toutes les communautés, mais cet effet est beaucoup moins important dans la communauté planctonique eucaryote que les autres. Cela signifie donc que les communautés sessiles étaient initialement composées d'une moins grande diversité d'ASVs, mais que ces ASVs avaient une abondance relativement similaire alors que les communautés planctoniques étaient composées d'une plus grande diversité d'ASVs, mais que ces ASVs avaient une abondance relative moins similaire entre elles puisque l'équité des communautés planctoniques est inférieure. La richesse plus élevée des communautés planctoniques était attendue, mais sa diminution rapide des premiers jours est surprenante puisqu'il n'était pas attendu que cette communauté soit particulièrement active. Il est toutefois difficile de départager la proportion des microorganismes planctoniques étant actifs et ceux en dormance ayant été éjectée de la communauté sessile. Les

différents microorganismes planctoniques issus de la communauté sessile ont également probablement été ajoutés à la communauté planctonique à une vitesse variable en fonction des conditions biotiques et abiotiques de leur environnement et du taxon concerné. Il serait aussi possible que, suite à leur exclusion ou leur éjection de la communauté sessile, les microorganismes colonisant l'environnement vierge planctonique colonisent les sédiments présents dans l'eau. Se retrouvant maintenant dans un environnement plus favorable que l'environnement planctonique, ils pourraient recommencer à se multiplier plus rapidement. Il serait donc primordial d'analyser le niveau d'activité des microorganismes sessiles et planctoniques, de même que leur abondance absolue, afin de mieux comprendre les dynamiques spécifiques à la communauté planctonique et de pouvoir départager les microorganismes planctoniques actifs, les microorganismes planctoniques dormants et ceux étant en fait sessiles et vivant sur des sédiments.

En ce qui a trait à la colonisation du milieu sessile, les différents genres provenant de la communauté planctonique ont colonisé cet environnement vierge et semblent avoir montré une abondance relative similaire pour les deux premiers jours. L'équité de la communauté était donc élevée. Suite à ces deux jours, peut-être une fois que les conditions requises à la production d'un biofilm ont été atteintes, il semble plausible que les microorganismes capables d'y contribuer ont eu un avantage compétitif et ont pu prendre beaucoup de place dans la communauté sans réussir à complètement éliminer les autres microorganismes. Il y a donc eu une diminution de l'équité, mais une richesse restant relativement similaire.

Le changement extrêmement rapide de diversité bêta observé entre le premier jour pour les bactéries ou les deux premiers jours pour les eucaryotes et les jours suivants représente probablement la phase d'assemblage de la communauté (Fillinger *et al.*, 2019). Ce changement rapide semble suivre un gradient temporel et est observable dans toutes les communautés. Les communautés planctoniques semblent toutefois avoir été plus fortement influencées par le temps que les communautés sessiles. Les communautés des premiers jours semblent plus similaires à celles des derniers jours que les communautés intermédiaires. Cette similarité peut seulement être observée sur la NMDS effectuée sur la matrice de dissimilarité de Bray-Curtis et est fortement réduite par une transformation de Hellinger. Dans les dendrogrammes, les échantillons des premiers et derniers jours se joignent avant les autres uniquement dans le bioréacteur 1 de la communauté bactérienne planctonique et ils se joignent à une dissimilarité élevée. Nous supposons donc que la proximité entre les premiers et derniers échantillons observés sur la NMDS s'explique, en fait, par un effet de fer à cheval. Cet artefact mathématique

proviendrait de la façon comment la dissimilarité entre deux sites est analysée; deux sites présentant une même espèce sont considérés comme étant similaires et seront donc plus près dans l'ordination. Il est toutefois impossible de savoir si deux sites ne présentant pas un ASV spécifique ne le présentent pas parce que les deux sites présentent à l'ASV des conditions similaires et défavorables ou s'ils présentent des conditions défavorables différentes. Puisque les communautés des premiers et derniers jours sont extrêmement différentes les unes des autres, elles ont vraisemblablement présenté un environnement défavorable à de nombreux ASVs, mais pour des raisons différentes. Ces deux communautés diamétralement opposées sont donc considérées par l'ordination comme étant plus près les unes des autres, parce que de nombreux ASV sont absents des deux communautés. En réalité, elles représentent deux extrêmes d'un même gradient (Borcard *et al.*, 2011). Dans notre cas, ce gradient représente probablement le temps. Seul l'échantillon du premier jour pour les bactéries ou des deux premiers jours pour les eucaryotes serait à une extrémité du fer à cheval et tous les autres échantillons suivraient un gradient relativement linéaire. Cette explication est appuyée par le fait que le temps explique une beaucoup plus grande part de la variation des communautés planctoniques que sessiles. Il est donc normal que l'effet de fer à cheval soit plus prononcé chez les communautés planctoniques. Comme mentionné précédemment, l'effet de fer à cheval disparaît en effectuant une transformation de Hellinger sur les données, mais la NMDS représentant la communauté sessile eucaryote devient illisible et le gradient temporel observé avec la matrice de dissimilarité de Bray-Curtis devient beaucoup moins visible. Suite à cette transformation, les communautés bactériennes semblent arrangées de façon très chaotique et aucune tendance ne semble en émerger. Pour les eucaryotes planctoniques, les premiers et certains des derniers jours se démarquent de la masse, mais tous les autres sont agglomérés dans un même amas. En combinant les observations des deux types de visualisation de la NMDS, il est donc possible d'observer que le gradient temporel semble être l'élément majeur de la succession de tous les microorganismes, mais particulièrement des microorganismes planctoniques. On note aussi un changement extrêmement prononcé dans la variation de diversité bêta entre les deux premiers jours et les jours suivants. Puisque la fin de la deuxième phase de la succession observée dans toutes les communautés n'a pas été observée, il serait intéressant d'analyser plus précisément la deuxième phase en retirant les 2 premiers jours de l'analyse et en les comparant aux variations observées dans les communautés sources (les communautés planctoniques de l'*E1*) dans le but de déterminer si les communautés de la deuxième phase de la succession primaire (les communautés planctoniques de l'*E2)* ont un comportement similaire à celui des communautés matures d'âge inconnu (les communautés planctoniques de l'*E1*).

Les divers milieux et méthodes utilisés dans la littérature pour analyser les communautés de la subsurface rendent l'extrapolation des résultats d'une étude à une autre hasardeuse, mais l'étude la plus similaire à la nôtre du côté méthodologique, mais ayant lieu dans un environnement très différent (Sharma *et al.*, 2024) a observé une augmentation rapide de l'abondance absolue de la communauté bactérienne jusqu'au jour 4 suivi d'une stabilisation jusqu'au jour 28 avant de recommencer à augmenter. Ce groupe de recherche n'a toutefois pas fait d'analyse temporelle de la diversité alpha, il n'est donc pas possible de comparer les variations temporelles dans la diversité alpha de ces deux études. De plus, le fait que l'amplification de la PCRd ait fonctionné pour eux, mais pas pour nous, alors que les durées de croissance étaient similaires indique qu'il serait peut-être approprié d'essayer les amorces utilisées par ce groupe de recherche ou à tout le moins, une plus grande diversité d'amorces pour la PCRd. Il est toutefois important de noter que Sharma *et al*. ont échantillonné des communautés vivant dans un environnement géologique composé de calcaire et d'argile, le pH était donc vraisemblablement beaucoup plus élevé que celui de notre aquifère et contenait potentiellement moins de contaminants (Cravotta Iii et Trahan, 1999).

L'activité de la population planctonique, quant à elle, était censée être très faible. La variation progressive d'abondance de divers genres et le passage de genres bactériens autotrophes à hétérotrophes nous poussent toutefois à croire qu'il y a soit eu une forte variation des microorganismes sessiles émis dans l'environnement planctonique, mais qui étaient en trop faible abondance pour être inclus comme source dans le FEAST ou qu'il y a eu, contrairement à ce qui était attendu, le développement d'une communauté microbienne active. Le séquençage de l'ARN présent dans la communauté serait pertinent pour répondre à cette question, mais il avait été choisi de se concentrer sur l'ADN plutôt que l'ARN puisque nous n'étions pas confiants de l'abondance d'acides nucléiques et de leur pureté par rapport aux contaminants co-extraits avec les acides nucléiques. L'analyse de l'ADN semblait donc être un choix plus fiable que celle de l'ARN, ou encore de la coextraction de l'ADN et de l'ARN. En rétrospective, en voyant que de nombreux échantillons étaient fortement contaminés et que leurs concentrations en ADN étaient parfois extrêmement faibles, ce choix semble avoir été judicieux. Il va toutefois de soi que, dans un environnement moins contaminé et avec des techniques d'extractions et de séparation des acides nucléiques plus performantes, la capacité d'analyser les ratios d'ADN et d'ARN serait un ajout indéniable à ce type d'étude, permettant de déterminer avec plus de certitude le degré d'activité de chaque microorganisme présent dans la communauté.

Le fait que chaque communauté ait tiré presque exclusivement sa source des jours précédents suite à la colonisation initiale semble indiquer que chaque communauté devient relativement rapidement isolée de l'autre sauf dans le cas des eucaryotes sessiles. Il serait maintenant intéressant de changer l'eau de l'aquifère alimentant les bioréacteurs tout en gardant des paramètres physico-chimiques relativement stables pour voir si les nouvelles communautés planctoniques entrant en interaction avec les communautés sessiles établies pourraient à leur tour agir de source ou si, une fois établies, les communautés sessiles sont réellement isolées des influences extérieures. Il serait également intéressant d'étudier si, dans un même système, les communautés sessiles d'un endroit ont la capacité de migrer vers une autre communauté sessile en créant un système où un premier bioréacteur est connecté à un deuxième. Cela permettrait de voir si la communauté sessile se développant dans le premier pourrait agir comme source pour la deuxième ou si deux communautés indépendantes se développeraient.

Deux genres microbiens (*Alkanindiges* et *Novosphingobium*) capables de dégrader divers hydrocarbures ont également été identifiés parmi les microorganismes les plus abondants de ces communautés. Aucune contamination de l'aquifère aux hydrocarbures n'est connue, mais ce résultat, de même que la concentration anormalement élevée en DOC de l'aquifère et des résultats préliminaires de métagénomique (N. Ortiz, candidat MSc, communication personnelle) provenant du même aquifère, indiquent que cet aquifère réputé propre est potentiellement contaminé et colonisé par des bactéries dégradant divers types d'hydrocarbures. L'identification des divers types de carbones organiques présents dans l'aquifère plutôt que la simple quantification de la concentration de carbone organique pourrait également permettre d'étudier l'effet de chaque espèce de carbone organique et, potentiellement, d'hydrocarbure, sur les communautés plutôt que l'effet global de toutes les sortes de carbone combinées.

Comme mentionné tout au long de l'article, l'échantillon B1 eucaryote sessile du jour 24 s'est comporté de façon distinctement différente des autres échantillons. Nous n'avons toutefois rien noté durant les incubations ou le reste du laboratoire nous menant à déduire quoi que ce soit qui permettrait de classer l'échantillon comme étant aberrant. Il a donc été conservé tout au long des analyses, mais il semble très probable qu'il y ait eu une erreur de manipulation ou autre, puisque son comportement est extrêmement différent des autres et uniquement pour un échantillon.

Finalement, la présence d'une aussi grande proportion de microorganismes photosynthétiques ou mixotrophes eucaryotes capables de dominer les eucaryotes hétérotrophes, mais l'absence

d'importants taxons bactériens ayant des capacités similaires est également surprenante. Bien que la résolution taxonomique et nos connaissances actuelles ne permettent pas de déterminer s'il s'agissait de microorganismes photosynthétiques ou mixotrophes, il est possible que certaines sources lumineuses aient permis une croissance photosynthétique. Par exemple, l'ordinateur portable situé dans la chambre de croissance fournissant un éclairage très faible, mais constant. Les portes de la chambre de croissance devaient également être ouvertes régulièrement à des fins d'échantillonnage, exposant les systèmes d'incubation à la lumière artificielle de la pièce où la chambre de croissance se situait. Il était supposé que ces sources ne seraient pas suffisamment importantes pour permettre la croissance puisque les bouteilles contenant l'eau de l'aquifère étaient opaques, les tuyaux connectant les bouteilles contenant l'eau de l'aquifère et les bioréacteurs étaient relativement opaques et les bioréacteurs étaient enveloppés d'aluminium, mais les bouteilles collectant l'eau sortant des bioréacteurs et la tubulure connectant les bioréacteurs et ces bouteilles n'étaient pas nécessairement opaques. De plus, suite à leur retrait de la chambre de croissance, les pastilles de roche encore humides étaient exposées à la lumière pour plusieurs minutes, le temps de les apporter au congélateur, et la filtration des échantillons d'eau pouvait durer plusieurs heures, durant lesquelles les échantillons étaient exposés à la lumière. Cette exposition aura donc potentiellement été suffisante pour favoriser la croissance des microorganismes photosynthétiques. Il n'y a toutefois pas eu de bactéries photosynthétiques parmi les taxons bactériens dominants malgré la faible présence de bactéries photosynthétiques dans les échantillons telle que des cyanobactéries. Puisque les deux domaines ont coexisté pour l'ensemble de l'incubation, l'apport de lumière ayant permis aux eucaryotes photosynthétiques de se développer aurait aussi potentiellement dû permettre aux bactéries photosynthétiques de se développer, mais ça ne semble pas avoir été le cas. Le fait qu'un comportement similaire ait été observé dans l'expérience de Patel *et al.* (2024) semble indiquer qu'il s'agit d'un phénomène normal, mais sa source reste à déterminer. Il semble très probable, comme discuté précédemment, qu'il s'agisse de microorganismes mixotrophes, auquel cas ils n'auraient pas eu besoin de lumière, mais il reste surprenant que ces microorganismes eucaryotes aient eu la capacité de croître de façon beaucoup plus importante que leurs pairs hétérotrophes malgré l'abondance de carbone organique alors qu'un comportement similaire n'a pas été observé chez les bactéries. Le fait qu'un comportement similaire ait été observé dans l'article de Patel *et al*. (2024) semble aussi éliminer la possibilité que ce soit un biais introduit par le choix de base de données puisqu'ils ont utilisé la base de données Silva 138.1 et que nous avons utilisé $PR^2$.

## Conclusion

Dû à sa difficulté d'accès, la subsurface est un milieu qui est toujours peu connu malgré le fait que nous interagissions avec de multiples façons. Ce milieu présente également le potentiel de nous aider dans notre lutte contre les changements climatiques en permettant d'y séquestrer, entre autres, du $CO_2$ ou de l'hydrogène. Toutefois, vu le peu de connaissances des communautés microbiennes omniprésentes dans cet environnement et leur capacité à interagir avec un nombre impressionnant de composés présents dans la subsurface, les risques de répercussions imprévues provenant de notre utilisation de la subsurface sont bien réels. Il nous semble donc essentiel de mieux comprendre ces communautés et comment elles changent à travers le temps et en fonction de divers facteurs géochimiques. Notre étude visait donc, à travers la reproduction d'un système d'aquifères en laboratoire, à mieux comprendre la succession primaire ayant lieu dans un aquifère non-contaminé et l'effet que certains paramètres géochimiques pouvaient avoir sur cette succession. Suite à cette étude, nous avons observé ce qui semble être deux phases distinctes dans les changements taxonomiques ayant lieu dans les communautés bactériennes et eucaryotes ayant un mode de vie sessile ou planctonique. La première phase, ayant lieu dans les quelques premiers jours, est caractérisée par des changement rapides et prononcés dans les communautés et la seconde phase consiste en l'établissement d'une communauté plus stable qui changera plus progressivement à travers le temps.

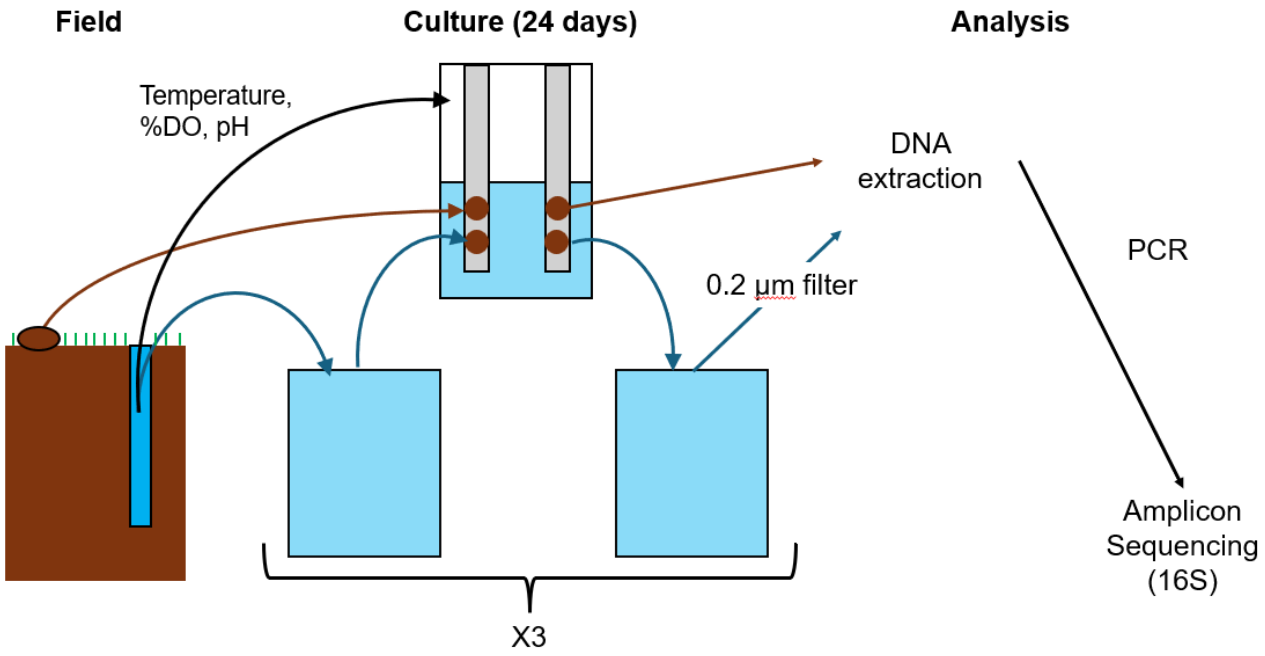
En ce qui a trait à l'effet des nutriments, l'azote a probablement été le facteur limitant, nous avons donc observé très peu de résultats concluants quant à l'effet du carbone. L'effet du carbone organique n'a pas pu être étudié sur la succession primaire des communautés planctoniques, mais sur les communautés sessiles, il n'a pas eu d'effet significatif. Nous supposons que c'est dû à sa concentration très élevée; il était probablement présent en plus grande quantité que ce que les communautés nécessitaient. Leur croissance a donc été limitée par d'autres facteurs que le carbone organique tel que l'azote ou les autres électrolytes. Le carbone inorganique, quant à lui, a seulement eu un effet sur les communautés bactériennes planctoniques et seulement à haute concentration. Nous supposons que cet effet est, en fait, l'effet que l'acide carbonique a sur le pH de l'environnement de la communauté puisque la communauté était très majoritairement composée de bactéries hétérotrophes. Ces bactéries n'utilisaient donc pas de carbone inorganique pour leurs besoins métaboliques et il ne semble pas y avoir d'explication biologique directe de l'effet du carbone inorganique sur des microorganismes hétérotrophes. Nous avons également observé une présence importante d'eucaryotes vraisemblablement mixotrophes dans nos deux incubations, mais l'état actuel des connaissances, la

résolution taxonomique obtenue par le séquençage et les méthodes utilisées ne permettent pas de mieux comprendre le rôle de ces microorganismes et leur interaction avec les bactéries dans un environnement où la lumière est presqu'absente et où la compétition est féroce.

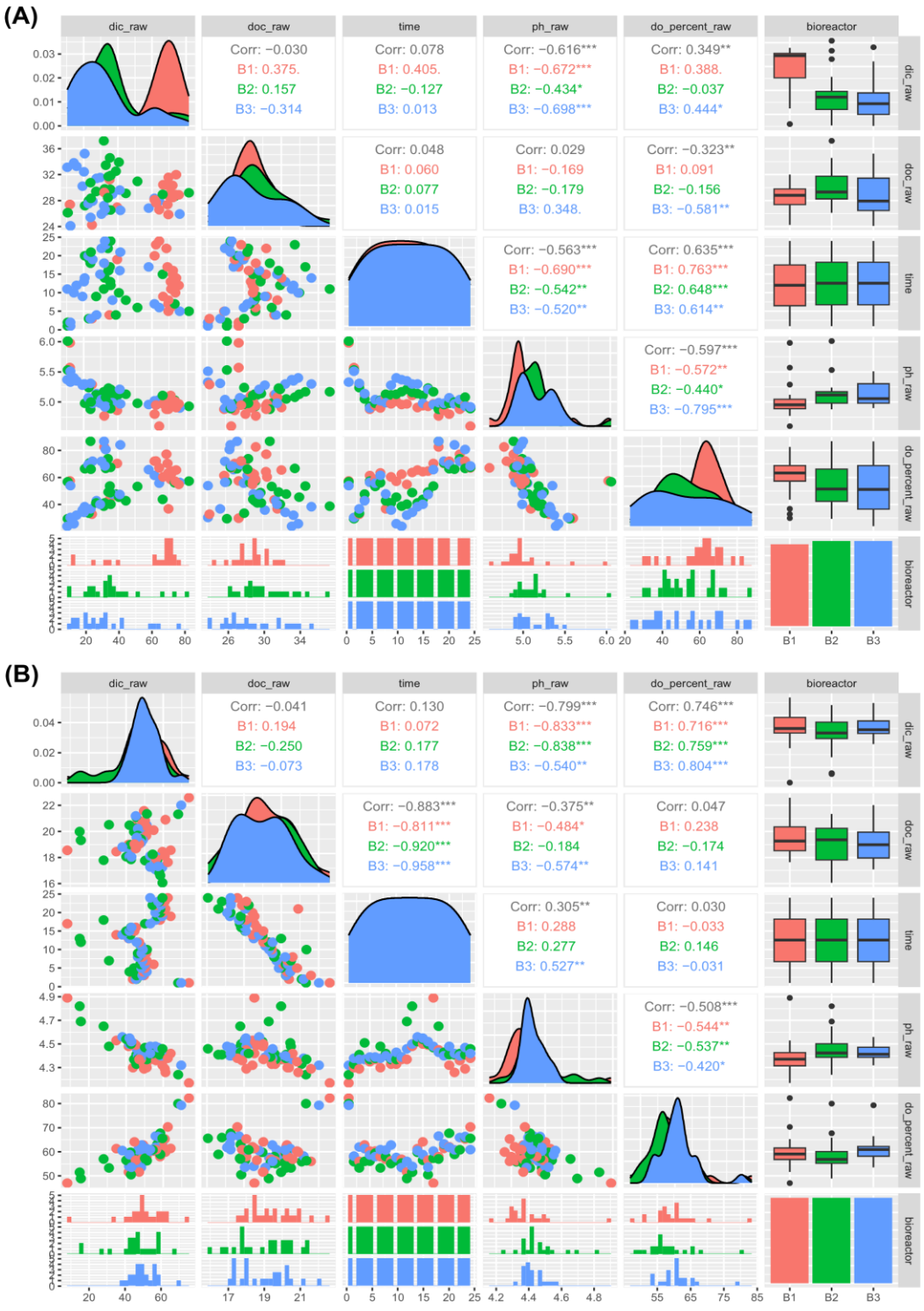
Finalement, la détection de microorganismes dégradant des hydrocarbures et d'autres recherches en cours démontrent le potentiel que pourraient avoir des méthodes de séquençage pour déterminer si un aquifère est contaminé par divers hydrocarbures et diverses méthodes biologiques qui pourraient être utilisées pour éliminer la contamination.

## Annexe A: Supplementary Material

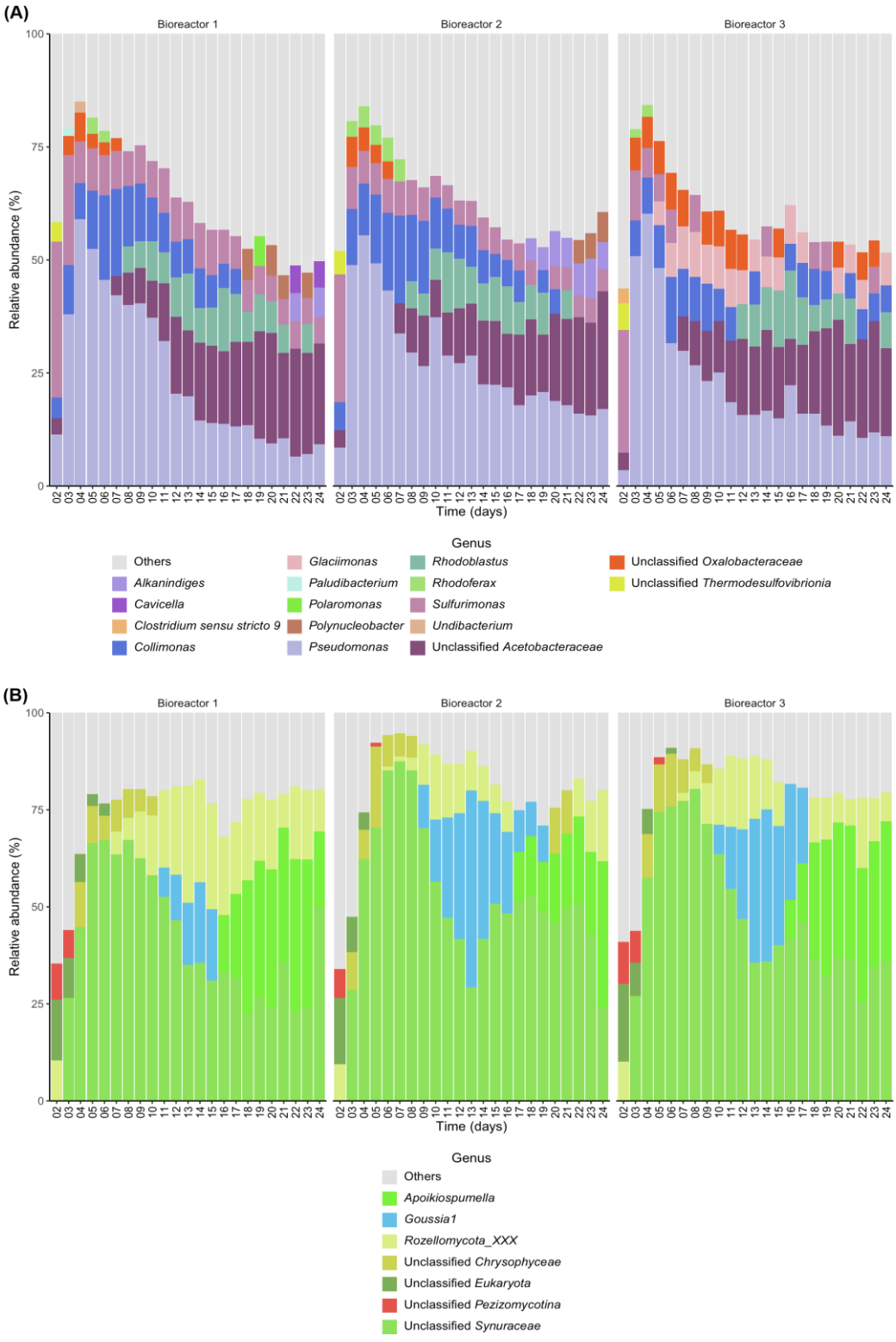
### Supplementary Figures




**Supplementary Figure 1.** Visual summary of the experimental set-up of the incubation. There were 24 pellets by bioreactor, but only 4 are shown for simplicity.

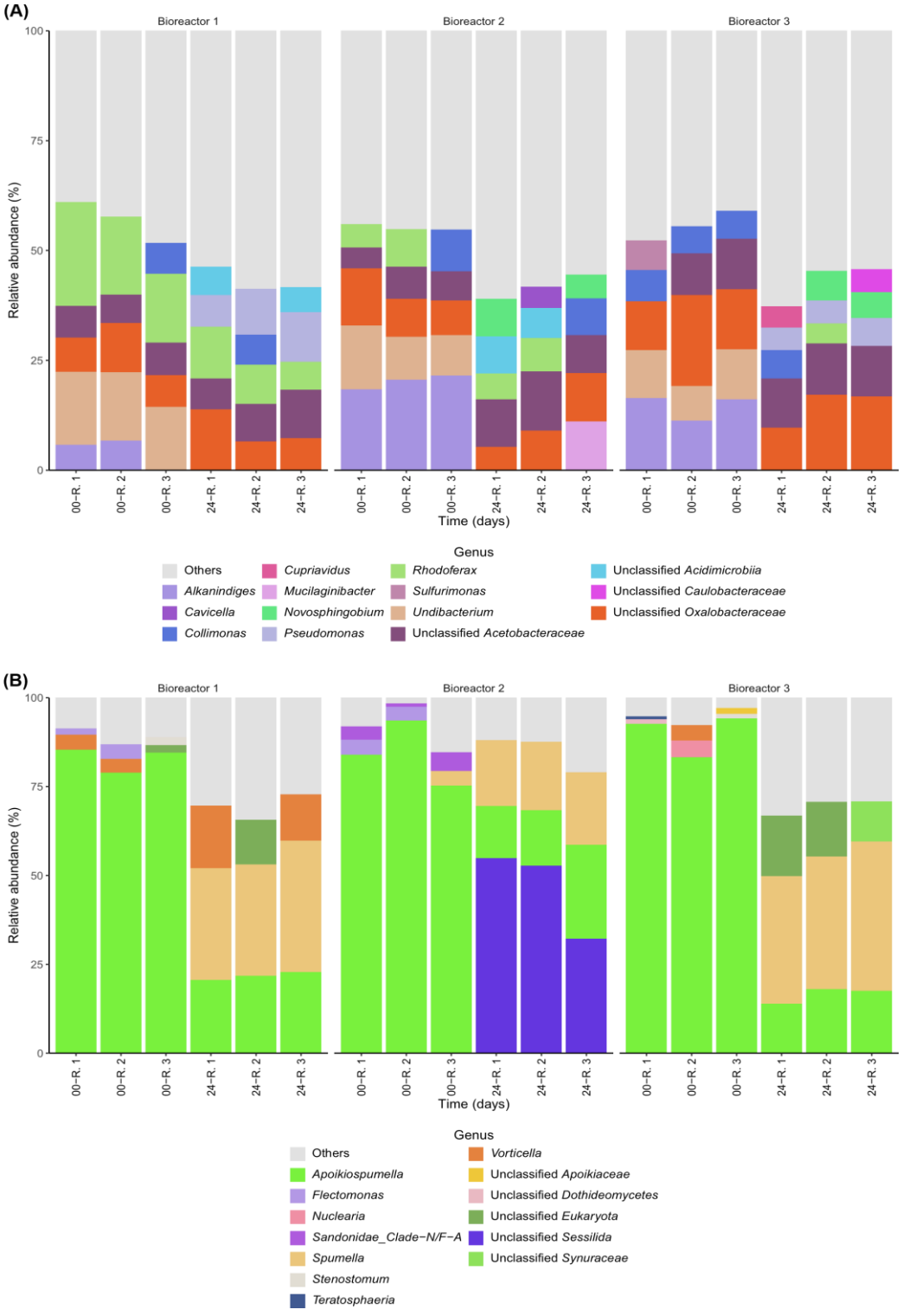



**Supplementary Figure 2.** Plots displaying Pearson correlation between the physico- and geo-chemical variables of experiment *E1* (panel A), and *E2* (panel B). Corr., Pearson correlation coefficients; dic_raw, dissolved inorganic carbon; doc_raw, dissolved organic carbon; do_percent_raw, dissolved oxygen.

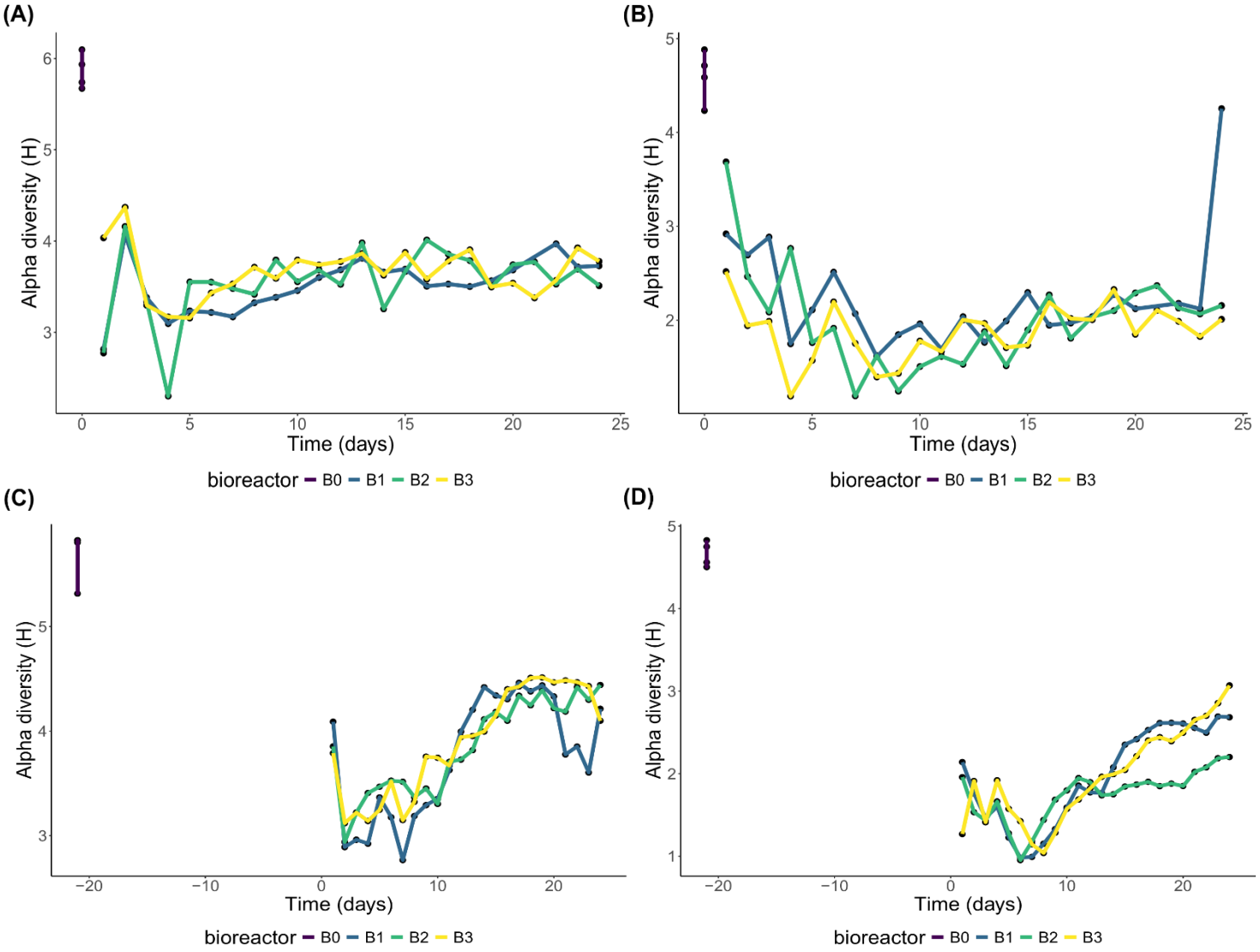



**Supplementary Figure 3.** Stacked bar chart showing the taxonomic composition of the planktonic communities of *E1* based on 16S/18S rRNA gene sequencing. Only the 5 most abundant bacterial genera

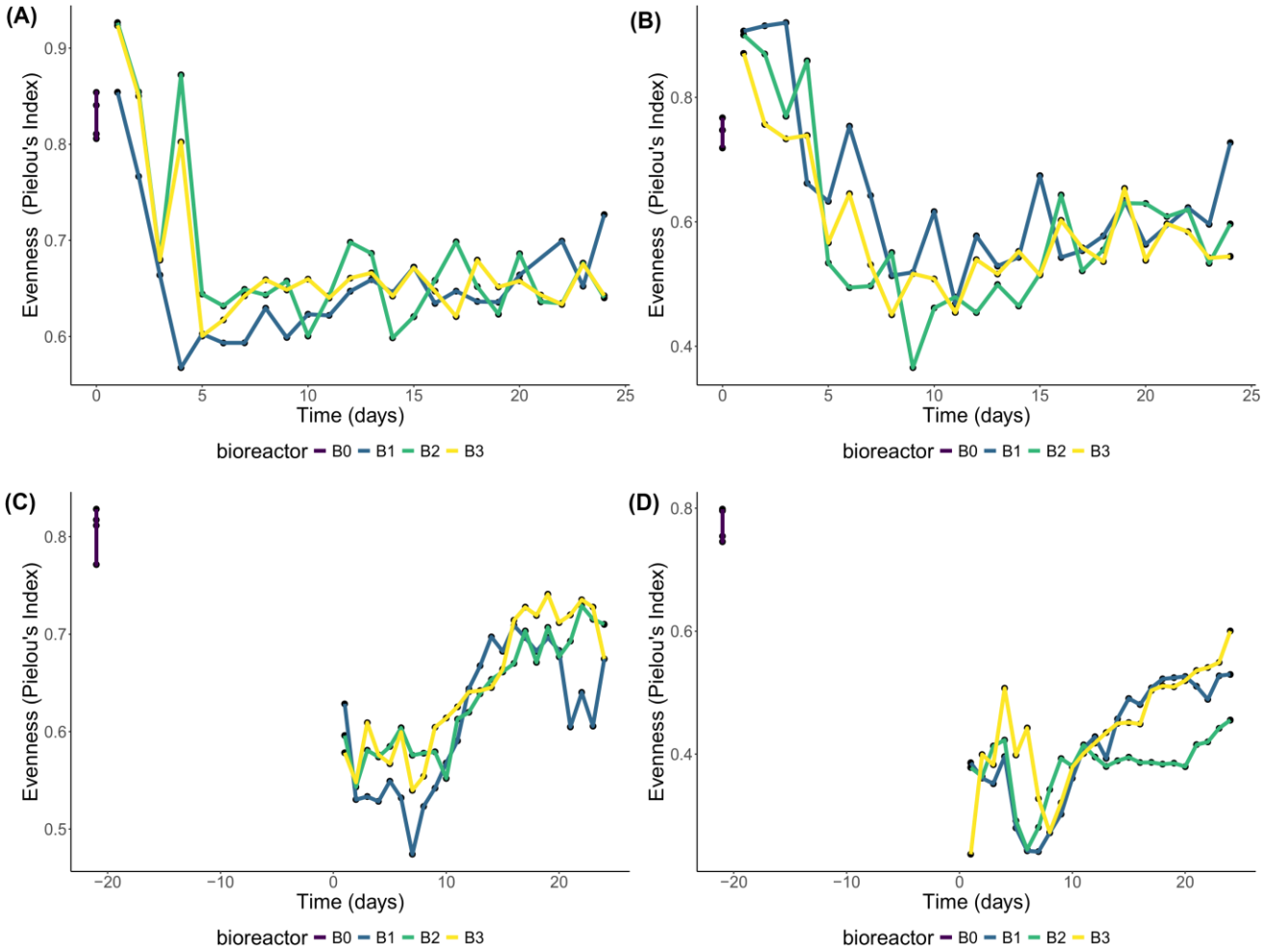
and 3 most abundant eukaryote genera of each day are shown. A) Bacterial community; B) Eukaryotic community.



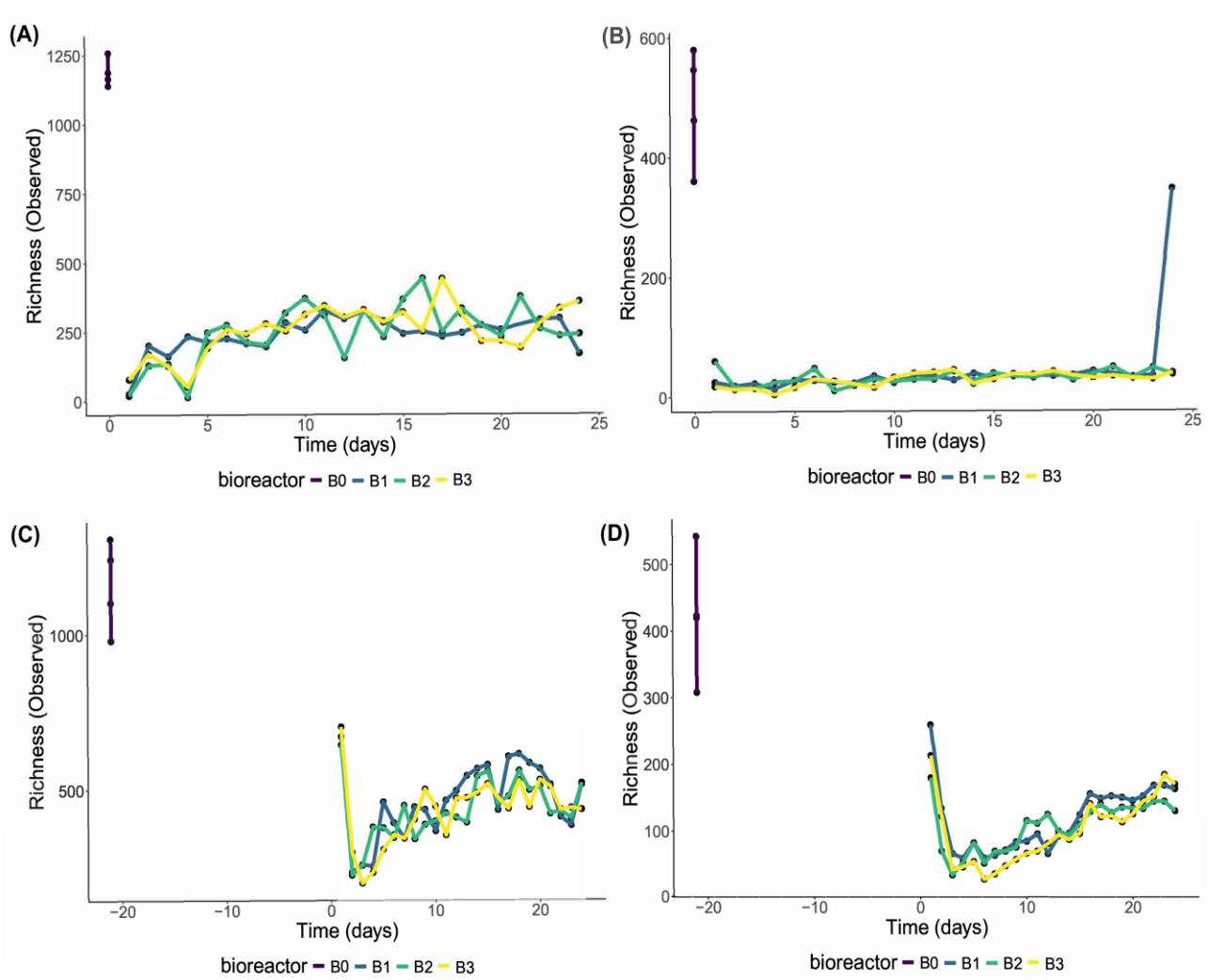
**Supplementary Figure 4.** Stacked bar chart showing the taxonomic composition of each sessile community of *E2* based on 16S/18S rRNA sequencing. Only the 5 most abundant bacterial genera and 3 most abundant eukaryote genera of each day are shown. A) Sessile bacterial community for *E2*, B) Sessile eukaryotic community for *E2*.



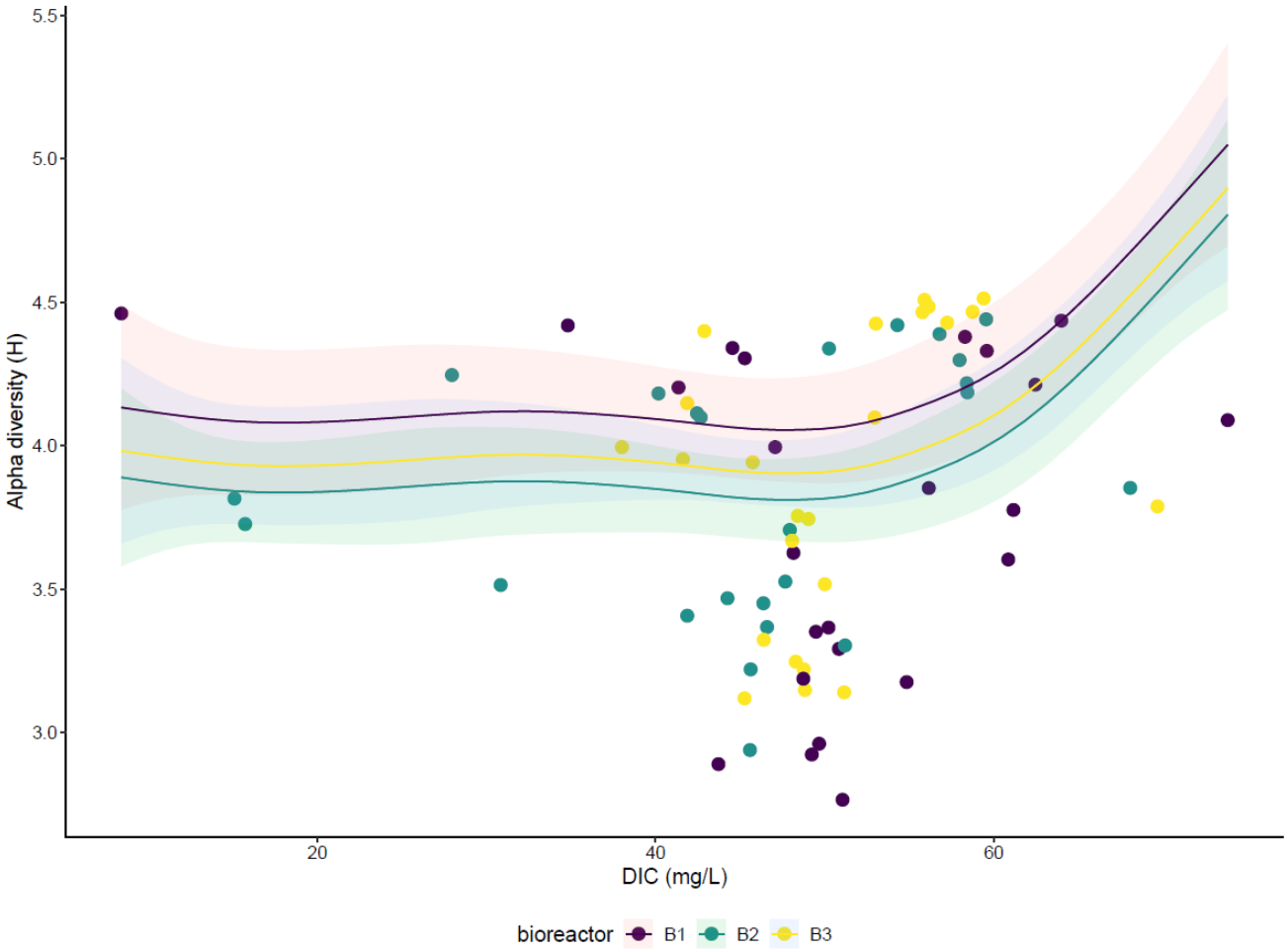

**Supplementary Figure 5.** Variation of alpha diversity (Shannon index) through time for each bioreactor (B1, B2, and B3) and of B0, the water collected in-situ. The gap between the in-situ sampling and the incubation for fig. C and D represent the first phase of the second experiment where no samples were collected. A) sessile bacteria experiment *E1*; B) sessile eukaryotes *E1*; C) planktonic bacteria *E2*; and D) planktonic eukaryotes *E2*.



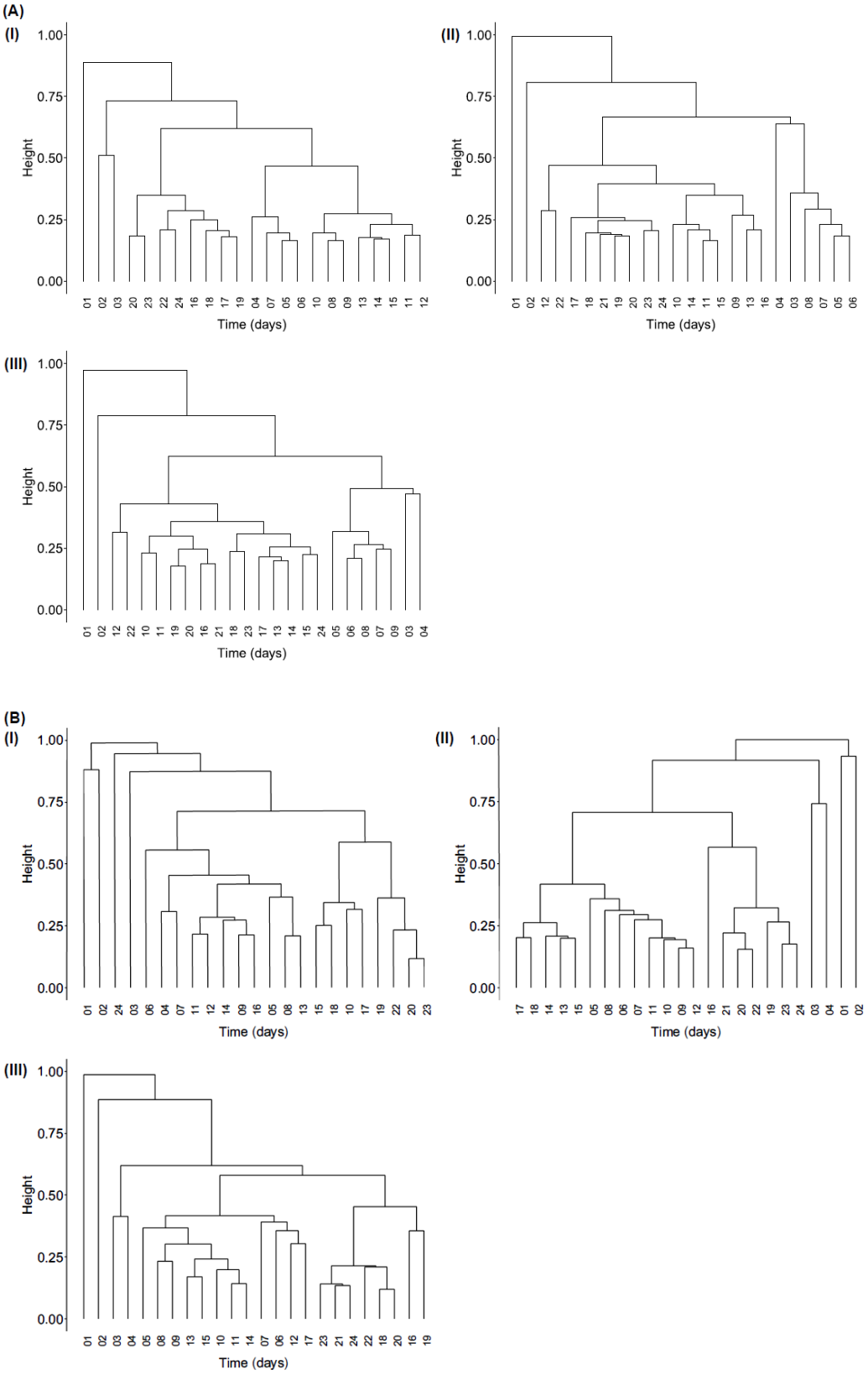

**Supplementary Figure 6.** The variation of evenness (Pielou's Index) through time for each bioreactor (B1, B2, and B3) and of B0, the water collected in-situ. The gap between the in-situ sampling and the incubation for fig. C and D represent the first phase of the second experiment where no samples were collected. A) sessile bacteria experiment *E1*; B) sessile eukaryotes *E1*; C) planktonic bacteria *E2*; and D) planktonic eukaryotes E2.

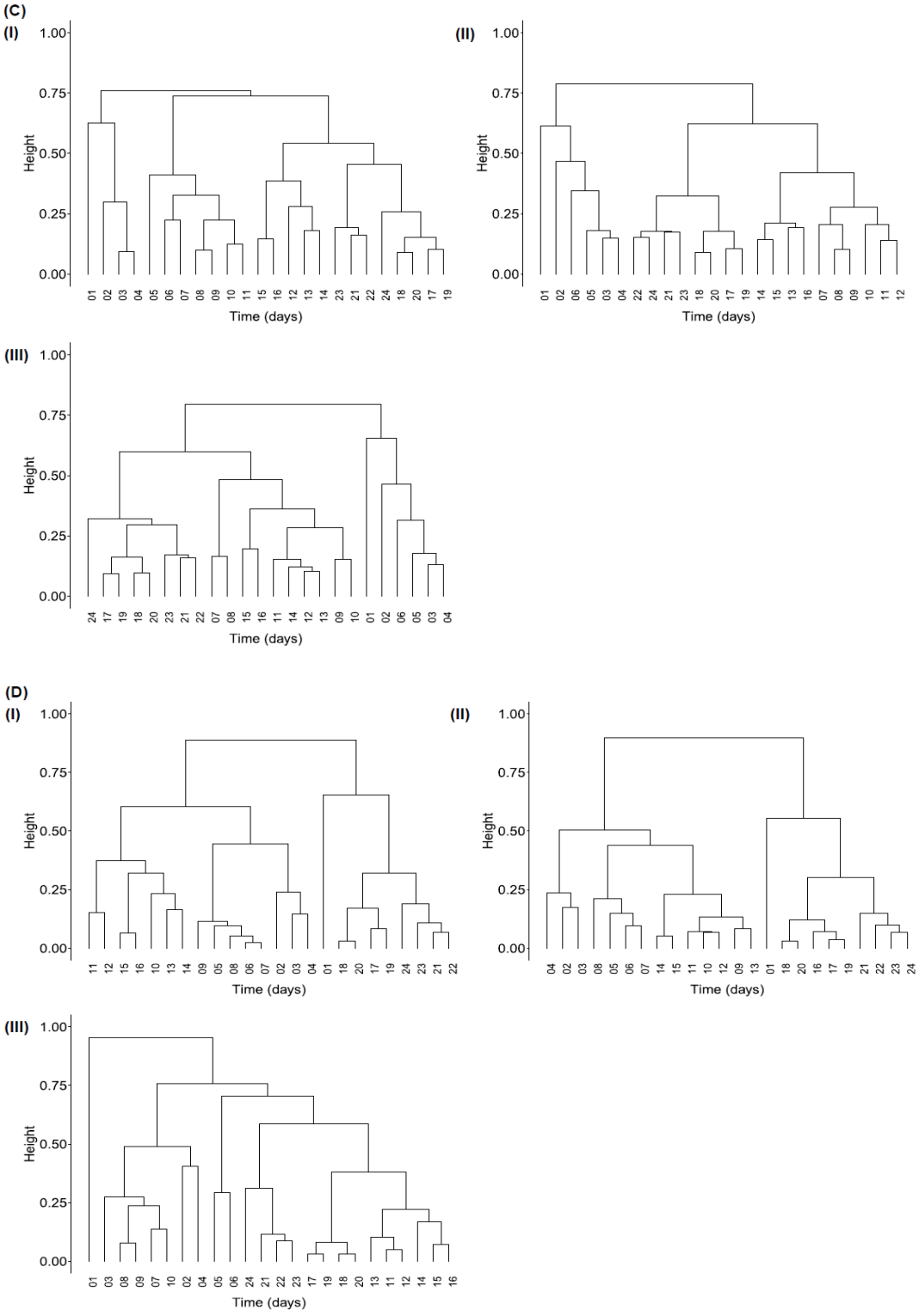



**Supplementary Figure 7.** The variation of observed richness through time for each bioreactor (B1, B2, and B3) and of B0, the water collected in-situ. The gap between the in-situ sampling and the incubation for fig. C and D represent the first phase of the second experiment where no samples were collected. A) sessile bacteria experiment *E1*; B) sessile eukaryotes *E1*; C) planktonic bacteria *E2*; and D) planktonic eukaryotes, *E2*.

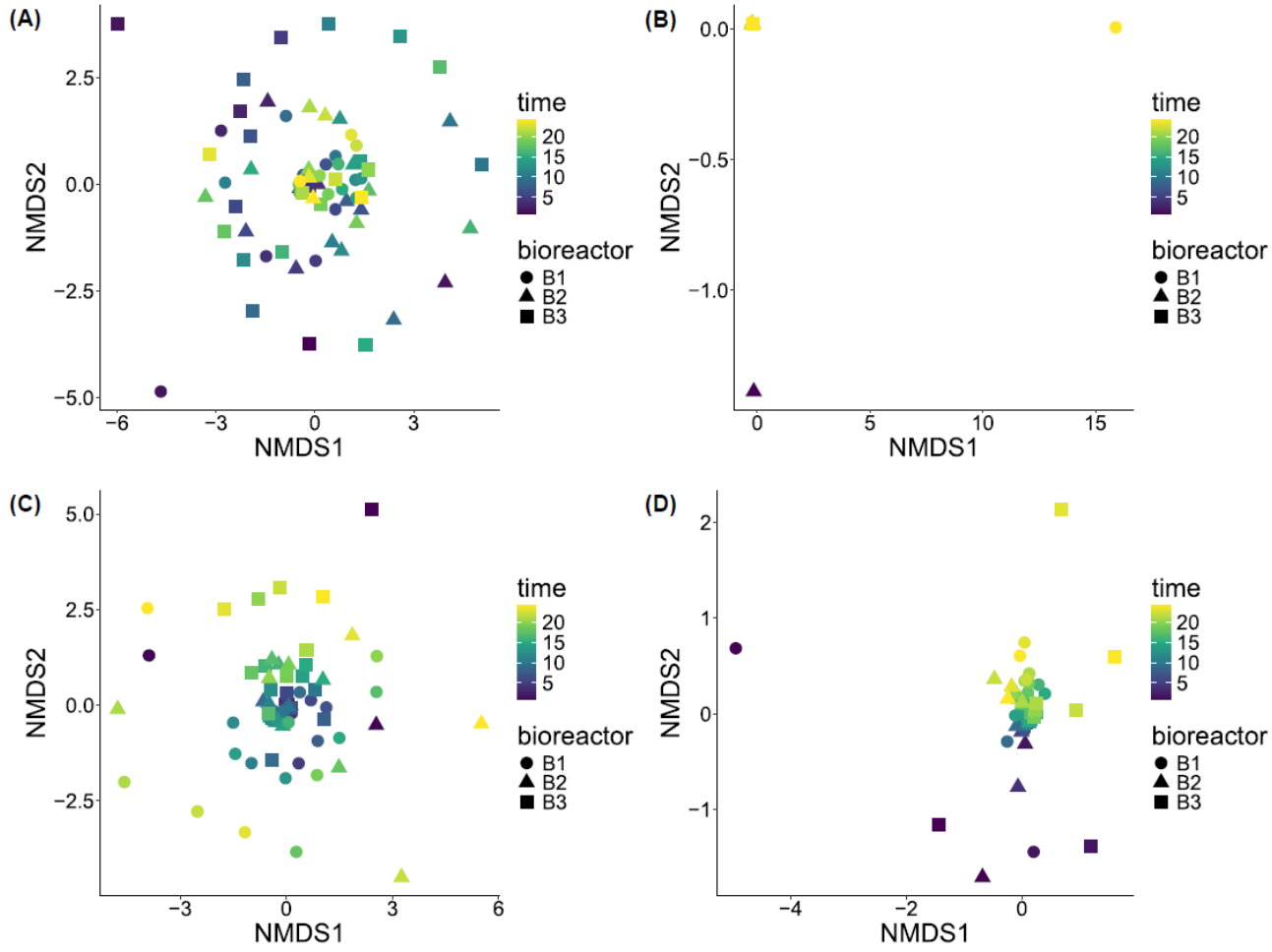



**Supplementary Figure 8.** Partial effect of DIC concentration on the Shannon index of the planktonic bacterial community of *E2*.

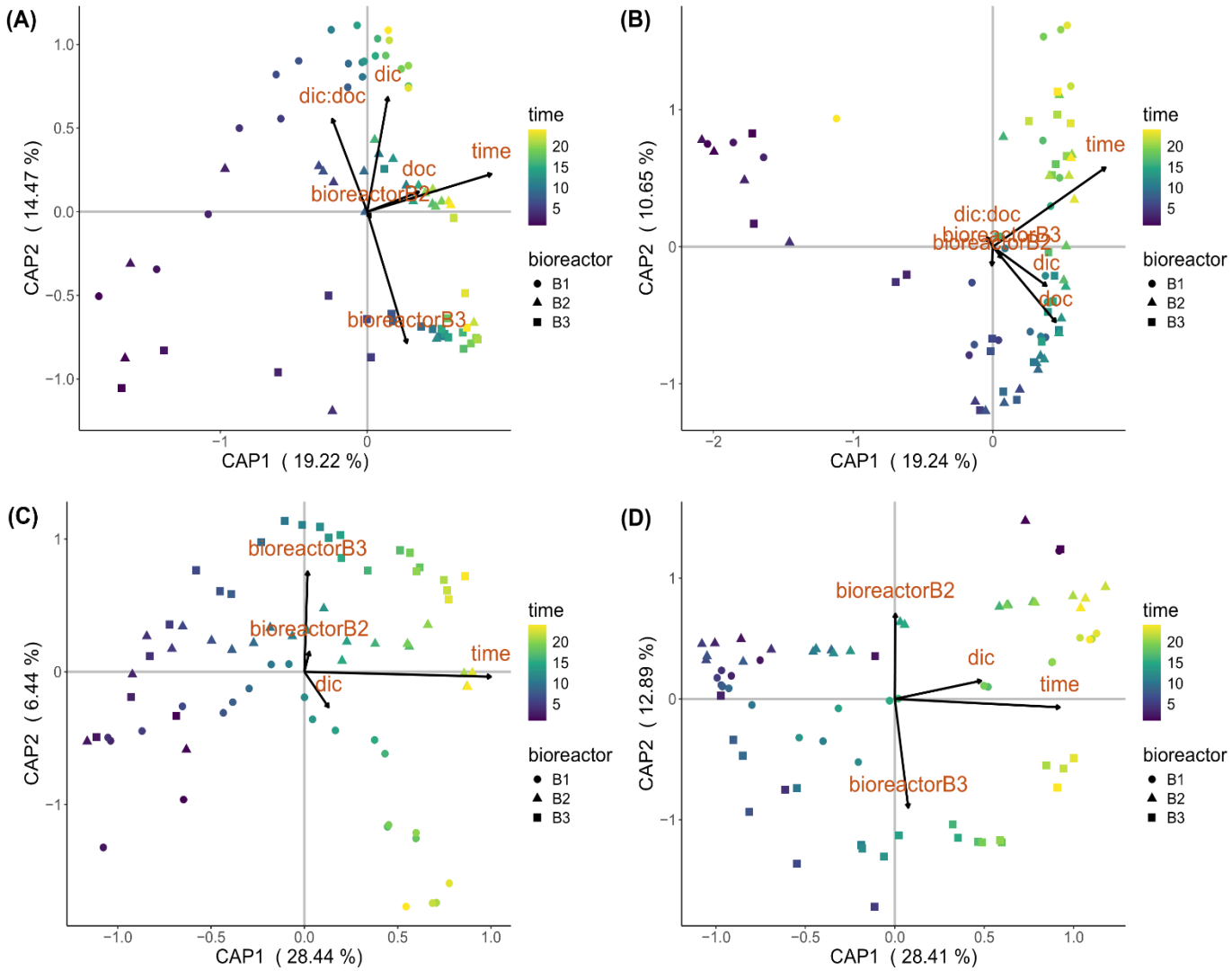

(A)
(I)
(II)
(III)
(B)
(I)
(II)
(III)
Height
Time (days)


(C)
(I)
(II)
(III)
(D)
(I)
(II)
(III)
Height
Time (days)

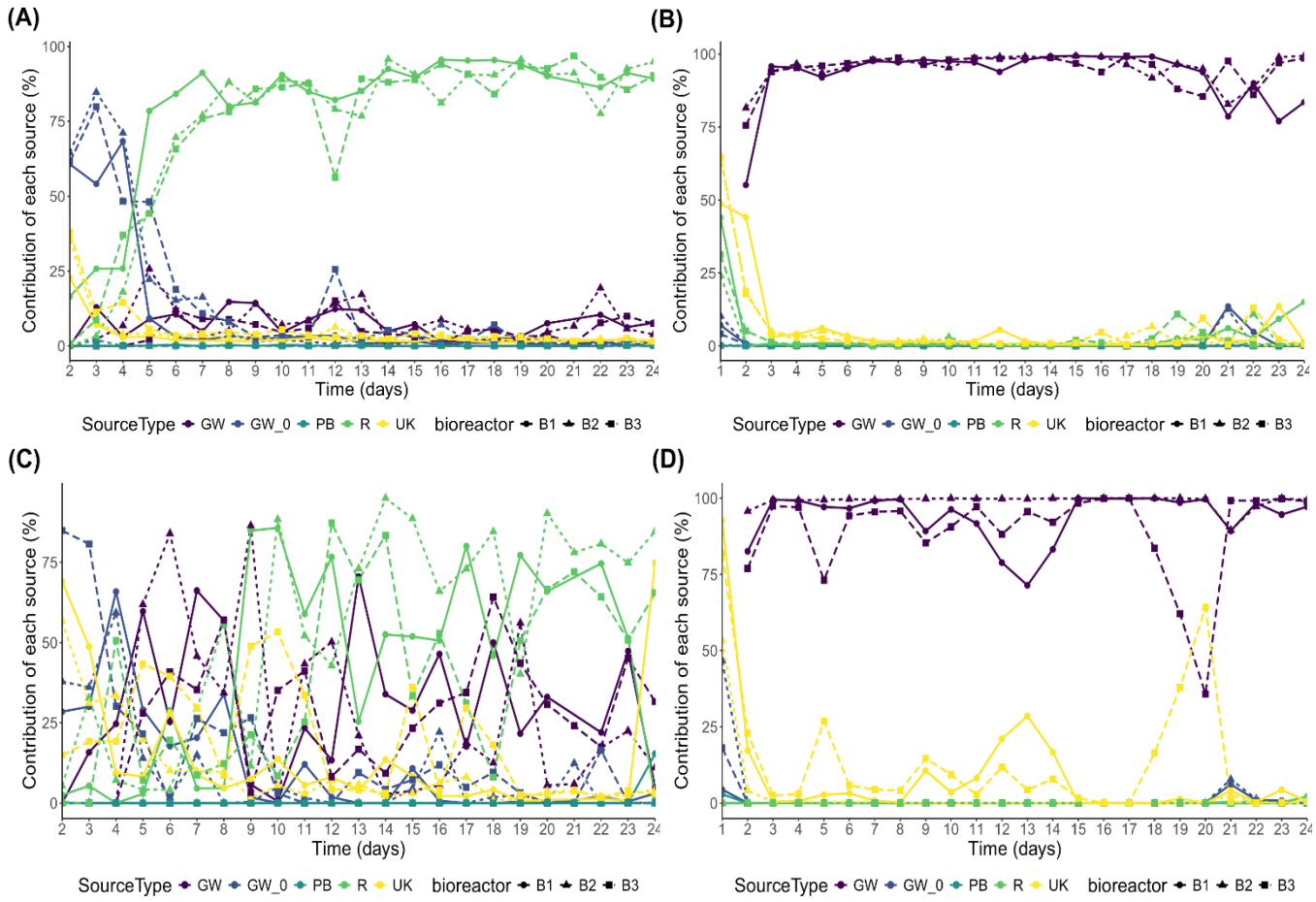
**Supplementary Figure 9.** Cluster dendrograms of the communities in each bioreactor. A) sessile bacteria experiment *E1*; B) sessile eukaryotes *E1*; C) planktonic bacteria *E2*; D) planktonic eukaryotes *E2*; I) Bioreactor B1; II) Bioreactor B2; and III) Bioreactor B3.




**Supplementary Figure 10.** NMDS of the beta diversity (Hellinger transformation) of all communities. A) sessile bacteria *E1*, stress = 0.0905; B) sessile eukaryotes *E1*, stress = 8.56*10^-5; C) planktonic bacteria, *E2*, stress = 0.1079; D) planktonic eukaryotes, *E2*, stress = 0.114.




**Supplementary Figure 21.** Db-RDA graph showing the correlation of physico- and geo-chemical parameters (DIC, DOC, interaction of DIC and DOC, time and each individual bioreactor) with the beta diversity of all communities (Bray-Curtis matrix) through time. A) sessile bacteria experiment *E1*; B) sessile eukaryotes *E1*; C) planktonic bacteria *E2*; and D) planktonic eukaryotes E2. DOC, dissolved organic carbon; DIC, dissolved inorganic carbon. B1 is not displayed as B2 and B3 are compared to B1.




**Supplementary Figure 32.** Summed cumulative contribution percentages of each source through time for A) sessile community of Bacteria experiment *E1*; B) planktonic community of bacteria *E2*; C); The sessile community of Eukaryotes *E1*; and D) planktonic community of Eukaryotes *E2*. GW, planktonic community in the incubation water; GW_0, planktonic community in the groundwater collected at the sampling site; PB, community of the peat bog at the sampling site; R, sessile community; and UK, remainder of the variation that is attributed to an unknown source.

**Supplementary Tables**

**Supplementary Table 1**. PCR conditions used for 16S or 18S rRNA gene amplification.

| Domain | | Denaturation | Annealing | Extension | Final Extension |
|---|---|---|---|---|---|
| Bacteria | Nb of cycles | | 35 | | 1 |
| | Time (s) | 30 | 60 | | 30 |
| | Temperature (℃) | 98 | 57 | | 98 |
| Eukaryote | Nb of cycles | | 35 | | 1 |
| | Time (s) | 30 | 30 | | 30 |
| | Temperature (℃) | 98 | 61 | | 98 |
| Archaea | Nb of cycles | | 35 | | 1 |
| | Time (s) | 30 | 30 | 60 | 600 |
| | Temperature (℃) | 98 | 67 | 72 | 72 |

**Supplementary Table 2.** Summary of the geochemical characteristics of each experiment: A) *E1*, B) *E2*; and C) Raw geochemical measurements for all samples.

A)

| | pH | Dissolved $O_2$ (%) | DIC (mg/L) | DOC (mg/L) |
|---|---|---|---|---|
| Min. | 4.600 | 23.900 | 10.350 | 24.110 |
| 1st Qu. | 4.955 | 42.000 | 24.090 | 27.320 |
| Median | 5.030 | 56.800 | 35.320 | 28.900 |
| Mean | 5.096 | 55.340 | 41.780 | 29.170 |
| 3rd Qu. | 5.190 | 66.900 | 65.260 | 30.870 |
| Max. | 6.010 | 86.800 | 81.680 | 37.210 |

B)

| | pH | Dissolved $O_2$ (%) | DIC (mg/L) | DOC (mg/L) |
|---|---|---|---|---|
| Min. | 4.170 | 47.000 | 8.410 | 16.060 |
| 1st Qu. | 4.370 | 56.250 | 45.090 | 18.060 |
| Median | 4.410 | 58.850 | 48.940 | 19.230 |
| Mean | 4.425 | 59.700 | 48.930 | 19.160 |
| 3rd Qu. | 4.480 | 61.850 | 56.150 | 20.250 |
| Max. | 4.890 | 82.300 | 73.830 | 22.590 |

C)

| sample name | Sample type | Rock pellet position | Temperature (℃) | pH | Dissolved $O_2$ (%) | DIC (mg/L) | DOC (mg/L) |
|---|---|---|---|---|---|---|---|
| E1-R-T01-B1-1 | Rock | 1 | 13-14 | 5.98 | 57.5 | | |
| E1-R-T01-B2-1 | Rock | 1 | 13-14 | 6.01 | 56.8 | | |
| E1-R-T01-B3-1 | Rock | 1 | 13-14 | 5.33 | 53.9 | | |
| E1-R-T02-B1-1 | Rock | 2 | 13-14 | 5.57 | 29.9 | 11.85 | 27.405 |
| E1-R-T02-B2-1 | Rock | 2 | 13-14 | 5.53 | 29.5 | 10.62 | 26.145 |
| E1-R-T02-B3-1 | Rock | 2 | 13-14 | 5.51 | 30.4 | 12.215 | 24.105 |
| E1-R-T03-B1-1 | Rock | 3 | 13-14 | 5.29 | 32.9 | 24.845 | 24.265 |
| E1-R-T03-B2-1 | Rock | 3 | 13-14 | 5.24 | 33.6 | 22.965 | 28.005 |
| E1-R-T03-B3-1 | Rock | 3 | 13-14 | 5.31 | 36.8 | 24 | 25.415 |
| E1-R-T04-B1-1 | Rock | 1 | 13-14 | 5.12 | 43.5 | 35.11 | 31.25 |
| E1-R-T04-B2-1 | Rock | 1 | 13-14 | 5.05 | 47.7 | 38.83 | 32.53 |
| E1-R-T04-B3-1 | Rock | 1 | 13-14 | 5.14 | 42.8 | 27.495 | 31.435 |
| E1-R-T05-B1-1 | Rock | 2 | 13-14 | 4.88 | 61.4 | 69.61 | 25.855 |
| E1-R-T05-B2-1 | Rock | 2 | 13-14 | 4.93 | 57.3 | 81.68 | 29.215 |
| E1-R-T05-B3-1 | Rock | 2 | 13-14 | 4.9 | 56.4 | 76.255 | 25.945 |
| E1-R-T06-B1-1 | Rock | 3 | 13-14 | 4.94 | 58.6 | 70.925 | 29.225 |
| E1-R-T06-B2-1 | Rock | 3 | 13-14 | 4.98 | 53.3 | 51.485 | 30.97 |
| E1-R-T06-B3-1 | Rock | 3 | 13-14 | 5.04 | 55.8 | 64.275 | 27.96 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| E1-R-T07-B1-1 | Rock | 1 | 13-14 | 4.9 | 63.4 | 69.6 | 28.795 |
| E1-R-T07-B2-1 | Rock | 1 | 13-14 | 5.03 | 56.1 | 73.305 | 28.635 |
| E1-R-T07-B3-1 | Rock | 1 | 13-14 | 5.02 | 61.5 | 61.44 | 28.35 |
| E1-R-T08-B1-1 | Rock | 2 | 13-14 | 5.08 | 36.8 | 69.675 | 31.58 |
| E1-R-T08-B2-1 | Rock | 2 | 13-14 | 5.13 | 42.8 | 44.685 | 29.12 |
| E1-R-T08-B3-1 | Rock | 2 | 13-14 | 4.99 | 47.8 | 36.86 | 29.01 |
| E1-R-T09-B1-1 | Rock | 3 | 13-14 | 4.97 | 60.4 | 66.505 | 30.46 |
| E1-R-T09-B2-1 | Rock | 3 | 13-14 | 5.12 | 41.2 | 27.3 | 29.42 |
| E1-R-T09-B3-1 | Rock | 3 | 13-14 | 5.28 | 35.9 | 19.305 | 30.235 |
| E1-R-T10-B1-1 | Rock | 1 | 13-14 | 5.01 | 57.1 | 73.745 | 28.94 |
| E1-R-T10-B2-1 | Rock | 1 | 13-14 | 5.13 | 45.5 | 40.6 | 33.93 |
| E1-R-T10-B3-1 | Rock | 1 | 13-14 | 5.4 | 25.8 | 14.215 | 33.82 |
| E1-R-T11-B1-1 | Rock | 2 | 13-14 | 4.96 | 62.4 | 71.205 | 29.07 |
| E1-R-T11-B2-1 | Rock | 2 | 13-14 | 5.17 | 38.8 | 31.755 | 37.21 |
| E1-R-T11-B3-1 | Rock | 2 | 13-14 | 5.37 | 23.9 | 10.345 | 33.135 |
| E1-R-T12-B1-1 | Rock | 3 | 13-14 | 4.97 | 54.9 | 75.71 | 28.9 |
| E1-R-T12-B2-1 | Rock | 3 | 13-14 | 5.21 | 40.2 | 35.765 | 31.88 |
| E1-R-T12-B3-1 | Rock | 3 | 13-14 | 5.35 | 32.1 | 15.775 | 33.305 |
| E1-R-T13-B1-1 | Rock | 1 | 13-14 | 5.11 | 63.7 | 70.91 | 28.73 |
| E1-R-T13-B2-1 | Rock | 1 | 13-14 | 5.16 | 48.8 | 35.505 | 29.765 |
| E1-R-T13-B3-1 | Rock | 1 | 13-14 | 5.3 | 34.2 | 20.155 | 32.435 |
| E1-R-T14-B1-1 | Rock | 2 | 13-14 | 4.91 | 65.5 | 74.165 | 32.01 |
| E1-R-T14-B2-1 | Rock | 2 | 13-14 | 5.14 | 41.2 | 32.28 | 29.82 |
| E1-R-T14-B3-1 | Rock | 2 | 13-14 | 5.3 | 38.4 | 20.525 | 35.245 |
| E1-R-T15-B1-1 | Rock | 3 | 13-14 | 4.94 | 64.3 | 72.075 | 30.35 |
| E1-R-T15-B2-1 | Rock | 3 | 13-14 | 5.11 | 43.8 | 66.23 | 28.965 |
| E1-R-T15-B3-1 | Rock | 3 | 13-14 | 4.98 | 40.1 | 29.625 | 31.555 |
| E1-R-T16-B1-1 | Rock | 1 | 13-14 | 4.93 | 64.7 | 72.365 | 28.5 |
| E1-R-T16-B2-1 | Rock | 1 | 13-14 | 5.23 | 49.2 | 34.48 | 31.72 |
| E1-R-T16-B3-1 | Rock | 1 | 13-14 | 5.21 | 47.4 | 41.105 | 29.625 |
| E1-R-T17-B1-1 | Rock | 2 | 13-14 | 4.97 | 70.4 | 69.1 | 27.4 |
| E1-R-T17-B2-1 | Rock | 2 | 13-14 | 5.17 | 53.7 | 35.32 | 34.59 |
| E1-R-T17-B3-1 | Rock | 2 | 13-14 | 4.96 | 65.8 | 58.075 | 26.855 |
| E1-R-T18-B1-1 | Rock | 3 | 13-14 | 4.77 | 82.6 | 32.045 | 29.26 |
| E1-R-T18-B2-1 | Rock | 3 | 13-14 | 4.88 | 86.8 | 23.98 | 30.765 |
| E1-R-T18-B3-1 | Rock | 3 | 13-14 | 4.99 | 86.7 | 31.94 | 27.24 |
| E1-R-T19-B1-1 | Rock | 1 | 13-14 | 4.86 | 70.4 | 39.62 | 26.985 |
| E1-R-T19-B2-1 | Rock | 1 | 13-14 | 4.96 | 70.7 | 23.295 | 27.92 |
| E1-R-T19-B3-1 | Rock | 1 | 13-14 | 4.98 | 72 | 25.165 | 27.93 |
| E1-R-T20-B1-1 | Rock | 2 | 13-14 | 4.8 | 72 | 61.54 | 28.145 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| E1-R-T20-B2-1 | Rock | 2 | 13-14 | 4.98 | 66.5 | 20.68 | 27.23 |
| E1-R-T20-B3-1 | Rock | 2 | 13-14 | 5.07 | 69.5 | 22.7 | 27.535 |
| E1-R-T21-B2-1 | Rock | 3 | 13-14 | 5 | 66.1 | 24.17 | 28.355 |
| E1-R-T21-B3-1 | Rock | 3 | 13-14 | 4.97 | 84.1 | 41.435 | 26.515 |
| E1-R-T22-B1-1 | Rock | 1 | 13-14 | 4.88 | 77.2 | 68.485 | 30.38 |
| E1-R-T22-B2-1 | Rock | 1 | 13-14 | 4.98 | 73.8 | 34.175 | 28.44 |
| E1-R-T22-B3-1 | Rock | 1 | 13-14 | 5.04 | 71.9 | 30.34 | 25.935 |
| E1-R-T23-B1-1 | Rock | 2 | 13-14 | 4.95 | 72.1 | 62.315 | 27.675 |
| E1-R-T23-B2-1 | Rock | 2 | 13-14 | 5.08 | 66.7 | 32.875 | 33.46 |
| E1-R-T23-B3-1 | Rock | 2 | 13-14 | 4.93 | 80.7 | 32.845 | 26.425 |
| E1-R-T24-B1-1 | Rock | 3 | 13-14 | 4.6 | 67.1 | 64.285 | 26.61 |
| E1-R-T24-B2-1 | Rock | 3 | 13-14 | 4.89 | 67.5 | 35.715 | 26.55 |
| E1-R-T24-B3-1 | Rock | 3 | 13-14 | 4.92 | 68.5 | 32.44 | 26.65 |
| E1-W-T02-B1-1 | Groundwater | 2 | 13-14 | 5.57 | 29.9 | 11.85 | 27.405 |
| E1-W-T02-B2-1 | Groundwater | 2 | 13-14 | 5.53 | 29.5 | 10.62 | 26.145 |
| E1-W-T02-B3-1 | Groundwater | 2 | 13-14 | 5.51 | 30.4 | 12.215 | 24.105 |
| E1-W-T03-B1-1 | Groundwater | 3 | 13-14 | 5.29 | 32.9 | 24.845 | 24.265 |
| E1-W-T03-B2-1 | Groundwater | 3 | 13-14 | 5.24 | 33.6 | 22.965 | 28.005 |
| E1-W-T03-B3-1 | Groundwater | 3 | 13-14 | 5.31 | 36.8 | 24 | 25.415 |
| E1-W-T04-B1-1 | Groundwater | 1 | 13-14 | 5.12 | 43.5 | 35.11 | 31.25 |
| E1-W-T04-B2-1 | Groundwater | 1 | 13-14 | 5.05 | 47.7 | 38.83 | 32.53 |
| E1-W-T04-B3-1 | Groundwater | 1 | 13-14 | 5.14 | 42.8 | 27.495 | 31.435 |
| E1-W-T05-B1-1 | Groundwater | 2 | 13-14 | 4.88 | 61.4 | 69.61 | 25.855 |
| E1-W-T05-B2-1 | Groundwater | 2 | 13-14 | 4.93 | 57.3 | 81.68 | 29.215 |
| E1-W-T05-B3-1 | Groundwater | 2 | 13-14 | 4.9 | 56.4 | 76.255 | 25.945 |
| E1-W-T06-B1-1 | Groundwater | 3 | 13-14 | 4.94 | 58.6 | 70.925 | 29.225 |
| E1-W-T06-B2-1 | Groundwater | 3 | 13-14 | 4.98 | 53.3 | 51.485 | 30.97 |
| E1-W-T06-B3-1 | Groundwater | 3 | 13-14 | 5.04 | 55.8 | 64.275 | 27.96 |
| E1-W-T07-B1-1 | Groundwater | 1 | 13-14 | 4.9 | 63.4 | 69.6 | 28.795 |
| E1-W-T07-B2-1 | Groundwater | 1 | 13-14 | 5.03 | 56.1 | 73.305 | 28.635 |
| E1-W-T07-B3-1 | Groundwater | 1 | 13-14 | 5.02 | 61.5 | 61.44 | 28.35 |
| E1-W-T08-B1-1 | Groundwater | 2 | 13-14 | 5.08 | 36.8 | 69.675 | 31.58 |
| E1-W-T08-B2-1 | Groundwater | 2 | 13-14 | 5.13 | 42.8 | 44.685 | 29.12 |
| E1-W-T08-B3-1 | Groundwater | 2 | 13-14 | 4.99 | 47.8 | 36.86 | 29.01 |
| E1-W-T09-B1-1 | Groundwater | 3 | 13-14 | 4.97 | 60.4 | 66.505 | 30.46 |
| E1-W-T09-B2-1 | Groundwater | 3 | 13-14 | 5.12 | 41.2 | 27.3 | 29.42 |
| E1-W-T09-B3-1 | Groundwater | 3 | 13-14 | 5.28 | 35.9 | 19.305 | 30.235 |
| E1-W-T10-B1-1 | Groundwater | 1 | 13-14 | 5.01 | 57.1 | 73.745 | 28.94 |
| E1-W-T10-B2-1 | Groundwater | 1 | 13-14 | 5.13 | 45.5 | 40.6 | 33.93 |
| E1-W-T10-B3-1 | Groundwater | 1 | 13-14 | 5.4 | 25.8 | 14.215 | 33.82 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| E1-W-T11-B1-1 | Groundwater | 2 | 13-14 | 4.96 | 62.4 | 71.205 | 29.07 |
| E1-W-T11-B2-1 | Groundwater | 2 | 13-14 | 5.17 | 38.8 | 31.755 | 37.21 |
| E1-W-T11-B3-1 | Groundwater | 2 | 13-14 | 5.37 | 23.9 | 10.345 | 33.135 |
| E1-W-T12-B1-1 | Groundwater | 3 | 13-14 | 4.97 | 54.9 | 75.71 | 28.9 |
| E1-W-T12-B2-1 | Groundwater | 3 | 13-14 | 5.21 | 40.2 | 35.765 | 31.88 |
| E1-W-T12-B3-1 | Groundwater | 3 | 13-14 | 5.35 | 32.1 | 15.775 | 33.305 |
| E1-W-T13-B1-1 | Groundwater | 1 | 13-14 | 5.11 | 63.7 | 70.91 | 28.73 |
| E1-W-T13-B2-1 | Groundwater | 1 | 13-14 | 5.16 | 48.8 | 35.505 | 29.765 |
| E1-W-T13-B3-1 | Groundwater | 1 | 13-14 | 5.3 | 34.2 | 20.155 | 32.435 |
| E1-W-T14-B1-1 | Groundwater | 2 | 13-14 | 4.91 | 65.5 | 74.165 | 32.01 |
| E1-W-T14-B2-1 | Groundwater | 2 | 13-14 | 5.14 | 41.2 | 32.28 | 29.82 |
| E1-W-T14-B3-1 | Groundwater | 2 | 13-14 | 5.3 | 38.4 | 20.525 | 35.245 |
| E1-W-T15-B1-1 | Groundwater | 3 | 13-14 | 4.94 | 64.3 | 72.075 | 30.35 |
| E1-W-T15-B2-1 | Groundwater | 3 | 13-14 | 5.11 | 43.8 | 66.23 | 28.965 |
| E1-W-T15-B3-1 | Groundwater | 3 | 13-14 | 4.98 | 40.1 | 29.625 | 31.555 |
| E1-W-T16-B1-1 | Groundwater | 1 | 13-14 | 4.93 | 64.7 | 72.365 | 28.5 |
| E1-W-T16-B2-1 | Groundwater | 1 | 13-14 | 5.23 | 49.2 | 34.48 | 31.72 |
| E1-W-T16-B3-1 | Groundwater | 1 | 13-14 | 5.21 | 47.4 | 41.105 | 29.625 |
| E1-W-T17-B1-1 | Groundwater | 2 | 13-14 | 4.97 | 70.4 | 69.1 | 27.4 |
| E1-W-T17-B2-1 | Groundwater | 2 | 13-14 | 5.17 | 53.7 | 35.32 | 34.59 |
| E1-W-T17-B3-1 | Groundwater | 2 | 13-14 | 4.96 | 65.8 | 58.075 | 26.855 |
| E1-W-T18-B1-1 | Groundwater | 3 | 13-14 | 4.77 | 82.6 | 32.045 | 29.26 |
| E1-W-T18-B2-1 | Groundwater | 3 | 13-14 | 4.88 | 86.8 | 23.98 | 30.765 |
| E1-W-T18-B3-1 | Groundwater | 3 | 13-14 | 4.99 | 86.7 | 31.94 | 27.24 |
| E1-W-T19-B1-1 | Groundwater | 1 | 13-14 | 4.86 | 70.4 | 39.62 | 26.985 |
| E1-W-T19-B2-1 | Groundwater | 1 | 13-14 | 4.96 | 70.7 | 23.295 | 27.92 |
| E1-W-T19-B3-1 | Groundwater | 1 | 13-14 | 4.98 | 72 | 25.165 | 27.93 |
| E1-W-T20-B1-1 | Groundwater | 2 | 13-14 | 4.8 | 72 | 61.54 | 28.145 |
| E1-W-T20-B2-1 | Groundwater | 2 | 13-14 | 4.98 | 66.5 | 20.68 | 27.23 |
| E1-W-T20-B3-1 | Groundwater | 2 | 13-14 | 5.07 | 69.5 | 22.7 | 27.535 |
| E1-W-T21-B1-1 | Groundwater | 3 | 13-14 | 4.78 | 63.8 | 64.765 | 29.2 |
| E1-W-T21-B2-1 | Groundwater | 3 | 13-14 | 5 | 66.1 | 24.17 | 28.355 |
| E1-W-T21-B3-1 | Groundwater | 3 | 13-14 | 4.97 | 84.1 | 41.435 | 26.515 |
| E1-W-T22-B1-1 | Groundwater | 1 | 13-14 | 4.88 | 77.2 | 68.485 | 30.38 |
| E1-W-T22-B2-1 | Groundwater | 1 | 13-14 | 4.98 | 73.8 | 34.175 | 28.44 |
| E1-W-T22-B3-1 | Groundwater | 1 | 13-14 | 5.04 | 71.9 | 30.34 | 25.935 |
| E1-W-T23-B1-1 | Groundwater | 2 | 13-14 | 4.95 | 72.1 | 62.315 | 27.675 |
| E1-W-T23-B2-1 | Groundwater | 2 | 13-14 | 5.08 | 66.7 | 32.875 | 33.46 |
| E1-W-T23-B3-1 | Groundwater | 2 | 13-14 | 4.93 | 80.7 | 32.845 | 26.425 |
| E1-W-T24-B1-1 | Groundwater | 3 | 13-14 | 4.6 | 67.1 | 64.285 | 26.61 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| E1-W-T24-B2-1 | Groundwater | 3 | 13-14 | 4.89 | 67.5 | 35.715 | 26.55 |
| E1-W-T24-B3-1 | Groundwater | 3 | 13-14 | 4.92 | 68.5 | 32.44 | 26.65 |
| E2-R-T21-B1-1 | Rock | | 18-19 | 4.1 | 62.4 | 66.865 | 25.02 |
| E2-R-T21-B1-2 | Rock | | 18-19 | 4.1 | 62.4 | 66.865 | 25.02 |
| E2-R-T21-B1-3 | Rock | | 18-19 | 4.1 | 62.4 | 66.865 | 25.02 |
| E2-R-T21-B2-1 | Rock | | 18-19 | 4.14 | 58.8 | 67.665 | 22.48 |
| E2-R-T21-B2-2 | Rock | | 18-19 | 4.14 | 58.8 | 67.665 | 22.48 |
| E2-R-T21-B2-3 | Rock | | 18-19 | 4.14 | 58.8 | 67.665 | 22.48 |
| E2-R-T21-B3-1 | Rock | | 18-19 | 4.24 | 63.5 | 67.825 | 22.895 |
| E2-R-T21-B3-2 | Rock | | 18-19 | 4.24 | 63.5 | 67.825 | 22.895 |
| E2-R-T21-B3-3 | Rock | | 18-19 | 4.24 | 63.5 | 67.825 | 22.895 |
| E2-R-T45-B1-1 | Rock | | 18-19 | 4.38 | 70.3 | 62.46 | 17.635 |
| E2-R-T45-B1-2 | Rock | | 18-19 | 4.38 | 70.3 | 62.46 | 17.635 |
| E2-R-T45-B1-3 | Rock | | 18-19 | 4.38 | 70.3 | 62.46 | 17.635 |
| E2-R-T45-B2-1 | Rock | | 18-19 | 4.46 | 65.6 | 59.55 | 16.06 |
| E2-R-T45-B2-2 | Rock | | 18-19 | 4.46 | 65.6 | 59.55 | 16.06 |
| E2-R-T45-B2-3 | Rock | | 18-19 | 4.46 | 65.6 | 59.55 | 16.06 |
| E2-R-T45-B3-1 | Rock | | 18-19 | 4.42 | 60.9 | 52.96 | 17.275 |
| E2-R-T45-B3-2 | Rock | | 18-19 | 4.42 | 60.9 | 52.96 | 17.275 |
| E2-R-T45-B3-3 | Rock | | 18-19 | 4.42 | 60.9 | 52.96 | 17.275 |
| E2-W-T22-B3-1 | Groundwater | | 18-19 | 4.32 | 79.3 | 69.68 | 22.015 |
| E2-W-T22-B2-1 | Groundwater | | 18-19 | 4.24 | 80 | 68.07 | 21.32 |
| E2-W-T22-B1-1 | Groundwater | | 18-19 | 4.17 | 82.3 | 73.835 | 22.59 |
| E2-W-T23-B3-1 | Groundwater | | 18-19 | 4.38 | 58.2 | 45.265 | 21.19 |
| E2-W-T23-B2-1 | Groundwater | | 18-19 | 4.33 | 55.9 | 45.585 | 21.085 |
| E2-W-T23-B1-1 | Groundwater | | 18-19 | 4.3 | 57.8 | 43.72 | 20.41 |
| E2-W-T24-B3-1 | Groundwater | | 18-19 | 4.37 | 62 | 48.765 | 20.26 |
| E2-W-T24-B2-1 | Groundwater | | 18-19 | 4.41 | 53.3 | 45.625 | 20.245 |
| E2-W-T24-B1-1 | Groundwater | | 18-19 | 4.29 | 56 | 49.675 | 21.585 |
| E2-W-T25-B3-1 | Groundwater | | 18-19 | 4.37 | 61.3 | 51.155 | 20.48 |
| E2-W-T25-B2-1 | Groundwater | | 18-19 | 4.51 | 56 | 41.875 | 20.48 |
| E2-W-T25-B1-1 | Groundwater | | 18-19 | 4.37 | 62.8 | 49.24 | 20.295 |
| E2-W-T26-B3-1 | Groundwater | | 18-19 | 4.39 | 58.7 | 48.29 | 19.86 |
| E2-W-T26-B2-1 | Groundwater | | 18-19 | 4.37 | 56.1 | 44.255 | 19.975 |
| E2-W-T26-B1-1 | Groundwater | | 18-19 | 4.35 | 60.3 | 50.23 | 20.84 |
| E2-W-T27-B3-1 | Groundwater | | 18-19 | 4.39 | 58 | 50.01 | 20.345 |
| E2-W-T27-B2-1 | Groundwater | | 18-19 | 4.38 | 59.4 | 47.675 | 20.24 |
| E2-W-T27-B1-1 | Groundwater | | 18-19 | 4.37 | 60.3 | 54.85 | 20.095 |
| E2-W-T28-B3-1 | Groundwater | | 18-19 | 4.38 | 60.9 | 48.835 | 19.74 |
| E2-W-T28-B2-1 | Groundwater | | 18-19 | 4.48 | 53.3 | 30.845 | 20.51 |

| | | | | | | |
|---|---|---|---|---|---|---|
| E2-W-T28-B1-1 | Groundwater | 18-19 | 4.3 | 58 | 51.06 | 20.33 |
| E2-W-T29-B3-1 | Groundwater | 18-19 | 4.4 | 60.7 | 46.4 | 20.205 |
| E2-W-T29-B2-1 | Groundwater | 18-19 | 4.42 | 58 | 46.595 | 19.615 |
| E2-W-T29-B1-1 | Groundwater | 18-19 | 4.42 | 56.9 | 48.74 | 20.605 |
| E2-W-T30-B3-1 | Groundwater | 18-19 | 4.38 | 62.8 | 48.41 | 19.515 |
| E2-W-T30-B2-1 | Groundwater | 18-19 | 4.37 | 55.1 | 46.38 | 19.685 |
| E2-W-T30-B1-1 | Groundwater | 18-19 | 4.33 | 56.3 | 50.845 | 19.67 |
| E2-W-T31-B3-1 | Groundwater | 18-19 | 4.43 | 57.6 | 49.04 | 19.345 |
| E2-W-T31-B2-1 | Groundwater | 18-19 | 4.37 | 56.3 | 51.21 | 21.345 |
| E2-W-T31-B1-1 | Groundwater | 18-19 | 4.33 | 56.9 | 49.485 | 19.48 |
| E2-W-T32-B3-1 | Groundwater | 18-19 | 4.39 | 53.5 | 48.075 | 19.135 |
| E2-W-T32-B2-1 | Groundwater | 18-19 | 4.39 | 55.3 | 47.94 | 19.41 |
| E2-W-T32-B1-1 | Groundwater | 18-19 | 4.37 | 56.9 | 48.155 | 19.44 |
| E2-W-T33-B3-1 | Groundwater | 18-19 | 4.46 | 56 | 45.745 | 19.53 |
| E2-W-T33-B2-1 | Groundwater | 18-19 | 4.69 | 48.9 | 15.735 | 19.32 |
| E2-W-T33-B1-1 | Groundwater | 18-19 | 4.37 | 51.6 | 47.075 | 19.035 |
| E2-W-T34-B3-1 | Groundwater | 18-19 | 4.5 | 54 | 41.615 | 18.515 |
| E2-W-T34-B2-1 | Groundwater | 18-19 | 4.82 | 51.5 | 15.1 | 19.985 |
| E2-W-T34-B1-1 | Groundwater | 18-19 | 4.48 | 51.6 | 41.36 | 19.005 |
| E2-W-T35-B3-1 | Groundwater | 18-19 | 4.56 | 60.5 | 38.015 | 18.805 |
| E2-W-T35-B2-1 | Groundwater | 18-19 | 4.52 | 59.8 | 42.45 | 18.98 |
| E2-W-T35-B1-1 | Groundwater | 18-19 | 4.53 | 61.3 | 34.815 | 18.45 |
| E2-W-T36-B3-1 | Groundwater | 18-19 | 4.55 | 58.8 | 41.875 | 18.09 |
| E2-W-T36-B2-1 | Groundwater | 18-19 | 4.54 | 56.5 | 40.175 | 18.055 |
| E2-W-T36-B1-1 | Groundwater | 18-19 | 4.5 | 64.1 | 44.55 | 19.03 |
| E2-W-T37-B3-1 | Groundwater | 18-19 | 4.52 | 53.6 | 42.885 | 18.04 |
| E2-W-T37-B2-1 | Groundwater | 18-19 | 4.5 | 50.5 | 42.67 | 17.845 |
| E2-W-T37-B1-1 | Groundwater | 18-19 | 4.49 | 56.5 | 45.28 | 18.685 |
| E2-W-T38-B3-1 | Groundwater | 18-19 | 4.48 | 60.3 | 53.03 | 18.115 |
| E2-W-T38-B2-1 | Groundwater | 18-19 | 4.47 | 57.3 | 50.26 | 17.765 |
| E2-W-T38-B1-1 | Groundwater | 18-19 | 4.89 | 47 | 8.41 | 18.55 |
| E2-W-T39-B3-1 | Groundwater | 18-19 | 4.43 | 66.5 | 55.905 | 17.72 |
| E2-W-T39-B2-1 | Groundwater | 18-19 | 4.65 | 57 | 27.96 | 17.79 |
| E2-W-T39-B1-1 | Groundwater | 18-19 | 4.45 | 58 | 58.3 | 18.11 |
| E2-W-T40-B3-1 | Groundwater | 18-19 | 4.4 | 63.3 | 59.405 | 17.235 |
| E2-W-T40-B2-1 | Groundwater | 18-19 | 4.43 | 63.1 | 56.795 | 17.815 |
| E2-W-T40-B1-1 | Groundwater | 18-19 | 4.42 | 61.4 | 63.995 | 18.555 |
| E2-W-T41-B3-1 | Groundwater | 18-19 | 4.48 | 61.8 | 55.785 | 17.085 |
| E2-W-T41-B2-1 | Groundwater | 18-19 | 4.41 | 67.7 | 58.405 | 17.27 |
| E2-W-T41-B1-1 | Groundwater | 18-19 | 4.32 | 62.1 | 59.59 | 18.445 |

| | | | | | | |
|---|---|---|---|---|---|---|
| E2-W-T42-B3-1 | Groundwater | 18-19 | 4.42 | 60.8 | 56.15 | 18.065 |
| E2-W-T42-B2-1 | Groundwater | 18-19 | 4.41 | 58.9 | 58.44 | 16.62 |
| E2-W-T42-B1-1 | Groundwater | 18-19 | 4.26 | 59.9 | 61.165 | 20.9 |
| E2-W-T43-B3-1 | Groundwater | 18-19 | 4.4 | 65.9 | 58.75 | 17.28 |
| E2-W-T43-B2-1 | Groundwater | 18-19 | 4.42 | 60.8 | 54.3 | 18.24 |
| E2-W-T43-B1-1 | Groundwater | 18-19 | 4.36 | 60.9 | 56.16 | 17.89 |
| E2-W-T44-B3-1 | Groundwater | 18-19 | 4.47 | 66.3 | 57.245 | 17.31 |
| E2-W-T44-B2-1 | Groundwater | 18-19 | 4.41 | 65.9 | 57.98 | 16.805 |
| E2-W-T44-B1-1 | Groundwater | 18-19 | 4.29 | 65.3 | 60.86 | 18.12 |
| E2-W-T45-B3-1 | Groundwater | 18-19 | 4.42 | 60.9 | 52.96 | 17.275 |
| E2-W-T45-B2-1 | Groundwater | 18-19 | 4.46 | 65.6 | 59.55 | 16.06 |
| E2-W-T45-B1-1 | Groundwater | 18-19 | 4.38 | 70.3 | 62.46 | 17.635 |

**Supplementary Table 3**. P-values of the alpha diversity time series analysis' parameters and the deviance explained by each model. Significant values are in bold.

| | Bacteria *E1* | Bacteria *E2* | Eukaryote *E1* | Eukaryote *E2* |
|---|---|---|---|---|
| DIC*DOC or DIC (p-value) | 0.99 | **$9.10*10^{-7}$** | 0.15 | 0.94 |
| Time (p-value) | **0.0027** | **$<2*10^{-16}$** | **$<2*10^{-16}$** | **$<2*10^{-16}$** |
| Time:B1 (p-value) | 0.52 | **$2.93*10^{-5}$** | 0.83 | **$7.69*10^{4}$** |
| Time:B2 (p-value) | 0.44 | 0.087 | 0.39 | **$2.87*10^{-5}$** |
| Time:B3 (p-value) | 0.30 | 0.33 | **0.031** | **$1.70*10^{-5}$** |
| Bioreactor, bs = 're' (p-value) | 0.36 | **$6.69*10^{-5}$** | **0.0055** | **$9.59*10^{-7}$** |
| Deviance explained (%) | 37 | 94.2 | 62.2 | 94.9 |
| Variations between minimum and maximum | 0.6 | 1.25 | 1 | 1.25 |

**Supplementary Table 4**. Wilcoxon rank sum test comparing the evenness of sessile and planktonic communities for both the Bacteria and Eukaryote domains. Significant values are in bold.

| | W | p-value |
|---|---|---|
| Bacteria | 3123 | **0.02218** |
| Eukaryote | 4738 | **$<2.2*10^{-16}$** |

**Supplementary Table 5.** Anova by contrast (999 permutations) on the Bray-Curtis dissimilarity index of each domain. Significant values are in bold. A) Bacteria; B) Eukaryote.

A)

| Parameters | Df | SumOfSqs | F | Pr(>F) |
|---|---|---|---|---|
| Sample_typeRock | 1 | 6.132631 | 47.099015 | **0.001** |
| experienceE2 | 1 | 11.4686371 | 88.079899 | **0.001** |
| bioreactorB2 | 1 | 0.5819618 | 4.469505 | **0.002** |
| bioreactorB3 | 1 | 1.5382856 | 11.814136 | **0.001** |
| Sample_typeRock:experienceE2 | 1 | 2.3743153 | 18.2349 | **0.001** |
| Residual | 224 | 29.1664131 | NA | NA |

B)

| Parameters | Df | SumOfSqs | F | Pr(>F) |
|---|---|---|---|---|
| Sample_typeRock | 1 | 4.323 | 23.7693 | **0.001** |
| experienceE2 | 1 | 20.069 | 110.3425 | **0.001** |
| bioreactorB2 | 1 | 0.495 | 2.7222 | **0.015** |
| bioreactorB3 | 1 | 0.859 | 4.7243 | **0.002** |
| Sample_typeRock:experienceE2 | 1 | 2.225 | 12.2356 | **0.001** |
| Residual | 224 | 40.74 | | |

**Supplementary Table 6**. Anova and variance partitioning results on the Bray-Curtis dissimilarity index of each community. The percentage of the variance explained by the bioreactors is for all bioreactors whereas the p-values are in contrast to B1. In addition to the values displayed in the table, the variance partitioning test could not disentangle the following effects: Bacteria *E1* shared 3% between bioreactor and DIC, and Eukaryote *E2* shared 2% between DIC and time. Significant values are in bold.

| Predictor variable | Bacteria *E1* | | Bacteria *E2* | | Eukaryote *E1* | | Eukaryote *E2* | |
|---|---|---|---|---|---|---|---|---|
| | Var. part (%) | p-value | Var. part (%) | p-value | Var. part (%) | p-value | Var. part (%) | p-value |
| DIC | 4 | **0.001** | 3 | **0.001** | 6 | **0.001** | 8 | **0.001** |
| DOC | 3 | **0.001** | NA | NA | 7 | **0.001** | NA | NA |
| DIC:DOC | NA | 0.062 | NA | NA | NA | 0.274 | NA | NA |
| Time | 15 | **0.001** | 29 | **0.001** | 16 | **0.001** | 25 | **0.001** |
| Bioreactors | 14 | | 8 | | 4 | | 20 | |
| B2 | | **0.001** | | **0.007** | | 0.218 | | **0.001** |
| B3 | | **0.001** | | **0.001** | | **0.001** | | **0.001** |

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