Sorbonne Université

École Doctorale des Sciences de l'Environnement d'Ile-de-France

Laboratoire d'Océanographie Microbienne (UMR 7621)

Consequences of environmental disturbances on community structure and functioning of aquatic prokaryotes

Par **Angel Rain Franco**

Thèse de doctorat de Sciences de l'environnement (ED129)

Dirigée par Camila Fernandez et Sara Beier

Présentée et soutenue publiquement le 12 novembre 2021

Devant un jury composé de :

Josep Gasol, ICM, Barcelona, Spain, Rapporteur

Jarone Pinhassi , Linnaeus University , Sweden, Rapporteur

Hannes Peter, École polytechnique fédérale de Lausanne, Switzerland, Examinateur

Clara Ruiz-Gonzalez, ICM, Barcelona, Spain, Examinatrice

Ingrid Obernosterer, LOMIC, Banyuls-sur-Mer, France, Examinatrice

Camila Fernandez, LOMIC, Banyuls-sur-Mer, France, Directrice de thèse

Sara Beier, IOW, Germany, Co-Directrice de thèse

ACKNOWLEDGEMENTS

I would like to thank my two supervisors for their guidance, support, and encouragement in the development of my doctoral thesis. I want to thank Camila for trusting in my capacities and giving me the chance to develop my carrier in this amazing place. I want to specially thanks Sara for the courage to take this train with me and for all time you invested in my scientific training and your efforts to provide an adequate environment of cooperation around our team.

I want to thank Pep Gasol and Jarone Pinhassi to accept to be reporters of my thesis. I also thank Clara Ruiz-Gonzalez and Ingrid Obernosterer for been part of the jury in my thesis defense. Thanks also to Hannes Peter and Cyrille Violle for being part my thesis committees and for the valuable comments during this project.

In the first place, I want to thanks Laurence and Josiane from the “Verrerie”, their invaluable help in the laboratory affairs but also for the introduction to the French language, because of you, my first months in the lab are memorable. I want to thank Laurent and Nyree from the BIO2MAR platform for always friendly help on the operation of molecular devices. I also thank Christophe and David from the BioPIC platform for their help in the flow cytometer. I thanks Paul and Eric the “Service d’observation” and Gilles for their unlimited patience related to the favors I asked you.

I want to thank my friends Jingguang, Justine, Nawal and Jana, Yan and Valerie for sharing incredible moments, you were always there to discuss and help. I will especially remember our amazing Fridays eating pizza at the beach and our “very very good” evenings together, I love you guys. I thank Lydvina for always been herself, an incredible and always very energetic person.

I also thank my friends Eva, Kim Joel, and Bantú, and of course, Magda, Alba, Laura, Raphael, and to my unexpected Chilean neighbors Claudia, Jared, Maritza, and the half-Chilean Joffrey. I cannot forget to mention my foster family Vanda and Eric for the infinite love they have for the people around them. I thank Pame for her support during all this process, you are very special friend.

I thank to Nathalie for her good vibes and her to help in one of the most stressful moments of my thesis.

Thanks to the Chilean Agency of Research and development (ANID) for the four years of funding that allow me the pursued studies in France.

I thank my lovely family Fernanda and Mario for supporting me in this journey, I will always love you.

Table of Contents

List of Figures

List of Tables

Introduction

Chapter 1

Chapter 2

Chapter 3

Chapter 4

ABBREVIATIONS

%TF	Fraction of transcription factors
16s rRNA	Bacterial small subunit ribosomal ribonucleic acid
ANOSIM	Analysis of similarities
ANOVA	Analysis of variance
ARISA	Automated Ribosomal Intergenic Spacer Analysis
ASV	Amplicon sequence variant
ASW	Artificial seawater
BEF	Biodiversity-ecosystem functioning
BGE	Bacterial growth efficiency
Chl-a	Chlorophyll-a
CRS	Competitors-Ruderals-Stress tolerant
CUB	Codon usage bias
CV	Coefficient of variation
CWM	Community weighted mean
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
DOP	Dissolved organic phosphorous
FC	Flow cytometry
FDA	Fluorescein diacetate
GTBD	Genome Taxonomy Database
IDH	Intermediate disturbance hypothesis
KEGG	Kyoto Encyclopedia of Genes and Genomes
L2FC	Log2-fold-change
LB medium	Luria-Bertani medium
ML	Machine learning
MLM	Mixed linear models
NB	Niche breadth
NTI	Nearest taxon index
OD	Optical density
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
RF	Random forest
RI	Resistance Index
rmANOVA	Repeated-measurements analyses of variances
RNA	Ribonucleic acid
RRN	Number of 16s rRNA gene copies
RT qPCR	Quantitative reverse transcription PCR
TCA	Trichloroacetic acid

SUMMARY

Microbes are impacted by environmental disturbances and the functional stability of microbial community members after exposure to environmental change eventually affects community dynamics and ecosystem functioning. However, the responses of microbial communities to environmental disturbances are complex, difficult to elucidate and the mechanics of functional stability are still poorly understood. In this thesis, I investigated microbial responses to environmental disturbances from single populations to complex communities. For the single population approach, we addressed the transcriptional response of single bacterial populations with varying niche breadths along an environmental gradient (Chapter 1). To address the consequences of disturbances at the community level, we have established and tested a protocol for cryopreserving complex microbial communities. This work aimed to improve the replicability of experimental studies with natural microbial aquatic community assemblies as inoculum sources (Chapter 2). Furthermore, we have experimentally exposed complex aquatic microbial communities to pulsed disturbances to study the consequences of such disturbances on community structural changes and broad functional parameters, such as bacterial growth efficiency (Chapter 3). Finally, we have inspected in more detail the consequences of pulsed disturbances on processes involved in nitrogen cycling (Chapter 4).

The Chapter 1 assessed the transcriptional regulation of fitness-related and adaptation-related genes of 11 bacterial strains concerning their niche breadth and stress exposure level under changing salinity conditions. The results suggested that transcriptional regulation of fitness- and adaptation-related genes correlated with the niche breadth and/or the stress exposure of the inspected strains. we further identified a shortlist of candidate stress marker genes that could be used in future studies to monitor the susceptibility of bacterial populations or communities to environmental change.

The Chapter 2 developed a method for the preservation and resuscitation of natural aquatic prokaryote assemblages. we studied the impact of inoculum size, processing time, and storage time on the success of resuscitation. We further assessed the effect of growth media supplemented with two different dissolved organic matter (DOM) sources on the recovery of the initially cryopreserved communities obtained from two contrasting sites in terms of trophic status and environmental heterogeneity. Our results demonstrated that the variability of the resuscitation process among replicates decreased with increasing inoculum size. In line with our results, cryopreservation appears as a promising tool to advance experimental research in the field of microbial ecology.

In Chapter 3, the aim was to studied the impact of pulsed disturbances on community functional characteristics and community assembly. We performed a 41-days continuous culture experiment under two disturbance regimes (weekly pulse disturbances and undisturbed controls) that were crossed with two levels of resource availability. The results pointed to a large contribution of stochastic assemblage processes that were evident from deviations in species-level community composition among replicates. In contrast, we found consistent patterns of overall community structural patterns and functional characteristics, such as species diversity, trait distributions, and functional resistance levels in response to the treatments. Community weighted means of genomic traits indicated a high prevalence of resistant and simultaneously fast-growing species particularly in the disturbed communities under high nutrient availability. Furthermore, it was detected an elevated species diversity in this compared to the other treatments. These findings concerning the trait distributions in the community and species diversity patterns likely explain the high functional resistance that was measured in the pulse disturbed communities grown under resource-rich conditions.

The Chapter 4 examined whether the pulse disturbances from the above described continuous culture experiment impacted reactions of the nitrogen cycles, and if so whether this was due to the selection of specific taxa involved in nitrogen cycling. We observed significantly lower nitrate net consumption rates towards the experiment in the disturbed compared to the control treatments. However, concentrations of other nitrogen compounds were not significantly affected by the disturbance regime. We applied a machine learning (ML) approach to explore potential relationships between community composition and nitrate concentrations. The resulting ML-based models predict nitrate consumption with high precision from community composition data (Rho=0.7), demonstrating that changes in community composition were an important driver for the measured nitrate concentrations. We interpreted from the dynamics of nitrogenous compounds in the experiment, in combination with a set of bacterial taxa that had the highest importance for the ML model that reduced denitrification activity under pulse disturbances was the most likely mechanism to explain the observed differences in nitrate consumption rates. Coupled nitrification/denitrification activity in coastal waters are responsible for large scale nitrogen removal, and our finding concerning changed nitrogen cycling under salinity disturbances may have consequences on the magnitude of the nitrogen inputs into the oceans.

In all chapters, I particularly focused on isolates and communities that originated from coastal aquatic habitats that provide important ecosystem services.

RÉSUMÉ

CONTEXTE DE L'ETUDE

Perturbations dans un environnement changeant

Les changements globaux causés par les activités humaines affectent toutes les formes de vie sur terre : par exemple, les activités agricoles, l'industrie et la croissance démographique, et ont provoqué une perte sans précédent de la biodiversité (Barnosky et al., 2011). L'un des aspects les plus menaçants des changements planétaires est le réchauffement climatique (GIEC, 2021) qui est induit par l'émission de gaz à effet de serre via la combustion de combustibles fossiles, la respiration, la méthanogénèse et la dénitrification, ainsi que par des changements dans les réactions qui affectent les flux de carbone mondiaux en raison de la séquestration du carbone (Cavicchioli et al., 2019).

Les perturbations causées par les changements environnementaux contribuent à l'hétérogénéité de l'environnement et l'impact des perturbations peut dépendre de l'état de l'environnement au moment où la perturbation se produit (Hillebrand et Kunze, 2020). Les perturbations sont des événements écologiquement complexes et leurs effets peuvent être observés à différents niveaux trophiques (par exemple, espèce, population, communauté, écosystème) mais aussi en fonction du type de perturbation (par exemple, biotique, abiotique ; Sousa, 1984).

Les perturbations peuvent être classées en fonction de leur gravité dans le temps en perturbations pulsées ou en perturbations continues. Les perturbations pulsées sont des événements à court terme dont l'intensité diminue rapidement jusqu'à une condition de référence antérieure (Lake, 2000), comme les incendies, les tsunamis, les tempêtes, les sécheresses, les fortes précipitations ou les inondations. Les perturbations de type continues sont caractérisées par un changement brusque ou régulier, mais leur pression reste constante dans le temps (Lake, 2000). Par exemple, l'élévation du niveau de la mer causée par la fonte des couvertures neigeuses et des stocks de glace en raison du réchauffement climatique (Pereira et al., 2019) ou la salinisation progressive des écosystèmes côtiers (Williams, 2001) peuvent être classées comme des perturbations continues.

Importance des perturbations dans les environnements marins côtiers et pélagiques

Les côtes du monde, comme les estuaires, les lagunes, les mangroves et les zones humides, forment une zone d'interface étroite entre les zones marines et terrestres dans laquelle se trouvent 40 % de la population humaine et de l'activité économique mondiale (Maul et Duedall, 2019). Les zones côtières comprennent des écosystèmes abritant de nombreuses espèces menacées, et tout en fournissant d'importants services écosystémiques, tels que la protection des côtes, la pêche, des terres agricoles riches, des zones à haute valeur touristique, ainsi que le cycle des nutriments et la séquestration du carbone (Barbier et al., 2011).

Les zones côtières sont également soumises à une forte hétérogénéité environnementale naturelle. Par conséquent, ces systèmes sont affectés à la fois par des perturbations naturelles (par exemple, le débit des rivières, les remontées d'eau, les marées, les vagues) et anthropiques (utilisation des terres, charges en nutriments, perturbations liées au changement climatique). Mais aussi, du fait de l'activité anthropique, les zones côtières sont menacées en raison de l'élévation du niveau de la mer (Fagherazzi et al., 2019) mais aussi de l'augmentation des apports d'azote produits par les populations humaines (Damashek et Francis, 2018). L'excès de libération d'azote dû à la fertilisation a provoqué une eutrophisation accrue de nombreux écosystèmes estuariens (Herbert, 1999) entraînant un ensemble de problèmes environnementaux dont l'appauvrissement en oxygène ou la prolifération d'algues (Gruber et Galloway, 2008). Les estuaires sont des zones cruciales pour le traitement de l'azote, notamment en ce qui concerne la nitrification et la dénitrification couplées (Seitzinger et al., 2006), et les sédiments des systèmes aquatiques côtiers peuvent contribuer de manière substantielle à l'élimination de l'azote qui atteint dans certains cas entre 20 et 50 % des apports d'azote (Seitzinger, 1988).

La salinité comme facteur de stress modèle

L'évolution des conditions de salinité est particulièrement pertinente dans les écosystèmes côtiers où les habitats d'eau douce rencontrent des sites marins. Elles sont en outre intéressantes car les scénarios de changement climatique prévoient l'occurrence croissante d'événements, tels que les sécheresses, les tempêtes, les inondations et l'augmentation du ruissellement fluvial après de fortes précipitations, qui ont un impact sur les concentrations de sel (Seneviratne et al., 2012). L'application des changements de salinité comme facteur de stress modèle est donc très pertinente pour étudier les réponses des écosystèmes des sites côtiers dans le cadre des scénarios de changement climatique.

Des effets de la modification de la salinité sur les cycles biogéochimiques ont été signalés, notamment en ce qui concerne le cycle du carbone et de l'azote : par exemple, l'intrusion d'eaux plus salées peut-elle diminuer les taux d'assimilation du carbone dans les zones humides et avoir ainsi un impact sur les réservoirs de carbone côtiers (Pezeshki et al., 1990 ; Fagherazzi et al., 2019). D'autre part, des réponses contrastées ont été rapportées pour les processus du cycle de l'azote dans les zones humides côtières, où il a été démontré que les taux de dénitrification augmentent ou diminuent en raison des intrusions d'eau de mer (Rysgaard et al., 1999 ; Wu et al., 2008 ; Marks et al., 2016). Des preuves expérimentales ont en outre montré que les taux de nitrification sont perturbés en réponse à l'augmentation simultanée de la température et à la diminution des niveaux de salinité après les précipitations (Horz et al., 2004).

Importance des procaryotes pour le cycle global des nutriments et d'autres services écosystémiques

Les procaryotes sont ubiquistes et remplissent des fonctions importantes dans tous les écosystèmes car ils sont impliqués dans tous les cycles biogéochimiques (Falkowski et al., 2008). Plusieurs processus biogéochimiques clés (par exemple, la nitrification, la dénitrification ou la fixation de l'azote) sont exclusivement médiés par les procaryotes (Galloway et al., 2004; Koch et al., 2019). Les procaryotes fournissent des services écosystémiques essentiels pour la santé des plantes et des animaux ainsi que pour l'agriculture (Cavicchioli et al., 2019). Ils sont cruciaux pour la régulation du climat via leur impact sur le stockage du carbone : par exemple, les producteurs primaires procaryotes marins contribuent à ~50% de la production primaire nette mondiale (Field et al., 1998). Ils contribuent aussi largement aux réserves d'azote biologiquement disponibles par la fixation de l'azote (Codispoti, 2007), constituant ainsi la base des réseaux alimentaires (Azam et Malfatti, 2007). Les procaryotes sont également les principaux décomposeurs de la matière organique dans les écosystèmes terrestres et aquatiques, où les nutriments libérés servent aux plantes supérieures pour leur croissance ou sont transférés à des niveaux trophiques supérieurs via la boucle microbienne (Azam et al., 1983). Le recyclage de la matière organique peut également entraîner la libération de gaz à effet de serre tels que le CO2, le CH4 ou le N2O, qui ont un impact sur le climat mondial (Falkowski et al., 2008). La dynamique des procaryotes dans le fonctionnement et l'assemblage des communautés est donc essentielle pour les processus et le fonctionnement de l'écosystème dans son ensemble.

Les micro-organismes comme modèle en ecologie experimentale

Au-delà du rôle crucial qu'ils jouent dans le fonctionnement des écosystèmes, les systèmes microbiens sont des systèmes modèles très appropriés pour tester les théories écologiques ou évolutives dans des conditions contrôlées. En effet, les micro-organismes possèdent des caractéristiques qui facilitent l'expérimentation par rapport aux macro-organismes, comme leur petite taille et leur temps de génération court (Jessup et al., 2004). Les études expérimentales ont donc joué un rôle central en écologie microbienne pour générer une compréhension critique de la dynamique et des processus microbiens ou pour tester la théorie écologique générale (Duarte et al., 1997 ; Shade et al., 2012). Des efforts ont été déployés pour généraliser les résultats écologiques des études microécologiques aux études macroécologiques, et il a été démontré que, par exemple, la similarité distance-décroissance ou les relations espèce-espace sont valables à la fois pour les microorganismes et les organismes plus grands (Horner-Devine et al., 2004 ; Soininen, 2012 ; Clark et al., 2021).

Les systèmes microbiens permettent, par exemple, d'exposer des communautés différentes et bien caractérisées à des régimes de perturbation multiples et reproductibles tout en contrôlant d'autres facteurs, ce qui permet d'étudier systématiquement l'impact des perturbations sur les systèmes microbiens.

La cryoconservation est une méthode courante utilisée pour conserver des souches microbiennes cultivables axéniques et non axéniques (Nagai et al., 2005 ; Heylen et al., 2012). L'application récente de protocoles de cryoconservation sur des communautés de bioréacteurs a démontré que non seulement des souches uniques mais aussi des communautés microbiennes pouvaient être récupérées avec succès en termes de composition et de fonctionnalité après cryoconservation (Kerckhof et al., 2014). L'application des protocoles de cryoconservation à des communautés complexes et naturelles pourrait donc être utilisée comme une alternative aux assemblages de communautés artificielles. La préparation de multiples aliquotes de communautés cryoconservées pourrait garantir la reproductibilité des conditions initiales dans les installations expérimentales tout en évitant ou du moins en réduisant les inconvénients mentionnés ci-dessus, inhérents aux études basées sur des communautés artificielles.

Caractérisation des procaryotes via leur matériel génomique

Pour les écologistes microbiens, l'avènement des techniques de séquençage des acides nucléiques a permis de décrire l'immense diversité des communautés procaryotes par le biais de marqueurs taxonomiques offrant une grande opportunité d'étudier la distribution des procaryotes dans l'espace et le temps (Hanson et al., 2012 ; Shade et al., 2013). Les analyses de données de séquençage permettent en outre une caractérisation complète des capacités fonctionnelles potentielles ou de l'activité actuelle des individus, des populations ou des communautés procaryotes qui sont médiées par des changements dans leur contenu en ADN et en ARN.

Le contenu génomique des micro-organismes autres que les organismes plus grands qui peuvent acquérir un répertoire de réponses au cours de leur vie (Fahimipour et Gross, 2020) est étroitement lié aux réponses aux changements environnementaux, et les caractéristiques génétiquement codées pourraient être interprétées comme de potentiels. Ces traits génomiques pourraient être appliqués pour déduire les changements dans la performance et la composition des communautés microbiennes dans divers scénarios (Krause et al., 2014 ; Malik et al., 2020). Par exemple, une fraction élevée de facteurs de transcription ainsi qu'une grande taille du génome peuvent être considérées comme des traits génomiques reflétant une plus grande plasticité phénotypique, car ces caractéristiques génomiques confèrent des capacités majeures pour faire face à des habitats dissemblables en raison du potentiel (Kostadinov et al., 2011 ; Bentkowski et al., 2015). En revanche, il a été suggéré que le biais d'utilisation des codons ou le nombre de gènes d'ARNr 16s étaient associés à la longueur de la phase de latence et/ou aux taux de croissance maximaux (Klappenbach et al., 2000a ; Vieira-Silva et Rocha, 2010 ; Weissman et al., 2021).

Réponses des communautés procaryotes aux perturbations

Les perturbations sont connues comme étant des facteurs importants de la composition et du fonctionnement des communautés (Bardgett et Caruso, 2020) et la compréhension des mécanismes sous-jacents aux réponses des communautés aux perturbations environnementales est une clé pour prédire leur dynamique et les conséquences sur les services écosystémiques (Allison et Martiny, 2008). La stabilité des communautés en réponse aux perturbations est déterminée par leur niveau de résistance et/ou de résilience, où la résistance est définie comme le degré d'insensibilité d'une communauté aux perturbations, et la résilience est la vitesse à laquelle une communauté revient à un état antérieur à la perturbation (Shade et al., 2012).

Les structures globales de la communauté, telles que la diversité des espèces et les modèles d'interaction entre les espèces, peuvent avoir un impact sur le degré de stabilité

(Griffiths et Philippot, 2013). En outre, les propriétés des membres individuels de la communauté, telles que leur tolérance aux perturbations (spécialiste ou généraliste) peuvent avoir un impact sur la stabilité de la communauté dans un environnement fluctuant (Pandit et al., 2009 ; Matias et al., 2013).

L'impact des structures communautaires globales sur la stabilité de la communauté

La biodiversité est un facteur clé du fonctionnement des écosystèmes et les relations entre la biodiversité et le fonctionnement des écosystèmes (BEF) ont été intensivement étudiées dans des travaux antérieurs (par exemple, Naeem et al., 2000 ; Loreau et al., 2001 ; Bell et al., 2005). L'hypothèse d'assurance stipule que la stabilité des communautés après des perturbations augmente avec la diversité des espèces (Yachi et Loreau, 1999). En effet, les communautés très riches ont plus de chances d'abriter des espèces fonctionnellement redondantes qui peuvent compenser le fonctionnement des espèces qui disparaissent ou dont l'abondance diminue après une perturbation environnementale. En outre, des études de réseau basées sur des modèles ont prédit que des rétroactions positives limitées et de faibles interactions positives entre espèces ont un effet stabilisateur sur les communautés exposées à des perturbations (Coyte et al., 2015 ; Ratzke et al., 2020).

Stratégies d'histoire de vie

L'histoire de vie des organismes détermine comment les espèces répartissent l'énergie disponible entre des caractéristiques telles que le taux de croissance, la reproduction, l'efficacité de l'utilisation des ressources, la capacité à éviter la prédation ou la résistance au stress (Stearns, 1977 ; Ferenci, 2016). Comme l'énergie disponible est limitée, on a observé que différentes caractéristiques du cycle de vie, comme le taux de croissance et l'efficacité de l'utilisation des ressources, s'équilibrent (Fierer et al., 2007 ; Roller et al., 2016).

Une option pour caractériser le cycle de vie des espèces consiste à les classer le long du continuum binaire spécialiste-généraliste : les généralistes sont des espèces dont la valeur adaptative est répartie uniformément dans plusieurs environnements, et les spécialistes sont des espèces dont la valeur adaptative est limitée à quelques environnements (Levins, 1968). Cette capacité à coloniser plusieurs environnements est liée au concept de "jack of all trade master of none", selon lequel certaines espèces sont capables de remplir plusieurs fonctions, mais à un niveau sous-optimal. À l'inverse, les spécialistes ne maîtrisent que certaines fonctions, mais sont capables de supplanter les espèces généralistes dans leur habitat optimal (MacArthur, 1972). Le concept de généraliste-spécialiste est étroitement lié à l'étendue de la niche (Sexton et al., 2017) qui peut être estimée comme la gamme d'occupation en fonction des environnements ou en mesurant directement la performance des espèces dans des conditions changeantes.

Cependant, la classification des espèces le long du continuum généraliste-spécialiste reste difficile car elle dépend du contexte utilisé pour définir le type de "généraliste" qui nous intéresse. Bell et Bell 2021 ont expliqué que les classifications généralistes-spécialistes peuvent se référer à une multitude de dimensions potentielles, telles que les conditions abiotiques (utilisation des substrats, habitat) et biotiques (interactions), où l'utilisation des substrats est le facteur le plus couramment étudié et où une espèce qui est, par exemple, un spécialiste de la salinité peut être en même temps un généraliste des ressources (Bell et Bell, 2021).

Il a été discuté que la tolérance aux changements environnementaux est liée aux exigences énergétiques, ce qui suggère que pour les généralistes il devrait y avoir un coût métabolique (Dall et Cuthill, 1997 ; Jasmin et Kassen, 2007 ; Bell et Bell, 2021). Par exemple, les ressources peuvent limiter la réponse des phénotypes généralistes aux facteurs de stress abiotiques (DeWitt et al., 1998 ; Kassen, 2002 ; Hall et al., 2018). Cependant, malgré des discussions approfondies, il n'existe aucune preuve expérimentale démontrant ce paradigme de longue date.

Au-delà des approches binaires, le cadre Compétiteurs-Rudéraux-Tolérants au stress (CRS) qui a été initialement conçu pour les plantes (Grime, 1977). Le schéma CRS classe les espèces en fonction de leur présence dans des habitats soumis à des niveaux élevés de compétition dans des habitats riches en ressources (C), à des niveaux élevés de stress causés par exemple par une faible disponibilité des ressources ou d'autres facteurs de stress environnementaux (S), et à une fréquence ou une intensité élevée de perturbation (R). Ce cadre intègre ainsi une dimension de disponibilité des nutriments avec une dimension qui comprend la réponse des espèces le long d'un gradient de perturbation. Selon la théorie du SRC, les organismes peuvent être affectés aux différentes catégories en fonction de leurs traits, et plusieurs études ont proposé d'appliquer le cadre du SRC également aux procaryotes (par exemple, Prosser et al., 2007 ; Krause et al., 2014 ; Fierer, 2017).

Une étude récente suggère toutefois qu'il n'est pas possible d'effectuer un tri globalement valable des espèces procaryotes selon le schéma CSR, car les compromis chez les procaryotes ne sont pas cohérents (Beier et al., 2021). Au lieu de cela, une classification résistance-résilience a été suggérée, où le compromis entre les traits varie en fonction de la taille du génome : alors que les traits liés à la résistance et à la résilience dans les génomes jusqu'à environ 5 Mbp covarient positivement, le contraire est vrai pour les génomes plus grands. Il est prouvé que la taille moyenne du génome des communautés microbiennes dépend des habitats dans lesquels elles vivent, les procaryotes des habitats aquatiques présentant généralement des génomes de taille plus petite que ceux des habitats terrestres (Giovannoni et al., 2014). Par conséquent, le compromis qui façonne l'assemblage des communautés de procaryotes peut dépendre de leur type d'habitat.

L'hypothèse du rapport de masse soutient que les traits dominants, plutôt que le nombre d'espèces, déterminent le fonctionnement de l'écosystème (Grime, 1998) et prédit que les variations des traits entre les individus peuvent être mises à l'échelle et influencer les communautés par le biais de la moyenne pondérée de la communauté (Violle et al., 2007). L'application des moyennes pondérées par la communauté permet d'agréger la multitude de valeurs de traits individuels d'une communauté en un indicateur communautaire général. Les moyennes pondérées communautaires ont été largement utilisées pour décrire les communautés de macroorganismes (Díaz et al., 2007) et il a été suggéré de les appliquer également pour caractériser les communautés microbiennes (Fierer et al., 2014).

Historique des rassemblements et des perturbations de la communauté

Si les structures globales des communautés et les distributions des traits sont déterminantes pour la réponse immédiate des communautés aux perturbations, l'historique des perturbations d'un environnement façonne également ces paramètres communautaires et donc à nouveau les réponses fonctionnelles (Reichstein et al., 2013). Par exemple, il a été démontré que les sites marins présentant une hétérogénéité environnementale accrue sélectionnent les micro-organismes ayant une proportion plus élevée de facteurs de transcription (Kostadinov et al., 2011). De même, un modèle de simulation informatique de l'évolution a démontré que des habitats de plus en plus perturbés entraînent l'évolution de procaryotes dont le génome est plus grand (Bentkowski et al., 2015).

Les covariations positives ou négatives des traits liés à la résistance et à la résilience, qui dépendent de l'habitat (Beier et al. 2021), devraient, selon la théorie écologique, avoir un impact sur l'assemblage des structures de diversité de la communauté : alors qu'une diminution ou une augmentation simultanée de la résistance et de la résilience devrait diminuer ou augmenter la diversité des espèces, la diversité des espèces devrait être moins variable dans les systèmes où la résistance et la résilience se compensent (Nimmo et al., 2015).

Des recherches antérieures ont indiqué l'apparition d'une succession de communautés après plusieurs perturbations pulsées consécutives : alors qu'un nombre croissant d'espèces opportunistes a été observé après la première perturbation, l'exposition continue aux perturbations pulsées a favorisé les communautés dont les membres sont résistants (Evans et Wallenstein, 2014). Ces changements successifs dans la composition des communautés étaient liés à l'augmentation de la stabilité fonctionnelle par rapport aux témoins (Evans et Wallenstein, 2012). De même, la fréquence et l'intensité des perturbations peuvent affecter la diversité selon l'hypothèse de perturbation intermédiaire (IDH). Selon cette hypothèse, les niveaux de perturbation intermédiaires (en fréquence et en intensité) limitent l'exclusion compétitive ou les taux d'extinction, ce qui entraîne une augmentation de la richesse locale par rapport aux niveaux de perturbation faibles ou élevés (Connell, 1978). En retour, des niveaux élevés de diversité des espèces, en accord avec l'hypothèse d'assurance (Yachi et Loreau, 1999), devraient conduire à une augmentation de la stabilité de la communauté.

Les objectifs de la thèse

L'objectif de cette thèse de doctorat était d'étudier les conséquences des perturbations environnementales sur les micro-organismes au niveau des populations uniques ainsi que des communautés complexes. Pour l'approche au niveau d'une seule population, j'ai étudié la réponse transcriptionnelle de populations bactériennes uniques ayant une largeur de niche variable le long d'un gradient environnemental (chapitre 1). Pour aborder les conséquences des perturbations au niveau de la communauté, j'ai établi et testé un protocole de cryoconservation de communautés microbiennes complexes afin d'améliorer la reproductibilité expérimentale des assemblages de communautés naturelles (chapitre 2). J'ai en outre exposé des communautés microbiennes complexes à des perturbations pulsées afin d'étudier les conséquences de ces perturbations sur les changements structurels et le fonctionnement de la communauté (chapitre 3). J'ai également examiné si les perturbations pulsées avaient un impact sur les réactions du cycle de l'azote, et si oui, si cela était dû à la sélection de taxons spécifiques impliqués dans le cycle de l'azote (Chapitre 4).

CHAPITRE 1 : L'ETENDUE DE LA NICHE AFFECTE LES MODELES DE TRANSCRIPTION BACTERIENNE LE LONG D'UN GRADIENT DE SALINITE.

Objectif

Dans ce chapitre, j'ai testé l'hypothèse selon laquelle les espèces généralistes exposées à un changement de salinité présentent une régulation transcriptionnelle plus élevée des gènes impliqués dans l'adaptation à la salinité par rapport aux souches spécialisées. En revanche, je m'attends à ce que les gènes impliqués dans les fonctions cellulaires de base, comme la production de biomasse, soient moins régulés chez les généralistes que chez les spécialistes. Pour tester cette hypothèse, j'ai évalué la régulation transcriptionnelle des gènes liés à l'aptitude et à l'adaptation de 11 souches bactériennes en fonction de l'étendue de leur niche et de leur niveau d'exposition au stress dans des conditions de salinité changeantes.

Methodologie

Pour évaluer l'effet de la largeur de niche (NB) sur l'activité transcriptionnelle bactérienne, nous avons inclus 11 souches bactériennes modèles dans notre étude. Ces souches appartiennent à une collection d'isolats provenant de plusieurs environnements aquatiques avec des salinités différentes (Matias et al., 2013). Pour évaluer leur tolérance face aux changements de salinité, les cellules des souches ont été incubées en milieu LB standard stérile avec différentes concentrations de NaCl (10, 20, 30, 40, 50, 60, 70, 80, 90 et 100 g L^{-1}). La croissance des souches à 25ºC a été suivie dans chaque puits en mesurant toutes les heures leurs densités cellulaires via la densité optique (DO) à 600 nm dans un lecteur de microplaques Paradigm (Molecular Devices, LLC., California, USA) jusqu'à ce que la phase de plateau soit atteinte.

Pour étudier la réponse transcriptionnelle aux changements de niveaux de salinité, les extractions d'ARN des souches modèles ont été effectuées comme suit : Chaque souche a été cultivée en double à trois niveaux de salinité et à 25ºC (100 rpm) dans des tubes de verre stériles contenant 15 ou 50 mL de milieu LB. Nous cultivons les souches pour cette expérience autour de leurs niveaux de salinité optimaux, ainsi que deux niveaux de salinité, chacun situé symétriquement 15 g L^{-1} NaCl du côté hyper- et hypoosmotique de l'optimum. De plus, 1200 µL de la culture ont été fixés avec du glutaraldéhyde pour le dénombrement des cellules.

Afin de tester les hypothèses H1 selon lesquelles les gènes ayant un lien direct avec la valeur adaptative d'un organisme sont plus fortement régulés dans les souches à NB étroit, nous avons construit 3 catégories de gènes putativement liés à la valeur adaptative codant pour les protéines suivantes : 1) les enzymes ADN polymérases, qui sont essentielles à la réplication de l'ADN et à la prolifération cellulaire. 2) Les ARN polymérases, qui transcrivent les gènes en ARNm et constituent donc la première étape de la production de biomasse cellulaire via la synthèse des protéines. 3) Les protéines ribosomales, qui sont des unités essentielles des ribosomes qui catalysent la traduction de l'ARNm en protéines et qui constituent donc la deuxième étape de la production de biomasse cellulaire par la synthèse des protéines.

Afin de tester les hypothèses H2 selon lesquelles les gènes codant pour l'adaptation cellulaire contre le stress salin sont plus fortement régulés dans les souches avec un NB plus large, nous avons construit 2 autres catégories avec des gènes potentiels liés à l'adaptation : 4) Gènes codant pour le transport de composés osmoprotecteurs, incluant principalement les solutés compatibles, mais aussi d'autres osmolytes, comme l'urée. 5) Les protéines de choc thermique (HSP), qui catalysent le repliement et le dépliage des macromolécules et sont donc impliquées dans la réparation des macromolécules endommagées (Kültz, 2003 ; Lindquist & Craig, 1988).

Nous avons utilisé des modèles linéaires mixtes (MLM) pour vérifier si les niveaux de régulation transcriptionnelle des gènes dans chacune des catégories décrites ci-dessus étaient corrélés dans la direction prédite avec le NB (log-transformé) ou l'exposition au stress le long du gradient de salinité respectif des souches individuelles.

Principaux résultats

Alors qu'aucune tendance significative n'a été notée pour la régulation des gènes codant pour les ADN polymérases, la régulation des gènes codant pour les protéines ribosomiques et les ARN polymérases a diminué en accord avec H1 de manière significative avec l'augmentation du NB ($P=0,003$ et $P=0,041$, respectivement). La direction de la relation s'est inversée lorsque l'on considère l'exposition au stress plutôt que le NB comme variable explicative de la régulation des gènes. Cependant, seuls les gènes codant pour la protéine ribosomale étaient significativement régulés avec l'augmentation des niveaux de stress ($P<0,001$). Une inspection de la direction de la régulation des gènes a révélé une régulation ascendante significative des gènes codant pour les protéines ribosomiques et les ARN polymérases en cas d'exposition croissante au stress et une tendance non significative pour la régulation ascendante des gènes codant pour les ADN polymérases. Cependant, dans les trois catégories, des gènes individuels ont également été régulés à la baisse sous l'effet du stress, en particulier si les niveaux de stress étaient extraits de plages de salinité dépassant l'optimum de salinité.

Nous signalons ici 7 gènes qui présentaient des valeurs P significatives (P<0,05). Trois des sept gènes candidats étaient régulés à la baisse en réponse à l'augmentation des niveaux de stress et les quatre autres gènes étaient régulés à la hausse. Deux des gènes candidats qui étaient régulés à la hausse en réponse à l'augmentation des niveaux de stress (ybeB et rpsI) se retrouvent dans >75% de tous les génomes procaryotes répertoriés dans la base de données KEGG, tandis que l'occurrence des autres gènes candidats était moins conservée parmi les procaryotes.

Discussion

Dans l'ensemble, nos résultats confirment que les schémas généraux de la régulation transcriptionnelle bactérienne peuvent permettre de distinguer les modes de vie généralistes et spécialistes. Les forces de corrélation variables des niveaux de régulation des gènes par rapport au NB et au stress dans les catégories respectives impliquent une covariation étroite des traits liés à la forme physique, mais aussi des gènes HSP avec les niveaux de forme physique sur l'axe y des courbes de forme physique. En revanche, les niveaux de régulation des gènes des transporteurs d'osmoprotectants étaient liés au NB mais à un moindre degré au stress. La régulation des transporteurs d'osmoprotectants covariait donc plutôt avec les conditions environnementales changeantes affichées sur l'axe des x des courbes de fitness, qu'avec le fitness. Dans tous les cas, en tenant compte du fonctionnement physiologique des gènes dans leurs catégories respectives, ces observations représentent des réponses significatives et bien interprétables.

Nous avons également proposé une liste de gènes candidats marqueurs de stress dont la régulation est corrélée avec les niveaux d'exposition au stress dans notre étude. Nous suggérons que ces gènes soient testés dans des études futures afin de valider leur applicabilité universelle pour détecter les niveaux de stress, soit dans des populations individuelles, soit dans des communautés exposées à des conditions changeantes. L'exposition au stress des espèces dans une communauté a été considérée comme l'un des trois principaux axes environnementaux définissant la distribution des traits dans une communauté (JP Grime, 1977 ; Malik et al., 2020) et est donc un paramètre clé pour le fonctionnement de la communauté et les processus d'assemblage (Romero, Acuña, & Sabater, 2020). L'approche basée sur les gènes suggérée ici pour la surveillance du stress dans les communautés microbiennes peut donc être incorporée dans des modèles pour prédire les flux de carbone, comme suggéré ailleurs (Malik et al., 2020) et accomplir une approche basée sur les taxons proposée précédemment pour définir les bioindicateurs potentiels du stress (Rocca et al., 2019).

CHAPITRE 2 : CRYOCONSERVATION ET RESSUSCITATION DES COMMUNAUTES PROCARYOTIQUES AQUATIQUES NATURELLES

Objectif

L'objectif de ce chapitre est l'établissement d'une méthode pour la préservation et la ressuscitation des communautés naturelles de procaryotes aquatiques. J'ai testé plusieurs aspects du protocole de cryoconservation : i) l'impact de la taille de l'inoculum sur la ressuscitation, ii) dans quelle mesure la diversité et la composition de la communauté peuvent être maintenues en fonction des caractéristiques de l'inoculum et du milieu de culture, iii) l'effet du temps de manipulation pendant la préparation des aliquotes de la communauté, et iv) l'effet du temps de stockage des aliquotes cryoconservées sur la composition de la communauté après cryoconservation.

Méthodologie

Les communautés procaryotiques ont été échantillonnées dans la station côtière SOLA au nord-ouest de la Méditerranée et dans la lagune de Canet. Les deux sites sont situés sur la côte sud du golfe de Lyon dans la région Occitanie, dans le sud de la France. Ces deux sites ont été choisis en raison de leurs conditions environnementales contrastées : SOLA représente un site oligotrophe avec des concentrations de Chlorophylle-a (Chl-a) variant entre 0.01-4.39 µg L^{-1} avec des concentrations de salinité plutôt stables (33.20-39.99 PSU) et Canet est une lagune côtière peu profonde caractérisée par une forte productivité et des changements de salinité à grande échelle (1.09-125.76 µg L^{-1} Chl-a, 2.20- 43.00 PSU) au cours de l'année.

Les apports en matière organique dissoute (MOD) qui ont servi de substrats aux communautés ressuscitées ont été obtenus soit sur le site d'échantillonnage SOLA, soit dans l'embouchure de la rivière Warnow dans la mer Baltique, à Warnemünde, en Allemagne. L'embouchure de la rivière Warnow présente une salinité comprise entre 5 et 18 PSU en raison du mélange avec les masses d'eau de la mer Baltique (Schernewski et al., 2019). L'estuaire de Warnow est très productif en raison des charges terrestres, avec des valeurs de Chl-a comprises entre 18 et 65 µg L^{-1} (Freese et al., 2007).

On a utilisé comme milieu soit de l'eau stérile filtrée de la station SOLA, ci-après F-SEA, filtrée à travers un filtre en cellulose de 0,22 µm, 47 mm (Millipore, Massachusetts, USA), soit un milieu basique inorganique d'eau de mer artificielle (ASW, Eguchi et al., 1996) supplémenté en DOM. Outre les suppléments de DOM, aucun autre carbone, azote, phosphore ou vitamines n'a été ajouté au milieu ASW.

Nous avons adapté la procédure de cryoconservation développée par Vekeman et Heylen (2017) pour la cryoconservation de souches uniques ou de cultures mixtes pour cryoconserver les communautés aquatiques naturelles. Ce protocole précédemment publié avait été appliqué par les auteurs exclusivement à des cultures cellulaires de laboratoire cultivées à des densités cellulaires élevées (>108 cellules/mL, Vekeman et Heylen, 2017) alors que les densités cellulaires des communautés bactériennes marines varient généralement de 105 à 106 cellules par mL (Kirchman, 2008). Pour cette raison, mais aussi pour minimiser le report des substrats dans les nouveaux milieux de culture, une étape de concentration des cellules présentes dans les échantillons aquatiques avant cryoconservation a été ajoutée.

Entre 0,1 et 1,5 L d'eau pré-filtrée a ensuite été filtrée sur un filtre de 0,22 µm à l'aide d'une pompe à vide (~600 mg Hg) afin de concentrer les cellules procaryotes. Le séchage des filtres a été soigneusement évité pendant la filtration, sauf à sa fin, lorsque la pression a été réglée au minimum, afin d'éviter d'endommager les cellules, et le volume d'eau final recouvrant le filtre a été lentement filtré. Immédiatement après le retrait de l'eau de l'échantillon, la pompe a été arrêtée et 0,5 ml de milieu inorganique ASW a été ajouté au sommet du filtre, qui a ensuite été utilisé pour remettre en suspension les cellules du filtre en les pipettant de haut en bas. Ce volume a ensuite été transféré dans des tubes de 2 mL préalablement préparés avec 0,5 mL de cryoprotecteur contenant 10% (v/v) de DMSO filtré stérile dans de l'ASW inorganique, équilibré à 4°C (concentration finale de DMSO : 5%). Ensuite, les filtres ont été complètement immergés dans le milieu avec le cryoprotecteur et les cellules remises en suspension et les tubes ont été maintenus à 4°C pendant 15 min pour l'équilibrage (Vekeman et Heylen, 2017). Les tubes ont ensuite été congelés au flash dans l'azote liquide et transférés à -80°C pour un stockage à long terme.

Pour ressusciter les communautés cryoconservées, les échantillons cryoconservés ont été décongelés à température ambiante (~30 min) puis ajoutés (avec le filtre le cas échéant) au milieu de culture. Les cellules ont été incubées pendant 5-6 jours à une température proche de la température in-situ (15-18°C, Tableau 2) dans des bouteilles en verre sous agitation avec des agitateurs magnétiques. Au total, quatre expériences ont été réalisées pour tester et évaluer la cryoconservation et la réanimation des communautés naturelles de procaryotes aquatiques. Dans toutes les expériences de réanimation, le nombre de cellules a été contrôlé quotidiennement par cytométrie de flux, comme indiqué ci-dessus.

Dans la première expérience, nous avons évalué l'effet du nombre initial de cellules sur la croissance cellulaire après ressuscitation, en incluant à la fois des inocula concentrés par filtration cryoconservés (expérience 1a), et des aliquots d'eau cryoconservés provenant d'eau de mer SOLA pré-filtrée à 0,8 µm (expérience 1b). Ensuite, l'expérience 2 a été réalisée dans le but d'évaluer la similarité des communautés ressuscitées avec les communautés initiales de départ en fonction du milieu de croissance et de la source d'inoculum. Dans l'expérience 3, un ensemble de trois inocula concentrés par filtration provenant de SOLA a été cryoconservé immédiatement après l'étape de pré-filtration et un autre ensemble de cette même communauté a été cryoconservé 7,5 heures plus tard. Dans l'expérience 4, trois ensembles de communautés cryoconservées concentrées par filtration provenant du SOLA (4,12 x108 cellules) ont été ressuscités après une cryoconservation de 1,5, 6 et 12 mois.

Principaux résultats

La ressuscitation et l'incubation ultérieure des inocula cryoconservés ont réussi indépendamment du type d'inoculum (concentré par filtration ou eau d'échantillon) et de la taille de l'inoculum. Les deux types d'inoculum ont présenté des phases de latence plus longues (trois jours) pour les plus grands inocula, tandis que la phase de latence n'a pas dépassé deux jours dans tous les autres traitements. De plus, la capacité de charge de toutes les incubations a dépassé le nombre de cellules détectées dans l'eau SOLA initiale pré-filtrée à 0,8 µm (0,66 ± 0,05x106 cellules mL^{-1}), et la différence était généralement plus importante dans les incubations avec un plus petit inoculum, sauf pour le plus petit inoculum de l'expérience 1a. Nous avons observé pour les deux types d'inoculum que la variance entre les répétitions avait tendance à diminuer avec l'augmentation de la taille de l'inoculum. De plus, nous avons observé une diminution significative du coefficient de variation pour les inocula de filtres cryoconservés. En revanche, les incubations d'inoculums d'eau ont montré une tendance à l'augmentation du coefficient de variation avec des inoculums plus grands.

Les communautés cryoconservées de la station SOLA et de Canet qui ont été ressuscitées dans l'expérience 2 ont montré une croissance dans tous les milieux testés (F-SEA, S-DOM, et SW-DOM). Les tendances de changement de composition au niveau de l'ordre décrites ici pour les deux incubations F-SEA avec une grande similarité avec les échantillons initiaux du SOLA ont également été observées, à un niveau plus prononcé, pour la communauté du SOLA incubée dans les milieux S-DOM ou SW-DOM, ainsi que pour l'une des incubations F-SEA. Alors que les communautés de Canet ressuscitées ressemblaient remarquablement à la communauté de Canet initiale au niveau de l'ordre, quel que soit le milieu utilisé.

Les communautés de SOLA ressuscitées qui ont été traitées soit directement après l'étape de préfiltration, soit après un délai de plusieurs heures, ont présenté des courbes de croissance similaires avec une phase de latence de deux à trois jours. La composition des communautés provenant d'inocula cryopréservés traités avec un délai était moins similaire (distances de Bray-Curtis 0,741 ± 0,246). Deux réplicats se sont regroupés étroitement tandis que la composition de la communauté du troisième réplicat semblait être plus similaire aux communautés provenant des autres incubations.

Les courbes de croissance des communautés cryoconservées qui ont été ressuscitées après une période de stockage de 1,5 et 12 mois étaient similaires, avec des phases de latence de trois à quatre jours et des capacités de charge comprises entre 0,65 ± 0,14 x106 cellules mL^{-1} et 0,91 ± 0,23 x106 cellules mL^{-1}. Les comparaisons par paires post-hoc ont indiqué que les différences possibles dans la composition de la communauté se sont produites principalement entre les échantillons avec une durée de stockage de 1,5 mois et les échantillons avec 6 et 12 mois de stockage. La comparaison par paire entre les échantillons stockés pendant 6 et 12 mois n'a pas indiqué d'influence de la durée de stockage sur la composition de la communauté dans ce cas. En outre, un test de mantel n'a pas confirmé une augmentation significative de la dissimilarité des communautés avec l'augmentation du temps de stockage des inocula cryoconservés.

Discussion

Cette étude fournit des preuves de la faisabilité d'une méthode de cryoconservation et de ressuscitation d'assemblages naturels de procaryotes aquatiques. Plusieurs aspects de la procédure de cryoconservation (taille de l'inoculum, durée de la procédure expérimentale et durée de stockage) ont été évalués de manière critique afin d'offrir de nouvelles opportunités pour de futures recherches en écologie microbienne basées sur la manipulation de communautés naturelles. La ressuscitation a été réalisée pour des communautés contrastées récoltées dans les eaux côtières de la mer Méditerranée du Nord-Ouest. Sur la base des résultats de cette étude, nous recommandons d'utiliser des aliquotes de communautés suffisamment grandes pour la cryoconservation afin d'améliorer la reproductibilité, tout en évitant d'autre part des temps de manipulation prolongés. Enfin, les temps de stockage à long terme, de 6 à 12 mois, ne semblent pas causer de changements majeurs dans la composition des communautés ressuscitées.

CHAPITRE 3 : LE COUT DE L'ADAPTABILITE : LA DISPONIBILITE DES RESSOURCES CONTRAINT LA STABILITE FONCTIONNELLE SOUS DES PERTURBATIONS PULSEES

Objectif

Dans ce chapitre, nous avons testé l'hypothèse selon laquelle l'exposition à long terme de communautés à des perturbations pulsées de salinité favoriserait la sélection de membres de la communauté ayant des capacités de résistance et/ou de résilience plus élevées, en particulier lorsque les niveaux de ressources sont élevés. Je m'attendais également à ce que ce processus de sélection conduise, dans les communautés exposées à des perturbations pulsées vers la fin de l'expérience et en particulier à des niveaux de ressources élevés, à une stabilité fonctionnelle accrue au niveau de la communauté en réponse aux perturbations. J'ai étudié des assemblages bactériens soumis à des perturbations pulsées hebdomadaires et j'ai mesuré les changements de composition et de fonction dans une expérience de culture continue de 41 jours sous deux régimes de perturbation (perturbations pulsées et contrôle non perturbé) croisés avec deux niveaux de disponibilité des ressources.

Méthodologie

Les inocula pour l'expérience de culture continue ont été obtenus à partir d'aliquotes de communautés microbiennes cryoconservées. Les environnements aquatiques dans lesquels les aliquotes de communautés ont été prélevés comprennent plusieurs sites dans le sud du Golfe de Lyon, dans le sud de la France. Les sites comprenant la station de terrain méditerranéenne SOLA et les lagunes côtières de La Palme et Gruissan sont caractérisés par une variabilité environnementale contrastée. Des aliquotes de communautés cryoconservées provenant de ces sites ont été regroupées afin d'obtenir une communauté " super-diversifiée " dans le but que les mécanismes de sélection lors des traitements expérimentaux en aval puissent agir sur une possible grande collection d'espèces aux traits complémentaires. Nous avons utilisé cette communauté pour réaliser une expérience de culture continue de 41 jours sous deux régimes de perturbation (perturbations pulsées et contrôle non perturbé) croisés avec deux niveaux de disponibilité des ressources (LDOM et HDOM). Au cours de l'expérience, les assemblées bactériennes aquatiques ont été soumises à des perturbations pulsées hebdomadaires, et les changements compositionnels (via l'ARNr 16S) et fonctionnels (production, respiration, activité) ont été évalués. Nous avons estimé les traits génomiques à l'aide du logiciel PICRUSt2 (Douglas et al., 2020), ils ont été classés en traits liés à la résilience comme le nombre de copies de gènes (RRN) et le temps de génération, et en traits liés à la résistance comme le % de facteurs de transcription (%TF) et la taille du génome.

Principaux résultats

L'activité microbienne et les taux de production ainsi que le BGE étaient significativement plus résistants aux perturbations de la salinité dans le HDOM par rapport au LDOM. Ce schéma n'a cependant pas été détecté pour les mesures de respiration. La résistance aux perturbations de la salinité a augmenté de manière significative dans le temps pour la respiration et le BGE au HDOM, cependant, pour les autres mesures de résistance, aucun changement temporel significatif des niveaux de résistance n'a été détecté.

Le facteur de structuration le plus fort pour la composition de la communauté phylogénétique était le temps ($F=60{,}49$; $P\leq 0{,}001$), suivi par l'apport de DOM ($F=9{,}89$, $P\leq 0{,}001$) et ensuite le régime de perturbation ($F=3{,}68$; $P=0{,}009$), alors qu'aucun des effets d'interaction n'était significatif. Lors de l'inspection des traits génomiques, les traits liés à la résilience et à la résistance ont évolué dans le temps tout au long de l'expérience, indépendamment du régime de perturbation et du niveau de DOM. En outre, dans les deux conditions de DOM, les traits liés à la résilience ont changé de manière significative en réponse au régime de perturbation. Les perturbations ont induit des RRN et des taux de croissance significativement plus élevés dans les traitements perturbés par rapport aux contrôles. Les traits liés à la résistance n'ont pas montré une augmentation significative en réponse au régime de perturbation dans le niveau LDOM. En revanche, les traitements perturbés ont présenté un %TF et une taille de génome significativement plus élevés dans les conditions HDOM.

Discussion

Dans l'ensemble, l'étude expérimentale présentée a confirmé les hypothèses testées concernant la sélection d'espèces tolérantes dans des environnements fréquemment perturbés en fonction de la disponibilité des ressources. Le fait que cela semble être indépendant des modèles à petite échelle de la composition de la communauté au niveau des espèces augmente encore la généralité de nos résultats. Nous pensons que notre expérience dans des conditions contrôlées montre que la dynamique des communautés microbiennes hautement diversifiées est généralement prévisible si l'on se concentre sur les distributions des traits des histoires de vie et des structures de diversité qui sont à leur tour des paramètres pivots pour le fonctionnement de la communauté.

Notre étude fournit pour la première fois des preuves expérimentales des mécanismes moléculaires et des coûts associés responsables de la stabilité des communautés lors de perturbations fréquentes. Bien que nos données indiquent une contribution importante des événements stochastiques au cours de l'assemblage de la communauté au niveau de l'espèce, nous avons trouvé des modèles très cohérents de modèles de communauté superordonnés et de caractéristiques fonctionnelles. Nos résultats suggèrent donc l'existence de différentes solutions alternatives de composition ayant des propriétés fonctionnelles similaires et provenant de la même communauté initiale.

CHAPITRE 4 : LES PERTURBATIONS PULSEES DE L'EXPOSITION A LONG TERME PERTURBENT LE CYCLE DES NITRATES

Objectif

Ce chapitre a examiné l'effet des perturbations pulsées fréquentes sur les processus clés du cycle de l'azote et si les différences dues au régime de perturbation ont été la conséquence du renouvellement de la communauté. Des différences significatives dans les concentrations de nitrate sous différents régimes de perturbation ont suggéré que les perturbations pulsées de salinité ont effectivement affecté le cycle de l'azote. Une grande précision dans la prédiction des concentrations de nitrate à partir des données de composition de la communauté par le biais d'un algorithme d'apprentissage automatique a également suggéré que la présence et/ou l'abondance de taxons clés dans le cycle de l'azote était un facteur majeur de la consommation nette de nitrate observée.

Methodologie

L'expérience de culture continue a été réalisée pendant 41 jours tout en combinant les régimes de perturbation et les niveaux de nutriments. Le dispositif expérimental est décrit en détail dans le chapitre III de la présente thèse. En bref, les milieux de culture étaient constitués d'eau de mer artificielle (ASW ; la salinité de 38 g L^{-1} et le pH 8 ; Eguchi et al., 1996) qui ont été amendés avec les deux suppléments de DOM qui ont servi de source exclusive d'azote : faible concentration de DOM (ci-après : LDOM, DON 2,24 µM) et forte concentration de DOM (ci-après : HDOM, DON 4,87 µM). Les niveaux de DON dans le milieu HDOM ressemblaient à la concentration moyenne de DON en surface dans le bassin occidental de la mer Méditerranée (Pujo-Pay et al. 2011).

Nous avons ajouté deux puces poreuses (polyéthylène, Mutag BioChip 25™, MUTAG, Chemnitz, Allemagne) à chaque récipient pour fournir une surface potentielle pour la formation de biofilms microbiens. Le biofilm sur ces puces a servi de refuge spatial car les cellules qui y sont attachées ne sont pas lavées dans la culture continue et peuvent recoloniser le milieu. Nous avons décidé d'ajouter ces puces pour réduire le risque potentiel d'un effondrement de la culture continue pendant l'expérience à long terme.

Les échantillons destinés à quantifier l'abondance bactérienne par cytométrie en flux (FC) ont été prélevés directement dans les échantillons d'ADN pour quantifier les abondances cellulaires, mais aussi dans des aliquotes des récipients de chémostat en parallèle avec l'échantillonnage chimique. Les échantillons pour les mesures chimiques ont été prélevés chaque semaine directement dans les récipients d'incubation, 3 jours après les prélèvements hebdomadaires d'ADN. Les concentrations d'azote organique dissous (DON), de nitrate (NO_3^+), de nitrite (NO_2^-) et d'ammonium (NH_4^+) ont été quantifiées dans les milieux de culture stériles ainsi que dans les cultures continues échantillonnées chaque semaine.

Nous avons utilisé l'algorithme d'apprentissage automatique Random Forests (RF) (Breiman 2001) pour tester si la consommation nette de nitrate dans le milieu pouvait être prédite à partir des données de composition de la communauté. Nous avons considéré soit les données de composition de la communauté basées sur la FC (Wanderley et al. 2019) échantillonnées le même jour que les données chimiques, soit les données de composition de la communauté du gène de l'ARNr 16S (Callahan et al. 2016) dans le débit sortant, qui ont été collectées trois jours après l'échantillonnage des données chimiques.

Principaux résultats

Des changements temporels dans tous les composés azotés ont été observés. Les concentrations mesurées d'ammonium et de nitrate indiquent une consommation nette de ces composés dans toutes les cultures continues. En revanche, les concentrations de nitrite et de DON étaient pour la plupart proches des concentrations mesurées dans les milieux d'origine. Seules les valeurs de DON dans le milieu LDOM ont indiqué une certaine production nette de ce composé pendant les incubations. Des changements temporels significatifs ont été observés pour l'ammonium et le nitrate dans les régimes HDOM. Cependant, un effet significatif ($P < 0{,}001$) ou proche d'un effet significatif ($P=0{,}050$) du régime de perturbation a été détecté uniquement pour le nitrate net.

Les modèles basés sur la FR pour les données sur le gène FC et le gène de l'ARNr 16S ont prédit de manière significative la concentration de nitrate à partir des données sur la composition de la communauté, tandis que le modèle basé sur le FC a donné lieu à une meilleure prédiction (Spearman rho=0,75) par rapport au modèle basé sur le gène de l'ARNr 16s (Spearman rho=0,58). Le modèle FC et le modèle basé sur le gène de l'ARNr 16s ont tous deux classé les taxons indicateurs les plus importants, définis ci-après comme des taxons ayant une grande importance dans le modèle RF et abondants soit au début de l'expérience, soit au milieu, soit vers la fin. En général, nous avons observé de grandes variances entre les traitements répétés.

Discussion

Des changements significatifs dans les taux de consommation nette de nitrate dans la phase finale de l'expérience à long terme ont confirmé que les changements fréquents de salinité avaient un impact sur le cycle de l'azote. La possibilité de prédire de manière significative le nitrate net à partir des données de composition de la communauté a également démontré que la sélection différentielle des espèces dans les différentes conditions de traitement était un facteur important des taux de consommation nette de nitrate observés.

Notre étude démontre que le cycle de l'azote (nitrification et/ou dénitrification) peut être affecté par une fréquence accrue des perturbations de la salinité dans les zones côtières, comme le prévoient les scénarios de changement climatique. Les zones côtières conduisent généralement, par le biais de taux de nitrification-dénitrification couplés importants, à une élimination à grande échelle de l'azote des eaux de ruissellement dans les océans (Boyer et Howarth 2008). Une diminution de l'efficacité de l'élimination de l'azote due à des changements de salinité de plus en plus fréquents pourrait avoir des conséquences importantes sur l'écologie des systèmes marins qui sont souvent limités en azote (Howarth and Marino 2006).

CONCLUSIONS ET PERSPECTIVES

Ma thèse portait notamment sur la façon dont les traits exprimés au niveau de la population ou la moyenne pondérée des traits de la communauté sont liés aux niveaux de résistance mesurés physiologiquement, évalués par exemple via les taux de croissance, la densité cellulaire maximale ou l'efficacité de la croissance. Dans ce contexte, j'ai confirmé l'association précédemment prédite de la covariation des niveaux de résistance et de résilience avec la diversité des espèces (Nimmo et al., 2015), qui à son tour peut avoir eu un impact sur la stabilité de la communauté. Cependant, au-delà des propriétés des espèces et de la diversité des communautés, les interactions entre espèces ont également été mises en évidence dans des études antérieures comme un facteur important pour la stabilité des communautés (Griffiths et Philippot, 2013). Les interactions biologiques peuvent influencer le degré de stabilité de la communauté face aux perturbations. Bien qu'elles n'aient pas encore été abordées, les données chronologiques sur les communautés présentées au chapitre 3 pourraient être utilisées à l'avenir pour évaluer l'interaction des espèces via les modèles de cooccurrence des espèces et les questions suivantes pourraient être posées : (i) Comment les perturbations pulsées sous différents régimes de nutriments ont-elles eu un impact sur le réseau d'interaction ? et (ii) les traitements avec moins d'interactions positives entre les espèces présenteront-ils une résistance fonctionnelle accrue, comme cela a été proposé précédemment (Coyte et al., 2015) ?

Au cours de cette thèse, l'accent a également été mis sur les paramètres expérimentaux contrôlés avec la salinité comme seul facteur de stress modèle. Cependant, l'application de la salinité comme seul facteur de perturbation ne reflète pas la complexité des systèmes naturels, où les communautés sont généralement soumises à de multiples facteurs de stress se produisant simultanément : par exemple, lorsque le ruissellement augmente, des eaux de salinité différente sont mélangées et ces masses d'eau sont susceptibles de différer non seulement en salinité mais aussi dans d'autres paramètres, tels que la température ou le pH, entre autres. Les réponses des communautés à chaque facteur de stress peuvent cependant être obscurcies si l'on expose la communauté simultanément à plusieurs facteurs de stress, qui peuvent avoir des effets antagonistes ou synergiques. Ainsi, dans les futurs montages expérimentaux, l'exposition de communautés complexes à de multiples facteurs de stress est nécessaire pour imiter des conditions plus naturelles. Les questions pertinentes auxquelles il faut répondre pourraient par exemple être les suivantes : (i) Quel serait l'effet d'une combinaison de facteurs de stress anthropogéniques (par exemple, les pesticides, les métaux lourds) et naturels (par exemple, la salinité, la température, le pH) sur la stabilité fonctionnelle et compositionnelle à long terme des procaryotes ? ou (ii) Quel serait l'effet de la pré-exposition des communautés à un certain facteur de stress sur la résistance à un autre facteur de stress ? Alors que l'on pourrait s'attendre à ce que l'énorme diversité métabolique des communautés microbiennes les rende intrinsèquement résistantes ou résilientes aux perturbations, les recherches suggèrent que les communautés microbiennes sont généralement sensibles aux perturbations (Shade et al., 2012). Cela soulève la question de savoir quelles sont les limites de la stabilité des communautés jusqu'à la catastrophe écologique ? Ou encore, quelles intensités ou fréquences de perturbation provoquent des changements dans les services écosystémiques ? Les résultats du chapitre 4 ont démontré que les perturbations de la salinité affectent le cycle de l'azote dans un système de culture continue. Des effets similaires de changement de salinité dans un système côtier naturel peuvent avoir des conséquences sur l'équilibre sensible des différents processus de transformation de l'azote qui déterminent la quantité d'azote transportée dans les océans souvent limités en azote.

Les études menées dans des conditions expérimentales contrôlées permettent d'étudier les communautés microbiennes dans des systèmes simplifiés et sont essentielles pour démêler l'impact des différents facteurs qui influencent leur dynamique. Toutefois, pour être en mesure de comprendre et de prévoir les processus dans les habitats naturels, des études d'observation sur le terrain sont également nécessaires. Les séries chronologiques régulières sont particulièrement précieuses car elles couvrent la variabilité naturelle et peuvent saisir simultanément les perturbations pulsées et continues ainsi que les réponses de la communauté à ces perturbations. En combinaison avec les approches basées sur les marqueurs ADN ou ARN de cette thèse, les proxies des histoires de vie de la distribution pourraient être évalués dans le contexte d'une hétérogénéité environnementale quantifiée simultanément. De plus, les séries temporelles à long terme peuvent révéler des tendances saisonnières à long terme ainsi que la variabilité à court terme, ce qui, en combinaison avec les données sur la composition et les fonctions des communautés, peut donner des indications sur les échelles de stabilité et les différents états alternatifs des écosystèmes.

Cette thèse contribue à la compréhension de l'expression et de la distribution à l'échelle de la communauté des traits des procaryotes comme indicateurs de leur histoire de vie et de leurs implications pour la stabilité de la communauté. Les marqueurs moléculaires basés sur l'ADN ou l'ARN qui ont été décrits dans cette thèse peuvent être utilisés pour cribler les communautés afin de tirer des conclusions sur la susceptibilité des communautés microbiennes aux perturbations et de prédire la dynamique des systèmes écologiques. Une forte hétérogénéité environnementale dans des conditions riches en nutriments a conduit à une communauté dont les traits génomiques indiquent une forte prévalence de généralistes à croissance rapide. Cela a probablement causé les capacités de résistance élevées mesurées de la communauté. Ces résultats sont particulièrement importants face à l'augmentation prévue des perturbations et à la nécessité de mieux administrer l'intervention anthropique sur les écosystèmes pour maintenir les services écologiques critiques. Ma thèse fournit en outre des preuves expérimentales que l'établissement de généralistes ayant une tolérance accrue aux changements environnementaux est lié à des coûts métaboliques qui pourraient être surmontés par une plus grande disponibilité des ressources.

INTRODUCTION

INTRODUCTION

1. Ecosystem disturbances

1.1. Changing environment and disturbances

Global changes caused by human activities affect all forms of life on earth: for instance, agricultural activities, industry, and population growth have caused an unprecedented loss in biodiversity (Barnosky et al., 2011). One of the most threatening aspects of global changes is global warming (IPCC, 2021) which is mediated through the emission of greenhouse gases via the burning of fossil fuels, respiration, methanogenesis, and denitrification as well as through changes in reactions that affect global carbon fluxes due to carbon sequestration (Cavicchioli et al., 2019).

Concomitantly with climate change effects, also the effects of discontinuous physical landscape characteristics or environmental heterogeneity (Dronova, 2017) have been a central topic to ecologists to investigate the mechanisms that impact ecosystem stability under environmental change scenarios (Allison and Martiny, 2008; Shade et al., 2012; Bardgett and Caruso, 2020).

Disturbances caused by environmental changes contribute to environmental heterogeneity and the impact of disturbance may depend on the state of the environment when the disturbance occurs (Hillebrand and Kunze, 2020). Disturbances are ecologically complex events and their effects can be observed at different trophic levels (e.g., species, population, community, ecosystem) but also depending on the type of disturbance (e.g., biotic, abiotic; Sousa, 1984). Due to the high environmental complexity and organism life span, different approaches have been considered to define disturbances (White and Pickett, 1985): an early definition for disturbance states that a disturbance can be considered an external factor causing the partial or total destruction of biomass in a certain environment, while stress is defined as an environmental variable that restricts the growth of the organism (Grime, 1977). A later, more generalized definition claims that a disturbance can be any discrete event in time that disrupts populations, communities, or ecosystems and changes resources, substrate availability, or the physical environment (White and Pickett, 1985).

Disturbances can be can classified considering their severity over time in either pulse disturbances or press disturbances (Fig. 1). Pulse disturbances are short-term events that rapidly decrease in intensity to a previous baseline reference condition (Fig. 1A; Lake, 2000) as fires, tsunamis, storms, droughts, heavy precipitation events, or floods.

Press disturbances are characterized by a sharp or smooth change but their pressure remains constant over time (Fig. 1B; Lake, 2000). For example, the sea level rise caused by the melting of snow covers and ice stocks as a result of global warming (Pereira et al., 2019) or the gradual salinization of the coastal ecosystems (Williams, 2001) can be classified as press disturbances.

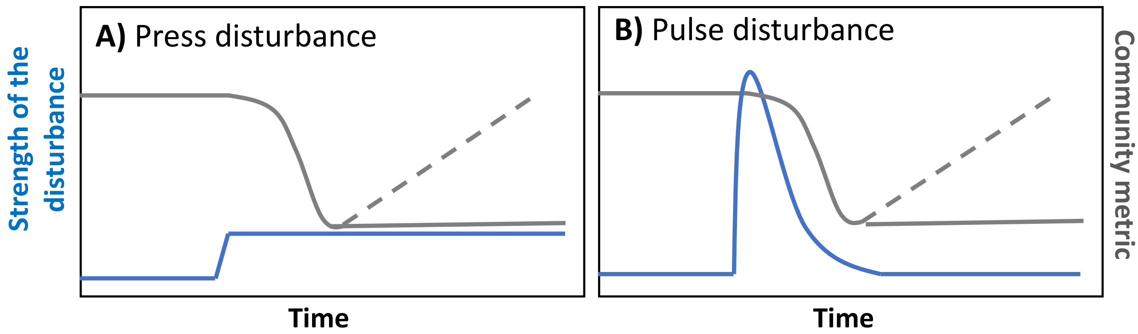

Figure 1. Schematic illustration of pulse and press disturbances and the expected ecological response. *A) Press disturbances and B) pulse disturbances. The dashed grey line represents the trajectory of the community ecological metric if the community recovers to pre-disturbance conditions, while the solid grey line indicates an alternative stable state. Adapted from Shade et al (2012).*

1.2. Importance of disturbances in coastal and pelagic marine environments

The coasts of the world, such as estuaries, lagoons, mangroves, and wetlands, form a narrow interface zone between marine and terrestrial areas in which 40 % of the human population and global economic activity are located (Maul and Duedall, 2019). Coastal areas comprise ecosystems with many endangered species, and while providing important ecosystem services, such as coastal protection, fisheries, rich agricultural lands, areas of high touristic value, as well as nutrient cycling and carbon sequestration (Barbier et al., 2011).

As a result of the anthropogenic activity, the coastal zones are threatened due to sea-level rise (Fagherazzi et al., 2019) but also due to the increase of nitrogen inputs produced by the growth of human populations (Damashek and Francis, 2018). The excess of nitrogen release due to fertilization has caused increased eutrophication of numerous estuarine ecosystems (Herbert, 1999) resulting in a suite of environmental problems including oxygen depletion or the proliferation of algae blooms (Gruber and Galloway, 2008). Estuaries are crucial zones for nitrogen processing, particularly concerning coupled nitrification and denitrification (Seitzinger et al., 2006), and sediments in coastal aquatic systems can contribute substantially to N removal that reaches in some cases between 20-50% of nitrogen inputs (Seitzinger, 1988).

Coastal zones are also subject to elevated natural environmental heterogeneity. As a result, these systems are affected by both, natural (e.g. river discharges, upwelling events, tides, waves) and anthropogenic disturbances (land use change, nutrient loads, climate change-related disturbances). Natural disturbances can modify the biogeochemical properties, such as pH and salinity of the coastal systems at several temporal scales (from hours to years; Raymond and Cole, 2003; Duarte et al., 2013; Pérez-Ruzafa et al., 2019). In addition to natural environmental variability, also long-term anthropogenic effects, such as the change of land use from forest to agricultural use may impact coastal systems. This intervention can intensify the weathering on land, which, for example, leads to increased carbonate exports to the coastal oceans (Raymond and Cole, 2003). Natural and anthropogenic disturbances in coastal systems may occur independently or synergic in space and time (He and Silliman, 2019).

Although environmental heterogeneity and disturbances are of particular relevance for coastal systems, other oceanic habitats are also impacted by environmental variability, including seasonal, interannual, and interdecadal variability: this is exemplified in long-term time-series data, such as the Bermuda Atlantic Time-series Study (http://bats.bios.edu/). Here, global warming represents a slow but strong motor of change of the ocean properties with important effects on the chemistry of the ocean (Bates and Johnson, 2020), for example, by stimulating denitrification rates (Veraart et al., 2011). Also, short-term variability has been observed to increase in intensity and frequency in surface waters, exemplified in the increasing occurrence of positive temperature and salinity anomalies in the last decades (Fig. 2).

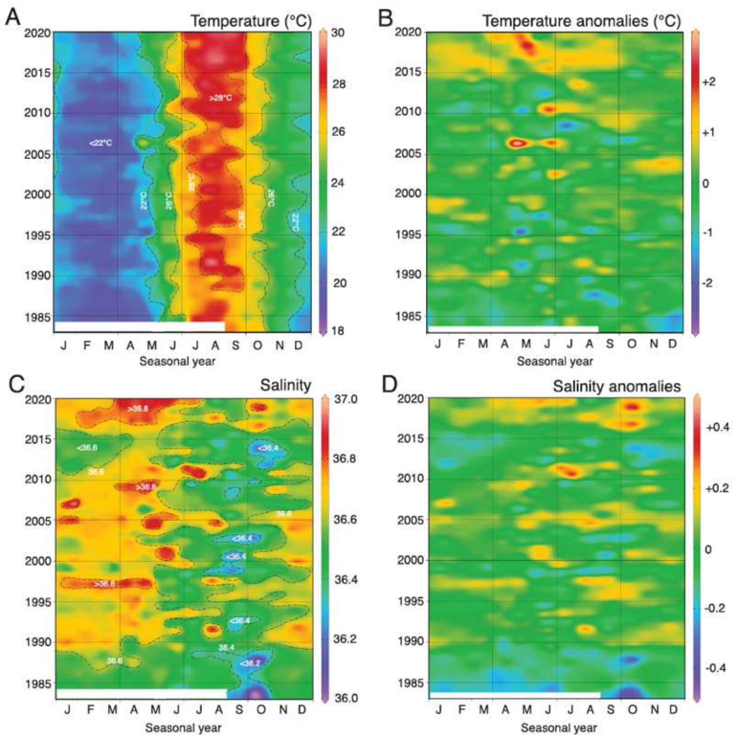

***Figure 2. Long-term water surface properties at Bermuda Atlantic Time-series Study from 1995-2020** (from Bates and Johnson, 2020). A) Surface temperature, B) Surface temperature anomaly, C) Surface salinity. D) Surface salinity anomaly. Anomalies were calculated as the monthly data minus the mean value between 1983-2020. The Y-axis represents the years of the time series. The X-axis represents the month of the year.*

1.2. Salinity as a model stressor

Changing salinity conditions are particularly relevant in coastal ecosystems where freshwater habitats encounter marine sites. They are furthermore of interest because climate change scenarios predict the increasing occurrence of events, such as droughts, storms, floods, and increased river run-off after heavy rainfall events, that impact salt concentrations (Seneviratne et al., 2012). The application of salinity changes as a model stressor is therefore highly relevant to study ecosystem responses of coastal sites under climate change scenarios.

Effects of changing salinity on the biogeochemical cycles have been reported, particularly concerning carbon and nitrogen cycling: for instance, may the intrusion of saltier waters decrease carbon assimilation rates in wetlands and thereby impact coastal carbon reservoirs (Pezeshki et al., 1990; Fagherazzi et al., 2019). On the other hand were contrasting responses reported for nitrogen cycle processes in coastal wetlands, where denitrification rates were shown to increase or decrease due to seawater intrusions (Rysgaard et al., 1999; Wu et al., 2008; Marks et al., 2016). Experimental evidence has furthermore shown that nitrification rates are disrupted in response to the simultaneous increase of temperature and decrease of salinity levels after precipitation (Horz et al., 2004).

Salinity changes are considered to be critical for a wide range of organisms, such as plants (Chinnusamy et al., 2005) or aquatic organisms (Lozupone and Knight, 2007; Kültz, 2015), and constraints caused by changing salinity concentrations varied from disruption of the membrane stability to osmotic stress (Wood, 2015). Organisms in natural habitats will be exposed to a large variety of different stressors. Interestingly, some physiological responses that protect cells against salinity changes, such as the intracellular accumulation of compatible solutes, may also protect cells against other stressors such as temperature changes, droughts, or oxidative stress (Singh et al., 2015).

Salinity variations have been shown to cause changes in functioning (Rath and Rousk, 2015) and community composition (Herlemann et al., 2011) of prokaryote communities. More generally it has been suggested that salinity is one of the major drivers of prokaryote community assembly that has a stronger impact on community composition than other environment variables, such as temperature or pH changes (Lozupone and Knight, 2007).

2. Prokaryotes in ecological studies

2.1. Importance of prokaryotes for global nutrient cycling and other ecosystem services

Prokaryotes are ubiquitous and perform important functions in all ecosystems as they are involved in all biogeochemical cycles (Falkowski et al., 2008). Several biogeochemical key processes (e.g. nitrification, denitrification, or nitrogen fixation) are exclusively mediated by prokaryotes (Galloway et al., 2004; Koch et al., 2019). Prokaryotes provide essential ecosystem services for plant and animal health as well as agriculture (Cavicchioli et al., 2019). They are crucial for climate regulation via their impact on carbon storage: for example, marine prokaryote primary producers contribute to ~50% of the global net primary production (Field et al., 1998). They also contribute largely to the biologically available nitrogen pools via nitrogen fixation (Codispoti, 2007) building thereby the basis of the food webs (Azam and Malfatti, 2007). Prokaryotes are also the main decomposers of the organic matter in both, terrestrial and aquatic ecosystems, where the released nutrients serve higher plants for growth or are transferred to superior trophic levels via microbial loop (Azam et al., 1983). Organic matter recycling may further cause the release of greenhouse-relevant gases such as carbon dioxide (CO_2), methane (CH_4), or Nitrous oxide (N_2O) that impact the global climate (Falkowski et al., 2008). Prokaryote dynamics in functioning and community assembly are accordingly pivotal for the overall ecosystem processes and functioning.

2.2. Microbes as models in experimental ecology

Beyond their above-mentioned crucial role on ecosystem functioning, microbial systems are highly appropriate as model systems to test ecological or evolutionary theory under controlled conditions. This is because microbes possess characteristics that facilitate experimentation when compared to macroorganisms, such as, their small size and short generation times (Jessup et al., 2004). Experimental studies have accordingly played a central role in microbial ecology to generate a critical comprehension of microbial dynamics and processes or to test general ecological theory (Duarte et al., 1997; Shade et al., 2012). There are efforts to generalize ecological findings from micro- to macroecological studies, and it was shown that for example, the distance-decay similarity or the species-area relationships are valid for both microbes and larger organisms (Horner-Devine et al., 2004; Soininen, 2012; Clark et al., 2021).

Microbial systems enable for instance the exposure of different and well-characterized communities to multiple, and reproducible disturbance regimes while controlling for other factors and allow thereby to systematically address the impact of disturbances on microbial systems.

However, the confidence in experimental ecology relies on maintaining comparable initial conditions, which includes the identity and density of organisms in replicable starting communities. Differences in initial community composition and inherent environmental variability may be propagated and amplified during experiments (Fukami, 2015), effectively affecting both the final community composition and functioning (Bloesch et al., 1988). For this reason, the assembly of strains in artificial communities has been applied in multiple studies during the past 20 years to test ecological theory (Table 1). Artificially assembled communities have for instance been used to address questions concerning biodiversity-ecosystem functioning (Bell et al., 2005; Matias et al., 2013; García et al., 2018). However, even though the reduced complexity (richness) and phylogenetic diversity of the artificial communities allow the elucidation of the main drivers behind community performance and stability (Großkopf and Soyer, 2014), these species do not represent naturally co-occurring microorganisms. Accordingly may the impact of species interactions in natural systems that are crucial for ecosystem functioning not be well reflected in these setups (Bell et al., 2009).

The exclusive use of culturable strains further raises a degree of uncertainty whether the natural diversity is appropriately represented. This is of particular concern for abundant and widely spread species, such as the SAR11 clade (Morris et al., 2002) that are adapted to oligotrophic conditions (Giovannoni et al., 2005) and difficult to culture. Isolated prokaryotic strains that were used in artificial communities typically represent copiotrophic taxa because standard culture media, for example, Marine-Broth do not properly represent the ocean nutrient levels in terms of carbon, nitrogen, and phosphorous concentration (Giovannoni and Stingl, 2007).

Cryopreservation is a common method used to conserve axenic and non-axenic culturable microbial strains (Nagai et al., 2005; Heylen et al., 2012). The recent application of cryopreservation protocols on bioreactor communities demonstrated that not only single strains but also microbial communities could be recovered successfully in terms of composition and functionality after cryopreservation (Kerckhof et al., 2014). The application of cryopreservation protocols to complex and naturally occurring communities could therefore be used as an alternative to artificial community assemblies. The preparation of multiple cryopreserved community aliquots could ensure the reproducibility of initial conditions in experimental setups while avoiding or at least reducing the above-mentioned drawbacks that are inherent to studies based on artificial communities.

2.3. Characterization of prokaryotes via their genomic material

For microbial ecologists, the advent of nucleic acid sequencing techniques has made it possible to describe the immense diversity of prokaryote communities through taxonomic markers offering a great opportunity to study the distribution of prokaryotes in space and time (Hanson et al., 2012; Shade et al., 2013). Sequence data analyses additionally enable a comprehensive characterization of the potential functional capacities or current activity of prokaryotic individuals, populations, or communities that are mediated via changes in their DNA and RNA content.

Genome sequence data describe the overall prokaryote identity and its potential adaptation capacity to heterologous conditions. In contrast reflect transcriptome sequence of prokaryotes their activity in the context of a specific situation (Muller, 2019).

The genomic content of microbes other than in larger organisms that can acquire a repertory of responses during their lifetime (Fahimipour and Gross, 2020) is tightly linked to the responses to environmental change, and genetically encoded features could be interpreted as potential traits. Such genomic traits could be applied to infer changes in microbial community performance and composition under diverse scenarios (Krause et al., 2014; Malik et al., 2020). For example, a high fraction of transcription factors as well as a large genome size may be considered as genomic traits that reflect higher phenotypic plasticity since these genomic features confer major capabilities to face dissimilar habitats due to potentially (Kostadinov et al., 2011; Bentkowski et al., 2015). In contrast, the codon usage bias or the number of 16s rRNA genes were suggested to be associated with the length of the lag phase and/ or the maximal growth rates (Klappenbach et al., 2000a; Vieira-Silva and Rocha, 2010; Weissman et al., 2021)

Table 1. *Examples of studies applying artificial microbial communities to address ecological theory.*

Year	Richness	Organism Domains	Taxonomic diversity (Class)	Environments of isolation	Reference
2000	1,2,4,8	Bacteria; Algae	Bacteria: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacilli; Algae: Chlorophyceae, Chlorophyceae, Zygnematophyceae, Trebouxiophyceae, Euglenoidea	Not informed	Naeem et al., 2000
2002	1,6	Bacteria	Alphaproteobacteria, Gammaproteobacteria	River sediments, Bioreactor effluents	Canstein et al., 2002
2004	1,2,4,8	Bacteria	Bacilli, Deinococci, Cocci, Actinomycetia, Gammaproteobacteria	Submerged leaf litter at a single location in the James River, Virginia	Wohl et al., 2004
2005	72	Bacteria	Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Bacilli, Flavobacteriia, Gammaproteobacteria, Sphingobacteria	Freshwater isolated from rain-filled pools (*Fagus sylvatica*)	Bell et al., 2005
2010	1,2,11	Protist	Heterotrichea, Oligohymenophorea, Oligotrichea, Ciliatea, Colpodea	Freshwater bacterivorous protists	Violle et al., 2010
2013	1,2,4	Bacteria	Betaproteobacteria, Gamaproteobacteria	Freshwater, lagoons	Matias et al., 2013
2013	64	Bacteria-Archaea	Bacteria: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacilli; Algae: Chlorophyceae, Chlorophyceae, Zygnematophyceae, Trebouxiophyceae, Euglenoidea	Human body, marine, and terrestrial aquatic environments, soils, and the subsurface	Shakya et al 2013
2014	14	Bacteria	Gammaproteobacteria	*E. coli* mutants	Mee et al., 2014
2014	6	Bacteria	Gammaproteobacteria	Soils	Celiker and Gore 2014
2016	2,50	Bacteria	Gammaproteobacteria, Alphaproteobacteria, Betaproteobacteria, Actinomycetia, Flavobacteriia, Bacilli	Freshwater sediments	Yu et al., 2016
2016	1, 2, 4, 8 and 16	Bacteria	Gammaproteobacteria, Alphaproteobacteria, Betaproteobacteria, Actinomycetia, Flavobacteriia, Bacilli, Sphingobacteriia	Freshwater isolated from rain-filled pools (*Fagus sylvatica*)	Rivett et al., 2016
2018	2,4,8,16,24	Bacteria	Bacilli, Betaproteobacteria, Flavobacteriia, Gammaproteobacteria	Biofilms	García et al., 2018
2018	33	Bacteria	Alphaproteobacteria, Betaproteobacteria, Chitinophagia, Flavobacteriia, Gammaproteobacteria, Sphingobacteria	University of Helsinki culture collection (HAMBI): Soil and aquatic systems, animal and plants, and industry	Cairns et al., 2018
2018	12	Bacteria	Bacteroidia, Clostridia, Coriobacteriia, Deltaproteobacteria	Human body	Venturelli et al., 2018

3. Responses of prokaryote communities to disturbances

Disturbances are known as important drivers of community composition and functioning (Bardgett and Caruso, 2020) and understanding the mechanisms underlying community responses to environmental disturbances is a key to predict their dynamic and the consequences on ecosystem services (Allison and Martiny, 2008). The stability of communities in response to disturbances is hereby driven by their level of resistance and/or resilience, where resistance is defined as the degree to which a community is insensitive to disturbances, and resilience is the rate at which a community returns to a pre-disturbance condition (Shade et al., 2012).

Overall community structures, such as species diversity and species interaction patterns can impact the degree of stability (Fig. 3; Griffiths and Philippot, 2013). Beyond this also the properties of individual community members, such as their tolerance against disturbances (specialist vs generalist) may impact the community's stability in a fluctuating environment (Pandit et al., 2009; Matias et al., 2013).

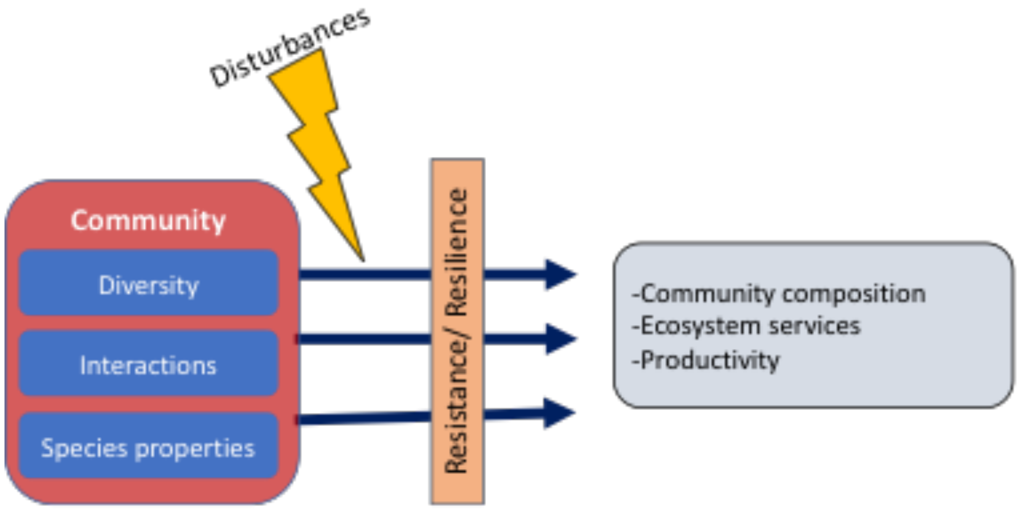

Figure 3. Factors determining the response of community stability to disturbances. *Modified from Griffiths and Phillippot, 2013.*

3.1. The impact of overall community structures on community stability

Biodiversity is a key driver of ecosystem functioning and relationships between biodiversity and ecosystem functioning (BEF) have been intensively addressed in earlier work. (e.g., Naeem et al., 2000; Loreau et al., 2001; Bell et al., 2005). The insurance hypothesis states that the stability of communities after disturbances increases along with increasing species diversity (Yachi and Loreau, 1999). This is because communities with a high richness increase the chance that functionally redundant species are present that can compensate for the functioning of species that disappear or decrease in abundance after an environmental disturbance.

Model-based network studies further predicted that limited positive feedback and weak positive species interactions have a stabilizing effect on communities that are exposed to disturbances (Coyte et al., 2015; Ratzke et al., 2020). An explanation for this is that with the increasing number of obligatory (i.e. strong) dependencies of one species on others the probability increases that at least one of them is not tolerant against environmental change. This in turn would then not only affect the non-tolerant species but all other species that depend on it. Thus, the theory predicts that communities with strong cooperative networks would be more sensitive to disturbances.

3.2. The impact of individual species properties on community stability

3.2.1. Ecological niche

The ecological niche is a fundamental concept in environmental sciences and is defined as the range of biotic and abiotic factors necessary for the persistence of the species in geographical space. This space can the influence by physical conditions (i.e. temperature, salinity, solar radiation), as well as biological parameters (i.e. predation, competition, commensalism). Each of these variables can be ordinated in a theoretical cube of n-dimensions where each dimension represents a single variable of this hypervolume (Hutchinson, 1959). The fundamental niche can be considered as the extent of the n-dimensional space, in which the organism can occur. However, since habitats are usually occupied by several species, under a combination of several environmental factors and source distributions, species are finally constrained to a smaller portion of this multidimensional space, which is referred to as the "realized niche". Accordingly, one can consider that each of these dimensions represent an axis of variability where the expressed traits of an organism in dependence of other community members determine its position in the niche space and thereby their realized niche. Thus, assessing the combination of all traits inherent to a species would capture the full set of species properties and allow to predict the growth of a species in a given environment. However, it stays practically impossible to comprehensively assess all traits associated with a species. Therefore, instead of measuring a plethora of traits a focus on superordinate traits, such as the traits that determine the life history of a species may be an appropriate solution to characterize species properties with high informative value concerning the specie's response to disturbances.

3.2.2. Life history strategies

The life history of organisms determines how species allocate the available energy among characteristics, such as growth rate, reproduction, resource usage efficiency, the capacity to avoid predation, or stress resistance (Stearns, 1977; Ferenci, 2016). Because the available energy is limited, different life history characteristics as growth rate versus resource usage efficiency have been observed to trade off (Fierer et al., 2007; Roller et al., 2016).

One option to characterize the life history of species is to classify them along the binary specialist-generalist continuum: generalists are species whose fitness is distributed evenly across multiple environments, and specialists are species whose fitness is restricted in a few environments (Levins, 1968). This capacity to colonize several environments is related to the "jack of all trade master of none" concept, where some species are capable to perform multiple functions, but at a suboptimal level. In contrast, specialists master only some functions, while they are capable to outcompete generalist species in their optimum habitat (MacArthur, 1972). The generalist-specialist concept is tightly linked to the niche breadth (Sexton et al., 2017) that can be estimated as the range of occupancy along with environments or by directly measuring species performance under changing conditions.

However, the species classification along the generalist-specialist continuum is still challenging as it depends on the context used to define what kind of "generalist" we are interested in. Bell and Bell 2021 discussed that generalist-specialist classifications can refer to a multitude of potential dimensions, such as abiotic (substrates usage, habitat), biotic conditions (interactions), where the substrate usage is the most commonly studied factor and species that is, for example, a salinity specialist can be at the same time a resource generalist (Bell and Bell, 2021).

It has been discussed that tolerance against environmental change is linked to energetic requirements, suggesting that for generalists there should be metabolic cost (Dall and Cuthill, 1997; Jasmin and Kassen, 2007; Bell and Bell, 2021). For instance, resources can constrain, the response of generalist phenotypes to abiotic stressors (DeWitt et al., 1998; Kassen, 2002; Hall et al., 2018). However, despite extensive discussions, there is no experimental evidence demonstrating this long-lasting paradigm.

A further binary life history schema is the r/K strategy theory that states that r-strategists have a short life span, higher growth rates, and abundant offspring compared to K-strategists and at the cost of resources invested in the offspring (Gadgil and Solbrig, 1972). While all microbes could be considered as r-strategists in comparison to larger organisms, microbes can be classified among them according to the r/K selection framework: microbial r-strategists are fast growers with short lag phases at the cost of low competitiveness and low carbon usage efficiency (Klappenbach et al., 2000b; Roller et al., 2016). They can be accordingly be considered opportunist species that react fast to favorable habitat changes or recover fast after disturbances.

Beyond the above described binary approaches, the Competitors-Ruderals-Stress tolerant (CRS) framework that was early designed for plants (Grime, 1977). The CRS schema sorts species based on their occurrence in habitats under high competition levels in resource-rich habitats (C), high levels of stress caused for instance by low resource availability or other environmental stressors (S), and under elevated disturbance frequency or intensity (R). This framework thereby integrates a dimension of nutrient availability with a dimension that comprises the response of species along a disturbance gradient. According to the CRS theory, organisms can be assigned to the different categories based on their traits, and several studies proposed applying the CSR framework also to prokaryotes (e.g., Prosser et al., 2007; Krause et al., 2014; Fierer, 2017).

A recent study however suggests that a globally valid sorting of prokaryote species along the CSR schema is not possible because tradeoffs in prokaryotes are not consistent (Beier et al., 2021). Instead, a resistance-resilience classification has been suggested, where the tradeoff among traits varied in relationship with genome size: while resistance and resilience-related traits in genomes up to roughly 5 Mbp covaried positively, the opposite was true for larger genomes. There is evidence that the average genome size of microbial communities depends on the habitats they are living in, while prokaryotes in aquatic habitats typically feature in average smaller genome sizes compared to soil habitats (Giovannoni et al., 2014).

Accordingly, the tradeoff shaping prokaryote community assembly may depend on their habitat type.

The Mass Ratio Hypothesis argues that dominant traits rather than species numbers drive ecosystem functioning (Grime, 1998) and predicts that variations in traits among individuals can be scaled up and influence communities via the community weighted mean (CWM; Violle et al., 2007). The application of community weighted means allows to aggregate the multitude of individual trait values of a community into a general community indicator. Community weighted means have been used extensively to describe communities of macroorganisms (Díaz et al., 2007) and it has been suggested to apply them also to characterize microbial communities (Fierer et al., 2014).

4. Community assembly and disturbance history

While community overall structures and trait distributions are decisive for the immediate response of communities to disturbances, the disturbance history of an environment also shapes these community parameters and thereby again functional responses (Reichstein et al., 2013). For instance, it has been shown that marine sites with increased environmental heterogeneity selected for microbes with a higher proportion of transcription factors (Kostadinov et al., 2011). Similarly, an evolutionary computer-based simulation model demonstrated that increasingly disturbed habitats result in the evolution of prokaryotes with larger genome sizes (Bentkowski et al., 2015).

Habitat dependent either positive or negative covariations of resistance and resilient related traits (Beier et al. 2021) should according to ecological theory also impact the assembly of community diversity structures: while a simultaneous decrease or increase in both, resistance and resilience is expected to decrease or increase species diversity, species diversity is expected to be less variable in systems where resistance and resilience trade-off (Nimmo et al., 2015).

Earlier research indicated the occurrence of community succession after several consecutive pulse disturbances: while an increasing number of opportunist species was observed after the first disturbance, the continuous exposition to pulse disturbances favored communities with resistant community members (Evans and Wallenstein, 2014). These successional changes in community composition were linked to the increase of functional stability when compared to the controls (Evans and Wallenstein, 2012).

Also, the frequency and intensity of disturbance may affect diversity according to the intermediate disturbance hypothesis (IDH). The IDH states that intermediate disturbance levels (in frequency and intensity) constrain competitive exclusion or extinction rates resulting in an increased local richness compared to low or high disturbance levels (Connell, 1978). In turn, should high species diversity levels in agreement with the insurance hypothesis (Yachi and Loreau, 1999) lead to an increase in community stability.

As a consequence, oligotrophic aquatic environments with low exposure to environmental heterogeneity and where nutrient limitation should restrict the selection of generalist life strategists, disturbances are expected to lead to a loss in richness. Thus, a more pristine and stable system would be more vulnerable to increased frequency of disturbance events compared to more impacted/heterogeneous environments, for example, coastal areas.

THESIS OBJECTIVES

The objective of this doctoral thesis was to study the consequences of environmental disturbances on microbes at the level of single populations as well as complex communities. For the single population approach, I studied the transcriptional response of single bacterial populations with varying niche breadths along an environmental gradient (Chapter 1). To address the consequences of disturbances at the community level, I have established and tested a protocol for cryopreservation of complex microbial communities to improve the experimental replicability for natural community assemblies (Chapter 2). I have furthermore exposed complex microbial communities to pulsed disturbances to investigate the consequences of such disturbances on community structural changes and functioning (Chapter 3). I have also examined whether the pulse disturbances impacted reactions of the nitrogen cycle, and if so whether this was due to the selection of specific taxa involved in nitrogen cycling (Chapter 4).

Chapter 1: Niche breadth affects bacterial transcription patterns along a salinity gradient

Angel Rain-Franco, Nicolas Mouquet, Claire Gougat-Barbera, Thierry Bouvier & Sara Beier

Under revision in Molecular Ecology (minor revisions)

The aim of this chapter was to test the hypothesis that generalist species exposed to changing salinity exhibit higher transcriptional regulation of genes involved in the adaptation against salinity compared to specialist strains. In contrast, we expect that genes involved in core cellular functions such as biomass production will be less regulated in generalists compared to specialists. To test this hypothesis, we assessed the transcriptional regulation of fitness-related and adaptation-related genes of 11 bacterial strains according to their niche breadth and stress exposure level under changing salinity conditions.

Author contributions:

The experimental setup for the isolation experiment was discussed and designed by SB, TB, and NM. NM and CB collected and isolated the bacterial strains. ARF and SR performed laboratory work and analyzed the data with the support of NM. ARF and SB wrote the manuscript and all the authors commented on the manuscript.

Chapter 2: Cryopreservation and Resuscitation of Natural Aquatic Prokaryotic Communities

Angel Rain-Franco, Guilherme Pavan de Moraes & Sara Beier

Published in Frontiers in Microbiology (2021) 11:3633.

https://doi.org/10.3389/fmicb.2020.597653

Chapter 2 describes a method for the preservation and resuscitation of natural aquatic prokaryote communities. We tested several aspects of the cryopreservation protocol: i) the impact of inoculum size on the resuscitation, ii) to which extent diversity and community composition can be maintained in dependence on the inoculum characteristics and culture medium, iii) the effect of handling time during preparation of community aliquots, and iv) the effect of storage time of the cryopreserved aliquots on the community composition after cryopreservation.

Author contribution:

SB and GPM designed the study. SB, GPM, and AR-F collected samples, performed the experiments, and analyzed the data. AR-F and SB mainly wrote the manuscript, while GPM also contributed. All authors commented on the manuscript, and read and approved the final version of the manuscript.

Chapter 3: The cost of adaptability: resource availability constrains functional stability under pulsed disturbances

Angel Rain-Franco, Hannes Peter, Guilherme Pavan de Moraes & Sara Beier

In preparation

This chapter tested the hypothesis that the long-term exposition of communities to salt pulsed disturbances would favor the selection of community members with higher capabilities for resistance and/or resilience, particularly under high resource levels. We further expected that this selection process would lead in communities exposed to pulsed disturbances towards the experiment end and particularly under high resource levels to increased community-level functional stability in response to disturbances. We studied bacterial assemblies subjected to weekly pulse disturbances and measured compositional and functional changes in a 41-days continuous culture experiment under two disturbance regimes (pulse disturbances and undisturbed control) crossed with two levels of resource availability.

Author contribution:

SB, AR and GPM designed the study. The continuous culture system was set up following the instructions and advice from HP. SB, GPM, and ARF collected samples and performed the experiments. SB and ARF analyzed the data. The manuscript was mainly written by AR and SB, while GPM also contributed.

Chapter 4: Long-term exposition pulse disturbances disrupt nitrate cycling

Angel Rain-Franco, Camila Fernandez & Sara Beier

In preparation

The aimed of this chapter was to answer the questions, (i) does the frequent exposure of microbial communities to salinity pulse disturbances affect nitrogen cycling and if so, (ii) is this due to the selection of specific taxa involved in nitrogen cycling or rather to their activity independent on their presence or abundance? To answer these questions, we performed a long-term continuous culture experiment where pulse disturbed and non-disturbed control treatments. We examined whether these changes of nitrogen compounds in the culture media were linked to changes in the microbial community composition.

Author contribution:

SB and ARF designed the study, collected samples and performed the experiments. SB and ARF and CF analyzed the data. ARF and SB mainly wrote the manuscript, CF also contributed. All authors approved the current version of the manuscript.

REFERENCES

Allison, S. D., and Martiny, J. B. H. (2008). Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci. U. S. A.* 105, 11512–11519. doi:10.1073/pnas.0801925105.

Azam, F., Fenchel, T., Field, J., Gray, J., Meyerreil, L., and Thingstad, F. (1983). The Ecological Role of Water-Column Microbes in the Sea. *Mar. Ecol.-Prog. Ser.* 10, 257–263. doi:10.3354/meps010257.

Azam, F., and Malfatti, F. (2007). Microbial structuring of marine ecosystems. *Nat Rev Micro* 5, 782–791. doi:10.1038/nrmicro1747.

Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C., and Silliman, B. R. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs* 81, 169–193. doi:10.1890/10-1510.1.

Bardgett, R. D., and Caruso, T. (2020). Soil microbial community responses to climate extremes: resistance, resilience and transitions to alternative states. *Philosophical Transactions of the Royal Society B: Biological Sciences* 375, 20190112. doi:10.1098/rstb.2019.0112.

Barnosky, A. D., Matzke, N., Tomiya, S., Wogan, G. O. U., Swartz, B., Quental, T. B., et al. (2011). Has the Earth's sixth mass extinction already arrived? *Nature* 471, 51–57. doi:10.1038/nature09678.

Bates, N. R., and Johnson, R. J. (2020). Acceleration of ocean warming, salinification, deoxygenation and acidification in the surface subtropical North Atlantic Ocean. *Communications Earth & Environment* 1, 1–12. doi:10.1038/s43247-020-00030-5.

Beier, S., Werner, J., Bouvier, T., Mouquet, N., and Violle, C. (2021). Trait-trait relationships and functional tradeoffs vary with genome size in prokaryotes. *bioRxiv*, 2021.07.23.453341. doi:10.1101/2021.07.23.453341.

Bell, T., Gessner, M. O., Griffiths, R. I., McLaren, J., Morin, P. J., van der Heijden, M., et al. (2009). "Microbial biodiversity and ecosystem functioning under controlled conditions and in the wild," in *Biodiversity, Ecosystem Functioning, and Human Wellbeing: An Ecological and Economic Perspective*, eds. S. Naeem, D. E. Bunker, A. Hector, M. Loreau, and C. Perrings (Oxford University Press).

Bell, T. H., and Bell, T. (2021). Many roads to bacterial generalism. *FEMS Microbiology Ecology* 97. doi:10.1093/femsec/fiaa240.

Bell, T., Newman, J. A., Silverman, B. W., Turner, S. L., and Lilley, A. K. (2005). The contribution of species richness and composition to bacterial services. *Nature* 436, 1157–1160.

Bentkowski, P., Van Oosterhout, C., and Mock, T. (2015). A Model of Genome Size Evolution for Prokaryotes in Stable and Fluctuating Environments. *Genome Biol. Evol.* 7, 2344–2351. doi:10.1093/gbe/evv148.

Bloesch, J., Bossard, P., Bührer, H., Bürgi, H. R., and Uehlinger, U. (1988). Can results from limnocorral experiments be transferred to in situ conditions? *Hydrobiologia* 159, 297–308. doi:10.1007/BF00008242.

Cavicchioli, R., Ripple, W. J., Timmis, K. N., Azam, F., Bakken, L. R., Baylis, M., et al. (2019). Scientists' warning to humanity: microorganisms and climate change. *Nat Rev Microbiol* 17, 569–586. doi:10.1038/s41579-019-0222-5.

Chinnusamy, V., Jagendorf, A., and Zhu, J.-K. (2005). Understanding and Improving Salt Tolerance in Plants. *Crop Science* 45, 437–448. doi:10.2135/cropsci2005.0437.

Clark, D. R., Underwood, G. J. C., McGenity, T. J., and Dumbrell, A. J. (2021). What drives study-dependent differences in distance–decay relationships of microbial communities? *Global Ecology and Biogeography* 30, 811–825. doi:10.1111/geb.13266.

Codispoti, L. A. (2007). An oceanic fixed nitrogen sink exceeding 400 Tg N $a^{\text{−1}}$ vs the concept of homeostasis in the fixed-nitrogen inventory. *Biogeosciences* 4, 233–253. doi:10.5194/bg-4-233-2007.

Connell, J. H. (1978). Diversity in Tropical Rain Forests and Coral Reefs. *Science* 199, 1302. doi:10.1126/science.199.4335.1302.

Coyte, K. Z., Schluter, J., and Foster, K. R. (2015). The ecology of the microbiome: Networks, competition, and stability. *Science* 350, 663–666. doi:10.1126/science.aad2602.

Dall, S., and Cuthill, I. (1997). The Information Costs of Generalism. *Oikos* 80, 197–202. doi:10.2307/3546535.

Damashek, J., and Francis, C. A. (2018). Microbial Nitrogen Cycling in Estuaries: From Genes to Ecosystem Processes. *Estuaries and Coasts* 41, 626–660. doi:10.1007/s12237-017-0306-2.

DeWitt, T. J., Sih, A., and Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution* 13, 77–81. doi:10.1016/S0169-5347(97)01274-3.

Díaz, S., Lavorel, S., Bello, F. de, Quétier, F., Grigulis, K., and Robson, T. M. (2007). Incorporating plant functional diversity effects in ecosystem service assessments. *PNAS* 104, 20684–20689. doi:10.1073/pnas.0704716104.

Dronova, I. (2017). Environmental heterogeneity as a bridge between ecosystem service and visual quality objectives in management, planning and design. *Landscape and Urban Planning* 163, 90–106. doi:10.1016/j.landurbplan.2017.03.005.

Duarte, C. M., Gasol, J. M., and Vaqué, D. (1997). Role of experimental approaches in marine microbial ecology. *Aquat Microb Ecol* 13, 101–111.

Duarte, C. M., Hendriks, I. E., Moore, T. S., Olsen, Y. S., Steckbauer, A., Ramajo, L., et al. (2013). Is Ocean Acidification an Open-Ocean Syndrome? Understanding Anthropogenic Impacts on Seawater pH. *Estuaries and Coasts* 36, 221–236. doi:10.1007/s12237-013-9594-3.

Evans, S. E., and Wallenstein, M. D. (2012). Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? *Biogeochemistry* 109, 101–116. doi:10.1007/s10533-011-9638-3.

Evans, S. E., and Wallenstein, M. D. (2014). Climate change alters ecological strategies of soil bacteria. *Ecology Letters* 17, 155–164. doi:https://doi.org/10.1111/ele.12206.

Fagherazzi, S., Anisfeld, S. C., Blum, L. K., Long, E. V., Feagin, R. A., Fernandes, A., et al. (2019). Sea Level Rise and the Dynamics of the Marsh-Upland Boundary. *Front. Environ. Sci.* 0. doi:10.3389/fenvs.2019.00025.

Fahimipour, A. K., and Gross, T. (2020). Mapping the bacterial metabolic niche space. *Nat Commun* 11, 4887. doi:10.1038/s41467-020-18695-z.

Falkowski, P. G., Fenchel, T., and Delong, E. F. (2008). The microbial engines that drive Earth's biogeochemical cycles. *Science* 320, 1034–1039. doi:10.1126/science.1153213.

Ferenci, T. (2016). Trade-off Mechanisms Shaping the Diversity of Bacteria. *Trends in Microbiology* 24, 209–223. doi:10.1016/j.tim.2015.11.009.

Field, C. B., Behrenfeld, M. J., Randerson, J. T., and Falkowski, P. (1998). Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. *Science* 281, 237–240. doi:10.1126/science.281.5374.237.
Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* 15, 579–590. doi:10.1038/nrmicro.2017.87.
Fierer, N., Barberán, A., and Laughlin, D. C. (2014). Seeing the forest for the genes: using metagenomics to infer the aggregated traits of microbial communities. *Frontiers in Microbiology* 5, 614. doi:10.3389/fmicb.2014.00614.
Fierer, N., Bradford, M. A., and Jackson, R. B. (2007). Toward an ecological classification of soil bacteria. *Ecology* 88, 1354–1364. doi:10.1890/05-1839.
Fukami, T. (2015). Historical Contingency in Community Assembly: Integrating Niches, Species Pools, and Priority Effects. *Annu. Rev. Ecol. Evol. Syst.* 46, 1–23. doi:10.1146/annurev-ecolsys-110411-160340.
Gadgil, M., and Solbrig, O. T. (1972). The Concept of r- and K-Selection: Evidence from Wild Flowers and Some Theoretical Considerations. *The American Naturalist* 106, 14–31. doi:10.1086/282748.
Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., et al. (2004). Nitrogen Cycles: Past, Present, and Future. *Biogeochemistry* 70, 153–226. doi:10.1007/s10533-004-0370-0.
García, F. C., Bestion, E., Warfield, R., and Yvon-Durocher, G. (2018). Changes in temperature alter the relationship between biodiversity and ecosystem functioning. *Proc Natl Acad Sci USA* 115, 10989. doi:10.1073/pnas.1805518115.
Giovannoni, S. J., Thrash, J. C., and Temperton, B. (2014). Implications of streamlining theory for microbial ecology. *ISME J.* 8, 1553–1565. doi:10.1038/ismej.2014.60.
Giovannoni, S. J., Tripp, H. J., Givan, S., Podar, M., Vergin, K. L., Baptista, D., et al. (2005). Genome streamlining in a cosmopolitan oceanic bacterium. *Science* 309, 1242–1245. doi:10.1126/science.1114057.
Giovannoni, S., and Stingl, U. (2007). The importance of culturing bacterioplankton in the "omics" age. *Nature Reviews Microbiology* 5, 820–826. doi:10.1038/nrmicro1752.
Griffiths, B. S., and Philippot, L. (2013). Insights into the resistance and resilience of the soil microbial community. *FEMS Microbiology Reviews* 37, 112–129. doi:10.1111/j.1574-6976.2012.00343.x.
Grime, J. (1977). Evidence for Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory. *Am. Nat.* 111, 1169–1194. doi:10.1086/283244.
Grime, J. P. (1998). Benefits of Plant Diversity to Ecosystems: Immediate, Filter and Founder Effects. *Journal of Ecology* 86, 902–910.
Großkopf, T., and Soyer, O. S. (2014). Synthetic microbial communities. *Current Opinion in Microbiology* 18, 72–77. doi:10.1016/j.mib.2014.02.002.
Gruber, N., and Galloway, J. N. (2008). An Earth-system perspective of the global nitrogen cycle. *Nature* 451, 293–296. doi:10.1038/nature06592.
Hall, J. P. J., Harrison, E., and Brockhurst, M. A. (2018). Competitive species interactions constrain abiotic adaptation in a bacterial soil community. *Evolution Letters* 2, 580–589. doi:https://doi.org/10.1002/evl3.83.
Hanson, C. A., Fuhrman, J. A., Horner-Devine, M. C., and Martiny, J. B. H. (2012). Beyond biogeographic patterns: processes shaping the microbial landscape. *Nature Reviews Microbiology* 10, 497–506. doi:10.1038/nrmicro2795.

He, Q., and Silliman, B. R. (2019). Climate Change, Human Impacts, and Coastal Ecosystems in the Anthropocene. *Current Biology* 29, R1021–R1035. doi:10.1016/j.cub.2019.08.042.

Herbert, R. A. (1999). Nitrogen cycling in coastal marine ecosystems. *FEMS Microbiology Reviews* 23, 563–590. doi:10.1111/j.1574-6976.1999.tb00414.x.

Herlemann, D. P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J., and Andersson, A. F. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J* 5, 1571–1579.

Heylen, K., Ettwig, K., Hu, Z., Jetten, M., and Kartal, B. (2012). Rapid and Simple Cryopreservation of Anaerobic Ammonium-Oxidizing Bacteria. *Appl. Environ. Microbiol.* 78, 3010. doi:10.1128/AEM.07501-11.

Hillebrand, H., and Kunze, C. (2020). Meta-analysis on pulse disturbances reveals differences in functional and compositional recovery across ecosystems. *Ecology Letters* 23, 575–585. doi:10.1111/ele.13457.

Horner-Devine, M. C., Lage, M., Hughes, J. B., and Bohannan, B. J. (2004). A taxa-area relationship for bacteria. *Nature* 432, 750–3.

Horz, H.-P., Barbrook, A., Field, C. B., and Bohannan, B. J. M. (2004). Ammonia-oxidizing bacteria respond to multifactorial global change. *PNAS* 101, 15136–15141. doi:10.1073/pnas.0406616101.

Hutchinson, G. E. (1959). Homage to Santa Rosalia or Why Are There So Many Kinds of Animals? *The American Naturalist* 93, 145–159. doi:10.1086/282070.

IPCC (2021). *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change.* , eds. V. Masson-Delmotte, A. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, et al. Cambridge University Press.

Jasmin, J.-N., and Kassen, R. (2007). On the experimental evolution of specialization and diversity in heterogeneous environments. *Ecology Letters* 10, 272–281. doi:https://doi.org/10.1111/j.1461-0248.2007.01021.x.

Jessup, C. M., Kassen, R., Forde, S. E., Kerr, B., Buckling, A., Rainey, P. B., et al. (2004). Big questions, small worlds: microbial model systems in ecology. *Trends in Ecology & Evolution* 19, 189–197. doi:10.1016/j.tree.2004.01.008.

Kassen, R. (2002). The experimental evolution of specialists, generalists, and the maintenance of diversity. *J. Evol. Biol.* 15, 173–190. doi:10.1046/j.1420-9101.2002.00377.x.

Kerckhof, F.-M., Courtens, E. N. P., Geirnaert, A., Hoefman, S., Ho, A., Vilchez-Vargas, R., et al. (2014). Optimized Cryopreservation of Mixed Microbial Communities for Conserved Functionality and Diversity. *PLOS ONE* 9, e99517. doi:10.1371/journal.pone.0099517.

Klappenbach, J. A., Dunbar, J. M., and Schmidt, T. M. (2000a). RRNA operon copy number reflects ecological strategies of bacteria. *Appl. Environ. Microbiol.* 66, 1328–1333. doi:10.1128/AEM.66.4.1328-1333.2000.

Klappenbach, J. A., Dunbar, J. M., and Schmidt, T. M. (2000b). rRNA Operon Copy Number Reflects Ecological Strategies of Bacteria. *Appl. Environ. Microbiol.* 66, 1328–1333. doi:10.1128/AEM.66.4.1328-1333.2000.

Koch, H., van Kessel, M. A. H. J., and Lücker, S. (2019). Complete nitrification: insights into the ecophysiology of comammox Nitrospira. *Appl Microbiol Biotechnol* 103, 177–189. doi:10.1007/s00253-018-9486-3.

Kostadinov, I., Kottmann, R., Ramette, A., Waldmann, J., Buttigieg, P. L., and Glöckner, F. O. (2011). Quantifying the effect of environment stability on the transcription factor

repertoire of marine microbes. *Microbial Informatics and Experimentation* 1, 9. doi:10.1186/2042-5783-1-9.
Krause, S., Le Roux, X., Niklaus, P. A., Van Bodegom, P. M., Lennon, J. T., Bertilsson, S., et al. (2014). Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. *Front. Microbiol.* 5. doi:10.3389/fmicb.2014.00251.
Kültz, D. (2015). Physiological mechanisms used by fish to cope with salinity stress. *Journal of Experimental Biology* 218, 1907–1914. doi:10.1242/jeb.118695.
Lake, P. S. (2000). Disturbance, patchiness, and diversity in streams. *Journal of the North American Benthological Society* 19, 573–592. doi:10.2307/1468118.
Levins, R. (1968). *Evolution in Changing Environments: Some Theoretical Explorations. (MPB-2)*. Princeton University Press doi:10.2307/j.ctvx5wbbh.
Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., et al. (2001). Ecology - Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science* 294, 804–808. doi:10.1126/science.1064088.
Lozupone, C. A., and Knight, R. (2007). Global patterns in bacterial diversity. *Proceedings of the National Academy of Sciences of the United States of America* 104, 11436–11440.
MacArthur, R. (1972). *Geographical Ecology: Patterns in the Distribution of Species*. Available at: https://press.princeton.edu/books/paperback/9780691023823/geographical-ecology [Accessed August 15, 2021].
Malik, A. A., Martiny, J. B. H., Brodie, E. L., Martiny, A. C., Treseder, K. K., and Allison, S. D. (2020). Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. *The ISME Journal* 14, 1–9. doi:10.1038/s41396-019-0510-0.
Marks, B. M., Chambers, L., and White, J. R. (2016). Effect of Fluctuating Salinity on Potential Denitrification in Coastal Wetland Soil and Sediments. *Soil Science Society of America Journal* 80, 516–526. doi:10.2136/sssaj2015.07.0265.
Matias, M. G., Combe, M., Barbera, C., and Mouquet, N. (2013). Ecological strategies shape the insurance potential of biodiversity. *Front. Microbiol.* 3, 432. doi:10.3389/fmicb.2012.00432.
Maul, G. A., and Duedall, I. W. (2019). "Demography of Coastal Populations," in *Encyclopedia of Coastal Science*, eds. C. W. Finkl and C. Makowski (Cham: Springer International Publishing), 692–700. doi:10.1007/978-3-319-93806-6_115.
Morris, R. M., Rappe, M. S., Connon, S. A., Vergin, K. L., Siebold, W. A., Carlson, C. A., et al. (2002). SAR11 clade dominates ocean surface bacterioplankton communities. *Nature* 420, 806–810. doi:10.1038/nature01240.
Muller, E. E. L. (2019). Determining Microbial Niche Breadth in the Environment for Better Ecosystem Fate Predictions. *mSystems* 4, e00080-19. doi:10.1128/mSystems.00080-19.
Naeem, S., Hahn, D. R., and Schuurman, G. (2000). Producer–decomposer co-dependency influences biodiversity effects. *Nature* 403, 762–764. doi:10.1038/35001568.
Nagai, T., Tomioka, K., Takeuchi, K., Iida, M., Kawada, M., and Sato, T. (2005). Evaluation of Preservation Techniques of Microorganism Resources in the MAFF Genebank. *Japan Agricultural Research Quarterly: JARQ* 39, 19–27. doi:10.6090/jarq.39.19.
Pandit, S. N., Kolasa, J., and Cottenie, K. (2009). Contrasts between habitat generalists and specialists: an empirical extension to the basic metacommunity framework. *Ecology* 90, 2253–2262. doi:10.1890/08-0851.1.

Pereira, C. S., Lopes, I., Abrantes, I., Sousa, J. P., and Chelinho, S. (2019). Salinization effects on coastal ecosystems: a terrestrial model ecosystem approach. *Philosophical Transactions of the Royal Society B: Biological Sciences* 374, 20180251. doi:10.1098/rstb.2018.0251.

Pérez-Ruzafa, A., Pérez-Ruzafa, I. M., Newton, A., and Marcos, C. (2019). "Chapter 15 - Coastal Lagoons: Environmental Variability, Ecosystem Complexity, and Goods and Services Uniformity," in *Coasts and Estuaries*, eds. E. Wolanski, J. W. Day, M. Elliott, and R. Ramachandran (Elsevier), 253–276. doi:10.1016/B978-0-12-814003-1.00015-0.

Pezeshki, S. R., Delaune, R. D., and Patrick, W. H. (1990). Flooding and saltwater intrusion: Potential effects on survival and productivity of wetland forests along the U.S. Gulf Coast. *Forest Ecology and Management* 33–34, 287–301. doi:10.1016/0378-1127(90)90199-L.

Prosser, J. I., Bohannan, B. J. M., Curtis, T. P., Ellis, R. J., Firestone, M. K., Freckleton, R. P., et al. (2007). The role of ecological theory in microbial ecology. *Nature Reviews Microbiology* 5, 384–392. doi:10.1038/nrmicro1643.

Rath, K. M., and Rousk, J. (2015). Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: A review. *Soil Biology and Biochemistry* 81, 108–123. doi:10.1016/j.soilbio.2014.11.001.

Ratzke, C., Barrere, J., and Gore, J. (2020). Strength of species interactions determines biodiversity and stability in microbial communities. *Nat Ecol Evol* 4, 376–383. doi:10.1038/s41559-020-1099-4.

Raymond, P. A., and Cole, J. J. (2003). Increase in the Export of Alkalinity from North America's Largest River. *Science* 301, 88–91. doi:10.1126/science.1083788.

Reichstein, M., Bahn, M., Ciais, P., Frank, D., Mahecha, M. D., Seneviratne, S. I., et al. (2013). Climate extremes and the carbon cycle. *Nature* 500, 287–295. doi:10.1038/nature12350.

Roller, B. R. K., Stoddard, S. F., and Schmidt, T. M. (2016). Exploiting rRNA operon copy number to investigate bacterial reproductive strategies. *Nat Microbiol* 1, 1–7. doi:10.1038/nmicrobiol.2016.160.

Rysgaard, S., Thastum, P., Dalsgaard, T., Christensen, P. B., and Sloth, N. P. (1999). Effects of salinity on NH4+ adsorption capacity, nitrification, and denitrification in Danish estuarine sediments. *Estuaries* 22, 21–30. doi:10.2307/1352923.

Seitzinger, S., Harrison, J. A., Böhlke, J. K., Bouwman, A. F., Lowrance, R., Peterson, B., et al. (2006). Denitrification Across Landscapes and Waterscapes: A Synthesis. *Ecological Applications* 16, 2064–2090. doi:10.1890/1051-0761(2006)016[2064:DALAWA]2.0.CO;2.

Seitzinger, S. P. (1988). Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnology and Oceanography* 33, 702–724. doi:10.4319/lo.1988.33.4part2.0702.

Seneviratne, S., Nicholls, N., Easterling, D., Goodess, C., Kanae, S., Kossin, J., et al. (2012). Changes in climate extremes and their impacts on the natural physical environment. 109–230. doi:10.7916/d8-6nbt-s431.

Sexton, J. P., Montiel, J., Shay, J. E., Stephens, M. R., and Slatyer, R. A. (2017). Evolution of Ecological Niche Breadth. *Annu. Rev. Ecol. Evol. Syst.* 48, 183–206. doi:10.1146/annurev-ecolsys-110316-023003.

Shade, A., Gregory Caporaso, J., Handelsman, J., Knight, R., and Fierer, N. (2013). A meta-analysis of changes in bacterial and archaeal communities with time. *The ISME Journal* 7, 1493–1506. doi:10.1038/ismej.2013.54.

Shade, A., Peter, H., Allison, S. D., Baho, D. L., Berga, M., Buergmann, H., et al. (2012). Fundamentals of microbial community resistance and resilience. *Front. Microbiol.* 3, 417. doi:10.3389/fmicb.2012.00417.

Singh, M., Kumar, J., Singh, S., Singh, V. P., and Prasad, S. M. (2015). Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. *Rev Environ Sci Biotechnol* 14, 407–426. doi:10.1007/s11157-015-9372-8.

Soininen, J. (2012). Macroecology of unicellular organisms – patterns and processes. *Environmental Microbiology Reports* 4, 10–22. doi:10.1111/j.1758-2229.2011.00308.x.

Sousa, W. P. (1984). The Role of Disturbance in Natural Communities. *Annual Review of Ecology and Systematics* 15, 353–391.

Stearns, S. C. (1977). The Evolution of Life History Traits: A Critique of the Theory and a Review of the Data. *Annu. Rev. Ecol. Syst.* 8, 145–171. doi:10.1146/annurev.es.08.110177.001045.

Veraart, A. J., Klein, J. J. M. de, and Scheffer, M. (2011). Warming Can Boost Denitrification Disproportionately Due to Altered Oxygen Dynamics. *PLOS ONE* 6, e18508. doi:10.1371/journal.pone.0018508.

Vieira-Silva, S., and Rocha, E. P. C. (2010). The Systemic Imprint of Growth and Its Uses in Ecological (Meta) Genomics. *PLoS Genet.* 6, e1000808. doi:10.1371/journal.pgen.1000808.

Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., et al. (2007). Let the concept of trait be functional! *Oikos* 116, 882–892. doi:10.1111/j.2007.0030-1299.15559.x.

Weissman, J. L., Hou, S., and Fuhrman, J. A. (2021). Estimating maximal microbial growth rates from cultures, metagenomes, and single cells via codon usage patterns. *PNAS* 118. doi:10.1073/pnas.2016810118.

White, P. S., and Pickett, S. T. A. (1985). “Chapter 1 - Natural Disturbance and Patch Dynamics: An Introduction,” in *The Ecology of Natural Disturbance and Patch Dynamics*, eds. S. T. A. Pickett and P. S. White (San Diego: Academic Press), 3–13. doi:10.1016/B978-0-12-554520-4.50006-X.

Williams, W. D. (2001). “Anthropogenic salinisation of inland waters,” in *Saline Lakes: Publications from the 7th International Conference on Salt Lakes, held in Death Valley National Park, California, U.S.A., September 1999* Developments in Hydrobiology., eds. J. M. Melack, R. Jellison, and D. B. Herbst (Dordrecht: Springer Netherlands), 329–337. doi:10.1007/978-94-017-2934-5_30.

Wood, J. M. (2015). Bacterial responses to osmotic challenges. *Journal of General Physiology* 145, 381–388. doi:10.1085/jgp.201411296.

Wu, Y., Tam, N. F. Y., and Wong, M. H. (2008). Effects of salinity on treatment of municipal wastewater by constructed mangrove wetland microcosms. *Marine Pollution Bulletin* 57, 727–734. doi:10.1016/j.marpolbul.2008.02.026.

Yachi, S., and Loreau, M. (1999). Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proc. Natl. Acad. Sci. U. S. A.* 96, 1463–1468. doi:10.1073/pnas.96.4.1463.

Chapter 1

Niche breadth affects bacterial transcription patterns along a salinity gradient

Angel Rain-Franco[1], Nicolas Mouquet[2], Claire Gougat-Barbera[3], Thierry Bouvier[2], Sara Beier[4]

[1] Sorbonne Université, CNRS, Laboratoire d'Océanographie Microbienne, LOMIC, F-66650 Banyuls/mer, France.

[2] MARBEC, Université de Montpellier, CNRS, Ifremer, IRD, Montpellier, 34095, FRANCE.

[3] ISEM, University of Montpellier, CNRS, EPHE, IRD, Montpellier, 34095, France

[4] Leibniz Institute for Baltic Sea Research, Warnemünde, Germany.

Under revision in Molecular Ecology (minor revisions)

ABSTRACT

Understanding molecular mechanisms that determine the species life history is important for predicting their susceptibility to environmental change. While specialist species with a narrow niche breadth (NB) maximize their fitness in their optimum habitat, generalists with broad NB adapt to multiple environments. The main objective of this study was to identify general transcriptional patterns that would distinguish bacterial strains characterized by contrasted NBs along a salinity gradient. More specifically, we hypothesized that genes encoding fitness-related traits, such as biomass production have a higher degree of transcriptional regulation in specialists than in generalists, because the fitness of specialists is more variable under environmental change. By contrast, we expected that generalists would exhibit enhanced transcriptional regulation of genes encoding traits that protect them against cellular damage. To test these hypotheses, we assessed the transcriptional regulation of fitness-related and adaptation-related genes of 11 bacterial strains in relation to their NB and stress exposure under changing salinity conditions. The results suggested that transcriptional regulation levels of fitness- and adaptation-related genes correlated with the NB and/or the stress exposure of the inspected strains. We further identified a shortlist of candidate stress marker genes that could be used in future studies to monitor the susceptibility of bacterial populations or communities to environmental changes.

INTRODUCTION

Environmental changes and disturbances caused by anthropogenic activities are an increasing threat to natural ecosystems (Grimm et al., 2013). For understanding the effect of environmental disturbances on the functioning and composition of species assemblages it is essential to examine the response and adaptions of individual species in a changing environment. In this context, the assessment of niche dimensions has been one of the most relevant questions in ecology for understanding the biological adaptation of individual species to environmental change (Slatyer, Hirst, & Sexton, 2013). The ecological niche is a fundamental concept in ecology and is defined as the set of environmental conditions, under which species can live and reproduce (Hutchinson, 1957). The niche breadth (NB) is defined as the width of an organism's fitness curve over an environmental gradient and can be used to discriminate between generalist and specialist species (Devictor et al., 2010; Lynch & Gabriel, 1987). Specialists with a narrow niche possess traits that optimize fitness in their optimum habitat at expenses of the performance under suboptimal conditions, while, generalists with a broad niche are considered to be less competitive under optimal growth conditions but feature higher resistance against changing conditions (Huey & Slatkin, 1976; Jasmin & Kassen, 2007; MacArthur, 1972). Accordingly, specialists are locally adapted organisms and are considered to be particularly endangered by environmental disturbances that are predicted to occur more frequently under climate change scenarios (Colles, Liow, & Prinzing, 2009; Planton, Déqué, Chauvin, & Terray, 2008; Thuiller, Lavorel, & Araújo, 2005).

Different approaches for NB estimations have been used in previous research, that were based for example on the number of different resources an organism can use, the number of abiotic associations and biological interactions an organism is involved, or its tolerance against changing environmental conditions (Sexton, Montiel, Shay, Stephens, & Slatyer, 2017). Among these aspects, environmental tolerance has been demonstrated to be one of the most important factors determining the geographical distribution of species (Slatyer et al., 2013).

Stress refers directly to the decrease of an organism's fitness caused by external constraints, for example, nutrient limitation, but also physical factors, such as temperature or salinity changes (P. Grime J. & Pierce, 2012). Stress is accordingly linked to the NB, where species with a narrow NB exhibit a larger variability in fitness under the same environmental change than do species with a broad NB (e.g. Matias et al. 2013). However, while NB is a constant parameter for a given species and environmental parameter, the stress exposure of this species depends on the environmental gradient under inspection (Fig.1).

Microbes have been used widely over the past few decades to test general ecological theory (Bell et al., 2009) and including the integration of functional perspectives has helped to elucidate the link between traits and niche-related processes (Krause et al., 2014). The short generation times of microbes and their small size allow to perform controlled and replicable experiments at different spatial and temporal scales (Jessup et al., 2004). In combination with powerful sequencing techniques, microbes can be used as a relevant model system for studying molecular mechanisms that are associated with the adaptation of species to environmental change concerning their NB. Besides, microorganisms are the main drivers of carbon and nutrient cycling in all environments and therefore relevant to the function of ecosystems (Konopka, Lindemann, & Fredrickson, 2015).

The impact of the NB distribution in communities on the biodiversity-ecosystem-function and biodiversity-insurance relationships (Gravel et al., 2011; Matias, Combe, Barbera, & Mouquet, 2013) or dispersal and community assembly mechanisms (Shen, Juergens, & Beier, 2018; Szekely, Berga, & Langenheder, 2013) underlines the importance of the NB concept to understand community functional and compositional dynamics. Transcriptome data of microbial organisms represent a blueprint of their response and tolerance to environmental change and could be used as a tool to understand the molecular mechanisms behind resistance but also to identify NB distributions in more complex species assemblies. Our main objective was therefore to investigate whether there are commonly valid transcriptional regulation mechanisms that are related to the tolerance-based NB of microorganisms. We are aware of one earlier study addressing general differences in the transcriptional regulation patterns of oligotrophic versus copiotrophic aquatic bacterial strains (Cottrell & Kirchman, 2016). However, to our knowledge, this is the first study with the aim to identify transcriptional patterns that allow to discriminate between generalist and specialist life histories and detect their exposure to different stress levels, in order to learn more about the susceptibility of organisms to environmental change.

By definition, the fitness of specialists is more variable along an environmental gradient than that of generalists (Lynch & Gabriel, 1987). We, therefore, hypothesized that genes that are directly involved in fitness-related traits, such as growth or biomass production, have a higher level of transcriptional regulation in specialists than in generalists under changing environmental conditions. Due to a negative relationship between NB and stress exposure and the causal link between stress and fitness, we expect to detect the opposite relationship between regulation and stress exposure levels (H1). We furthermore hypothesized that generalist species would exhibit higher transcriptional regulation of genes encoding traits involved in the physiological adaption to changing environmental conditions than specialists (H2). This hypothesis refers to all genes that prevent damage from cells under suboptimal environmental conditions.

To test these hypotheses we incubated 11 bacterial strains with varying tolerance against salinity changes at different salinity levels and tested correlations of transcriptional regulation patterns of fitness and adaptation-related genes against NB and stress. We furthermore present a list of candidate stress marker genes whose transcriptional regulation correlated to stress exposure and which may be applied in future studies to monitor stress in microbial populations or communities.

We have chosen changing salinity as a stressor because it has been described as one of the major abiotic drivers of microbial community composition across several environments (Lozupone and Knight, 2007). Changing salinity conditions are furthermore environmentally relevant because climate change scenarios predict an increasing occurrence of salinity changes caused by events, such as droughts, storms, floods, and river run-off (Seneviratne et al., 2012).

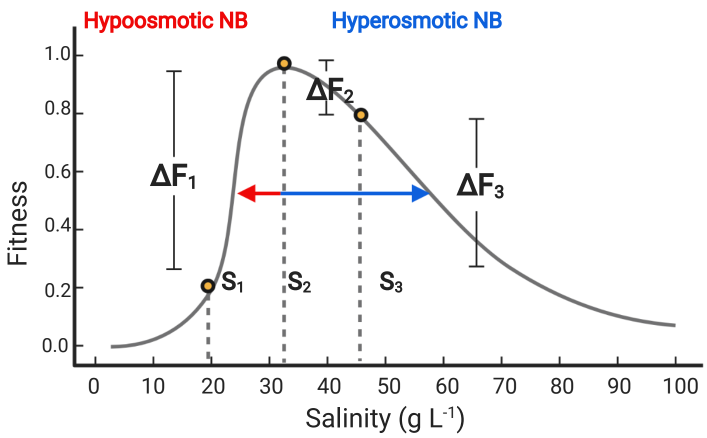

Figure 1. Schematic illustration for side depend NBs and stress exposure. *Most of the 11 model strains were characterized by asymmetric fitness curves. The hyper- and hypoosmotic NBs are indicated with red and blue arrows, respectively. Stress exposure levels along salinity gradients (here S1:S2,S2:3 and S1:S3) can be estimated by subtracting fitness values at the start and end point of the salinity gradients under consideration (here ΔF_1, ΔF_2 or ΔF_3). Different then the NBs, stress exposure is not a constant parameter for a given species but depends on the position and length of the salinity gradient under inspection (i.e. the position of S1, S2 or S3).*

METHODS

Bacterial fitness curves

To assess the effect of the NB on bacterial transcriptional activity, we included 11 bacterial model strains in our study (Table 1, Table S1). These strains belong to a collection of isolates originating from several aquatic environments with different salinities (Matias et al., 2013). To ensure the purity of all isolates, cryopreserved cells from all 11 strains were re-isolated after plating on standard LB agar to which 20 g L^{-1} NaCl was added (final salinity of 30 g L^{-1} NaCl). A single colony was picked and transferred into a tube containing 2 mL of liquid LB medium (final salinity of 30 g L^{-1} NaCl), incubated at room temperature, and then cryopreserved using glycerol as cryoprotectant for downstream applications.

Table 1. *Summary strains used in this study and their niche breadth (NB) characteristics.*

Strain ID	Phylogeny (Class | Order | Family | Genus)	NB (NaCl g L^{-1})	Optimum modeled Salinity (NaCl g L^{-1})	Skewness	Hypo-osmotic NB	Hyper-osmotic NB
S331	Gammaproteobacteria | Pseudomonadales | Halomonadaceae | Halomonas	143	-5	0.93	45	98
S337	Gammaproteobacteria| Pseudomonadales | Marinomonadaceae | Marinomonas	48	42	1.14	17	31
S338	Gammaproteobacteria| Pseudomonadales | Marinomonadaceae | Marinomonas	53	33	1.44	17	36
S366	Gammaproteobacteria| Pseudomonadales | Marinomonadaceae | Marinomonas	56	35	1.32	18	38
S374	Gammaproteobacteria| Pseudomonadales | Marinomonadaceae |Marinomonas	58	35	1.21	18	40
S432	Gammaproteobacteria |Enterobacterales | Alteromonadaceae| Pseudoalteromonas	50	44	1.02	18	32
S479	Gammaproteobacteria| Enterobacterales | Alteromonadaceae| Pseudoalteromonas	58	53	0.15	29	29
S490	Gammaproteobacteria| Enterobacterales | Alteromonadaceae| Pseudoalteromonas	51	42	1.22	18	33
S599	Alphaproteobacteria | Rhodobacterales | Rhodobacteraceae | Celeribacter	29	49	0.49	12	17
S618	Gammaproteobacteria | Enterobacterales | Vibrionaceae | Vibrio	67	21	1.23	14	53
S630	Actinobacteria | Actinomycetales | Micrococcaceae | Nesterenkonia	140	41	0.61	29	111

To assess their tolerance against salinity changes, cells from the strains were defrosted for 15 min at room temperature and used to inoculate sterile standard LB medium (Carl Roth, Karlsruhe, Germany; final salinity of 30 g L^{-1} NaCl), in which they were incubated for 2 days at 25ºC. Next, 300 µL sterile standard LB medium (Carl Roth, Karlsruhe, Germany) with different NaCl concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 g L^{-1}) were distributed in 96-well microplates and inoculated with the same number of cells from the individual strains, respectively (6 replicates per strain and salt concentration). The growth of the strains at 25ºC was monitored in each well by hourly measuring their cell densities via the optical density (OD) at 600 nm (after shaking at medium intensity, with orbital shaking set to 20) in a Paradigm Microplate reader (Molecular Devices, LLC., California, USA) until the plateau phase was reached (Fig. S1).

The fitness of strains under the different salt concentrations was assessed based on their growth curves in each medium by using their maximum cell densities and their growth rates. Growth rates were estimated by fitting a logistic growth equation as detailed elsewhere (García, Bestion, Warfield, & Yvon-Durocher, 2018) until maximal cell densities were reached. We further excluded the lag-phases defined by the period until initial OD values had doubled from growth rate estimations because the lag-phases were likely impacted by an acclimation phase only in those media that differed from the salinity in the pre-culture medium. The growth curves of some strains differed over the salinity gradient mainly in their maximal cell densities, while others differed more strongly in the incubation time after which the maximal growth density was reached and accordingly in their growth rates (Fig. S1). To integrate these different aspects of fitness we created a combined fitness index from the product of maximum cell densities and growth rates for each strain in the given medium. This combined fitness index was used for all downstream analyses. Fitness curves were fitted by applying either a lognormal or a gaussian model to the fitness values along the salinity gradient, while the best model was selected by the Akaike information criterion (Fig. 2; Table S1).

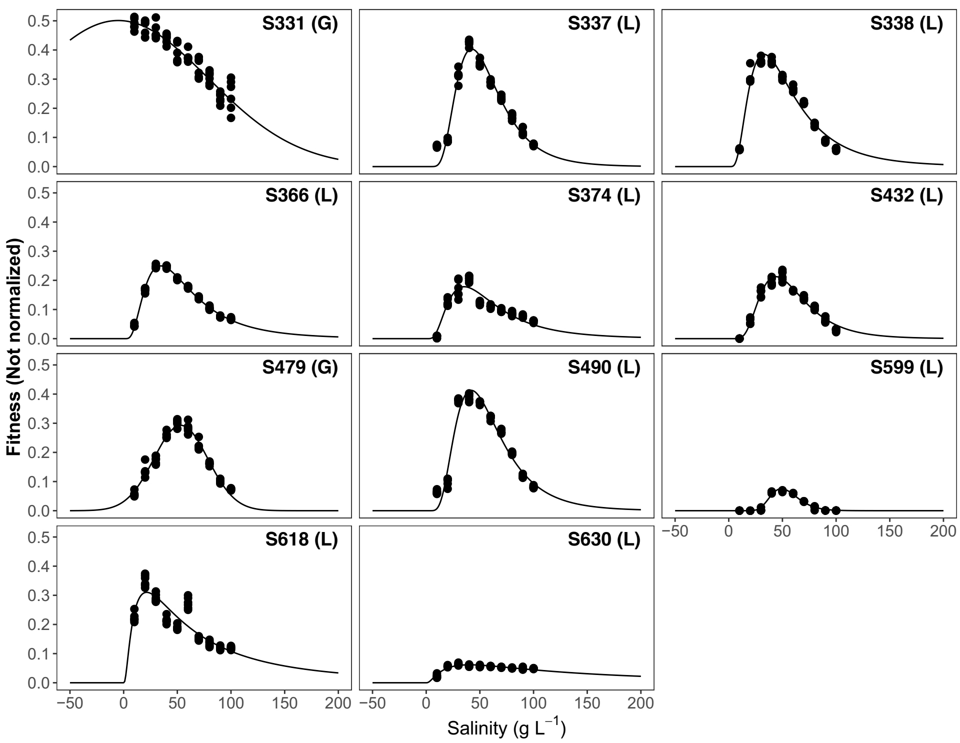

Figure 2. Fitness estimations and modelled curves. *Black points lines indicate the fitness indices from 6 replicates per strain at each salinity level, respectively. Gray lines indicate the fitness curves fitted according to the Akaike information criterion either via a lognormal (L) or gaussian (G) model.*

The NB of the strains was calculated after normalizing the fitness curves by dividing by the maximal modeled fitness value as the salinity range in g L^{-1} NaCl where the individual strains reached at least 50% of the extrapolated normalized fitness. The resulting fitness curves featured in most cases asymmetric shapes (Skewness > 0; Table 1), implying that the strains were characterized by differential tolerances against hypoosmotic and hyperosmotic stress (Fig. 2). For this purpose, we divided the NBs between the right (hyperosmotic) and left (hypoosmotic) sides relative to the modeled optimal salt concentration to obtain a side-dependent hyperosmotic and hypoosmotic NB for each strain (Fig. 1; Table 1). Beyond NB estimations, fitness curves allow also to delineate stress exposure levels in response to changing salinity, by subtracting the fitness values at the start and end points of a salinity gradient (Fig. 1). NBs are negatively related to stress levels if the stress values are assessed for salinity gradients that are located symmetrically around the optimum fitness value (Fig. 3). However, stress values can be determined for every arbitrary salinity gradient, within which the growth of a strain is detected. Accordingly, for any given strain and independently of its NB, all stress values between zero and one can be obtained in dependence on the position and strength of the salinity gradient of interest.

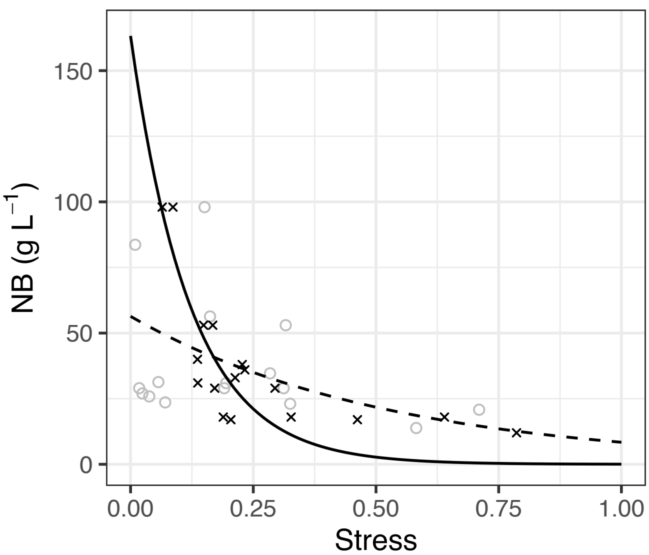

Figure 3. Regression between side-dependent NB and stress estimations for the 11 model strains. *The black cross-marks (and solid black trend line: R^2=0.73, P<0.001) represent stress levels assessed for salinity gradients that were located ±15 g L^{-1} NaCl symmetrically around the optimum fitness value and the corresponding side-dependent NB that were used for mixed model regressions against the NB. The gray circles represent stress levels between salinity concentrations where the optimum was crossed and/or delineated from a salinity gradient of 30 g L^{-1} NaCl. The corresponding NB values were in this case estimated from the proportional contribution of the hypo or hyperosmotic NB that was covered by the respectively considered salinity gradient. The dotted trend line was fitted by including all data points (R^2=0.23, P<0.021).*

Transcriptional response to changing salinity levels

For downstream RNA extractions, each strain was grown in duplicates at three salinity levels and 25ºC (100 rpm) in sterile glass tubes containing 15 or 50 mL LB medium (Table S2). We originally had planned to grow the strains for this experiment at their optimal salinity levels (S_2), as well as two salinity levels, each located symmetrically 15 g L^{-1} NaCl at the hyper- and hypoosmotic side from the optimum (S_1, S_3) (Fig. 1). However, in several cases, the incubation salinity levels were shifted relative to the optimum salinity, because we had optimized the fitness models after performing the experiment (Fig. 4).

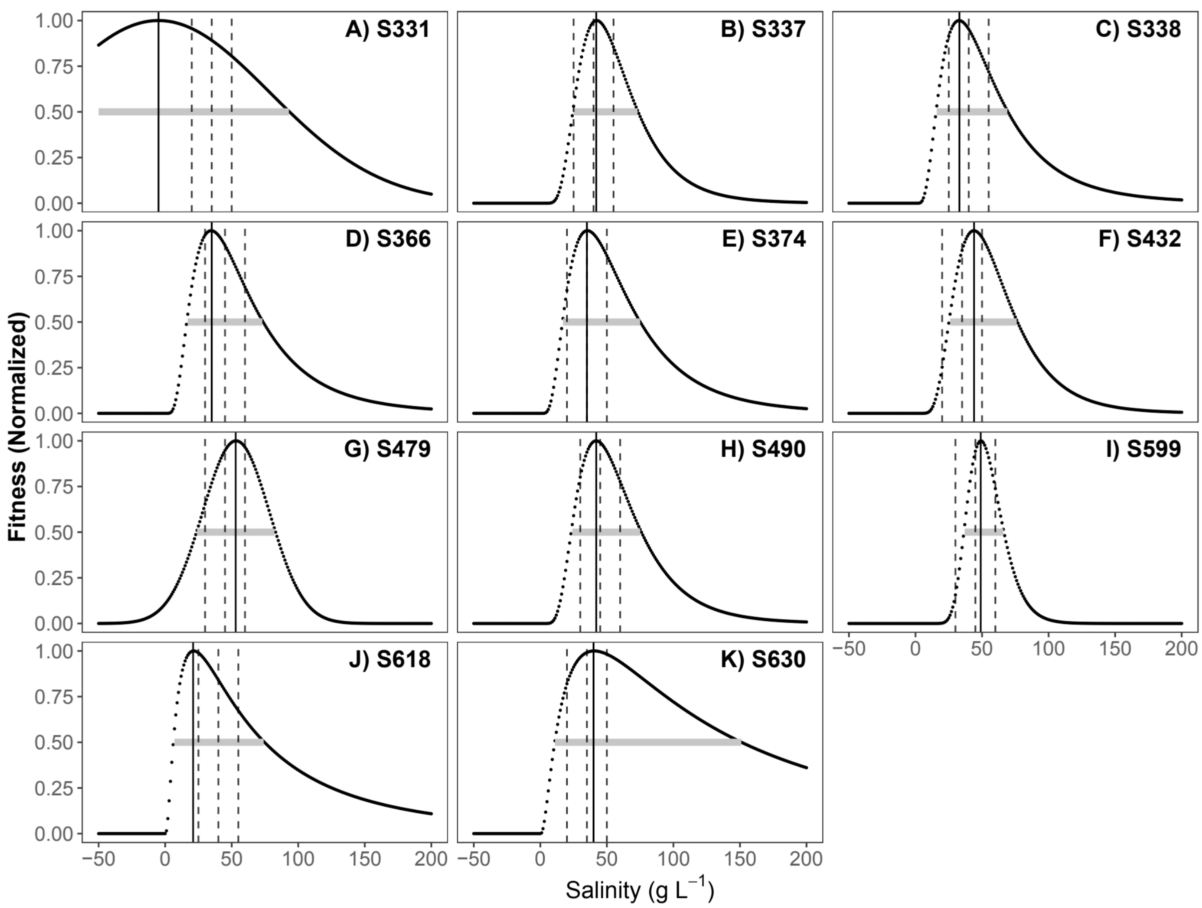

Figure 4. Modeled fitness for the 11 strains used in this study. *A-K) Fitted fitness curves and the sampled salinity levels (dashed black lines, from left to right S_1, S_2, S_3 in each panel) to estimate the transcriptional response to changing salinity relative to the fitness curves. The vertical black line indicates the optimal fitness of each strain.*

To monitor the growth of the strains during the experiments, 300 μL aliquots from the inoculated medium were pipetted in 96-well microplates. The microplates were incubated at the same temperature as in the glass tubes either in a Paradigm Microplate reader (Molecular

Devices, LLC., California, USA) or in a VICTOR Multilabel Plate Reader (PerkinElmer, Massachusetts, USA), where hourly measurements for OD (600 nm) were performed after 5s shaking. During the exponential phase that was monitored in real-time for each bacterial strain via OD measurements, samples for RNA and cell enumeration were harvested from the glass tubes. In order to stop the incubations, 13.5 or 48.5 mL of the cultured bacteria were fixed by adding 1.5 or 5.4 mL of a 5% solution of Phenol:Chloroform 5:1 (Sigma-Aldrich, Missouri, USA) diluted in absolute ethanol (Sigma, Missouri, USA) (Feike et al., 2012). The fixed cell cultures were centrifuged (8500 g for 5 min) and the supernatant was carefully discarded. Next, 300 of µL RNA later (Sigma-Aldrich, Missouri, USA) was added to the pelleted cells that were resuspended by pipetting up and down. The cell suspension was transferred into a 2 mL tube, flash-frozen in liquid nitrogen and stored at -80ºC for later RNA extraction (Table S2 for incubation details).

From the remaining volume in the incubation tubes, 1200 µL were fixed with glutaraldehyde (0.1% final concentration) and kept at -80°C until later analysis for cell enumeration. The number of cells was estimated by flow cytometry in the cytometer Cytoflex Flow Cytometer (Beckman Coulter, California, USA) using a standard procedure described elsewhere (Marie, Simon, Guillou, Partensky, & Vaulot, 2000).

DNA extractions for genome sequencing

The strains were incubated in 2 mL LB (salinity of 30 g L^{-1} NaCl) for 5 days at room temperature, pelleted and stored at -30ºC until DNA extraction. DNA was extracted following the standard protocol of the Gentra Puregene Cell Kit (QIAGEN, Hilden, Germany). The concentration and quality of the eluted DNA were tested by electrophoresis (1% agarose gel) and Quantus fluorometer using the PicoGreen assay (Promega, Wisconsin, USA). The extracted DNA was sent for genome sequencing (2 ×150 bp reads, Illumina NextSeq 500 V2). Library demultiplexing of libraries via the Illumina bcl2fastq 2.17.1.14 software (2 mismatches allowed, minimum length <20), was carried out by the sequencing company.

RNA extractions for transcriptome sequencing

The cells which were harvested during their exponential phase from the incubation experiment at different salt concentrations were defrosted at room temperature, pelleted by centrifugation and the supernatant containing RNA later solution was discarded. The RNA extractions were performed for all strains using the Direct-zol™ RNA-Miniprep Plus (Zymo

Research, California, USA) following the manufacturer's instructions, except for strain S630. For downstream absolute transcript quantification (Satinsky, Gifford, Crump, & Moran, 2013), 5 ng of a spiked-in RNA standard was added to the cell lysis solution (TRIzol reagent, the first step of the extraction protocol). In the case of strain S630, it was not possible to obtain sufficient RNA yield when using the Direct-zol™ RNA-Miniprep Plus kit for RNA extraction. For this reason, we implemented the SNAP™ protocol for gram-positive bacteria published elsewhere (Stead et al., 2012) with some modifications. In short, cell pellets were resuspended in 300 µl SNAP™ RNA extraction solution and 5 ng of the internal RNA standard was added for downstream absolute transcript quantification. After the addition of low binding zirconium beads (OPS diagnostics, Lebanon, USA) the suspension was treated at 6 ms^{-1} for 2 × 45 s in a FastPrep-24™ 5G MP (Biomedicals, California, USA) for mechanical cell disruption and subsequently heated for 7 min at 95°C. To enhance the efficiency of the SNAP™ method, we furthermore placed tubes with the extraction solution in a beaker filled with 500 mL water and microwaved them for 45 sec (Brandt Microwave, model SM2602B, Saskatchewan, Canada) at the defrosting mode (~300 W). While a similar microwaving protocol was originally developed to permeabilize cell walls for downstream fluorescence in situ hybridization (FISH) applications (Tischer et al., 2012), this additional step also increased the RNA yield for strain S630. The extracted RNA in the SNAP™ RNA extraction solution was purified with the RNA Clean & Concentrator™-5 kit including the optional on-column DNA digestion step (Zymo Research, California, USA).

An additional DNA removal step was performed for all RNA extracts using the TURBO DNA-free™ Kit (Invitrogen, California, USA). Then, the eluted RNA was concentrated by applying the RNA Clean & Concentrator™-5 kit (Zymo Research, California, USA). The concentration of the eluted RNA was estimated in a NanoVue Plus™ Spectrophotometer (Biochrom, Cambridge, UK), while RNA quality was visually evaluated by inspecting RNA molecule length profiles using an Agilent 2100 Bioanalyzer (Agilent Technologies, California, USA, RNA6000 NanoKit).

To economize sequencing costs, RNA extracts from 3 or 4 strains, that according to taxonomic annotations of genome sequences (see section bioinformatic processing) affiliated with different genera were pooled equimolarly (Table S2). The extracted RNA was sent for genome sequencing (2 ×150 bp reads, Illumina NextSeq 500 V2).

Bioinformatic processing

Library demultiplexing of all obtained raw reads via the Illumina bcl2fastq 2.17.1.14 software (2 mismatches allowed, minimum length <20), was carried out by the sequencing company. The resulting adapter-free paired-end reads were trimmed using the SICKLE software v1.33 (quality-type sanger, quality-threshold 20, length-threshold 75) (Joshi & Fass, 2001).

The trimmed reads from the genome sequences were then assembled using the SPAdes genome assembler v3.13.0 (Bankevich et al., 2012) with the following settings: k-mers from 21 to 99, 4 nucleotides steps. Taxonomic assignations for the assembled genomes were performed using the GTDB-Tk v0.2.2 software (Chaumeil, Mussig, Hugenholtz, & Parks, 2020) against the GTBD database. Genes on the assembled contigs were predicted via the PRODIGAL v2.6.3 software (Hyatt et al., 2010). The predicted genes were functionally annotated via DIAMOND blast v0.8.22 against genes in the KEGG database (Kanehisa *et al.*, 2007, downloaded May 2016) with an e-value cutoff of 1e-5.

The SORTMERNA v1.9 software (Kopylova, Noe, & Touzet, 2012) was used to separate the trimmed non-protein coding RNA from protein-coding reads. Spike-in standard reads in each sample were detected using the LASTAL software v.393 (Kielbasa *et al.*, 2011), and counted for downstream absolute transcript quantification. Quality-trimmed protein-coding reads were mapped on the predicted genes from the assembled genomes using the BOWTIE2 v2.3.4.3 software set to very-sensitive-local (Langmead & Salzberg, 2012) and summarized by the FEATURECOUNTS v1.4.6-p2 software (Liao, Smyth, & Shi, 2014).

Transcriptional regulation patterns

Per cell transcriptional regulation was calculated as log2 fold-changes of per cell transcription levels between two salinity levels and was determined for the individual genes annotated from each strain and each pair of salinity conditions using the R package DESeq2 (Love, Huber, & Anders, 2014). The regulation patterns were delineated from the absolute transcript per cell transcription levels, similar as detailed elsewhere (Beier et al., 2019). In short, raw count data for individual genes were normalized using the count data of the added spike-in standard that were multiplied with the total number of cells subjected to RNA extraction and divided by the amount of added standard via the "controlGene" option of the DESeq2 "estimateSizeFactors" function. As expected, we found an almost perfect linear correlation between values for total transcripts per cell calculated with a previously published formula (Satinsky et al., 2013) and the total transcripts per cell variable obtained by summing up count data after the DESeq2 normalization step (r = 1.00; Fig. S2). We did not specifically select genes based on the DESeq2 significance levels for transcriptional regulation, because the downstream regression analyses were designed to also include genes that exhibited low or no transcriptional regulation.

We used the value for total transcripts per cell (DESeq2-estimated) to test the overall correlations between total per cell transcription levels against NB and stress estimations, to screen for potential relationships that could bias our hypothesis testing.

Definition of gene categories

In order to test the hypotheses H1 that genes with a direct link to an organism's fitness are stronger regulated in strains with narrow NB, we built 3 categories of putatively fitness-related genes encoding the following proteins: 1) DNA polymerases enzymes, which are essential for DNA replication and cell proliferation. This category included all genes containing the text string "DNA polymerase" in the gene description. 2) RNA polymerases, which transcribe genes into mRNA and are accordingly the first step in cellular biomass production via protein synthesis. RNA polymerase genes were defined as the genes classified as protein-coding genes in the KEGG pathway ko03020. 3) Ribosomal proteins, which are essential units of the ribosomes that catalyze the translation of mRNA into proteins and are accordingly the second step in cellular biomass production via protein synthesis. We defined all genes annotated to protein-coding genes in the KEGG pathway ko03010 as ribosomal proteins.

These three categories comprise core genes that are highly conserved across organisms in all three domains of life (Kültz, 2003) and the individual encoded proteins in these categories, e.g. ribosomal proteins, exhibit their catalytic activity as subunits in a larger assembly of catalytic active units. We therefore selected the individual genes in each category from the pool of genes shared by all of the 11 model strains (253 shared genes; Table S3) and considered the transcriptional regulation of the individual genes in each category as replicates for downstream statistical analyses.

In order to test the hypotheses H2 that genes encoding cell adaption against salt stress are stronger regulated in strains with broader NB, we constructed another 2 categories with potential adaptation-related genes: 4) Genes encoding the transport of osmoprotective compounds, mainly including compatible solutes, but also other osmolytes, such as urea. Compatible solutes are small organic molecules such as sugars or amino acids that do not have detrimental effects on cell functions (Welsh, 2000). However, the use of compatible solutes as osmoprotectants is highly species-specific (Bougouffa, Radovanovic, Essack, & Bajic, 2014; Sévin, Stählin, Pollak, Kuehne, & Sauer, 2016). For instance, glycine betaine, which serves as an osmoprotectant in organisms of all domains (Empadinhas & Viete-Vallejo, 2008) was not observed to protect the archaeon *S. solfataricus* against salt stress (Park & Lee, 2000). We, therefore, selected from all transporter genes that were expressed in at least one of the model strains a list of potential osmoprotectant transporters (Table S4). 5) Heat-shock proteins (HSP), which catalyze the folding and unfolding of macromolecules and are therefore involved in the repair of damaged macromolecules (Kültz, 2003; Lindquist & Craig, 1988). However, different from the osmoprotectant genes listed above, genes in this category do not initially prevent cells from damage but step into action only after damage occurred. This category included all genes containing the text strings "heat-shock" or "chaperone" in the gene description.

Different than in the categories encoding fitness-related proteins, we assumed that the individual genes in the categories of the adaptation-related proteins act independent from each other and their protective effect is additive. For instance, the more different osmoprotectants are transported the stronger the protection against osmotic stress. Genes encoding the adaptive response against osmotic stress are furthermore and in contrast to fitness-related genes species-specific (Sévin et al., 2016), and only two among the selected potential osmoprotectant transporters (K15268, K02030) were shared among all model strains from this study (Table S3). For this reason, we considered the additive transcriptional response by summing up the fold change of the individual genes in categories 4 and 5, respectively for downstream statistical analyses.

Regression of gene regulation patterns against NB and stress levels

We applied mixed linear models (MLM) to test if the transcriptional regulation levels of genes within each of the above-described categories were correlated in the predicted direction with either the NB (log-transformed) or the stress exposure along the respective salinity gradient of the individual strains. The MLMs were implemented in R using the package nlme (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2020) and the R-package olsrr

(Hebbali, 2020) was applied to verify the normal distribution of the residuals in all MLM using Kolmogorov-Smirnov tests. In a few cases where a normal distribution of the residuals could not be verified transcriptional regulation levels were subjected to a square root or inverse transformation.

In the case of regressions against NB, we considered regulation patterns along salinity gradients of 15 g L^{-1} NaCl, but only if the salinity optimum (± 4.5 g L^{-1} NaCl equivalent to 30% of the total change) of the respective strain was not passed. With this rule, maximal two values for gene regulation per strain and gene were considered (Table 1; Table S1). Because these regulation values referred to either the hyper or the hypoosmotic side, MLM was performed against the respective values for side-dependent NBs, with the side-dependent NB as a fixed factor. Replicate regulation values per strain (retrieved from different salinity gradients on either of the two sides and, where applicable, individual genes in a category that were shared by all 11 strains) were used as random factors. An analogous approach was used, to test if the degree of gene regulation increased or decreased with increasing stress exposure. However, in this case, we considered stress levels between all sampled salinity gradients per strain as replication level and accordingly as a random factor in the MLM (3 regulation values per strain and gene: ΔF_1, ΔF_2,ΔF_3; Fig. 2; Table 1). Stress levels featured a pronounced negative correlation with NB, if only stress values from salinity range 15 g L^{-1} NaCl and not crossing the optimum were considered (i.e. those data points considered for the regressions against NB; Fig. 3). However, a reduced correlation strength between NB and stress levels was observed when all data points were considered (Fig. 3).

While our hypotheses referred only to the extent of regulation activity regardless of its direction, we also inspected regressions taking into account the regulation direction to see whether genes in the categories were either up or down-regulated in response to stress.

The slopes of the MLM, as well as P-values, were retrieved to describe the direction of the regression and test the hypotheses. To evaluate the proportion of variance that was explained by the fitted MLM, pseudo R^2 values for the overall model were estimated using the r.squaredGLMM function from the R-package MunIn (Bartoń, 2020).

Detection of stress marker genes

To detect potential individual stress marker genes, we applied MLM on each of the 253 genes shared by the 11 strains using stress as a fixed factor and the three replicate stress levels per strain as random factors. In this case, we performed the regression analyses considering the direction of the gene regulation under stress conditions (upregulation or downregulation). The resulting P-values were adjusted to account for false discovery rates after multiple comparisons (Benjamini & Hochberg, 1995).

The R codes used for the statistical evaluations that were performed for this study are available on GitHub (https://github.com/sarabeier/Strains.NB).

RESULTS

Strain characteristics and total transcript regulation

The bacterial model strains covered several phylogenetic lineages (*Alphaproteobacteria*, *Gammaproteobacteria* and *Actinobacteria*) with representatives affiliated to the orders *Pseudomonadales*, *Enterobacterales*, *Rhodobacterales* and *Actinomycetales* (Table 1). The strains exhibited side-dependent NBs that ranged from 12 to 111 g L^{-1} NaCl. Along the NB continuum, shorter NBs were dominated by hypoosmotic estimations, while most broader NBs corresponded to hyperosmotic values (Fig. 5).

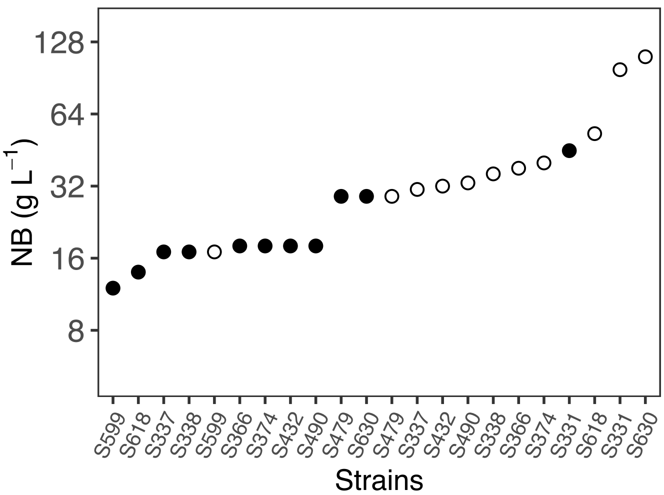

Figure 5. Ranking of the side dependent niche breadths (NB) for the 11 model strains. *Black dots indicate hyperosmotic NB, while white dots indicate hypoosmotic NB. The y-axis is displayed in log-scale.*

Per cell transcription levels were highly variable among strains, differing up to a factor of 5 (i.e. S630 vs S490, Fig. S3). However, the overall per cell transcriptional regulation did neither correlate significantly with NB nor with stress estimations and R^2 values were consistently low (Table 2, Fig. 6A-C).

Regulation of fitness-related genes

We detected only two genes that were shared across strains in the DNA and RNA polymerases categories, respectively. In contrast, the ribosomal protein category contained 32 genes shared by all strains (Table 2). While no significant trend was noted for the regulation of genes encoding DNA polymerases (Table 2), the regulation of genes encoding ribosomal proteins and RNA polymerases decreased in agreement with H1 significantly with increasing NB ($P=0.003$ and $P=0.041$, respectively; Fig. 6G, J, Table 2). The direction of the relationship turned when considering stress exposure instead of NB as an explanatory variable for gene regulation. However only genes encoding ribosomal protein were significantly regulated with increasing stress levels ($P<0.001$; Fig. 6I). An inspection of the direction of gene regulation revealed a significant upregulation of genes encoding ribosomal proteins and RNA polymerases under increasing stress exposure (Fig. 6L; Table 2) and a not significant trend for upregulation of DNA polymerases encoding genes (Fig. 6F; Table 2). However, in all three categories, individual genes were also downregulated under stress, particularly if stress levels were retrieved from salinity ranges crossing the salinity optimum (Fig. 6C, F, I).

Regulation of adaptation-related genes

The number of potential osmoprotectant transporter genes per strain varied between 20 and 93 (Table 2). The summed upregulation level of these genes along with increasing NB exhibited a positive slope, which however was not significant considering the significance level $P<0.05$ ($P=0.076$; Table2; Fig. 6M). On the other hand, stress did not seem to affect the absolute regulation of these genes nor the direction of the regulation (Table 2; Fig. 6N, O).

While the regulation of genes encoding HSP did not exhibit a significant correlation with NB (Table 2; Fig. 6P), a significant increase of the regulation along with increasing stress levels was detected ($P=0.030$; Table 2; Fig. 6Q). Similar to the osmoprotectant transporter category, no significant trend for an up-or downregulation of HSP under stress was detected (Table 2; Fig. 6R).

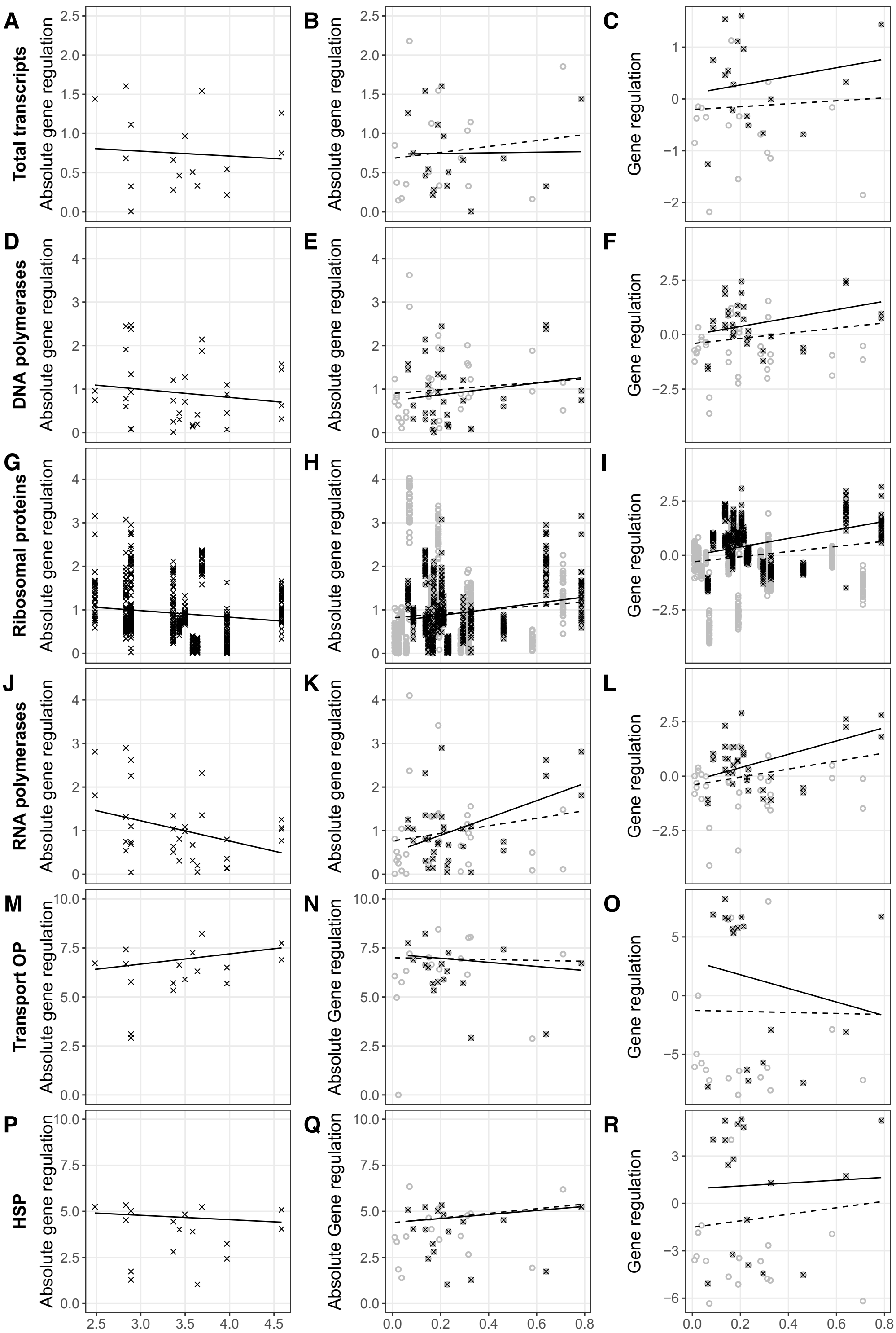

Figure 6. Regressions of gene regulation against side-dependent niche breadth and stress. *MLM of the absolute gene regulation against NB (log-transformed, left panels) and stress (center panels) , and directional gene regulation against stress (right panels). A, B, C) Total transcripts per cell, D, E, F) DNA polymerases, G, H, I) Ribosomal proteins, J, K, L) RNA polymerases, M, N, O) Transport of osmoprotectants and P, Q, R) HSP. Black cross-marks (and the black solid trend lines) represent pairwise comparisons that did not cross the optimum fitness for NB and stress. Gray circles present all pairwise comparisons between salinity concentrations where the optimum was crossed and/or delineated from a salinity gradient of 30 g L^{-1} NaCl. The dotted trend lines were fitted by including all data points.*

Candidate marker genes for stress

Only one of the individual genes shared among all 11 model strains exhibited a significant relationship with stress levels after P-value adjustment for multiple testing (Padj <0.1). We, however, report here further 6 genes that exhibited significant P-values (P<0.05) prior to P-value correction as potential candidate stress marker genes (Fig. 7, Table 3). Three out of the seven candidate genes were downregulated in response to increasing stress levels and the remaining four genes were upregulated (Fig. 7, Table 3). Two of the candidate genes that were upregulated with increasing stress levels (ybeB and rpsI) occur in >75% of all prokaryotic genomes listed in the KEGG database while the occurrence of the other candidate genes was less conserved across prokaryotes (Table 3, Beier *et al.*, 2020; KEGG version 2016).

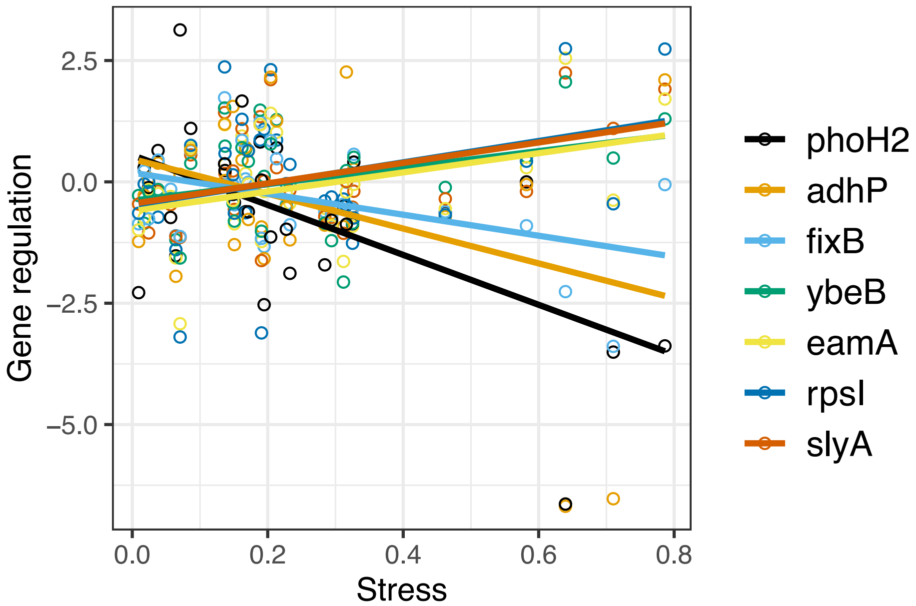

Figure 7. Regression of candidate stress marker gene regulation against stress exposure levels. *This graphic displays the regression of 7 candidate stress marker genes that were selected because of their significant correlation (P<0.05, no adjustments for multiple testing) against the exposure of the 11 model strain to osmotic stress.*

Table 2. *Summary for mixed linear models for gene regulation against niche breadth (NB) and stress including genes grouped into categories.*

			Absolute Gene Regulation vs NB			Absolute Gene Regulation vs Stress§			Gene Regulation vs Stress§		
Classification	Functional categories	Number of considered genes per strain	Slope	R^2	P	Slope	R^2	P	Slope	R^2	P
	Total transcripts	1 †	-0.06	0.01	0.768	0.38	0.02	0.458	0.28	0.07	0.749
Fitness-related genes	DNA polymerases	2 †	-0.19	0.02	0.399	0.42	0.01	0.399	1.19	0.13	0.151
	Ribosomal proteins	32 †	-0.08	0.09	**0.003***	0.30	0.02	**0.000*****	1.19	0.13	**0.000*****
	RNA polymerases	2 †	-0.46	0.12	**0.041***	0.87	0.04	0.113	1.84	0.14	**0.030***
Adaptation-related genes	Transport of osmoprotectants	25-93 ‡	0.52	0.19	**0.076•**	-0.23	0.00	0.767	-0.44	0.02	0.940
	HSP	6-17 ‡	-0.24	0.06	0.332	1.26	0.14	**0.030***	2.06	0.17	0.571

† MLMs were performed using the individual genes as a random factor
‡ MLMs were performed using the additive transcriptional regulation on individual genes
§ The displayed statistical output parameters refer to regressions including all stress exposure data points. The statistical output parameters for regressions including only data based on 15 NaCl L^{-1} salinity gradients in which the performance optimum was not passed and which correspond to the datapoints included in the NB regressions are displayed in Table S5
***:P-value <0.001 / *:P-value <0.05 / •:P-value <0.1 and the regression followed the predicted direction

Table 3. *Summary for significant mixed linear models of regulation of shared genes against stress levels.*

KEGG ID	Gene ID	Gene description	Slope	R^2	P	Padj	Occurrence in prokaryotes†
K07175	phoH2	PhoH-like ATPase	-5.14	0.04	**0.000*****	0.088	34%
K13953	adhP	Alcohol dehydrogenase, propanol-preferring	-3.57	0.09	0.038*	0.982	49%
K03522	fixB, etfA	Electron transfer flavoprotein	-2.10	0.15	0.015*	0.982	53%
K09710	ybeB	Ribosome-associated protein	2.06	0.04	0.018*	0.982	86%
K15268	eamA	O-acetylserine/cysteine efflux transporter	2.17	0.06	0.025*	0.982	16%
K02996	rpsI	Small subunit ribosomal protein S9	2.48	0.08	0.045*	0.982	99%
K06075	slyA	MarR family transcriptional regulator	2.50	0.08	0.004*	0.450	20%

† Indicates the fraction of prokaryotic genomes in the KEGG database (version 2016) carrying the gene
***:P-value <0.001/ *:P-value <0.05

DISCUSSION

We experimentally evaluated gene regulation patterns for genes encoding either fitness-related or adaptation-related genes, and their relationship with NB and stress exposure levels of 11 bacterial strains. We believe that evaluating the transcriptional response patterns of individual strains, taking into account concepts of trait research in ecology, is a prerequisite to better understand the currently difficult to interpret community metatranscriptome data (Prosser, 2015; Prosser & Martiny, 2020).

Our results suggest that overall per cell transcriptional regulation level changes across the model strains were independent from the strains' NBs or the stress levels to which the strains were exposed to (Table 2). The variability of cellular transcription levels could be interpreted as a measure for transcriptional plasticity and thus, if assuming that transcriptional activity is manifested in the expression of traits, also as a form of phenotypic plasticity (Beier, Rivers, Moran, & Obernosterer, 2015). It has been discussed that high plasticity enlarges the NB of organisms (Kellermann, van Heerwaarden, Sgrò, & Hoffmann, 2009; Sultan, 2001; Van Buskirk, 2002), which was accordingly not supported by our findings concerning the overall per cell transcriptional levels. However, it has also been argued that the kind of trait matters for relating it to the response of organisms to disturbances (Hooper et al., 2005). Therefore, in order to address the relationship between transcriptional regulation levels and NB or stress, we have differentiated between genes encoding fitness-related traits and genes encoding traits related to the adaption of organisms to changing environmental conditions.

Indeed, in agreement with our hypotheses, results suggested that transcriptional regulation levels of either fitness or adaptation-related genes if inspected separately, impacted the NB, and therefore the life history of species as well as stress levels that cells were exposed to.

Although the regulation levels of DNA polymerases did not decrease significantly with increasing NB, our analyses confirmed as expected in H1 a significant decrease of RNA polymerases as well as ribosomal protein transcription levels along with increasing NB (Table 2). Both, RNA polymerases and ribosomal proteins are directly involved in the cellular translation activity and accordingly in biomass production. Unlike NB, which is a constant for a given strain, cellular fitness and stress levels are closely linked, even if environmental gradients of different lengths and exceeding the point of optimal cellular performance are taken into account (Fig. 2), as was the case in our analyses. The direct involvement of RNA polymerases and ribosomal proteins transcriptional regulation in cellular fitness was therefore also reflected in the close to significant ($P= 0.113$, Table 2) or even highly significant positive relationship of gene regulation patterns with stress levels ($P<0.001$, Table 2). While a downregulation of genes encoding biomass is to be expected intuitively under increasing stress and correspondingly decreasing fitness levels, we observed the opposite trend. This was particularly pronounced if environmental ranges that did not pass the fitness optimum were considered (Fig. 6F, I, L). This observation suggests that the detected transcriptional upregulation of genes involved in translation activity under elevated stress levels could be linked to mechanisms for replacing or repairing damaged proteins or other macromolecules, as it has been outlined elsewhere (Evans & Hofmann, 2012).

Our results further give certain evidence in support of H2, where we expected an increased transcriptional regulation of genes encoding the adaption to changing salinity along with increasing NB. The expected positive relationship was observed for a list of candidate genes encoding the transport of osmoprotectant substances (Fig. 6M). Although the P-value for this correlation was not below the significance level of $P < 0.05$ we argue that the reported P-value ($P = 0.076$, Table 2) still provides statistical support for the hypothesis H2: this hypothesis did not only include the assumption of a correlation, but also the expected direction of the correlation and an one-tailed instead of the more stringent two-tailed test would have been an appropriate solution. However, because the R-package nlme v3.1-148 does not enable to set up mixed models using one-tailed test designs we report here the more stringed P-value from the two-tailed test design.

The lower (not significant) relationship of the regulation of osmoprotectant transporters and cellular stress levels was not unexpected (Table 2), at least not if stress levels of salinity ranges passing the optimum were included in the regression because within the same organism different osmoregulation mechanisms may be relevant under hypo- or hyperosmotic stress (Deole & Hoff, 2020; Lin, Liang, Yan, & Luo, 2017).

Also, as a consequence of the species-specific use of different osmoprotectants, may the uptake of compounds such as certain amino acids serve in one species for osmoregulation (Park & Lee, 2000) while the uptake of the same amino acid could be instead associated with biomass production in another species. This blurred separation of transport mechanisms that may either represent adaptation-related traits via their association with osmoregulation or be related to cellular fitness likely introduced noise into the performed regression analyses. The positive regression found between regulation and NB (Fig. 6M) shows, however, that the role of the listed candidate osmoprotectant transporters (Table S4) in our experimental setup actually lied largely in their participation in cellular osmoregulation.

We also inspected regulation patterns of HSP that are recognized as classical stress response proteins that due to their folding and unfolding function catalyze the repair of damaged proteins (Roncarati & Scarlato, 2017; Sørensen, Kristensen, & Loeschcke, 2003). Their expression may increase the resistance of cells to stress and thereby impact an organism's life history (Sørensen et al., 2003). We, therefore, classified HSP as genes encoding an adaption to environmental change and assumed to detect a more pronounced regulation of HSP in strains with broader NB (H2). This, however, was not the case. Even though not significant, indeed an opposite than expected trend for the relationship of HSP regulation and NB was observed (Fig. 6P). Although elevated HSP expression may, on the one hand, impact the resistance of cells against stress (Sørensen et al., 2003), expression levels of HSP have also been shown to increase under stress (Evans & Hofmann, 2012), which was reflected in a close to a significant positive correlation between stress levels and HSP regulation (Table 2). However, in our sample design, stress levels correlated negatively with NB (Fig. 3), and obviously the stress-induced regulation of HSPs masked the possible effect on NB-dependent regulation. If applying a sample design where we would have chosen salinity ranges to keep the stress levels instead of the environmental distance constant, we might have been able to detect the predicted positive correlation between NB and HSP regulation.

Beyond testing the hypotheses H1 and H2 we also screened absolute regulation patterns of the genes shared across all strains without any a priori hypothesis for their correlation with stress exposure and present a list of candidate stress marker genes. Except for the phoH-like ATPase phoH2, we though did not observe any individual genes whose regulation correlated after adjustment for multiple comparisons significantly to stress levels ($P_{adj} < 0.01$). We anyway reported here all genes with the not adjusted P-value < 0.05 to be considered as potential candidate stress marker genes. The gene rpsI encodes a ribosomal protein and is consequently involved in biomass production and was within the group of genes considered for hypothesis testing. The upregulation of rpsI under elevated stress levels is furthermore in agreement with the overall regulation patterns observed for other ribosomal proteins (Fig. 6G) and might be explained by enhanced transcriptional activity under stress to induce the replacement of damaged proteins (Evans & Hofmann, 2012). Although not considered as a ribosomal protein, also ybeB encodes a ribosome-associated protein, which has been described as a ribosomal silencing factor that downregulates protein synthesis when cells enter the stationary phase (Häuser et al., 2012). This agrees with observations that mechanisms that induce growth arrest are typically upregulated under stress (Kültz, 2003). Both rpsI and ybeB can be considered as fitness-related genes to which hypothesis H2 applies and meaningful mechanisms can explain the observed correlation of gene regulation patterns with stress levels. Accordingly, also the non-adjusted P-value may in these cases provide sufficient statistical evidence to support the classification of these genes as stress markers. Furthermore, both genes are highly conserved and encoded by almost all bacteria (Häuser et al., 2012; Lecompte, Ripp, Thierry, Moras, & Poch, 2002), which allows the design of primers or probes to monitor transcriptional expression levels specifically of target genes e.g. via quantitative PCR approaches (Beier, Gálvez, et al., 2015). On the other hand, it would also be possible to use metatranscriptome approaches to taxonomically assign different rpsI or ybeB transcript variants and thereby allow to track taxon-specific stress levels. Still, in the case of rpsI, it must be taken into account that we detected transcriptional upregulation in response stress exposure by comparing populations during their exponential phase. Stress due to nutrient restrictions and an associated switch from the exponential to the stationary growth phase can instead lead to a downregulation of ribosomal protein transcription (Aseev, Koledinskaya, & Boni, 2016), and regulation patterns of rpsI may not apply well as stress marker in all situations. In contrast, ybeB was analogously to results in our study also upregulated during the stationary growth phase in response to nutrient limitation (Häuser et al., 2012). The upregulation of ybeB may therefore be universally associated with exposure of bacterial cells to multiple stressors.

The presence of the remaining listed candidate stress marker genes was less conserved across prokaryote genomes (Table 3) and the exact mechanisms that could explain the detected correlations of regulation patterns with stress levels were less evident. However, several of them were observed earlier in connection to some kind of stress response (sylA: Alekshun and Levy, 1999; eamA: Ohtsu et al., 2015; phoH2: Andrews and Arcus, 2020). The genes adhP and fixB, are both involved in anaerobic energy production processes (Rao & Stokes, 1953; Weidenhaupt, Rossi, Beck, Fischer, & Hennecke, 1996) and their downregulation along with increasing stress levels is more difficult to interpret.

The 11 model strains considered in our study comprised members of the classes Alphaproteobacteria, Gammaproteobacteria and Actinobacteria (Table 1), which represent quantitatively important and ecologically relevant taxonomic groups in aquatic environments (Hoshino et al., 2020; Lambert et al., 2019; Rojas-Jimenez et al., 2021). However, we assume that the model strains represent rather copiotrophic strains, while particularly marine habitats are often dominated by oligotrophic strains (Giovannoni, 2017). Earlier research suggested that streamlined oligotroph marine bacterial strains may feature reduced transcriptional regulation compared to copiotrophic strains (Cottrell & Kirchman, 2016). Instead, post-transcriptional regulation mechanisms, e.g. mediated via riboswitches seemed to be particularly common in the highly abundant and oligotroph SAR11 clade (Kazanov, Vitreschak, & Gelfand, 2007). On the other hand, at least the regulation of SAR11 ribosomal proteins has been shown to be under transcriptional control (Ottesen et al., 2013). Consequently, there is no particular reason to assume that the stress-related regulation of ribosomal proteins in SAR11 should not follow the patterns that we detected in this study.

While we used changing NaCl concentrations to induce osmotic stress, organisms in natural habitats will be exposed to a large variety of different stressors. However, by definition all stressors interact with the fitness of organisms. It seems therefore plausible that the detected patterns concerning the fitness-related genes screened in this study are not specific for a certain stressor, but could be more universally linked to the stress tolerance (i.e. tolerance-related NB) of bacterial organisms. Also, the employment of compatible solutes has been shown to protect cells not only against osmotic stress but also against temperature changes, droughts, or oxidative stress (Singh, Kumar, Singh, Singh, & Prasad, 2015).

CONCLUSIONS

Overall, the performed MLM against NB analyses supported that general patterns of bacterial transcriptional regulation can discriminate between generalist and specialist lifestyles. Varying correlation strengths of gene regulation levels against NB and stress in the respective categories implied a close covariation of fitness-related traits, but also HSP genes with fitness levels on the y-axis of the fitness curves (Fig. 1). In contrast, gene regulation levels of osmoprotectant transporters were related to NB but at a lesser degree to stress. The regulation of osmoprotectant transporters covaried accordingly rather with the changing environmental conditions displayed on the x-axis of the fitness curves, than with fitness (Fig. 1). In all cases, taking into account the physiological functioning of the genes in their respective categories, these observations represent meaningful and well interpretable responses.

We further proposed a list of candidate stress marker genes whose regulation correlated with the stress exposure levels in our study. We suggest that these genes may be tested in future studies to validate their universal applicability to detect stress levels, either in individual populations or also communities that were exposed to changing conditions. The stress exposure of species in a community has been considered as one of three main environmental axis defining trait distribution in a community (JP Grime, 1977; Malik et al., 2020) and is therefore a key parameter for community functioning and assembly processes (Romero, Acuña, & Sabater, 2020). The here suggested gene-based approach for stress monitoring in microbial communities may accordingly be incorporated into models to predict carbon fluxes as suggested elsewhere (Malik et al., 2020) and accomplish an earlier proposed taxon-based approach to define potential bioindicators for stress (Rocca et al., 2019).

The application of transcriptome analyses is an appropriate tool to characterize the expression of traits in microorganisms, while this method may be less useful to assess the functional properties of larger organisms. Still, general ecological rules, such as species-area relationships (Horner-Devine, Lage, Hughes, & Bohannan, 2004) have often been shown to be valid across all domains of life . We argue that our findings, concerning the negative or positive correlation of the plasticity of either fitness or adaption related traits with an organism's NB should be a more general ecological pattern and its application to macroorganisms which have different response timescales remains to be investigated.

ACKNOWLEDGEMENTS

The study was supported by a grant from the German Science Foundation (DFG) awarded to SB (BE 5937/2-1). ARF was funded by a scholarship from the Chilean National Agency for Research and Development (ANID) / Scholarship Program / DOCTORADO BECAS CHILE/2017 – 72180448. The bioinformatic work was supported by the BMBF-funded de.NBI Cloud within the German Network for Bioinformatics Infrastructure (de.NBI) (031A532B, 031A533A, 031A533B, 031A534A, 031A535A, 031A537A, 031A537B, 031A537C, 031A537D, 031A538A). We thank the Bio2mar platform for the access and the technical support in their molecular biology facilities. We are further thankful to the MARBEC department at Montpellier University to provide laboratory space and facilities for performing initial work on the strains and for the technical support of Corinne Bouvier and Patrice Got.

DATA ACCESSIBILITY

The sequence data for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB43309 (https://www.ebi.ac.uk/ena/browser/view/PRJEB43309). R-scripts that were used for the data analyses have been published on GitHub (https://github.com/sarabeier/Strains.NB).

AUTHOR CONTRIBUTIONS

The experimental setup for the isolation experiment was discussed and designed by SB, TB and NM. NM and CB collected and isolated the bacterial strains. ARF and SR performed laboratory work and analyzed the data with support of NM. ARF and SB wrote the manuscript and all the authors commented on the manuscript.

REFERENCES

Alekshun, M. N., & Levy, S. B. (1999). The mar regulon: Multiple resistance to antibiotics and other toxic chemicals. Trends in Microbiology, 7(10), 410–413. doi: 10.1016/S0966-842X(99)01589-9

Andrews, E. S. V., & Arcus, V. L. (2020). PhoH2 proteins couple RNA helicase and RNAse activities. Protein Science, 29(4), 883–892. doi: https://doi.org/10.1002/pro.3814

Aseev, L. V., Koledinskaya, L. S., & Boni, I. V. (2016). Regulation of Ribosomal Protein Operons rplM-rpsI, rpmB-rpmG, and rplU-rpmA at the Transcriptional and Translational Levels. Journal of Bacteriology, 198(18), 2494–2502. doi: 10.1128/JB.00187-16

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., … Pevzner, P. A. (2012). SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. Journal of Computational Biology, 19(5), 455–477. doi: 10.1089/cmb.2012.0021

Bartoń, K. (2020). MuMIn: Multi-Model Inference [R package version 1.43.17]. Retrieved from https://CRAN.R-project.org/package=MuMIn

Beier, S., Andersson, A. F., Galand, P. E., Hochart, C., Logue, J. B., McMahon, K., & Bertilsson, S. (2020). The environment drives microbial trait variability in aquatic habitats. Molecular Ecology, n/a(n/a). doi: 10.1111/mec.15656

Beier, S., Gálvez, M. J., Molina, V., Sarthou, G., Queroue, F., Blain, S., & Obernosterer, I. (2015). The transcriptional regulation of the glyoxylate cycle in SAR11 in response to iron fertilization in the Southern Ocean. Environmental Microbiology Reports, 7(3), 427–434. doi: 10.1111/1758-2229.12267

Beier, S., Holtermann, P. L., Numberger, D., Schott, T., Umlauf, L., & Jürgens, K. (2019). A metatranscriptomics-based assessment of small-scale mixing of sulfidic and oxic waters on redoxcline prokaryotic communities. Environmental Microbiology, 21(2), 584–602. doi: 10.1111/1462-2920.14499

Beier, S., Rivers, A. R., Moran, M. A., & Obernosterer, I. (2015). Phenotypic plasticity in heterotrophic marine microbial communities in continuous cultures. Isme Journal, 9(5), 1141–1151. doi: 10.1038/ismej.2014.206

Bell, T., Gessner, M. O., Griffiths, R. I., McLaren, J., Morin, P. J., van der Heijden, M., & van der Putten, W. (2009). Microbial biodiversity and ecosystem functioning under controlled conditions and in the wild. In S. Naeem, D. E. Bunker, A. Hector, M. Loreau, & C. Perrings (Eds.), Biodiversity, Ecosystem Functioning, and Human Wellbeing: An Ecological and Economic Perspective. Oxford University Press.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society. Series B (Methodological), 57(1), 289–300. JSTOR. Retrieved from JSTOR.

Bougouffa, S., Radovanovic, A., Essack, M., & Bajic, V. B. (2014). DEOP: A database on osmoprotectants and associated pathways. Database, 2014(bau100). doi: 10.1093/database/bau100

Chaumeil, P.-A., Mussig, A. J., Hugenholtz, P., & Parks, D. H. (2020). GTDB-Tk: A toolkit to classify genomes with the Genome Taxonomy Database. Bioinformatics, 36(6), 1925–1927. doi: 10.1093/bioinformatics/btz848

Colles, A., Liow, L. H., & Prinzing, A. (2009). Are specialists at risk under environmental change? Neoecological, paleoecological and phylogenetic approaches. Ecology Letters, 12(8), 849–863. doi: https://doi.org/10.1111/j.1461-0248.2009.01336.x

Cottrell, M. T., & Kirchman, D. L. (2016). Transcriptional Control in Marine Copiotrophic and Oligotrophic Bacteria with Streamlined Genomes. Applied and Environmental Microbiology, 82(19), 6010–6018. doi: 10.1128/AEM.01299-16

Deole, R., & Hoff, W. D. (2020). A potassium chloride to glycine betaine osmoprotectant switch in the extreme halophile Halorhodospira halophila. Scientific Reports, 10. doi: 10.1038/s41598-020-59231-9

Devictor, V., Clavel, J., Julliard, R., Lavergne, S., Mouillot, D., Thuiller, W., … Mouquet, N. (2010). Defining and measuring ecological specialization. Journal of Applied Ecology, 47(1), 15–25. doi: https://doi.org/10.1111/j.1365-2664.2009.01744.x

Empadinhas, N., & Viete-Vallejo, O. (2008). Osmoadaptation mechanisms in prokaryotes: Distribution of compatible solutes. International Microbiology, (11), 151–161. doi: 10.2436/20.1501.01.55

Evans, T. G., & Hofmann, G. E. (2012). Defining the limits of physiological plasticity: How gene expression can assess and predict the consequences of ocean change. Philosophical Transactions of the Royal Society B-Biological Sciences, 367(1596), 1733–1745. doi: 10.1098/rstb.2012.0019

Feike, J., Juergens, K., Hollibaugh, J. T., Krueger, S., Jost, G., & Labrenz, M. (2012). Measuring unbiased metatranscriptomics in suboxic waters of the central Baltic Sea using a new in situ fixation system. Isme Journal, 6(2), 461–470. doi: 10.1038/ismej.2011.94

García, F. C., Bestion, E., Warfield, R., & Yvon-Durocher, G. (2018). Changes in temperature alter the relationship between biodiversity and ecosystem functioning. Proceedings of the National Academy of Sciences, 115(43), 10989. doi: 10.1073/pnas.1805518115

Giovannoni, S. J. (2017). SAR11 Bacteria: The Most Abundant Plankton in the Oceans. Annual Review of Marine Science, 9(1), 231–255. doi: 10.1146/annurev-marine-010814-015934

Gravel, D., Bell, T., Barbera, C., Bouvier, T., Pommier, T., Venail, P., & Mouquet, N. (2011). Experimental niche evolution alters the strength of the diversity-productivity relationship. Nature, 469(7328), 89-U1601. doi: 10.1038/nature09592

Grime, J. (1977). Evidence for Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory. American Naturalist, 111(982), 1169–1194. doi: 10.1086/283244

Grime, P., J., & Pierce, S. (2012). The Evolutionary Strategies that Shape Ecosystems. Wiley-Blackwell.

Grimm, N. B., Chapin, F. S., Bierwagen, B., Gonzalez, P., Groffman, P. M., Luo, Y., … Williamson, C. E. (2013). The impacts of climate change on ecosystem structure and function. Frontiers in Ecology and the Environment, 11(9), 474–482. doi: 10.1890/120282

Häuser, R., Pech, M., Kijek, J., Yamamoto, H., Titz, B., Naeve, F., … Uetz, P. (2012). RsfA (YbeB) Proteins Are Conserved Ribosomal Silencing Factors. Plos Genetics, 8(7), e1002815. doi: 10.1371/journal.pgen.1002815

Hebbali, A. (2020). olsrr: Tools for Building OLS Regression Models [R package version 0.5.3]. Retrieved from https://CRAN.R-project.org/package=olsrr

Hooper, D. U., Chapin, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., … Wardle, D. A. (2005). Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs, 75(1), 3–35. doi: 10.1890/04-0922

Horner-Devine, M. C., Lage, M., Hughes, J. B., & Bohannan, B. J. (2004). A taxa-area relationship for bacteria. Nature, 432(7018), 750–753.

Hoshino, T., Doi, H., Uramoto, G.-I., Wörmer, L., Adhikari, R. R., Xiao, N., … Inagaki, F. (2020). Global diversity of microbial communities in marine sediment. Proceedings of the National Academy of Sciences, 117(44), 27587–27597. doi: 10.1073/pnas.1919139117

Huey, R. B., & Slatkin, M. (1976). Cost and Benefits of Lizard Thermoregulation. The Quarterly Review of Biology, 51(3), 363–384. doi: 10.1086/409470

Hutchinson, G. E. (1957). Concluding Remarks. 22, 415–427. doi: doi:10.1101/SQB.1957.022.01.039

Hyatt, D., Chen, G.-L., LoCascio, P. F., Land, M. L., Larimer, F. W., & Hauser, L. J. (2010). Prodigal: Prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics, 11(1), 119. doi: 10.1186/1471-2105-11-119

Jasmin, J.-N., & Kassen, R. (2007). On the experimental evolution of specialization and diversity in heterogeneous environments. Ecology Letters, 10(4), 272–281. doi: https://doi.org/10.1111/j.1461-0248.2007.01021.x

Jessup, C. M., Kassen, R., Forde, S. E., Kerr, B., Buckling, A., Rainey, P. B., & Bohannan, B. J. M. (2004). Big questions, small worlds: Microbial model systems in ecology. Trends in Ecology & Evolution, 19(4), 189–197. doi: 10.1016/j.tree.2004.01.008

Joshi, N., & Fass, J. (2001). Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files (Version 1.33) [Software]. Available at https://github.com/najoshi/sickle.

Kanehisa, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., … Yamanishi, Y. (2007). KEGG for linking genomes to life and the environment. Nucleic Acids Research, 36(Database), D480–D484. doi: 10.1093/nar/gkm882

Kazanov, M. D., Vitreschak, A. G., & Gelfand, M. S. (2007). Abundance and functional diversity of riboswitches in microbial communities. BMC Genomics, 8(1), 347. doi: 10.1186/1471-2164-8-347

Kellermann, V., van Heerwaarden, B., Sgrò, C. M., & Hoffmann, A. A. (2009). Fundamental Evolutionary Limits in Ecological Traits Drive Drosophila Species Distributions. Science, 325(5945), 1244. doi: 10.1126/science.1175443

Kielbasa, S. M., Wan, R., Sato, K., Horton, P., & Frith, M. C. (2011). Adaptive seeds tame genomic sequence comparison. Genome Research, 21(3), 487–493. doi: 10.1101/gr.113985.110

Konopka, A., Lindemann, S., & Fredrickson, J. (2015). Dynamics in microbial communities: Unraveling mechanisms to identify principles. The ISME Journal, 9(7), 1488–1495. doi: 10.1038/ismej.2014.251

Kopylova, E., Noe, L., & Touzet, H. (2012). SortMeRNA: Fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. Bioinformatics, 28(24), 3211–3217. doi: 10.1093/bioinformatics/bts611

Krause, S., Le Roux, X., Niklaus, P. A., Bodegom, P. V., Lennon, J. T., Bertilsson, S., … Bodelier, P. (2014). Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. Aquatic Microbiology, 5, 251. doi: 10.3389/fmicb.2014.00251

Kültz, D. (2003). Evolution of the cellular stress proteome: From monophyletic origin to ubiquitous function. Journal of Experimental Biology, 206(18), 3119–3124. doi: 10.1242/jeb.00549

Lambert, S., Tragin, M., Lozano, J.-C., Ghiglione, J.-F., Vaulot, D., Bouget, F.-Y., & Galand, P. E. (2019). Rhythmicity of coastal marine picoeukaryotes, bacteria and archaea despite irregular environmental perturbations. The ISME Journal, 13(2), 388–401. doi: 10.1038/s41396-018-0281-z

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. Nature Methods, 9(4), 357-U54. doi: 10.1038/NMETH.1923

Lecompte, O., Ripp, R., Thierry, J. C., Moras, D., & Poch, O. (2002). Comparative analysis of ribosomal proteins in complete genomes: An example of reductive evolution at the domain scale. Nucleic Acids Research, 30(24), 5382–5390. doi: 10.1093/nar/gkf693

Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: An efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics, 30(7), 923–930. doi: 10.1093/bioinformatics/btt656

Lin, J., Liang, H., Yan, J., & Luo, L. (2017). The molecular mechanism and post-transcriptional regulation characteristic of Tetragenococcus halophilus acclimation to osmotic stress revealed by quantitative proteomics. Journal of Proteomics, 168, 1–14. doi: 10.1016/j.jprot.2017.08.014

Lindquist, S., & Craig, E. A. (1988). The heat-shock proteins. Annual Review of Genetics, 22(1), 631–677. doi: 10.1146/annurev.ge.22.120188.003215

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology, 15(12), 550. doi: 10.1186/s13059-014-0550-8

Lynch, M., & Gabriel, W. (1987). Environmental Tolerance. American Naturalist, 129(2), 283–303. doi: 10.1086/284635

MacArthur, R., H. (1972). Geographical Ecology. Princeton (N.J.) : Princeton university press.

Malik, A. A., Martiny, J. B. H., Brodie, E. L., Martiny, A. C., Treseder, K. K., & Allison, S. D. (2020). Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. The ISME Journal, 14(1), 1–9. doi: 10.1038/s41396-019-0510-0

Marie, D., Simon, N., Guillou, L., Partensky, F., & Vaulot, D. (2000). Flow Cytometry Analysis of Marine Picoplankton. In R. A. Diamond & S. Demaggio (Eds.), In Living Color: Protocols in Flow Cytometry and Cell Sorting (pp. 421–454). Berlin, Heidelberg: Springer. doi: 10.1007/978-3-642-57049-0_34

Matias, M. G., Combe, M., Barbera, C., & Mouquet, N. (2013). Ecological strategies shape the insurance potential of biodiversity. Frontiers in Microbiology, 3, 432. doi: 10.3389/fmicb.2012.00432

Ohtsu, I., Kawano, Y., Suzuki, M., Morigasaki, S., Saiki, K., Yamazaki, S., … Takagi, H. (2015). Uptake of L-cystine via an ABC transporter contributes defense of oxidative stress in the L-cystine export-dependent manner in Escherichia coli. PLOS ONE, 10(4), e0120619. doi: 10.1371/journal.pone.0120619

Ottesen, E. A., Young, C. R., Eppley, J. M., Ryan, J. P., Chavez, F. P., Scholin, C. A., & DeLong, E. F. (2013). Pattern and synchrony of gene expression among sympatric marine microbial populations. Proceedings of the National Academy of Sciences of the United States of America, 110(6), E488–E497. doi: 10.1073/pnas.1222099110

Park, C. B., & Lee, S. B. (2000). Effects of exogenous compatible solutes on growth of the hyperthermophilic archaeon Sulfolobus solfataricus. Journal of Bioscience and Bioengineering, 89(4), 318–322. doi: 10.1016/S1389-1723(00)88952-5

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2020). {nlme}: Linear and Nonlinear Mixed Effects Models [R package version 3.1-148]. Retrieved from https://CRAN.R-project.org/package=nlme

Planton, S., Déqué, M., Chauvin, F., & Terray, L. (2008). Expected impacts of climate change on extreme climate events. Comptes Rendus Geoscience, 340(9), 564–574. doi: 10.1016/j.crte.2008.07.009

Prosser, J. I. (2015). Dispersing misconceptions and identifying opportunities for the use of “omics” in soil microbial ecology. Nature Reviews Microbiology, 13(7), 439–446. doi: 10.1038/nrmicro3468

Prosser, J. I., & Martiny, J. B. H. (2020). Conceptual challenges in microbial community ecology. Philosophical Transactions of the Royal Society B-Biological Sciences, 375(1798), 20190241. doi: 10.1098/rstb.2019.0241

Rao, M. R. R., & Stokes, J. L. (1953). Utilization of ethanol by acetic acid bacteria. Journal of Bacteriology, 66(6), 634–638.

Rocca, J. D., Simonin, M., Blaszczak, J. R., Ernakovich, J. G., Gibbons, S. M., Midani, F. S., & Washburne, A. D. (2019). The Microbiome Stress Project: Toward a Global Meta-Analysis of Environmental Stressors and Their Effects on Microbial Communities. Frontiers in Microbiology, 9. doi: 10.3389/fmicb.2018.03272

Rojas-Jimenez, K., Araya-Lobo, A., Quesada-Perez, F., Akerman-Sanchez, J., Delgado-Duran, B., Ganzert, L., … Grossart, H.-P. (2021). Variation of bacterial communities along the vertical gradient in Lake Issyk Kul, Kyrgyzstan. Environmental Microbiology Reports, n/a(n/a). doi: https://doi.org/10.1111/1758-2229.12935

Romero, F., Acuña, V., & Sabater, S. (2020). Multiple Stressors Determine Community Structure and Estimated Function of River Biofilm Bacteria. Applied and Environmental Microbiology, 86(12). doi: 10.1128/AEM.00291-20

Roncarati, D., & Scarlato, V. (2017). Regulation of heat-shock genes in bacteria: From signal sensing to gene expression output. FEMS Microbiology Reviews, 41(4), 549–574. doi: 10.1093/femsre/fux015

Satinsky, B. M., Gifford, S. M., Crump, B. C., & Moran, M. A. (2013). Use of internal standards for quantitative metatranscriptome and metagenome analysis. Methods in Enzymology, 531, 237–250. doi: 10.1016/B978-0-12-407863-5.00012-5

Seneviratne, S., Nicholls, N., Easterling, D., Goodess, C., Kanae, S., Kossin, J., … Zwiers, F. W. (2012). Changes in climate extremes and their impacts on the natural physical environment. 109–230. doi: 10.7916/d8-6nbt-s431

Sévin, D. C., Stählin, J. N., Pollak, G. R., Kuehne, A., & Sauer, U. (2016). Global Metabolic Responses to Salt Stress in Fifteen Species. PLOS ONE, 11(2), e0148888. doi: 10.1371/journal.pone.0148888

Sexton, J. P., Montiel, J., Shay, J. E., Stephens, M. R., & Slatyer, R. A. (2017). Evolution of Ecological Niche Breadth. Annual Review of Ecology, Evolution, and Systematics, 48(1), 183–206. doi: 10.1146/annurev-ecolsys-110316-023003

Shen, D., Juergens, K., & Beier, S. (2018). Experimental insights into the importance of ecologically dissimilar bacteria to community assembly along a salinity gradient. Environmental Microbiology, 20(3), 1170–1184. doi: 10.1111/1462-2920.14059

Singh, M., Kumar, J., Singh, S., Singh, V. P., & Prasad, S. M. (2015). Roles of osmoprotectants in improving salinity and drought tolerance in plants: A review. Reviews in Environmental Science and Bio/Technology, 14(3), 407–426. doi: 10.1007/s11157-015-9372-8

Slatyer, R. A., Hirst, M., & Sexton, J. P. (2013). Niche breadth predicts geographical range size: A general ecological pattern. Ecology Letters, 16(8), 1104–1114. doi: 10.1111/ele.12140

Sørensen, J. G., Kristensen, T. N., & Loeschcke, V. (2003). The evolutionary and ecological role of heat shock proteins. Ecology Letters, 6(11), 1025–1037. doi: 10.1046/j.1461-0248.2003.00528.x

Stead, M. B., Agrawal, A., Bowden, K. E., Nasir, R., Mohanty, B. K., Meagher, R. B., & Kushner, S. R. (2012). RNAsnap (TM): A rapid, quantitative and inexpensive, method for isolating total RNA from bacteria. Nucleic Acids Research, 40(20), e156. doi: 10.1093/nar/gks680

Sultan, S. E. (2001). Phenotypic Plasticity for Fitness Components in Polygonum Species of Contrasting Ecological Breadth. Ecology, 82(2), 328–343. doi: https://doi.org/10.1890/0012-9658(2001)082[0328:PPFFCI]2.0.CO;2

Szekely, A. J., Berga, M., & Langenheder, S. (2013). Mechanisms determining the fate of dispersed bacterial communities in new environments. Isme Journal, 7(1), 61–71. doi: 10.1038/ismej.2012.80

Thuiller, W., Lavorel, S., & Araújo, M. B. (2005). Niche properties and geographical extent as predictors of species sensitivity to climate change. Global Ecology and Biogeography, 14(4), 347–357. doi: https://doi.org/10.1111/j.1466-822X.2005.00162.x

Tischer, K., Zeder, M., Klug, R., Pernthaler, J., Schattenhofer, M., Harms, H., & Wendeberg, A. (2012). Fluorescence in situ hybridization (CARD-FISH) of microorganisms in hydrocarbon contaminated aquifer sediment samples. Systematic and Applied Microbiology, 35(8), 526–532. doi: 10.1016/j.syapm.2012.01.004

Van Buskirk, J. (2002). A Comparative Test of the Adaptive Plasticity Hypothesis: Relationships between Habitat and Phenotype in Anuran Larvae. The American Naturalist, 160(1), 87–102. doi: 10.1086/340599

Weidenhaupt, M., Rossi, P., Beck, C., Fischer, H.-M., & Hennecke, H. (1996). Bradyrhizobium japonicum possesses two discrete sets of electron transfer flavoprotein genes: FixA, fixB and etfS, etfL. Archives of Microbiology, 165(3), 169–178. doi: 10.1007/BF01692858

Welsh, D. T. (2000). Ecological significance of compatible solute accumulation by micro-organisms: From single cells to global climate. FEMS Microbiology Reviews, 24(3), 263–290. doi: 10.1111/j.1574-6976.2000.tb00542.x

SUPPLEMENTARY MATERIALS

Table S1. *Details strains characteristics. Sampling locations and environmental conditions, NB and stress estimations.*

Strain ID	Isolation Site	Environment	Salinity (NaCl g L^{-1})	Latitude (°N)	Longitude (°E)	Isolation Year	Isolation Month	Bacterial type	Fitness (ODXGR) model Selected	Niche breadth (NaCl g L^{-1})	Optimum modeled Salinity (NaCl g L^{-1})	Mean (g L^{-1})	Standard deviation (g L^{-1})	Skewness	Kurtosis	Hypoosmotic NB	Hyperosmotic NB	sided NB S2S1†	sided NB S2S3†	Stress (Δ1)	Stress (Δ2)	Stress (Δ3)
S331	Frontignan, France	Hypersaline lagoon	30	43,271	3,470	2010	2	Gram-negative	Gaussian	143	-5	64,21	48,78	0,93	0,49	45	98	98	98	0,064	0,086	0,151
S337	Frontignan, France	Hypersaline lagoon	30	43,271	3,470	2010	2	Gram-negative	Log-normal	48	42	58,14	27,18	1,14	1,49	17	31	17	31	0,462	0,137	0,325
S338	Frontignan, France	Hypersaline lagoon	30	43,271	3,470	2010	2	Gram-negative	Log-normal	53	33	58,58	37,26	1,44	2,32	17	36	NA	36	0,038	0,233	0,195
S366	Frontignan, France	Hypersaline lagoon	20	43,271	3,470	2010	2	Gram-negative	Log-normal	56	35	61,58	38,41	1,32	1,71	18	38	NA	38	0,057	0,228	0,284
S374	Frontignan, France	Hypersaline lagoon	20	43,271	3,470	2010	2	Gram-negative	Log-normal	58	35	62,08	37,61	1,21	1,25	18	40	18	40	0,327	0,136	0,191
S432	Magdalen Islands, Canada	Marine	33	47,261	-61,492	2010	3	Gram-negative	Log-normal	50	44	60,24	27,82	1,02	0,93	18	32	18	NA	0,640	0,071	0,710
S479	North Sea, Kiel, Germany	Marine	12,6	54,264	10,141	2010	4	Gram-negative	Gaussian	58	53	54,27	23,88	0,15	-0,35	29	29	29	NA	0,294	0,018	0,312
S490	North Sea, Kiel, Germany	Marine	12,6	54,264	10,141	2010	4	Gram-negative	Log-normal	51	42	60,57	30,55	1,22	1,69	18	33	18	33	0,189	0,213	0,024
S599	Apalache Bay, USA	Marine	33	30,015	-84,053	2010	5	Gram-negative	Log-normal	29	49	53,69	12,92	0,49	-0,21	12	17	12	17	0,786	0,204	0,582
S618	Apalache Bay, USA	Marine	33	30,015	-84,053	2010	5	Gram-negative	Log-normal	67	21	80,86	66,72	1,23	0,88	14	53	53	53	0,149	0,167	0,316
S630	Frontignan, France	Hypersaline lagoon	340	43,271	3,470	2010	7	Gram-positive	Log-normal	140	41	115,18	75,46	0,61	-0,66	29	111	29	NA	0,171	0,009	0,162

Table S2. *Summary transcriptional response to changing salinity levels experiment and RNA extraction procedure.*

#ID	Strain ID	Sampling point	Rep	Sampling points Salinity (NaCl g L^{-1})	Volume incubated (mL)	Abundance (cell mL^{-1})	RNA Extraction	DNA purification	RNA cleaning and concentration	Transcriptome mix	ID library	Std added (ng)	Mapped reads
1	S337	S_1	R_1	15	15	4,98E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1L1	5	3 893 269
2	S337	S_1	R_2	15	15	6,32E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1L2	5	6 125 259
3	S337	S_2	R_1	30	15	4,26E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1M1	5	2 878 190
4	S337	S_2	R_2	30	15	5,62E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1M2	5	2 565 092
5	S337	S_3	R_1	45	15	5,48E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1H1	5	1 692 221
6	S337	S_3	R_2	45	15	5,27E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1H2	5	2 896 371
7	S432	S_1	R_1	10	15	5,35E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1L1	5	4 074 285
8	S432	S_1	R_2	10	15	6,08E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1L2	5	4 404 748
9	S432	S_2	R_1	25	15	5,69E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1M1	5	810 159
10	S432	S_2	R_2	25	15	6,00E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1M2	5	1 243 933
11	S432	S_3	R_1	40	15	4,04E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1H1	5	1 393 241
12	S432	S_3	R_2	40	15	4,13E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1H2	5	2 136 366
13	S599	S_1	R_1	20	50	6,25E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1L1	5	4 308 213
14	S599	S_1	R_2	20	50	6,19E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1L2	5	3 395 799
15	S599	S_2	R_1	35	50	9,18E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1M1	5	977 975
16	S599	S_2	R_2	35	50	9,26E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1M2	5	1 210 536
17	S599	S_3	R_1	50	50	1,62E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1H1	5	755 681
18	S599	S_3	R_2	50	50	1,20E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M1	M1H2	5	1 068 989
19	S366	S_1	R_1	20	15	8,45E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2L1	5	1 770 775
20	S366	S_1	R_2	20	15	7,96E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2L2	5	1 184 844
21	S366	S_2	R_1	35	15	8,25E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2M1	5	650 139
22	S366	S_2	R_2	35	15	7,26E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2M2	5	2 688 563
23	S366	S_3	R_1	50	15	9,52E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2H1	5	2 278 776
24	S366	S_3	R_2	50	15	8,55E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2H2	5	1 163 570
25	S490	S_1	R_1	20	15	3,51E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2L1	5	2 600 024
26	S490	S_1	R_2	20	15	3,60E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2L2	5	1 148 480
27	S490	S_2	R_1	35	15	3,69E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2M1	5	1 000 328
28	S490	S_2	R_2	35	15	3,44E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2M2	5	1 968 709
29	S490	S_3	R_1	50	15	3,15E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2H1	5	4 309 222
30	S490	S_3	R_2	50	15	3,31E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2H2	5	1 504 304
31	S618	S_1	R_1	15	15	5,31E+08	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2L1	5	935 596
32	S618	S_1	R_2	15	15	6,46E+08	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2L2	5	697 323
33	S618	S_2	R_1	30	15	4,25E+08	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2M1	5	475 975

34	S618	S_2	R_2	30	15	4,06E+08	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2M2	5	1 425 276
35	S618	S_3	R_1	45	15	3,47E+08	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2H1	5	2 870 859
36	S618	S_3	R_2	45	15	3,26E+08	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M2	M2H2	5	1 381 460
37	S331	S_1	R_1	10	15	1,67E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3L1	5	2 606 672
38	S331	S_1	R_2	10	15	1,72E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3L2	5	2 008 936
39	S331	S_2	R_1	25	15	1,35E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3M1	5	1 759 940
40	S331	S_2	R_2	25	15	1,62E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3M2	5	1 244 024
41	S331	S_3	R_1	40	15	9,68E+06	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3H1	5	1 165 788
42	S331	S_3	R_2	40	15	1,04E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3H2	5	908 016
43	S338	S_1	R_1	15	15	3,59E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3L1	5	1 576 549
44	S338	S_1	R_2	15	15	3,83E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3L2	5	1 481 603
45	S338	S_2	R_1	30	15	3,92E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3M1	5	2 038 404
46	S338	S_2	R_2	30	15	3,97E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3M2	5	1 444 402
47	S338	S_3	R_1	45	15	3,38E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3H1	5	1 391 056
48	S338	S_3	R_2	45	15	3,38E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3H2	5	1 405 709
49	S374	S_1	R_1	10	15	9,91E+08	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3L1	5	1 203 362
50	S374	S_1	R_2	10	15	9,20E+08	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3L2	5	1 026 834
51	S374	S_2	R_1	25	15	7,75E+08	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3M1	5	1 063 681
52	S374	S_2	R_2	25	15	8,40E+08	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3M2	5	956 621
53	S374	S_3	R_1	40	15	3,36E+08	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3H1	5	985 259
54	S374	S_3	R_2	40	15	2,99E+08	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3H2	5	782 123
55	S479	S_1	R_1	20	15	4,87E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3L1	5	587 937
56	S479	S_1	R_2	20	15	4,41E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3L2	5	571 350
57	S479	S_2	R_1	35	15	4,39E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3M1	5	857 300
58	S479	S_2	R_2	35	15	4,14E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3M2	5	807 126
59	S479	S_3	R_1	50	15	4,24E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3H1	5	1 743 651
60	S479	S_3	R_2	50	15	4,58E+07	Direct-zol™ RNA-Miniprep Plus	TURBO DNA-free™ Kit	RNA Clean & Concentrator™-5 kit	M3	M3H2	5	953 015
61	S630	S_1	R_1	10	50	1,90E+08	SNAP™	TURBO DNA-free™ Kit	RNA Clean & Concentrator™	M2	M2L1	5	1 175 862
62	S630	S_1	R_2	10	50	1,73E+08	SNAP™	TURBO DNA-free™ Kit	RNA Clean & Concentrator™	M2	M2L2	5	383 019
63	S630	S_2	R_1	25	50	1,75E+08	SNAP™	TURBO DNA-free™ Kit	RNA Clean & Concentrator™	M2	M2M1	5	388 208
64	S630	S_2	R_2	25	50	1,73E+08	SNAP™	TURBO DNA-free™ Kit	RNA Clean & Concentrator™	M2	M2M2	5	1 028 203
65	S630	S_3	R_1	40	50	1,89E+08	SNAP™	TURBO DNA-free™ Kit	RNA Clean & Concentrator™	M2	M2H1	5	1 454 551
66	S630	S_3	R_2	40	50	2,07E+08	SNAP™	TURBO DNA-free™ Kit	RNA Clean & Concentrator™	M2	M2H2	5	642 666

Table S3. *Shared genes across the 11 strains used in this study. The full version of the table can be found online in* *https://www.dropbox.com/sh/1swn1wcfse8dnfx/AABmeIlTihIlWXefvqzUqCWLa?dl=0*

#	KEGG ID	Gene description	DNA polymerases	Ribosomal proteins	RNA polymerases	Potential osmolyte transport	HSP
1	K00001	E1.1.1.1, adh; alcohol dehydrogenase [EC:1.1.1.1]	FAUX	FAUX	FAUX	FAUX	FAUX
2	K00012	UGDH, ugd; UDPglucose 6-dehydrogenase [EC:1.1.1.22]	FAUX	FAUX	FAUX	FAUX	FAUX
3	K00014	aroE; shikimate dehydrogenase [EC:1.1.1.25]	FAUX	FAUX	FAUX	FAUX	FAUX
4	K00052	leuB; 3-isopropylmalate dehydrogenase [EC:1.1.1.85]	FAUX	FAUX	FAUX	FAUX	FAUX
5	K00057	gpsA; glycerol-3-phosphate dehydrogenase (NAD(P)+) [EC:1.1.1.94]	FAUX	FAUX	FAUX	FAUX	FAUX
6	K00059	fabG; 3-oxoacyl-[acyl-carrier protein] reductase [EC:1.1.1.100]	FAUX	FAUX	FAUX	FAUX	FAUX
7	K00117	gcd; quinoprotein glucose dehydrogenase [EC:1.1.5.2]	FAUX	FAUX	FAUX	FAUX	FAUX
8	K00121	frmA, ADH5, adhC; S-(hydroxymethyl)glutathione dehydrogenase / alcohol dehydrogenase [EC:1.1.1.284 1.1.1.1]	FAUX	FAUX	FAUX	FAUX	FAUX
9	K00135	gabD; succinate-semialdehyde dehydrogenase / glutarate-semialdehyde dehydrogenase [EC:1.2.1.16 1.2.1.79 1.2.1.20]	FAUX	FAUX	FAUX	FAUX	FAUX
10	K00147	proA; glutamate-5-semialdehyde dehydrogenase [EC:1.2.1.41]	FAUX	FAUX	FAUX	FAUX	FAUX
11	K00242	sdhD, frdD; succinate dehydrogenase / fumarate reductase, membrane anchor subunit	FAUX	FAUX	FAUX	FAUX	FAUX
12	K00286	proC; pyrroline-5-carboxylate reductase [EC:1.5.1.2]	FAUX	FAUX	FAUX	FAUX	FAUX
13	K00311	ETFDH; electron-transferring-flavoprotein dehydrogenase [EC:1.5.5.1]	FAUX	FAUX	FAUX	FAUX	FAUX
14	K00344	qor, CRYZ; NADPH2:quinone reductase [EC:1.6.5.5]	FAUX	FAUX	FAUX	FAUX	FAUX
15	K00375	K00375; GntR family transcriptional regulator / MocR family aminotransferase	FAUX	FAUX	FAUX	FAUX	FAUX
16	K00384	trxB; thioredoxin reductase (NADPH) [EC:1.8.1.9]	FAUX	FAUX	FAUX	FAUX	FAUX
17	K00564	rsmC; 16S rRNA (guanine1207-N2)-methyltransferase [EC:2.1.1.172]	FAUX	FAUX	FAUX	FAUX	FAUX
18	K00568	ubiG; 2-polyprenyl-6-hydroxyphenyl methylase / 3-demethylubiquinone-9 3-methyltransferase [EC:2.1.1.222 2.1.1.64]	FAUX	FAUX	FAUX	FAUX	FAUX
19	K00575	cheR; chemotaxis protein methyltransferase CheR [EC:2.1.1.80]	FAUX	FAUX	FAUX	FAUX	FAUX
20	K00611	OTC, argF, argI; ornithine carbamoyltransferase [EC:2.1.3.3]	FAUX	FAUX	FAUX	FAUX	FAUX
21	K00627	DLAT, aceF, pdhC; pyruvate dehydrogenase E2 component (dihydrolipoamide acetyltransferase) [EC:2.3.1.12]	FAUX	FAUX	FAUX	FAUX	FAUX
22	K00645	fabD; [acyl-carrier-protein] S-malonyltransferase [EC:2.3.1.39]	FAUX	FAUX	FAUX	FAUX	FAUX
23	K00655	plsC; 1-acyl-sn-glycerol-3-phosphate acyltransferase [EC:2.3.1.51]	FAUX	FAUX	FAUX	FAUX	FAUX
24	K00658	DLST, sucB; 2-oxoglutarate dehydrogenase E2 component (dihydrolipoamide succinyltransferase) [EC:2.3.1.61]	FAUX	FAUX	FAUX	FAUX	FAUX
25	K00674	dapD; 2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase [EC:2.3.1.117]	FAUX	FAUX	FAUX	FAUX	FAUX
26	K00762	pyrE; orotate phosphoribosyltransferase [EC:2.4.2.10]	FAUX	FAUX	FAUX	FAUX	FAUX
27	K00766	trpD; anthranilate phosphoribosyltransferase [EC:2.4.2.18]	FAUX	FAUX	FAUX	FAUX	FAUX
28	K00799	GST, gst; glutathione S-transferase [EC:2.5.1.18]	FAUX	FAUX	FAUX	FAUX	FAUX
29	K00800	aroA; 3-phosphoshikimate 1-carboxyvinyltransferase [EC:2.5.1.19]	FAUX	FAUX	FAUX	FAUX	FAUX
30	K00847	E2.7.1.4, scrK; fructokinase [EC:2.7.1.4]	FAUX	FAUX	FAUX	FAUX	FAUX
31	K00858	ppnK, NADK; NAD+ kinase [EC:2.7.1.23]	FAUX	FAUX	FAUX	FAUX	FAUX
32	K00859	coaE; dephospho-CoA kinase [EC:2.7.1.24]	FAUX	FAUX	FAUX	FAUX	FAUX

Table S4. *Potential osmolytes transport genes used in this study. The full version of the table can be found online in* *https://www.dropbox.com/sh/1swn1wcfse8dnfx/AABmeIlTihIlWXefvqzUqCWLa?dl=0*

KEGG ID	**Gene description**	**KEGG path ID**	**Potential osmolyte transport**
K09969	aapJ, bztA; general L-amino acid transport system substrate-binding protein	ko02010	VRAI
K09970	aapQ, bztB; general L-amino acid transport system permease protein	ko02010	VRAI
K09971	aapM, bztC; general L-amino acid transport system permease protein	ko02010	VRAI
K09972	aapP, bztD; general L-amino acid transport system ATP-binding protein [EC:3.6.3.-]	ko02010	VRAI
K02025	ABC.MS.P; multiple sugar transport system permease protein	NA	VRAI
K02026	ABC.MS.P1; multiple sugar transport system permease protein	NA	VRAI
K02027	ABC.MS.S; multiple sugar transport system substrate-binding protein	NA	VRAI
K02028	ABC.PA.A; polar amino acid transport system ATP-binding protein [EC:3.6.3.21]	NA	VRAI
K02029	ABC.PA.P; polar amino acid transport system permease protein	NA	VRAI
K02030	ABC.PA.S; polar amino acid transport system substrate-binding protein	NA	VRAI
K02052	ABC.SP.A; putative spermidine/putrescine transport system ATP-binding protein	NA	VRAI
K02053	ABC.SP.P; putative spermidine/putrescine transport system permease protein	NA	VRAI
K02054	ABC.SP.P1; putative spermidine/putrescine transport system permease protein	NA	VRAI
K02055	ABC.SP.S; putative spermidine/putrescine transport system substrate-binding protein	NA	VRAI
K02056	ABC.SS.A; simple sugar transport system ATP-binding protein [EC:3.6.3.17]	NA	VRAI
K02057	ABC.SS.P; simple sugar transport system permease protein	NA	VRAI
K02058	ABC.SS.S; simple sugar transport system substrate-binding protein	NA	VRAI
K05777	ABC.VB1X.S; putative thiamine transport system substrate-binding protein	NA	VRAI
K05778	ABC.VB1X.P; putative thiamine transport system permease protein	NA	VRAI
K05779	ABC.VB1X.A; putative thiamine transport system ATP-binding protein	NA	VRAI
K10232	aglE, ggtB; alpha-glucoside transport system substrate-binding protein	ko02010	VRAI
K10233	aglF, ggtC; alpha-glucoside transport system permease protein	ko02010	VRAI
K10234	aglG, ggtD; alpha-glucoside transport system permease protein	ko02010	VRAI
K10235	aglK; alpha-glucoside transport system ATP-binding protein	ko02010	VRAI
K17241	aguE; alpha-1,4-digalacturonate transport system substrate-binding protein	ko02010	VRAI
K17242	aguF; alpha-1,4-digalacturonate transport system permease protein	ko02010	VRAI
K17243	aguG; alpha-1,4-digalacturonate transport system permease protein	ko02010	VRAI
K10022	aotJ; arginine/ornithine transport system substrate-binding protein	ko02010	VRAI
K10023	aotM; arginine/ornithine transport system permease protein	ko02010	VRAI
K10024	aotQ; arginine/ornithine transport system permease protein	ko02010	VRAI

Table S5. *The statistical output for MLMs including data based on 15 NaCl L^{-1} salinity gradients in which the performance optimum was not passed.*

			Absolute Gene Regulation vs NB			**Absolute Gene Regulation vs Stress§**			**Gene Regulation vs Stress§**		
Classification	**Functional categories**	**Number of considered genes per strain**	**Slope**	**R^2**	**P**	**Slope**	**R^2**	**P**	**Slope**	**R^2**	**P**
	Total transcripts	1 †	-0,06	0,01	0,768	0.38(0.04)	0.02(0.00)	0.458(0.952)	0.28(0.83)	0.07(0.13)	0.749(0.482)
Fitness-related genes	DNA polymerases	2 †	-0,19	0,02	0,399	0.42(0.67)	0.01(0.03)	0.399(0.330)	1.19(1.93)	0.13(0.29)	0.151(0.066)
	Ribosomal proteins	32 †	-0,08	0,09	**0.003***	0.30(0.27)	0.02(0.08)	**0.000***(0.001**)**	1.19(-0.15)	0.13(0.06)	**0.000***(0.002**)**
	RNA polymerases	2 †	-0,46	0,12	**0.041***	0.87(1.98)	0.04(0.23)	0.113(**0.004***)	1.84(3.06)	0.14(0.38)	**0.03(0.006*)**
Adaptation-related genes	Transport of osmoprotectants	20-93 ‡	0,52	0,19	**0.076•**	-0.23(-1.04)	0.00(0.08)	0.767(0.271)	-0.44(-5.81)	0.02(0.03)	0.940(0.495)
	HSP	6-17 ‡	-0,24	0,06	0,332	1.26(1.09)	0.14(0.13)	**0.030***(0.143)	2.06(0.91)	0.17(0.00)	0.571(0.866)

† MLMs were performed using the individual genes as a random factor

‡ MLMs were performed using the additive transcriptional regulation on individual genes.

§ The displayed statistical output parameters refer to regressions including all stress exposure data points, while only data based on 15 NaCl L^{-1} salinity gradients in which the performance optimum was not passed are displayed in parenthesis.

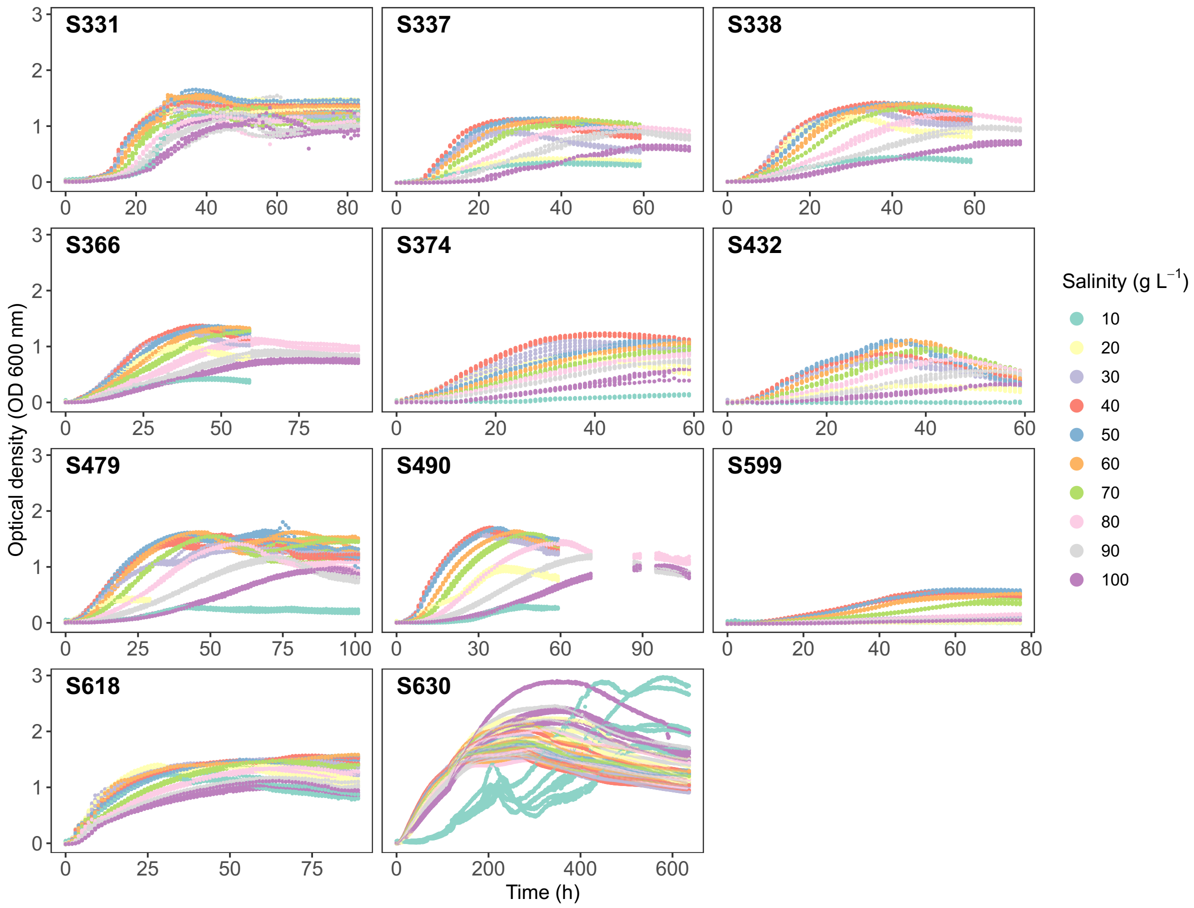

Figure S1. Growth curves of 11 strains at 10 different salt conditions used to estimate fitness indices. *In the case of strain S630 grown at 10 g L^{-1}, we decided to consider the first maximum reached after roughly 200 hours incubations time to estimate the fitness index. We assumed that the later increase in cell densities after extended incubation time that in some replicates surpassed those values measured at salinities with overall highest fitness estimates might have been due to the selection of genetically adapted cells that had evolved under high physiological pressure.*

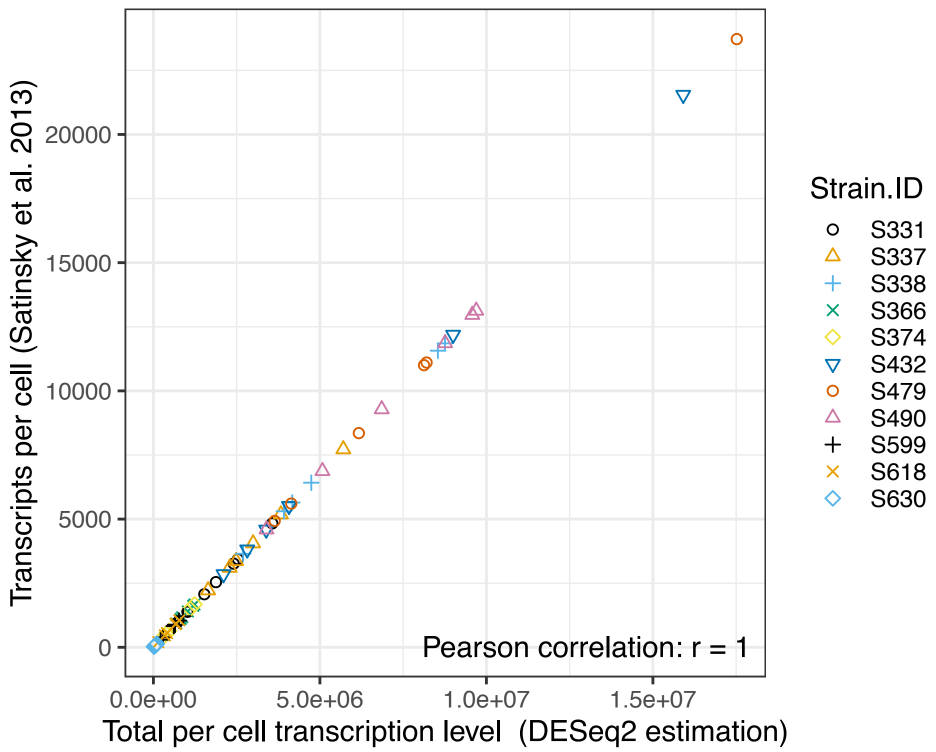

Figure S2. Regression of the number transcripts per cell estimated as published elsewhere (Satinsky et al., 2013) against a variable for the total per cell transcription level estimated using the DESeq2 package (R2=1.00, P<0.001, Pearson correlation)*. In order to obtain the DESeq2 transcription level variable, count data for all individual genes in each transcriptome library that were obtained after the normalization step using the estimateSizeFactors function in DeSeq2 were summed up.*

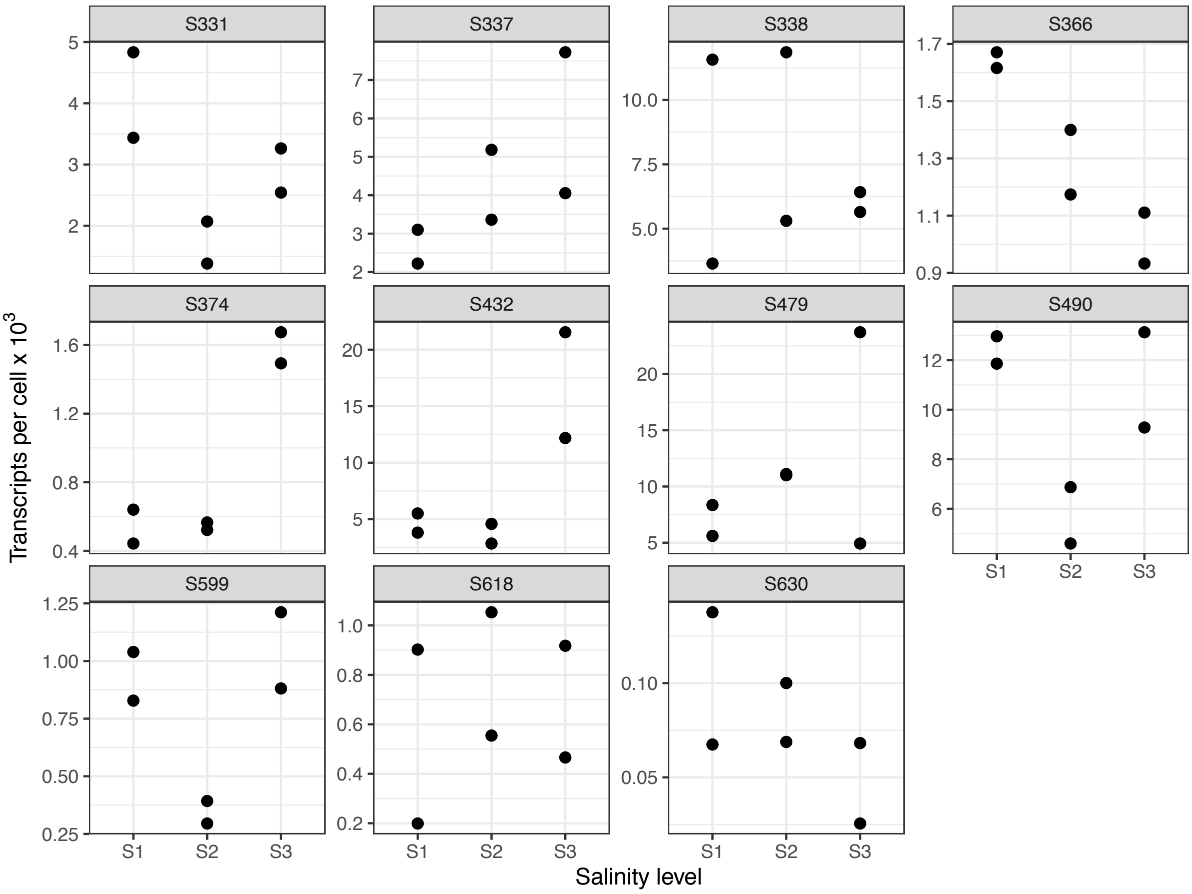

Figure S3. Total transcript per cell by strain at the three sampling options from the transcriptional regulation experiment. *Sampling points are indicated as S1,S2 and S3.*

Chapter 2

Cryopreservation and resuscitation of natural aquatic prokaryotic communities

Rain-Franco, Angel[1,+], Pavan de Moraes, Guilherme [1,2,3,+] & Beier, Sara[1,3,*]

[1] UMR 7621 Laboratoire d'Océanographie Microbienne, Observatoire Océanologique de Banyuls-sur-Mer, Sorbonne Université, Banyuls-sur-Mer, France.

[2] Graduate Program in Ecology and Natural Resources (PPGERN), Laboratory of Phycology, Department of Botany (DB), Universidade Federal de Sao Carlos (UFSCar), Sao Carlos, SP, Brazil.

[3] Department of Biological Oceanography, Leibniz Institute for Baltic Sea Research Warnemünde, Rostock, Germany

[+] These authors contributed equally to this work.

* Corresponding author.

*Published in Frontiers in Microbiology under doi:*10.3389/fmicb.2020.597653

ABSTRACT

Experimental reproducibility in aquatic microbial ecology is critical to predict the dynamics of microbial communities. However, controlling the initial composition of naturally occurring microbial communities that will be used as the inoculum in experimental setups is challenging, because a proper method for the preservation of those communities is lacking. To provide a feasible method for preservation and resuscitation of natural aquatic prokaryote assemblages, we developed a cryopreservation procedure applied to natural aquatic prokaryotic communities. We studied the impact of inoculum size, processing time and storage time on the success of resuscitation. We further assessed the effect of different growth media supplemented with DOM prepared from naturally occurring microorganisms on the recovery of the initially cryopreserved communities obtained from two sites that have contrasting trophic status and environmental heterogeneity. Our results demonstrated that the variability of the resuscitation process among replicates decreased with increasing inoculum size. The degree of similarity between initial and resuscitated communities was influenced by both the growth medium and origin of the community. We further demonstrated that depending on the inoculum source, 45-72% of the abundant species in the initially natural microbial communities could be detected as viable cells after cryopreservation. Processing time and long-term storage up to 12 months did not significantly influence the community composition after resuscitation. However, based on our results we recommend keeping handling time to a minimum and ensure identical incubation conditions for repeated resuscitations from cryo-preserved aliquots at different time points. Given our results, we recommend cryopreservation as a promising tool to advance experimental research in the field of microbial ecology.

INTRODUCTION

Experimental studies have played a central role in the field of microbial ecology over the past 55 years (Duarte et al., 1997; Shade et al., 2012) and have been critical to our comprehension of microbial dynamics and processes in aquatic ecosystems (Bell et al., 2009). Such studies are particularly valuable to reduce the complexity of natural systems by artificially controlling conditions while separately assessing the impact of selected parameters on community dynamics and functioning.

However, their confidence and reproducibility rely on maintaining comparable initial conditions, which includes the identity and density of organisms in the starting community. Differences in community initial composition and inherent environmental variability may be propagated and amplified during the course of an experiment (Fukami, 2015), effectively affecting both the final community composition and functioning (Bloesch et al., 1988). Particularly, the option to perform temporally delayed follow-up experiments, for instance with the aim of changing certain parameters of interest based on the outcome of an initial experiment, depends on the availability of standardized starting communities.

The assembly of bacterial strains into artificial communities to be used as starting communities in experimental setups is one option to circumvent these caveats and it has been applied in multiple studies to test ecological theory, such as the biodiversity-ecosystem functioning (BEF) relationships (e.g., Bell et al., 2005; Matias et al., 2013; García et al., 2018). Experimental ecological studies usually aim to elucidate generally valid ecological patterns that do not depend on the presence of particular species. Therefore, deviations in the species composition of experimental communities compared to naturally occurring communities are usually accepted. However, while experimental approaches based on artificial communities did, to our knowledge, maximally include 72 different species (Bell et al., 2005; Shakya et al., 2013; Singer et al., 2016; Yu et al., 2016; Cairns et al., 2018), natural microbial communities are usually highly diverse assemblages containing several hundreds of species (Ibarbalz et al., 2019; Salazar et al., 2019). Even though the reduced complexity of the artificial communities allows the elucidation of the main drivers behind community performance and stability (Großkopf and Soyer, 2014), it is unknown whether the complexity of natural microbial communities is accurately represented in this approach (Wolfe, 2018). For instance, it has been observed that community functioning is strongly linked to changing species diversity in low diversity mock communities, while BEF relationships are less predictable in highly diverse communities, which is typical for natural microbial communities (Bell et al., 2009).

To better link the findings from experiments performed on a limited number of strains with processes taking place in natural environments, experimental manipulation of naturally co-occurring microbes is a valuable tool (e.g., Bell et al., 2005). However, while the control over the composition and density of starting communities of simple consortia comprised of few species is rather easy, controlling the initial composition of complex, naturally occurring microbial communities is challenging.

The composition of natural microbial communities is highly dynamic and time-space dependent, with concomitant short and long-time phenomena affecting their succession at synoptical and seasonal scales or due to regime shifts (Faust et al., 2015). We suggest that cryopreservation could be one option to control the obvious lack of standardization of the initial composition of complex microbial communities in experimental setups, as it has been shown to be a promising way to preserve both community composition and functioning (Kerckhof et al., 2014).

Cryopreservation is the preservation of biological material at cryogenic ultralow temperatures (-80 °C or -196 °C) for its optimal long-term preservation (Prakash et al., 2013). It is usually carried out with the addition of cryoprotectants, which help to reduce biophysical damage of cells by preventing the formation of intracellular ice crystals, resulting in increased recovery yields upon resuscitation (Fuller, 2004). Among a variety of different cryoprotectants, dimethyl sulphoxide (DMSO) has been described as one of the most successful and commonly used cryoprotectants in microbiology (Hubálek, 2003; Chian, 2010).

The effectivity of cryopreservation has been verified for both axenic and non-axenic cultures during the last decades (Nagai et al., 2005; Heylen et al., 2012a). More recently it has also been demonstrated for bioreactor communities, which successfully recovered their composition and functionality after cryopreservation (Kerckhof et al., 2014). So far, cryopreservation has been mainly used in applications linked to biotechnology and not yet applied to conserve highly complex and dynamic communities from natural aquatic environments.

Even if cells remain viable after cryopreservation, appropriate thawing and culturing conditions are required to recover the activity of individual community members and effectively resuscitate the preserved communities (Heylen et al., 2012b). However, most prokaryotes occurring in natural habitats have not yet been successfully cultured (Steen et al., 2019), rendering the resuscitation process particularly challenging for cryopreserved natural communities. Shortcomings of prokaryotes culturing can be associated with their narrow and mostly unknown physiological requirements (Rappé et al., 2002; Giovannoni, 2017), such as their adaption to low nutrient levels, the frequent occurrence of temporarily inactive cellular states (i.e. starvation, dormancy), and the complexity of inter-species interactions (Pagnier et al., 2015; Overmann et al., 2017). Widely applied culture media, such as Marine Broth (ZoBell, 1941), are in striking contrast to global ocean nutrient levels and do not represent properly the environmental conditions (Giovannoni and Stingl, 2007).

Culture media based on the original environmental conditions, such as filtered seawater and artificial fresh- or sea-water amended with low concentration of nutrients, have been shown to increase the culturing success (Overmann et al., 2017). It has further been suggested that complex carbon substrates, can promote collaborative interactions among species in mixed cultures (Deng and Wang, 2016). However, the extremely high diversity and reactivity of the molecules constituting the natural dissolved organic matter (DOM) (Hansell, 2013) makes its reproduction in laboratory conditions challenging. To tackle this difficulty, representative DOM sources, such as leachates obtained from representative carbon sources (e.g, plant litter or leaves) or artificially created DOM substrates with contrasting chemical reactivities (e.g., monomers vs oligomers) have been applied in earlier studies to assess the effect of several DOM pools on the microbial community composition and activity (Lechtenfeld et al., 2015; D'Andrilli et al., 2019; Morán et al., 2020).

In this study, we propose a method to improve the control of initial microbial communities of experiments through the cryopreservation and resuscitation of natural aquatic microbial assemblages in either sterile filtered seawater or artificial seawater with DOM supplements that were prepared from the particulate organic fraction of seawater (Figure 1). The method was tested on communities harvested from a coastal station and a closely located coastal lagoon on the Northwest coast of the Mediterranean Sea, which have contrasting trophic status and environmental variability. More specifically we assessed the (i) impact of inoculum size on the resuscitation and (ii) to which extent diversity and community composition can be maintained according to inoculum characteristics and culture medium. We further evaluated the effect of (iii) handling time during preparation of community aliquots and (iv) storage time of the cryopreserved aliquots on the community composition of the resuscitated communities.

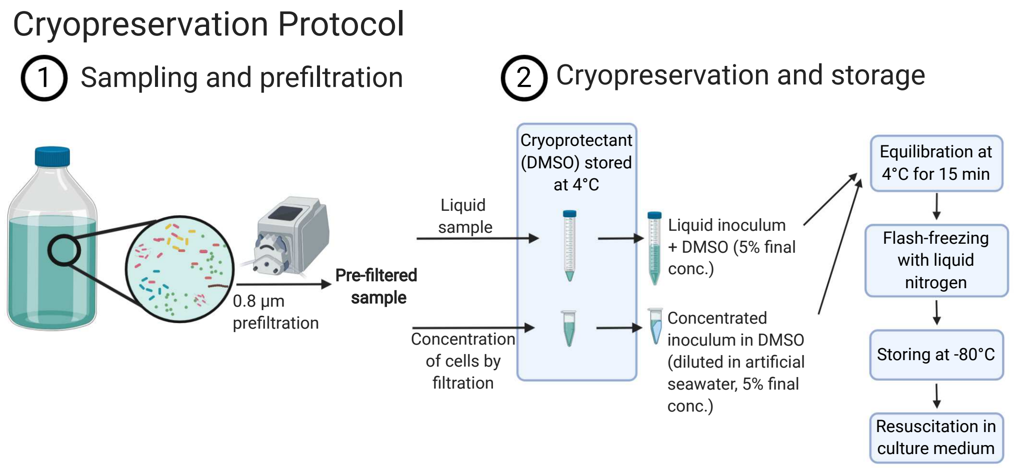

Figure 1. *Schema displaying the cryopreservation procedure (created with BioRender.com).*

MATERIALS AND METHODS

Sampling locations

Prokaryotic communities were sampled from the SOLA coastal station in the northwest Mediterranean Sea and the Canet Lagoon. Both sites are located on the southern coast of the Gulf of Lyon in the region of Occitanie, South France (Figure 2A, Table 1). These two locations were chosen because of their contrasting environmental conditions: SOLA represents an oligotrophic site with Chlorophyll-a (Chl-a) concentrations varying between 0.01-4.39 µg L^{-1} with rather stable salinity concentrations (33.20-39.99 PSU) and Canet is a shallow coastal Lagoon characterized by high productivity and large-scale salinity changes (1.09-125.76 µg L^{-1} Chl-a, 2.20- 43.00 PSU; Figure 2B, C) over the year.

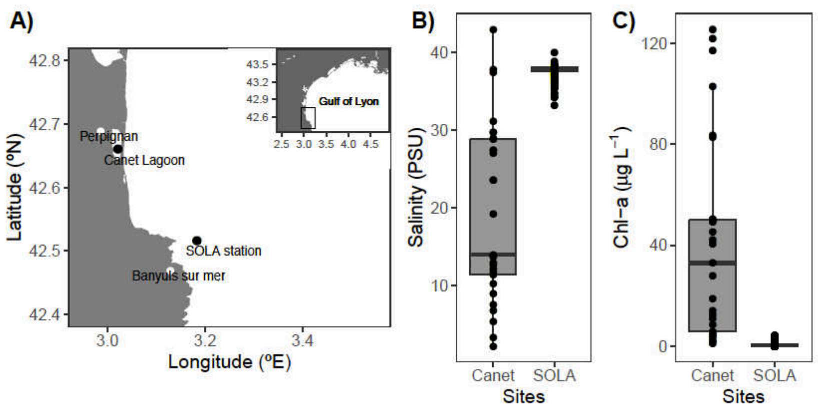

Figure 2. *Sampling sites and representative environmental conditions. A) Sampling location of the SOLA coastal station and the Canet Lagoon. B) Salinity (PSU) and C) Chlorophyll-a (µg L^{-1}) for the Canet lagoon and SOLA station. Salinity and chlorophyll data for SOLA were provided by the SOMLIT program (http://somlit-db.epoc.u-bordeaux1.fr/bdd.php; 03/05/2005-30/10/2018, n=595). Salinity and chlorophyll data for the Canet lagoon were extracted from the IFREMER Surval service (https://wwz.ifremer.fr/surval/, 26/06/2001-07/09/2006, n=87.*

Dissolved organic matter (DOM) supplements that served as substrates for the resuscitated communities were obtained from either the SOLA sample site or from the Warnow River Mouth into the Baltic Sea, in Warnemünde, Germany (Table 1), as detailed below. The Warnow River Mouth has a salinity range of 5–18 PSU due to the mixing with water masses from the Baltic Sea (Schernewski et al., 2019). The Warnow estuary is highly productive due to terrestrial loads, with Chl-a values ranging between 18-65 µg L^{-1} (Freese et al., 2007).

Table 1. *Overview of sampling locations and dates.*

Location	Sampling type	Latitude (°N)	Longitude (°E)	Sampling date
SOLA	Community[a] / DOM[b]	42° 29.0'	03° 08.7'	03/07/2018, 18/02/2020
Canet	Community	42° 40.0'	03° 01.0'	07/08/2018
Warnow	DOM	54°10.2’	12°06.3’	10/08/2017

[a] Community = Samples for cryopreserved communities.
[b] DOM = Samples for DOM extracts.

Preparation of culture media and dissolved organic matter (DOM) supplements

Either sterile filtered water from the SOLA station, hereafter F-SEA, filtered through 0.22 µm, 47 mm cellulose filter (Millipore, Massachusetts, USA) or inorganic artificial seawater basic medium (ASW, Eguchi et al., 1996) supplemented with DOM were used as medium. Besides the DOM supplements, no further carbon, nitrogen, phosphorus, or vitamins were added to the ASW medium. Trace metals, Fe and EDTA were added 100 times less concentrated than in the ASW original recipe (Eguchi et al., 1996), with final salinity of 35 g L^{-1} and a pH of 8. For the preparation of DOM supplements, water from the SOLA station and the Warnow river were sampled in 25 L polycarbonate carboys previously rinsed with HCl 10% and Milli-Q water. A total of 65 L and 13.6 L of the Warnow and SOLA water, respectively, were filtered through 0.22 µm Cellulose Acetate filters (OE 66, 142 mm, Whatman, Buckinghamshire, UK) to retain bacteria and all particles in larger size classes. These filters were then stored frozen (-20°C) until further processing.

To prepare the DOM used as supplement, the filters were ground in a stainless-steel mortar and pestle (Bel-Art™, Fisher Scientific, Strasbourg, France) while immersed in liquid nitrogen. Next, the filter pieces were resuspended in a known volume of ultrapure water (60 mL) and autoclaved at 120°C for 20 min. Subsequently, the sterile suspensions containing the filter pieces were filtered through pre-combusted (450°C, 6 h) GF/F 47 mm filters (Whatman, Buckinghamshire, UK) to remove particles. Finally, the obtained DOM supplements were stored frozen at -20°C until use.

For this study, two kinds of DOM supplements were used: one containing exclusively DOM from SOLA (hereafter S-DOM) and the second containing mixed DOM from SOLA and the Warnow River (hereafter SW-DOM). S-DOM obtained from the equivalent of 0.1 L of sample water was added to 1 L of ASW medium, while DOM supplements from the equivalent of 0.05 L from the sample water obtained from SOLA and 0.17 L from the Warnow River were added to 1 L ASW medium in the SW-DOM medium.

Chemical characterization of DOM supplements

To characterize the DOM supplements, the concentration of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), dissolved organic phosphorous (DOP), and nutrients (NO_3^-, NO_2^-, PO_4^{3-}) were determined. Aliquots of the water samples and DOM concentrates were filtered through 2 pre-combusted (450°C, 6h) 25 mm GF/F filters (pore size 0.7 µm, Whatman, Buckinghamshire, UK). Aliquots for DOC measurements (10 mL) were stored in combusted glass ampoules and acidified (85% H_3PO_4, final pH = 2). The ampoules were closed and maintained at room temperature until analyzed on a Shimadzu TOC-V following a protocol published elsewhere (Cauwet, 1994). Aliquots for DON and DOP (20 mL) were collected in Teflon bottles and, stored at -20°C. DON and DOP were simultaneously determined using the wet oxidation method (Pujo-Pay and Raimbault, 1994). Nitrate, (NO_3^-), nitrite (NO_2^-), and phosphate (PO_4^{3-}) were determined by standard colorimetric technique (Bran Luebbe autoanalyzer) (Aminot and Kérouel, 2007).

Cryopreservation of natural prokaryotic communities

We have adapted the cryopreservation procedure developed by Vekeman and Heylen (2017) for the cryopreservation of single strains or mixed cultures to cryopreserve natural aquatic communities. This previously published protocol had been applied by the authors exclusively to laboratory cell cultures grown in high cell densities (>10^8 cells/mL, Vekeman and Heylen, 2017) while cell densities in marine bacterial communities range typically from 10^5 to 10^6 cell per mL (Kirchman, 2008). For this reason, but also to minimize the carryover of substrates to new culture media, a step to concentrate the cells present in aquatic samples before cryopreservation was added.

Previous to the cryopreservation protocol and in order to remove potentially present bacterivorous protists, which may also be cryopreserved in DMSO (Supplementary Figure S1; Liu et al., 2009), the sample water was pre-filtered using 0.8 µm pore size filters (47 mm mixed cellulose esters, Millipore, Massachusetts, USA). We have performed this prefiltration step as it is a common practice in experimental work with natural aquatic microbial assemblies, to avoid protist grazing during incubations (e.g., Giorgio et al., 1996; Beardsley et al., 2003; Szekely et al., 2013; Kujawinski et al., 2016). However, studies with a focus only on the first incubation days before protists start to proliferate (approximately 4 days after prokaryotes start to proliferate, Fig. S1), or that are particularly interested in incubations containing protists, may skip the prefiltration step.

Between 0.1 and 1.5 L of pre-filtered water was subsequently filtered through 0.22 µm filter (hydrophilic PVDF, 25 mm filter, Millipore, Massachusetts, USA) using a vacuum pump (~600 mg Hg) in order to concentrate prokaryotic cells. Drying of the filters was carefully avoided during the filtration, except at its end, when the pressure was set to a minimum, to prevent cell damage, and the final water volume covering the filter was slowly filtered. Immediately after removal of the sample water, the pump was turned off and 0.5 mL of inorganic ASW medium was added to the top of the filter, which was then used to resuspend the cells from the filter by pipetting up and down. This volume was subsequently transferred into previously prepared 2 mL tubes with 0.5 mL cryoprotectant containing 10% (v/v) sterile-filtered DMSO (ACS reagent, ≥99.9%, Sigma-Aldrich, Missouri, USA) in inorganic ASW, equilibrated at 4°C (DMSO final concentration: 5%). Next, the filters were completely immersed into the medium with cryoprotectant and resuspend cells and the tubes were kept at 4°C for 15 min for equilibration (Vekeman and Heylen, 2017). The tubes were then flash-frozen in liquid nitrogen and transferred to -80°C for long-term storage.

We additionally prepared 10 mL aliquots of 0.8 µm pre-filtered water samples without prior cell concentration via filtration. Sterile-filtered DMSO was added as cryoprotectant (5% final concentration). Then, the aliquots were kept for 15 min at 4°C before flash-freezing in liquid nitrogen and transfer to -80°C for long-term storage (Figure 1; for a step-by-step protocol see Supplementary Text S1).

The abundance of prokaryotic cells in the 0.8 µm pre-filtered inoculum water of SOLA and Canet was estimated via flow cytometry as detailed elsewhere (Marie et al., 2000). In short, aliquots (1350 µL) were fixed with glutaraldehyde (0.1% final concentration) and stored at -80°C until analysis. Samples were analyzed using either the cytometer FACSCanto II (BD

Biosciences, New Jersey, USA) or the Cytoflex Flow Cytometer (Beckman Coulter, California, USA) (Table 2).

Additionally to the preparation of filters with cryopreserved communities, 1.5 L of the 0.8 µm (47 mm mixed cellulose esters, Millipore, Massachusetts, USA) pre-filtered water from both locations was filtered onto 0.22 µm filters (47 mm cellulose filters, Millipore, Massachusetts, USA) for later DNA extraction and 16s rRNA gene sequencing to characterize the initial communities.

Resuscitation of natural prokaryotic communities and experimental designs

To resuscitate the cryopreserved communities, the cryopreserved samples were thawed at room temperature (~30 min) and then added (together with the filter where applicable) to the culture medium. The cells were incubated for 5-6 days close to the *in-situ* temperature (15-18°C, Table 2) in glass bottles under agitation with magnetic stirrers. In total, four experiments were performed to test and evaluate the cryopreservation and resuscitation of the natural aquatic prokaryote communities (Table 2). In all resuscitation experiments, cell numbers were monitored daily via flow cytometry as detailed above.

Table 2. *Overview of the performed experiments*

ID experiment	Description	Inoculum source	Inoculum size (10^6 cells)	Temperature (°C)	Growth medium	Inc. time (days)	Variables analyzed
1a	Effect of the Inoculum size (filters)	SOLA	66, 132, 330, 495, 990	15	S-DOM	6	Microbial abundance [b]
1b	Effect of the inoculum size (water aliquot)	SOLA	1.32, 3.30, 6.60, 9.90	15	S-DOM	6	Microbial abundance [b]
2	Similarity between resuscitated and original communities (filters)	SOLA, Canet	SOLA: 412, Canet: 804	18	F-SEA, S-DOM, SW-DOM	5	Microbial abundance [a], Community composition (16S rRNA sequencing)
3	Handling time (filters)	SOLA	412	18	S-DOM	6	Microbial abundance [a], Community composition (ARISA fingerprinting)
4	Storage time (filters)	SOLA	412	18	S-DOM	5	Microbial abundance [b], Community composition (ARISA fingerprinting)

[a] Flow cytometer FACSCanto II (BD Biosciences)
[b] Flow cytometer Cytoflex (Beckman Coulter)

In the first experiment, we assessed the effect of initial cell numbers on cell growth after resuscitation, including both cryopreserved filter-concentrated inocula (experiment 1a), and cryopreserved water aliquots from 0.8 µm pre-filtered SOLA seawater (experiment 1b). For experiment 1a, filters containing concentrated cryopreserved communities were prepared from 100, 200, 500, 750, and 1500 mL 0.8 µm pre-filtered SOLA seawater. These inocula contained, according to the results from flow cytometry analyses, 66, 132, 330, 495, and 990 million cells in total. Triplicate cryopreserved communities of each volume were resuscitated in specific volumes of S-DOM medium (60, 120, 300, 450, and 900 mL, respectively) in order to ensure constant initial cell abundance in the incubations (1.1×10^6 cells mL^{-1}). For experiment 1b, cryopreserved water aliquots (2, 5, 10, and 15 mL) from the 0.8 µm-prefiltered SOLA seawater were diluted 10-fold and resuscitated in triplicates in S-DOM medium (initial abundance of 0.07×10^6 cells mL^{-1}). The starting inocula in this experiment contained 1.32, 3.30, 6.60, and 9.90 million cells, respectively. The resuscitated cells in both experiments were incubated for 6 days (Table 2). Since larger inocula resulted in improved similarity among resuscitation replicates, all downstream experiments were performed using filter-concentrated inocula containing $\geq 412 \times 10^6$ cells.

Next, experiment 2 was performed with the aim to evaluate the similarity of resuscitated communities to the initial starting communities in dependence of the growth medium and inoculum source. Cryopreserved filter-concentrated inocula from SOLA (4.12×10^8 cells) and Canet (8.04×10^8 cells) were incubated in 900 mL medium in 1 L Duran Schott bottles. Triplicate SOLA inocula were incubated in F-SEA, ASW supplemented with S-DOM extract, and ASW supplemented with SW-DOM extract. Triplicate Canet inocula were incubated only in ASW supplemented with S-DOM or SW-DOM. The water of the F-SEA treatment was prepared at the same day as the cryopreserved SOLA community and inoculated on the evening of that same day after the communities had been cryopreserved for a few hours. This was done to avoid or minimize chemical changes in the F-SEA medium that could occur during prolonged storage time. The incubation of SOLA communities in F-SEA was performed to test the resuscitation procedure in an environment as close as possible to the natural environment of the initial SOLA community. Incubations in the S-DOM and SW-DOM media differed in their C:N:P ratio (Table 3) and represent media with different trophic status. Not surprisingly, the C:N:P ratios of S-DOM and SW-DOM were similar to the C:N:P ratios of cell contents reported in previous reports (45:9:1; Goldman et al., 1987), while F-SEA was more depleted in both nitrogen and phosphorous. The communities reached stationary phase on the fifth day, when they were collected onto 0.22 µm filters (Millipore, Massachusetts, USA) for later DNA extraction and 16s rRNA gene amplicon sequencing.

Table 3. *Characterization of DOM extracts used as substrates in the resuscitation experiments.*

	F-SEA (µmol L^{-1})	S-DOM (µmol L^{-1})	SW-DOM (µmol L^{-1})
DOC	78.20[a]	0.708	1.952
DON	6.52[b]	0.046	0.524
DOP	0.07[b]	0.011	0.071
C:N:P	1050:84:1	60:4:1	25:7:1

[a] Concentration corresponds to July 2013. Data provided by Sánchez-Pérez et al., 2020.
[b] Estimation based on the Redfield ratio described by Pujo-Pay et al., 2011 for the western surface Mediterranean waters.

The impact of the handling time to prepare cryopreserved community aliquots and the impact of the storage time of these aliquots on the resuscitated communities were tested in experiments 3 and 4, respectively.

In experiment 3, a set of triplicate filter-concentrated inocula from SOLA was cryopreserved immediately after the pre-filtration step and a further set of that same community was cryopreserved 7.5 hours later. Cryopreserved communities from both sets (4.12 x10^8 cells) were resuscitated in 900 mL of S-DOM medium. In experiment 4, three sets of cryopreserved filter-concentrated communities from SOLA (4.12 x10^8 cells) were resuscitated after cryopreservation for 1.5, 6 and 12 months. The resuscitation was performed in 900 mL S-DOM medium in 1 L Duran Schott bottles. In both experiments the communities were incubated for 5-6 days (Table 2), before they were collected onto 0.22 µm pore size filters (Millipore, Massachusetts, USA) for later DNA extraction and 16s rRNA gene fingerprinting via Automated Ribosomal Intergenic Spacer Analysis (ARISA) (Cardinale et al., 2004).

DNA extraction

Filters for DNA extractions were stored at -30°C. DNA extraction was performed after cutting the filters in small pieces and using a modified protocol of the QIAmp DNA Mini Kit (QIAGEN, Hilden, Germany) with an initial bead-beating step in ATL buffer using a FastPrep-24 ™ 5G (MP Biomedical, California, USA). Concentration and quality of the eluted DNA were tested by electrophoresis (1% agarose gel) and Quantus fluorometer using the PicoGreen assay (Promega, Wisconsin, USA).

16s rRNA gene amplicon sequencing and bioinformatic evaluation

DNA samples from experiment 2 from the initial 0.8 µm pre-filtered SOLA and Canet communities were sent for 16s rRNA gene amplicon sequencing (300 base pairs paired-end read, Illumina Miseq V3) using the primers pair 515yf-926r (Parada et al., 2016). For the SOLA and Canet initial communities, only one DNA extract from each was available and we performed triplicate library preparation in order to obtain replicates that reflect the technical variance introduced during library preparation and sequencing. Demultiplexing of libraries via the Illumina bcl2fastq 2.17.1.14 software, removal of reads with length <100 bases, as well as primer clipping (up to 3 mismatches per primer), were performed by the sequencing company (LGC Genomics GmbH). Further processing of the sequence reads was done in R using the DADA2 pipeline (Callahan et al., 2016) applying settings given in the instructions (https://benjjneb.github.io/dada2/tutorial.html), with some modifications (R-code is available in https://github.com/angelrainf/gesifus.cryo.dada2). A total of 700 amplicon sequence variants (ASVs) were identified across all sequenced samples and the obtained ASVs were taxonomically assigned using the Genome Taxonomy Database (GTDB) (Parks et al., 2018). Raw sequence data are available at European Nucleotide Archive under the accession number PRJEB38947.

ARISA fingerprinting

Differences in the community composition of incubations in experiments 3 and 4 were estimated using ARISA analysis. Extracted DNA from the communities was amplified with KAPA2G Fast Ready Mix (Sigma-Aldrich, Missouri, USA) in 20 µl reactions using 10 µM of the primers 1406F and 23SRY (Frossard et al., 2012) and 1 µl of DNA template. Reactions mixtures were initially held at 94°C for 3 min, followed by 35 amplification cycles of 94°C for 15 s, 55 °C for 15 s, and 72°C for 30 s, with a final extension of 72°C for 1 minute.

PCR products were subsequently purified through Sephadex G-50 fine (Sigma-Aldrich, Missouri, USA) and quantified with a Quantus fluorometer with the Picogreen assay (Promega, Wisconsin, USA). Then, 2.5 µL of purified PCR products were mixed with 0.2 µL of MapMarker1000 (Bioventures, Tennessee, USA) and 15 µL of formamide (Sigma-Aldrich, Missouri, USA) and, denatured at 95°C for 3 minutes. Samples were then sequenced in a Sequencer 3130XL (Applied Biosystems®, California, USA).

Terminal restriction fragments (T-RFs) were identified using the GeneMapper software (Applied Biosystems®, California, USA) and the areas of the identified T-RF peaks were imported into the T-Rex (T-RFLP analysis EXpedited) online software for noise filtering, alignment, and binning by defaults parameters (Culman et al., 2009). ARISA data were normalized by dividing the area of the individual peak by the total peak area of the sample.

Statistical analysis and data acquisition

All statistical analysis were performed using the R platform (R Core Team, 2018) employing the packages vegan (Oksanen et al., 2019), lme4 (Bates et al., 2015), and lmerTest (Kuznetsova et al., 2017). Sample variation descriptors given after mean values correspond to the standard deviation if not otherwise specified.

For describing the growth curves during incubations, carrying capacity was estimated by fitting a logistic growth equation (García et al., 2018). To test the impact of inoculum size on the variability of cell growth after resuscitation (experiment 1a-b), we estimated the variance and the coefficient of variation (CV) of the cell abundances of resuscitated inocula after 4,5, and 6 days of incubation. We fitted a mixed linear model for both the variance and the CV, using the incubation day as random factors and the inoculum sizes as fixed factors. The CV of cell abundance was estimated for experiments 2, 3, and 4 from the time point with maximum abundance. We further compared the variability of cell growth among simultaneously performed triplicate incubations in our study with triplicate incubations of diluted, non-cryopreserved natural aquatic microbial communities published elsewhere (Morán et al., 2020). For this purpose, CV values from the latter incubations were estimated from digitalized figures in Morán et al., 2020 by the Engauge Digitizer Program (Mitchell et al., 2019).

In experiment 2, we compared initial communities of SOLA and Canet with resuscitated communities after several days of incubation. However, while triplicate values in the initial communities represent technical replicates, triplicates after resuscitation represent biological replicates with presumably larger variance. Differences in variance among compared datasets violate assumptions for two-sample t-tests. We accordingly used a one-sample t-test (two-tailed) to statistically compare the Shannon diversity between initial and resuscitated communities. For this purpose, we used the mean Shannon diversity of the technical replicates from the initial community as a reference value and we subtracted Shannon diversities from the triplicate incubations from this mean. The one-sample t-test was then applied to test the null hypothesis that the difference in diversity between the initial sample used as inoculum and the incubations did not differ from zero.

To assess and subsequently visualize the similarity of resuscitated communities to the initial starting communities in dependence of the growth medium and inoculum (experiment 2), pairwise Bray-Curtis distance indices between all communities were calculated. This was done based on the relative abundance of ASVs in each community obtained after rarefication to the minimum number of processed reads obtained for the individual libraries (Canet initial, 13200 reads, Supplementary Table S1).

We further evaluated the number and proportion of initially abundant ASVs (>0.5% relative abundance in the initial pre-filtered samples of SOLA and Canet) that exhibited cell growth and that were thus viable during the incubations. In this analysis, we compared absolute abundance estimates of individual ASVs in the initial communities with estimates obtained from the resuscitated communities. Absolute abundances of individual ASVs were calculated by multiplying the relative abundances of each ASV by the total cell counts of the communities from which they were identified. Because of the biases during DNA extraction and/or amplification, this is not a proper estimation of the absolute cell numbers of individual ASVs within a community (Andersson et al., 2010). However, the dynamics of absolute cell numbers, such as the fold change and, thus, the cell growth of specific ASVs between two communities can be approximated because the biases among compared samples are similar (Props et al., 2017; Ward et al., 2017; Shen et al., 2018a).

Log2-fold-change (L2FC) of the absolute abundances of each ASV exceeding 0.5% relative abundance in the initial sample between the incubation start (day 0) and the final incubation day were used to estimate their growth in each incubation. Positive L2FC values indicate that the tested ASV exhibited more cells in the incubation medium after five days than in the starting community, implying that cells affiliating with this ASV were actively replicating.

To statistically evaluate the growth of individual ASVs originating from the SOLA and Canet inocula after the cryopreservation and resuscitation in the different media, we applied similarly as above a one-sample t-tests (two-tailed) to the replicate L2FC values of each AVS per treatment (SOLA: F-SEA, S-DOM, SW-DOM; Canet: S-DOM, SW-DOM). In the F-SEA incubations, we omitted one of the three replicates from the t-test, where the community was different from the other two replicates due to the overgrow of members of the Enterobacterales order. ASVs with (significantly) positive L2FC in at least one medium were considered as viable. P-values were adjusted with the Benjamini-Hochberg correction to account for false discovery rates during multiple comparisons (Benjamini and Hochberg, 1995).

To visualize the effect of handling and storage time, Hierarchical Clustering (Ward.D2) was performed on the normalized ARISA community fingerprint data based on Bray-Curtis distances from the incubations obtained in experiments 3 and 4. Analyses of similarities (ANOSIM, permutations=999) were performed to evaluate the effect of the handling and storage time. In the case of the storage time analysis, an additional post hoc pairwise ANOSIM was performed between each of the three tested storage time followed by a Bonferroni correction for multiple comparisons. For the samples of experiment 4 (storage time), we furthermore applied a Mantel test between the Bray-Curtis distance matrix and the Euclidean distance matrix of storage time to evaluate the effect of storage time on the dissimilarity among samples.

RESULTS

Effect of the inoculum size on the resuscitation procedure (experiment 1a/b)

The resuscitation and subsequent incubation of cryopreserved inocula in S-DOM media succeeded independently from the inoculum type (concentrated by filtration or sample water) and inoculum size (Figure 3A,B). Both inoculum types displayed longer lag-phases (three days) for the largest inocula, while the lag phase did not exceed two days in all remaining treatments. Furthermore, the carrying capacity of all incubations surpassed the cell numbers detected in the initial 0.8 µm pre-filtered SOLA water ($0.66 \pm 0.05 \times 10^6$ cell mL^{-1}), and the difference was generally greater in incubations with smaller inoculum, except for the smallest inoculum in experiment 1a (Table 4).

We observed for both inoculum types that the variance among replicates tended to decrease with increasing inoculum size (Figure 3C,D). Moreover, we observed a significant decrease in the CV for cryopreserved filter inocula. In contrast, water inocula incubations displayed a trend for the CV to increase with larger inocula (Figure 3E,F, Table 4).

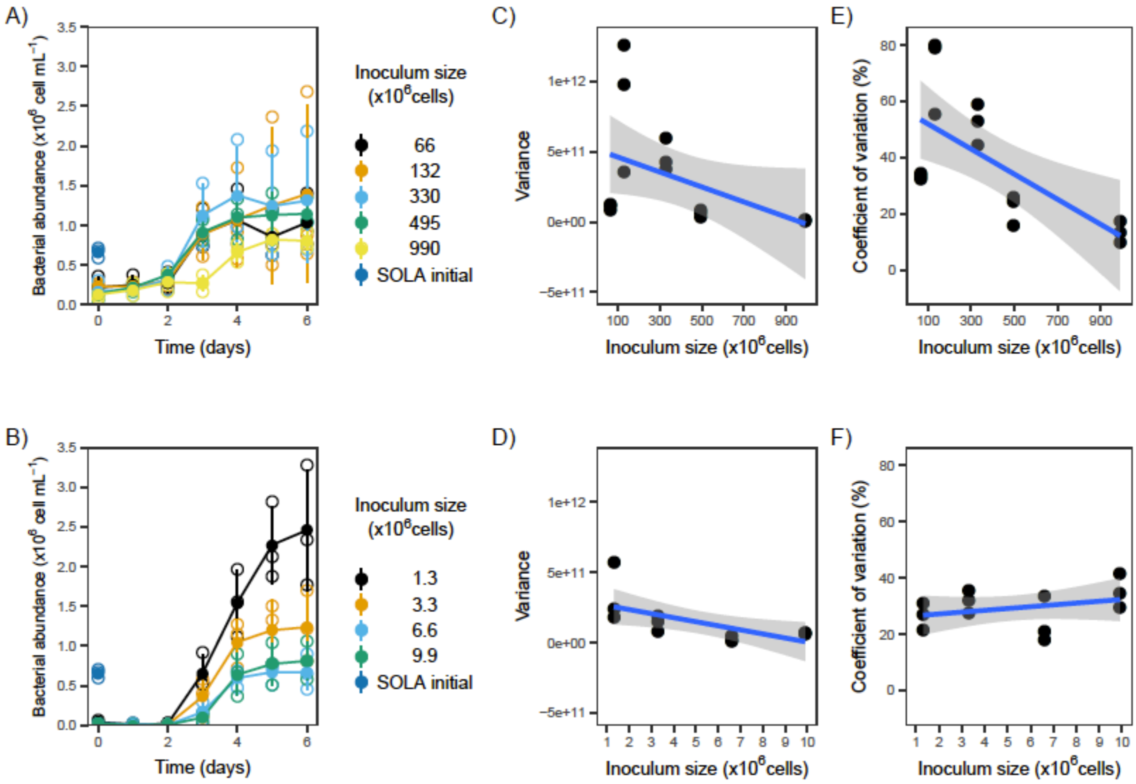

Figure 3. Growth and variability of resuscitated communities from experiment 1a (filter concentrated inocula; upper panels) and 1b (water inocula; lower panels). *A, B) Growth curves. Empty circles represented the individual replicates, while filled circles represent the replicates mean (n=3). C, D) Variance, and E, F) coefficient of variation for bacterial cells detected after 4, 5 and 6 incubation days in dependence on the inoculum size in triplicate incubations.*

Table 4. *Carrying capacities and statistical parameters for experiment 1.*

Experiment	Inoculum size (10^6 cells)	Carrying capacity (x10^6 cell mL^{-1})	Mixed linear model		
			Variable	Slope[a]	P
1a) Filter-concentrated inocula	66	0.99 ± 0.31	Variance	-5.35 x10^8 (± 2.77 x10^8)	0.081
	132	1.51 ± 1.27			
	330	1.34 ± 0.68	CV	-0.04 (± 0.01)	0.025
	495	1.17 ± 0.25			
	990	0.99 ± 0.14			
1b) Water inocula	1.32	2.49 ± 0.68	Variance	-2.89 x10^{10} (± 1.42 x10^{10})	0.168
	3.3	1.22 ± 0.42			
	6.6	0.67 ± 0.17	CV	0.65 (± 0.63)	0.355
	9.9	0.80 ± 0.25			

[a] Numbers in brackets indicate the standard error.

Community similarity of initial and resuscitated communities in dependence of media and inoculum source (experiment 2)

Cryopreserved communities from the SOLA field station and Canet that were resuscitated in experiment 2 exhibited growth in all tested media (F-SEA, S-DOM, and SW-DOM, Fig. 4). The carrying capacity of SOLA resuscitated communities in F-SEA was highly variable (0.93 ± 0.42 x10^{6} cell mL^{-1}) compared to the S-DOM (0.80 ± 0.26 x10^{6} cell mL^{-1}) and SW-DOM (0.43 ± 0.13 x10^{6} cell mL^{-1}), a difference caused by one of the F-SEA triplicate incubations reaching much higher abundances than the other two replicates (Figure 4A). The incubations of Canet cryopreserved communities exhibited carrying capacities of 1.05 ± 0.07 x10^{6} cell mL^{-1} and 0.92 ± 0.29 x10^{6} cell mL^{-1} in S-DOM and SW-DOM medium, respectively, with larger differences among the replicates in the SW-DOM medium (Figure 4B). Lag-phases lasting for three days were observed in the resuscitated SOLA communities incubated in the S-DOM or SW-DOM media, while lag-phases were neither detected for the resuscitated SOLA communities growing in the F-SEA medium nor for the resuscitated Canet communities growing in both tested media (Figure 4).

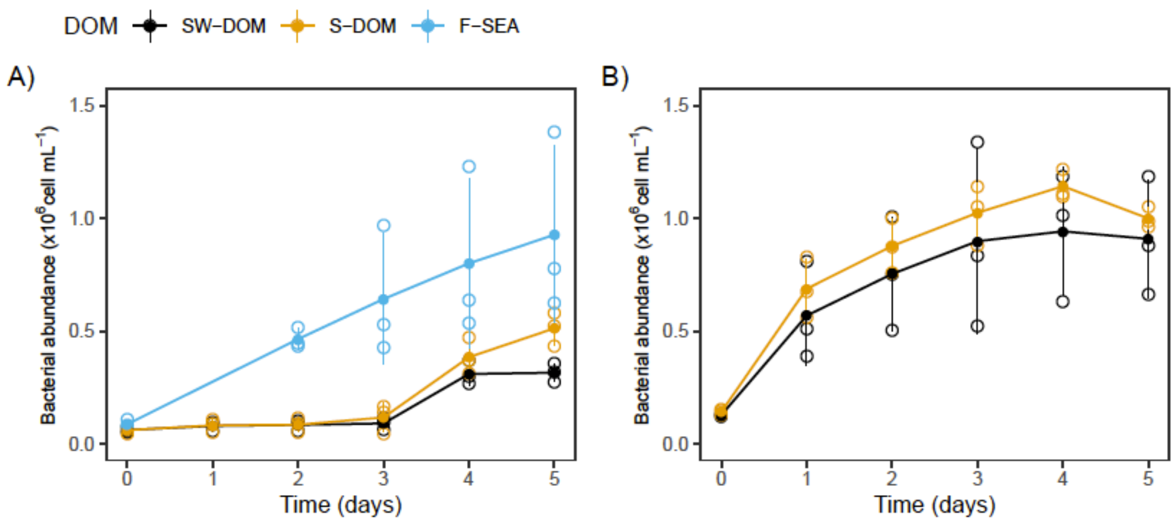

*Figure 4. **Growth of resuscitated communities incubated in different media from experiment 2.** Growth curves of resuscitated communities originating from A) SOLA, and B) the Canet lagoon. Error bars represent standard deviation among replicates (n=3). Empty circles illustrate the individual replicates, while filled circles represent the mean of triplicate incubations.*

The ASV composition of the F-SEA treatment was moderately similar to the initial SOLA community in two replicates (average Bray-Curtis dissimilarity: 0.62 ± 0.08, Figure 5A), while the replicate characterized by high cell numbers differed at a higher degree (Bray-Curtis dissimilarity: 0.93, Figure 5A). Also, S-DOM and SW-DOM treatments resulted in high dissimilarity relative to the initial community (average Bray-Curtis dissimilarity: 0.96 ± 0.01 and 0.92 ± 0.02, respectively, Figure 5A). In contrast, resuscitated Canet communities exhibited comparatively high similarity to the initial Canet community regardless of the growth medium used, with an average Bray-Curtis dissimilarity of 0.50 ± 0.04 and 0.52 ± 0.01 for S-DOM and SW-DOM, respectively (Figure 5A), even though the DOM supplements did not originate from the Canet Lagoon (S-DOM, SW-DOM).

There were considerable similarities between the community composition of two replicates of the F-SEA communities, at the order level, and the initial SOLA community (Figure 5A,B). However, members of the Pelagibacterales, which represented approximately 34% of all ASVs in the initial SOLA community, were particularly underrepresented in these two F-SEA incubations (~15%). Likewise, members of the orders Balneolales, Puniceispirillales, and SAR86 clade that represented between 7 and 13% decreased to 1.6 – 2.7%. Conversely, members of the orders Flavobacteriales, Rhodobacterales, and, Pseudomonadales were overrepresented in these two F-SEA incubations (Figure 5B). Members of the Enterobacterales, mostly affiliated with the genus Alteromonas (Supplementary Table S2), displayed low abundance in the initial SOLA community (~0.03%) but increased substantially and even dominated in one of the incubations (Figure 5B).

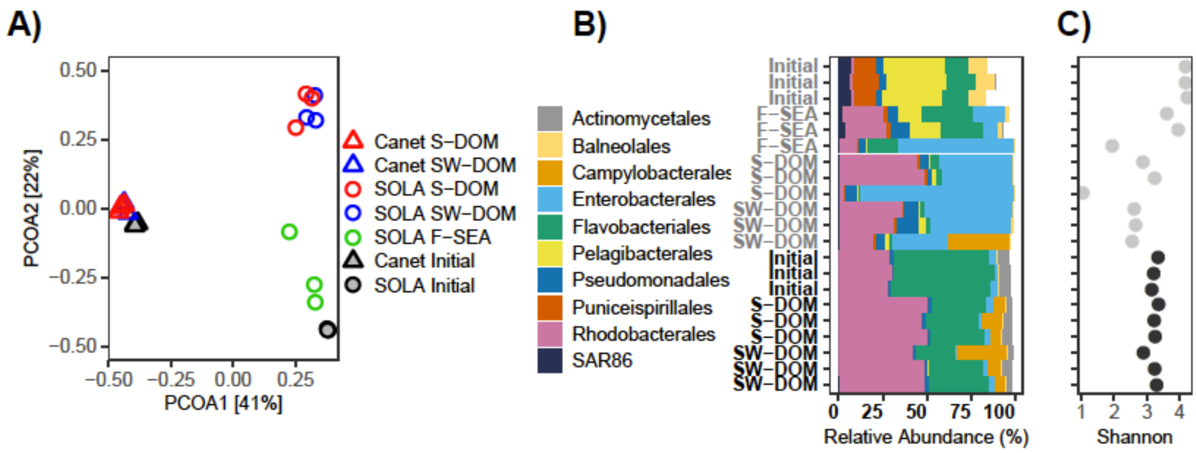

Figure 5. Initial and resuscitated microbial assemblies from experiment 2. *A) PCoA biplot displaying the community similarities (based on ASVs) among the initial and resuscitated communities in different media. B) Relative contribution of the 10 most abundant orders in the initial communities as well as in the resuscitated communities after 5 days incubation in different media, and C) Shannon diversity indices. The panels B and C share the same y-axis labels.*

The trends in compositional change at the order level described here for the two F-SEA incubations with high similarity to the initial SOLA samples were also observed, at a more pronounced level, for the SOLA community incubated in S-DOM or SW-DOM media, as well as for one of the F-SEA incubations. In one of the SW-DOM incubations, we additionally detected members of the order Campylobacterales that were not detected in any of the other SOLA communities. The detection of Campylobacterales in a single SOLA SW-DOM incubation may have resulted from contamination from the Canet communities, where Campylobacterales were abundant.

Resuscitated Canet communities remarkably resembled the initial Canet community at the order level, regardless of the medium used (Figure 5C). Members of Actinomycetales and Flavobacteriales orders, which represented around 6 to 56 % of all the ASVs in the initial community, decreased to ~3 and 30% in the resuscitated S-DOM and SW-DOM incubations, respectively. In contrast, Rhodobacterales increased their abundances in all the resuscitated treatments, from ~29% in the initial Canet community to ~48%. Strikingly, Campylobacterales members that affiliated exclusively with the genus Arcobacter (Supplementary Table S2) were present in low abundances in the initial Canet community (~0.7%) and increased to up to 28% abundance in the resuscitated communities. Members of the Enterobacterales order were similarly represented in both the initial and resuscitated communities (~3%).

SOLA communities resuscitated in the F-SEA medium exhibited two incubations with Shannon entropy values that were very similar to those estimated for the initial SOLA community (One sample t-test, $P=0.238$, Figure 5C). For resuscitation of SOLA inocula in S-DOM, no significant differences relative to the initial SOLA community Shannon diversity were detected (One sample t-test, $P=0.113$). On the other hand, the resuscitation of SOLA inocula in SW-DOM resulted in a significant decrease in Shannon diversity compared to the initial SOLA community (One sample t-test, $P<0.001$, Figure 5C). Similar levels of Shannon diversity were found in all resuscitated Canet inocula in comparison to the initial Canet community (One sample t-test, $P=0.489$, and $P=0.591$ for S-DOM and SW-DOM, respectively, Figure 5C).

Growth analyses of the initially abundant SOLA ASVs in the resuscitated communities revealed that at least 45% (One sample t-test, adjusted P-value cutoff: 0.1) were viable (Figure 6A). Among ASVs that were initially abundant in the Canet community, at least 72% (One sample t-test, adjusted P-value cutoff 0.1) were viable (Figure 6B). ASVs in the SOLA communities for which no active growth could be verified included representatives of the class

Puniceispirillales as well as members of the orders Flavobacteriales, Balneolales, the SAR86 clade, and members of the class Alphaproteobacteria (Figure 6C). In the Canet resuscitations, ASVs affiliating with the Flavobacteriales order did not exhibit detectable growth (Figure 6D).

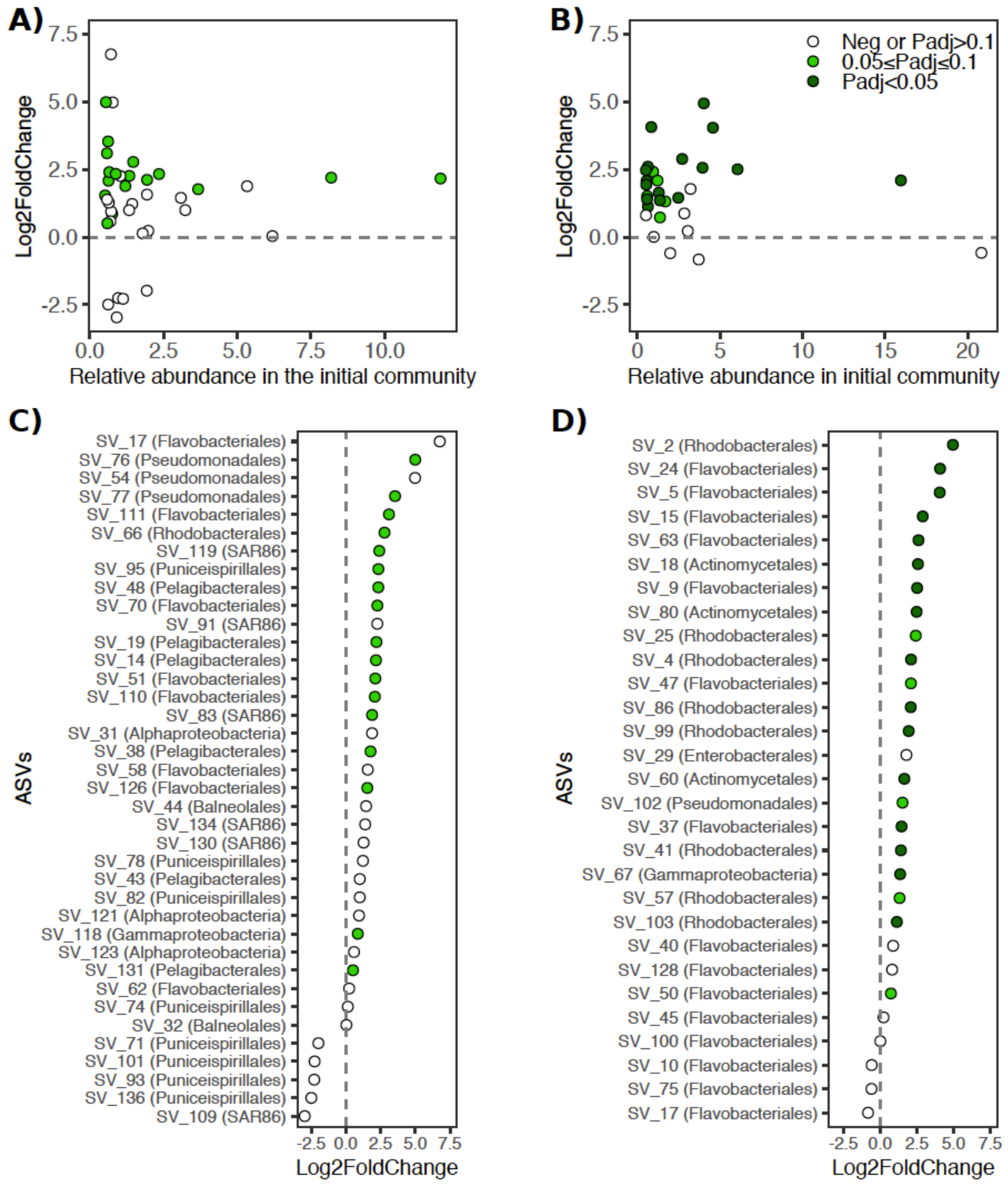

Figure 6. L2FC (Log2-fold-changes) of absolute abundances of ASVs (> 0.5% relative abundance in the initial inoculum) between the initial inoculum the final incubation day. *L2FC values of ASVs against their relative abundances in the initial sample from A) SOLA and B) Canet, respectively. Ranked L2FC values of ASVs and the assigned taxonomy at the order level if available or otherwise at the class level for C) SOLA and D) Canet, respectively. For each ASV only the L2FC in one medium is displayed: we selected for each ASV among the media with positive L2FC the one exhibiting the highest statistical support for growth (lowest P-value). For ASVs that exhibited in all media exclusively negative L2FC values we displayed the one with the lowest p-value. Negative L2FC values or positive L2FC values with Padj > 0.1 are displayed in empty circles. Positive L2FC values with 0.05 ≤Padj < 0.1 are displayed in light green and positive L2FC values with P < 0.05 are displayed in dark green.*

Effect of handling time on the resuscitation of cryopreserved prokaryotic communities (experiment 3)

Resuscitated SOLA communities that were processed either directly after the pre-filtration step or after a delay of several hours exhibited similar growth curves with a lag phase of two to three days. They also reached similar carrying capacities, varying between 0.52 ± 0.01 $x10^6$ cell mL^{-1} and 0.54 ± 0.02 $x10^6$ cell mL^{-1} for 0h and 7.5h treatment, respectively (Figure 7A).

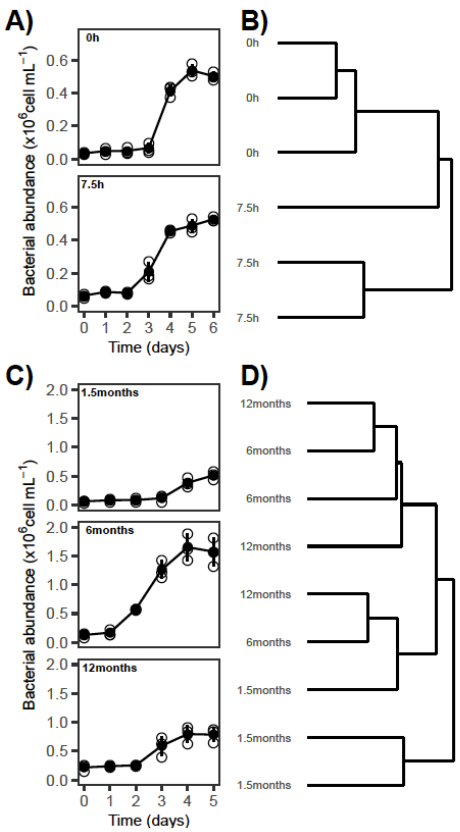

Figure 7. Growth of resuscitated communities and ARISA-based dendrogram displaying community composition similarity from experiment 3 and 4. *A) Growth curves and B) dendrogram illustrating the influence of handling time (0h and 7.5h) during the preparation of inocula for cryopreservation on community composition 5 days after resuscitation. C) Growth curves, and D) dendrogram illustrating the influence of storage time of cryopreserved inocula at -80 °C (1.5, 6, and 12 months) on community composition 5 days after resuscitation. Empty circles in A and C indicate the individual replicates, while, filled circles represent the mean value. Vertical lines represent the standard deviation.*

Differences in community composition between the resuscitated communities were assessed by ARISA fingerprinting, and the hierarchical cluster analysis revealed that communities that had been processed directly after the pre-filtration step clustered closely together (Bray-Curtis distances 0.362 ± 0.074). However, the composition of communities originating from cryopreserved inocula processed with delay was less similar (Bray-Curtis distances 0.741 ± 0.246). Two replicates clustered closely together while the community composition of the third replicate seemed to be more similar to communities from the other incubations (Figure 7B). Further, the ANOSIM did not indicate significant differences in the community composition in dependence of the handling time at an alpha error level of 5% (Table 5).

Table 5. *Statistical parameters from ANOSIM and Mantel tests for experiment 3 and 4.*

Experiment	Comparison	Statistical results	P
Experiment 3 (Handling time)	ANOSIM (all samples)	R=0.333	0.100
Experiment 4 (Storage time)	ANOSIM (all samples)	R=0.276	0.069
	ANOSIM (1.5 months vs 6 months)	R=0.482	0.300 [a]
	ANOSIM (1.5months vs 12 months)	R=0.370	0.300 [a]
	ANOSIM (6 months vs 12 months)	R=0.000	1.000 [a]
	Mantel test	R=0.203	0.117

[a] P values were adjusted for multiple pair-wise testing via the Bonferroni correction.

Effect of storage time on the resuscitation of cryopreserved prokaryotic communities (experiment 4)

Growth curves of cryopreserved communities that were resuscitated after a storage time of 1.5 and 12 months were similar, with lag phases of three to four days and carrying capacities between 0.65 ± 0.14 x10^6 cell mL^{-1} and 0.91 ± 0.23 x10^6 cell mL^{-1}. However, because of a slightly longer lag phase of communities originating from inocula stored for 1.5 months, the communities were, in this case, possibly sampled just before reaching the plateau phase. Incubations from aliquots with a storage time of 6 or 12 months were in contrast sampled shortly after reaching the maximal cell densities (Figure 7C). Different than the other treatments, cryopreserved communities that were resuscitated after 6 months revealed a lag phase of only one day and a higher carrying capacity (1.66 ± 0.21 x10^6 cell mL^{-1}).

However, these differences in the growth curves of the storage time of 6 months versus storage times of 1.5 or 12 months were less evident in the community composition. Here, three clusters in the hierarchical cluster analysis could be observed: one cluster contained two replicates from inocula stored for 1.5 months. A second cluster contained one replicate of each storage time. The third cluster contained two replicates from inocula stored for 6 months and 12 months, respectively (Figure 7D). ANOSIM analyses did not reveal significant differences in community composition among samples with different storage time (Table 5). However, the reported significance level ($p=0.07$, Table 5) was close to the p-value cutoff of $p<0.05$ and could point to an effect of the storage time on community composition. Post-hoc pairwise comparisons indicated that possible differences in the community composition occurred primarily between the samples with a storage time of 1.5 months versus the samples with 6 and 12 months of storage. Pairwise comparison between samples with 6 and 12 months storage time did not indicate any influence of storage time on community composition in this case. Furthermore, a mantel test did not support a significant increase in community dissimilarity with increasing storage time of the cryopreserved inocula (Table 5).

DISCUSSION

The cryopreservation of microorganisms has been considered common practice for the long-term storage of isolated strains and, more recently, of relatively simple and usually artificial prokaryotic assemblages. However, cryopreservation techniques for the preservation of complex natural microbial communities have, to our knowledge, not yet been applied.

Experimental setups based on freshly sampled natural microbial communities lack reproducibility in follow up experiments because the starting community cannot be conserved. We suggested that the cryopreservation of natural prokaryotic communities could circumvent the caveats presented by the lack of standardized initial communities. To the best of our knowledge, this is the first study that tested and evaluated protocols for cryopreservation and resuscitation of highly complex natural prokaryotic assemblages from aquatic habitats. Here, we were able to cryopreserve and resuscitate two distinct and naturally occurring communities preserving most of their diversity.

We interpreted the general trend of lower cell number variances with increasing inoculum size (Figure 3B,D) and a significant decrease in the CV in the experiment 1a (filter-concentrated inocula; Figure 3C) as indication that the variability among replicates of the cryopreservation-resuscitation process decreased with increasing inoculum size. The opposite trend for the CV in experiment 1b (not concentrated inocula, Figure 3F) resulted apparently from the increase of both the cell numbers and their variance in the incubations with smaller inocula sizes. These parameters are effectively counteracting each other, since the CV is the standard deviation normalized by the mean of the measured variable.

Increased cell growth and biomass production for smaller inocula, and corresponding lowered biodiversity, contradicts general predictions and observations in biodiversity-ecosystem functioning research (Yachi and Loreau, 1999; Duffy et al., 2017). Possibly, in the specific case of experiment 1b, the reduction of biodiversity below diversity levels in experiment 1a caused the removal of antagonistic species interactions: fast growers that were suddenly under less competitive pressure may have caused the observed biomass increase in incubations with small inocula and, accordingly, low diversity. The reduction of species diversity in small inocula may further be a reasonable explanation for higher variability of cell growth during the incubations.

There is indeed evidence that small sampling volumes from aquatic environments do not represent adequately the prokaryote composition due to microbial microscale heterogeneity (Seymour et al., 2000; Long and Azam, 2001), which supports the notion of stochastic events in small inocula. For instance, rare species responsible for keystone functions that further influence the succession of communities in the incubations may, in small inocula, stochastically cross a critical abundance threshold that allows them to proliferate during the incubation. Our observations are also in agreement with a recent study where starting communities with lower cell numbers resulted in higher variability of community succession in an artificial three species consortium (Liu et al., 2020).

Our results suggest that the less time-consuming option to directly freeze water samples (up to 10 million cells) without a preceding filtration step to concentrate cells resulted in higher CVs and similar variances. The concentration of cells on a filter further allows the complete removal of the initial water sample, avoiding the carry-over of substrates into the incubation medium and, therefore, providing additional control over initial the substrate conditions during the resuscitation process. Additionally, since the concentrated cells were cryopreserved in a liquid volume of only 2 mL, the amount of DMSO needed as a cryoprotectant is reduced to a minimum. This could be important since the ability to metabolize DMSO to dimethyl sulfide is widespread among microorganisms (Griebler and Slezak, 2000), and may bias the microbial growth of the preserved inocula after resuscitation towards species that can use DMSO as substrate. The concentration of cells via filtration before cryopreservation seems to be of particular interest for studies where carry-over of substrates from the initial sample water are undesirable or if a possible bias towards the growth of species that use DMSO as a substrate should be minimized.

Overall, the CV observed among triplicates for larger inocula in our experiments (CV=3-35% excluding one outlier from the F-SEA incubation; Supplementary Table S4) were similar to values reported in a recently published work with triplicate incubations under controlled conditions using original, not cryopreserved natural aquatic communities as inocula (CV=3-18%) (Morán et al., 2020; Supplementary Table S4). We therefore conclude that as long as the inocula are sufficiently large, the steps involved in the cryopreservation resulted in only limited amount of further added variation into downstream incubations than the use of natural communities as starting inocula. In this sense, increased reproducibility of starting communities in time-shifted experimental designs might bring some level of variability to simultaneously incubated replicates. This observation is, however, not surprising since additional handling steps, such as the steps required for cryopreservation, typically add variation to the outcome of experiments.

Experiment 2 was designed to test the extent of the diversity and community composition of the initial community that can be maintained after the resuscitation of cryopreserved inocula. We found that the inoculum community, the culture medium, and the interaction of both factors were relevant.

In incubations with SOLA communities, an oligotrophic and stable environment, a high degree of similarity of community composition relative to the initial SOLA community was only found in sterile-filtered original seawater treatment (F-SEA medium). This was evident in the analyses by individual ASVs (Figure 5A), ASVs grouped by order (Figure 5B), and Shannon diversity (Figure 5C). The added DOM supplements, even if reflecting to some degree the stoichiometry of the cellular content of microbes from the SOLA field station, seemingly did not promote the growth of many of the taxa present in the initial SOLA community. We assume that DOM prepared from the microbial community's cellular contents could have been mainly composed of labile molecules, which is reflected in the higher C:N or C:P ratios as compared to the environmental DOM present at the SOLA station (Table 3). Therefore, these DOM amended treatments may have added a greater fraction of labile compounds than what is naturally found in the aquatic environment, where the recalcitrant fraction of the DOM dominates (Hansell, 2013). This likely promoted the growth of opportunists, such as members of the Alteromonadaceae family.

Conversely, Canet resuscitated communities, which originated from a highly variable source environment, attained in all incubations a comparably high degrees of similarity of the community composition as well as diversity levels relative to the initial community. This result is further striking because Canet inocula were not incubated in sterile-filtered Canet water, but exclusively in media with DOM supplements that did not even originate from the Canet Lagoon (Figure 5). We speculate that these findings are linked to the adaptation of prokaryote communities to high habitat heterogeneity of the Canet Lagoon. These conditions may favor the selection of generalist species (Büchi and Vuilleumier, 2014) that accordingly grow well in a wide range of conditions, including our incubations with S-DOM or SW-DOM as growth substrates. In contrast, the oligotroph SOLA field station, featuring low habitat heterogeneity, may select for specialist species that grow well only under the specific conditions present in the initial sample water (F-SEA).

The overrepresentation of taxa in the incubations such as ASVs affiliating with the genera Alteromonas (Enterobacterales) and Arcobacter (Campylobacterales) likely reflect their niche adaptation as opportunists within the SOLA and Canet communities, respectively. Members of the genus Alteromonas are known for their ability to exploit complex organic substrates (McCarren et al., 2010) and their capability of rapid growth at high nutrient levels while outcompeting species that are abundant in natural aquatic environments (Eilers et al., 2000; Tada et al., 2011). Similarly, representatives of the genus Arcobacter were described as metabolically versatile organisms able to endure distinct habitats (Campbell et al., 2006; Snelling et al., 2006).

In contrast, Pelagibacterales, the SAR86 clade, and Puniceispirillales, which were abundant in the SOLA initial community, were generally underrepresented in the resuscitated communities, particularly those grown in ASW with DOM supplements. This is not surprising as representatives of the order Pelagibacterales are notoriously hard to grow under laboratory conditions (e.g Sharma et al., 2013; Shen et al., 2018b). Nevertheless, members of the Pelagibacterales were actively growing, particularly in the F-SEA medium, as supported by higher cell abundances during the final incubation day compared to the incubation start (Figure 6C; positive log2-fold changes, padj <0.1). This observation is in concordance with the cultivability of the oligotrophic SAR11 clade in low nutrient sterile-seawater (Rappé et al., 2002). Similarly, members of the ubiquitous but so far uncultured marine SAR86 clade, which has streamlined metabolism (Dupont et al., 2012; Swan et al., 2013), were actively growing even if still underrepresented in F-SEA incubations. Finally, the limited growth of Puniceispirillales, that have been described as photoheterotrophs (Oh et al., 2010), may be the consequence of our incubations setup in dark conditions.

Overall, our analyses suggested that a high proportion of abundant ASVs in the initial SOLA and Canet samples was viable (Figure 6, Supplementary Table S3). Our estimation for viability was based on the statistically tested growth of individual ASVs in triplicate incubations. In some cases, the variance of ASV abundances among the tested triplicates was large, but the growth of an ASV in a single replicate would have been sufficient to prove its viability. However, we did not have technical replicates to statistically test for the growth of ASVs in each incubation, which likely resulted in an underestimation of statistical significance used as an indicator for viability. We additionally may have underestimated the number of viable cells because we analyzed community composition based on a single data point at the end of short-term experiments, while growth for certain ASVs may have been detected only during earlier time points or during an extended incubation. Moreover, besides using filtered seawater as growth medium in one of the treatments, we did not provide growth conditions that fully reflected those present at the sample site, e.g., we incubated in dark excluded particle attached cells via the prefiltration step in the starting communities, where the latter may have removed species interactions that were essential for some of the remaining community members. ASVs for which no growth was detected could therefore still have been viable after the cryopreservation and resuscitation procedure. Therefore, we consider the reported numbers of viable ASVs as a minimum estimate of ASVs that were effectively viable after cryopreservation.

The analyses of experiment 3 did not verify an impact of handling time during the preparation of aliquots for cryopreservation on the composition of resuscitated communities at an alpha error cut-off of 0.05. Still, our results cannot exclude that handling time may potentially have impacted the succession in community composition during the incubations: even though not significant, a trend of clustering of samples in dependency of the handling time was visible (Figure 7A,B). We therefore suggest reducing the handling time to a minimum when preparing community aliquots for cryopreservation. The handling time may influence the downstream incubations because during this period specific community members may change their activity levels compared to *in situ* conditions or even start to proliferate. It indeed had been observed that the transcriptional activities of marine prokaryotes changed differentially for different taxa at the scale of hours after sampling marine ecosystems while maintaining *in situ* conditions such as temperature and oxygen levels (Stewart et al., 2012).

Our data further indicated that long term storage did not significantly impact community composition after the resuscitation process (Figure 7D). It needs to be pointed out that in experiment 4, differently of the other experiments, the compared incubations could not be performed simultaneously. Incubation conditions, particularly temperature, may have changed slightly since we did not use an incubation chamber for our incubations, but instead an air-conditioning system to control the temperature in the laboratory, which is less effective in maintaining exact temperatures. We believe that the observed differences in the duration of the lag-phases, as well as the time to reach the plateau (Figure 7C), may be rather a consequence of small differences in the incubation conditions than due to gradually increasing damage of cells during storage. This assumption is supported by the fact that the lag time did not successively increase with storage time, but displayed a maximum for samples with the shortest storage time, decreased to a minimum at 6 months storage, and subsequently increased again for samples stored for 12 months. In agreement with this, it has been shown previously that the incubation temperature can affect the shape of prokaryotic growth curves (Mellefont and Ross, 2003; Morán et al., 2020).

The potential differences in community composition after 5 days incubation observed between samples stored for 1.5 months and samples stored for 6 or 12 months could also be a consequence that the samples with the shortest storage time were sampled before the maximum cell density was reached, while the two latter were sampled during the plateau phase. Based on these results, we highlight the importance to ensure absolute identical incubation conditions to improve the reproducibility of cryopreserved prokaryote communities.

While we did not test storage times exceeding 12 months, it has however been reported that the storage of cryopreserved cells at -80°C does not fully prevent the gradual formation of ice crystals that could decrease the viability of cells after extended long-term storage times (Brockbank et al., 1992). Therefore, it seems reasonable to assume that after extended long-term storage (>>12 months) at -80°C a gradually increasing number of cells in the inoculum will be harmed, resulting in consequences for the community composition and reproducibility of incubations after resuscitation. This can be avoided by long-term storage of the cryopreserved communities at -196°C, in liquid nitrogen. Under these conditions, the cells are more stable by maintaining a vitrified state, and no damage caused by ice crystals can occur (Benson, 2008). For instance, a full recovery of the physiological capabilities of microalgae was verified after extended storage times at -196°C (Kapoore et al., 2019).

In conclusion, this study provides evidence of a feasible method for the cryopreservation and resuscitation of natural aquatic prokaryote assemblages. Several aspects of the cryopreservation procedure (inoculum size, experimental procedure time, and storage time) were critically assessed to offer new opportunities for future research in microbial ecology based on the manipulation of natural communities. Resuscitation was achieved for contrasting communities harvested from the coastal waters of the Northwest Mediterranean Sea. Based on the findings of this study, we recommend using sufficiently large community aliquots for cryopreservation to improve reproducibility, while on the other hand avoiding extended handling time. Finally, also long-term storage times from 6 up to 12 months did not seem to cause major changes in the community composition of the resuscitated communities.

The cryopreservation protocol presented in this study can be applied to current challenges in microbial ecology since it enables to control the starting community in various experimental setups based on complex natural communities. Our method will for instance allow all kinds of experimental setups, where communities are mixed after a time delay and possibly in different ratios to address the priority effect after community coalescence events. Experiments could also be repeated retrospectively after the evaluation of a first experimental series, with the option to change a specific parameter in the setup while controlling for the starting community. This, for instance, could be applied to consecutively scrutinize culturing settings that cause initially rare opportunistic species to overgrow other members of their communities, with the aim to develop protocols for improved culturing of natural communities under laboratory conditions. The opportunity to completely remove the original sample medium makes the presented method furthermore highly feasible for transplant experiments, where the carry-over of substances in the original sample water should be avoided. The implementation of cryopreservation to natural communities in combination with high-throughput sequencing may furthermore increase the success for the cultivation of target organisms: preceding analyses of meta-'omic' data can be used to identify samples, where a certain target taxon is abundant and may, after the application of genome binning approaches, additionally reveal genes of the target taxon that indicate specific growth requirements. Cryopreserved communities from selected samples can then in retrospect be resuscitated under optimized conditions to enrich or even isolate the target taxon.

Overall, we believe that cryopreservation techniques applied to natural microbial communities have a high potential to advance the research field of microbial ecology. The presented tests and analyses can guide researches on the methodological aspects that should be considered for the cryopreservation of natural aquatic communities in dependency of the research question to be addressed.

ACKNOWLEDGMENTS

We thank the Service d'Observation en Milieu Littoral (INSU-CNRS, Banyuls sur mer) for providing data, as well as the facilities to collect water samples. The facilities for sampling the Warnow river were provided by the Leibniz Institute for Baltic Sea Research and we thank Uwe Hehl for his support during sampling. We thank Olivier Crispy, Jenny Jeschek and Madleen Dierken for their help in the analysis of dissolved organic compounds and nutrients.

We are grateful to the Bio2Mar platform (http://bio2mar.obs-banyuls.fr) for providing technical help and support. We also thank BIOPIC platform at the Oceanological Observatory of Banyuls sur mer for access to flow cytometry facilities and Philippe Catala for performing the counting of heterotroph flagellates.. We thank Inessa Lacativa Bagatini for her help in the data analyses and her input on earlier versions of the manuscript.

AUTHOR CONTRIBUTIONS

SB and GPM designed the study. SB, GPM and AR collected samples and performed experiments. GPM, AR and SB analyzed the data. The manuscript was mainly written by AR and SB, while also GPM contributed. All authors have commented on the manuscript and have read and approved the final manuscript version.

FUNDING

The study was supported by a grant from the German Science Foundation (DFG) awarded to SB (BE 5937/2-1). AR was funded by the National Agency for Research and Development (ANID) / Scholarship Program / DOCTORADO BECAS CHILE/2017 – 72180448. GPM was funded by the doctorate scholarship program of the Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil and a financial support via the above-mentioned DFG funding.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The sequence datasets generated for this study are available the European Nucleotide Archive (ENA, https://www.ebi.ac.uk/ena/browser/home) under the accession number PRJEB38947.

REFERENCES

Aminot, A., and Kérouel, R. (2007). Dosage automatique des nutriments dans les eaux marines: méthodes en flux continu. Editions Quae.

Andersson, A. F., Riemann, L., and Bertilsson, S. (2010). Pyrosequencing reveals contrasting seasonal dynamics of taxa within Baltic Sea bacterioplankton communities. The ISME Journal 4, 171–181. doi:10.1038/ismej.2009.108.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, Articles 67, 1–48. doi:10.18637/jss.v067.i01.

Beardsley, C., Pernthaler, J., Wosniok, W., and Amann, R. (2003). Are Readily Culturable Bacteria in Coastal North Sea Waters Suppressed by Selective Grazing Mortality? Appl. Environ. Microbiol. 69, 2624–2630. doi:10.1128/AEM.69.5.2624-2630.2003.

Bell, T., Gessner, M. O., Griffiths, R. I., McLaren, J., Morin, P. J., van der Heijden, M., et al. (2009). "Microbial biodiversity and ecosystem functioning under controlled conditions and in the wild," in Biodiversity, Ecosystem Functioning, and Human Wellbeing: An Ecological and Economic Perspective, eds. S. Naeem, D. E. Bunker, A. Hector, M. Loreau, and C. Perrings (Oxford University Press). Available at: http://www.oup.com/us/catalog/general/subject/LifeSciences/Ecology/ConservationBiology/?view=usa&sf=toc&ci=9780199547951 [Accessed December 20, 2017].

Bell, T., Newman, J. A., Silverman, B. W., Turner, S. L., and Lilley, A. K. (2005). The contribution of species richness and composition to bacterial services. Nature 436, 1157–1160.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society. Series B (Methodological) 57, 289–300.

Benson, E. E. (2008). Cryopreservation of Phytodiversity: A Critical Appraisal of Theory & Practice. Critical Reviews in Plant Sciences 27, 141–219. doi:10.1080/07352680802202034.

Bloesch, J., Bossard, P., Bührer, H., Bürgi, H. R., and Uehlinger, U. (1988). Can results from limnocorral experiments be transferred to in situ conditions? Hydrobiologia 159, 297–308. doi:10.1007/BF00008242.

Brockbank, K. G. M., Carpenter, J. F., and Dawson, P. E. (1992). Effects of storage temperature on viable bioprosthetic heart valves. Cryobiology 29, 537–542. doi:10.1016/0011-2240(92)90058-A.

Büchi, L., and Vuilleumier, S. (2014). Coexistence of Specialist and Generalist Species Is Shaped by Dispersal and Environmental Factors. The American Naturalist 183, 612–624. doi:10.1086/675756.

Cairns, J., Jokela, R., Hultman, J., Tamminen, M., Virta, M., and Hiltunen, T. (2018). Construction and Characterization of Synthetic Bacterial Community for Experimental Ecology and Evolution. Front. Genet. 9. doi:10.3389/fgene.2018.00312.

Callahan, B. J., Mcmurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2 : High-resolution sample inference from Illumina amplicon data. 13. doi:10.1038/nmeth.3869.

Campbell, B. J., Engel, A. S., Porter, M. L., and Takai, K. (2006). The versatile ε-proteobacteria: key players in sulphidic habitats. Nature Reviews Microbiology 4, 458–468. doi:10.1038/nrmicro1414.

Cardinale, M., Brusetti, L., Quatrini, P., Borin, S., Puglia, A. M., Rizzi, A., et al. (2004). Comparison of Different Primer Sets for Use in Automated Ribosomal Intergenic Spacer Analysis of Complex Bacterial Communities. Appl. Environ. Microbiol. 70, 6147. doi:10.1128/AEM.70.10.6147-6156.2004.

Cauwet, G. (1994). HTCO method for dissolved organic carbon analysis in seawater: influence of catalyst on blank estimation. Marine Chemistry 47, 55–64. doi:10.1016/0304-4203(94)90013-2.

Chian, R.-C. (2010). "Cryobiology: an overview," in Fertility Cryopreservation, eds. P. Quinn and R.-C. Chian (Cambridge: Cambridge University Press), 1–9. doi:10.1017/CBO9780511730207.002.

Christaki, U., Courties, C., Massana, R., Catala, P., Lebaron, P., Gasol, J. M., et al. (2011). Optimized routine flow cytometric enumeration of heterotrophic flagellates using SYBR Green I. Limnology and Oceanography: Methods 9, 329–339. Available at: https://aslopubs.onlinelibrary.wiley.com/doi/abs/10.4319/lom.2011.9.329 [Accessed December 9, 2020].

Culman, S. W., Bukowski, R., Gauch, H. G., Cadillo-Quiroz, H., and Buckley, D. H. (2009). T-REX: software for the processing and analysis of T-RFLP data. BMC Bioinformatics 10, 171. doi:10.1186/1471-2105-10-171.

D'Andrilli, J., Junker, J. R., Smith, H. J., Scholl, E. A., and Foreman, C. M. (2019). DOM composition alters ecosystem function during microbial processing of isolated sources. Biogeochemistry 142, 281–298. doi:10.1007/s10533-018-00534-5.

Deng, Y.-J., and Wang, S. Y. (2016). Synergistic growth in bacteria depends on substrate complexity. Journal of Microbiology 54, 23–30. doi:10.1007/s12275-016-5461-9.

Duarte, C. M., Gasol, J. M., and Vaqué, D. (1997). Role of experimental approaches in marine microbial ecology. Aquat Microb Ecol 13, 101–111.

Duffy, J. E., Godwin, C. M., and Cardinale, B. J. (2017). Biodiversity effects in the wild are common and as strong as key drivers of productivity. Nature 549, 261-+. doi:10.1038/nature23886.

Dupont, C. L., Rusch, D. B., Yooseph, S., Lombardo, M.-J., Alexander Richter, R., Valas, R., et al. (2012). Genomic insights to SAR86, an abundant and uncultivated marine bacterial lineage. The ISME Journal 6, 1186–1199. doi:10.1038/ismej.2011.189.

Eguchi, M., Nishikawa, T., Macdonald, K., Cavicchioli, R., Gottschal, J. C., and Kjelleberg, S. (1996). Responses to Stress and Nutrient Availability by the Marine Ultramicrobacterium Sphingomonas sp. Strain RB2256. Appl. Environ. Microbiol. 62, 1287.

Eilers, H., Pernthaler, J., and Amann, R. (2000). Succession of Pelagic Marine Bacteria during Enrichment: a Close Look at Cultivation-Induced Shifts. Appl. Environ. Microbiol. 66, 4634. doi:10.1128/AEM.66.11.4634-4640.2000.

Faust, K., Lahti, L., Gonze, D., de Vos, W. M., and Raes, J. (2015). Metagenomics meets time series analysis: unraveling microbial community dynamics. Current Opinion in Microbiology 25, 56–66. doi:10.1016/j.mib.2015.04.004.

Freese, H. M., Görs, S., Karsten, U., and Schumann, R. (2007). Dissolved inorganic nutrients and organic substrates in the River Warnow (North-Eastern Germany) – Utilisation by bacterioplankton. Limnologica 37, 264–277. doi:10.1016/j.limno.2007.03.001.

Frossard, A., Gerull, L., Mutz, M., and Gessner, M. O. (2012). Fungal importance extends beyond litter decomposition in experimental early-successional streams. Environmental Microbiology 14, 2971–2983. doi:10.1111/j.1462-2920.2012.02865.x.

Fukami, T. (2015). Historical Contingency in Community Assembly: Integrating Niches, Species Pools, and Priority Effects. Annu. Rev. Ecol. Evol. Syst. 46, 1–23. doi:10.1146/annurev-ecolsys-110411-160340.

Fuller, B. J. (2004). Cryoprotectants: the essential antifreezes to protect life in the frozen state. CryoLetters 25, 375–388.

García, F. C., Bestion, E., Warfield, R., and Yvon-Durocher, G. (2018). Changes in temperature alter the relationship between biodiversity and ecosystem functioning. Proc Natl Acad Sci USA 115, 10989. doi:10.1073/pnas.1805518115.

Giorgio, P. A. del, Gasol, J. M., Vaqué, D., Mura, P., Agustí, S., and Duarte, C. M. (1996). Bacterioplankton community structure: Protists control net production and the proportion of active bacteria in a coastal marine community. Limnology and Oceanography 41, 1169–1179. doi:https://doi.org/10.4319/lo.1996.41.6.1169.

Giovannoni, S. J. (2017). “SAR11 Bacteria: The Most Abundant Plankton in the Oceans,” in Annual Review of Marine Sciences, Vol 9 (Palo Alto: Annual Reviews), 231–255.
Giovannoni, S., and Stingl, U. (2007). The importance of culturing bacterioplankton in the “omics” age. Nature Reviews Microbiology 5, 820–826. doi:10.1038/nrmicro1752.
Goldman, J. C., Caron, D. A., and Dennett, M. R. (1987). Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C: N ratio1. Limnology and Oceanography 32, 1239–1252. doi:https://doi.org/10.4319/lo.1987.32.6.1239.
Griebler, C., and Slezak, D. (2000). Microbial DMSO reduction is widespread among microorganisms and is therefore proposed as a reliable activity parameter. SIL Proceedings, 1922-2010 27, 2492–2497. doi:10.1080/03680770.1998.11901690.
Großkopf, T., and Soyer, O. S. (2014). Synthetic microbial communities. Current Opinion in Microbiology 18, 72–77. doi:10.1016/j.mib.2014.02.002.
Hansell, D. A. (2013). “Recalcitrant Dissolved Organic Carbon Fractions,” in Annual Review of Marine Science, Vol 5, eds. C. A. Carlson and S. J. Giovannoni (Palo Alto: Annual Reviews), 421–445.
Heylen, K., Ettwig, K., Hu, Z., Jetten, M., and Kartal, B. (2012a). Rapid and Simple Cryopreservation of Anaerobic Ammonium-Oxidizing Bacteria. Appl. Environ. Microbiol. 78, 3010. doi:10.1128/AEM.07501-11.
Heylen, K., Hoefman, S., Vekeman, B., Peiren, J., and De Vos, P. (2012b). Safeguarding bacterial resources promotes biotechnological innovation. Applied Microbiology and Biotechnology 94, 565–574. doi:10.1007/s00253-011-3797-y.
Hubálek, Z. (2003). Protectants used in the cryopreservation of microorganisms. Cryobiology 46, 205–229. doi:10.1016/S0011-2240(03)00046-4.
Ibarbalz, F. M., Henry, N., Brandão, M. C., Martini, S., Busseni, G., Byrne, H., et al. (2019). Global Trends in Marine Plankton Diversity across Kingdoms of Life. Cell 179, 1084-1097.e21. doi:10.1016/j.cell.2019.10.008.
Kapoore, R. V., Huete-Ortega, M., Day, J. G., Okurowska, K., Slocombe, S. P., Stanley, M. S., et al. (2019). Effects of cryopreservation on viability and functional stability of an industrially relevant alga. Scientific Reports 9, 2093. doi:10.1038/s41598-019-38588-6.
Kerckhof, F.-M., Courtens, E. N. P., Geirnaert, A., Hoefman, S., Ho, A., Vilchez-Vargas, R., et al. (2014). Optimized Cryopreservation of Mixed Microbial Communities for Conserved Functionality and Diversity. PLOS ONE 9, e99517. doi:10.1371/journal.pone.0099517.
Kirchman, D. L. (2008). “Introduction and Overview,” in Microbial Ecology of the Oceans (John Wiley & Sons, Ltd), 1–26. doi:10.1002/9780470281840.ch1.
Kujawinski, E. B., Longnecker, K., Barott, K. L., Weber, R. J. M., and Kido Soule, M. C. (2016). Microbial Community Structure Affects Marine Dissolved Organic Matter Composition. Front. Mar. Sci. 3. doi:10.3389/fmars.2016.00045.
Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. Journal of Statistical Software 82, 1–26. doi:10.18637/jss.v082.i13.
Lechtenfeld, O. J., Hertkorn, N., Shen, Y., Witt, M., and Benner, R. (2015). Marine sequestration of carbon in bacterial metabolites. Nature Communications 6, 6711. doi:10.1038/ncomms7711.
Liu, F., Mao, J., Kong, W., Hua, Q., Feng, Y., Bashir, R., et al. (2020). Interaction variability shapes succession of synthetic microbial ecosystems. Nature Communications 11, 309. doi:10.1038/s41467-019-13986-6.
Liu, K., Raghavan, S., Nelesen, S., Linder, C. R., and Warnow, T. (2009). Rapid and Accurate Large-Scale Coestimation of Sequence Alignments and Phylogenetic Trees. Science 324, 1561–1564. doi:10.1126/science.1171243.
Long, R. A., and Azam, F. (2001). Microscale patchiness of bacterioplankton assemblage richness in seawater. Aquat Microb Ecol 26, 103–113.

Marie, D., Simon, N., Guillou, L., Partensky, F., and Vaulot, D. (2000). "Flow Cytometry Analysis of Marine Picoplankton," in In Living Color: Protocols in Flow Cytometry and Cell Sorting Springer Lab Manuals., eds. R. A. Diamond and S. Demaggio (Berlin, Heidelberg: Springer), 421–454. doi:10.1007/978-3-642-57049-0_34.

Matias, M. G., Combe, M., Barbera, C., and Mouquet, N. (2013). Ecological strategies shape the insurance potential of biodiversity. Front. Microbiol. 3, 432. doi:10.3389/fmicb.2012.00432.

McCarren, J., Becker, J. W., Repeta, D. J., Shi, Y., Young, C. R., Malmstrom, R. R., et al. (2010). Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. Proc. Natl. Acad. Sci. U. S. A. 107, 16420–16427. doi:10.1073/pnas.1010732107.

Mellefont, L. A., and Ross, T. (2003). The effect of abrupt shifts in temperature on the lag phase duration of Escherichia coli and Klebsiella oxytoca. International Journal of Food Microbiology 83, 295–305. doi:10.1016/S0168-1605(02)00378-1.

Mitchell, M., Muftakhidinov, B., Winchen, T., van Schaik, B., Wilms, A., kylesower, et al. (2019). Engauge Digitizer Software. Available at: http://doi.org/10.5281/zenodo.3558440 [Accessed June 26, 2020].

Morán, X. A. G., Baltar, F., Carreira, C., and Lønborg, C. (2020). Responses of physiological groups of tropical heterotrophic bacteria to temperature and dissolved organic matter additions: food matters more than warming. Environmental Microbiology 22, 1930–1943. doi:10.1111/1462-2920.15007.

Nagai, T., Tomioka, K., Takeuchi, K., Iida, M., Kawada, M., and Sato, T. (2005). Evaluation of Preservation Techniques of Microorganism Resources in the MAFF Genebank. Japan Agricultural Research Quarterly: JARQ 39, 19–27. doi:10.6090/jarq.39.19.

Oh, H.-M., Kwon, K. K., Kang, I., Kang, S. G., Lee, J.-H., Kim, S.-J., et al. (2010). Complete Genome Sequence of "Candidatus Puniceispirillum marinum" IMCC1322, a Representative of the SAR116 Clade in the Alphaproteobacteria. J. Bacteriol. 192, 3240. doi:10.1128/JB.00347-10.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2019). vegan: Community Ecology Package. Available at: https://CRAN.R-project.org/package=vegan.

Overmann, J., Abt, B., and Sikorski, J. (2017). Present and Future of Culturing Bacteria. Annu. Rev. Microbiol. 71, 711–730. doi:10.1146/annurev-micro-090816-093449.

Pagnier, I., Yutin, N., Croce, O., Makarova, K. S., Wolf, Y. I., Benamar, S., et al. (2015). Babela massiliensis, a representative of a widespread bacterial phylum with unusual adaptations to parasitism in amoebae. Biology Direct 10, 13. doi:10.1186/s13062-015-0043-z.

Parada, A. E., Needham, D. M., and Fuhrman, J. A. (2016). Every base matters : assessing small subunit rRNA primers for marine microbiomes with mock communities , time series and global field samples. 18, 1403–1414. doi:10.1111/1462-2920.13023.

Parks, D. H., Chuvochina, M., Waite, D. W., Rinke, C., Skarshewski, A., Chaumeil, P.-A., et al. (2018). A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nature Biotechnology 36, 996–1004. doi:10.1038/nbt.4229.

Prakash, O., Nimonkar, Y., and Shouche, Y. S. (2013). Practice and prospects of microbial preservation. FEMS Microbiology Letters 339, 1–9. doi:10.1111/1574-6968.12034.

Props, R., Kerckhof, F.-M., Rubbens, P., De Vrieze, J., Hernandez Sanabria, E., Waegeman, W., et al. (2017). Absolute quantification of microbial taxon abundances. The ISME Journal 11, 584–587. doi:10.1038/ismej.2016.117.

Pujo-Pay, M., Conan, P., Oriol, L., Cornet-Barthaux, V., Falco, C., Ghiglione, J.-F., et al. (2011). Integrated survey of elemental stoichiometry (C, N, P) from the western to eastern Mediterranean Sea. Biogeosciences 8, 883–899. doi:10.5194/bg-8-883-2011.

Pujo-Pay, M., and Raimbault, P. (1994). Improvement of the wet-oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters. Marine Ecology Progress Series 105, 203–207.

R Core Team (2018). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing Available at: https://www.R-project.org/.

Rappé, M. S., Connon, S. A., Vergin, K. L., and Giovannoni, S. J. (2002). Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature 418, 630–633. doi:10.1038/nature00917.

Salazar, G., Paoli, L., Alberti, A., Huerta-Cepas, J., Ruscheweyh, H.-J., Cuenca, M., et al. (2019). Gene Expression Changes and Community Turnover Differentially Shape the Global Ocean Metatranscriptome. Cell 179, 1068-1083.e21. doi:10.1016/j.cell.2019.10.014.

Sánchez-Pérez, E. D., Ortega-Retuerta, E., Conan, P., Pujo-Pay, M., and Marrasé, C. (2020). Mismatched dynamics of dissolved organic carbon and chromophoric dissolved organic matter in the coastal NW Mediterranean Sea. Science of The Total Environment, 141190. doi:10.1016/j.scitotenv.2020.141190.

Schernewski, G., Paysen, P., Robbe, E., Inácio, M., and Schumacher, J. (2019). Ecosystem Service Assessments in Water Policy Implementation: An Analysis in Urban and Rural Estuaries. Frontiers in Marine Science 6, 183. doi:10.3389/fmars.2019.00183.

Seymour, J. R., Mitchell, J. G., Pearson, L., and Waters, R. L. (2000). Heterogeneity in bacterioplankton abundance from 4.5 millimetre resolution sampling. Aquat Microb Ecol 22, 143–153.

Shade, A., Peter, H., Allison, S. D., Baho, D. L., Berga, M., Burgmann, H., et al. (2012). Fundamentals of microbial community resistance and resilience. Frontiers in microbiology 3. doi:10.3389/fmicb.2012.00417.

Shakya, M., Quince, C., Campbell, J. H., Yang, Z. K., Schadt, C. W., and Podar, M. (2013). Comparative metagenomic and rRNA microbial diversity characterization using archaeal and bacterial synthetic communities. Environmental Microbiology 15, 1882–1899. doi:https://doi.org/10.1111/1462-2920.12086.

Sharma, A. K., Becker, J. W., Ottesen, E. A., Bryant, J. A., Duhamel, S., Karl, D. M., et al. (2013). Distinct dissolved organic matter sources induce rapid transcriptional responses in coexisting populations of Prochlorococcus, Pelagibacter and the OM60 clade. Environmental Microbiology, n/a–n/a. doi:10.1111/1462-2920.12254.

Shen, D., Juergens, K., and Beier, S. (2018a). Experimental insights into the importance of ecologically dissimilar bacteria to community assembly along a salinity gradient. Environ. Microbiol. 20, 1170–1184. doi:10.1111/1462-2920.14059.

Shen, D., Langenheder, S., and Jürgens, K. (2018b). Dispersal Modifies the Diversity and Composition of Active Bacterial Communities in Response to a Salinity Disturbance. Frontiers in Microbiology 9, 2188. doi:10.3389/fmicb.2018.02188.

Singer, E., Andreopoulos, B., Bowers, R. M., Lee, J., Deshpande, S., Chiniquy, J., et al. (2016). Next generation sequencing data of a defined microbial mock community. Scientific Data 3, 160081. doi:10.1038/sdata.2016.81.

Snelling, W. J., Matsuda, M., Moore, J. E., and Dooley, J. S. G. (2006). Under the Microscope: Arcobacter. Letters in Applied Microbiology 42, 7–14. doi:10.1111/j.1472-765X.2005.01841.x.

Steen, A. D., Crits-Christoph, A., Carini, P., DeAngelis, K. M., Fierer, N., Lloyd, K. G., et al. (2019). High proportions of bacteria and archaea across most biomes remain uncultured. The ISME Journal 13, 3126–3130. doi:10.1038/s41396-019-0484-y.

Stewart, F. J., Ulloa, O., and DeLong, E. F. (2012). Microbial metatranscriptomics in a permanent marine oxygen minimum zone. Environ. Microbiol. 14, 23–40. doi:10.1111/j.1462-2920.2010.02400.x.

Swan, B. K., Tupper, B., Sczyrba, A., Lauro, F. M., Martinez-Garcia, M., González, J. M., et al. (2013). Prevalent genome streamlining and latitudinal divergence of planktonic bacteria in the surface ocean. PNAS 110, 11463–11468. doi:10.1073/pnas.1304246110.

Szekely, A. J., Berga, M., and Langenheder, S. (2013). Mechanisms determining the fate of dispersed bacterial communities in new environments. ISME J. 7, 61–71. doi:10.1038/ismej.2012.80.

Tada, Y., Taniguchi, A., Nagao, I., Miki, T., Uematsu, M., Tsuda, A., et al. (2011). Differing Growth Responses of Major Phylogenetic Groups of Marine Bacteria to Natural Phytoplankton Blooms in the Western North Pacific Ocean. Appl. Environ. Microbiol. 77, 4055. doi:10.1128/AEM.02952-10.

Vekeman, B., and Heylen, K. (2017). "Preservation of Microbial Pure Cultures and Mixed Communities," in Hydrocarbon and Lipid Microbiology Protocols: Isolation and Cultivation, eds. T. J. McGenity, K. N. Timmis, and B. Nogales (Berlin, Heidelberg: Springer Berlin Heidelberg), 299–315. doi:10.1007/8623_2015_51.

Ward, C. S., Yung, C.-M., Davis, K. M., Blinebry, S. K., Williams, T. C., Johnson, Z. I., et al. (2017). Annual community patterns are driven by seasonal switching between closely related marine bacteria. The ISME Journal 11, 1412–1422. doi:10.1038/ismej.2017.4.

Wolfe, B. E. (2018). Using Cultivated Microbial Communities To Dissect Microbiome Assembly: Challenges, Limitations, and the Path Ahead. mSystems 3, e00161-17. doi:10.1128/mSystems.00161-17.

Yachi, S., and Loreau, M. (1999). Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. Proc. Natl. Acad. Sci. U. S. A. 96, 1463–1468. doi:10.1073/pnas.96.4.1463.

Yu, Z., Krause, S. M. B., Beck, D. A. C., and Chistoserdova, L. (2016). A Synthetic Ecology Perspective: How Well Does Behavior of Model Organisms in the Laboratory Predict Microbial Activities in Natural Habitats? Front. Microbiol. 7. doi:10.3389/fmicb.2016.00946.

ZoBell, C. (1941). Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. J. Mar. Res. 4, 42–75.

SUPPLEMENTARY MATERIALS

***Table S1**. Summary sample processing of r16S amplicon sequences via the DADA2 pipeline (experiment 2).*

Location	Treatment	Replicate	Raw sequence read pairs	Filtered read pairs	Denoised forward reads	Denoised reverse reads	Merged read pairs	Nonchimeric
SOLA	Initial	1	30757	25601	25394	25427	23553	20500
SOLA	Initial	2	30059	25282	25063	25065	22746	19146
SOLA	Initial	3	42909	39254	38824	38822	35043	29144
SOLA	F-SEA	1	79859	60964	60696	60843	59448	55305
SOLA	F-SEA	2	28985	23271	23120	23167	22078	19048
SOLA	F-SEA	3	26886	20265	20155	20199	19345	16745
SOLA	S-DOM	1	84959	60178	60103	60135	59683	55086
SOLA	S-DOM	2	60463	45681	45570	45613	44729	34313
SOLA	S-DOM	3	80173	59075	58990	59026	58181	45057
SOLA	SW-DOM	1	58457	42588	42483	42486	41361	36067
SOLA	SW-DOM	2	63983	47108	46999	47029	45952	37610
SOLA	SW-DOM	3	48602	33819	33759	33772	33137	27209
Canet	Initial	1	29409	23290	23076	23139	21832	15102
Canet	Initial	2	39365	32441	32228	32272	30948	22832
Canet	Initial	3	20181	17248	17114	17132	16572	13200
Canet	S-DOM	1	55352	44292	44119	44172	43340	33802
Canet	S-DOM	2	42024	31896	31743	31800	30918	23084
Canet	S-DOM	3	35430	27169	27074	27119	26613	20620
Canet	SW-DOM	1	30842	22712	22610	22646	22062	16314
Canet	SW-DOM	2	53018	40342	40182	40246	39369	30783
Canet	SW-DOM	3	51221	39331	39190	39246	38241	31243

Table S2. *GTBD Taxonomic assignation and average proportion per treatment for each ASV (experiment 2). Full table available in https://www.frontiersin.org/articles/10.3389/fmicb.2020.597653/full#supplementary-material*

Kingdom	Phylum	Class	Order	Family	Genus	Species	ASV	CANET _initial	CANET _S-DOM	CANET _SW-DOM	SOLA_i nitial	SOLA_ F-SEA	SOLA_ S-DOM	SOLA_ SW-DOM
Bacteria	**Proteobacteria**	**Gammaproteobacteria**	**Enterobacterales**	**Alteromonadaceae**	**Alteromonas**	**(RS_GCF_001953635.1)**	**SV_1**	**0.0025**	**0.0126**	**0.0379**	**0.0000**	**0.0114**	**35.6566**	**11.1995**
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Litoreibacter	Litoreibacter_janthinus(RS_GCF_900111945.1)	**SV_2**	3.9874	17.3308	16.2273	0.0000	0.1780	0.7828	0.4369
Bacteria	**Proteobacteria**	**Gammaproteobacteria**	**Enterobacterales**	**Alteromonadaceae**	*NA*	*NA*	**SV_3**	**0.0051**	**0.0025**	**0.0000**	**0.0177**	**12.2955**	**0.0101**	**0.0000**
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	HIMB11	(RS_GCF_000472185.1)	**SV_4**	15.9242	9.6894	9.7172	0.0000	0.0265	0.0152	0.0303
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum	*NA*	**SV_5**	0.2399	0.2576	0.2652	0.0000	0.0000	0.0000	0.0025
Bacteria	**Proteobacteria**	**Gammaproteobacteria**	**Enterobacterales**	**Alteromonadaceae**	**Alteromonas**	*NA*	**SV_6**	**0.0000**	**0.5404**	**0.7879**	**0.0000**	**0.0000**	**3.9495**	**22.3434**
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Jannaschia	Jannaschia_faecimaris(RS_GCF_900107415.1)	**SV_7**	0.1717	0.8182	0.4899	0.0202	2.1364	13.7753	2.8864
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*NA*	*NA*	**SV_8**	0.3258	8.3535	9.7828	0.0000	0.0000	0.0000	0.0025
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	*NA*	*NA*	**SV_9**	6.0530	4.9293	5.4773	025	0.1023	0.0025	0.0000
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	MS024-2A	(RS_GCF_000173095.1)	**SV_10**	0.0000	0.0000	0.0000	0.4646	0.6932	0.0631	0.1212
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*NA*	*NA*	**SV_11**	0.0000	0.0000	0.0051	0.0025	0.0000	1.2828	13.5707
Bacteria	**Campylobacterota**	**Campylobacteria**	**Campylobacterales**	**Arcobacteraceae**	**Arcobacter**	**Arcobacter_lekithochrous(RS_GCF_001878855.1)**	**SV_12**	**0.0000**	**7.7677**	**4.0960**	**0.0000**	**0.0000**	**0.0000**	**0.0101**
Bacteria	**Campylobacterota**	**Campylobacteria**	**Campylobacterales**	**Arcobacteraceae**	**Arcobacter**	**Arcobacter_lekithochrous(RS_GCF_001878855.1)**	**SV_13**	**0.0000**	**0.0000**	**0.0025**	**0.0000**	**0.0000**	**0.0000**	**11.5657**
Bacteria	Proteobacteria	Alphaproteobacteria	Pelagibacterales	Pelagibacteraceae	Pelagibacter	*NA*	**SV_14**	0.0000	0.0000	0.0000	11.8737	5.8750	0.3207	0.9672
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	*NA*	*NA*	**SV_15**	1.9924	0.2096	0.1869	0.0000	0.0000	0.0025	0.0000
Bacteria	**Proteobacteria**	**Gammaproteobacteria**	**Enterobacterales**	**Alteromonadaceae**	**Alteromonas**	*NA*	**SV_16**	**0.0000**	**0.0429**	**0.1061**	**0.0000**	**0.0000**	**6.2374**	**1.7247**
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Hel3-A1-48	(RS_GCF_001735715.1)	**SV_17**	0.0152	0.0101	0.0000	0.1111	1.3977	0.0126	0.0328
Bacteria	Actinobacteriota	Actinobacteria	Actinomycetales	Microbacteriaceae	UBA1487	(GB_GCA_002325245.1)	**SV_18**	3.8990	3.3990	2.8636	0.0051	0.0000	0.0000	0.0051
Bacteria	Proteobacteria	Alphaproteobacteria	Pelagibacterales	Pelagibacteraceae	Pelagibacter	Pelagibacter_ubique_D(RS_GCF_001180225.1)	**SV_19**	0.0480	0.0606	0.1136	8.1793	4.1477	0.2424	0.5480
Bacteria	**Campylobacterota**	**Campylobacteria**	**Campylobacterales**	**Arcobacteraceae**	**Arcobacter**	**Arcobacter_nitrofigilis(RS_GCF_000092245.1)**	**SV_20**	**0.0000**	**0.0000**	**9.0707**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
Bacteria	**Proteobacteria**	**Gammaproteobacteria**	**Enterobacterales**	**Alteromonadaceae**	**Alteromonas**	**Alteromonas_australica(RS_GCF_000730385.1)**	**SV_21**	**0.0000**	**0.0025**	**0.0000**	**0.0025**	**0.0152**	**3.8081**	**3.2727**
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Nereida	Nereida_ignava(RS_GCF_900114125.1)	**SV_22**	0.1667	0.0505	0.0379	0.0833	5.7538	2.0253	0.4848
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Saccharospirillaceae	Oleibacter	Oleibacter_marinus(RS_GCF_900156675.1)	**SV_23**	0.0000	0.4394	0.3965	0.0000	0.0038	1.5808	3.9899
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	UBA7446	(GB_GCA_002478685.1)	**SV_24**	0.0000	0.0000	0.0000	1.3030	0.7273	0.1162	0.2475
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*NA*	*NA*	**SV_25**	0.9773	0.7475	0.6414	0.0253	6.7992	0.5934	0.2955
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Thalassobacter	Thalassobacter_stenotrophicus(RS_GCF_001458315.1)	**SV_26**	0.4949	3.6061	1.4369	0.0000	0.0000	0.0076	0.0025
Bacteria	**Proteobacteria**	**Gammaproteobacteria**	**Enterobacterales**	**Alteromonadaceae**	**Alteromonas**	**(RS_GCF_001953635.1)**	**SV_27**	**0.0000**	**0.0025**	**0.0000**	**0.0000**	**0.0000**	**2.7854**	**2.6692**
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ruegeria	Ruegeria_profundi(RS_GCF_001507545.1)	**SV_28**	0.0000	0.0025	0.0000	0.0000	0.0000	0.6869	5.4747
Bacteria	**Proteobacteria**	**Gammaproteobacteria**	**Enterobacterales**	**Alteromonadaceae**	**Alteromonas**	*NA*	**SV_29**	**3.1970**	**1.8535**	**1.3232**	**0.0000**	**0.0265**	**0.0025**	**0.0025**
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	MS024-2A	(RS_GCF_000173095.1)	**SV_30**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Bacteria	Proteobacteria	Alphaproteobacteria	*NA*	*NA*	*NA*	*NA*	**SV_31**	0.0000	0.0000	0.0000	5.3157	2.5720	0.0581	0.1692
Bacteria	Bacteroidota	Rhodothermia	Balneolales	Balneolaceae	UBA1275	(GB_GCA_002689185.1)	**SV_32**	0.0051	0.0000	0.0025	6.2121	0.6667	0.0354	0.1818
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Leisingera	*NA*	**SV_33**	0.0000	0.0076	0.0000	0.0000	0.0000	0.1439	4.2929
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Pseudophaeobacter	(RS_GCF_000152965.1)	**SV_34**	0.0000	0.0051	0.0000	0.0000	0.0038	4.4192	0.0000
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*NA*	*NA*	**SV_35**	0.4242	1.5202	1.5227	0.0000	0.0000	0.0227	0.0000
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Pseudoruegeria_A	Pseudoruegeria_A_aquimaris(RS_GCF_900172235.1)	**SV_36**	0.2576	1.3359	1.4444	0.0000	0.0000	0.0000	0.0025
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	UBA7446	(GB_GCA_002478685.1)	**SV_37**	0.0000	0.0000	0.0000	0.0758	2.2083	0.0227	0.0379
Bacteria	Proteobacteria	Alphaproteobacteria	Pelagibacterales	Pelagibacteraceae	Pelagibacter	*NA*	**SV_38**	0.0000	0.0000	0.0000	3.7298	1.3902	0.1389	0.3157
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	*NA*	*NA*	*NA*	**SV_39**	0.0000	0.0126	0.0000	0.0000	0.0000	1.2854	0.6717
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Cryomorphaceae	Coccinistipes	*NA*	**SV_40**	0.1515	0.6944	0.5833	0.0000	0.0000	0.0000	0.0025
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	HIMB11	(RS_GCF_000472185.1)	**SV_41**	0.5631	0.2247	0.2045	0.2601	5.2462	0.1490	0.2146
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ruegeria_A	(RS_GCF_000014065.1)	**SV_42**	0.0000	0.1768	0.1616	0.0000	0.0000	2.4520	0.0278
Bacteria	Proteobacteria	Alphaproteobacteria	Pelagibacterales	Pelagibacteraceae	Pelagibacter_A	Pelagibacter_A_ubique(GB_GCA_000419545.1)	**SV_43**	0.0000	0.0000	0.0000	3.2551	0.7727	0.1086	0.2399
Bacteria	Bacteroidota	Rhodothermia	Balneolales	Balneolaceae	UBA1275	(GB_GCA_002689185.1)	**SV_44**	0.0808	0.0429	0.0429	3.1187	0.9545	0.0581	0.0909
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	*NA*	*NA*	**SV_45**	0.6313	0.5202	0.5758	0.0000	0.0000	0.0000	0.0025
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Hahellaceae	Marinobacter	*NA*	**SV_46**	0.0000	0.0530	0.0303	0.0000	0.0000	0.5379	1.7449
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum	(RS_GCF_002700005.1)	**SV_47**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Bacteria	Proteobacteria	Alphaproteobacteria	Pelagibacterales	Pelagibacteraceae	Pelagibacter	*NA*	**SV_48**	0.0051	0.0025	0.0025	2.3737	1.2917	0.0884	0.2121
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	MS024-2A	(RS_GCF_000173095.1)	**SV_49**	0.0000	0.0025	0.0000	1.9697	0.2727	0.0657	0.1641
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	*NA*	*NA*	**SV_50**	0.0000	0.0000	0.0000	0.0051	0.0038	0.0025	0.0000
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum	(RS_GCF_000518405.1)	**SV_51**	0.0000	0.0000	0.0000	0.0025	0.0000	0.0000	0.0000
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	*NA*	*NA*	*NA*	**SV_52**	0.0000	0.0000	0.0000	0.0000	0.0189	0.0000	0.0000
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ruegeria_B	Ruegeria_B_pomeroyi(RS_GCF_000011965.2)	**SV_53**	0.1641	1.0758	1.1338	0.0000	0.0038	0.0076	0.0025
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Halieaceae	Luminiphilus	(RS_GCF_000227505.1)	**SV_54**	0.0000	0.0000	0.0000	0.7374	3.0492	0.0152	0.0429
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Thalassobacter	Thalassobacter_stenotrophicus(RS_GCF_001458315.1)	**SV_55**	0.0101	0.0530	0.0051	0.0000	0.1477	1.5960	0.0000
Bacteria	**Proteobacteria**	**Gammaproteobacteria**	**Enterobacterales**	**Alteromonadaceae**	**Alteromonas**	*NA*	**SV_56**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**1.5556**	**0.3712**
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	HIMB11	(RS_GCF_000472185.1)	**SV_57**	1.6641	0.5783	0.5152	0.0076	0.0341	0.0000	0.0101
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Weeksellaceae	Chryseobacterium	Chryseobacterium_arthrosphaerae(RS_GCF_001684965.1)	**SV_58**	0.0000	0.0000	0.0000	0.0076	0.0000	0.0000	0.0000
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	*NA*	*NA*	*NA*	**SV_59**	0.0000	0.0051	0.0076	0.0000	0.0000	0.0000	0.0000
Bacteria	Actinobacteriota	Actinobacteria	Actinomycetales	Microbacteriaceae	Aquiluna	Aquiluna_sp1(RS_GCF_000257665.1)	**SV_60**	1.2955	0.5732	0.4798	0.0000	0.0000	0.0000	0.0000
Bacteria	**Proteobacteria**	**Gammaproteobacteria**	**Enterobacterales**	**Alteromonadaceae**	**Alteromonas**	*NA*	**SV_61**	**0.0505**	**0.0808**	**0.0455**	**0.0025**	**0.5417**	**0.0859**	**0.0227**
Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Tenacibaculum	(RS_GCF_000518405.1)	**SV_62**	0.0000	0.0025	0.0000	0.0101	0.9735	0.8409	0.1187

Table S3. *L2FC and corresponding P-values indicating cell growth (viability) for each initially abundant ASV (>0.5 % relative abundance) in the treatment with highest evidence for growth.*

Location	ASV	Mean Log2FC	Relative abundance in initial sample	P	Padj	Treatment
SOLA	SV_101 (Puniceispirillales)	-2.256	0.010	0.089	0.106	S-DOM
SOLA	SV_109 (SAR86)	-2.969	0.009	0.038	0.058	S-DOM
SOLA	SV_110 (Flavobacteriales)	2.085	0.006	0.040	0.096	F-SEA
SOLA	SV_111 (Flavobacteriales)	3.101	0.006	0.022	0.089	F-SEA
SOLA	SV_118 (Gammaproteobacteria)	0.855	0.008	0.018	0.086	F-SEA
SOLA	SV_119 (SAR86)	2.404	0.007	0.024	0.089	F-SEA
SOLA	SV_121 (Alphaproteobacteria)	0.946	0.007	0.177	0.217	F-SEA
SOLA	SV_123 (Alphaproteobacteria)	0.585	0.007	0.136	0.178	F-SEA
SOLA	SV_126 (Flavobacteriales)	1.541	0.005	0.013	0.086	F-SEA
SOLA	SV_130 (SAR86)	1.273	0.006	0.071	0.112	F-SEA
SOLA	SV_131 (Pelagibacterales)	0.510	0.006	0.028	0.089	F-SEA
SOLA	SV_134 (SAR86)	1.388	0.006	0.103	0.145	F-SEA
SOLA	SV_136 (Puniceispirillales)	-2.494	0.006	0.005	0.048	S-DOM
SOLA	SV_14 (Pelagibacterales)	2.167	0.119	0.012	0.086	F-SEA
SOLA	SV_17 (Flavobacteriales)	6.762	0.007	0.071	0.112	F-SEA
SOLA	SV_19 (Pelagibacterales)	2.198	0.082	0.007	0.086	F-SEA
SOLA	SV_31 (Alphaproteobacteria)	1.883	0.053	0.220	0.261	F-SEA
SOLA	SV_32 (Balneolales)	0.037	0.062	0.794	0.815	F-SEA
SOLA	SV_38 (Pelagibacterales)	1.772	0.037	0.005	0.086	F-SEA
SOLA	SV_43 (Pelagibacterales)	0.998	0.032	0.056	0.101	F-SEA
SOLA	SV_44 (Balneolales)	1.451	0.031	0.056	0.101	F-SEA
SOLA	SV_48 (Pelagibacterales)	2.333	0.023	0.030	0.089	F-SEA
SOLA	SV_51 (Flavobacteriales)	2.118	0.019	0.017	0.086	F-SEA
SOLA	SV_54 (Pseudomonadales)	4.981	0.008	0.056	0.101	F-SEA
SOLA	SV_58 (Flavobacteriales)	1.573	0.019	0.048	0.101	F-SEA
SOLA	SV_62 (Flavobacteriales)	0.232	0.020	0.144	0.183	F-SEA
SOLA	SV_66 (Rhodobacterales)	2.779	0.015	0.031	0.089	F-SEA
SOLA	SV_70 (Flavobacteriales)	2.265	0.013	0.044	0.099	F-SEA
SOLA	SV_71 (Puniceispirillales)	-1.983	0.019	0.011	0.048	S-DOM
SOLA	SV_74 (Puniceispirillales)	0.137	0.018	0.674	0.732	F-SEA
SOLA	SV_76 (Pseudomonadales)	4.991	0.005	0.037	0.096	F-SEA
SOLA	SV_77 (Pseudomonadales)	3.538	0.006	0.009	0.086	F-SEA
SOLA	SV_78 (Puniceispirillales)	1.226	0.014	0.130	0.177	F-SEA
SOLA	SV_82 (Puniceispirillales)	0.996	0.013	0.074	0.112	F-SEA

SOLA	SV_83 (SAR86)	1.887	0.012	0.018	0.086	F-SEA
SOLA	SV_91 (SAR86)	2.261	0.010	0.070	0.112	F-SEA
SOLA	SV_93 (Puniceispirillales)	-2.283	0.011	0.010	0.048	S-DOM
SOLA	SV_95 (Puniceispirillales)	2.338	0.009	0.041	0.096	F-SEA
Canet	SV_10 (Flavobacteriales)	-0.582	0.208	0.131	0.166	SW-DOM
Canet	SV_100 (Flavobacteriales)	0.008	0.010	0.975	0.975	S-DOM
Canet	SV_102 (Pseudomonadales)	1.515	0.006	0.036	0.085	SW-DOM
Canet	SV_103 (Rhodobacterales)	1.130	0.006	0.019	0.035	S-DOM
Canet	SV_128 (Flavobacteriales)	0.808	0.005	0.121	0.159	S-DOM
Canet	SV_15 (Flavobacteriales)	2.891	0.027	0.001	0.008	S-DOM
Canet	SV_17 (Flavobacteriales)	-0.829	0.037	0.128	0.166	SW-DOM
Canet	SV_18 (Actinomycetales)	2.564	0.039	0.008	0.017	S-DOM
Canet	SV_2 (Rhodobacterales)	4.946	0.040	0.000	0.005	S-DOM
Canet	SV_24 (Flavobacteriales)	4.071	0.008	0.001	0.008	S-DOM
Canet	SV_25 (Rhodobacterales)	2.418	0.009	0.035	0.056	S-DOM
Canet	SV_29 (Enterobacterales)	1.781	0.032	0.134	0.168	S-DOM
Canet	SV_37 (Flavobacteriales)	1.453	0.025	0.002	0.009	S-DOM
Canet	SV_4 (Rhodobacterales)	2.097	0.159	0.004	0.011	S-DOM
Canet	SV_40 (Flavobacteriales)	0.875	0.028	0.085	0.118	S-DOM
Canet	SV_41 (Rhodobacterales)	1.407	0.006	0.017	0.034	S-DOM
Canet	SV_45 (Flavobacteriales)	0.224	0.030	0.457	0.473	S-DOM
Canet	SV_47 (Flavobacteriales)	2.091	0.012	0.033	0.056	S-DOM
Canet	SV_5 (Flavobacteriales)	4.046	0.046	0.000	0.005	S-DOM
Canet	SV_50 (Flavobacteriales)	0.727	0.014	0.015	0.074	SW-DOM
Canet	SV_57 (Rhodobacterales)	1.318	0.017	0.048	0.073	S-DOM
Canet	SV_60 (Actinomycetales)	1.641	0.013	0.002	0.009	S-DOM
Canet	SV_63 (Flavobacteriales)	2.605	0.006	0.002	0.009	S-DOM
Canet	SV_67 (Gammaproteobacteria)	1.357	0.014	0.004	0.011	S-DOM
Canet	SV_75 (Flavobacteriales)	-0.596	0.020	0.232	0.259	SW-DOM
Canet	SV_80 (Actinomycetales)	2.477	0.005	0.002	0.009	S-DOM
Canet	SV_86 (Rhodobacterales)	2.076	0.005	0.006	0.013	S-DOM
Canet	SV_9 (Flavobacteriales)	2.509	0.061	0.005	0.011	S-DOM
Canet	SV_99 (Rhodobacterales)	1.937	0.005	0.004	0.011	S-DOM

***Table S4**. Coefficients of variation of maximal cell density for all incubations as well as for incubations incubation reported by Morán and colleagues (Morán et al., 2020).*

ID experiment	Description	Inoculum source	Inoculum size (x10^6 cells)	Growth medium	Treatment	Day (max abundance)	CV (%)
1a	Effect of the Inoculum size (filters)	SOLA	66	S-DOM		4	33.1
		SOLA	132	S-DOM		6	80.2
		SOLA	330	S-DOM		4	44.2
		SOLA	495	S-DOM		6	15.8
		SOLA	990	S-DOM		5	9.9
1b	Effect of the inoculum size (water aliquot)	SOLA	1.3	S-DOM		6	30.8
		SOLA	3.3	S-DOM		6	35.5
		SOLA	6.6	S-DOM		5	20.8
		SOLA	9.9	S-DOM		6	29.4
2	Similarity between resuscitated and original communities (filters)	SOLA	412	F-SEA		5	43.1(14.8)[a]
		SOLA	412	S-DOM		5	14.2
		SOLA	412	SW-DOM		5	12.8
		Canet	804	S-DOM		5	4.9
		Canet	804	SW-DOM		4	30.2
3	Handling time (filters)	SOLA	412	S-DOM	T0h	5	7.1
		SOLA	412	S-DOM	T7,5h	6	2.5
4	Storage time (filters)	SOLA	412	S-DOM	T1.5 months	5	14.2
		SOLA	412	S-DOM	T6 months	4	18.9
		SOLA	412	S-DOM	T12 months	4	14.2
	Moran et al., 2020	Marine		Seawater witout any DOM addition	Temperature: *in situ* -3°C	3	4.9
		Marine		Seawater witout any DOM addition	Temperature: *in situ*	3	10.2
		Marine		Seawater witout any DOM addition	Temperature: *in situ* +3°C	3	8.7
		Marine		Seawater + Glucose	Temperature: *in situ* -3°C	3	5.2
		Marine		Seawater + Glucose	Temperature: *in situ*	3	6.1
		Marine		Seawater + Glucose	Temperature: *in situ* +3°C	3	8.8
		Marine		Seawater + Mangrove	Temperature: *in situ* -3°C	3	3.3
		Marine		Seawater + Mangrove	Temperature: *in situ*	3	3.0
		Marine		Seawater + Mangrove	Temperature: *in situ* +3°C	3	5.1
		Marine		Seawater + Seagrass	Temperature: *in situ* -3°C	3	17.1
		Marine		Seawater + Seagrass	Temperature: *in situ*	3	12.4
		Marine		Seawater + Seagrass	Temperature: *in situ* +3°C	3	14.2

[a]CV value excluding replicate 1

[b]raw cells counts used to calculate CV values were estimated by the Engauge Digitizer program from figures published in Moran et al. 2020.

Supplementary Text S1. Step-by-step protocol for cryopreservation and resuscitation procedure

I) Prepare a sterile stock solution of Dimethyl sulfoxide (DMSO, ACS reagent, ≥99.9%, Sigma-Aldrich, Missouri, USA) by sterile filtration (0.22 µm filter, hydrophilic PVDF, 25 mm filter, Millipore, Massachusetts, USA).

For cryopreservation of filters proceed to step IIa, while for cryopreserved water samples proceed to step IIb.

IIa) Cryopreservation of concentrated inocula:

1. Prepare a DMSO working solution by adding the filtered DMSO to fresh artificial seawater (ASW) medium reflecting the salinity of the original sample (alternatively, sterile filtered seawater can be considered) to a 5% (v/v) concentration.
2. Add 1000 µL DMSO working solution into standard 2 mL tubes (one tube per filter), keep the tubes at 4°C until use.
3. Filter sample water from a volume containing ≥ 500 x10^6 cells (after 0.8 µm prefiltration to remove protists) with a vacuum pump or a peristaltic pump through 0.22 µm filter (e.g. hydrophilic PVDF, 25 mm filter, Millipore, Massachusetts, USA) (vacuum pump: ~600 mg Hg; peristaltic pump: (~60 mL min^{-1}). If using a vacuum pump carefully avoid drying of the filters, by reducing the pressure at the end of the filtration to a minimum to prevent cell damage[1].
4. Next, immerse the filter completely into the DMSO working solution resuspended cells attached to the filter by pipetting up and down. Keep the tube 15 min at 4°C for equilibration.

IIb) Cryopreservation of aquatic microbial communities for sample water:

1. Prepare aliquots of 0.8 µm pre-filtered water samples without prior cell concentration via filtration.
2. Add filtered DMSO to the aliquots prepared in the preceding step (DMSO 5% final concentration).
3. Keep the aliquots for 15 min at 4°C.

III) Flash-freeze the community aliquots in liquid nitrogen, then transfer it to -80°C for long-term storage.

IV) Thaw the cryopreserved samples at room temperature for about 30-45 min to initiate the resuscitation.

V) Add the whole content of the defrosted tubes (filter together with the DMSO solution and the resuspended cells; step IIa) or the frozen liquid sample aliquots (step IIb) into freshly prepared growth medium to regrow the cryopreserved communities.

[1] This step slightly differs from the protocol performed on samples presented in the manuscript where we resuspended the cells by pipetting up and down ASW medium on the filter while it was still placed on the filter device. For later preparation of cryopreserved community aliquots, we however followed the protocol as detailed here.

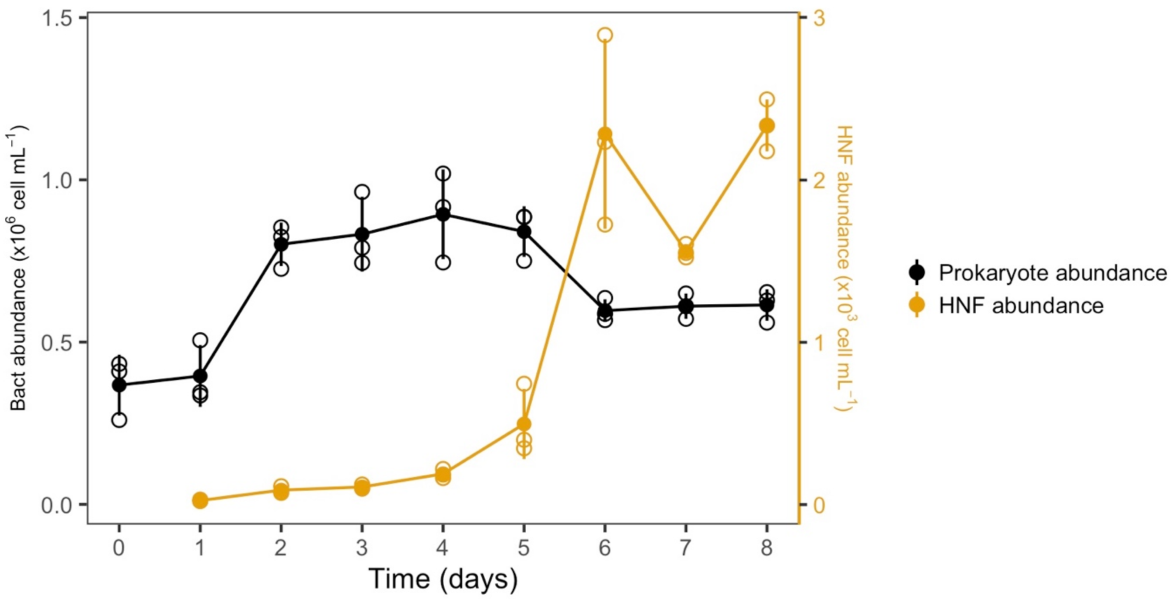

Figure S1. Growth of resuscitated bacterial and heterotrophic nanoflagellates (HNF) in cryopreserved not prefiltered communities from the SOLA station. *Error bars represent the standard deviation among replicates (n=3). Filled circles represent the mean of triplicate incubations, while empty circles illustrate individual replicates.*

Unfiltered surface seawater (1 L) from the SOLA station (sample day: 07/10/2020) was passed in triplicates through a 0.22 µm filter (hydrophilic PVDF, 47 mm filter, Millipore, Massachusetts, USA) using a peristaltic pump (60 mL min^{-1}) to concentrate the cells. Filters were carefully transferred and completely immersed into the previously prepared 2 mL tubes with 1 mL cryoprotectant containing sterile-filtered DMSO (ACS reagent, ≥99.9%, Sigma-Aldrich, Missouri, USA) in 0.22 µm filter seawater. The tubes were kept at 4°C for 15 min for equilibration and then flash-frozen in liquid nitrogen and transferred to -80°C. Resuscitation of cryopreserved communities was done as described in the main method section. Briefly, the cells were incubated for 8 days in triplicate incubations at in situ temperature (~18°C) in 900 mL 0.22 µm-filtered seawater and in dark. Daily samples were fixed with glutaraldehyde (0.1% final concentration) and stored at -80°C until flow cytometry analyses. The abundance of prokaryotes was quantified as described in the main manuscript text. The abundance of HNF cells was analyzed using the cytometer FACSCanto II (BD Biosciences, New Jersey, USA) as described elsewhere (Christaki et al., 2011).

Chapter 3

The cost of adaptability: Resource availability constrains functional stability under pulsed disturbances

Rain-Franco Angel[1], Peter Hannes[2], Pavan de Moraes Guilherme[1,3,4] & Beier Sara[1,4]

[1]UMR 7621 Laboratoire d'Océanographie Microbienne, Observatoire Océanologique de Banyuls-sur-Mer, Sorbonne Université, Banyuls-sur-Mer, France

[2]Stream Biofilm and Ecosystem Research Laboratory, Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland.

[3]Graduate Program in Ecology and Natural Resources (PPGERN), Laboratory of Phycology, Department of Botany, Universidade Federal de São Carlos, São Carlos, Brazil.

[4]Department of Biological Oceanography, Leibniz Institute for Baltic Sea Research Warnemünde, Rostock, Germany .

Manuscript in preparation

ABSTRACT

Disturbances are events that disrupt ecosystems and have been described as major drivers of microbial community composition and functioning. Functional stability (resistance and resilience) can be influenced by the properties of individual community members, which can be assessed via the distributions of genomic traits in the community. The functional stability can be maintained through the development of environmental tolerance traits, may present metabolic costs. To study the impact of pulsed disturbances on community assembly and functioning, we performed a 41-days continuous culture experiment under two disturbance regimes (weekly disturbed and undisturbed controls) crossed with two levels of resource availability. Changes in community composition and functional resistance to the disturbances were assessed weekly. Results revealed a large contribution of stochastic assembly processes that were evident from deviations in species-level composition among replicates. In contrast, we found consistent patterns of superordinate community structural patterns and functional characteristics, such as levels of species diversity, traits distribution, and functional resistance in response to the treatments. Genomic traits indicated a high prevalence of species with resistant- and resilience-related traits, particularly in the disturbed communities under high nutrient availability, which displayed higher diversity than the other treatments. The findings related to trait distributions and species diversity patterns likely explain the high functional resistance measured in the pulse disturbed communities grown under resource-rich conditions.

INTRODUCTION

Disturbances are major drivers of microbial community composition and functioning (Bardgett and Caruso, 2020) and understanding the mechanisms that determine the response of microbial communities to disturbances is key for predicting their dynamics. Disturbances are defined in ecology as abiotic or biotic events that disrupt ecosystems, communities, or populations (Rykiel, 1985). They are categorized as short-term (pulse) or continuous (press) disturbances (Shade et al., 2012). The response of communities to disturbances is driven by their level of resistance and/or resilience, where resistance is defined as the degree to which a community is insensitive to a disturbance while resilience is the rate at which a community returns to a pre-disturbance condition (Shade et al., 2012). These responses are quantified relative to the community composition or to any of several functional measurements (e.g., respiration, production).

It has been suggested that the disturbance regime of an environment shapes microbial communities. For instance, while the number of opportunist species increased after an initial disturbance, the continuous exposition to pulse disturbances favored communities with resistant members and resulted in increased functional stability (Evans and Wallenstein, 2012). Furthermore, the Biodiversity-ecosystem-functioning (BEF) research states that species diversity increases the stability of communities when facing disturbances by increasing the functional redundancy among its members (Yachi and Loreau, 1999).

The impact of diversity patterns on ecosystem functioning is thereby mediated via the kind, diversity, and distribution of the species' traits (Griffiths and Philippot, 2013). Therefore, there has been a recent upsurge in the research interest to the so-called trait-based approaches that assess community dynamics in response to environmental gradients (Ackerly and Cornwell, 2007; Cornwell and Ackerly, 2009; Enquist et al., 2015). Life-history traits associated with resistance (i.e. attributes that confer stress tolerance and facilitate adaptation) and resilience (i.e. attributes related to growth capacity and reproduction) (Nimmo et al., 2015) are of particular interest to predict the response of communities to disturbance.

Several studies suggested that traits that can be inferred from the genomic material of microbial communities are appropriate proxies for the life histories of community members (Beier et al., 2021; Westoby et al., 2021). For instance, the fraction of transcription factors (%TF) and genome size were linked to classifications in the generalist-specialist continuum, since more genes and regulation capabilities potentially enhance the ability to face environmental change (Kostadinov et al., 2011; Bentkowski et al., 2015). These genomic traits may consequently be considered resistance-related traits. In contrast, the codon usage bias or the number of 16s rRNA gene copies (RRN) are associated with the maximal growth rates and/or the length of the lag-phase (Klappenbach et al., 2000; Vieira-Silva and Rocha, 2010; Weissman et al., 2021) and may accordingly be classified as resilience-related traits.

High resistance at the compositional level can be linked to both high and low functional resistance, depending on which set of traits are assessed. For example, it has recently been demonstrated that high compositional resistance was linked to low variability (i.e., high resistance) in the expression patterns of fitness-related traits, such as growth rate and biomass production, but to higher variability in the expression of adaptation-related traits that, for instance, prevent cell damage after an environmental change (Rain-Franco et al.; Chapter 1). Thus, it seems plausible that functional resistance and resilience levels, particularly of fitness-related traits, are tightly linked to community compositional dynamics and, consequently, also to compositional resistance and resilience levels.

It has been debated that a generalist lifestyle should be associated with higher metabolic costs that allow species to tolerate environmental change (Dall and Cuthill, 1997; DeWitt et al., 1998; Bell and Bell, 2021). It has been suggested that high potential growth rates and, accordingly, high resilience can have a tradeoff regarding resource usage efficiency (Fierer et al., 2007; Roller et al., 2016). Indeed, the investment required to develop adaptation-related traits that prevent cellular damage under stressful conditions, such as the production of compatible solutes or heat-shock proteins (Rain-Franco et al., Chapter 1) may be associated with metabolic costs. To compensate for these costs, species have to re-allocate the available energy, for example, from protein synthesis to amino acid acquisition (Schimel et al., 2007). However, little direct experimental evidence supports the idea that the expression of generalist phenotypes is constrained by resource availability (Kassen and Bell, 1998; Magalhães et al., 2009; Hall et al., 2018).

To test the impact of pulsed disturbances and resource availability on community assembly and community functional characteristics, we performed a 41-days continuous culture experiment under two disturbance regimes (pulse disturbances and undisturbed control) crossed with two resource availability levels. During the experiment, aquatic bacterial assemblies were subjected to weekly pulse disturbances, and compositional and functional changes were assessed.

Based on trait-trait covariation patterns it has recently been proposed that resistance and resilience-related traits are positively related, particularly in aquatic habitats (Beier et al., 2021). We, therefore, hypothesized that the long-term exposition of communities to salt pulse disturbances favor the growth of community members with higher capabilities for resistance and resilience under high resource levels. Simultaneously, high resistance and resilience levels should lead to species gain in disturbed environments (Nimmo et al., 2015), thus we accordingly expect increased diversity levels under these conditions. Finally, we expected that the selection process in communities exposed to pulsed disturbances, particularly under high resource levels, would lead to increased community-level functional stability in response to disturbances.

To our knowledge, this is the first study addressing the impact of frequent disturbances on microbial community resistance in dependency of the resource availability applying a full-factorial design. The induced salinity pulse-disturbances are also relevant under climate change scenarios, as the pulse disturbances events, especially those associated with salt concentration, are predicted to increase in frequency due to global warming (Planton et al., 2008).

METHODS

Starting communities

Inocula for the continuous culture experiment were obtained from cryopreserved microbial community aliquots that had been prepared as previously described (Rain-Franco et al., 2021). These communities were sampled from several aquatic environments in the south of the Gulf of Lyon, South France. The Mediterranean field station SOLA and the coastal lagoons La Palme and Gruissan are characterized by contrasting environmental variability (Fig. 1; Table 1). Cryopreserved community aliquots from these sites were pooled to obtain a 'super-diverse' community to allow for selection mechanisms to act on a possibly large collection species that have complementary traits.

Table 1. *Information on sample sites for the source communities used in this study.*

Location	Latitude (°N)	Longitude (°E)	Depth (m)	Sampling date
SOLA	42° 29.0'	03° 08.7'	3	11/06/2019
Gruissan Lagoon	43° 06.7'	04° 28.9'	1	25/05/2019
La Palme Lagoon	42° 57.2'	03° 03.4'	1	25/05/2019

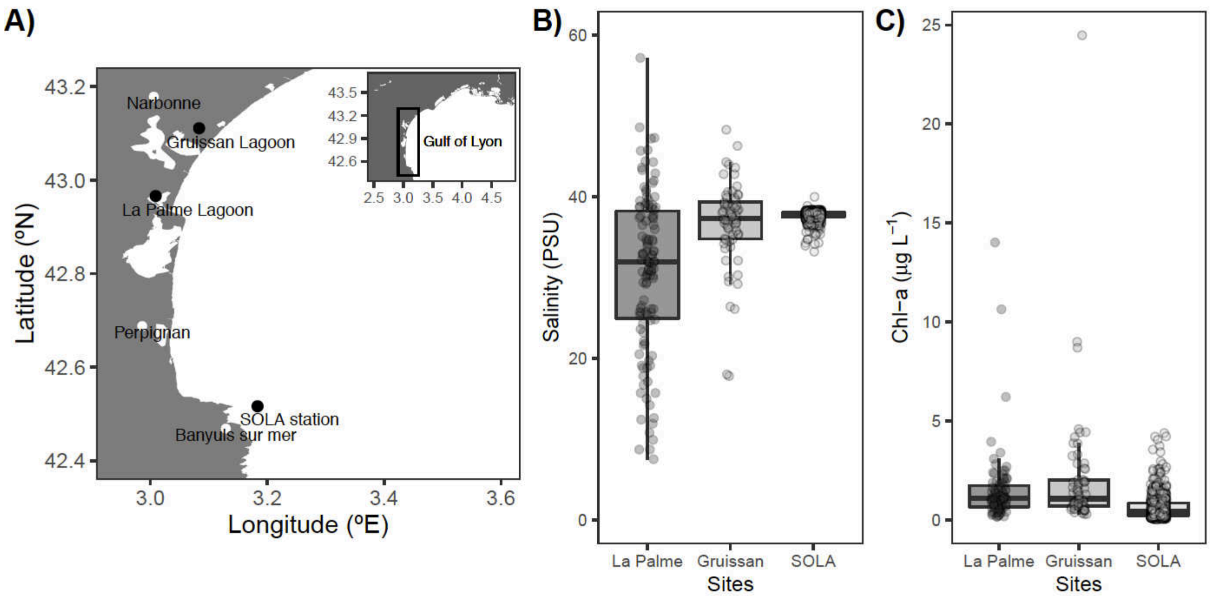

Figure 1. Sampling locations and environmental characteristics of the sample sites, from which the starting inocula were obtained. *A) Geographic locations of the La Palme and Gruissan lagoons as well as the Mediterranean coastal SOLA field station. B) Temporal salinity (PSU) and C) Chlorophyll-a (µg l^{-1}) variability. Time series environmental data for La Palme (23/09/1989-11/09/2020, n = 206) and Gruissan (23/09/1989-08/08/2019, n = 128) were obtained from https://wwz.ifremer.fr/surval. Environmental data for the SOLA station was provided by the SOMLIT program (https://www.somlit.fr/; 04/01/2005-24/11/2020, n = 1443).*

Culturing media

Cultures media in this study were based on artificial seawater (ASW; Eguchi *et al.*, 1996) with a salinity of 38 g l^{-1} and pH 8. Trace metals, Fe, and EDTA were added 100-fold less concentrated than originally published. The ASW was amended with DOM supplements as the sole source of carbon, nitrogen, and phosphorus, with no vitamins addition.

DOM supplements were prepared from particular material retained after filtration of water from seven different aquatic environments that differed in their trophic status (Table S1) as described elsewhere (Rain-Franco et al., 2021). Based on several pre-tests, we combined these DOM supplements into low and high nutrient DOM supplements (referred to as LDOM and HDOM, respectively, Table 2). The levels of DOM in these supplements supported the growth of cell densities as typically found in oligo- to mesotrophic conditions (LDOM) or eutrophic conditions (HDOM). Since we prepared concentrated LDOM and HDOM stock solutions, we could repeatedly and reproducibly prepare the large volumes of media that were necessary for the long-term continuous culture experiment. HDOM media were additionally amended with yeast extract (0.28 mg l^{-1}; Sigma–Aldrich, St. Louis, MO, United States) to add a highly labile DOM compound. Complex and diverse DOM sources as the DOM supplements for LDOM and HDOM media have been discussed to support the assembly of diverse prokaryotic communities (Morán et al., 2020).

Table 2. *Chemical composition of the two DOM mixes used as supplements in the continuous culture experiment.*

DOM sources	**LDOM**	**HDOM**
Samples of origin	SOLA; Gruissan Lagoon; La Palme Lagoon	Canet lagoon; Baltic Sea: Warnow River and coastal; Yeast extract
DOC (µmol l^{-1})	529	3627
DON (µmol l^{-1})	2.24	4.87
DOP (µmol l^{-1})	B.D.L	B.D.L
NO3 (µmol l^{-1})	1.4	1.41
NH_4^+ (µmol l^{-1})	12.91	13.32
PO_4 (µmol l^{-1})	0.1	0.13
C:N	236:1	745:1

B.D.L= Below detection limit

Experimental setup and design

We started the long-term experiment with a batch preculture. For this purpose, cryopreserved communities from SOLA, Gruissan, and La Palme were resuscitated in 6 l ASW to which all seven DOM supplements were added, each of them in half concentration compared to LDOM and HDOM media used later. This preculture was incubated for three days at 18°C under agitation by a magnetic stirrer. Subsequently, this preculture was split into two volumes. We added another half portion of either the LDOM or the HDOM media supplements to each of the volumes, respectively, except for the yeast extract, and maintained these cultures for three more days in batch condition before distributing them into the continuous system. After six days in batch mode, 300 ml of both precultures were distributed into six reactor vessels, respectively (Figure S1).

The inflow of LDOM and HDOM media was activated to fill the vessels reaching a total volume of 400 ml, then started the continuous mode. From the six chemostats assigned to each DOM condition, three were used as control and three for the pulse disturbance treatment. The medium was pumped from six 2 l bottles (three containing LDOM and three containing HDOM medium) into the culture vessels using a peristaltic pump (ISMATEC, Cole-Parmer GmbH, Wertheim, Germany), with each bottle feeding into one control and one disturbance, respectively. The flow rate was set to 70 µl min^{-1} to approximate the generation time measured for prokaryote communities from the Mediterranean sea (~2.75 days, Landa et al., 2013).

The cultures were mixed constantly with magnetic stirrers and ventilated through venting filters (0.2 µm, 64 mm diameter, Midisart® 2000, Sartorius, Göttingen, Germany) attached to the lids. Syringes (50 ml) were connected to the culture vessels via stainless steel needles and were used either to add saturated salt solutions to the disturbance treatments or to sample small volumes for flow cytometry (FC), salinity, and functional rate measurements. The continuous culture's outflow were pooled for two consecutive days in a sterile 1 l bottle, totaling volumes of approximately 200 ml, and used for downstream DNA extractions. Overall, the setup of the continuous culture system resembled that used in an earlier continuous culture study (Fig. S1; Baho et al., 2012). We further added two polyethylene biochips (Mutag BioChip 25™, MUTAG, Chemnitz, Germany) into each vessel to provide a surface for biofilm formation. The biofilm can act as a spatial refuge, for some bacterial cells, which would not be washed out and may recolonize the medium. We decided to add these chips to lower the risk of a potential collapse of the continuous culture during the long-term experiment.

After filling the reactor vessels, the cultures were stabilized in continuous mode for two days before the first pulse disturbance. Salt pulse disturbances were applied once per week to the disturbance treatments by adding ~18 ml of a saturated NaCl solution after the same volume was removed from the reactor vessels (+13 gl^{-1} NaCl). The exact added volume was calculated immediately prior to every salinity pulse disturbance for each treatment vessel from the salinity measured in the respective vessel, to avoid a potential continuous raise of the baseline salinity and ensure that the final salinity after each peak did not exceed a salinity of ~54 gl^{-1} NaCl. As a consequence of the continuous culture mode of operation, the salt was continuously diluted throughout the following seven days, before the next pulse disturbance was introduced, approximately returning to the baseline salinity (Fig. 2A). In total, six pulse disturbances were applied within 41 days of continuous flow mode (Fig. 2A; Table S2).

The incubations were kept at 18°C in dark during the entire experiment. All components of the continuous culture system were autoclaved before the experiment start. We exchanged the medium feed and the tubing system every 3-4 days to avoid potential contamination. We further tested the medium regularly for potential contamination after it had passed the tubing system, just before the inlet into the continuous culture, via FC. While eventual contaminations were detected, these never exceeded 0.09×10^6 cells per ml^{-1} (Table S3). In case of contamination, the whole tubing system and the medium feed of the affected culture vessels were replaced by newly sterilized material.

Biological measurements

Cell numbers in the continuous culture were estimated by FC as detailed elsewhere (Marie et al., 2000). Briefly, aliquots (1350 µl) were sampled using sterile syringes, fixed with glutaraldehyde (0.1% final concentration) and stored at -80°C. Samples were analyzed in the Cytoflex Flow Cytometer (Beckman Coulter, California, USA).

Samples for DNA extraction and downstream metabarcoding were taken from the continuous culture outflow and filtered onto 0.22 µm filters (cellulose filters, Millipore, MA, United States) (Table S2). To obtain a sufficiently large water volume, a sterile water bottle was attached to the outflow approximately 48 h before sampling. Sampling was performed at least once per week. In week 3, an additional set of samples was obtained as the continuous flow had accidentally stopped for some hours in two vessels (vessel 2 and 5; Fig. S1). Also in week 6 two additional sets of DNA were sampled (Table S2). In total, we obtained DNA samples from 9 sample days. Filters for DNA extractions were stored at -30°C until further processing.

Chemical analysis of the media

Concentrations of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), dissolved organic phosphorous (DOP), nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3-}) and ammonium (NH_4^+) were measured in both culture media. The media were filtered through two pre-combusted GFF filters (0.7 µm, 25 mm, Whatman, Buckinghamshire, UK). For the DOC analyses, 10 ml aliquots were stored in combusted flasks and acidified (85% H_3PO_4, final pH=2). The flasks were closed and maintained at room temperature until the samples were analyzed in a Shimadzu TOC-V following the standard protocol (Cauwet, 1994). Samples for DON and DOP (20 ml) were collected in Teflon bottles and stored at -20°C. DON and DOP were simultaneously quantified using the wet oxidation protocol (Pujo-Pay and Raimbault, 1994). NO_3^-, NO_2^-, and PO_4^{3-} were determined by standard colorimetric technique (Bran Luebbe autoanalyzer) (Aminot and Kérouel, 2007), while NH_4^+ samples were analyzed by a fluorometric method (Holmes et al., 1999).

Community functioning measurements

Functional resistance of the continuous culture was estimated for treatments and controls once per week by measuring the below-described bulk community functional rates before and 1 hour after each induced disturbance (Table S2). Heterotroph bacterial production (BP) was assessed via ^{3}H-leucine incorporation: 100 µL of a working solution containing 1 part ^{3}H-leucine (125.6 Ci mmol^{-1}, Perkin Elmer™, Massachusetts, USA) and 4 parts cold leucine were added to 1.5 ml sample water (final leucin concentration: 40 nM) and incubated in dark at 18°C. The incubations were stopped after 1.5 hours by adding 150 µl 50% trichloroacetic acid (TCA). Two technical replicates and one blanc control with 50% TCA added before the incubation were performed for each reactor vessel and measure point. The incubations were then processed using the microcentrifuge method as published elsewhere (Smith and Azam, 1992), and quantified using a liquid scintillation counter (Hidex 300SL HIDEX, Turku, Finland). The theoretical conversion factor of 1.55 kg C mol Leu^{-1} was used to convert leucine incorporation to carbon production (Kirchman, 1993).

The microbial activity was determined via fluorescein diacetate (FDA) hydrolysis (Battin, 1997; Adam and Duncan, 2001). FDA diffuses freely into intact cells and can be hydrolyzed by non-specific esterases that are widespread in microbes. We applied the FDA assay following the procedure published elsewhere (Adam and Duncan, 2001). In short, Phosphate buffer (60 mM, pH 7.6) was prepared by adding 8.7 g of K_2HPO_4 and 1.3 g of KH_2PO_4 (Sigma–Aldrich, St. Louis, MO, United States) to 1 l of Milli-Q water. An aliquot of 150 µl of the sample was incubated with 150 µl Phosphate buffer and 4 µl of FDA (20 mM, Sigma–Aldrich, St. Louis, MO, United States) dissolved in acetone (ACS reagent, Sigma–Aldrich, St. Louis, MO, United States). Triplicate incubations were performed for each reactor vessel and sampling time. The assays were incubated in 96-well microplates in a VICTOR Multilabel Plate Reader (Excitation: 535 nm Emission: 485 nm; PerkinElmer, Massachusetts, United States) every 3 min for 1.5 h at room temperature. Two sets of triplicate blank incubations were prepared as the reaction mix but instead of the sample, a sterile culture medium with the respective salinity was added. A calibration curve (0.03, 0.07, 0.10,0.14 µM of fluorescein diacetate) was prepared for each measuring point for the two corresponding salt concentrations using Fluorescein (Sigma–Aldrich, St. Louis, MO, United States) diluted in acetone to calculate FDA production in the incubations and interpreted as a measure for bulk cellular activity.

Respiration was quantified as the oxygen consumption estimated in 5 ml glass vials equipped with an OxoDish using a SensorDish reader (PreSens, Regensburg, Germany). The tubes were filled with sample water and closed with an air-tight lid while avoiding the enclosure of air bubbles. The filled vials were subsequently placed in an incubator at 18°C in dark.

Respiration was estimated as the rate of oxygen decrease from a linear fitting from oxygen measurements taken every 3 min during 14 h. Bacterial growth efficiency (BGE in %) was estimated by dividing BP by the sum of BP and respiration, while the respiration rates were transformed to carbon consumption considering a respiratory quotient of 0.89 (Williams and Giorgio, 2005).

Community functional resistance index

Log-response ratios (lnR) are widely used metrics to measure the relative impact of a treatment on a given variable, independently of its metric units (Hedges et al., 1999). In this study, we applied the lnR (Hillebrand and Gurevitch, 2016) to quantify the relative change of the functional rate F before and after the pulse disturbance in the disturbed communities (lnR_D) and in the corresponding controls where no disturbance was introduced (lnR_C) at the same time intervals. Values of the lnR close to 0 represent a low variability of the measured functional rate, while, deviations from 0 indicate an increase (decrease) of functional rates reflected in positive (negative) lnR during the interval of consideration. We considered the difference between both ratios as resistance index for F (RI_F) which is similar to the effect size measurement published previously by Osenberg et al (1997). Since we were interested in the resistance metric independent from the direction of change, we used differently from the originally published metric the absolute difference in this study. We also did not normalize by time, as the considered time interval was equal for lnR_D and lnR_C.

$$RI_F = |lnR_C - lnR_D|$$

If RI_F approximates zero, the temporal change of the functional response F was similar in magnitude and direction in both, the disturbed and control communities. In this case, F was only marginally impacted by the induced salinity pulse pointing to a high resistance level. The larger the deviation of RI_F from zero, the lower/higher the functional resistance/sensitivity.

For the lnR_C, the average of the triplicates was calculated to cover the total range of variability of the response variable.

DNA extraction and sequencing

DNA extractions were performed using a QIAmp DNA Mini Kit (QIAGEN, Hilden, Germany) with an initial bead-beating step in ATL buffer using a FastPrep-24™ 5G (MP Biomedical, California, USA). The concentration and quality of the eluted DNA were tested using a DS11-FX+ microvolume spectrophotometer (DeNovix, Delaware, USA).

DNA samples from the continuous culture were sent for 16s rRNA gene amplicon sequencing (300 base pairs paired-end read, Illumina Miseq V3) using the primers pair 515yf-926r (Parada et al., 2016). Libraries were demultiplexed by the sequencing company (LGC Genomics GmbH) using the BCL2FASTQ software v2.17.1.14 and removing reads with length <100 bases, as well as primer clipping (up to 3 mismatches per primer). Sequence processing was performed using the DADA2 for R (Callahan et al., 2016) by slightly modifying the standard pipeline (truncLen=c(250,180); https://benjjneb.github.io/dada2/tutorial.html). A total of 1678 amplicon sequence variants (ASVs) were identified across samples. Retrieved ASVs were taxonomically assigned using the Genome Taxonomy Database (GTDB) (Parks et al., 2018).

Assembly mechanisms

We computed the nearest taxon indexes (NTIs) to evaluate assembly mechanisms (Webb, 2000; Stegen et al., 2012) in response to the disturbance regimes under the different DOM levels. NTIs >0 indicate phylogenetic over-clustering of community members due to deterministic assembly processes as a result of environmental filtering. NTIs <0 point to phylogenetic over-dispersion due to deterministic assembly processes as a result of species interactions, such as competitive exclusion. The closer NTIs are to 0, the larger the impact of neutral assembly processes. NTIs were calculated via the iCAMP R-package (v1.3.4, Ning et al., 2020). All NTIs were estimated relative to the species pool in the incubations under the same DOM regime sampled during a single time point (=6 samples). This reference species pool was chosen because we aimed to focus on assembly mechanisms due to the disturbance regime for each of the DOM levels separately while excluding successional aspects.

Community diversity and genomic trait distributions

The Shannon diversity index was estimated from daily measured FC data by applying the "FlowDiv" R-package (Wanderley et al., 2019) through the SSC-, FL1-H and FL3-H channels. The reason to use FC instead of metabarcoding data to estimate diversity for downstream statistics was that both estimates correlated significantly (Fig. S2; R^2=0.38, P<0.001) while more data points were available for the FC data. FC measurements furthermore refer in contrast to the DNA samples that were collected from the outflow directly to the community within the reaction vessels.

Genomic traits that are indicative of the life history of prokaryotes feature significant phylogenetic signals and can be extrapolated from closely related relatives via taxonomic marker genes of uncultured species in a community (Beier et al., 2021). Genomic traits were predicted for each ASV using the hidden state prediction option included in the PICRUSt2 software (Douglas et al., 2020). They were classified into resilience-related (RRN and generation time (d)) and resistance-related traits (%TF and genome size). The average phylogenetic distances to the closest relatives in the PICRUSt2 default reference database ranged from 0.003-0.116 (abundance weighted NSTI, Table S4). For each sample and genomic trait, the community weighted mean (CWM) was used for downstream statistical analyses. The relative abundance of ASVs in each sample was calculated after rarefication to the minimum number of reads obtained for the individual libraries (9,439 reads, Table S5).

The estimation of d was based on the codon usage bias as detailed elsewhere (Weissman et al., 2021). Weissmann et al. suggested that only d up to 5 h can be reliable predicted from the codon usage bias, while the prediction of larger d becomes increasingly inaccurate. CWMs of d in our samples were in most cases ≤ 5h and reached maximally 6.2 h. We, therefore, decided that d estimations were sufficiently accurate.

Statistical analysis

Statistical analyses were performed in R (R Core Team, 2018). At the end of the long-term experiment (day 41), each one replicate of the disturbance treatment under HDOM and LDOM was affected by a sudden community collapse (Fig. 2F). We have therefore considered data obtained from FC for statistical analyses only until day 40. Data obtained from the final DNA sampling event at day 41 were included in the downstream statistical analyses as it was sampled early during day 41 and most of the water volume was derived from day 39 or 40.

The phylogenetic compositional structure of the communities was assessed using a pairwise abundance weighted unifrac metric. To test the effect of time, disturbance treatment, and DOM regime on the phylogenetic community composition, we performed a Multivariate Analysis of Variance (PERMANOVA) using the function adonis2 available in the "vegan" R-package (permutations=1000, Oksanen et al., 2019).

To assess the hypothesis of the gained functional resistance under high resource conditions we performed two-ways repeated-measurements analyses of variances (rmANOVAs) on the resistance indices (RI_F) considering time and DOM level. We additionally fitted a mixed linear model (MLM) on the RI by DOM level to evaluate the direction of potentially detected trends over time, using the "nlme" R-package (Pinheiro et al., 2020). For this model, we considered time as a fixed factor and the replicates as random factors.

We furthermore performed two-way rmANOVAs to assess the effect of time and the disturbance regime on bacterial abundances, Shannon diversity, and genomic traits in each of the DOM regimes, separately. In our analysis, we aimed to test the one-tailed hypotheses that disturbances should select for taxa with larger genomes, higher RRN, higher %TF and lower d as well as higher species diversity when genomic trait distributions indicated a simultaneous increase of resistance and resilience levels. As a consequence of formulating one-tailed hypotheses, we considered a P-value <0.1 as significant, if the hypothesized direction of the response values was observed. Normality and homogeneity of variance of the data were tested by the Kolmogorov-Smirnov and the Levene test, respectively. Overall no violations of the ANOVA assumptions concerning normality and homogeneity of variance were detected, except for bacterial abundance, bulk microbial activity, and community respiration. In these cases, a square root transformation was applied to the data to fulfill the assumptions.

RESULTS

Community functioning

Microbial activity and production rates as well as the BGE were significantly more resistant against salinity disturbances in HDOM compared to LDOM (Fig. 2B-E; Table 3). This pattern was however not detected for the respiration measurements. The resistance against salinity disturbances increased significantly over time for respiration and BGE at HDOM (decreasing RI, Fig. 2D-E; Table 3), however, for the remaining resistance measurements, no significant temporal changes of resistance levels were detected.

Table 3. *Summary of the rmANOVA to test the effect of the DOM regime on the RI. Mean represent the mean value of the respective RI for LDOM and HDOM condition respectively. Slopes from mixed linear models were included to illustrate the direction of temporal trends of the RIs.*

rmANOVA	**LDOM**	**HDOM**	**RM ANOVA**				**MLM_{LDOM}**	**MLM_{HDOM}**	
RIs Bulk functioning	**Mean**	**Mean**	**F_{Time}**	**P_{Time}**	**F_{DOM}**	**P_{DOM}**	**Slope**	**Slope**	**n**
Enzymatic activity	0.6	0.3	3.15	**0.026***	18.26	**0.000*****	0.02	0.04	6
Heretotrophic production	0.4	0.2	0.57	0.723	11.87	**0.002***	-0.01	0.03	6
Respiration	1.7	1.4	3.02	**0.031***	0.41	0.527	-0.19	-0.60	6
BGE	0.9	0.4	3.30	**0.022***	6.12	**0.021***	-0.24	-0.15	6

*Indicates P-values <0.05
***Indicates P-values<0.001

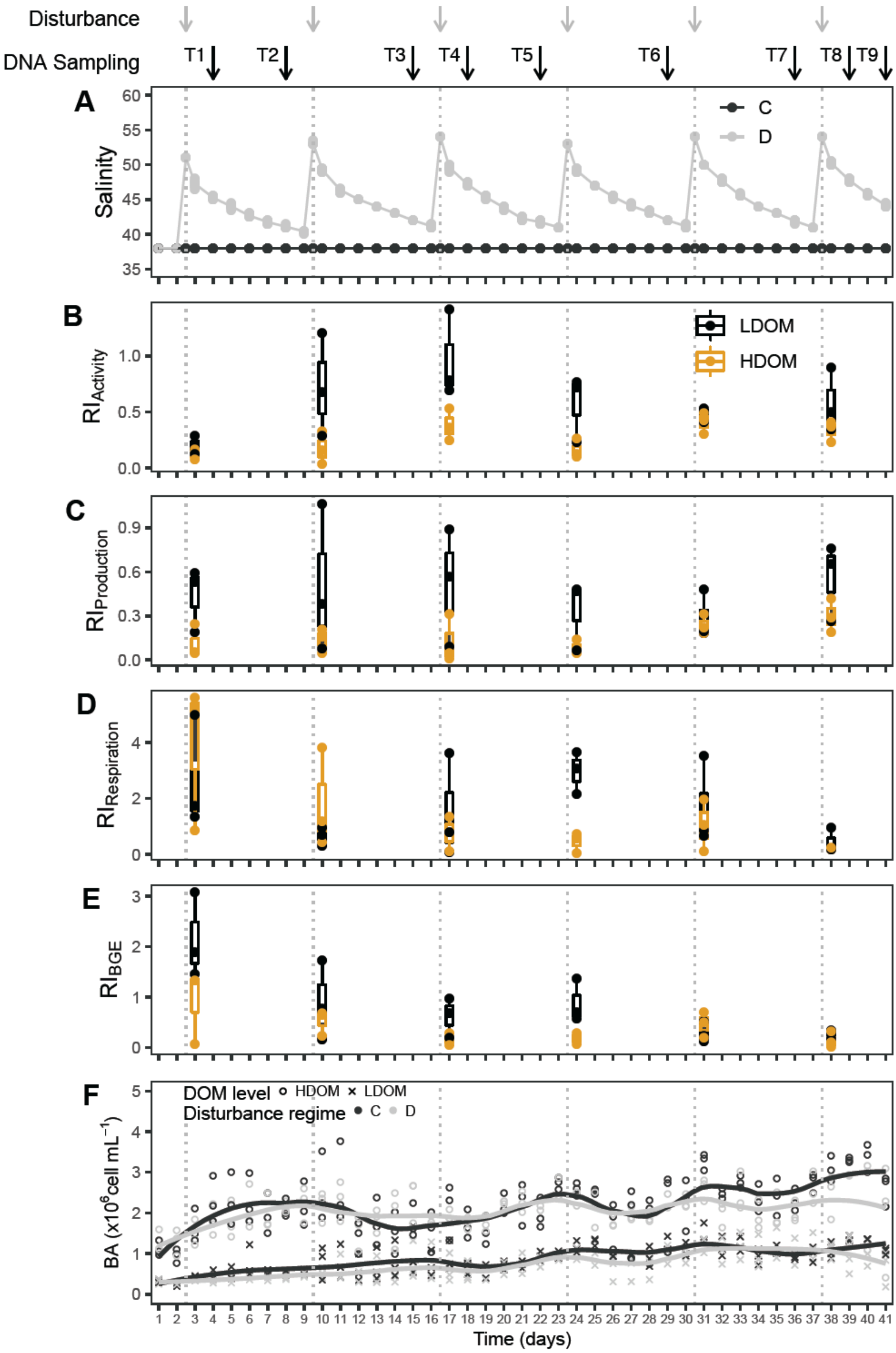

Figure 2. Pulse disturbance salinity regime and community functioning parameters *A) Salinity in the continuous cultures. level. Dots represent the salinity measured in the individual replicates, black and grey lines correspond to the mean value per time point for the disturbed and control regimes, respectively. Boxplots of the RI and pulse disturbances for B) microbial activity C) heterotrophic production, D) community respiration and E) BGE. F) Bacterial abundance, lines indicate locally fitted values (loess smoothing) under each disturbance and DOM regime.*

HDOM promoted markedly higher bacterial abundances than LDOM (Fig. 2F). No significant differences in bacterial abundances were found between the control and disturbance treatments in the HDOM media (Table 4). In contrast, the disturbance treatment resulted in significantly lower bacterial abundances compared to the control at LDOM media ($P<0.001$; Table 4).

Species assembly

The strongest structuring factor for the phylogenetic community composition was the time ($F=60.49$; $P\leq0.001$) followed by the DOM supply ($F=9.89$, $P\leq0.001$) and then the disturbance regime ($F=3.68$; $P=0.009$), while none of the interaction effects were significant. This was illustrated in the community PCoA biplots, where a clear shift in the community composition was observed after 2 weeks, and also during later succession stages a rough clustering of samples according to the sample time was visible (Fig. 3A). The impact of the DOM or disturbance regimes was by far less obvious from visual inspection of the PCoA biplots (Fig. 3B). Very similar patterns were observed also for community composition patterns evaluated based on Bray-Curtis dissimilarities (Fig. S3).

Early stages during the succession were dominated by members of Flavobacteriales and Enterobacterales orders at LDOM, as well as Rhodobacterales under the HDOM regime (Fig. 3C-D). At intermediate succession stages, Rhodobacterales and Caulobacterales increased in abundance. At the experiment end diverse taxonomic structures were observed and depending on the culture vessel for instance members of the Caulobacterales, Sphingomonadales, Rhodospirillales, or Enterobacterales dominated the community.

While the effect of the disturbance regime on the community composition, although significant seemed to have a large stochastic compound, we detected a clearer impact of disturbances concerning diversity structures: Pulse disturbances triggered significantly higher diversity levels in the HDOM culture compared to the controls, while no significant trend was observed for the LDOM culture that however tended to exhibit lower diversity levels (Table 4, Fig. 3E).

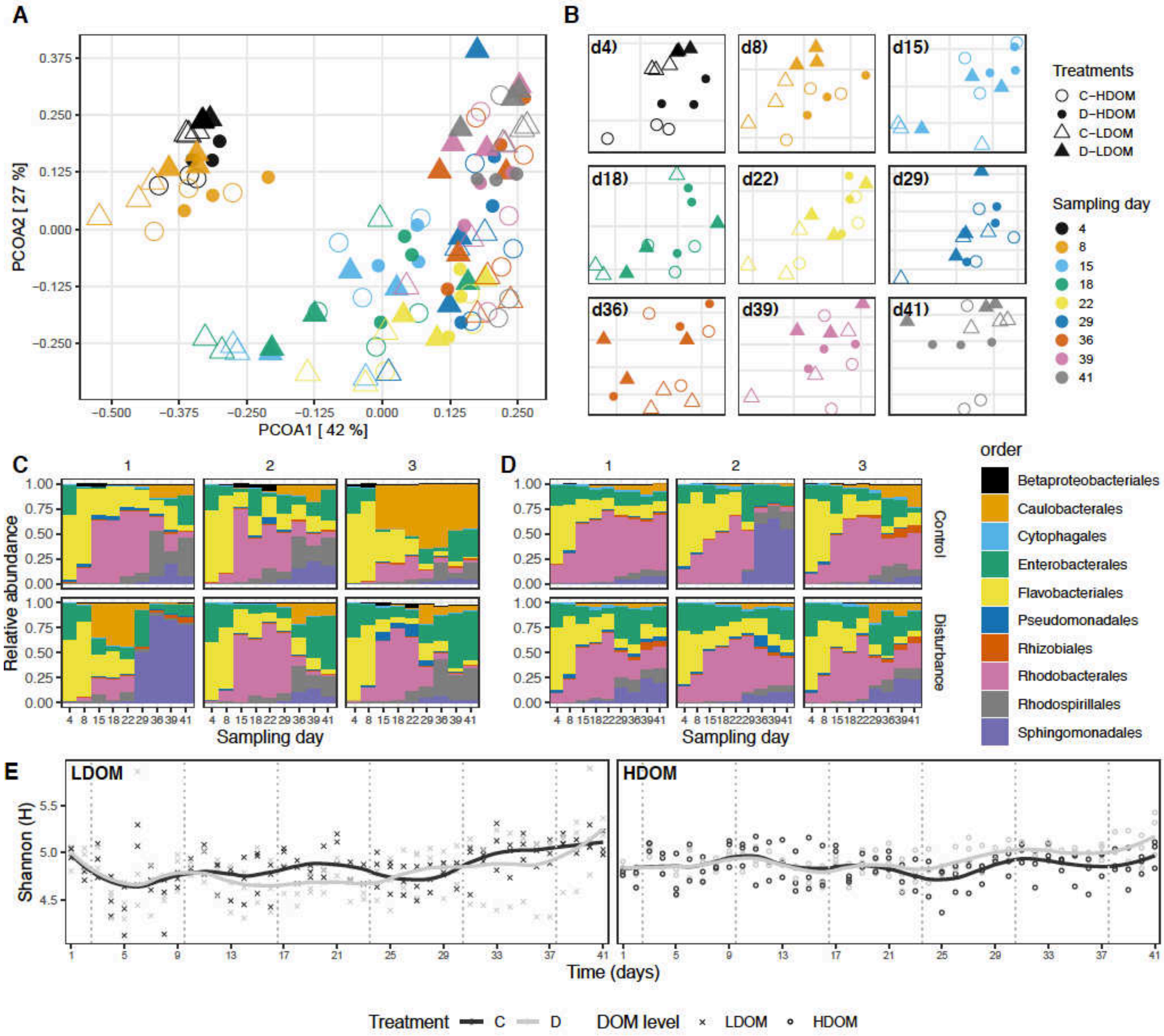

Figure 3. Community structure overview. *A) Overview PCoA biplot including all data points (weighted Unifrac distances). B) PCoAs (weighted Unifrac distances) for individual sampling days; axes of the individual plots are differently scaled as indicated by the grid lines. Relative abundance of the 10 most abundant orders in the initial communities as well as in the resuscitated communities at C) LDOM and D) HDOM levels at each sampling day. E) Daily Shannon diversity index; lines represented the locally fitted mean (loess smoothing) under each disturbance and DOM regime.*

Estimated NTIs for each sample time, were under both DOM regimes on average greater than 0 ($p<0.001$, Fig. 4). While we observed similar NTI distribution in response to the disturbance regime, the NTIs under HDOM were on average higher (0.98 ± 0.45) than under LDOM (0.55 ± 0.47).

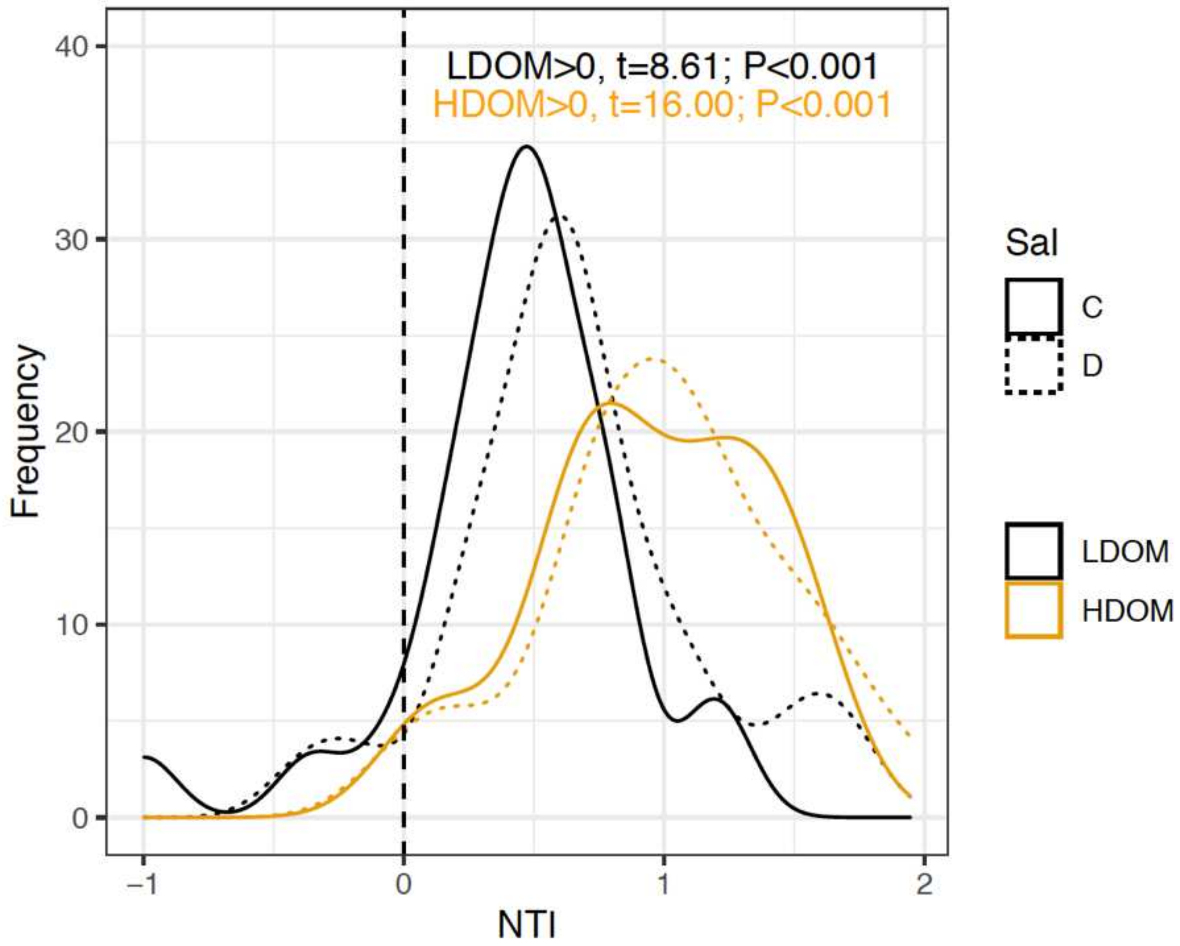

Figure 4. Nearest taxon indexes (NTI) estimated for the disturbance and DOM regimes in the continuous cultures. *Statistical values show the result of one-sample t-tests to reject the null hypothesis that NTI is equal to zero.*

Table 4. *Summary of the repeated measurements ANOVAs to test the effect of disturbance on community traits under the two DOM regimes. Mean Control/Dist represent the mean value of the respective trait for the control and disturbance treatment respectively.*

	LDOM	**HDOM**	**RM ANOVA$_{LDOM}$**				**RM ANOVA$_{HDOM}$**					
Community variables	**Mean Control/Dist**	**Mean Control/Dist**	**F_{Time}**	**P_{Time}**	**$F_{disturbance}$**	**$P_{disturbance}$**	**F_{Time}**	**P_{Time}**	**$F_{disturbance}$**	**$P_{disturbance}$**	**Dataset**	**n**
Bacterial abundance (x10^6)	0.86/0.72	2.18/2.05	286.00	**0.000*** **	20.90	**0.000*** **	64.03	**0.000*** **	2.25	0.135	FC	40
Shannon (H)	4.84/4.79	4.85/4.91	24.16	**0.000*** **	2.77	0.097	7.28	**0.007***	8.36	**0.004***	FC	40
RRN (Resilience)	2.00/2.25	1.90/2.15	19.07	**0.000*** **	5.98	**0.020***	23.32	**0.000*** **	10.64	**0.003***	16S rRNA	9
Generation time (Resilience)	4.75/4.35	4.31/4.08	28.28	**0.000*** **	10.57	**0.003***	8.74	**0.000*** **	3.93	0.056•	16S rRNA	9
%TF (Resistance)	2.77/2.78	2.75/2.82	3.59	**0.004***	0.08	0.779	2.50	**0.030***	4.45	**0.042***	16S rRNA	9
Genome size (Resistance)	3.85/3.85	3.82/3.87	4.71	**0.001***	0.01	0.908	12.65	**0.000*** **	3.51	0.070•	16S rRNA	9

•Indicates P-values <0.1
*Indicates P-values <0.05
***Indicates P-values<0.001

Genomic trait distributions

Both resilience and resistance-related traits changed over time throughout the experiment independently of the disturbance regime and DOM level (Fig. 5; Table 4). Furthermore, under both DOM conditions resilience-related traits changed significantly in response to the disturbance regime (Table 4). Disturbances induced significantly higher RRN and growth rates (i.e. shorter d) in the disturbed treatments compared to the controls (Table 4).

The resistance-related traits did not exhibit a significant increase in response to the disturbance regime in the LDOM level (Table 4). In contrast, the disturbed treatments featured a significantly greater %TF and genome size under HDOM conditions (Fig. 5; Table 4).

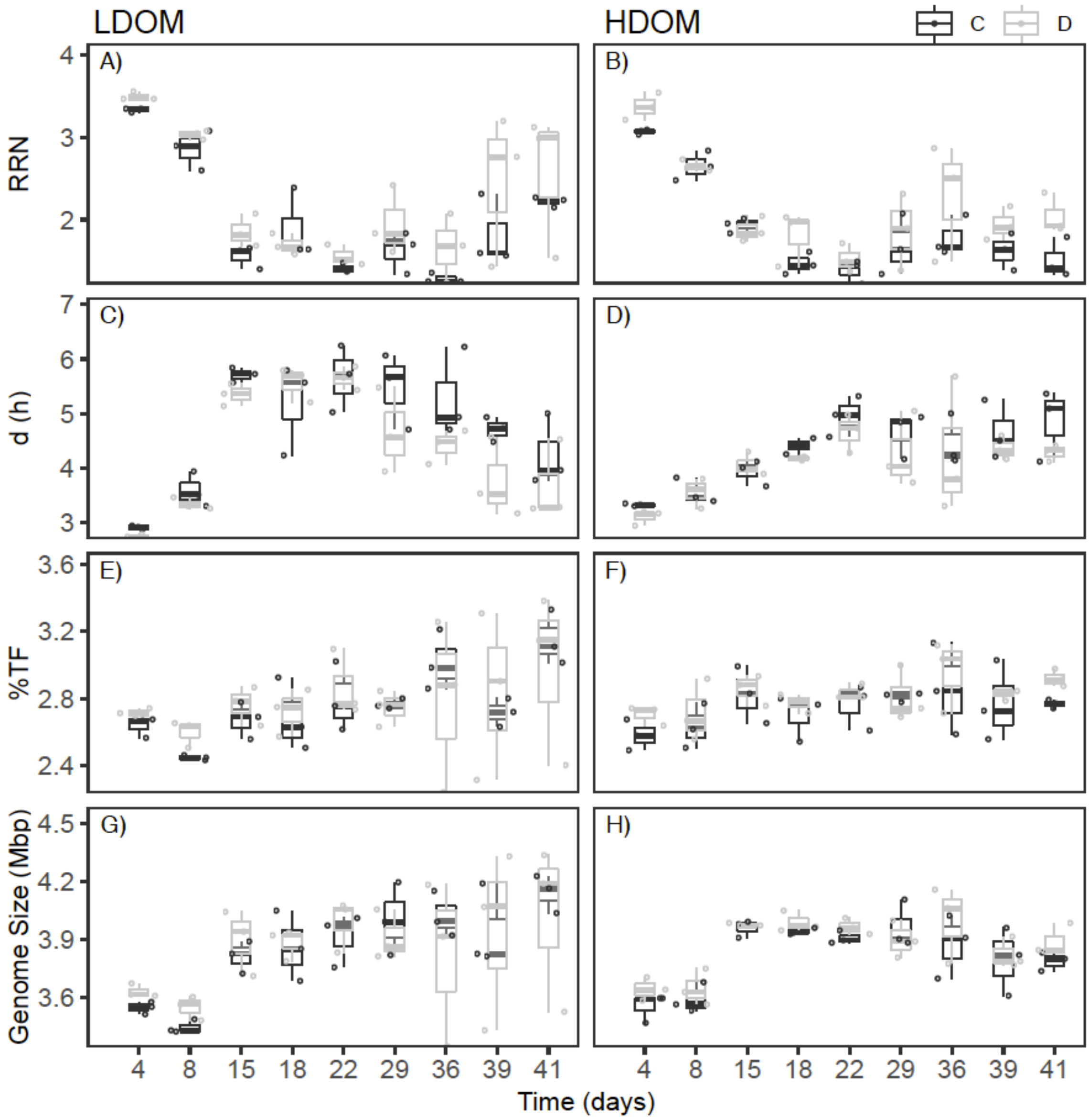

***Figure 5. Boxplots displaying CWMs of genomic traits**. A,B) RRN, C,D) generation time (d), E,F) %TF and G,H) genome size.*

DISCUSSION

Our study aimed to assess community assembly and succession of a highly diverse model microbial community under different disturbance regimes crossed with two resource regimes and the impact of selection processes on the community functional performance.

While we observed different successional stages in community composition during the experiment that were consistent across the treatments, treatment-specific effects were, although significant, by far less evident. Instead, replicate treatments exhibited mostly distinct communities. Long-term continuous cultures are generally prone to contaminations, which could be one explanation for the diverging development of replicates. However, although we sporadically detected contaminations, these were minor compared to cell concentrations in the vessels (<6.6%, Table S3) and are unlikely to explain the striking divergence in community composition among replicates from all treatments after the second experimental week (Fig. 3).

Results from an earlier continuous culture experiment suggested that higher species diversities in the starting communities led to a higher contribution of stochastic events during community assembly (Ayarza and Erijman, 2011). We, therefore, argue that the setup of our continuous culture with highly diverse initial communities induced stochastic events during the assembly processes and was the major reason for the observed divergent community composition among replicated treatments. In agreement with our observation concerning the divergence of replicate samples, we detected a low phylogenetic over-clustering, with NTIs around 0.5 or 1 in the LDOM and HDOM treatments respectively. Compared to other studies applying this metric and that detected positive NTIs up to 5.6 (Stegen et al., 2012; Evans and Wallenstein, 2014; Shen et al., 2018) our values were low and accordingly indicated a large contribution of stochastic events on the community assembly in this study, while also environmental filtering influenced the community assembly to a certain degree.

Strikingly, beyond the limited impact of the disturbance regime on the (phylogenetic) community compositional structures of individual taxa, pronounced and consistent effects were apparent on superordinate community structures, such as species diversity patterns, trait distributions, and ultimately also on the functional community characteristics.

In agreement with our hypothesis, we found that disturbances favored the simultaneous selection of genomic traits reflecting the life histories of fast-growing opportunists and traits reflecting the life history of generalists under high resource availability (HDOM). In contrast, under low resource availability (LDOM), only resilience, but not resistance-related genomic traits were significantly elevated in the disturbed treatments (Table 4). Our results confirm findings demonstrating the selection for bacterial generalist species with larger genomes and higher %TF under environmental fluctuations (Kostadinov et al., 2011; Bentkowski et al., 2015). Data from this study however additionally emphasize the existence of costs related to a generalist lifestyle: generalists with large genomes and elevated %TF had only competitive advantages if they were simultaneously exposed to pulsed disturbances and high resource availability. These findings support our hypothesis that the necessity for increased regulation under fluctuating environmental conditions is translated into energetic costs of organisms that by their physiological properties (i.e. large genomes and high %TF) are capable to adapt to variable environments.

The coinciding increase of resilience and resistance-related traits furthermore approves the recently posed suggestion that resistance and resilience of prokaryotic communities are commonly positively related in aquatic habitats, while they trade-off in soil environments (Beier et al., 2021). A simultaneous increase of resistance and resilience should according to Nimmo (2015) cause a species gain, and the detected increased species diversity, particularly in the disturbed HDOM incubations, supports also this hypothesis.

In agreement with our expectations, we did not only observe the selection of species with genomic traits that indicate a higher potential for resistance in the HDOM disturbances treatments. We simultaneously measured also higher functional resistance of microbial activity and production as well as BGE under HDOM compared to LDOM conditions (Fig. 2). It seems likely that the hypothesized and observed higher functional resistance in the HDOM compared to the LDOM cultures was a consequence of the increased prevalence of generalist species in HDOM conditions. Particularly, the reduced resistance of BGE in the LDOM compared to HDOM treatments may have caused reduced growth rates and accordingly lower cell numbers that were observed in the disturbed compared to the control LDOM treatments. The increase of BGE resistance levels over time in the disturbed LDOM incubations furthermore were reflected in the reduced growth mainly evident during the first 4 experimental weeks (Fig. 2F).

While we could establish a link between the CWM of resistance-related genomic traits with functional resistance, we did not explicitly measure functional resilience. We can therefore not experimentally prove that the observed distribution of resilience-related traits had functional consequences. Still, there is ample evidence from earlier literature that elevated RRN and codon usage biases are indeed associated with increased maximal growth rates and short lag phases, which in turn is linked to resilience (Klappenbach et al., 2000; Vieira-Silva and Rocha, 2010; Long et al., 2021; Weissman et al., 2021)

While not hypothesized, our data indicated a strong successional effect on community composition and trait distributions, that was independent of the treatments. The high prevalence of resilience-related traits during the early successional stages of microbial communities observed in our experiment verifies earlier findings (Nemergut et al., 2016). Notably, at the experiment end, RRN approached particularly in the HDOM control incubations values in the range of those measured in aquatic habitats (Gao and Wu, 2018). Although we only sporadically detected typically abundant marine oligotrophic bacteria such as members of the Pelagibacteriales, low RRN numbers could mean that the communities at the final successional stage feature similar properties to communities in natural aquatic habitats. Long-term continuous experiments could therefore be an appropriate tool to examine the dynamics of complex aquatic communities under controlled conditions.

CONCLUSION

Our study provides for the first time experimental evidence of the molecular mechanisms and the associated costs responsible for community stability under frequent disturbance events. Although our data point to a large contribution of stochastic events during community assembly at the species level, we found highly consistent patterns of superordinate community patterns and functional characteristics. Our findings, therefore, suggest the existence of different alternative compositional solutions with similar functional properties that originated from the same initial community.

Overall, the presented experimental study confirmed all tested hypotheses concerning the selection of tolerant species under frequently disturbed environments in dependency on resource availability. The fact that this seemed to be independent of small-scale patterns of the community composition at the species level even increases the generality of our findings. We believe that our experiment under controlled conditions shows that the dynamics of highly diverse microbial communities are generally predictable if focusing on trait distributions of life histories and diversity structures that are in turn pivotal parameters for community functioning.

ACKNOWLEDGMENTS

This study was financed by the German Science Foundation (DFG) granted SB (BE 5937/2-1). ARF was supported by a scholarship from the Chilean National Agency for Research and Development (ANID) / Scholarship Program / DOCTORADO BECAS CHILE/2017 – 72180448. GPM was supported by the doctorate scholarship program of the Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil, and the mentioned DFG funding. The bioinformatics work was supported by the BMBF-funded de.NBI Cloud within the German Network for Bioinformatics Infrastructure (de.NBI) (031A532B, 031A533A, 031A533B, 031A534A, 031A535A, 031A537A, 031A537B, 031A537C, 031A537D, 031A538A). We thank the Service d'Observation en Milieu Littoral (SOMLIT-Banyuls-sur-Mer) for providing data, and the facilities to collect water samples. We thank the BIO2MAR platform for the access and the technical support in their molecular biology facilities. We thank Olivier Crispy, Jenny Jeschek, and Madleen Dierken for their help in the analysis of dissolved organic compounds and nutrients. We also thank Fernanda González for supporting the sampling.

REFERENCES

Ackerly, D. D., and Cornwell, W. K. (2007). A trait-based approach to community assembly: partitioning of species trait values into within- and among-community components. Ecology Letters 10, 135–145. doi:https://doi.org/10.1111/j.1461-0248.2006.01006.x.

Adam, G., and Duncan, H. (2001). Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. Soil Biol. Biochem. 33, 943–951. doi:10.1016/S0038-0717(00)00244-3.

Aminot, A., and Kérouel, R. (2007). Dosage automatique des nutriments dans les eaux marines: méthodes en flux continu. Editions Quae.

Ayarza, J. M., and Erijman, L. (2011). Balance of Neutral and Deterministic Components in the Dynamics of Activated Sludge Floc Assembly. Microb. Ecol. 61, 486–495. doi:10.1007/s00248-010-9762-y.

Baho, D. L., Peter, H., and Tranvik, L. J. (2012). Resistance and resilience of microbial communities - temporal and spatial insurance against perturbations. Environ. Microbiol. 14, 2283–2292. doi:10.1111/j.1462-2920.2012.02754.x.

Bardgett, R. D., and Caruso, T. (2020). Soil microbial community responses to climate extremes: resistance, resilience and transitions to alternative states. Philosophical Transactions of the Royal Society B: Biological Sciences 375, 20190112. doi:10.1098/rstb.2019.0112.

Battin, T. J. (1997). Assessment of fluorescein diacetate hydrolysis as a measure of total esterase activity in natural stream sediment biofilms. Sci. Total Environ. 198, 51–60. doi:10.1016/S0048-9697(97)05441-7.

Beier, S., Werner, J., Bouvier, T., Mouquet, N., and Violle, C. (2021). Trait-trait relationships and functional tradeoffs vary with genome size in prokaryotes. *bioRxiv*, 2021.07.23.453341. doi:10.1101/2021.07.23.453341.

Bell, T. H., and Bell, T. (2021). Many roads to bacterial generalism. FEMS Microbiology Ecology 97. doi:10.1093/femsec/fiaa240.

Bentkowski, P., Van Oosterhout, C., and Mock, T. (2015). A Model of Genome Size Evolution for Prokaryotes in Stable and Fluctuating Environments. Genome Biol. Evol. 7, 2344–2351. doi:10.1093/gbe/evv148.

Callahan, B. J., Mcmurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2 : High-resolution sample inference from Illumina amplicon data. 13. doi:10.1038/nmeth.3869.

Cauwet, G. (1994). HTCO method for dissolved organic carbon analysis in seawater: influence of catalyst on blank estimation. Marine Chemistry 47, 55–64. doi:10.1016/0304-4203(94)90013-2.

Cornwell, W. K., and Ackerly, D. D. (2009). Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. Ecological Monographs 79, 109–126. doi:https://doi.org/10.1890/07-1134.1.

Dall, S., and Cuthill, I. (1997). The Information Costs of Generalism. Oikos 80, 197–202. doi:10.2307/3546535.

DeWitt, T. J., Sih, A., and Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. Trends in Ecology & Evolution 13, 77–81. doi:10.1016/S0169-5347(97)01274-3.

Douglas, G. M., Maffei, V. J., Zaneveld, J. R., Yurgel, S. N., Brown, J. R., Taylor, C. M., et al. (2020). PICRUSt2 for prediction of metagenome functions. Nature Biotechnology 38, 685–688. doi:10.1038/s41587-020-0548-6.

Eguchi, M., Nishikawa, T., Macdonald, K., Cavicchioli, R., Gottschal, J. C., and Kjelleberg, S. (1996). Responses to Stress and Nutrient Availability by the Marine

Ultramicrobacterium Sphingomonas sp. Strain RB2256. Appl. Environ. Microbiol. 62, 1287.

Enquist, B. J., Norberg, J., Bonser, S. P., Violle, C., Webb, C. T., Henderson, A., et al. (2015). "Chapter Nine - Scaling from Traits to Ecosystems: Developing a General Trait Driver Theory via Integrating Trait-Based and Metabolic Scaling Theories," in Advances in Ecological Research Trait-Based Ecology - From Structure to Function., eds. S. Pawar, G. Woodward, and A. I. Dell (Academic Press), 249–318. doi:10.1016/bs.aecr.2015.02.001.

Evans, S. E., and Wallenstein, M. D. (2012). Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? Biogeochemistry 109, 101–116. doi:10.1007/s10533-011-9638-3.

Evans, S. E., and Wallenstein, M. D. (2014). Climate change alters ecological strategies of soil bacteria. Ecology Letters 17, 155–164. doi:https://doi.org/10.1111/ele.12206.

Fierer, N., Bradford, M. A., and Jackson, R. B. (2007). Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364. doi:10.1890/05-1839.

Gao, Y., and Wu, M. (2018). Free-living Bacterial Communities Are Mostly Dominated by Oligotrophs. bioRxiv, 350348. doi:10.1101/350348.

Griffiths, B. S., and Philippot, L. (2013). Insights into the resistance and resilience of the soil microbial community. FEMS Microbiology Reviews 37, 112–129. doi:10.1111/j.1574-6976.2012.00343.x.

Hall, J. P. J., Harrison, E., and Brockhurst, M. A. (2018). Competitive species interactions constrain abiotic adaptation in a bacterial soil community. Evolution Letters 2, 580–589. doi:https://doi.org/10.1002/evl3.83.

Hedges, L. V., Gurevitch, J., and Curtis, P. S. (1999). The Meta-Analysis of Response Ratios in Experimental Ecology. Ecology 80, 1150–1156. doi:10.2307/177062.

Hillebrand, H., and Gurevitch, J. (2016). "Meta-Analysis and Systematic Reviews in Ecology," in eLS, ed.John Wiley & Sons Ltd (Chichester, UK: John Wiley & Sons, Ltd), 1–11. doi:10.1002/9780470015902.a0003272.pub2.

Holmes, R. M., Aminot, A., Kérouel, R., Hooker, B. A., and Peterson, B. J. (1999). A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Canadian Journal of Fisheries and Aquatic Sciences. doi:10.1139/f99-128.

Kassen, R., and Bell, G. (1998). Experimental evolution in Chlamydomonas . IV. Selection in environments that vary through time at different scales. Heredity 80, 732–741. doi:10.1046/j.1365-2540.1998.00329.x.

Kirchman, D. (1993). Handbook of Methods in Aquatic Microbial Ecology.

Klappenbach, J. A., Dunbar, J. M., and Schmidt, T. M. (2000). RRNA operon copy number reflects ecological strategies of bacteria. Appl. Environ. Microbiol. 66, 1328–1333. doi:10.1128/AEM.66.4.1328-1333.2000.

Kostadinov, I., Kottmann, R., Ramette, A., Waldmann, J., Buttigieg, P. L., and Glöckner, F. O. (2011). Quantifying the effect of environment stability on the transcription factor repertoire of marine microbes. Microbial Informatics and Experimentation 1, 9. doi:10.1186/2042-5783-1-9.

Lake, P. S. (2000). Disturbance, patchiness, and diversity in streams. Journal of the North American Benthological Society 19, 573–592. doi:10.2307/1468118.

Landa, M., Cottrell, M. T., Kirchman, D. L., Kaiser, K., Medeiros, P. M., Tremblay, L., et al. (2013). Phylogenetic and structural response of heterotrophic bacteria to dissolved organic matter of different chemical composition in a continuous culture study. Environmental Microbiology, n/a-n/a. doi:10.1111/1462-2920.12242.

Long, A. M., Hou, S., Ignacio-Espinoza, J. C., and Fuhrman, J. A. (2021). Benchmarking microbial growth rate predictions from metagenomes. ISME J. 15, 183–195. doi:10.1038/s41396-020-00773-1.

Magalhães, S., Blanchet, E., Egas, M., and Olivieri, I. (2009). Are adaptation costs necessary to build up a local adaptation pattern? BMC Evolutionary Biology 9, 182. doi:10.1186/1471-2148-9-182.

Marie, D., Simon, N., Guillou, L., Partensky, F., and Vaulot, D. (2000). "Flow Cytometry Analysis of Marine Picoplankton," in In Living Color: Protocols in Flow Cytometry and Cell Sorting Springer Lab Manuals., eds. R. A. Diamond and S. Demaggio (Berlin, Heidelberg: Springer), 421–454. doi:10.1007/978-3-642-57049-0_34.

Morán, X. A. G., Baltar, F., Carreira, C., and Lønborg, C. (2020). Responses of physiological groups of tropical heterotrophic bacteria to temperature and dissolved organic matter additions: food matters more than warming. Environmental Microbiology 22, 1930–1943. doi:10.1111/1462-2920.15007.

Nemergut, D. R., Knelman, J. E., Ferrenberg, S., Bilinski, T., Melbourne, B., Jiang, L., et al. (2016). Decreases in average bacterial community rRNA operon copy number during succession. ISME J. 10, 1147–1156. doi:10.1038/ismej.2015.191.

Nimmo, D. G., Nally, R. M., Cunningham, S. C., Haslem, A., and Bennett, A. F. (2015). Vive la résistance: reviving resistance for 21st century conservation. Trends in Ecology & Evolution 30, 516–523. doi:10.1016/j.tree.2015.07.008.

Ning, D., Yuan, M., Wu, L., Zhang, Y., Guo, X., Zhou, X., et al. (2020). A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming. Nature Communications 11, 4717. doi:10.1038/s41467-020-18560-z.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2019). vegan: Community Ecology Package. R package version 2.5-6. Available at: https://CRAN.R-project.org/package=vegan.

Osenberg, C. W., Sarnelle, O., and Cooper, S. D. (1997). Effect Size in Ecological Experiments: The Application of Biological Models in Meta-Analysis. The American Naturalist 150, 798–812. doi:10.1086/286095.

Parada, A. E., Needham, D. M., and Fuhrman, J. A. (2016). Every base matters : assessing small subunit rRNA primers for marine microbiomes with mock communities , time series and global field samples. 18, 1403–1414. doi:10.1111/1462-2920.13023.

Parks, D. H., Chuvochina, M., Waite, D. W., Rinke, C., Skarshewski, A., Chaumeil, P.-A., et al. (2018). A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nature Biotechnology 36, 996–1004. doi:10.1038/nbt.4229.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team (2020). {nlme}: Linear and Nonlinear Mixed Effects Models. Available at: https://CRAN.R-project.org/package=nlme.

Planton, S., Déqué, M., Chauvin, F., and Terray, L. (2008). Expected impacts of climate change on extreme climate events. Comptes Rendus Geoscience 340, 564–574. doi:10.1016/j.crte.2008.07.009.

Pujo-Pay, M., and Raimbault, P. (1994). Improvement of the wet-oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters. Marine Ecology Progress Series 105, 203–207.

R Core Team (2018). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing Available at: https://www.R-project.org/.

Rain-Franco, A., de Moraes, G. P., and Beier, S. (2021). Cryopreservation and Resuscitation of Natural Aquatic Prokaryotic Communities. Frontiers in Microbiology 11, 3633. doi:10.3389/fmicb.2020.597653.

Roller, B. R. K., Stoddard, S. F., and Schmidt, T. M. (2016). Exploiting rRNA operon copy number to investigate bacterial reproductive strategies. Nat Microbiol 1, 1–7. doi:10.1038/nmicrobiol.2016.160.

Rubbens, P., Props, R., Kerckhof, F.-M., Boon, N., and Waegeman, W. (2021). PhenoGMM: Gaussian Mixture Modeling of Cytometry Data Quantifies Changes in Microbial Community Structure. mSphere 6. doi:10.1128/mSphere.00530-20.

Rykiel, E. J. (1985). Towards a definition of ecological disturbance. Australian Journal of Ecology 10, 361–365. doi:https://doi.org/10.1111/j.1442-9993.1985.tb00897.x.

Schimel, J., Balser, T. C., and Wallenstein, M. (2007). Microbial Stress-Response Physiology and Its Implications for Ecosystem Function. Ecology 88, 1386–1394. doi:https://doi.org/10.1890/06-0219.

Shade, A., Peter, H., Allison, S. D., Baho, D. L., Berga, M., Buergmann, H., et al. (2012). Fundamentals of microbial community resistance and resilience. Front. Microbiol. 3, 417. doi:10.3389/fmicb.2012.00417.

Shen, D., Juergens, K., and Beier, S. (2018). Experimental insights into the importance of ecologically dissimilar bacteria to community assembly along a salinity gradient. Environ. Microbiol. 20, 1170–1184. doi:10.1111/1462-2920.14059.

Smith, D., and Azam, F. (1992). A simple economical method for measuring bacterial protein synthesis rates in seawater using 3H-Leucine. Marine Microbial Food Webs 6, 107–114.

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

Vieira-Silva, S., and Rocha, E. P. C. (2010). The Systemic Imprint of Growth and Its Uses in Ecological (Meta) Genomics. PLoS Genet. 6, e1000808. doi:10.1371/journal.pgen.1000808.

Wanderley, B. M. S., A. Araújo, D. S., Quiroga, M. V., Amado, A. M., Neto, A. D. D., Sarmento, H., et al. (2019). flowDiv: a new pipeline for analyzing flow cytometric diversity. BMC Bioinformatics 20, 274. doi:10.1186/s12859-019-2787-4.

Webb, C. O. (2000). Exploring the Phylogenetic Structure of Ecological Communities: An Example for Rain Forest Trees. The American Naturalist 156, 145–155. doi:10.1086/303378.

Weissman, J. L., Hou, S., and Fuhrman, J. A. (2021). Estimating maximal microbial growth rates from cultures, metagenomes, and single cells via codon usage patterns. PNAS 118. doi:10.1073/pnas.2016810118.

Westoby, M., Gillings, M. R., Madin, J. S., Nielsen, D. A., Paulsen, I. T., and Tetu, S. G. (2021). Trait dimensions in bacteria and archaea compared to vascular plants. Ecology Letters n/a. doi:https://doi.org/10.1111/ele.13742.

Williams, P. J. le B., and Giorgio, P. A. del (2005). Respiration in aquatic ecosystems: history and background. Oxford University Press Available at: https://oxford.universitypressscholarship.com/view/10.1093/acprof:oso/9780198527084.001.0001/acprof-9780198527084-chapter-1 [Accessed June 8, 2021].

Yachi, S., and Loreau, M. (1999). Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. Proc. Natl. Acad. Sci. U. S. A. 96, 1463–1468. doi:10.1073/pnas.96.4.1463.

SUPPLEMENTARY MATERIALS

Table S1. *Summary sampling sites for DOM supplements the chemostat experiment.*

Location	Latitude (°N)	Longitude (°E)	Depth (m)	Sampling date	Salinity (PSU) (min-max)	Chlo-α (μg L^{-1}) mean±sd[a]
Gruissan Lagoon[1]	43° 06.7'	04° 28.9'	1	25/05/2019	17.80-48.30	0.30-24.48
La Palme Lagoon[1]	42° 57.2'	03° 03.4'	1	25/05/2019	7.50-57.20	0.16-14.02
MOLA station[2]	42° 27.2'	03° 41.3'	1	21/06/2018	37.5-38.2	0.10-0.90
Canet Lagoon[3]	42° 40.0'	03° 01.0'	1	05/10/2019	2.20–43.00	1.09-125.76
Baltic Warnow River[4]	54° 10.2'	12° 06.3'	1	10/08/2017	5.00–18.00	18.00-65.00
Baltic Coastal[5]	54° 12.5'	12° 05.9'	1	10/08/2017	5.08-26.81	

[1]https://wwz.ifremer.fr/surval

[2]Gonzalez M-L, Blain S, Obernosterer I. Seasonal freshening of NW Mediterranean surface water impacts microbial heterotrophic activity and dissolved organic matter. Estuarine, Coastal and Shelf Science 2019; 230: 106448.

[3]Rain-Franco A, de Moraes GP, Beier S. Cryopreservation and Resuscitation of Natural Aquatic Prokaryotic Communities. Frontiers in Microbiology 2021; 11: 3633.

[4]Freese HM, Görs S, Karsten U, Schumann R. Dissolved inorganic nutrients and organic substrates in the River Warnow (North-Eastern Germany) – Utilisation by bacterioplankton. Limnologica 2007; 37: 264–277.

[5]Feistel R, Weinreben S, Wolf H, Seitz S, Spitzer P, Adel B, et al. Density and Absolute Salinity of the Baltic Sea 2006–2009. Ocean Science 2010; 6: 3–24.

Table S2. *Summary sampling frequency in the continuous culture experiments.*

Week	Day	Date	Salinity	FC	Pulse disturbance	Heterotrophic production	Microbial activity	Bulk respiration	DNA samples	Exchange tubing system	FC tubing contamination control
1	1	24/09/2019	x	x							
	2	25/09/2019	x	x	x	x	x	x			
	3	26/09/2019	x	x						x	x
	4	27/09/2019	x	x					x		
	5	28/09/2019	x	x							
	6	29/09/2019	x	x							
2	7	30/09/2019	x	x						x	
	8	01/10/2019	x	x					x		
	9	02/10/2019	x	x	x	x	x	x			
	10	03/10/2019	x	x							x
	11	04/10/2019	x	x						x	
	12	05/10/2019	x	x							
	13	06/10/2019	x	x							
3	14	07/10/2019	x	x							
	15	08/10/2019	x	x					x	x	
	16	09/10/2019	x	x	x	x	x	x			
	17	10/10/2019	x	x							x
	18	11/10/2019	x	x							
	19	12/10/2019	x	x					x	x	
	20	13/10/2019	x	x							
4	21	14/10/2019	x	x							
	22	15/10/2019	x	x					x		
	23	16/10/2019	x	x	x	x	x	x		x	
	24	17/10/2019	x	x							x
	25	18/10/2019	x	x							
	26	19/10/2019	x	x							
	27	20/10/2019	x	x						x	
5	28	21/10/2019	x	x							
	29	22/10/2019	x	x					x		x
	30	23/10/2019	x	x	x	x	x	x			
	31	24/10/2019	x	x						x	
	32	25/10/2019	x	x							
	33	26/10/2019	x	x							
	34	27/10/2019	x	x							
6	35	28/10/2019	x	x						x	
	36	29/10/2019	x	x					x		
	37	30/10/2019	x	x	x	x	x	x			
	38	31/10/2019	x	x							x
	39	01/11/2019	x	x					x	x	
	40	02/11/2019	x	x							
	41	03/11/2019	x	x					x		

Table S3. *Summary bacterial abundance (10^6 cell ml^{-1}) estimated via flow cytometry from the culture medium inflow and the continuous culture vessels.*

		Abundance Inflow											
Date	**Day**	**Vessel 1**	**Vessel 2**	**Vessel 3**	**Vessel 4**	**Vessel 5**	**Vessel 6**	**Vessel 7**	**Vessel 8**	**Vessel 9**	**Vessel 10**	**Vessel 11**	**Vessel 12**
26/09/2019	3	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
03/10/2019	10	0.03	-0.01	0.00	-0.01	0.06	0.00	0.04	0.00	0.00	-0.01	0.00	0.01
10/10/2019	17	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.00	0.00
17/10/2019	24	0.06	-0.10	-0.07	-0.11	-0.08	-0.10	0.03	-0.03	-0.03	-0.04	-0.04	-0.02
24/10/2019	31	0.01	0.03	0.01	0.02	0.02	0.02	0.07	0.01	0.02	0.07	0.00	0.00
31/10/2019	38	-0.01	0.00	0.00	0.00	0.00	0.08	0.00	0.09	-0.01	0.01	0.00	-0.01
		Abundance Vessel											
26/09/2019	3	0.43	0.33	0.45	0.40	0.43	0.35	2.10	1.83	1.47	1.33	1.31	1.20
03/10/2019	10	1.13	0.41	0.35	0.51	0.93	0.64	2.24	2.35	3.51	2.47	1.88	1.74
10/10/2019	17	0.74	0.35	0.62	0.48	1.33	1.01	2.30	1.86	2.62	2.09	1.31	1.85
17/10/2019	24	1.12	1.06	1.03	0.86	1.32	1.14	2.72	2.47	2.58	2.18	2.40	2.50
24/10/2019	31	1.27	1.02	1.76	1.36	1.33	1.34	3.33	2.78	3.42	2.70	3.05	2.28
31/10/2019	38	0.89	0.85	1.29	1.22	1.00	1.16	2.77	1.83	3.04	2.86	3.39	2.64
		% of the abundance Inflow/Vessel											
26/09/2019	3	2.17	2.66	0.46	0.12	-0.43	-0.27	0.15	1.85	0.02	0.16	0.16	0.15
03/10/2019	10	3.01	-3.11	-0.92	-2.37	6.15	0.58	1.94	-0.10	0.03	-0.27	0.20	0.33
10/10/2019	17	-0.12	-0.46	0.18	-0.70	-0.36	-0.22	0.59	-0.10	-0.09	0.72	-0.05	-0.11
17/10/2019	24	5.47	-9.02	-6.44	-13.31	-5.72	-9.01	1.28	-1.07	-0.99	-1.94	-1.75	-0.60
24/10/2019	31	0.80	2.52	0.78	1.16	1.70	1.64	2.09	0.45	0.66	2.45	0.01	0.15
31/10/2019	38	-1.23	-0.42	0.07	0.25	0.04	6.61	0.17	4.66	-0.19	0.19	-0.07	-0.29

Table S4. *Community weighted mean NSTI by sample*

Day	Replicate	DOM level	Treatment	Community weighted mean NSTI
4	1	HDOM	Control	0.011
8	1	HDOM	Control	0.021
15	1	HDOM	Control	0.079
18	1	HDOM	Control	0.089
22	1	HDOM	Control	0.086
29	1	HDOM	Control	0.069
36	1	HDOM	Control	0.069
39	1	HDOM	Control	0.074
41	1	HDOM	Control	0.065
4	2	HDOM	Control	0.007
8	2	HDOM	Control	0.018
15	2	HDOM	Control	0.079
18	2	HDOM	Control	0.103
22	2	HDOM	Control	0.082
29	2	HDOM	Control	0.090
36	2	HDOM	Control	0.048
39	2	HDOM	Control	0.059
41	2	HDOM	Control	0.077
4	3	HDOM	Control	0.011
8	3	HDOM	Control	0.023
15	3	HDOM	Control	0.101
18	3	HDOM	Control	0.116
22	3	HDOM	Control	0.095
29	3	HDOM	Control	0.047
36	3	HDOM	Control	0.020
39	3	HDOM	Control	0.017
41	3	HDOM	Control	0.015
4	1	LDOM	Control	0.005
8	1	LDOM	Control	0.005
15	1	LDOM	Control	0.016
18	1	LDOM	Control	0.019
22	1	LDOM	Control	0.019
29	1	LDOM	Control	0.039
36	1	LDOM	Control	0.037

39	1	LDOM	Control	0.069
41	1	LDOM	Control	0.025
4	2	LDOM	Control	0.004
8	2	LDOM	Control	0.005
15	2	LDOM	Control	0.044
18	2	LDOM	Control	0.049
22	2	LDOM	Control	0.060
29	2	LDOM	Control	0.054
36	2	LDOM	Control	0.052
39	2	LDOM	Control	0.045
41	2	LDOM	Control	0.039
4	3	LDOM	Control	0.003
8	3	LDOM	Control	0.004
15	3	LDOM	Control	0.028
18	3	LDOM	Control	0.033
22	3	LDOM	Control	0.050
29	3	LDOM	Control	0.061
36	3	LDOM	Control	0.068
39	3	LDOM	Control	0.077
41	3	LDOM	Control	0.053
4	1	HDOM	Disturbance	0.011
8	1	HDOM	Disturbance	0.028
15	1	HDOM	Disturbance	0.078
18	1	HDOM	Disturbance	0.075
22	1	HDOM	Disturbance	0.075
29	1	HDOM	Disturbance	0.082
36	1	HDOM	Disturbance	0.085
39	1	HDOM	Disturbance	0.066
41	1	HDOM	Disturbance	0.063
4	2	HDOM	Disturbance	0.009
8	2	HDOM	Disturbance	0.031
15	2	HDOM	Disturbance	0.098
18	2	HDOM	Disturbance	0.116
22	2	HDOM	Disturbance	0.087
29	2	HDOM	Disturbance	0.055
36	2	HDOM	Disturbance	0.059
39	2	HDOM	Disturbance	0.082

41	2	HDOM	Disturbance	0.065
4	3	HDOM	Disturbance	0.011
8	3	HDOM	Disturbance	0.024
15	3	HDOM	Disturbance	0.097
18	3	HDOM	Disturbance	0.100
22	3	HDOM	Disturbance	0.092
29	3	HDOM	Disturbance	0.063
36	3	HDOM	Disturbance	0.031
39	3	HDOM	Disturbance	0.050
41	3	HDOM	Disturbance	0.048
4	1	LDOM	Disturbance	0.005
8	1	LDOM	Disturbance	0.006
15	1	LDOM	Disturbance	0.017
18	1	LDOM	Disturbance	0.019
22	1	LDOM	Disturbance	0.022
29	1	LDOM	Disturbance	0.035
36	1	LDOM	Disturbance	0.068
39	1	LDOM	Disturbance	0.052
41	1	LDOM	Disturbance	0.028
4	2	LDOM	Disturbance	0.004
8	2	LDOM	Disturbance	0.005
15	2	LDOM	Disturbance	0.035
18	2	LDOM	Disturbance	0.048
22	2	LDOM	Disturbance	0.051
29	2	LDOM	Disturbance	0.019
36	2	LDOM	Disturbance	0.019
39	2	LDOM	Disturbance	0.019
41	2	LDOM	Disturbance	0.018
4	3	LDOM	Disturbance	0.004
8	3	LDOM	Disturbance	0.006
15	3	LDOM	Disturbance	0.018
18	3	LDOM	Disturbance	0.020
22	3	LDOM	Disturbance	0.024
29	3	LDOM	Disturbance	0.075
36	3	LDOM	Disturbance	0.055
39	3	LDOM	Disturbance	0.027
41	3	LDOM	Disturbance	0.028

Table S5. *Summary read counts from DADA2 pipeline.*

Sample ID	Day	DOM	Treatment	Replicate	Raw reads	Filtered reads	Denoised forward reads	Denoised reverse reads	Merged read pairs	Nonchimeric
C10-1-01	4	LDOM	C	1	43678	34108	34049	34080	33733	31087
C10-1-02	4	LDOM	D	1	31154	25100	25058	25080	24841	22652
C10-1-03	4	LDOM	C	2	41955	34378	34327	34342	34043	31162
C10-1-04	4	LDOM	D	2	47617	39475	39406	39451	39067	35563
C10-1-05	4	LDOM	C	3	42499	33841	33781	33818	33498	30436
C10-1-06	4	LDOM	D	3	20688	16747	16713	16736	16573	14911
C10-1-07	4	HDOM	C	1	38154	31071	31015	31041	30673	27876
C10-1-08	4	HDOM	D	1	34999	28388	28344	28365	28122	26420
C10-1-09	4	HDOM	C	2	34047	27283	27232	27258	26965	24462
C10-1-10	4	HDOM	D	2	20039	15911	15885	15898	15737	14432
C10-1-11	4	HDOM	C	3	27053	22098	22063	22083	21906	21101
C10-1-12	8	HDOM	D	3	47258	37984	37918	37957	37589	34383
C10-2-01	8	LDOM	C	1	35301	28523	28473	28490	28249	27443
C10-2-02	8	LDOM	D	1	35163	28108	28054	28074	27840	26407
C10-2-03	8	LDOM	C	2	36119	30052	29999	30032	29835	28370
C10-2-04	8	LDOM	D	2	45120	36883	36821	36861	36613	35274
C10-2-05	8	LDOM	C	3	37718	29430	29365	29400	29154	27848
C10-2-06	8	LDOM	D	3	28050	22558	22520	22535	22361	20768
C10-2-07	8	HDOM	C	1	39808	31813	31763	31788	31343	27434
C10-2-08	8	HDOM	D	1	25518	20532	20507	20520	20342	18698
C10-2-09	8	HDOM	C	2	57365	44493	44404	44456	43902	38874
C10-2-10	8	HDOM	D	2	55407	47077	46979	47018	46310	40906
C10-2-11	8	HDOM	C	3	31235	25343	25297	25324	24958	21682
C10-2-12	8	HDOM	D	3	42706	34321	34260	34283	33782	29339
C10-3-01	15	LDOM	C	1	59514	45794	45761	45739	45208	42890
C10-3-02	15	LDOM	D	1	49835	42254	42218	42199	41892	39949
C10-3-03	15	LDOM	C	2	42231	34186	34145	34152	33865	31333
C10-3-04	15	LDOM	D	2	24528	16813	16800	16793	16634	14739
C10-3-05	15	LDOM	C	3	29708	22463	22429	22425	21952	18244
C10-3-06	15	LDOM	D	3	26745	21957	21923	21917	21459	18709
C10-3-07	15	HDOM	C	1	33166	26249	26216	26208	25708	20044
C10-3-08	15	HDOM	D	1	44758	34889	34846	34852	34371	29099
C10-3-09	15	HDOM	C	2	23547	17078	17060	17053	16741	13222
C10-3-10	15	HDOM	D	2	36921	30753	30691	30695	30118	24056
C10-3-11	15	HDOM	C	3	30125	22528	22483	22498	22122	18119
C10-3-12	15	HDOM	D	3	63792	53843	53787	53773	53211	45668
C10-4-01	18	LDOM	C	1	27083	20708	20693	20683	20441	19608

C10-4-02	18	LDOM	D	1	60702	51796	51747	51733	51309	48383
C10-4-03	18	LDOM	C	2	36534	28179	28156	28154	27884	25502
C10-4-04	18	LDOM	D	2	16399	13103	13093	13085	12934	11460
C10-4-05	18	LDOM	C	3	16637	12826	12805	12812	12565	9847
C10-4-06	18	LDOM	D	3	27676	21842	21827	21801	21537	19618
C10-4-07	18	HDOM	C	1	27089	21895	21867	21871	21611	18538
C10-4-08	18	HDOM	D	1	33686	27322	27289	27294	26933	22459
C10-4-09	18	HDOM	C	2	30771	24237	24201	24211	23727	18674
C10-4-10	18	HDOM	D	2	82847	65753	65657	65658	64718	55278
C10-4-11	18	HDOM	C	3	33746	27399	27360	27365	26990	22992
C10-4-12	18	HDOM	D	3	33896	26980	26965	26954	26691	23398
C10-5-01	22	LDOM	C	1	32933	26376	26349	26340	25989	23883
C10-5-02	22	LDOM	D	1	20593	16392	16386	16376	16240	15159
C10-5-03	22	LDOM	C	2	32377	26489	26471	26463	26190	23756
C10-5-04	22	LDOM	D	2	46673	36877	36857	36850	36654	33892
C10-5-05	22	LDOM	C	3	23641	18449	18430	18421	18030	15140
C10-5-06	22	LDOM	D	3	24048	18767	18742	18738	18374	16083
C10-5-07	22	HDOM	C	1	27736	22381	22358	22348	21999	18056
C10-5-08	22	HDOM	D	1	35788	28164	28127	28121	27682	23022
C10-5-09	22	HDOM	C	2	46493	34604	34547	34537	33619	25433
C10-5-10	22	HDOM	D	2	22727	18255	18230	18233	17881	14057
C10-5-11	22	HDOM	C	3	39265	31273	31233	31231	30843	26574
C10-5-12	22	HDOM	D	3	28005	22778	22762	22750	22523	19926
C10-6-01	29	LDOM	C	1	59947	46514	46476	46461	45874	41141
C10-6-02	29	LDOM	D	1	54880	46382	46351	46333	45977	42347
C10-6-03	29	LDOM	C	2	34792	27708	27688	27687	27380	23431
C10-6-04	29	LDOM	D	2	46214	36857	36841	36850	36647	32994
C10-6-05	29	LDOM	C	3	45729	34863	34839	34815	34379	29014
C10-6-06	29	LDOM	D	3	41045	34435	34361	34336	33599	26882
C10-6-07	29	HDOM	C	1	36190	29134	29101	29084	28626	23734
C10-6-08	29	HDOM	D	1	21476	16137	16128	16121	15991	14535
C10-6-09	29	HDOM	C	2	41124	31568	31514	31504	30852	24728
C10-6-10	29	HDOM	D	2	26080	21729	21698	21712	21481	18643
C10-6-11	29	HDOM	C	3	34601	27775	27757	27753	27539	24947
C10-6-12	29	HDOM	D	3	41431	33111	33093	33076	32809	29404
C10-7-01	36	LDOM	C	1	23237	17574	17554	17549	17186	13154
C10-7-02	36	LDOM	D	1	39240	33262	33216	33202	32658	25493
C10-7-03	36	LDOM	C	2	37099	28688	28665	28670	28348	24199
C10-7-04	36	LDOM	D	2	20999	16988	16981	16984	16905	15527
C10-7-05	36	LDOM	C	3	22055	16908	16889	16877	16493	12273
C10-7-06	36	LDOM	D	3	65617	55318	55223	55228	54527	46085

C10-7-07	36	HDOM	C	1	30867	23470	23427	23430	22853	17722
C10-7-08	36	HDOM	D	1	67476	57591	57453	57444	55897	44083
C10-7-09	36	HDOM	C	2	24886	19132	19080	19079	18400	13838
C10-7-10	36	HDOM	D	2	15982	12358	12333	12335	12058	9439
C10-7-11	36	HDOM	C	3	32742	26115	26092	26095	25853	22280
C10-7-12	36	HDOM	D	3	43391	33684	33629	33639	33098	27282
C10-8-01	38	LDOM	C	1	27122	21245	21206	21219	20858	16408
C10-8-02	38	LDOM	D	1	26702	20562	20542	20541	20344	17019
C10-8-03	38	LDOM	C	2	31975	25698	25669	25674	25330	21277
C10-8-04	38	LDOM	D	2	50916	40000	39982	39978	39846	37003
C10-8-05	38	LDOM	C	3	38677	28557	28508	28505	27807	20312
C10-8-06	38	LDOM	D	3	27784	21631	21618	21614	21419	18565
C10-8-07	38	HDOM	C	1	33537	26553	26511	26495	25897	20686
C10-8-08	38	HDOM	D	1	23653	18903	18878	18868	18553	15561
C10-8-09	38	HDOM	C	2	48693	37324	37253	37251	36387	29509
C10-8-10	38	HDOM	D	2	42394	35566	35484	35490	34736	28560
C10-8-11	38	HDOM	C	3	32293	25179	25164	25164	24969	21578
C10-8-12	38	HDOM	D	3	31621	25132	25112	25096	24802	21570
C10-9-01	41	LDOM	C	1	55766	42021	41957	41965	41267	32727
C10-9-02	41	LDOM	D	1	45767	37938	37889	37889	37377	30122
C10-9-03	41	LDOM	C	2	49536	39351	39287	39323	38780	31678
C10-9-04	41	LDOM	D	2	27160	21861	21857	21851	21769	20483
C10-9-05	41	LDOM	C	3	44770	33704	33662	33652	32936	26158
C10-9-06	41	LDOM	D	3	114566	89813	89732	89750	89171	79973
C10-9-07	41	HDOM	C	1	36008	28741	28695	28686	27966	21789
C10-9-08	41	HDOM	D	1	44185	34797	34763	34751	34352	30343
C10-9-09	41	HDOM	C	2	21978	16695	16652	16650	16074	12173
C10-9-10	41	HDOM	D	2	31241	26082	26010	26036	25348	19100
C10-9-11	41	HDOM	C	3	36606	27449	27420	27433	27056	22562
C10-9-12	41	HDOM	D	3	51069	43207	43143	43129	42319	35045

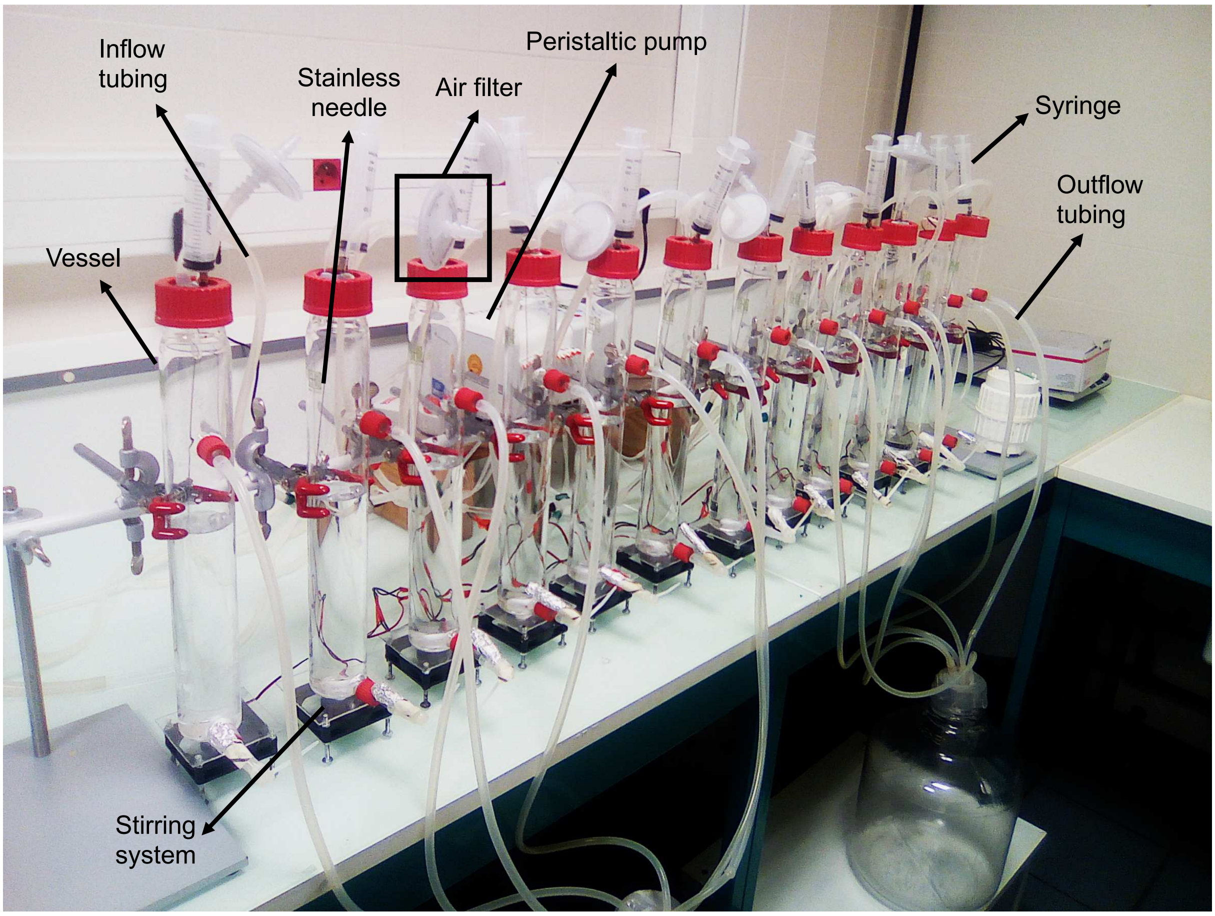

Figure S1. Continuous culture system with the incubation vessels 1-12 (from left to right) where arrows indicate the different parts of the continuous culture system. All odd-numbered vessels correspond to disturbed treatments, while the even-numbered vessels were control treatments. The vessels 1-6 were fed with LDOM and the vessels 7-12 were fed with HDOM. Every two vessels in the row (one disturbed vessel + one control vessel) were fed from the same inflow bottle.

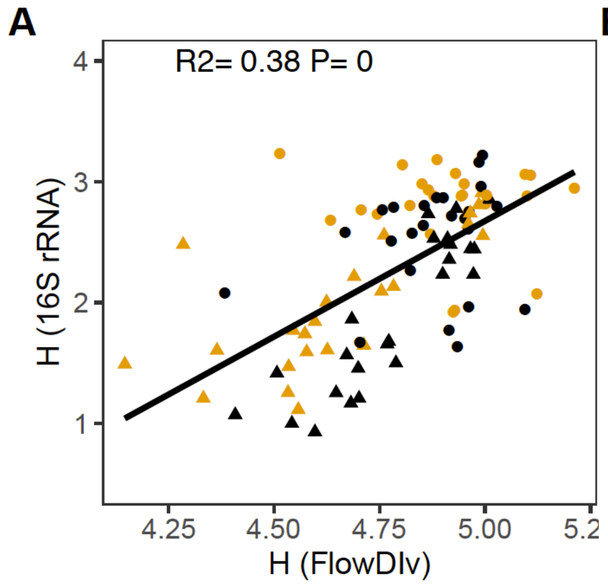

Figure S2. Comparison between the Shannon diversity indexes (H) that were obtained either from 16s rRNA gene amplicon samples or FC profiles. Latter were estimated from the same outflow water that was used also for DNA extraction. **A)** Regression of H (16s rRNA) against H estimated via the FlowDiv package (Wanderley et al., 2019) and **B)** H estimated using the PhenoGMM package (Rubbens et al., 2021). Triangles and circles represent the HDOM and LDOM treatment respectively. Black and orange symbols show the Control and Disturbed treatments.

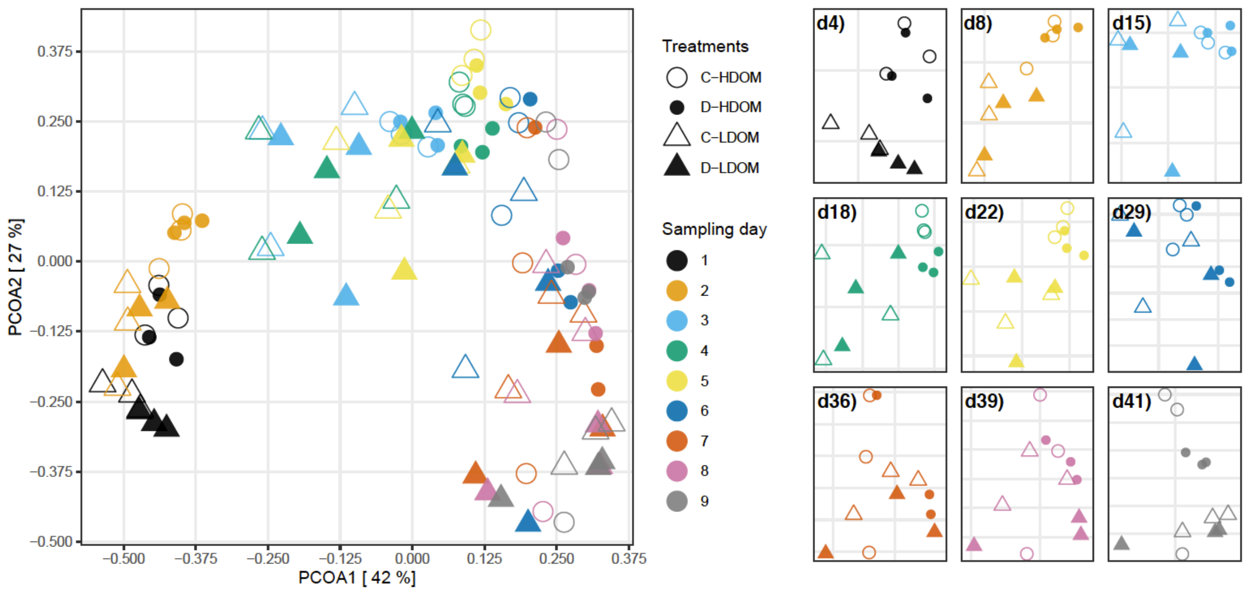

Figure S3. Overview PCoA biplot illustrating community composition (Bray-Curtis distance) and the subplots of the PCoA by the sampling day.

Chapter 4

Long-term exposition to pulse disturbances disrupts nitrate cycling

Rain-Franco Angel[1], Fernandez, Camila[1,2,34] & Sara Beier[1,5]

[1]UMR 7621 Laboratoire d'Océanographie Microbienne, Observatoire Océanologique de Banyuls-sur-Mer, Sorbonne Université, Banyuls-sur-Mer, France

[2]Interdisciplinary Center for Aquaculture Research (INCAR), University of Concepción, Concepción, Chile.

[3]COPAS SUR-AUSTRAL. Universidad de Concepción, Concepción, Chile.

[4]Research center Dynamic of High Latitude Marine Systems (IDEAL), Valdivia, Chile.

[5]Department of Biological Oceanography, Leibniz Institute for Baltic Sea Research Warnemünde, Rostock, Germany.

Manuscript in preparation

ABSTRACT

Microbial communities are the main drivers of nitrogen cycling, playing important roles in processes such as nitrification and denitrification. In this study we aimed to answer the questions, (i) does the frequent exposure of microbial communities to salinity pulse disturbances affect nitrogen cycling and if so, (ii) is this due to the selection of specific taxa involved in nitrogen cycling or rather to their activity independent on their presence or abundance? To answer these questions, we performed a long-term continuous culture experiment where pulse disturbed and non-disturbed control treatments with two different DOM substrates were combined in a full factorial design. We evaluated if the disturbance regime impacted the concentrations of nitrogen compounds in the culture media and whether this was linked to changes in the microbial community composition. We observed significant changes in nitrate net consumption under both DOM regimes after the exposure of several pulse disturbances. In contrast, the consumption or production of all other inspected N compounds was not significantly impacted by the disturbance regimes. The integration of advanced computational methods to decipher compositional changes, such as machine learning algorithms, have successfully been applied to microbial systems to reduce data dimensionality for predicting environmental parameters from community information. We could show via random forest (RF) based models that nitrate concentrations could be significantly predicated from community compositional data. This demonstrated that the differential selection of species under the different treatment conditions was an important driver of the observed nitrate net consumption rates.

INTRODUCTION

Nitrogen is one of the primary nutrients for organic molecules as such as nucleic acids, amino acids, and is essential for life on our planet as its concentration limits primary productivity in many ecosystems, including large parts of the oceans (Moore *et al.* 2013). The nitrogen cycle is composed of complex chemical transformations mediated by microorganisms. Nitrification is the oxidation of ammonium (NH_4^+), to nitrite (NO_2^-) and nitrate (NO_3^-) carried out by ammonia-oxidizing bacteria and archaea and nitrite-oxidizing bacteria (Kowalchuk and Stephen 2001), or complete ammonia oxidizers (Daims *et al.* 2015). Nitrogen is the one of the major nutrient for microbial life and nitrogen is transformed via assimilatory nitrate reduction to ammonium and incorporated into cells. Under anoxic or suboxic conditions, dissimilatory pathways transform NO_3^- via denitrification to N_2 or via the dissimilatory nitrate reduction (DNRA) pathway to ammonium (Fig. 1).

Coastal areas comprise diverse ecosystems which provide a multitude of important ecosystem services of high economical value, such as coastal protection from tides and tsunamis, fisheries and fish-farm activity, rich agricultural lands, areas of high touristic value, and nutrient cycling and carbon sequestration (Barbier *et al.* 2011). Coastal areas are exposed to a high level of natural environmental heterogeneity as well as anthropogenic impacts, such as the sea-level rise (Fagherazzi *et al.* 2019). In addition, the growth of human populations leads to an excess of N in coastal areas (Damashek and Francis 2018) causing eutrophication of numerous estuarine ecosystems (Herbert 1999). The estuaries, where denitrification in sediments can substantially contribute to the removal (20-50%) of nitrogen inputs, are crucial zones in terms of nitrogen cycling (Seitzinger 1988; Seitzinger *et al.* 2006): denitrification carried out in estuarine sediments or suspended particles is primarily responsible for controlling the magnitude of nitrogen that rivers deliver to the open oceans (Boyer and Howarth 2008).

In addition, pulse-like disturbance events, such as river floods and coastal storms are a natural attribute of coastal habitats that may affect the physiology of macro and microorganisms but also drive the colonization of habitats with new communities. Climate change scenarios predict the increasing occurrence of events, such as floods, and river run-offs, that impact salt concentrations (Seneviratne *et al.* 2012). While salt-related disturbances had contrasting effects on denitrification rates (Rysgaard *et al.* 1999; Magalhães *et al.* 2005; Wu, Tam and Wong 2008; Marks, Chambers and White 2016), it was shown that that the abundance of ammonium-oxidizers increased due to the simultaneous increase of temperature and the decrease of salinity due to precipitation (Horz *et al.* 2004).

The disturbance history, assessed as the exposure to previous disturbances can affect compositional and functional stability via acquired stress resistance (Renes *et al.* 2020). This may play a role in the important processes of the biogeochemical cycles. For instance, a comparison between sites exposed to different salt regimes (salt marsh vs fresh marsh) from the Mississippi River showed that intermediate salinity disturbances (15 ppt) may increase denitrification rates while more extreme changes (35 ppt) caused a decrease in measured rates (Marks, Chambers and White 2016). Indeed, previous studies demonstrated that multiple consecutively occurring disturbances were linked to successional compositional changes in microbial communities with consequences for organic matter cycling (Evans and Wallenstein 2012, 2014).

Microbial communities are the main drivers of nitrogen cycling, as several key processes for nitrogen cycling, such as nitrification and denitrification, are exclusively performed by prokaryotes. However, microbial communities are characterized by high compositional complexity and dynamics that are challenging to predict. The integration of advanced computational methods to decipher compositional changes, such as machine learning algorithms, have successfully been applied to microbial systems to reduce data dimensionality for predicting environmental parameters from community composition (e.g. Thompson *et al.* 2019). In this study, we attempted to explore potential relations between taxon turnover and the consumption or production of N-compounds following the exposition of microbial communities to several pulse disturbances. Thus, we aimed to answer the questions, (i) does the frequent exposure of microbial communities to salinity pulse disturbances affect nitrogen cycling and if so, (ii) is this due to the selection of specific taxa involved in nitrogen cycling or rather to their activity independent on their presence or abundance? We performed a long-term continuous culture experiment where pulse disturbed and non-disturbed control treatments were crossed with two different DOM substrates. To address these questions, we evaluated if the disturbance regime impacted the concentrations of nitrogen compounds in the culture media and whether this was linked to changes in the microbial community composition.

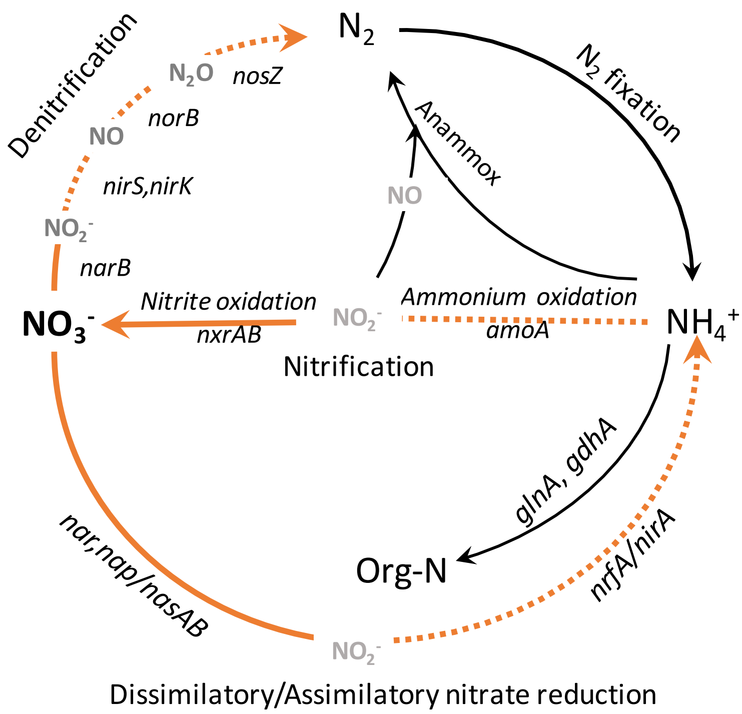

Figure 1. Overview of the nitrogen cycle. *Processes that impact nitrate balances are highlighted in orange, while the partial reactions that directly interact with nitrate changes are represented by solid lines and other partial reactions in dashed lines. Schema modified from Pajares and Ramos, 2019.*

METHODOLOGY

Setup of the continuous cultures system

The continuous culture experiment lasted 41 days and two disturbance regimes were combined with two nutrient levels in a full factorial design. The experimental setup is described in detail in Chapter III. In brief, culture media consisted of artificial seawater (ASW; the salinity of 38 g L^{-1} and pH 8; Eguchi *et al.*, 1996) that were amended with the two DOM supplements that served as an exclusive source for nitrogen: low DOM (hereafter: LDOM, DON 2.24 µM) and high DOM (hereafter: HDOM, DON 4.87 µM). DON levels in the HDOM medium resembled the average surface concentration of DON in the Western basin of the Mediterranean sea (Pujo-Pay *et al.* 2011).

The starting cultures were inoculated with cryopreserved communities from contrasting environmental conditions as detailed in Chapter III. The resuscitated microbial communities were incubated for 6 days at 18°C in batch culture mode, either in LDOM or HDOM media. Subsequently, the cultures were distributed in a continuous system. The four different treatments (LDOM-Disturbed, LDOM-Control; HDOM-Disturbed, HDOM-Control) were setup in triplicates, respectively.

We added two porous chips (polyethylene, Mutag BioChip 25™, MUTAG, Chemnitz, Germany) to each vessel to supply a potential surface for microbial biofilm formation. Biofilm on these chips served as a spatial refuge for microbial assemblages, because cells attached to it are not washed out in the continuous culture systems and may recolonize the medium. We decided to add these chips to lower the potential risk of a collapse of the continuous cultures during the long-term experiment. However, as these chips stimulate the formation of dense biofilms, they also promote the development of anoxic habitats, where reactions, such as denitrification can take place (Smriga, Ciccarese and Babbin 2021).

Pulse disturbances were applied once per week to the disturbance treatments by adding ~18 mL of a saturated NaCl solution after the same volume was removed from the reaction vessels (+13 gL^{-1} NaCl). In total, 6 pulse disturbances were applied during the experiment. Water samples (~200 mL) for DNA extractions were sampled weekly from the vessel outflow that was gathered during two consecutive days in sterile bottles (Fig. 2). Samples for chemical measurements were taken weekly directly from the incubation vessels, 3 days after the weekly DNA sampling events, except for the first DNA sampling, where chemical measurements were performed during the same day (Fig. 2). Samples to quantify bacterial abundance via flow cytometry (FC) were taken from outflow bottles used for DNA extraction as well as from the continuous culture vessels in parallel with the samples taken for chemical analyses (Fig. 2).

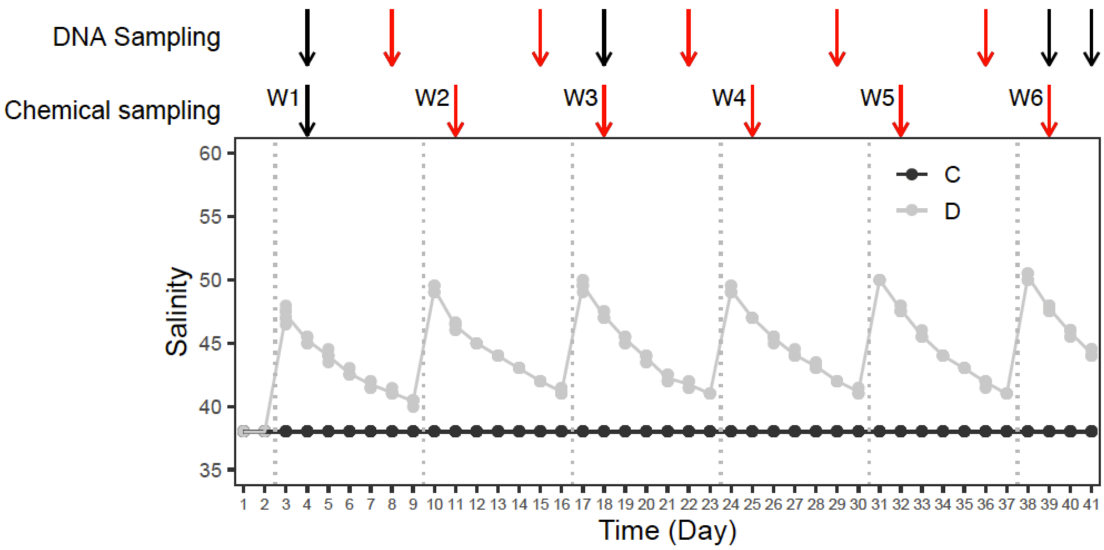

Figure 2. Salt pulse disturbances in the long-term continuous culture experiment. *Dots represent the salinity measured in the individual replicates, black and grey lines correspond to the mean value per time point for the Control and Disturbance regimes, respectively. Arrows indicate the sampling frequency for DNA and chemical variables. Red arrows represent the samples used in this study. W1-W6 indicate the experimental week 1 to week 6.*

Bacterial abundance and FC fingerprinting

The FC analyses were performed according to the standard protocol (Marie *et al.* 2000). In short, samples of 1350 µL were fixed with glutaraldehyde (0.1% final concentration) and stored at -80°C. All samples were analyzed in the Cytoflex Flow Cytometer (Beckman Coulter, California, USA) to obtain an estimate for cell counts. FC data that were sampled directly from the chemostat cultures were further analyzed by applying the R package "FlowDiv" (Wanderley *et al.* 2019) using the SSC-, FL1-H, and FL3-H channels to sort cells into phenotypic bins for community fingerprinting.

Chemical analyses

Concentrations of dissolved organic nitrogen (DON), nitrate, (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) were quantified in both sterile culture media as well as in the weekly sampled continuous cultures. Water samples were filtered through GFF filters (0.7 µm, 25 mm, Whatman, Buckinghamshire, UK). Samples for DON quantification (20 mL) were collected in autoclaved Teflon bottles and, stored at -20°C. DON was quantified using wet oxidation (Pujo-Pay and Raimbault 1994). NO_3^- and NO_2^- were determined by colorimetry (Bran Luebbe autoanalyzer; Aminot and Kérouel 2007), while NH_4^+ was measured by fluorometry (Holmes *et al.* 1999). Samples for the chemical analyses of the incubation media were taken one day after each pulse disturbance, while samples for DNA were taken 5 days after each pulse disturbance (Fig. 2).

DNA extraction and sequencing

DNA extraction, sequencing, and sequence processing were performed following the standard protocol as described in Chapter 3. In short, samples were taken for 16s rRNA gene amplicon sequencing (300 base pairs paired-end read, Illumina Miseq V3) using the primers pair 515yf-926r (Parada, Needham and Fuhrman 2016). Sequence processing was performed using the DADA2 software (Callahan *et al.* 2016) and applying the default settings (https://benjjneb.github.io/dada2/tutorial.html, truncLen=c(250,180)). In total 1678 amplicon sequence variants (ASVs) were retrieved across all samples. The taxonomic assignation was performed using the Genome Taxonomy Database (GTDB; Parks *et al.* 2018).We quantified the absolute abundances of individual ASVs by multiplying the relative abundances of each ASV with the total cell numbers of the communities in the outflow water. We used this absolute value as a proxy to describe the temporal dynamics of individual ASVs within each incubation. Biases introduced during the generation of 16s amplicon sequence data, e.g. during DNA extraction or amplification prevent the accurate estimation of cell numbers of single taxa within a sample (Props *et al.* 2017). However, evaluating changes in the absolute abundances of individual taxa across samples is more reliable because the introduced biases are similar among samples (Props *et al.* 2017; Shen, Juergens and Beier 2018).

Statistical analysis

Statistical analyses were performed in R (R Core Team 2018). To test the effect of disturbances on the concentration of nitrogen compounds (NH_4^+, NO_2^-, NO_3^-, DON) during the experiment, we performed 2-way repeated measurements analyses of variance (rmANOVAs) using the time and disturbance regime as factors for each of the DOM regimes separately. The concentration of organic and inorganic nitrogen compounds was normalized by subtracting raw concentrations values from the respective concentrations in the culture media to indicate net consumption or production levels. Depending on whether concentration of the measured N compounds were higher or lower in the incubation vessels compared to the concentration measured in the inflow medium we refer to either net production or net consumption.

Normality and homogeneity of variance were tested using the Kolmogorov-Smirnov and Levene test, respectively. No violation of the assumptions was detected.

As only N-compound, net nitrate concentrations exhibited a significant response to the disturbance regime (see details in the result part), the downstream analyses were accordingly applied only for net nitrate concentrations. We used the Random Forests (RF) machine learning algorithm (Breiman 2001) to test if net nitrate concentrations in the media could be predicted from community compositional data. We considered either FC-based community compositional data sampled on the same day as the chemical data or 16S rRNA gene community compositional data in the outflow, which was collected three days after the chemical data was sampled (Fig. 2). For DNA-based community composition, we included only samples after day 8 of the experiment when the time delay between samples nitrogen samples and DNA samples was constantly 3 days (n=60). In contrast, FC samples from all chemical measurement days were included (n=72; Fig. 2). To test for consistency between trends in DNA and FC-based community patterns we performed a Mantel test (R-package geosphere, Hijmans, 2019) to compare if beta diversity patterns assessed via pairwise Bray-Curtis distances from both datasets were significantly related to each other. The Mantel test was performed on DNA and FC based relative community compositional data that were obtained simultaneously from the outflow water collected for DNA extraction.

For RF an ensemble of decision trees is constructed, each tree predicts the value of a response variable (here net nitrate concentrations) based on the values of a set of features (here absolute phenotypic bin count data or absolute ASV count data). Each tree is trained on a different random subset of the samples and using a random subset of the features. An important advantage of RF-based modeling is that this algorithm also identifies the most important features for predicting changes of the response variable. Only ASVs and phenotypic bins presented in more than one sample were included in the analysis.

We evaluated the best setting for the number of features to be considered in each tree (mtry) based on a repeated cross-validation approach using the R-package "caret" (Kuhn 2020) and considering 70% of the samples as a training dataset. The final RF models were built on the training dataset with the R-package "randomForest" (Liaw and Wiener 2002) with mtry set to 35 or 75 for the 16s rRNA or the FC count data, respectively. These final models were subsequently used to predict NO_3 consumption in the remaining 30% of the samples in the test data set.

We furthermore inspected the top 10 ASVs and phenotypic bins with the highest importance for the respective model in more detail, to identify possible key species involved in nitrate transformations. These key taxa hereafter defined as top-ranked taxa presented high abundances either during the experiment start, in the middle, or towards the end of the experiment.

RESULTS

Changes in inorganic and organic nitrogen

Measured ammonium and nitrate concentrations pointed to a net consumption of these compounds in all continuous cultures (Fig. 3). In contrast, nitrite and DON concentrations were, at least in the HDOM incubations, mostly close to the concentrations measured in the original media. Only DON values in the LDOM media indicated a certain net production of this compound during the incubations (Fig. 3). Significant temporal changes were observed for ammonium and nitrate under HDOM regimes (Table 1). Ammonium concentration peaked at both DOM levels, but a continuous decrease of~30% towards the end of the experiment only at

HDOM independent of the disturbance regime (Fig. 3B). A significant ($P < 0.001$) or close to a significant ($P = 0.050$) effect of the disturbance regime was detected only for nitrate concentrations (Table 1). Here, nitrate levels of the disturbed and undisturbed incubations under both DOM regimes did not differ until week three. After week three nitrate levels diverged in response to the disturbance regime, while disturbed communities exhibited consistently higher net nitrate consumption than the control communities (Fig. 3E, F).

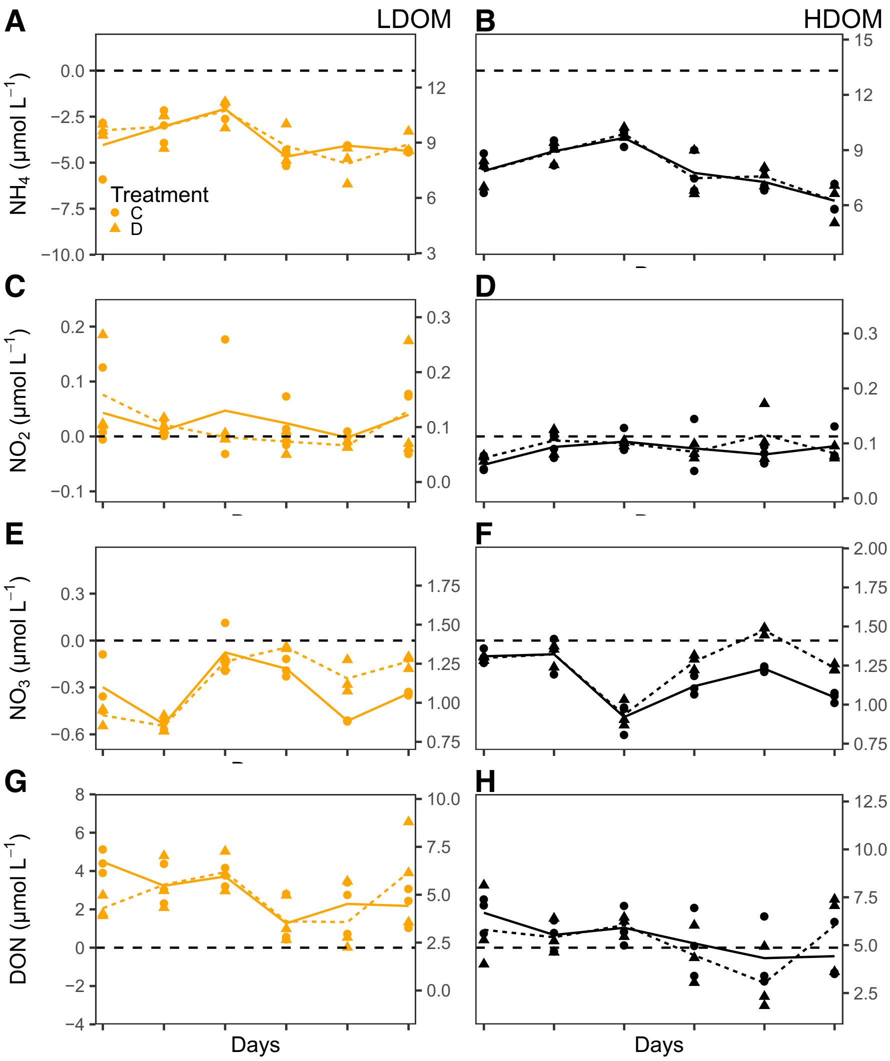

Figure 3. Variation of organic and inorganic nitrogen in the continuous cultures. *Points and triangles represent the values for the independent replicates for Control and Disturbance treatment, respectively. Solid and dashed lines represent the mean values for the Control and the Disturbance treatments, respectively. The left y-axis indicates values for the absolute concentrations. The right y-axis indicates the net consumption/production relative to the concentrations of each compound in the medium. The dashed horizontal line indicates the concentration of the respective N compounds in the inflow medium.*

Table 1. *Summarized output from the repeated measurement ANOVA to test the impact of time and disturbance regimes on the concentration of organic and inorganic nitrogen compounds in the continuous culture experiment by DOM level. Bold numbers indicate P≤ 0.05.*

	NH_4^+		NO_2^-		NO_3^-		DON	
LDOM	F	P	F	P	F	P	F	P
Time	9.18	**0.000*** **	0.97	0.456	23.38	**0.000*** **	3.09	**0.028***
Disturbance	0.19	0.668	0.14	0.711	4.30	**0.050**	0.21	0.651
Time x Disturbance	1.06	0.406	0.36	0.870	5.81	**0.001**	1.58	0.204
HDOM								
Time	13.57	**0.000*** **	1.50	0.229	51.22	**0.000*** **	2.20	0.089
Disturbance	0.01	0.925	0.55	0.466	29.94	**0.000*** **	0.15	0.701
Time x Disturbance	0.10	0.991	0.72	0.619	6.26	**0.001** **	0.67	0.649

*Indicates P-values <0.05
**Indicates P-values<0.01
***Indicates P-values<0.001

Prediction of nitrate from community compositional data and identification of taxa with high importance in the prediction model

The RF-based models for both, FC and 16S rRNA gene data significantly predicted nitrate concentration from community composition data, while the FC-based model resulted in a better prediction (Spearman rho=0.75) compared to the 16s rRNA gene-based model (Spearman rho=0.58; Fig. 4A, D). The top-ranked FC bins exhibited similar counts in the incubations under different DOM levels (Fig. 4C). The top-ranked ASVs were representatives of the orders *Enterobacterales*, *Flavobacteriales*, *Rhodobacterales*, and *Sphingomonadales* (Fig. 4E, Table 2). Some of these ASVs reached only high abundances in the HDOM incubations while featuring very low cell densities in the LDOM incubations (Fig. 4F). Generally, we observed large variances among replicate treatments.

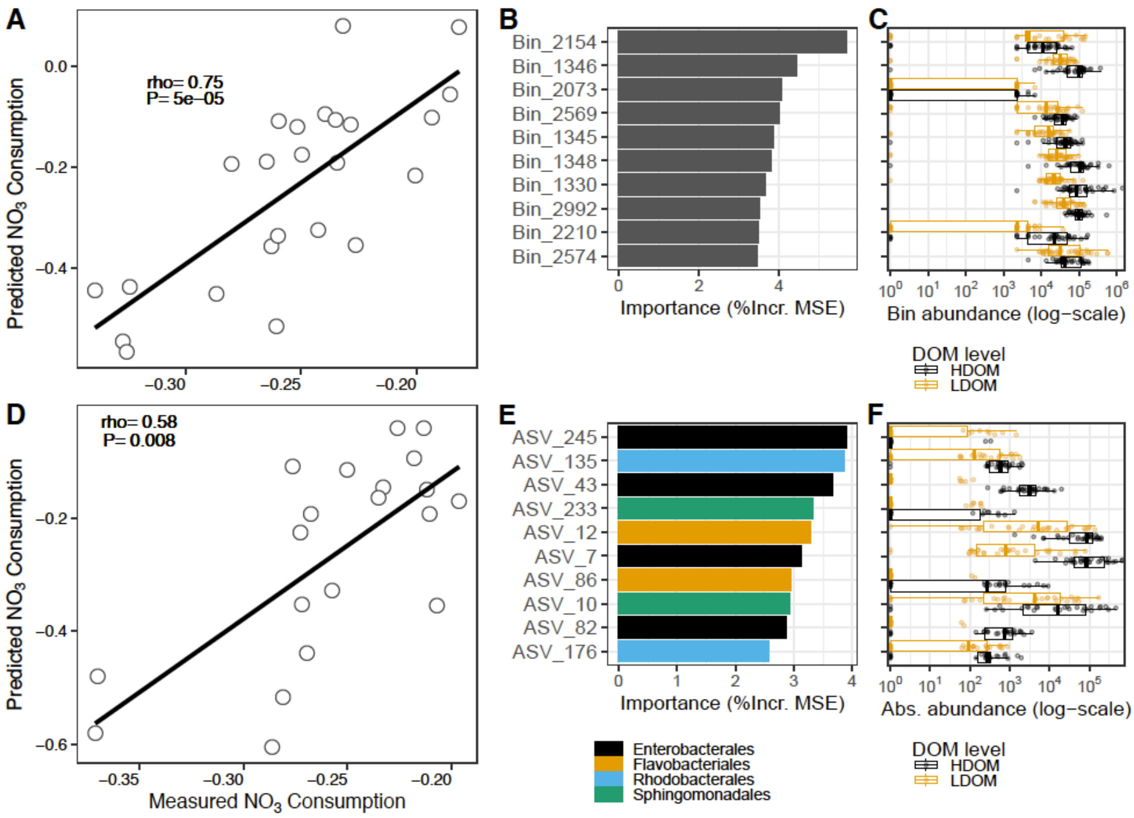

Figure 4. RF model predictions for the FC-derived phenotypic bins (upper panels) and ASVs (lower panels). *Spearman correlation between the observed NO_3^- and the predicted NO_3^- derived from the RF model for A) 16S rRNA and D) FC fingerprinting. Percentage of increase of the mean squared error MSE for B) indicator ASVs (MSE >2.5) and E) indicator phenotypic bins (MSE >3.475). C/F) Boxplot of the log10-transformed absolute abundance of ASVs /phenotypic bins by the DOM regime.*

Table 2. *Percentage MSE increase and taxonomic affiliation (GTDB database) for the top-ranked ASVs*

ASV	%Inc MSE	Phylum	Class	Order	Family	Genus	Species
ASV_245	3.9	Proteobacteria	Gammaproteobacteria	Enterobacterales	Alteromonadaceae	Alteromonas	NA
ASV_135	3.9	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Jannaschia	Jannaschia_faecimaris (RS_GCF_900107415.1)
ASV_43	3.7	Proteobacteria	Gammaproteobacteria	Enterobacterales	Vibrionaceae	Vibrio	NA
ASV_233	3.3	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Erythrobacter_A	NA
ASV_12	3.3	Bacteroidota	Bacteroidia	Flavobacteriales	Cryomorphaceae	Coccinistipes	NA
ASV_7	3.1	Proteobacteria	Gammaproteobacteria	Enterobacterales	Alteromonadaceae	Alteromonas	NA
ASV_86	2.9	Bacteroidota	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Muricauda	Muricauda_zhangzhouensis (RS_GCF_900102925.1)
ASV_10	2.9	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Erythrobacter_A	NA
ASV_82	2.9	Proteobacteria	Gammaproteobacteria	Enterobacterales	Vibrionaceae	Vibrio	NA
ASV_176	2.6	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Jannaschia	Jannaschia_faecimaris (RS_GCF_900107415.1)

Were most interested in the top-ranked taxa that contributed to net nitrate consumption towards the end of the experiment, where samples from the disturbed and control treatments differed concerning net nitrate concentrations. We will therefore particularly highlight those phenotypic bins or ASVs that were abundant during the last stage of the experiment.

Among the top-ranked taxa from the FC-based model, the bins X2154, X1330, and X2574 exhibited the highest abundances during the experimental end (Fig. 5). However, X1330 was only abundant in the HDOM treatments. The bins X2154 and X2574 were growing in all treatments at the experiment end with a trend for higher abundances in the control treatments (Fig. 5). This trend was particularly pronounced in the LDOM treatments and very weak in the X2574 HDOM treatment.

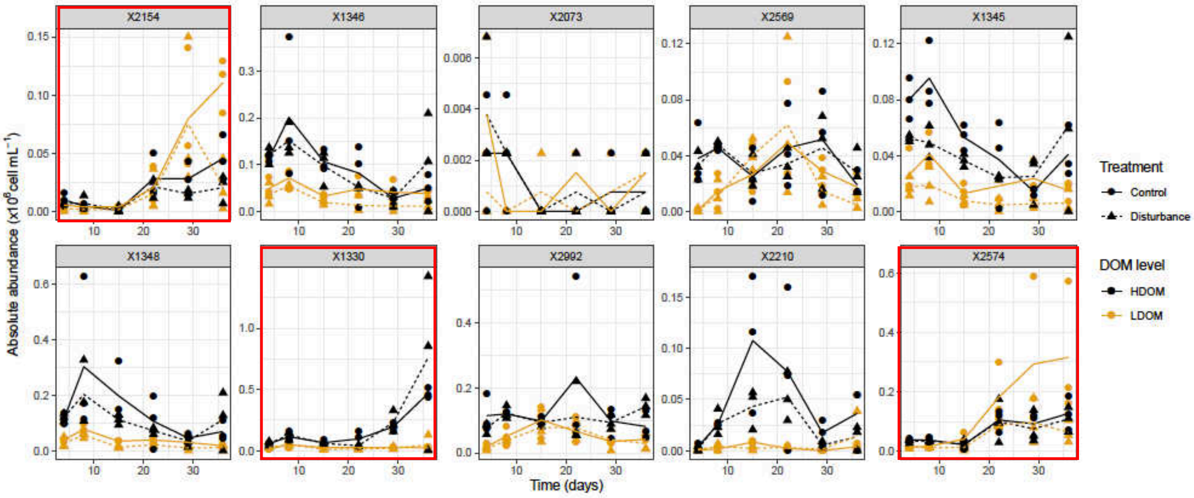

Figure 5. Temporal dynamics of the top-ranked phenotypic bins. Absolute abundance (x10^6 cell ml^{-1}) of the 10 top-ranked phenotypic bins. *Symbols represent individual replicates, while lines represent the mean values by disturbance regime. Bins are sorted from left to right by their importance. Red frames indicate the top-ranked bins that were abundant towards the experiment end.*

Among the top-ranked taxa from the 16s rRNA gene-based model, ASV_233, ASV_10, ASV_12, and ASV_86 exhibited high abundances towards the end of the experiment (Fig. 6). All four taxa were higher abundant in the HDOM treatments and tended to have higher abundances in all disturbed incubations in the case of ASV_233 and ASV_12, or only under HDOM conditions in the case of ASV_86 and ASV_10.

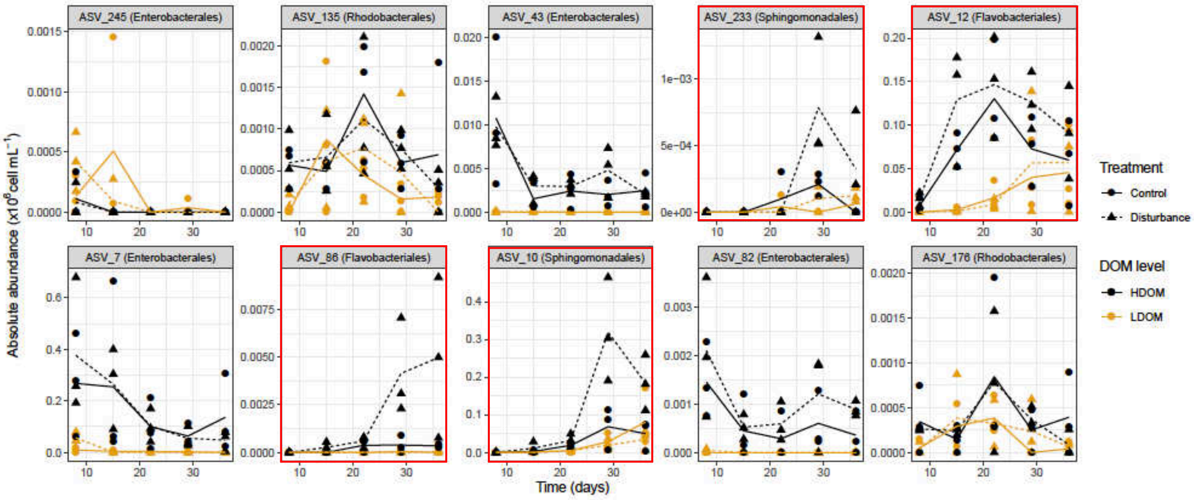

Figure 6. Temporal dynamics of top-ranked ASVs. Absolute abundance (x10^{6} cell ml^{-1}) of the 10 top-ranked taxa. *Symbols represent individual replicates, while lines represent the mean values by disturbance regime. ASVs are sorted from left to right by their importance. Red frames highlight the top-ranked taxa that were abundant towards the experiment end.*

DISCUSSION

We examined the effect of pulse disturbances on the nitrogen cycle by quantifying changes in nitrogen compounds in disturbed versus undisturbed control treatments of a continuous culture experiment.

The measured nitrate concentrations in the culture vessels represented the balance between the constant input of nitrate via the medium inflow, nitrate production via nitrification, and nitrogen removal processes such as denitrification or assimilatory nitrate reduction. Consistently and treatment independent lower nitrate concentrations in the chemostat vessels compared to the inflowing medium indicate that nitrate consumption must have taken place via either one of the above-mentioned denitrification-like processes.

However, all these processes start with nitrate reduction that only occurs under anaerobic conditions, while weekly oxygen measurements in the chemostat vessels indicated that these never turned into suboxic or anoxic cultures (Table S1). We assumed that the biochips added to the continuous culture represented a physical matrix that enabled the formation of dense biofilms creating microenvironments with suitable conditions for denitrification, as it had been observed elsewhere (Smriga, Ciccarese and Babbin 2021). Additionally, simultaneous nitrate reduction may have also occurred on particles. Indeed denitrification activity on particles had been shown to contribute up to 50% of nitrogen removal in some wastewater treatments even though oxic conditions were prevailing (Allegue *et al.* 2020). Nitrate concentrations measured at the initial experimental stage indicated higher nitrate reduction activity in the LDOM compared to the HDOM incubations, while the disturbance regime did not seem to impact nitrate reduction activity until week 3. In contrast, we observed a more pronounced nitrate net consumption in the control compared to the disturbed treatments after week 3. This could either be caused directly due to a decrease of denitrification rates in response to the pulse disturbance disturbances or indirectly due to elevated nitrification rates resulting in a decreased nitrate net consumption under equal denitrification rates in HDOM. The decrease of nitrate net consumption in the disturbed compared to the control treatments seemed to occur rather parallel independent from the DOM regime. However, trends for DOM dependent differences, for instance concerning net nitrite and net DON concentrations (Fig. 3), may indicate that different processes impacted the reduction of nitrate consumption under salinity disturbances in the HDOM and LDOM treatments, respectively.

We were able to significantly predict nitrate concentration from community compositional data with an explanatory power that was at least for the FC based prediction (Spearman rho=0.75) in the range of earlier published RF models that predicted environmental characteristics from microbial community data (Spearman rho between 0.67-0.79; Roguet *et al.* 2018; Tackmann *et al.* 2018; Thompson *et al.* 2019). This points to a major impact of community compositional changes on the dynamics of nitrate processing. Still, although compositional changes significantly predicted the nitrate dynamics in the culture media, we cannot exclude that beyond the abundance of nitrate transforming species, also their abundance-independent activities in response to salinity fluctuations contributed to nitrate processing.

The higher accuracy of the FC compared to the 16s rRNA gene-based model to predict nitrate net consumption was likely because the FC-based community patterns were sampled on the same day as the samples for chemical analyses. Instead, data for 16s rRNA gene analyses were sampled with a time delay and not even directly from the continuous cultures, but in the outflow. The latter was sampled after collecting the outflow during 48 h in a bottle with conditions differing compared to those in the culture vessels. However, while FC-based phenotypic proxies provided a fine resolution of compositional changes on the day of nitrate measurements, they lack other than 16s rRNA gene-based data information about species identity as a key aspect in microbiology. For this reason, we inspected top-ranked taxa from both, the FC and the 16s rRNA gene-based model, with the former providing a better match between the chemical and compositional data and the later providing information about the species identity.

Both models extracted top-ranked taxa that represented different community stages. This agrees with the pronounced successional patterns in the continuous culture communities that are outlined in Chapter 3. General community dynamics evaluated either via phenotypic binning or 16 rRNA amplicon data resulted in similar trends (Mantel test: Spearman rho=0.72, $P \leq 0.0001$) and a rough match in the abundance patterns of indicator taxa may point to a partial overlap among the extracted indicator phenotypic bins and the extracted indicator ASVs.

The FC-based indicator bins tended to have higher abundances in the control treatments. If assuming that the respective species are directly involved in nitrate transformations, this suggests that they were involved in nitrate removal, matching a higher net nitrate consumption in the controls compared to the disturbed treatments. On contrary, indicator ASVs tended to exhibit higher abundances in the disturbed treatments, which rather would point to their involvement in nitrite oxidation as a nitrate producing reaction: if nitrate production would be enhanced in the disturbed treatments, this likewise would cause higher nitrate net consumption in the controls.

For the above-outlined reasons, however, should faint patterns in the abundance dynamics, such as differences in abundances between disturbed and control treatment, be more reliable in the FC-based compared to the 16s rRNA gene-based indicator taxa. Furthermore, were the abundance data highly variable among replicates from the same treatments, and the discussed trends could not be statistically validated. This is in contrast to the comparable low variability of the replicate nitrate measurements. We assume that nitrate processing taxa were mainly active in biofilms on the chips and sporadically detached from there. This would explain a less even distribution of top-ranking taxa in the chemostat culture medium, compared to a more even distribution of these taxa attached to the chips leading possibly to the observed low variability in the nitrate concentrations among replicates.

The taxonomic affiliation of the top-ranked ASVs with high abundance during the experiment end may give some evidence about the process causing differential nitrate net consumption in the disturbed versus the control treatments: for instance, taxa affiliated with the genus *Erythrobacter* (ASV_10 and ASV_233) were detected in nitrite-oxidizing enriched cultures (Baskaran *et al.* 2020). However, members of this genus were also described to possess the ability for denitrification (Choi, Hwang and Lee 2021; Zhuang *et al.*). Members of genus *Municauda* (ASV_86) have been proposed as specialized N_2O oxidizers (Marchant *et al.* 2018). In the case of the genus *Coccinistipes* (ASV_12), we did not find any evidence linking this genus to nitrogen cycling (Brettar, Christen and Höfle 2012).

However, none of the indicator taxa were affiliated with phylogenetic lineages that have previously been described as autotrophic nitrite oxidizers. Indeed, nitrite oxidation has to our knowledge only been described for members affiliating with a short-list of bacterial groups, including *Nitrosospira*, *Nitrospina and Nitrobacter* (Ward 2008). Still, heterotrophic nitrification is a phylogenetically widespread process and nitrite oxidation may occur in representatives of lineages in which potential nitrification was not yet described (Stouthamer *et al.* 1997; Ward 2008). We therefore cannot exclude that the top-ranked ASVs may be able to contribute to nitrate production. As a consequence, the affiliation of top-ranked ASVs with either nitrate reducing or nitrite-oxidizing functional guilds stays at this point highly speculative**.**

In conclusion, significant changes in nitrate net consumption rates in the final phase of the long-term experiment confirmed that frequent salinity changes impacted nitrogen cycling. The possibility to significantly predict nitrate consumption rates from community composition data further demonstrated that the differential selection of species under the different treatment conditions was an important driver of the observed nitrate net consumption rates. Based on our data we assume that reduced nitrate reduction activity in the disturbed treatments is the most likely mechanism explaining lower net nitrate consumption rates in this treatment. However, this interpretation remains speculative. Ongoing analyses of metagenome data, that are available for the last DNA sample occasion of the continuous culture experiment can give more detailed insights about potential mechanisms contributing to differential net nitrate consumption rates in response to salinity disturbances: For instance, the abundance and potentially also taxonomic affiliations, at least at broader phylogenetic levels, of nitrite oxidoreductase (*nxrAB*) or nitrate reductase (*nar*, *nap*) genes can give evidence about the potential nitrate reduction versus nitrite oxidation activities (Fig. 1; Zehr and Kudela 2011; Pajares and Ramos 2019).

Our study in combination with earlier work (Seitzinger *et al.* 2006) demonstrates that nitrogen cycling (nitrification and/or denitrification) can be affected by an increased frequency of disturbances linked to salinity changes in coastal zones, as is predicted for climate change scenarios. Coastal zones typically lead via extensive coupled nitrification-denitrification rates to large-scale nitrogen removal from river-runoff into the oceans (Boyer and Howarth 2008). Decreased nitrogen removal rates due to increasingly occurring pulse disturbances may have important consequences for the ecology of marine systems that are often nitrogen limited (Howarth and Marino 2006).

ACKNOWLEDGMENTS

This study was funded by the German Science Foundation granted SB (BE 5937/2-1). ARF was supported by the Chilean National Agency for Research and Development (ANID) / Scholarship Program / DOCTORADO BECAS CHILE/2017 – 72180448. This study was also supported by the LIA MAST (LIA 1035). We thank the BIO2MAR platform for the access and the technical support in their molecular biology facilities. We thank Olivier Crispy, Jenny Jeschek, and Madleen Dierken for their help in the analysis of dissolved organic compounds and nutrients.

REFERENCES

Allegue T, Carballo-Costa MN, Fernandez-Gonzalez N et al. Simultaneous nitrogen and dissolved methane removal from an upflow anaerobic sludge blanket reactor effluent using an integrated fixed-film activated sludge system. Journal of Environmental Management 2020;263:110395.

Aminot A, Kérouel R. Dosage Automatique Des Nutriments Dans Les Eaux Marines: Méthodes En Flux Continu. Editions Quae, 2007.

Barbier EB, Hacker SD, Kennedy C et al. The value of estuarine and coastal ecosystem services. Ecological Monographs 2011;81:169–93.

Baskaran V, Patil PK, Antony ML et al. Microbial community profiling of ammonia and nitrite oxidizing bacterial enrichments from brackishwater ecosystems for mitigating nitrogen species. Sci Rep 2020;10:5201.

Boyer EW, Howarth RW. Nitrogen Fluxes from Rivers to the Coastal Oceans. Nitrogen in the Marine Environment 2008:1565–87.

Breiman L. Random Forests. Machine Learning 2001;45:5–32.

Brettar I, Christen R, Höfle MG. Analysis of bacterial core communities in the central Baltic by comparative RNA–DNA-based fingerprinting provides links to structure–function relationships. ISME J 2012;6:195–212.

Callahan BJ, Mcmurdie PJ, Rosen MJ et al. DADA2 : High-resolution sample inference from Illumina amplicon data. 2016;13, DOI: 10.1038/nmeth.3869.

Choi M-S, Hwang Y, Lee T-J. Nitrogen Removal Using a Membrane Bioreactor with Rubber Particles as the Fouling Reducer. Applied Sciences 2021;11:3578.

Daims H, Lebedeva EV, Pjevac P et al. Complete nitrification by Nitrospira bacteria. Nature 2015;528:504–9.

Damashek J, Francis CA. Microbial Nitrogen Cycling in Estuaries: From Genes to Ecosystem Processes. Estuaries and Coasts 2018;41:626–60.

Eguchi M, Nishikawa T, Macdonald K et al. Responses to Stress and Nutrient Availability by the Marine Ultramicrobacterium Sphingomonas sp. Strain RB2256. Appl Environ Microbiol 1996;62:1287.

Evans SE, Wallenstein MD. Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? Biogeochemistry 2012;109:101–16.

Evans SE, Wallenstein MD. Climate change alters ecological strategies of soil bacteria. Ecology Letters 2014;17:155–64.

Fagherazzi S, Anisfeld SC, Blum LK et al. Sea Level Rise and the Dynamics of the Marsh-Upland Boundary. Front Environ Sci 2019;0, DOI: 10.3389/fenvs.2019.00025.

Herbert RA. Nitrogen cycling in coastal marine ecosystems. FEMS Microbiology Reviews 1999;23:563–90.

Hijmans R J. Geosphere: Spherical Trigonometry., 2019.

Holmes RM, Aminot A, Kérouel R et al. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 1999, DOI: 10.1139/f99-128.

Horz H-P, Barbrook A, Field CB et al. Ammonia-oxidizing bacteria respond to multifactorial global change. PNAS 2004;101:15136–41.

Howarth RW, Marino R. Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades. Limnology and Oceanography 2006;51:364–76.

Kowalchuk GA, Stephen JR. Ammonia-oxidizing bacteria: A model for molecular microbial ecology. Annu Rev Microbiol 2001;55:485–529.

Kuhn M. Caret: Classification and Regression Training., 2020.

Liaw A, Wiener M. Classification and regression by randomForest. R news 2002;2:18–22.

Magalhães CM, Joye SB, Moreira RM et al. Effect of salinity and inorganic nitrogen concentrations on nitrification and denitrification rates in intertidal sediments and rocky biofilms of the Douro River estuary, Portugal. Water Research 2005;39:1783–94.
Marchant HK, Tegetmeyer HE, Ahmerkamp S et al. Metabolic specialization of denitrifiers in permeable sediments controls N2O emissions. Environmental Microbiology 2018;20:4486–502.
Marie D, Simon N, Guillou L et al. Flow Cytometry Analysis of Marine Picoplankton. In: Diamond RA, Demaggio S (eds.). In Living Color: Protocols in Flow Cytometry and Cell Sorting. Berlin, Heidelberg: Springer, 2000, 421–54.
Marks BM, Chambers L, White JR. Effect of Fluctuating Salinity on Potential Denitrification in Coastal Wetland Soil and Sediments. Soil Science Society of America Journal 2016;80:516–26.
Moore CM, Mills MM, Arrigo KR et al. Processes and patterns of oceanic nutrient limitation. Nature Geosci 2013;6:701–10.
Pajares S, Ramos R. Processes and Microorganisms Involved in the Marine Nitrogen Cycle: Knowledge and Gaps. Frontiers in Marine Science 2019;6:739.
Parada AE, Needham DM, Fuhrman JA. Every base matters : assessing small subunit rRNA primers for marine microbiomes with mock communities , time series and global field samples. 2016;18:1403–14.
Parks DH, Chuvochina M, Waite DW et al. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nature Biotechnology 2018;36:996–1004.
Props R, Kerckhof F-M, Rubbens P et al. Absolute quantification of microbial taxon abundances. The ISME Journal 2017;11:584–7.
Pujo-Pay M, Conan P, Oriol L et al. Integrated survey of elemental stoichiometry (C, N, P) from the western to eastern Mediterranean Sea. Biogeosciences 2011;8:883–99.
Pujo-Pay M, Raimbault P. Improvement of the wet-oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters. Marine Ecology Progress Series 1994;105:203–7.
R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing, 2018.
Renes SE, Sjöstedt J, Fetzer I et al. Disturbance history can increase functional stability in the face of both repeated disturbances of the same type and novel disturbances. Scientific Reports 2020;10:11333.
Roguet A, Eren AM, Newton RJ et al. Fecal source identification using random forest. Microbiome 2018;6:185.
Rysgaard S, Thastum P, Dalsgaard T et al. Effects of salinity on NH4+ adsorption capacity, nitrification, and denitrification in Danish estuarine sediments. Estuaries 1999;22:21–30.
Seitzinger S, Harrison JA, Böhlke JK et al. Denitrification Across Landscapes and Waterscapes: A Synthesis. Ecological Applications 2006;16:2064–90.
Seitzinger SP. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. Limnology and Oceanography 1988;33:702–24.
Seneviratne S, Nicholls N, Easterling D et al. Changes in climate extremes and their impacts on the natural physical environment. 2012:109–230.
Shen D, Juergens K, Beier S. Experimental insights into the importance of ecologically dissimilar bacteria to community assembly along a salinity gradient. Environ Microbiol 2018;20:1170–84.
Smriga S, Ciccarese D, Babbin AR. Denitrifying bacteria respond to and shape microscale gradients within particulate matrices. Commun Biol 2021;4:1–9.

Stouthamer AH, de Boer APN, van der Oost J et al. Emerging principles of inorganic nitrogen metabolism in Paracoccus denitrificans and related bacteria. Antonie van Leeuwenhoek 1997;71:33–41.

Tackmann J, Arora N, Schmidt TSB et al. Ecologically informed microbial biomarkers and accurate classification of mixed and unmixed samples in an extensive cross-study of human body sites. Microbiome 2018;6:192.

Thompson J, Johansen R, Dunbar J et al. Machine learning to predict microbial community functions: An analysis of dissolved organic carbon from litter decomposition. PLOS ONE 2019;14:e0215502.

Wanderley BMS, A. Araújo DS, Quiroga MV et al. flowDiv: a new pipeline for analyzing flow cytometric diversity. BMC Bioinformatics 2019;20:274.

Ward BB. Nitrification in Marine Systems. In: Capone DG, Bronk DA, Mulholland MR, et al. (eds.). Nitrogen in the Marine Environment (Second Edition). San Diego: Academic Press, 2008, 199–261.

Wu Y, Tam NFY, Wong MH. Effects of salinity on treatment of municipal wastewater by constructed mangrove wetland microcosms. Marine Pollution Bulletin 2008;57:727–34.

Zehr JP, Kudela RM. Nitrogen Cycle of the Open Ocean: From Genes to Ecosystems. Annu Rev Mar Sci 2011;3:197–225.

Zhuang L, Liu Y, Wang L et al. Erythrobacter atlanticus sp. nov., a bacterium from ocean sediment able to degrade polycyclic aromatic hydrocarbons. International Journal of Systematic and Evolutionary Microbiology 65:3714–9.

SUPPLEMENTARY MATERIALS

Table S1. *Oxygen concentration measured directly from the continuous culture vessels. Oxygen values obtained from the measurements used for community respiration estimations. Oxygen saturation was calculated online on the U.S. Geological Survey platform (USGS, https://water.usgs.gov/cgi-bin/dotables).*

	Vessel 1	Vessel 2	Vessel 3	Vessel 4	Vessel 5	Vessel 6	Vessel 7	Vessel 8	Vessel 9	Vessel 10	Vessel 11	Vessel 12
Date	Oxygen concentration (μmol L^{-1})											
24/09/2021	280	275	268	272	261	266	273	274	243	255	267	258
01/10/2021	279	268	261	270	263	276	264	274	264	257	264	253
08/10/2021	273	248	229	256	261	274	277	266	275	267	271	272
15/10/2021	305	276	260	309	272	280	288	300	288	275	289	280
22/10/2021	307	283	282	305	282	291	290	292	280	284	282	275
29/10/2021	308	281	273	294	298	296	294	297	290	278	290	276
	Oxygen concentration (mg L^{-1})											
24/09/2021	9.0	8.8	8.6	8.7	8.3	8.5	8.7	8.8	7.8	8.2	8.5	8.3
01/10/2021	8.9	8.6	8.4	8.6	8.4	8.8	8.4	8.8	8.5	8.2	8.4	8.1
08/10/2021	8.7	7.9	7.3	8.2	8.4	8.8	8.8	8.5	8.8	8.6	8.7	8.7
15/10/2021	9.8	8.8	8.3	9.9	8.7	9.0	9.2	9.6	9.2	8.8	9.3	9.0
22/10/2021	9.8	9.1	9.0	9.8	9.0	9.3	9.3	9.4	9.0	9.1	9.0	8.8
29/10/2021	9.9	9.0	8.7	9.4	9.5	9.5	9.4	9.5	9.3	8.9	9.3	8.8
	Oxygen saturation (%) salinity 13 at 18ºC											
24/09/2021	106	104	102	103	99	101	104	104	92	97	101	98
01/10/2021	106	102	99	102	100	105	100	104	100	97	100	96
08/10/2021	104	94	87	97	99	104	105	101	104	101	103	103
15/10/2021	116	105	99	117	103	106	109	114	109	104	110	106
22/10/2021	116	107	107	116	107	110	110	111	106	108	107	104
29/10/2021	117	107	104	112	113	112	111	113	110	105	110	105

GENERAL DISCUSSION

The aim of my thesis was to contribute to a better understanding of mechanisms that govern the response and adaptation of prokaryotes to environmental disturbances. To disentangle the effect of different individual and community mechanisms I applied several experimental approaches to study prokaryotes based on their life history properties inferred by molecular techniques. This thesis comprised a study based on single populations as systems with low complexity and subsequently moved on to examine complex communities, where both, the properties of single populations as well as community structural characteristics can impact the community-level response to disturbances.

1. Chapter synthesis

Chapter 1 demonstrated that transcriptional regulation and thus the transcriptional plasticity patterns of fitness- and adaptation-related genes of single strains in response to changing salinity are controlled in different directions by the life histories of these strains (i.e. their tolerance against salinity changes). We further extracted a shortlist of candidate stress marker genes. While this study was conducted at the population level, the expression of these potential stress marker genes may in the future also be used at the community level, either to monitor the stress exposure of individual community members or to evaluate the susceptibility of the combined community to environmental change, as detailed below (section 2 of the discussion).

The findings from this study may additionally give evidence on how functional resistance at the community level may translate into community compositional resistance. It has been argued that compositional resistance can be linked to enhanced functional plasticity that is needed to adapt to environmental change (Shade et al., 2012). For example, in heterotrophic bacteria, the differential expression of genes encoding the exploitation of different carbon substrates, such as citrate, sorbitol, mannitol, fructose, or cellobiose transporters along an environmental gradient can provide resistance against changing resource availability. However, this relationship specifically refers to genes that in our study were referred to adaptation-related genes. In contrast, it seems reasonable to believe that the functional resistance that is linked to the expression of fitness-related genes covaries positively compositional resistance. The data presented in this chapter, therefore, emphasize the importance of differentiating between adaptation- and fitness-related traits when relating functional to compositional resistance at the community level.

Chapter 2 describes the development of a method for the preservation and resuscitation of natural aquatic prokaryote assemblages, which was a prerequisite for setting up the downstream long-term pulse disturbance experiments (Chapter 3, 4): the preparation of cryopreserved community aliquots allowed to test the continuous culture systems while ensuring identical starting conditions for several shorter pre-experiments that were performed before the final long-term experiment. However, while the main purpose of this study was the development of the cryo-preservation method, the data give also evidence of differential community-level responses to disturbances depending on the disturbance history and trophic status of the source ecosystems. In this study, the cryopreservation itself in combination with the resuscitation procedure and the dilution of the community in media that differed from the original environment could be considered as a disturbance. While after the incubations, the initial community composition from the Canet lagoon was recovered at a high degree of similarity, this was not the case for the initial community from the SOLA station. Indeed, the community members from the hypereutrophic and highly variable Canet lagoon were accordingly more resistant against the induced disturbances compared to community members originating from the oligotrophic and less variable SOLA field station. (Chapter 3, Fig. 5). This finding can be interpreted as the elevated environmental heterogeneity in combination with high nutrient availability in the Canet lagoon selected for a high proportion of generalist species that were tolerant against environmental change.

In chapter 3 we thereupon systematically tested the impact of the disturbance history in combination with nutrient availability on community assembly including the selection of traits indicating life history, species diversity levels, and bulk community functional resistance. We particularly focused in this study on functional measurements, such as BGE that we considered to be tightly linked to the expression of fitness related traits or genes. In agreement with observations from chapter 2, experiments confirm that the long-term exposure of the community to heterogeneous environments in combination with high nutrient levels led to higher functional resistance. These conditions simultaneously favored the selection of species harboring resistance-related genomic traits. These findings accordingly provide experimental evidence for energetic costs that are linked to a high adaptability of biological systems to environmental change.

Chapter 4 explores how the exposure of communities to pulsed disturbances affected fluxes in the nitrogen cycle and I discuss the potential biogeochemical consequences of disturbances on nitrogen cycling.

2. Predictability of microbial systems: genomic and transcriptomic markers

A main focus of my thesis is on the assignment of life history properties to prokaryotes that were inferred by molecular markers. It has been argued earlier that the application of DNA or RNA markers can give information about the prevalence of different life histories in communities indicating the capacity of communities to adapt to environmental change (e.g., Krause et al., 2014; Fierer, 2017; Malik et al., 2020). In microbes, the multitude of responses to n-dimensional changes occurring in natural environments, represented for example, by biological interactions, resource availability, and potential stressors are coded in their genomic material. Thus, the DNA of a population is indicative of its potential performance under all possible conditions or its coverage of the n-dimensional niche space.

However, the lack to sufficiently understand the complexity of gene regulation from genome sequence data currently limits our capacity to exactly predict gene expression patterns and consequently the specific phenotype of a strain in a given environment. Still, it has been demonstrated that the presence of functional genes within genomes was tightly linked to the ecological niche occupied by corresponding prokaryotes, explaining ~50% of their niche variability in a high-dimensional niche space (Alneberg et al., 2020).

A more simplified possibility to characterize prokaryotes via their genomic data is to extract genomic traits, such as genome size or the codon usage bias (CUB) that have been linked to the life history of prokaryotes (Beier et al., 2021). For instance, has the genome size been interpreted as a trait that indicates the tolerance of species against environmental change. However, the assignment of species along the specialist-generalist gradient is highly context-dependent and a resource specialist might be at the same time be a salinity generalist (Bell and Bell, 2021). As a consequence, it is not possible to unambiguously characterize prokaryote species along the generalist-specialist continuum or predict their phenotypic response to a specific environmental change based on their genome size. However, the probability that a species is resistant against a specific environmental change increases along with its genome size. Similar, it is not possible to predict from a high CUB which was shown to correlate with high maximal growth rates that the corresponding strain grows fast in a given environment. Still, a high CUB the probability that it grows fast in a given environment.

Therefore, although the outline genomic traits are imprecise in predicting the phenotypic characteristics of an individual strain in a given environment, they affect the strain's likelihood to be tolerant or to grow fast in this environment. However, if a large number of different strains is considered, as is the case in the typically highly diverse prokaryotic communities, and genomic traits values are scale-up to their distribution at the community level, the predictability of functional consequences from genomic traits increases. Indeed, community weighted means (CWMs) of resistance and resilience related genomic traits could be meaningfully interpreted in terms of community assembly dynamics associated with community level resistance in response to community exposure pulse disturbances.

RNA markers represent in contrast to DNA markers the expressed activity in a specific moment and location and represent an organisms' phenotype in a given environment. To evaluate the response of a species to a (i) single environmental change or (ii) its tolerance along an environmental gradient, at least one reference value (i) or multiple data points (ii) are necessary to quantify the impact of the changing environment on the marker gene expression levels. In chapter 1, we particularly highlight *ybeB* as potentially universal bacterial stress marker gene in whose expression levels could be used in future as proxy for population or community-level susceptibility to environmental change. Due to the high sequence conservation of the *ybeB* gene and the fact that its phylogeny reflects those of other taxonomic markers (Häuser et al., 2012), *ybeB* gene variants from metatranscriptome time series data could inform about the stress status of individual taxa in bacterial communities. Alternatively, the design of *ybeB* gene specific primers would allow to monitor community level expression patterns for instance via RT qPCR approaches in order to evaluate stress exposure scaled-up to the community level. We suggest that RNA stress markers could be used to design warning alerts based on the microbial community's responses to environmental change in engineered systems or natural environments

Predictability and comparability are important factors in microbial ecology because ecosystem services mediated by microbes are fundamental for human populations (Cavicchioli et al., 2019). In this thesis, we have used transcriptomic and genomic traits to assess how communities respond to environmental change by estimating potential resistance/resilience via the selection of some specific markers.

3. Conclusions and perspectives

My thesis particularly addressed how expressed traits at the population level or trait CWMs are linked to physiologically measured resistance levels assessed for instance via growth rates, maximal cell density, or growth efficiency. In this context, I confirmed the previously predicted association of the covariation of resistance and resilience levels with species diversity (Nimmo et al., 2015), which in turn may have impacted community stability. However, beyond species properties and community diversity, also species interactions have been highlighted in earlier studies as an important factor for community stability (Griffiths and Philippot, 2013). Biological interactions can influence the degree of community's stability to disturbances. While not yet addressed, the time-series community data presented in chapter 3 may be used in the future to assess species interaction via species co-occurrence patterns and the following questions could be posed: (i) How did the pulse disturbances under different nutrient regimes impact the interaction network? and (ii) will treatments with fewer positive species interactions exhibit increased functional resistance, as it has been proposed earlier (Coyte et al., 2015)?

The focus during this thesis was also concentrated on controlled experimental settings with salinity as a single model stressor. However, the application of salinity as a sole disturbance factor doesn't reflect the complexity of natural systems, where communities are typically subject to multiple stressors occurring simultaneously: for instance, when runoff increases, waters with different salinity are mixed and these water bodies are likely to differ not only in salinity but also in other parameters, such as temperature or pH among others. The responses of communities to each stressor can however be obscured if exposing the community simultaneously to multiple stressors, that may have antagonistic or synergic effects. Thus, in future experimental setups the exposure of complex communities to multiple stressors is needed to mimic more natural conditions. Relevant questions to be answered could for instance be: (i) What would be the effect of combined anthropogenic (e.g. pesticides, heavy metals) and natural (e.g, salinity, temperature, pH) stressors on the long-term prokaryotic functional and compositional stability? or (ii) What would be the effect of the pre-exposition of communities to a certain stressor on providing resistance to another stressor? While one may expect that the huge metabolic diversity of microbial communities makes them inherently resistant or resilient to disturbances, research suggests that microbial communities are generally sensitive to disturbances (Shade et al., 2012). This raises the question about what are the limits of community stability until reaching ecological catastrophe? Or, which disturbance intensities or frequencies cause changes in ecosystem services? My results from chapter 4 demonstrated that salinity disturbances affected nitrogen cycling in a continuous culture system. Similar effects of changing salinity in a natural coastal system may have consequences for the sensitive balance of different nitrogen transforming processes that determine the amount of nitrogen transported into the often nitrogen limited oceans.

Studies under controlled experimental setups provide the opportunity to study microbial communities in simplified systems and are crucial to disentangle the impact of different drivers that influence their dynamics. However, to eventually being able to understand and predict processes in natural habitats also observational field studies are needed. Particularly regular time series are highly valuable because they cover natural variability and can simultaneously capture pulse and press disturbances as well as the community responses to these disturbances. In combination with the DNA or RNA marker-based approaches from this thesis, the distribution life histories proxies could be assessed in the context of simultaneously quantified environmental heterogeneity. Additionally, long-term time series can reveal seasonal long-term trends as well as short-term variability, which in combination with community compositional and functional data can give evidence about the stability scales and different alternative states of the ecosystems.

This thesis contributes to the understanding of the expression and community-wide distribution of prokaryotes traits as indicators for their life history and their implications for community stability. Both, DNA or RNA-based molecular markers that have been described in this thesis can be used to screen communities in order to draw conclusions about the susceptibility of microbial communities to disturbances and predict the dynamics of ecological systems. High environmental heterogeneity under nutrient-rich conditions led to a community with genomic traits indicating a high prevalence of fast-growing generalists. This likely caused the measured elevated community resistance capabilities. These results are particularly important in the face of the predicted increase of disturbances and the need to better administrate anthropogenic intervention on ecosystems to maintain critical ecological services. My thesis furthermore provides experimental evidence that the establishment of generalists with increased tolerance against environmental change is linked to metabolic costs that could be overcome by increased resource availability.

References

Alneberg, J., Bennke, C., Beier, S., Bunse, C., Quince, C., Ininbergs, K., et al. (2020). Ecosystem-wide metagenomic binning enables prediction of ecological niches from genomes. *Commun Biol* 3, 1–10. doi:10.1038/s42003-020-0856-x.

Beier, S., Werner, J., Bouvier, T., Mouquet, N., and Violle, C. (2021). Trait-trait relationships and functional tradeoffs vary with genome size in prokaryotes. *bioRxiv*, 2021.07.23.453341. doi:10.1101/2021.07.23.453341.

Cavicchioli, R., Ripple, W. J., Timmis, K. N., Azam, F., Bakken, L. R., Baylis, M., et al. (2019). Scientists' warning to humanity: microorganisms and climate change. *Nat Rev Microbiol* 17, 569–586. doi:10.1038/s41579-019-0222-5.

Coyte, K. Z., Schluter, J., and Foster, K. R. (2015). The ecology of the microbiome: Networks, competition, and stability. *Science* 350, 663–666. doi:10.1126/science.aad2602.

Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* 15, 579–590. doi:10.1038/nrmicro.2017.87.

Griffiths, B. S., and Philippot, L. (2013). Insights into the resistance and resilience of the soil microbial community. *FEMS Microbiology Reviews* 37, 112–129. doi:10.1111/j.1574-6976.2012.00343.x.

Häuser, R., Pech, M., Kijek, J., Yamamoto, H., Titz, B., Naeve, F., et al. (2012). RsfA (YbeB) Proteins Are Conserved Ribosomal Silencing Factors. *PLoS Genet.* 8, e1002815. doi:10.1371/journal.pgen.1002815.

Krause, S., Le Roux, X., Niklaus, P. A., Bodegom, P. V., Lennon, J. T., Bertilsson, S., et al. (2014). Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. *Front. Microbiol* 5, 251. doi:10.3389/fmicb.2014.00251.

Malik, A. A., Martiny, J. B. H., Brodie, E. L., Martiny, A. C., Treseder, K. K., and Allison, S. D. (2020). Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. *The ISME Journal* 14, 1–9. doi:10.1038/s41396-019-0510-0.

Nimmo, D. G., Nally, R. M., Cunningham, S. C., Haslem, A., and Bennett, A. F. (2015). Vive la résistance: reviving resistance for 21st century conservation. *Trends in Ecology & Evolution* 30, 516–523. doi:10.1016/j.tree.2015.07.008.

Shade, A., Peter, H., Allison, S. D., Baho, D. L., Berga, M., Buergmann, H., et al. (2012). Fundamentals of microbial community resistance and resilience. *Front. Microbiol.* 3, 417. doi:10.3389/fmicb.2012.00417.

Angel RAIN-FRANCO

Personal information

Date of birth: March 10th, 1989

Nationality: Chilean

Address: 41 Av. Pierre de Marca, 66650 Banyuls sur mer, France

E-mails: angel.rain@obs-banyuls.fr; angelrainf@gmail.com

https://www.researchgate.net/profile/Angel-Rain

Education

2017-2021 Current position: PhD candidate, Sorbonne Université. Laboratoire d'Océanographie Microbienne (LOMIC), UMR 7621, France.

Subject: *Effect of environmental disturbances on community composition and functioning of aquatic prokaryotes*. Supervised by Camila Fernandez & Sara Beier.

2013-2016 Master in Oceanography. University of Concepcion, Chile.

2007–2011 Bachelor in Marine Biology. University of Concepcion, Chile.

Academic Training

2019 July. Microbial Oceanography Course. University of Vienna and Laboratoire d'Oceanographie Microbienne (LOMIC), Observatoire Océanologique de Banyuls sur mer. Dr. Gerhard Herndl.

2019 April. "An Introduction to Metagenomics and Metabarcoding". Crete. Transmitting science. https://www.transmittingscience.org. Dr. M. Lisandra Zepeda-Mendoza.

2016 January. International Course "Eutrophication and microbial processes in the coastal ocean". Austral Summer Institute (ASI XVI). University of Concepcion. Dr. Hans Pearl (University of North Carolina at Chapel Hill).

2014 January. International Course "Ecology and Diversity of Marine Microorganisms, ECODIM". Austral Summer Institute (ASI XIV). Department of Oceanography. University of Concepcion. Dr. Kurt Hanselmann.

2013 January. International Course "Time series Analysis in Ecology and Oceanography". Austral Summer Institute (ASI XIII). Department of Oceanography. University of Concepcion. Dr Rodrigo Montes.

Research experience

Laboratory experience

2015-2017 Lab head in the International laboratory associated (LIA-MORFUN, CNRS & Universidad de Concepcion). Participation in Fondecyt Project 1150891 and Environmental Sustainability of FONDAP Interdisciplinary Center for Aquaculture Research (INCAR). University of Concepcion, Chile.

2012 Research assistant. Fondecyt Project 1100358: "Light inhibition of nitrification in the euphotic zone: testing a long lasting paradigm in a coastal upwelling ecosystem". CONICYT. Dra Camila Fernandez (PI). Chile.

Field experience

2017 Oceanographic Campaing Beagle Channel (~55 °S), Chile. Research Center Dynamics of High Latitude Marine Ecosystems (FONDAP-IDEAL).

2016 Oceanographic Campaign Puyuhuapi Channel., Chilean Patagonia (~45 °S). COPAS Sur Austral (CONICYT PIA).

2015-2016 Seasonal aquaculture monitoring, LLico bay (~37 °S), Concepcion, Chile. FONDAP-INCAR.

2015 Oceanographic Campaign for aquaculture monitoring, Chiloé Island (~42°S). FONDAP-INCAR.

2011-2012 Multiple participations in Time series *station 18* (~36 °S). FONDAP-COPAS.

Publications:

1. **Rain-Franco, A**., Fernandez C., & Beier, S. Long-term exposition pulse disturbances disrupt nitrate cycling. (*In preparation*).

2. **Rain-Franco, A**., Peter, H., de Moraes, G. P., & Beier, S. The cost of adaptation: Pulse disturbances drives community resistance (*In preparation*).

3. **Rain-Franco, A**., Mouquet, N., Barbera, C., Bouvier, T., & Beier, S. Niche breadth affects bacterial transcription patterns along a salinity gradient (*under revision in Molecular Ecology*).

4. **Rain-Franco, A**., de Moraes, G. P., & S, Beier. (2021). Cryopreservation and Resuscitation of Natural Aquatic Prokaryotic Communities. Frontiers in Microbiology 11, 3633. doi:10.3389/fmicb.2020.597653.

5. Fernandez, C, **Rain-Franco, A**, Rojas, C, & Molina, V. (2020) Ammonium release via dissolution and biological mineralization of food pellets used in salmon farming. Aquac Res.; 51: 779– 793. doi:10.1111/are.14428

6. **Rain-Franco, A.,** Sobarzo, M., Caparros, J., & Fernandez, C. (2018) Variability of chromophoric dissolved organic matter in three freshwater-influenced systems along central-southern Chile. Vol. 174:154-161. doi:10.1016/j.pocean.2018.09.009

7. **Rain-Franco, A.,** Rojas, C. & Fernandez, C. (2017) Potential effect of pesticides currently used in salmon farming on photo and chemoautotrophic carbon uptake in central – southern Chile. Aquaculture, Vol. 486: 3: 271-284, doi: 10.1016/j.aquaculture.2017.12.048

8. Levipan, H. A., Molina, V., Anguita, C., **Rain-Franco, A**., Belmar, L. & Fernandez, C. (2016), Variability of nitrifying communities in surface coastal waters of the Eastern South Pacific (~36° S). Environmental Microbiology Reports, 8: 851–864. doi:10.1111/1758-2229.12448

9. **Rain-Franco** A, Muñoz C., & Fernandez C (2014) Ammonium Production off Central Chile (36°S) by Photodegradation of Phytoplankton-Derived and Marine Dissolved Organic Matter. PLoS ONE 9(6): e100224. doi:10.1371/journal.pone.0100224

Grants and fellowships:

2019 Soutien Financier aux Jeunes Chercheurs. Association Francophone d'Ecologie Microbienne (AFEM)**.**

2017 Becas-Chile PhD. Chilean National Agency for Research and Development *(ANID).*

2013 Becas Master. Chilean National Agency for Research and Development *(ANID).*

Communications

Posters presentations

2018 Fernández C**., Rain-Franco A**., & Rojas, C. Potential effects of pesticides used in aquaculture on photo and chemoautotrophic carbon fixation in Central Chile. International Collaboration meetings: France-Chile. 19-21 September, 2018. LIA MORFUN AND LIA MAST. Banyuls sur mer, France.

2016 Fernandez, C. & **Rain-Franco, A**. Bacterial and archaea response to UV radiation and ammonium photoproduction in the coastal upwelling system off central Chile (36°S). Colloque LEFE-CYBER, CNRS. 17-23 August 2016. Toulouse, France.

2016 Fernández C., **Rain-Franco, A**. & Rojas, C. Effect of pesticides in the carbon fixation (photo and chemoautotrophic) in central Chile. XXXVI Congreso de Ciencias del Mar, Concepción, Chile.

Oral presentations

2019 **Rain-Franco A**., Peter, H., de Moraes, G., Fernandez, C. & Beier, S. Frequent pulse disturbances affect microbial community interactions and functioning: experimental evidence using chemostats. Potsdam, Germany.

2017 **Rain-Franco, A**. & Fernandez, C. Respuesta de la producción primaria al uso de pesticidas en la región centro y sur de Chile. VI Congreso Nacional de Acuicultura, Viña del Mar, Chile.

2015 **Rain-Franco A.**, Sobarzo, M. & Fernandez,C. Intradiurnal variation of CDOM in a coastal system influenced by a river. XXXV Congreso de Ciencias del Mar, Coquimbo, Chile.

Skills

Software: Unix-Shell scripting, R programing language, MATLAB

Languages: Spanish, English, French (Basic)

References

Dr. Sara Beier, IOW, Germany: sara.beier@io-warnemuende.de

Dr. Camila Fernandez, OOB, France: fernandez@obs-banyuls.fr