TAXONOMY, DIVERSITY, AND ECOLOGY OF BENTHIC MICROALGAE COMMUNITIES IN THE ARID DRÂA RIVER BASIN (SOUTHEASTERN MOROCCO): IMPACT OF SALINIZATION, BIOMONITORING, AND WATER QUALITY ASSESSMENT

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Accepted Dissertation thesis for the partial fulfillment of the requirements
for a Doctor of Natural Sciences
Fachbereich 7: Natur- und Umweltwissenschaften
RPTU Kaiserslautern-Landau
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Examination date: 14/07/ 2025

Declaration

I declare that I have written the dissertation entitled “Taxonomy, diversity, and ecology of benthic microalgae communities in the arid Drâa river basin (Southeastern Morocco): Impact of salinization, biomonitoring, and water quality assessment” independently, and all aids and sources used for the thesis have been indicated. Contributions from any collaborators and other authors are clearly labeled.

I declare that I have not utilized any paid mediation or counseling services, including those provided by doctoral advisors or others.

I confirm that apart from submitting the thesis to Cadi Ayyad University and RPTU Rhineland-Palatinate Technical Kaiserslautern-Landau University in the framework of the cotutelle, this thesis has not been submitted, in identical or substantially similar form, to any other state or academic examination in Germany or abroad. I also confirm that this dissertation has not been submitted to any other faculty or academic institution for a doctoral examination.

I am aware that a violation of any of the aforementioned points may result in the withdrawal of the doctoral degree and may also have further legal consequences.

I affirm that I used DeepL Translate [deepl.com] to translate key terms in preparing this assessment. I also used ChatGPT [https://chat.openai.com/], QuillBot, and Grammarly to refine my writing in this dissertation. I used the following features: clarity, paraphrasing, correctness, and readability. I carefully evaluated the feedback and revised the text using my own words and style.

Khawla Lazrak

Marrakesh, 03/09/2025

List of publications

This thesis comprises five articles, which are introduced below. The references for each chapter have been compiled into a single list at the end of this thesis. Funding for the research was provided by the "SALIDRAA 2" project under the Social-Ecological Research Programme of the German Federal Ministry of Education and Research (grant no. 01UU1906). Sequence data processing was carried out at the HPC cluster “elwetritsch” at the RPTU Kaiserslautern-Landau, supported by the DFG (INST 248/182–1 FUGG and INST 248/354–1 FUGG).

Article 1- Chapter 2 (Submitted)

Lazrak, K., Nothof, M., Filker, S., El Bouaidi,W. Berger, E., Loudiki,M. Water salinity impacts on the composition, structure and diversity of benthic microalgal communities in the arid Drâa river (Southeastern Morocco) using morphological and molecular metabarcoding. Science of the Total Environment.

Article 2- Chapter 3 (Unpublished)

Lazrak, K., Nothof, M., Filker, S., Berger, E., Loudiki,M. Water quality assessment and diatom-based biomonitoring of the ecological status of the Drâa river basin.

Article 3- Chapter 4 (Published)

Lazrak, K., Tazart, Z., Nothof, M., Filker, S., Hakkoum, Z., Kaczmarek, N., ... & Loudiki, M. (2025). Assessment of the short-term salinity effect on algal biofilm through field transfer in the Drâa river (Southeastern Morocco) using metabarcoding and morphological analyses. Environmental Monitoring and Assessment, *197*(4), 1-22.

Article 4- Chapter 5 (Published)

Lazrak, K., Nothof, M., Tazart, Z., Filker, S., Berger, E., Mouhri, K., & Loudiki, M. (2024). Salt stress responses of microalgae biofilm communities under controlled microcosm conditions. Algal Research, 103430.

Article 5- Chapter 6 (Unpublished)

Lazrak, K., Tazart, Z., Berger, E., Mouhri, K., & Loudiki, M. Salt stress responses of two diatom strains isolated from different saline habitats under laboratory batch cultures.

Dedication

I dedicate this work to
My dearest parents, *Latifa* and *Mohamed*,

Your unwavering support, patience, and encouragement have been the bedrock of my academic journey. The numerous sacrifices you've made, along with your unconditional love and steadfast presence, have contributed significantly to this success. No words of dedication can genuinely express the depth of love, respect, and gratitude I feel for you. Nothing in this world can match the relentless effort you have poured—day and night—into my education and well-being. I am incredibly thankful for everything you've done—thank you for being my steadfast strength and most dependable guide.

My dearest Brother, *Zakaria*, and Sister, *Ghita*.
You have been a constant source of strength, comfort, and joy during this journey. I sincerely appreciate your love, support, listening, and encouragement. Your presence means more to me than words can convey, and I completely share this achievement with you both.

My dearest Husband *Zakaria*,
Your unwavering support was a proper foundation of stability during my university journey. Your encouragement, understanding, and steadfast belief in me have significantly impacted every stage of this experience. I feel incredibly lucky to have you not just as my life partner, but also as my closest friend and colleague— a fellow traveler with whom I have shared the joys, challenges, and efforts of this academic endeavor.

To my beloved cousins, *Camelia* and *Naima*, and my Nephews *Ali*, *Sami*, and *Ghaith*
Your love, your support, and the wonderful memories we've shared truly make my life feel so rich and fulfilling.

To my dearest aunts and uncles,
To my dear in-laws
In memory of my aunt Rachida, my second mother, whom I lost during my PhD journey. Her love will always remain in my heart, and I miss her dearly every single day.
And in memory of my uncle Mbarak and my grandmother's grandfather's May Allah rest their souls in peace.

Acknowledgements

I thank Almighty God for granting me the strength to carry out this work, and I also thank myself for the perseverance and dedication that have brought me this far. I am also profoundly grateful to all those who have supported, helped, and encouraged me in completing my dissertation.

First, I would like to express my sincere gratitude to my supervisors, Professors **Mohammed Loudiki** and **Elisabeth Berger**, for their invaluable guidance and support throughout my thesis. Their expertise and encouragement shaped the direction and quality of my research. I especially appreciate the enriching discussions, constructive advice, and the opportunity to conduct my PhD thesis in the Salidraa Juj Project, which makes this journey meaningful and rewarding.

I would like to thank Professor **Mouhri Khadija** for her knowledge, expertise, and support. Her guidance has motivated me and fostered encouragement, enabling me to persevere until this endeavor's completion. I am truly thankful for your contributions.

Completing this thesis would not have been possible without the collaboration of Dr. **Sabine Filker** and my colleague and best friend, **Maren Nothof**, from the Molecular Ecology Group at RPTU Kaiserslautern-Landau in Kaiserslautern, Germany. I want to express my gratitude for their warm welcome. Their generosity in sharing their expertise and allowing me to collaborate within their esteemed research environment provided an enriching and instructional experience. I sincerely thank them for their hospitality and collaborative spirit, which significantly contributed to completing an essential aspect of my thesis.

I want to express my gratitude to the entire team of the “Socio-ecological Systems” working group and the Salidraa juj project, especially Pr. **Mohammed Znari**, Pr. **Mohammed Naimi**, Pr. **Mohammed Ghamizi**, Dr. **Lisa Bossenbroek**, Pr. **Oliver Frör**, and Pr. **Janpeter Schilling**. Throughout my PhD journey, I gained valuable knowledge and built wonderful friendships! I'm grateful to **Imane Mahjoubi**, **Maren Nothof**, **Abdelghani Aabid**, **Nils Kaczmarek**, **Mokhtar Benlasri**, **Luis Miguel Silva-Novoa Sánchez**, and **Merouane Yassine** for all the fantastic moments we shared together. Thank you for being such an important part of this experience!

I would also like to express my gratitude to Pr. **Ouazzani Naaila**, the director of the Laboratory of Water Sciences, Microbial Biotechnologies, and Sustainability of Natural Resources, for kindly agreeing to assess and chair the jury for my thesis defense.

I extend my warm thanks to the members of the jury. I am grateful to Pr. **Sabour Brahim**, Pr. **Kaaya Abderrazak**, and Pr. **Oudra Brahim** for agreeing to evaluate this work as reporters. I also appreciate Pr. **Naimi Mohamed** and Pr. **Douma Mountasser** for serving on the jury of this thesis and for evaluating this work.

I want to express my heartfelt gratitude to my friends, particularly to my best friend, **El Bouaidi Widad**. Her friendship has been a constant source of strength and joy throughout our journey, from undergraduate studies to my PhD. Widad’s kindness has had a profound impact on my academic and personal development. More than just a friend, she has been a solid pillar in my life. Her unwavering support, the joy we've shared, and the strength of our bond have guided me through my toughest moments. I am sincerely thankful to have Widad in my life—an invaluable ally with whom I've created unforgettable memories and a profound connection.

I also want to thank my best friend, **Soukaina El Amrani Zerrifi**, whom I met during my doctoral journey. We share traits that forged a deep bond. Her friendship is one of the most valuable gifts of this experience. Her wisdom and unwavering support have balanced and comforted me during challenging times. More than just a friend, she is an irreplaceable part of my life.

I appreciate **Abderrahim S'khifa** for his unwavering support during my PhD. In times of hardship, he was a true friend. His empathy, availability, and commitment exceeded expectations. He has been like a brother, providing comfort and encouragement. His generosity and friendship have made a lasting impact, and I will always be thankful for his help on this challenging journey.

To my loyal friend **Khaoula Bouziane**, despite the miles between us, she has consistently offered her support, friendship, and understanding.

I also want to express my sincere thanks to all Professors and colleagues from Laboratory of Water Sciences, Microbial Biotechnologies, and Sustainability of Natural Resources Faculty of Sciences Semlalia, Cadi Ayyad University, and friends, to all of them, Dr. **Minaoui Farah**, Dr **Hakkoum Zineb**, **Ouala Oumaima**, **Chabili Amer**, Dr. **Benrazzouk Karima**, Dr. **Tebaa Lamiaa**, Dr. **Haida Mohamed**, Dr. **Redouane Elmehdi**, Dr**. Mougani Richard**, **Essadki Yasser**, Dr. **Khadija Zidan**, and Dr. **Krimech Aafaf** which always showed the greatest support, generosity, availability and friendship during these years. It is much easier to work when you are part of a cohesive and dynamic team that provides you with all the necessary support.

I warmly thank all the administrative and technical staff of the FSSM, and in particular, those of the Departments of Biology, who were always available and supportive, as well as all my friends and colleagues.

To the Faculty of Sciences Semlalia for the real and invaluable support.

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Abbreviation list

%: Percentage

AFDW: Ash free dry weight

ANOVA: Analysis of variance APX: Ascorbate peroxidase

ASV: Amplicon Sequence Variant

BC: Bray Curtis

bp: base pairs CAT: Catalase

Chl-*a*: Chlorophyll *a*

Cm^2: Square centimeter

CO_2: Carbon dioxide

DADA2: Divisive Amplicon Denoising Algorithm 2

DO: Dissolved oxygen

DOC: Dissolved organic carbon

DW: Dry weight

EC: Conductivity

EPS: Extracellular polysaccharide

ES: Ecosystem services

Fig.: Figure

FS: Freshwater salinization

g/L: Gram per liter

g: Gram

GR: Glutathione reductase

h: Hour

H_2O_2: Hydrogen peroxide

IPPC: Intergovernmental Panel on Climate Change

IPS: Specific Polluo Sensitivity Index

IPS morpho: Specific Polluo Sensitivity Index using morphological data

IPS meta: Specific Polluo Sensitivity Index using DNA metabarcoding data

IR: Refractive index

IRES: Intermittent rivers and ephemeral streams

L: Liter

$lx.m^{-2}s^{-1}$: Lux per square meter per second

m: meter

M: Molarity

MDA: Malondialdehyde

ME: El Mansour Eddahbi dam

min: Minute

N: North

NaCl: Sodium chloride

NADPH: Nicotinamide adenine dinucleotide phosphate

NBT: Nitro blue tetrazolium chloride

NH_4^+: Ammonium

NMDS: Non-metric multidimensional scaling

NO_2^-: Nitrite

NO_3^-: Nitrate

ºC: Celsius degree

OD: Optical Density

OM: Organic matter

PCR: Polymerase Chain Reaction

PERMANOVA: Permutational Multivariate Analysis of Variance

PO_4^{3-}: Orthophosphate

POD: Peroxidase

rDNA: ribosomal Deoxyribonucleic Acid

ROS: Reactive oxygen species

rpm: Rotation per minute

RUBISCO: Ribulose-1,5-bisphosphate carboxylase/oxygenase

s: Second

SOD: Superoxide dismutase

TBA: Thiobarbituric acid

TCA: Trichloroacetic acid

U: Unit enzymatic activities

UNESCO: United Nations Educational, Scientific and Cultural Organization

UV: Ultraviolet light

VSEARCH: Vectorized search algorithm

W: West

WFD: Water Framework Directive

WI: water quality index

x1000: Microscope magnification

µS/cm: micro siemens per centimeter

Abstract

Many dryland and desert rivers, especially in North Africa, faced significant physicochemical and hydromorphological extreme changes due to climate change and anthropogenic pressures such as agriculture, mining, and tourism. These impacts are increasing the concentration of salts, threatening biodiversity, freshwater ecosystem functions and services, and raising vulnerability to water quality degradation, water security, and human well-being. While freshwater salinization is an escalating concern, understanding its effects on intermittent rivers and ephemeral streams (IRES) in the arid North Africa region is still limited and receives little scientific attention. The Drâa river (southeastern Morocco) is a human-regulated river facing multiple human pressures, resulting in reduced flow and increased water salinity. The main objective of this thesis is to investigate the composition, diversity, structure, and dynamics of benthic microalgae communities in the arid Drâa river, examine their responses to salinization effects, and assess water quality and ecological status using morphological versus molecular metabarcoding approaches.

The results showed that salinity increases from the upper to the lower Drâa, rising during dry periods due to reduced water flow and increased evaporation. Benthic microalgae samples from 19 sites during dry and wet periods revealed an important diversity of 159 diatom species, 40 green algae, and 48 cyanobacteria species identified morphologically. The morphological and DNA metabarcoding analysis of the benthic microalgae community reveals that salinity and hydrological conditions greatly affect their assemblages and composition, particularly for diatoms, with salinity emerging as the major influencing factor.

Examining water quality index through physicochemical parameters revealed that mineralization (conductivity and chloride) affects the lower and middle Drâa, while other factors influence the upper Drâa. This upstream part of the basin faced less pollution than the middle and lower Drâa, which are more impacted by anthropogenic pressures. The water quality index indicated worse values in the lower Drâa due to high point source pollution, while the IPS diatom index highlighted its reliability in capturing long-term environmental changes in the river. The diatom index IPS values from classical morphological and DNA metabarcoding analysis significantly correlated with similar ecological quality classes for over half of the samples, despite varying taxonomic compositions.

The biofilm field-transfer experiment demonstrated that increasing salinity in the Drâa river negatively affects benthic microalgae, both in terms of biomass and diversity, while lower salinity in brackish areas can benefit their growth. Similarly, the freshwater biofilm of the Drâa river was exposed to different salinity levels (1, 10, 30 g/L NaCl) using a microcosm experiment, demonstrating physiological and biochemical changes in response to salt stress for survival. Low salinity levels (1 g/L) promote biofilm development, whereas higher levels (10 and 30 g/L) hinder it. More specifically, the experimental batch culture evaluated the impact of increasing salinity on two diatom strains isolated from different salt habitats, showing that the halotolerant diatom species have greater physiological plasticity at higher salinity than the freshwater diatom strains.

The thesis results will enhance the understanding of the ecological impacts of freshwater salinization on benthic microalgal communities, predicting the consequences on aquatic ecosystem services, water quality, and implementing monitoring schemes, mitigation measures, and best management practices. These findings highlight the effectiveness of benthic diatoms as bioindicators for arid and desert river water quality and ecological assessments using both microscopic and molecular techniques.

Keywords: Drâa river, Benthic microalgae, Freshwater salinization, hydrological periods, Diatom index, Water quality, Ecological assessment, morphological identification, DNA metabarcoding.

Zusammenfassung

Viele Trocken- und Wüstenflüsse, vor allem in Nordafrika, sind aufgrund des Klimawandels und anthropogener Einflüsse wie Landwirtschaft, Bergbau und Tourismus erheblichen physikochemischen und hydromorphologischen Veränderungen ausgesetzt. Diese Auswirkungen erhöhen die Salzkonzentration, bedrohen die biologische Vielfalt, die Funktionen und Leistungen der Süßwasserökosysteme und erhöhen die Anfälligkeit für eine Verschlechterung der Wasserqualität, die Wassersicherheit und das menschliche Wohlergehen. Während die Versalzung des Süßwassers ein zunehmendes Problem darstellt, sind die Auswirkungen auf intermittierende Flüsse und ephemere Bäche (*engl.* intermittent rivers and ephemeral streams, IRES) in der trockenen Region Nordafrikas noch wenig erforscht und finden wenig wissenschaftliche Beachtung. Der Drâa-Fluss (Südostmarokko) ist ein vom Menschen regulierter Fluss, der mehrfachen Belastungen ausgesetzt ist, die zu einer Verringerung des Durchflusses und einem erhöhten Salzgehalt des Wassers führen. Das Hauptziel dieser Arbeit ist es, die Zusammensetzung, Vielfalt, Struktur und Dynamik benthischer Mikroalgengemeinschaften im Drâa-Fluss zu untersuchen, ihre Reaktionen auf Versalzungseffekte zu untersuchen und die Wasserqualität und den ökologischen Status mit Hilfe morphologischer und molekularer Metabarcoding-Ansätze zu bewerten.

Die Ergebnisse zeigten, dass der Salzgehalt vom oberen zum unteren Drâa ansteigt und in Trockenperioden aufgrund des geringeren Wasserflusses und der erhöhten Verdunstung, zunimmt. Benthische Mikroalgenproben von 19 Standorten gesammelt in Trocken- und Feuchtperioden, die morphologisch identifiziert wurden, ergaben 159 Kieselalgen-, 40 Grünalgen- und 48 Cyanobakterienarten. Die morphologische und DNA-Metabarcoding-Analyse der benthischen Mikroalgengemeinschaft zeigt, dass der Salzgehalt und die hydrologischen Bedingungen einen großen Einfluss auf ihre Zusammensetzung haben, insbesondere bei Kieselalgen, wobei sich der Salzgehalt als wichtigster Einflussfaktor herausstellte.

Die Untersuchung des Wasserqualitätsindex anhand physikalisch-chemischer Parameter ergab, dass die Mineralisierung (Leitfähigkeit und Chlorid) den unteren und mittleren Drâa beeinflusst, während andere Faktoren den oberen Drâa beeinflussen. Dieser flussaufwärts gelegene Teil des Einzugsgebiets ist weniger verschmutzt als dermittlere und untere Drâa, der stärker von anthropogenen Einflüssen betroffen ist. Der Wasserqualitätsindex wies in dem unteren Drâa aufgrund der starken Verschmutzung durch Punktquellen schlechtere Werte auf, während der IPS-Diatomeenindex seine Zuverlässigkeit bei der Feststellung langfristiger Umweltveränderungen im Fluss unterstrich. Die IPS-Diatomeen-Indexwerte aus klassischer Morphologie und DNA-Metabarcoding korrelierten in mehr als der Hälfte der Proben trotz unterschiedlicher taxonomischer Zusammensetzung signifikant mit ähnlichen ökologischen Qualitätsklassen.

Ein Biofilm-Feldtransfer-Experiment zeigte, dass sich der steigende Salzgehalt im Drâa-Fluss negativ auf benthische Mikroalgen auswirkt, sowohl hinsichtlich der Biomasse als auch der Vielfalt, während ein niedrigerer Salzgehalt in Brackwassergebieten ihr Wachstum begünstigen kann. In ähnlicher Weise wurde der Süßwasser-Biofilm des Drâa-Flusses in einem

Mikrokosmos-Experiment unterschiedlichen Salzgehalten (1, 10, 30 g/l NaCl) ausgesetzt, wobei physiologische und biochemische Veränderungen als Reaktion auf Salzstress zum Überleben nachgewiesen wurden, jedoch nur innerhalb einer bestimmten Schwelle. Niedrige Salzgehalte (1 g/l) förderten die Biofilmbildung, während höhere Gehalte (10 und 30 g/l) ihr schadeten. Genauer gesagt wurde in der experimentellen Batch-Kultur der Einfluss steigender Salzgehalte auf zwei aus verschiedenen Salzlebensräumen isolierten Diatomeenstämme untersucht, wobei sich zeigte, dass die halotoleranten Diatomeenarten bei höheren Salzgehalten eine größere physiologische Plastizität aufweisen als die Süßwasser-Diatomeenstämme.

Die Ergebnisse der Dissertation werden das Verständnis der ökologischen Auswirkungen der Versalzung von Süßwasser auf benthische Mikroalgen-Gemeinschaften verbessern und ermöglichen es, die Folgen für aquatische Ökosystemleistungen und die Wasserqualität vorherzusagen sowie Überwachungsprogramme, Minderungsmaßnahmen und bewährte Managementpraktiken umzusetzen. Diese Ergebnisse unterstreichen die Wirksamkeit benthischer Kieselalgen als Bioindikatoren für die Wasserqualität und ökologische Bewertungen von Flüssen in ariden und wüstenartigen Gebieten unter Verwendung sowohl mikroskopischer als auch molekularer Techniken.

Schlüsselwörter: Fluss Drâa, benthische Mikroalgen, Versalzung, hydrologische Perioden, Kieselalgen, Wasserqualität, ökologische Gewässerbewertung, morphologische Identifizierung, DNA-Metabarcoding.

Résumé

De nombreux cours d’eau des zones arides et désertiques, en particulier en Afrique du Nord, sont confrontées à des changements physicochimiques et hydromorphologiques extrêmes dus au changement climatique et aux pressions anthropiques telles que l'agriculture, l'exploitation minière et le tourisme. Ces impacts augmentent la concentration en sels, menacent la biodiversité, les fonctions et les services des écosystèmes d'eau douce et augmentent la vulnérabilité à la dégradation de la qualité de l'eau, à la sécurité hydrique et au bien-être humain. Alors que la salinisation de l'eau douce est une préoccupation croissante, la compréhension de ses effets sur les cours d'eau intermittents et éphémères des zones arides de l'Afrique du Nord est encore limitée et reçoit peu d'attention scientifique. Oued Drâa (sud-est du Maroc) est un cours d’eau régulée par l'homme qui subit de multiples pressions humaines, entraînant une réduction du débit et une augmentation de la salinité de l'eau. L'objectif principal de cette thèse est d'explorer la composition, la diversité, la structure et la dynamique des communautés de microalgues benthiques dans l’Oued Drâa, d'examiner leurs réponses aux effets de la salinisation et d'évaluer la qualité de l'eau et l'état écologique en utilisant des approches d’identification morphologique et moléculaire par métabarcording.

Les résultats ont montré que la salinité s'accroît du haut vers le bas Drâa, en particulier pendant les périodes sèches, en raison de la réduction du débit et de l'augmentation de l'évaporation. Des échantillons de microalgues benthiques prélevés sur 19 sites pendant des périodes sèches et humides ont révélé une diversité importante de 159 espèces de diatomées, 40 algues vertes et 48 espèces de cyanobactéries identifiées morphologiquement. L'analyse morphologique et le métabarcodage de la communauté microalgale benthique montrent que la salinité et les conditions hydrologiques affectent de manière significative leurs assemblages et leur composition, en particulier pour les diatomées, où la salinité semble être le principal facteur d'influence.

La qualité de l'eau à partir des paramètres physico-chimiques a révélé que la minéralisation (conductivité et chlorure) affecte le bas et moyen Drâa, tandis que le haut Drâa est influencé par d'autres facteurs. Cette partie amont du bassin est moins polluée que le moyen et bas Drâa, qui est plus touché par les pressions anthropiques. L'indice de qualité de l'eau a indiqué des valeurs plus mauvaises dans le bas Drâa en raison d'une forte pollution ponctuelle, tandis que l'indice diatomique IPS a mis en évidence sa fiabilité pour saisir les changements environnementaux à long terme dans le cours d’eau. Les valeurs de l'indice diatomique IPS obtenues par morphologie classique et par métabarcodage sont significativement corrélées avec des classes de qualité écologique similaires pour plus de la moitié des échantillons, malgré une composition taxonomique différente.

L'expérience de transfert in situ du biofilm a démontré que l'augmentation de la salinité dans l’Oued Drâa affecte négativement les microalgues benthiques, à la fois en termes de biomasse et de diversité, alors qu'une salinité plus faible dans les zones saumâtres peut être bénéfique à leur croissance. De même, le biofilm d’un affluent d’eau douce de l’Oued Drâa a été exposé à différents niveaux de salinité (1, 10, 30 g/L NaCl) à l'aide d'une expérience en microcosme, démontrant des changements physiologiques et biochimiques en réponse au stress salin pour la survie. Les faibles niveaux de salinité (1 g/L) favorisent le développement du biofilm, tandis que les niveaux plus élevés (10 et 30 g/L) lui nuisent. Plus précisément, la culture expérimentale par batch a évalué l'impact de l'augmentation de la salinité sur deux souches de diatomées isolées de différents habitats salins, montrant que les espèces de diatomées halotolérantes ont une plus grande plasticité physiologique à une salinité plus élevée que les souches de diatomées d'eau douce.

Les résultats de la thèse permettront de mieux comprendre les impacts écologiques de la salinisation sur les communautés de microalgues benthiques, de prévoir les conséquences sur les services des écosystèmes aquatiques, la qualité de l'eau, et de mettre en œuvre des schémas de surveillance, des mesures d'atténuation et des meilleures pratiques de gestion. Ces résultats soulignent l'efficacité des diatomées benthiques en tant que bioindicateurs pour la qualité de l'eau des wadis et les évaluations écologiques utilisant des techniques microscopiques et moléculaires.

Mots-clés : Oued Drâa, Microalgues benthiques, Salinisation, Périodes hydrologiques, Indice diatomique, Qualité de l'eau, Etat écologique, Identification morphologique, Métabarcoding.

ملخص

في السنوات الاخيرة ومع توالي سنوات الجفاف، تتعرض العديد من الأنهار في المناطق الجافة والصحراوية، لا سيما في شمال إفريقيا، لتحولات قصوى في خصائصها الفيزيائية والكيميائية والهيدرومورفولوجية، نتيجة للتغيرات المناخية والضغوط البشرية المتزايدة، كالتوسع الزراعي، والتعدين، والنشاط السياحي. وقد أدت هذه التأثيرات إلى ارتفاع تركيز الأملاح، مما يهدد التنوع البيولوجي، ويقوض وظائف وخدمات النظم البيئية للمياه العذبة، ويزيد من هشاشتها تجاه تدهور جودة المياه وأمنها، وبالتالي يؤثر سلباً على رفاهية الإنسان. ورغم تصاعد القلق العالمي حول ملوحة المياه العذبة، لا يزال فهم تأثيراتها على الأنهار المتقطعة والجداول المؤقتة في المناطق القاحلة من شمال إفريقيا محدوداً، ولا تحظى هذه الظاهرة بالاهتمام العلمي الكافي. في هذا السياق، يُعد نهر درعة (جنوب شرق المغرب) نموذجاً مهماً لدراسة هذه الظاهرة، حيث يخضع لتنظيم بشري مكثف، ويواجه ضغوطاً متعددة أدت إلى انخفاض تدفق المياه وارتفاع ملوحتها.

تهدف هذه الأطروحة إلى دراسة تركيب وتنوع وبنية ودينامية مجتمعات الطحالب الدقيقة القاعية في نهر درعة، وتحليل استجابتها لتغيرات الملوحة، بالإضافة إلى تقييم جودة المياه والحالة البيئية للنهر باستخدام منهجين متكاملين: التحليل المورفولوجي والتحليل الجزيئي بتقنية الميتاباركودينغ عبر دراسة تنوع الحمض النووي.

حيث أظهرت النتائج أن الملوحة تزداد تدريجياً من المنبع إلى المصب، لا سيما خلال الفترات الجافة التي تتميز بانخفاض تدفق المياه وارتفاع معدلات التبخر. كما كشفت العينات التي جُمعت من 19 موقعاً خلال الفترات الجافة والرطبة عن تنوع ملحوظ تمثل في 159 نوعاً من الدياتومات، و40 نوعاً من الطحالب الخضراء، و48 نوعاً من البكتيريا الزرقاء، تم تحديدها اعتماداً على الفحص المجهري.

في مأظهرت التحليلات المورفولوجية والجزيئية أن ملوحة المياه والظروف الهيدرولوجية تؤثر بشكل كبير على بنية مجتمعات الطحالب الدقيقة، حيث برزت الملوحة كأهم العوامل المحددة، خصوصاً بالنسبة للدياتومات. كما أظهرت مؤشرات جودة المياه، من خلال المعايير الفيزيائية والكيميائية، أن التمعدن (المتمثل في الموصلية والكلوريد) يُشكل تأثيراً أساسياً في المناطق الوسطى والسفلى من الحوض، في حين يتأثر الجزء العلوي بعوامل أخرى، ويعاني من تلوث أقل نسبياً مقارنةً بالمناطق الأخرى الأكثر تأثراً بالأنشطة البشرية.

أبرز مؤشر جودة المياه قيمًا متدهورة في الجزء السفلي من نهر درعة نتيجة للتلوث النقطي المرتفع، في حين أظهر مؤشر الدياتومات (IPS) فعاليته في رصد التغيرات البيئية طويلة المدى. وتبيّن أن القيم المحسوبة باستخدام المنهجين المورفولوجي والجزيئي تتقاطع في تصنيف الحالة البيئية لأكثر من نصف العينات، رغم التباين في التركيب التصنيفي.

أما التجارب الميدانية والمخبرية، فقد أظهرت أن زيادة الملوحة تؤثر سلباً على نمو وتنوع الطحالب القاعية، حيث أدى ارتفاع الملوحة إلى انخفاض الكتلة الحيوية، في حين ساهمت المستويات المنخفضة منها في تعزيز نموها. وعند تعريض الأغشية الحيوية لمستويات مختلفة من الملوحة (1، 10، 30 جم/لتر NaCl) في تجربة مجهرية، لوحظت تغيرات فسيولوجية وكيميائية حيوية تعكس استجابات تكيفية مع إجهاد الملوحة، ولكن فقط ضمن حدود معينة؛ فبينما دعمت الملوحة المنخفضة (1 جم/لتر) تطور الأغشية، سببت المستويات الأعلى (10 و30 جم/لتر) آثاراً سلبية واضحة.

وقد أظهرت تجارب الزرع المعزول أن الأنواع الدياتومية المتحملة للملوحة تُظهر مرونة فسيولوجية أكبر مقارنة بالأنواع التي تعيش في بيئات مياه عذبة، ما يؤكد أهمية التكيف البيئي في مواجهة التغيرات الملحية.

تُساهم نتائج هذه الدراسة في تعزيز الفهم العلمي لتأثير ملوحة المياه العذبة على مجتمعات الطحالب القاعية، وتُوفر قاعدة معرفية للتنبؤ بتداعيات هذه الظاهرة على خدمات النظم البيئية المائية وجودة المياه، مع دعم جهود الرصد البيئي وتطبيق التدابير الوقائية وأفضل ممارسات الإدارة. كما تؤكد النتائج فعالية الدياتومات القاعية كمؤشرات حيوية لتقييم جودة المياه في الأنهار الجافة والصحراوية، باستخدام كل من التقنيات المجهرية والتحليلات الجزيئية الحديثة.

الكلمات المفتاحية: واد درعة، الطحالب الدقيقة القاعية، تمليح المياه العذبة، الفترات الهيدرولوجية، مؤشر الدياتومات، جودة المياه، التقييم الإيكولوجي، التعريف المورفولوجي، التسلسل الجيني (ميتاباركودينغ).

General introduction

Desert rivers constitute a considerable proportion of the world's river ecosystems, occurring in arid, Mediterranean, and North African regions where they are commonly referred as “wadis” (Wheater and Al Weshah, 2002; Sen, 2008). These rivers belong to the intermittent river and ephemeral stream ecosystems (IRES) featuring fluctuating flow patterns and variable hydro-morphological and physicochemical characteristics (Kingsford and Thompson, 2006; Harms et al., 2018; Magand et al., 2020). Their flow, subject to hydrological occurrences such as droughts and floods, undergoes temporary interruptions in surface runoff, leading to substantial alterations in habitat across the hydrological cycle (Stanley et al., 1997; Datry et al., 2017c). They operate as vital arteries, providing shelter for diverse flora and fauna in a challenging desert environment (Arthington et al., 2014; Kerezsy et al., 2017; Stubbington et al., 2017b; Sabater et al., 2017). Moreover, human populations around these rivers rely strongly upon them for their subsistence and economic activities, including freshwater supply (drinking, domestic, and irrigation water), food, climate regulation, and recreation (Datry et al., 2018; Magand et al., 2020; Stubbington et al., 2020). Nevertheless, several anthropogenic factors (e.g., river dam regulation and water abstraction for agriculture) negatively impact the water quality of these rivers, notably salinization, which adversely affects aquatic life and ecosystem functions and services (Chiu et al., 2017; Magand et al., 2020; Acuña et al., 2020; Datry et al., 2023; Berger et al., 2019). Despite their important territorial value and remarkable functional biodiversity, IRES, primarily in North Africa, received considerably less research coverage and legal protection than temperate rivers worldwide (Clavero et al., 2015, 2017; Nikolaidis et al., 2013; Acuña et al., 2014; Chiu et al., 2017; Messager et al., 2021).

Generally, dryland rivers and streams are naturally salty due to high evaporation and geological causes, known as primary salinization (Berger et al., 2019). Furthermore, human activities exacerbate this problem, leading to secondary salinization (Cañedo-Argüelles et al., 2013; Iglesias, 2020; Cañedo-Argüelles et al., 2019; Thorslund et al., 2021; Kaushal et al., 2021; Cunillera-Montcusí et al., 2022). Over the past century, the problem of freshwater salinization has increased considerably due to the impacts of climate change, affecting mainly rivers, water security, and human well-being in arid and semi-arid regions (Cunillera-Montcusí et al., 2022; Jeppesen et al., 2020; Kaushal et al., 2018). It endangers the biodiversity of aquatic communities and undermines ecosystem functions and services, adversely affecting the entire food chain - from microalgae, the primary producers, to organisms at higher trophic levels - by triggering cascade interactions (Hintz et al., 2017; Ersoy et al., 2022). As primary producers in the food chain, benthic microalgae produce energy and oxygen through photosynthesis.

Ecosystem nitrogen cycling and energy transfer depend crucially on these microorganisms (Battin et al., 2016; Lowe and LaLiberte, 2017). Moreover, their ability to uptake nutrients and contaminants remains essential for water self-purification (Sabater et al., 2007; Wu, 2018; Battin et al., 2016). Additionally, benthic microalgae develop biofilms that supply food and shelter to many organisms, thereby stabilizing fluvial environments (Majdi et al., 2012). Considered the dominant group of benthic microalgae communities, diatoms have specific growth preferences and short reproductive cycles (Smol and Stoermer, 2010; Lowe and LaLiberte, 2017). Particularly, their ability to respond promptly to environmental changes renders them ideal bioindicators of water quality, including salinity (Rimet, 2012ab; Vidal et al., 2021; Kaddeche et al., 2024). Accordingly, benthic diatom-based assessments have been used globally to quantify river pollution and alteration, notably in EU countries and the USA (Charles et al., 2021).

One of the world's ten driest rivers, the Drâa basin in southeastern Morocco is the country's longest, spanning 115,000 km² and extending from the High Atlas to the Atlantic Ocean in the west and the Sahara Desert in the south (Revenga et al., 1998; Carrillo-Rivera et al., 2013). It is an essential natural and cultural heritage of the North African region, classified as a UNESCO World Heritage Site and a RAMSAR zone. Climate change and the overuse of water resources in this catchment area led to declining groundwater levels and an escalation of soil and river salinization. Furthermore, most rivers in the Drâa basin are temporary, relying on precipitation or snowmelt, and often present a natural salinity owing to high evaporation rates and geological influences, which is a process known as primary salinization (Speth et al., 2010; Johannsen et al., 2016). This situation worsens with human activities affecting the hydrological cycle, such as dam construction (e.g. Mansour Eddahbi) and land usage in the watershed, involving agricultural methods and extensive irrigation, particularly in downstream areas (secondary salinization) (Potapova and Charles, 2003; Cañedo-Argüelles et al., 2013; Nhiwatiwa et al., 2017). Increasing salinity in this river endangers the entire food chain, triggering a cascade of interactions at multiple levels. It alters the biota, reducing species diversity, functions, and services while threatening human well-being by impairing water quality and agricultural production (Schröder et al., 2015; Lazrak et al., 2022, 2024; Berger et al., 2019, 2021; Kaczmarek et al., 2023, 2024; Johannsen et al., 2016; Mahjoubi and Frör, 2024).

The increasing freshwater salinization (FS) in arid and semi-arid regions, including Northern Africa, poses a growing risk to the IRES, giving rise to considerable concern. This is particularly critical since the impact on essential photosynthetic producers, such as benthic microalgae, which are vital for maintaining the health of these aquatic ecosystems, is poorly documented (Cunillera-Montcusí et al., 2022). The knowledge gap is particularly pronounced in the Drâa basin, a representative example of many arid inland water systems in North Africa. In this area, research on benthic microalgae has been notably limited compared to other organisms. Moreover, there is a considerable lack of insight into how the composition and structure of benthic microalgae, specifically diatom communities, respond to hydrological shifts and salinization effects within IRES, including those in the Drâa river (Delgado et al., 2012; Wu et al., 2019; Falasco et al., 2021). Additionally, the potential of diatoms for biomonitoring freshwater ecosystems in Morocco remains untapped (Benhassane et al., 2020). Closing this knowledge gap is crucial for enhancing ecosystem assessments and supporting the use of diatoms in environmental monitoring within these fluctuating aquatic environments, especially in Morocco. In this context, the main objective of this thesis is to investigate the composition, diversity, structure, and dynamics of benthic microalgae communities in the Oued Drâa, examine their responses to salinization impact, and assess water quality and ecological status using morphological versus molecular metabarcoding approaches.

The thesis is presented in six chapters:

- **Chapter 1** provides an overview of the scientific literature on the physical, chemical, and ecological characteristics of intermittent rivers and ephemeral streams, highlighting the stress factors that cause various threats, including freshwater salinization. It specifically examines the impact of salinization on microalgae and takes the Drâa basin, which is representative of desert rivers in northwest Africa facing this issue, as a case study.

- **Chapter 2** presents an assessment of the composition, structure, and diversity of benthic microalgae assemblages from nineteen sampling sites in the arid Drâa basin, classified according to their water salinity during wet and dry seasons (hydrological periods), using morphological and DNA metabarcoding methods.

- **Chapter 3** highlights the water quality of the Drâa basin using physicochemical parameters and diatom-based biomonitoring of ecological status via morphological and DNA metabarcoding approaches.

- **Chapter 4** uses a biofilm field transfer experiment to investigate salinity's short-term effects on benthic microalgae communities in the Drâa river. Artificial substrates colonized by biofilms in the Drâa river were transferred between three sites of different salinity, and their responses in terms of biomass, alpha, beta diversity, and taxonomic composition were analyzed using morphological and DNA metabarcoding approaches.

- Chapter 5 investigates biofilms' responses to different salinity levels in a microcosm experiment aimed at understanding the potential impacts of freshwater salinization. Biofilms from freshwater in the Drâa basin exposed to progressively increasing concentrations of sodium chloride were assessed for biomass, productivity, biochemical and physiological responses, and oxidative and antioxidant activities.

- Chapter 6 outlines the increasing salinity effects on the growth and physiology of two diatom strains isolated from freshwater and saltwater habitats of the Drâa river in laboratory batch cultures.

Figure 1 presents a graphical abstract summarizing the main contents of this thesis, and the specific objectives of each chapter.

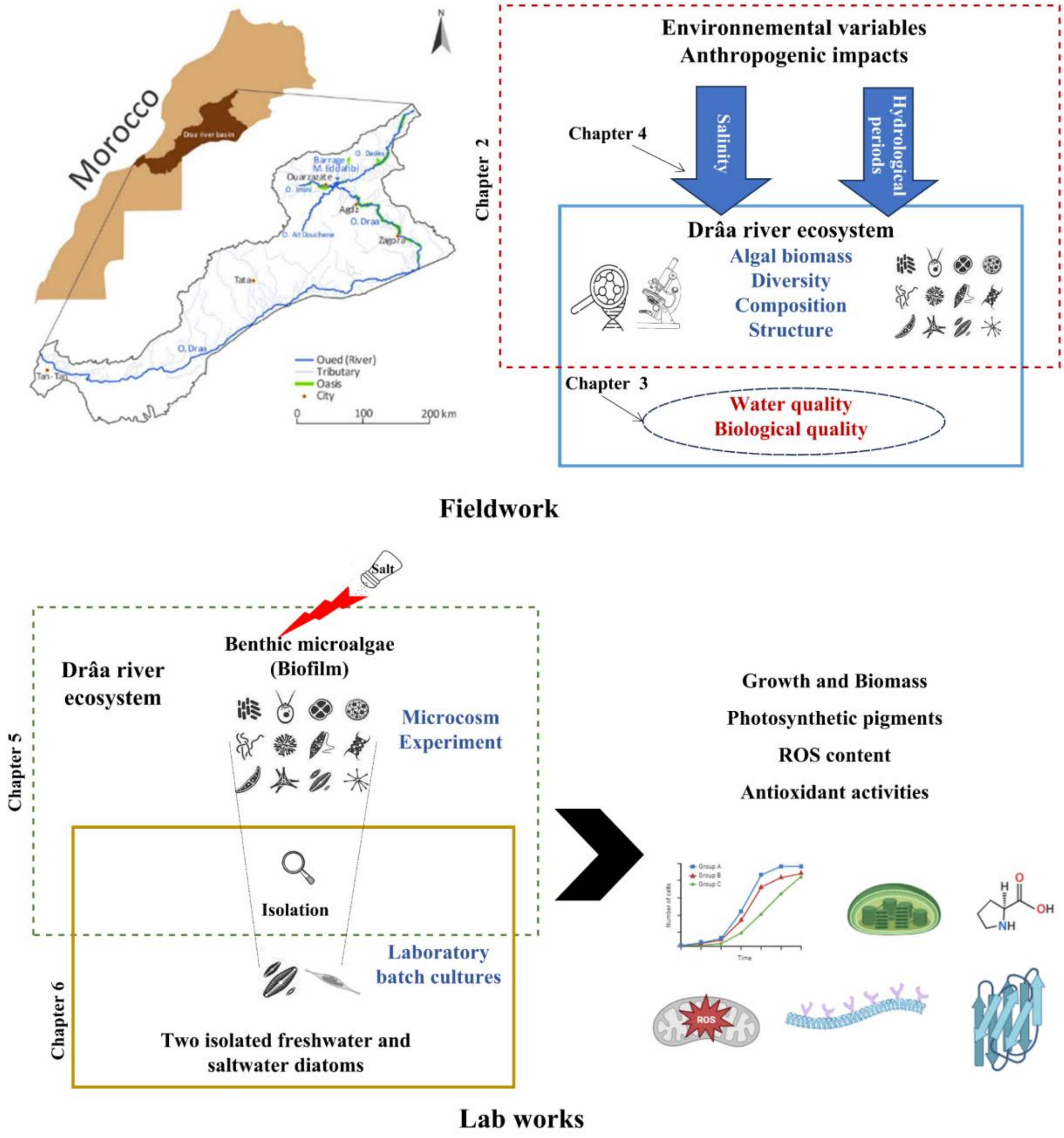

Figure 1: Graphical abstract summarizing the thesis content and the main objectives of each chapter.

Research hypothesis

In this study, we have investigated the following assumptions:

H0: An increase in water salt concentrations of the Drâa river can significantly affect the composition, diversity, abundance, and biomass of benthic microalgae communities, and can lead to changes in ecosystem diversity and functioning regardless of the hydrological periods.

H1: Water quality and ecological status of the Drâa river can be monitored using physicochemical and biological indices, especially the diatom-based index, via morphological and DNA metabarcoding approaches.

H2: Responses of benthic microalgae communities to short-term salinity changes in the Drâa river can be assessed in situ through a biofilm field transfer experiment. This could affect the structure and function of the river biofilm, influence ecosystem processes, and provide information on the ecological implications of freshwater salinization and its potential effects on river ecosystems.

H3: Increasing water salinity levels under controlled mesocosm conditions can negatively affect the composition and decrease the biomass of natural biofilm communities. Furthermore, microalgal cells will undergo physiological and biochemical changes in response to salt stress.

H4: Physiological responses of isolated diatom strains to saline stress can be assessed in laboratory batch culture, providing a better understanding of the tolerance mechanisms involved.

Thesis objectives

The main objective of this thesis is to assess the composition, diversity, structure, and dynamics of benthic microalgae communities in the Oued Drâa (Southeastern Morocco), examine their responses to salinization impact, and assess water quality and ecological status using morphological versus molecular metabarcoding approaches.

In light of this principal aim, specific objectives were defined:

1- To investigate the composition, diversity, abundance, and structure of benthic microalgae communities in the Drâa river as a function of water salinity during dry and wet periods using morphological and molecular methods.

2- To monitor water quality and ecological status in the Drâa river basin using a physicochemical and diatom-based indices via morphological and DNA metabarcoding approaches, and to develop appropriate assessment methods for ecological and water quality.

3- To assess the responses of benthic algal communities to salinity changes through in situ biofilm field transfer in the Drâa river, and to understand how algal biofilm responds to the salinity variations.

4- To evaluate salt stress responses in composition shifts, biomass, physiological, and biochemical processes of natural algal biofilm of the Drâa river under a controlled microcosm experiment.

5- To study the physiological responses to saline stress in laboratory batch cultures of two isolated diatom strains of Drâa river, and to determine their salinity tolerance thresholds and predict the dynamics of benthic diatoms in response to increasing salinity.

Chapter I. Bibliographic review and state-of-the-art

I. Intermittent rivers and ephemeral streams (IRES) ecosystems

Intermittent and ephemeral rivers (IRES) are watercourses that periodically cease to flow for part or all of their courses (Datry et al., 2017a; Messager et al., 2021; Sarremejane et al., 2022). These periods of temporary flow can vary from ephemeral streams that only flow for a few days after heavy rainfall to intermittent rivers that can dry up totally for a certain period of the year or be reduced to small pools (Magand et al., 2020). IRES account for between 51% and 60% of the world's rivers, with a more frequent occurrence in arid and semi-arid zones (Datry et al., 2014, 2017; Messager et al., 2021; Stubbington et al., 2017a; Costigan et al., 2017). Their number is expected to increase in the coming years due to the arid climate, human activities, and growing demand for water in these regions (de Graaf et al., 2019; Döll and Schmied, 2012; Magand et al., 2020). Their flow cessation periods generate mosaics of habitats (flowing, pools, and dry) that vary in space and time, affecting their physical and chemical processes, supporting high freshwater biodiversity, and providing ecosystem services. However, IRES are one of the world's most threatened ecosystems and require measures to better understand and preserve their biodiversity within functional ecosystems (Datry et al., 2021).

I.1. Processes and physical features in IRES

IRES have unique physical characteristics related to their flow patterns, hydrological connections, and hydromorphological variations. Water managers often neglect these features in contrast to their in-depth knowledge of perennial rivers, leading to poor water resource management and the degradation of these delicate ecosystems (Magand et al., 2020).

I.1.1. Flow regime and intermittence.

A river's flow regime corresponds to the temporal fluctuations in its water flow, (i.e., the quantity, timing, and variability in the discharge) **(**Poff et al., 1997; Nilsson and Renöfält, 2008; Sauquet et al., 2021**).** IRES have flow regimes characterized by extreme floods and droughts. Thus, these rivers alternate between wet and dry phases, and their lateral, vertical, and longitudinal hydrological connections with groundwater and terrestrial habitats are highly variable **(**Magand et al., 2020). As a result, periods of flow cessation (zero flow) at one or more points in the network define the flow regime in the

IRES. Depending on regional and local factors, these periods can vary from a few hours to several months or even years (Bhamjee and Lindsay, 2011; McDonough et al., 2011; Costigan et al., 2016). IRES are often only found upstream of rivers or headwaters in wet and temperate zones; however, they occupy a significant portion of the river's length and are dispersed throughout the system in many arid zones (Caruso and Haynes, 2011). Although intermittent flow in the IRES can occur naturally, it can also be altered or caused by anthropogenic activities such as dam construction, channel modification, and water abstraction (Magand et al., 2020).

I.1.2. Hydrological connectivity

Hydrological connectivity in all streams and rivers describes the natural processes of water movement that can transfer matter, energy, and organisms between different spatial dimensions, including longitudinal (from upstream to downstream), lateral (in riparian zones and floodplains), and vertical (between surface water and groundwater), which interact with a fourth dimension, time (Bracken and Croke, 2007; Freeman et al., 2007; Pringle, 2003; Ward, 1989; Ward and Stanford, 1995). In IRES, flow regime components (i.e., the size, frequency, duration, timing, and rate of flow change), the intermittence of streamflow, and geomorphology all have an impact on the complex and variable process of their hydrological connectivity (Boulton et al., 2017). In addition, land use and water abstraction changes, among other human activities, can substantially affect this connectivity, particularly in arid and semi-arid areas (Boulton et al., 2017; Ochoa et al., 2013).

Because of the fluctuating water levels in the IRES, six different aquatic states can be defined, each with a distinct number of zones (Gallart et al., 2012). These aquatic states are often summarized into three phases: flowing, isolated pools, and dry (Datry et al., 2016; Gallart et al., 2017). More specifically, they are, in order from wettest to driest states, hyperheic, eurheic, oligorheic, arheic, hyporheic, and edaphic (Bonada et al., 2020) (Figure 2). The duration of each of these states varies according to the type of soil, its permeability, and the amount of annual rainfall (Bonada et al., 2007).

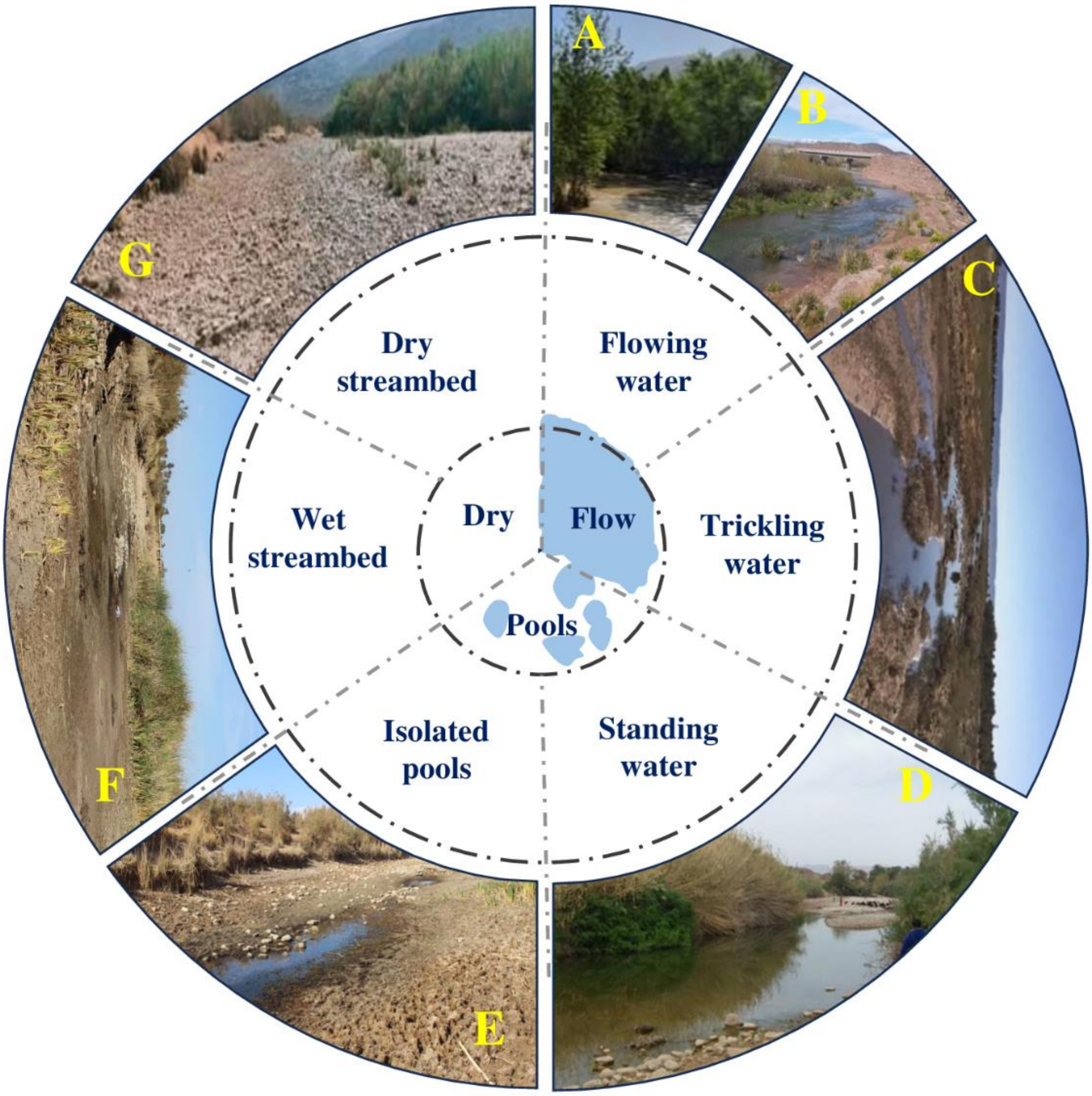

Figure 2. Summary of the different hydrological conditions that can occur in the IRES in terms of aquatic phases (flow, pools, and dry), flow categories (flowing, trickling, standing, isolated waters, and wet and dry streambed), and aquatic states (A, B, C, D, E, F and G).

A: Hyperheic; flood. **B:** Eurheic; significant flow, and the majority of mesohabitats connected. **C:** Oligorheic; rare flow, pool connected by water rivulets. **D and E:** Arheic; no flow, disconnected water pool. **F**: Hyporheic; no surface water with wet alluvium supports hyporheic life. **G:** Edaphic; no surface water with dry alluvium and no aquatic life.

I.1.3. Hydromorphological features and habitat heterogeneity.

The Water Framework Directive (WFD) first described hydromorphology as a group of features that can be used for ecological status assessments. These features include changes in aquatic and riparian habitats, water volume and flow, riverbeds, substrate, banks, and riparian zones (Akstinas et al., 2022). In the IRES, the hydromorphological features are diverse in space and time due to the intermittent nature of river flows, among other factors. When flow ceases, habitat fragmentation occurs, dividing a large part of it into numerous small patches separated from each other by a different habitat matrix (habitat heterogeneity) (Fahrig, 2003; Datry et al., 2017b). IRES often experience asynchronous patterns of dewatering and rewetting, giving rise to diverse mosaics of flowing (lotic), non-flowing (lentic), and terrestrial habitat patches, with implications for physicochemical processes and ecological dynamics (Boulton et al., 2017). Indeed, these different dry and wet habitat zones host dynamic biotic communities that evolve over time and space (Datry et al., 2014; Magand et al., 2020). Due to habitat fragmentation, the IRES ecosystem comprises many distinct microhabitats, each with its biota, ecological roles, and environmental variables (Larned et al., 2010; Datry et al., 2014, 2017b).

I.2. Chemical features in IRES

The hydrological characteristics of the IRES, such as intermittent flow and interrupted hydrological connectivity, significantly impact the chemical properties of this ecosystem, influencing its aquatic life (Gómez et al., 2017). These include water physicochemistry, nutrient dynamics, and organic matter dynamics.

I.2.1. Water physicochemistry

Physico-chemical variables—temperature, light, dissolved oxygen, pH, and salinity—influence biotic structure (species richness and diversity) and ecosystem functioning in watercourses. This applies to all aquatic environments, including intermittent rivers and ephemeral streams. However, the physical and chemical characteristics of these rivers change more over time and space than perennial watercourses, due to their intermittent flow and the cessation of surface runoff during dry periods (Boulton and Lake, 1990; Pires et al., 2000; Gómez et al., 2017; Rodrigues et al., 2022). During this drying phase, which significantly reduces river flow, hydrological connectivity throughout the catchment becomes fragmented and eventually halts

(Boulton et al., 2017; Magand et al., 2020). Consequently, the reduction in flow leads to a decrease in the dilution capacity of surface water from adjacent compartments, as well as an increase in water retention levels, and longer residence times in the watercourses (Gómez et al., 2017; Magand et al., 2020). Conversely, the rewetting phase, characterized by the first runoff events, re-establishes hydrological connections (Boulton et al., 2017; Magand et al., 2020). This process facilitates the movement and transport of substances accumulated in terrestrial soils and sediments in riverbeds during a drought, thus reducing the differences between the physicochemical properties of river water in different places (Gómez et al., 2017; Magand et al., 2020). Concerning temporal variations in physico-chemical conditions, the IRES is characterized by variations on several scales. These variations include daily, and seasonal fluctuations caused by a multitude of factors, such as glacial inputs, flow conditions (drying and rewetting cycles), and the activity of organisms (Wetzel, 2001; Vidal-Abarca et al., 2002; von Schiller et al., 2011; Arce et al., 2014, Al-Qudah et al., 2015). Here are the main trends in the temporal and spatial evolution of the principal physicochemical variables (Figure 3).

Temperature

Water temperature in IRES rivers varies with solar radiation, suspended particles, depth, water velocity, shading, upstream sources, groundwater inflow, and tributaries, influencing daily and seasonal temperature patterns (Gómez et al., 2017; Magand et al., 2020). Temporally, where surface flow decreases in the IRES, these factors rapidly warm and cool the water, causing diurnal oscillations. In addition, water temperatures rise during the drying cycle in areas of high solar irradiation coinciding with the cessation of flow, particularly in sections with open canopies (Hrachowitz et al., 2010; Gómez et al., 2017; Magand et al., 2020). Spatially, surface water fragmentation and remaining pools also affect mean water temperature and daily oscillations. The pool depth is also important, as shallow pools heat up faster than deeper ones so that thermal stratification can persist in deep residual pools (Turner and Erskine, 2005; Baldwin and Wallace, 2009; Gómez et al., 2017; Magand et al., 2020).

Dissolved oxygen (DO)

Photosynthesis, respiration, water turbulence, and temperature in all rivers and streams determine seasonal and daily dissolved oxygen (DO) content changes. In-stream photosynthesis raises DO; respiration lowers it. In heavily irradiated ecosystems, and particularly in the IRES, photosynthesis, and DO can surge during stream contraction, especially at mid-day, causing DO supersaturation (Boulton and Brock, 1999). However, due to microbial decomposition, organic matter (OM) accumulations in natural and human-derived sediments can lower DO concentrations, especially in the slow-flowing and drying phases. Moreover, the oxygen solubility in IRES decreases during river fragmentation, which occurs before the river dries up. This is due to increased temperature and salinity (Ylla et al., 2010). The oxygen solubility varies throughout this period depending on photosynthetic or respiration factors. Nevertheless, rewetting the river increases turbulence and raises dissolved oxygen (DO) (Ylla et al., 2010). After a rain, when flow resumes and metabolic activity increases, DO levels in the IRES can fall rapidly, leading to hypoxia or anoxia (Hladyz et al., 2011). Overall, groundwater infiltration and microbial activity in the hyporheic zone can affect dissolved oxygen (DO) levels, and excessive respiration rates can lead to hypoxia in the streambed (Gómez-Gener et al., 2021; Magand et al., 2020). Additionally, seasonal and daily DO concentrations vary in intermittent rivers, with higher spatial variability as surface flow discharge decreases (Gómez et al., 2017; Magand et al., 2020).

pH

The pH level is a crucial factor that impacts the accessibility of nutrients and metals in sediments and water. Several natural variables, such as seasonal changes in photosynthesis and respiration, fluctuations in the concentration of humic acids, and catchment lithology, can influence it (Magand et al., 2020). In addition, it can also be changed by human activities, including acid rain, mining, and soil clearance. Similar to DO fluctuations, the water pH in IRES tends to increase over the day during hydrological processes that support photosynthesis, such as creating isolated pools in open canopy habitats (Magand et al., 2020). However, higher respiration rates in these pools can cause a drop in pH as the stream dries out (Dahm et al., 2003; Hladyz et al., 2011). Furthermore, the resumption of flow can also lead to decreased pH in IRES because of microbial respiration fueled by high organic matter loads, particularly leaf litter (Magand et al., 2020).

Salinity

Multiple factors, such as climate, geology, hydrology, land use, and human activities determine salinity levels in the IRES. However, catchment lithology is crucial when evaluating high salinity levels and spatial variability in IRES, as not all are naturally saline (Magand et al., 2020). Salts are often present in arid and semi-arid areas, many experiencing problems with freshwater salinization (Berger et al., 2019). When riffles are fragmented (during periods of low flow), high evapotranspiration can cause surface waters in IRES to become very salty (Sarremejane et al., 2022). This leads to significant temporal variations in salinity in the IRES over daily, seasonal, and annual periods (Gómez et al., 2017). In naturally salty IRES, storms and melting snow and/or ice flush weathered minerals, increasing sulfate (SO_4^{2-}), sodium (Na^+), calcium (Ca^{2+}), silica (SiO_2), potassium (K^+), and chloride (Cl^-) (Al-Qudah et al., 2015; Robinson et al., 2016). However, rainfall events frequently dilute the high amounts of these dissolved ions; as a result, water salinity and conductivity decrease (Gómez et al., 2017). Furthermore, pollution from anthropogenic activities (agriculture, urban runoff, and industrial effluents) also increases the salinity of surface waters (Gómez et al., 2017, Berger et al., 2019). Understanding the spatial variability of surface water ionic composition also depends on the chemical composition of groundwater inputs. Indeed, when groundwater flows through water-soluble minerals or rocks of marine origin, surface water can become saltier (Herczeg et al., 2001).

I.2.2. Nutrients, and organic matter dynamics

The IRES has a unique biogeochemical rhythm, composed of varying amounts of nutrients (dissolved nitrogen and phosphorus) and organic matter (OM) input, transformed and transported downstream. Hydrological changes, low surface flows, and hydrological disconnection affect the quantities of nutrients and dissolved organic carbon (DOC). These changes occur through abiotic (dilution, adsorption, precipitation, and dissolution) and biotic (biological uptake and release, nitrification, and denitrification) processes. In addition, the stream channel, underground compartments, and adjacent terrestrial ecosystems also affect surface water quality (Gómez et al., 2017). Due to increased sun irradiation, photosynthesis in open canopy shallow reaches and pools may increase during low flow, decreasing nutrient concentration due to higher biological nutrient uptake demand (Austin et al., 2004; Baldwin and Mitchell, 2000; Fellman et al., 2013; Jacobson and Jacobson, 2013; Skoulikidis et al., 2017). However, water evaporation may raise nutrient concentrations if primary producers (aquatic plants and algae) are sparse. In addition, high water temperatures boost OM breakdown and surface water DOC content by stimulating microbial activity. Hypoxic and eutrophication conditions can increase as streams dry up, especially in organic matter-rich habitats such as pools. This typically results in a decrease in surface water nitrate due to denitrification.

When rivers are rewetted, the processes occurring in dried-up waterways significantly affect water quality (Gómez et al., 2017). Indeed, rewetted organic and inorganic substrates that have accumulated can be transported downstream (Corti and Datry, 2012; Obermann et al., 2007; Rosado et al., 2015). It also increases nitrification in dry sediments, disrupts aggregates physically, and promotes cell lysis due to osmotic shock. Furthermore, after flow resumption, extracellular enzyme activities significantly increase nitrogen, phosphorus, and dissolved organic carbon (Ylla et al., 2010; von Schiller et al., 2017).

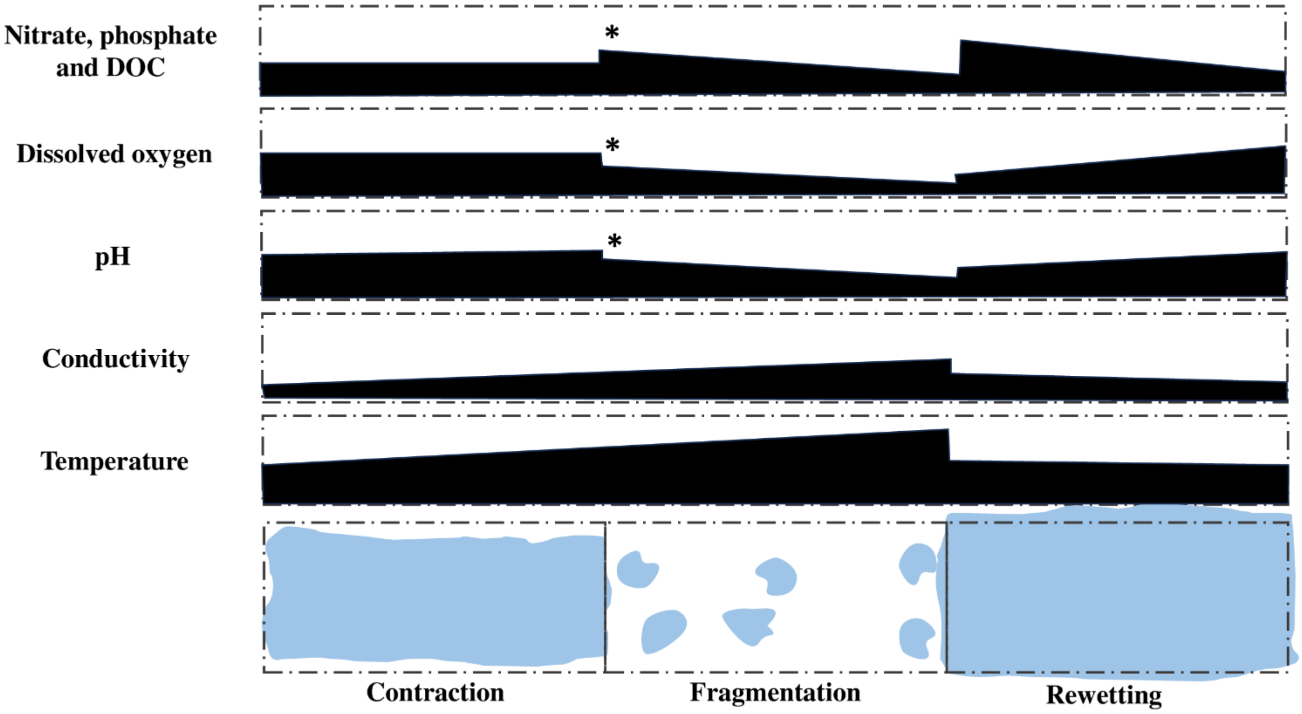

Figure 3. Temporal models of the main physico-chemical parameters during different hydrological phases.
* Denotes that changes occur under low sun irradiation conditions in a closed canopy reach; a reverse pattern can be expected in open canopy areas where photosynthetic activity is high due to high irradiation conditions (modified from Magand et al., 2020).

I.3. Ecological features and processes in IRES

As periodic drying out and reappearance, IRES are characterized by unique and important ecological processes, supporting specific biodiversity. These organisms interact within food webs and trophic interactions, influencing organic matter cycling and ecosystem services (Datry et al., 2018).

I.3.1. Biota and diversity of fauna

As IRES are known for their wet and dry habitats that change over time, they harbor a range of diverse biotic communities that vary along intermittent gradients. Indeed, species richness tends to decrease as the intensity of intermittence increases. Despite the decline in lotic species, the presence of ponds in arid regions enables the growth of communities with characteristics of lentic ecosystems and semi-aquatic habitats (Datry et al., 2014). Alternating wet and dry periods in IRES create a unique combination of aquatic, amphibious, and terrestrial associations, with a succession of biota occurring during the transition between the two periods. The particular conditions of the IRES exert strong selective pressure on the evolution of the resistance and resilience characteristics of the biota. This requires specific adaptations or increased tolerance to thrive in arid conditions, especially for species with high ecological valence (Steward et al., 2012). The high variability of IRES allows them to function as a biodiversity hotspot along their yearly cycle, harboring unique and diverse communities (Arthington et al., 2014). Among these communities are fish, invertebrates, aquatic and terrestrial plants, and various microorganisms such as bacteria and microalgae (Kerezsy et al.,2017; Stubbington et al.,2017b; Sabater et al., 2017). An important primary producer in the IRES, microalgae are a key element in the biota of these dynamic ecosystems. They support the diversity of aquatic life and have a major impact on the overall functioning of these ecosystems (McIntosh et al.,2017; Sabater et al., 2017).

I.3.2. Diversity of benthic microalgae

The abundance and diversity of benthic microalgae in the IRES vary depending on physicochemical characteristics, hydrological stages, and flow intermittence (Magand et al., 2020; Sabater et al., 2017). During flow periods, the microalgal composition and diversity in IRES can be similar to those in perennial ecosystems, particularly in the less severe IRES. However, during drought periods, the composition of these microorganisms changes considerably, and only species capable of or adapted (morphologically, physiologically, or genetically) to drought stress may remain (Sabater et al., 2017). The three main groups of benthic microalgae in these ecosystems are cyanobacteria, green algae, and diatoms.

I.3.2.1. Cyanobacteria

Cyanobacteria are autotrophic gram-negative bacteria known as blue-green algae because of their ability to produce energy through photosynthesis. They are among the oldest microorganisms on Earth, widespread, abundant, and have considerable morphological diversity (Scott and Marcarelli, 2012). Their filamentous forms dominate benthic environments (Lowe and LaLiberte, 2017). Indeed, *Anabaena spp.* and *Schizothrix spp.* are among the filamentous cyanobacteria found in IRES of dryland (the desert stream in Arizona) (Fisher et al., 1982; Sabater et al., 2017). Long periods without flow in IRES favor the growth of cyanobacteria, which are less appreciated by grazers than green algae and diatoms (Robson et al., 2008). When water returns, many cyanobacteria react quickly to moisture, particularly those with rapidly rehydrating sheaths (Sabater et al., 2000).

I.3.2.2. Green algae

Green algae are characterized by various forms, including unicellular, colonial, and filamentous. However, they are mainly found in filamentous forms, whether branched or unbranched, rather than colonial (Lowe and LaLiberte, 2017). Benthic green algae are more resistant to desiccation during the flow cessation in the IRES and can recover rapidly when rewetted (Holzinger and Karsten, 2013; Sabater et al., 2017). Indeed, some filamentous green algae (e.g., *Zygnema*, *Oedogonium*, and *Spirogyra*) can produce resilient sexual eggs known as zygospores. These zygospores remain inactive until the environment becomes favorable once again (Sabater et al., 2017). Among the filamentous green algae found in the IRES of dryland (the desert stream in Arizona) is *Cladophora glomerata* (Fisher et al., 1982; Sabater et al., 2017).

I.3.2.3. Diatoms

Diatoms are unicellular microalgae, usually brown, characterized by their siliceous cell walls forming two valves called frustules. These valves fit together as halves of a Petri dish, ornamented with striations or ribs. The frustule's shape, size, and ornamentation are specific to each species. Of all the benthic microalgae groups, they are the most widespread, abundant, and diverse (Lowe and LaLiberte, 2017). However, their species richness and alpha diversity are generally lower in IRES than in permanent rivers, particularly during a long period without water flow (e.g., over 100 days) (Tornés and Ruhí, 2013; Viktória et al., 2019; Tornés et al., 2021). Throughout the year, changes in the intermittence of flow in the IRES modify the composition of diatom communities, leading to a shift from species adapted to lotic habitats to those of lentic ones and finally to aerophilic species when the riverbed dries up (Datry et al., 2017b). Therefore, IRES only supports desiccation-tolerant species such as motile, pioneer, and aerophilic taxa, which could threaten desiccation-sensitive species with increased water intermittency. For instance, the *Cymbella* and *Gomphonema* genera can protect their cells by embedding them within a mucilage layer (Sabater et al., 2017). On the other hand, *Surirella*, *Nitzschia*, and *Navicula* genera contain motile species that can move towards safe areas on the substrate or penetrate the biofilm to reach the deeper layers (Falasco et al., 2016, 2018). After completely drying in IRES, only some subaerial diatoms, such as *Hantzschia amphioxys* and *Luticola mutica* remain. Nevertheless, many diatom species are typically less resistant to desiccation than green algae and cyanobacteria (Sabater et al., 2017).

Besides the intermittent nature of water in the IRES, physical alterations (Hill et al., 2000; Bona et al., 2008; Hlúbiková et al., 2014) and water quality changes in rivers (e.g., increased salinity) affect diatom composition by reducing their specific richness and diversity (Lazrak et al., 2022).

I.3.3. Role and function of benthic microalgae and trophic interactions

As primary producers, benthic microalgae form an important basis of the food web and are essential to IRES ecosystems' functioning, particularly in arid regions with sparsely vegetated riparian zones (McIntosh et al., 2017). Through photosynthesis, they generate oxygen and supply energy. Therefore, benthic microalgae play a crucial role in the ecosystem's nutrient cycle and energy transfer (Battin et al., 2016; Lowe and LaLiberte, 2017). Their ability to absorb nutrients and contaminants represents an essential component of water self-purification (Sabater et al., 2007; Wu et al., 2018; Battin et al., 2016). Additionally, benthic microalgae contribute significantly to the structural stability of river habitats by creating biofilms that serve as important food sources and offer shelter to various organisms (Majdi et al., 2012). Through the transfer of energy up the food web and the supply of oxygen to aerobic organisms, any alteration in the composition and structure of benthic microalgae can have a significant impact on the development, survival, growth, and reproduction of many organisms in aquatic ecosystems (Campeau et al., 1994; Viktória et al., 2019). Microalgae, particularly in the epilithic compartment, are most exposed to desiccation stress during periods of non-flow in the IRES but are remarkably resilient and recover immediately when water returns, highlighting their crucial role in maintaining food web balance and ecosystem functioning (Timoner et al., 2014).

I.3.4. Use of benthic diatoms as bio-indicators of water quality

Benthic diatoms, the most abundant of the main groups of microalgae, are known for their growth preferences and short reproductive cycles (Smol and Stoermer, 2010; Lowe and LaLiberte, 2017). Their ability to react quickly to environmental changes makes them an excellent bioindicator of water quality, including salinity (Rimet, 2012ab; Vidal et al., 2021; Kaddeche et al., 2024). As a result, they are included in the Water Framework Directive (WFD), Directive 2000/60/EC, as biomonitors for assessing the ecological status of river ecosystems. For many years, diatom indices based on morphological identification have been widely used to evaluate river water quality around the world (Lenoir and Coste,1996; Prygiel et al., 2002; Coste et al., 2009; Kelly and Whitton, 1995; Rott et al., 1997, 1999; Dell'Uomo, 2004; Szulc and Szulc, 2013) (Table 1).

Recently, great efforts have been made to develop complementary and alternative methods (molecular techniques) such as DNA metabarcoding for the ecological assessment using diatoms (Mann et al., 2010, Kermarrec et al., 2014, Visco et al., 2015, Bailet et al., 2019; Mortágua et al., 2019). This involves identifying taxa from environmental samples based on their DNA using standard genetic markers (barcodes) and determining their similarity to reference sequences (Zimmermann et al., 2015; Keck et al., 2018), offering an approach less dependent on taxonomists, and less time-consuming (Table 2).

Due to the dynamic nature of the IRES, standard biological indices are inadequate for their assessment, as they do not take into account periods of low water and drought. Scientific knowledge of the relationship between the structure and composition of diatom communities and the hydrological changes is insufficient (Delgado et al., 2012; Wu et al., 2019). It is important to fill this knowledge gap to develop specific assessments for IRES ecosystems (Falasco et al., 2021).

Table 1. Examples of diatom-based indices using the morphological approach in certain permanent and intermittent rivers in Mediterranean regions and their main results**.**

Study area	Index	Key finding	Reference
Coastal Central Constantine basin, Algeria (Mediterranean rivers).	- A new diatom-based index BDIAR, using water conductivity and diatom autoecological profiles. - Soda Pans (DISP) and Carayon indices	-Significant correlation between conductivity and BDIAR. - DISP and Carayon indices, although useful for deducing conductivity, are less accurate. - BDIAR can be a tool for biomonitoring in all freshwater ecosystems ecologically like Algerian rivers.	Kaddeche et al., 2024
Intermittent lowland stream (Létai-ér) in the Pannonian Ecoregion, Eastern Hungary.	-Specific pollution, sensitivity index (IPS), -Rott's saprobic index (SID), -Rott's trophic index (TID), -Hungarian phytobenthos metric (IPSITI); average of the above indices. - Diversity metrics: Taxa richness (Taxa_S), Shannon's H (effSH), and Rao's quadratic Entropy (FDQ).	- Significant variations in the hydrological regime (Flowing and standing conditions), with higher values observed in the flowing phase, except for SID. - No significant differences between diatom indices and habitats (natural and artificial). - No significant differences between hydrological conditions and diversity metrics. - High values of Taxon_S and effSH in artificial habitat. - Shift from flowing to pool conditions negatively impacts water quality, inducing changes in the composition of diatom assemblages.	Kiss et al., 2024
Guadiana, Sado and Mira and Ribeiras do Algarve streams, Southern Portugal.	-Specific Pollution Sensitivity Index (SPI) -Functional metrics (i.e. ecological guilds and life forms)	- SPI differentiated between mesohabitats (dry, pools, or flowing conditions) and sampling seasons. - Relative abundances of ecological guilds showed changes in response to aquatic states (Permanent, Intermittent-pools and Intermittent-dry).	Novais et al.,2020

		-Benthic diatom assemblages can be a bioindicator of ecological status during the dry period.	
Evrotas River (temporary stream), Southern Peloponnese, Greece.	-Specific Pollution Sensitivity Index (IPS)	-IPS score decreased with the increasing pollution, regardless of the level of water stress (temporary). -No significant interaction between the water stress and pollution responses on IPS. - Diatoms revealed greater impacts from pollution than water stress.	Karaouzas et al.,2018a
Rio Mannu di Porto Torres basin, northwestern Sardinia, Italy.	-Eutrophication/pollution index (EPI-D), - *Navicula Nitzschia Surirella* index (NNS') - Descy's index (DES), -Specific pollution index (IPS) - Generic diatom index (IDG), - European economic community index (CEE) - Biological diatom index (IBD), - Artois–Picardie diatom index (IDAP) - Swiss diatom index (DI-CH)	- EPI-D indicated mediocre to poor water quality, in line with environmental variables and intensive human use in the study area. - EPI-D showed significant correlations with IPS, CEE, IBD, and IDAP, but not with IDG, DES, and DI-CH. - NNS' revealed significant physical disturbances due to the Mediterranean climate, seasonal changes in water flow, and human activities.	Lai et al., 2014

Table 2. Some examples of comparison of diatom-based indices using morphological and molecular approaches in Mediterranean rivers and their main results.

Study area	**Index**	**Key finding**	**Reference**
Krka River (Dinaric region of Dalmatia, Croatia)	- Croatian Trophic Diatom Index (TDIHR) using both morphological and DNA metabarcoding approaches (with the application of a biovolume-based correction factor (CF) for molecular data quantification).	- 38.9% of samples shared the same ecological status for both approaches. - No correlation and a significant difference between the ecological status scores calculated from both methodologies. - Lack of a complete reference database, a major drawback of the molecular method.	Kulaš et al., 2022
Cyprus Rivers (an eastern Mediterranean country), Cyprus.	- Specific pollution sensitivity index (IPS) using both morphological and DNA metabarcoding approaches (with the application of (CF)).	-Two methods' IPS scores positively correlated, with over half of the samples in the same quality class. - Discrepancies between the two approaches (limitations of both methodologies). - *Fistulifera saprophila* negatively affects IPS scores in the molecular approach and is often overlooked by microscopy methods. - Gaps in DNA barcode reference databases. - DNA metabarcoding offsets morphological methods for river ecological quality assessment.	Pissaridou et al., 2021
Catalan (Mediterranean) Rivers, Catalonia (Nord Est Spain)	- Specific pollution sensitivity index (IPS) using both DNA metabarcoding (HTS with and without the application of (CF)) and light microscope-based (LM).	- High correlation between the IPS values of the two approaches. -Ecological status class in 10 sites changed from "good"/"high" with LM to "moderate"/"poor"/"low" with HTS. - IPS values obtained from morphology and CF-corrected HTS slightly higher correlated than without the application of CF.	Pérez-Burillo et al., 2020

		- Discrepancies in IPS between LM and HTS, mainly because LM misidentified and neglected some species such as *Fistulifera saprophila*. -Diatom DNA metabarcoding could be a useful method for WFD assessment of Catalan rivers.	
Portuguese rivers (Vouga, Mondego, and Lis basins)	-Specific pollution sensitivity index (IPS) using both morphological and DNA metabarcoding approaches (with and without the application of CF).	- Most sites showed "good" quality for all approaches. - Highest percentage of samples with class “Good” using a molecular approach without CF, followed by the morphological method. - Highest percentage of quality classes below "good" obtained using the molecular method with CF. - Molecular method with CF is closer to the morphological approach, with 57% of samples classified identically and 6% differently. -DNA metabarcoding could be a valid approach for assessing ecological quality, but it still needs to be improved before integrating this alternative approach into the WFD strategy.	Mortágua et al., 2019

I.4. Ecosystem services, economic values, and societal perceptions of IRES

Ecosystem services (ES) are described as "the benefits and advantages that people derive from ecosystems" (MEA, 2005; de Groot et al., 2002). IRES provide various ES, including provisioning services (e.g., food, wood, and freshwater supply), regulating services (e.g., climate, flood, and water regulation), supporting services (e.g., nutrient recycling, biodiversity maintenance, and primary productivity), and cultural services (e.g., stress relief and recreational value) (Datry et al., 2018; Magand et al., 2020; Stubbington et al., 2020). In dry and semi-arid zones where permanent watercourses are few or nonexistent, the freshwater supply by IRES is essential for agriculture, irrigation, and drinking water (Magand et al., 2020). The ES obtained by IRES are related to their hydrological phases; some ES are altered, diminished, or lost when flow ceases, while others are enhanced (Datry et al., 2018; Steward et al., 2012). For instance, non-flow and dewatering phases facilitate river access, and sand and gravel extraction, while the flow phase enhances fish habitats and sediment transfer (Boulton, 2014; Stubbington et al., 2020). The numerous ES offered by IRES contribute to economic values that can categorized into “use” and “nonuse”, but they are often underestimated and ignored by society, including those living near them, compared to perennial rivers (Armstrong et al., 2012; Datry et al., 2017c, Koundouri et al., 2017). Especially in Mediterranean regions, IRES are considered dangerous due to their flash flood potential (Koundouri et al., 2017), subjecting them to many stressors.

I.5. Stressors, threats, and management challenges of IRES

IRES, representing a significant proportion of the world's river systems, will become increasingly common, and demand for these water resources will intensify. They are exposed to numerous anthropogenic and natural stress factors, intensified by climate change (Magand et al., 2020; Datry et al., 2023). However, compared to their perennial counterparts, many IRES enjoy significantly less legal protection (Nikolaidis et al., 2013; Acuña et al., 2014; Chiu et al., 2017; Messager et al., 2021), which shows that society underestimates and misunderstands their environmental characteristics and the services they provide because their dry phase is not considered. Indeed, they are often regarded as terrestrial or permanent ecosystems. Due to a lack of appreciation and understanding, IRES are more susceptible to degradation than perennial systems (Chiu et al., 2017). To address these management challenges, it is essential to collaborate between researchers, stakeholders, and decision-makers in adopting interdisciplinary and holistic approaches to the conservation and sustainable management of these vulnerable IRES ecosystems (Datry et al., 2017c; Magand et al., 2020).

I.5.1. Human stressors

Over the last century, human activities have caused alterations to IRES (Acuña et al., 2020; Chiu et al., 2017). These alterations can be divided into hydrological, physical, chemical, and biological (Chiu et al., 2017). Hydrological changes result from dam flow regulation, water diversions for flood control, surface and groundwater extraction, irrigation, and land use changes (e.g., agriculture). These pressures generally strongly impact the IRES due to the characteristic variability of its flow, which alternates between periods of flood and drought (Poff et al., 2006; Chiu et al., 2017). Moreover, physical and chemical changes to the IRES caused by sediment extraction, and water pollution (e.g., urban, and industrial effluents) (Figure 4) harm the terrestrial and aquatic habitats of the IRES, reducing the abundance or causing the disappearance of species sensitive to water quality, including salinization (Chiu et al., 2017). Finally, biological alterations, particularly the introduction or invasion of species, threaten native communities (Clavero et al., 2015; Chiu et al., 2017). In addition, natural stress factors on the IRES, such as the intermittent nature of the flow, also exacerbate the effects of pollution, reducing the capacity for dilution and self-purification and ultimately influencing the physical, chemical, and biological characteristics of these ecosystems (Karaouzas et al., 2011, 2018b; Sabater et al., 2016).

Figure 4. Photos of physical and chemical alterations examples in the IRES (case of the Drâa basin, Southern Morocco).
A: Sediment extraction. **B**: Water pollution from carpet washing in the river.

I.5.2. Natural stressors and climate change

Intermittency is common in the IRES, as their water flows for only a few weeks or days a year (Vidal-Abarca et al., 2020; Sarremejane et al., 2022). Various natural drivers, such as meteorological, hydrological, geological, and geomorphological factors, influence intermittency's spatial and temporal patterns of IRES (Larned et al. 2010; Costigan et al. 2016; Hammond et al. 2021). According to a recent IPCC report (2021), severe droughts and other extreme weather events will likely occur more frequently in the coming years. Generally, the extent of river drought is strongly influenced by past rainfall patterns, which are expected to become more unpredictable due to climate change (Pendergrass et al., 2017). Compared to perennial rivers, IRES are highly sensitive to climatic variations and are predicted to face the greatest risk of changes in hydrological regimes (Dhungel et al., 2016). Understanding these factors influencing IRES dynamics and their response to climate variability is essential for predicting the future performance of these ecosystems in an unpredictable climate (Moidu et al., 2021).

II. Freshwater salinization challenge

Freshwater salinization is a growing global problem with significant impacts on the biodiversity, functioning, and services provided by freshwater ecosystems (Cunillera-Montcusí et al., 2022; Jeppesen et al., 2020; Kaushal et al., 2018). Various factors lead to an increase in the total concentration of the main ions in freshwater, which is at the root of this issue (Iglesias, 2020; Cunillera-Montcusí et al., 2022). Indeed, the salinity of many watercourses has increased considerably over the last century, particularly in arid zone rivers, whether intermittent or perennial (Cañedo-Argüelles et al., 2013; Kaushal et al., 2021; Berger et al., 2019). In addition, water scarcity intensifies the FS problem in arid and semi-arid regions (Cunillera-Montcusí et al., 2022). To cope with the rising impact of FS, it is essential to better understand its multiple drivers and serious environmental consequences (Cañedo-Argüelles et al., 2019; Kaushal et al., 2018, 2019, 2020, 2021, 2023; Cunillera-Montcusí et al., 2022).

II.1. Freshwater salinization drivers

A combination of natural processes and anthropogenic activities causes FS. Climate change further amplifies this issue (Figure 5) (IPCC, 2013; Warner et al., 2013;

Berger et al., 2019; Cañedo-Argüelles et al., 2019; Kaushal et al., 2021). Here are the most important, detailed factors contributing to FS.

II.1.1. Natural processes (Freshwater primary salinization)

FS is due to a series of natural processes that increase salt concentrations in freshwater ecosystems. In semi-arid and arid areas, rivers and streams are often naturally salty due to their geological composition and high evaporation rate (Berger et al., 2019). Additionally, glacial and interglacial cycles that have taken place throughout Earth's history control FS (Herbert et al**.,** 2015). Tidal effects, seasonal cycles, weather events, rock weathering, and aerosol deposits can also impact this process (Rengasamy, 2006**;** Williams, 2002; Millán et al., 2011). When these processes primarily contribute to salinization, it is known as *primary salinization*. However, human activities can accelerate natural processes by increasing ion transport to surface waters, known as *secondary salinization* **(**Steffen et al., 2007; Kaushal et al., 2018; 2021**).**

II.1.2. Human activities (Freshwater secondary salinization)

Freshwater secondary salinization, also called human-induced salinization, is a process by which anthropogenic activities increase salinity levels in freshwater ecosystems. Irrigation and rising groundwater tables are generally considered to be the main factors responsible for secondary salinization. This phenomenon is particularly widespread in arid and semi-arid regions, where agricultural production (e.g., watermelon cultivation) requires large water quantities (Cañedo-Argüelles et al., 2013; Lerotholi et al., 2004). In addition, *in these climate areas,* flow regime modifications (dams, resource extraction, power generation, water retention, and channelization), land use changes (agriculture and mining), and industrial and domestic wastewater effluents can significantly contribute to this issue (Malmqvist and Rundle, 2002; Vörösmarty et al., 2010; Cañedo-Argüelles et al., 2013; Warner et al., 2013; Iglesias, 2020; Cañedo-Argüelles et al., 2019; Thorslund et al., 2021; Kaushal et al., 2021; Cunillera-Montcusí et al., 2022).

II.1.3. Climate Change

Several research and scientific reports indicated, according to future climate models, that over the next few decades, freshwater ecosystems in the arid and semi-arid regions will become saltier as a result of global warming, which is leading to increased water evaporation, higher temperatures, and reduced rainfall (Sereda et al., 2011; IPCC, 2000, 2007, 2013, 2014; Cañedo-Argüelles et al., 2013; Warner et al., 2013; Kaushal et al., 2021; Cunillera-Montcusí et al., 2022). Furthermore, climate change and human disturbances to the hydrologic cycle will increase the extent and impact of FS (Herbert et al., 2015). In the future, the effects of climate change and increased water use, such as the growing water demand in human consumption, agriculture, and industries, will intensify the current water crisis (Sowers et al., 2011; Schilling et al., 2020). This will lead to even more severe water scarcity and exacerbate the problem of freshwater salinization by reducing dilution (Schindler, 1997; Schmandt, 2010; Jeppesen et al., 2020).

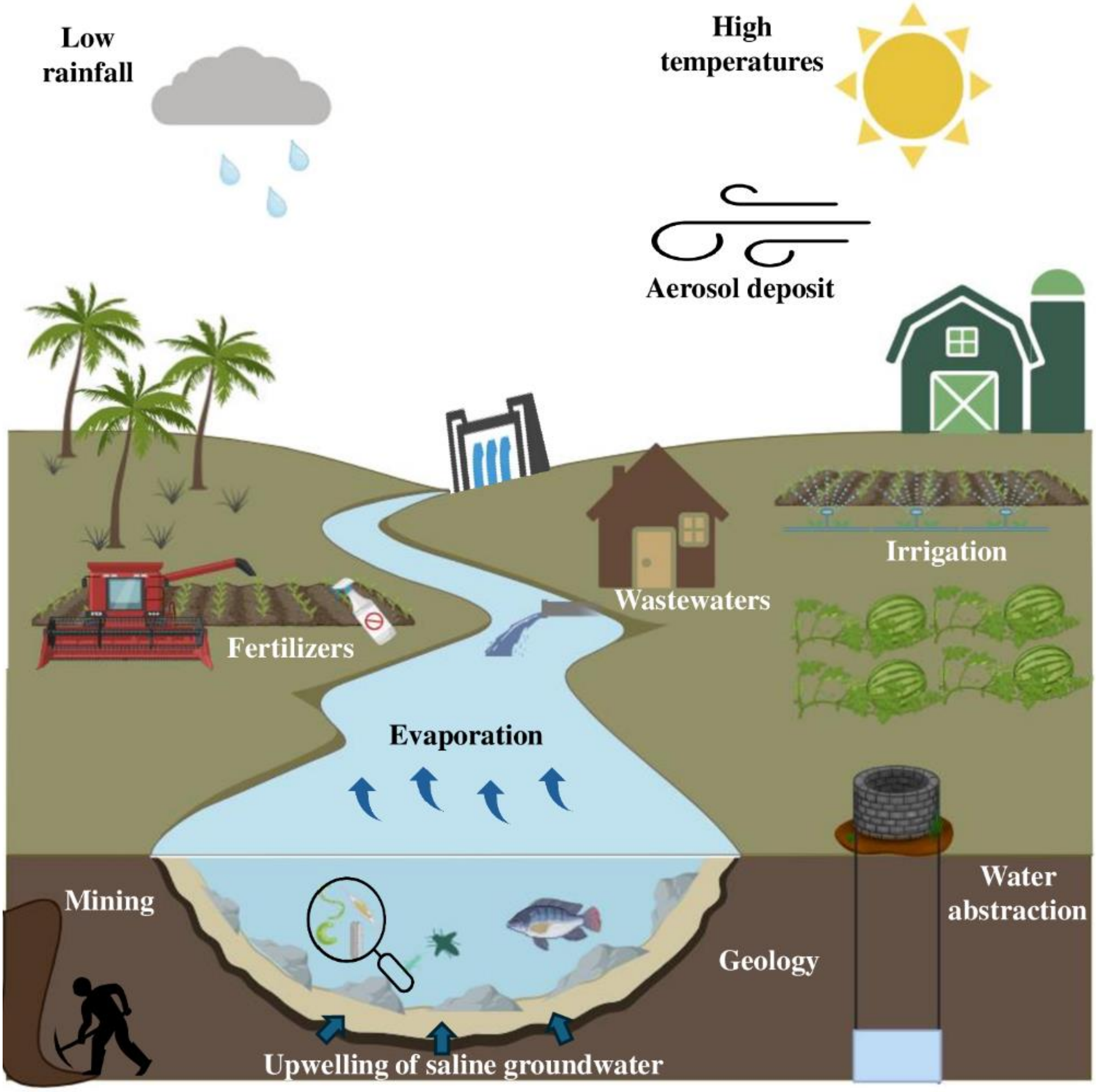

Figure 5. Overview of the freshwater salinization drivers in arid and semi-arid rivers.

II.2. Ecological impacts of freshwater salinization

Salinity has serious ecological effects on aquatic ecosystems. Indeed, higher salinity levels in rivers in arid zones can affect physical and chemical processes as well as bio-geochemical cycles involving carbon, nitrogen, phosphorus, and sulfur (Duan and Kaushel, 2015; Berger et al., 2019). In addition, it can affect various ecosystem processes, including leaf decomposition, metabolism, biomass production, and nutrient cycling (Pettorelli et al., 2017; Berger et al., 2019). These changes in freshwater wetlands influence their ability to provide ecosystem services, such as nutrient removal and carbon sequestration (Herbert et al., 2015). Furthermore, FS can also have negative effects on aquatic organisms, by increasing stress or mortality, reducing diversity, and, ultimately, disrupting the functioning of freshwater ecosystems (Berger et al., 2019; Cunillera-Montcusí et al., 2022). For instance, species richness decreases with increasing salinity, and many species disappear beyond a certain threshold (Cañedo-Argüelles et al., 2019; Lazrak et al., 2022). Starting from primary producers such as microalgae, salinization can have a substantial impact on the entire trophic food web within ecosystems, potentially triggering a trophic cascade interaction at different levels by affecting organisms higher up in the food web (Hintz et al., 2017; Ersoy et al., 2020).

II.2.1. Impact of salinity on the benthic microalgal communities (biofilm)

Salinity significantly impacts benthic microalgal communities, the primary producers in the freshwater ecosystem. At a community level, microalgae biofilms under salt stress change their composition to favor more salt-tolerant species. At the cellular level, high salinity affects the physiology and metabolism of benthic microalgae, demonstrating their capacity to develop various mechanisms in response to salt stress conditions.

II.2.1.1. Impact on algal composition and diversity

Higher salinity levels in aquatic ecosystems lead to changes in the composition of benthic microalgal communities (Vendrell-Puigmitja et al., 2021, 2022; Lazrak et al., 2022, 2024). Increasing salinity causes a shift from sensitive to more tolerant species (Zimmermann-Timm, 2007; Nuy et al., 2018). Specifically, some microalgae are considered halophilic, showing a natural preference for high-salinity environments (DasSarma and Arora, 2001; Indrayani et al., 2019). Others can tolerate high-salinity environments, known as halotolerant species (Ishika et al., 2017). Conversely, some species are halophobic and cannot survive in high salt conditions. Low-salinity environments allow certain microalgae to thrive, classifying them as oligohaline species (Lazrak et al., 2022). Some microalgae, known as euryhaline, exhibit multiple responses to changes in salinity, demonstrating their ability to thrive at different salt levels, from freshwater to highly saline environments (Roseli et al., 2023). These differences in salinity tolerance between microalgae lead to structural changes in high salt conditions by reducing species richness, abundance, and diversity (Vendrell-Puigmitja et al., 2021, 2022; Lazrak et al., 2022, 2024) (Table 3). These changes are also associated with physiological and metabolic modifications in the microalgal cells (Vendrell-Puigmitja et al., 2021, 2022; Lazrak et al., 2024).

II.2.1.2. Impact on physiology and metabolism

Salt stress significantly affects the physiological and biochemical processes involved in the growth and development of microalgae (Zhang et al., 2018; Ismaiel et al., 2018; Lazrak et al., 2024). High salinity can lead to overproduction of reactive oxygen species (ROS), which are products of altered cellular metabolism, resulting in ionic disruption, plasmolysis, changes in cell water potential, water input restriction, and oxidative damage. These ROS include hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), and hydroxyl radicals (^-OH) (Almeida et al., 2017; Verslues, 2016; Yun et al., 2019; Ma et al., 2020a). ROS typically act as secondary messengers in cell signaling pathways, triggering different modes of adaptation to environmental stresses, including salinity. However, their excess harms microalgae, damaging macromolecules such as proteins, lipids, and nucleic acids. This affects metabolic and physiological processes, namely photosynthesis, respiration, osmoregulation, and nutrient uptake (Čáp et al., 2012; Ma et al., 2020a; Kuo et al., 2020; Drira et al., 2021). Specifically, ROS are likely to reduce nutrient uptake, CO_2 flux, and increase NADPH synthesis (Srivastava et al., 2017). In addition, they can trigger lipid peroxidation in the thylakoid membrane, disrupting its structure and function, and photosynthesis. Similarly, osmotic stress induced by high salt concentrations minimizes chlorophyll synthesis and activates the chlorophyllase enzyme to degrade it (Ji et al., 2018). Furthermore, reduced chlorophyll levels could be linked to decreased RUBISCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) activity due to reduced CO_2 absorption (Rezayian et al., 2019; Hounslow et al., 2021). To mitigate damaging effects, microalgae can develop different strategies and mechanisms to protect themselves against salt stress (Shetty et al., 2019; Verma et al., 2019; Fal et al., 2022).

II.2.1.3. Adaptive strategy, resistance, and algal community resilience

When subjected to salt stress, microalgal communities employ various adaptive strategies, resistance, and resilience mechanisms (Borowitzka et al., 2018; Shetty et al., 2019). These include a wide range of morphological, biochemical, and molecular changes that promote their survival (Wang et al., 2018). Salt-tolerant microalgae have a remarkable ability to regulate ion transport, adjust membrane permeability, produce osmolytes and stress-related compounds, maintain turgor pressure, and defend against salinity-induced reactive oxygen species (ROS), enabling them to survive in new conditions (Zhang et al., 2018; Yancey, 2005).

They exhibit three salt tolerance phases: alarm reaction, regulation, and acclimation (Borowitzka et al. 2018). During the alarm response, microalgae cells increase cell volume and size due to water influx. As a result, osmolytes accumulate and Na^+ ions actively efflux from the algal cells to balance the internal osmotic pressure. During the regulation stage, post-translational modifications occur through phosphorylation/dephosphorylation of light-harvesting complexes, enzymes, or transporters, as well as activation or deactivation of existing enzymes (Lavaud 2007; Derks et al. 2015; Borowitzka, 2018). The acclimation phase induces changes in the control of gene and protein translation and Ca^{2+} signaling activation (Borowitzka, 2018; Einali, 2018). This process protects salt-stressed microalgal cells by repairing altered cell components and activating the antioxidant system (Borowitzka, 2018).

As part of the antioxidant defense, microalgae produce ROS-scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), and glutathione reductase (GR). They also accumulate non-enzymatic ROS scavengers including proline, betaine-glycine, carbohydrates, and others, which can also act as osmoprotective molecules (Figure 6) (Pancha et al., 2015; Ismaiel et al., 2018; Drira et al., 2021; Fal et al., 2022).

Microalgae may undergo genetic modifications known as adaptation in response to sustained exposure to new salinity conditions after initial acclimation. However, cell death may occur if the acclimation process requires more resources, especially energy than are available. Algal cells have alternative strategies aside from acclimation; some can form resistant cysts or spores to survive under stress (Borowitzka, 2018). For instance, diatoms typically have a valve structure at low salinities, whereas some have post-auxospores at high salinities (Schultz, 1971; Schmid, 1977, 1979). Only a few species of the genus *Craticula* (Levkov et al., 2016) have been observed to exhibit the appearance of "craticula" and "heribaudii" (normal vegetative cells produce four internal valves, two craticulae, and within these, two heribaudii; Round et al., 2000), which can develop during resting spore formation as a protection against high salt concentration or desiccation (Schmid, 1979, 2009).

Other diatom species (*Thalassiosira weissflogii*, *Achnanthidium minutissimum*, and *Navicula minima*) (Vrieling et al., 2007) can cover their cells with an organic casing to perform a similar function and protect the cell from osmotic pressure (Gélabert et al., 2004). Additionally, salt stress has significant repercussions on the morphology of microalgae, particularly diatoms, inducing a series of structural adaptations essential to their survival, such as reduced cell size, altered chain morphology, and the formation of teratological forms (altered number of fibulae, raphe system, and striation pattern) (Stenger-Kovács et al., 2023).

Overall, the responses of microalgae to salt stress are diverse and complex, depending on species-specific, developmental stages, metabolic states, magnitude, and duration of salt exposure (Borowitzka, 2018; Fal et al., 2022). Table 3 summarizes some examples of the observed effects of salinity on microalgae composition, diversity, physiology, morphology, and metabolism.

Table 3. Examples of observed salinity impacts on microalgae composition, diversity, physiology, morphology, and metabolism.

Microalgae	Approach	Salinity range	Impact	Key finding	Reference
Diatoms communities from Minno Creek, South Australian Stream	Mesocosm conditions	882, 1092, 1307, 1487, 1734, and 1958 µS cm^{-1} of conductivity using NaCl	**1**-Composition and diversity	**1**-Significant difference in diatom communities' composition between conductivity levels of 1490 µS cm−1 and 1730 µS cm−1. **1**-Negative correlations between species diversity, richness, and salinity.	Frost et al., 2023
Benthic diatom communities from freshwater pond, Spain	Laboratory batch culture	225 (control), 1042, 2053, and 10 073 µS.cm^{-1} of conductivity, obtained by adding sea salt (NaCl).	**1**-Composition and diversity **2**-Physiology responses **3**-Morphology changes	**1**- No changes in species richness or taxa diversity. **2**- A significant increase in total chl-*a* content at 1042 µS.cm-1 compared with controls, followed by a decrease in 2053, and 10,073 µS.cm-1 at the end of the experiment. **3**- No abnormal forms (teratological forms) in the control, while they increase with conductivity.	Noune et al., 2023
Green microalga *Chlamydomonas reinhardtii*	Laboratory batch culture	0 and 200 mM of NaCl	**1**-Physiology responses **2**- Metabolism	**1**- Significant reduction in growth rate, Chl *a*, and Chl *b* levels at 200 mM NaCl. **2**- 200 mM NaCl induced ROS generation with increased H_2O_2 content, leading to lipid peroxidation with elevated MDA levels. **2**- Increased carbohydrate and lipid accumulation under salt conditions. **2**- Accumulation of the main molecules involved in membrane remodeling under salt stress; alkenes, alkanes, phytosterols, linolenic acid (19.29%), and oleic acid (40.29%).	Fal et al., 2022

Benthic diatom communities	Field study on the Drâa river (Southeastern Morocco)	385.5, 1154, 2050, 3110, 4533, 5670, and 7090 µS.cm^{-1} of conductivity.	**1**-Composition and diversity	**1**-Halotolerant diatoms (*Nitzschia frustulum* and *Mastogloia smithii*) dominated the saltwater site, while halophobic and oligohaline species (*Gomphonema olivaceum* and *Cocconeis placentula)* characterized the freshwater stream. **1**-No significant correlation between diatom species richness and salinity.	Lazrak et al., 2022
Freshwater biofilm communities from pristine stream (Riera Major, Viladrau, Spain)	Microcosms conditions	Control, 3, 6, 15, 30 and 100 g.L^{-1} of salinity	**1**-Composition and diversity **2**-Physiology responses 3-Morphology changes	**1- Green algae** dominated all treatments but became much less abundant in 30 and 100 g.L^{-1} than in the control. **1- Cyanobacteria** and **diatoms** abundance increased over time in treatments above 6 g.L^{-1}. **1-**Significant decrease in species richness of **green algal** taxa with increasing salinity, and a shift in community composition, with an increase in *Selenastrum sp.* and a reduction in *Stigeoclonium sp.* above 6 g.L^{-1}. **1-**Diatom species richness (S) and Shannon diversity index (H′) decreased significantly along the salinity gradient. **1-**Higher abundances of some species such as *Platessa hustedtii, Navicula dealpina, Planothidium lanceolatum,* and *Nitzschia linearis* in treatments of 3, 6, 15, and 100 g.L^{-1}, reflecting a preference for these conditions. **1-**Significant decrease in **cyanobacteria** species diversity along the salinity gradient, with *Gleocapsa sp.* dominating at treatments of 3-15	Vendrell-Puigmitja et al., 2022

				g.L^{-1}, while *Chroococcus sp*. increased at 100 g.L^{-1}. **2**- Chl-*a* concentration at 30 and 100 g.L^{-1} treatments reduced significantly. **2**- Photosynthetic efficiency decreased in the 30 and 100 g.L^{-1} treatments by around 25% and 80% respectively, compared with control biofilms. **2**- Nutrient uptake capacity declined at 30 and 100 g.L^{-1} **3-** A small size of diatom species at 6 g.L^{-1}.	
Freshwater biofilm and diatom communities from pristine stream (Riera Major, Viladrau, Spain)	Microcosms conditions	Control, and 15 g.L^{-1} of salinity	**1**-Composition and diversity **2**-Physiology responses **3**-Morphology changes	**1**- Change in the diatom community of the biofilm exposed to salt, favoring salt-tolerant species such as *Navicula reichardtiana,* and *Nitzschia intermedia*. **1**-Diversity and richness of diatom species in the salt treatments declined significantly. **2**- After one day's exposure to salt, the efficiency of photosynthesis and phosphorus uptake in the salt-exposed biofilm decreased significantly. **3**- Cell size of diatom species reduced considerably in the salt-exposed biofilm.	Vendrell-Puigmitja et al., 2021
Halophilic Diatom *Amphora* sp. MUR258	Laboratory batch culture	7, 9, and 12% (w/v) NaCl	**1**-Physiology responses **2-**Metabolism	**1-**Maximum specific growth rate at 7%. **1-** Highest biomass productivity at 9% NaCl. **2-** Highest lipid content at 7% and the lowest at 12 %.	Indrayani et al., 2020
Cyanobacterium *Synechococcus* sp. PCC 7942	Laboratory batch culture	C, 10, 50, 100, 250, 500 mM of NaCl	**1**-Physiology responses	**1-**Maximum growth at 10mM but decreased with increasing salinity, (approx. 50%) at 500 mM.	Verma et al., 2019

			2- Morphology changes **3**-Metabolism	**2-** Cell deformation increases with salt concentration, peaking at 500 mM. **3-** MDA content showed an almost 10-fold increase in peroxidation at 500 mM compared with the control. **3-** Carbohydrates and lipids, respectively, increased 5- and 3-fold in 500 mM NaCl compared with the control.	
Benthic diatom communities	Field study on the United States' rivers (continental U.S., Alaska, and Hawaii)	Conductivity ranged from 10 to 14 500 µS.cm^{-1}	**1-**Composition and diversity	**1-**Conductivity and major ion (HCO_3, Cl, SO_4, Ca^{2+}, Mg^{2+}, Na^+, K^+) significantly influenced benthic diatom composition. **1-**Most variations in diatom assemblages ranged from waters dominated by Ca^{2+} and HCO_3^-+CO^{2-}_3 to those with more Na^+,K^+, and Cl^-.	Potapova and Charles, 2003

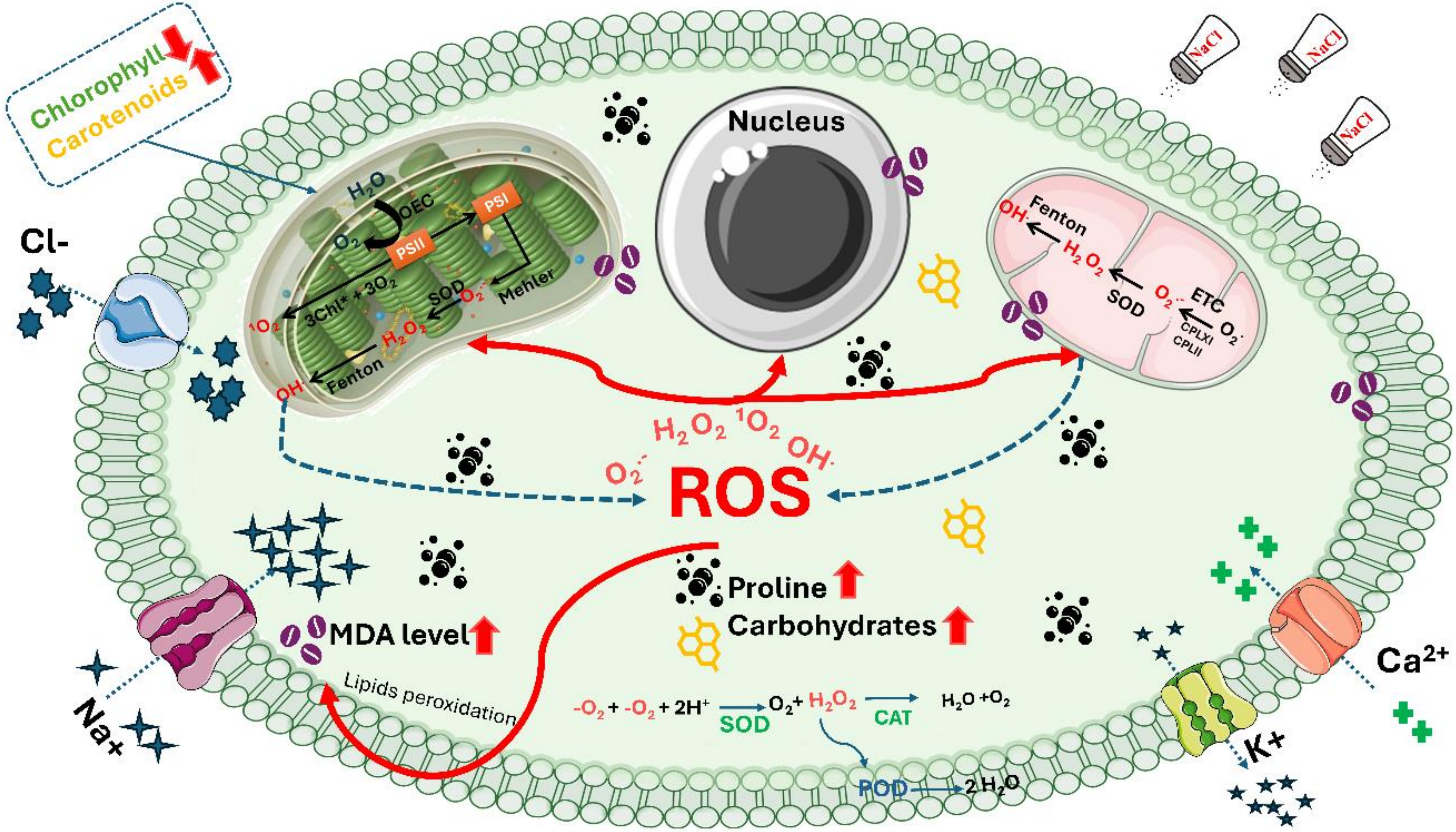

Figure 6. A model describing the salt stress response in microalgae.

Salt stress causes osmotic and ionic stress, eventually generating ROS in microalgae. Increased ROS production alters the ultrastructure of microalgae and causes membrane lipid peroxidation. To protect themselves from salt-induced ROS, microalgae produce more osmoprotectants (proline and carbohydrates) and activate antioxidant mechanisms (SOD, CAT, and POD).

II.3. Societal impacts of freshwater salinization

FS also has significant societal repercussions, affecting various aspects of human life and the environment (Cunillera-Montcusí et al., 2022). Economic costs are among the most important consequences of FS for society. Indeed, agricultural practices contributing to salinization can damage crops, reducing agricultural revenue (Brady and Hightower, 2014; Welle and Mauter, 2017; Berger et al., 2019). As a result, these economic losses can lead to changes in employment, restricted access to drinking water, and the deterioration of ecological services linked to freshwater, such as agriculture, fishing, and recreational opportunities, with considerable implications for the communities that depend on these services (Mahmuduzzaman et al., 2014). In addition to its economic consequences, FS also adversely affects human health. Specifically, salinized river water can render it unpalatable and unsafe (Gorostiza and Sauri, 2017; Kaushal et al., 2016), necessitating costly water treatment processes (Honey-Roses and Schneider, 2012). Thus, society needs to develop regulations and management strategies to address the effects of FS. Far-reaching legislation may be required to prevent human activities such as the diversion and abstraction of water for agriculture, which increase ion concentrations in freshwater systems (MacDonald and Dyack, 2004; Qadir, 2016). Considering emerging technologies and remedial efforts, these strategies must balance social, economic, and environmental implications. Their implementation needs interdisciplinary cooperation and stakeholder involvement (Armitage et al., 2009).

III. Freshwater salinization in desert rivers of Northwest Africa: Drâa river basin as a case study of social-ecological system

Desert rivers are highly dynamic ecosystems, alternating between dry periods with no flow and severe flash floods that shape their hydromorphological and physicochemical features (Kingsford and Thompson, 2006; Clavero et al., 2015; Harms et al., 2018). They are part of the IRES, which share the common features of water scarcity, cessation of surface flow at a particular point in time and space, and considerable spatial and temporal variability of habitats, resulting in high biodiversity during the hydrological cycle (Stanley et al., 1997; Datry et al., 2017c). Dryland rivers are widespread in the semi-arid and arid regions of the Mediterranean and North Africa and are commonly known as "Wadis" (Wheater and Al Weshah, 2002; Sen, 2008). Over the past century, the water of arid and semi-arid rivers has become saltier because of geological factors, high evaporation, and human activities such as intensive farming, irrigation, and water abstraction. This issue has been exacerbated by climate change and water scarcity (Cañedo-Argüelles et al., 2013; Kaushal et al., 2021; Berger et al., 2019; Cunillera-Montcusí et al., 2022).

Despite their great territorial importance and high functional biodiversity, desert rivers, particularly those in North Africa, have received less attention than temperate rivers worldwide (Clavero et al., 2015, 2017), with little understanding of the impact of freshwater salinization in these climatic regions (Cunillera-Montcusí et al., 2022). Among these rivers, the Drâa basin in southern Morocco is one of the ten driest rivers in the world (Revenga et al., 1998). It is of great importance to the nature and culture of the North African region (UNESCO World Heritage Site and RAMSAR zone).

III.1. Description of the Drâa basin and its hydrographic system

The Drâa River basin in southern Morocco, the longest river in the country (115,000 km^2), spans from the High Atlas Mountains to the Atlantic Ocean in the west and the Sahara Desert in the south (Carrillo-Rivera et al., 2013). This river is formed by the confluence of rivers and streams from the High Atlas, such as Dades, Imini, Iriri, and El Malleh, and from the Anti Atlas, namely Ait Douchen (Figure 7) (Warner et al., 2013). The Drâa basin is divided into three sub-basins: upper, middle, and lower Drâa. The upper Drâa sub-basin belongs to Ouarzazate province and extends from the peaks of the High Atlas to the outlet of the Mansour-Eddahbi (ME) dam. The middle Drâa is part of Zagora province and is drained from the ME dam to the M'hamid oasis before transforming into the lower Drâa and flowing towards the Atlantic Ocean (Schulz et al., 2008). These two sub-basins generally remain dry throughout the year, except for occasional periods of heavy rain, snowmelt, or the release of dams. This implies that most of the rivers in the Drâa basin are temporary, and only a few are permanent (Warner et al., 2013; Berger et al., 2021). The basin's altitude varies from 450 to 4071 meters above sea level (Diekkrüger et al., 2012). The region becomes increasingly arid towards the north-south-east, with average annual rainfall varying from 600 to 700 mm on the southern slopes of the High Atlas in the north to 200 mm in the valley and 60 mm in the south. Evaporation is high, at around 2,000 to 3,000 mm per year (Karmaoui et al., 2015). In terms of the hydrological regime of the Drâa, the upper Drâa valley retains an undisturbed natural hydrological regime of the semi-arid subtropics, except for the Iriri stream, which was regulated by the Sultan Moulay Ali Cherif dam. In contrast, water releases from the ME dam mainly control the middle Drâa sub-catchments.

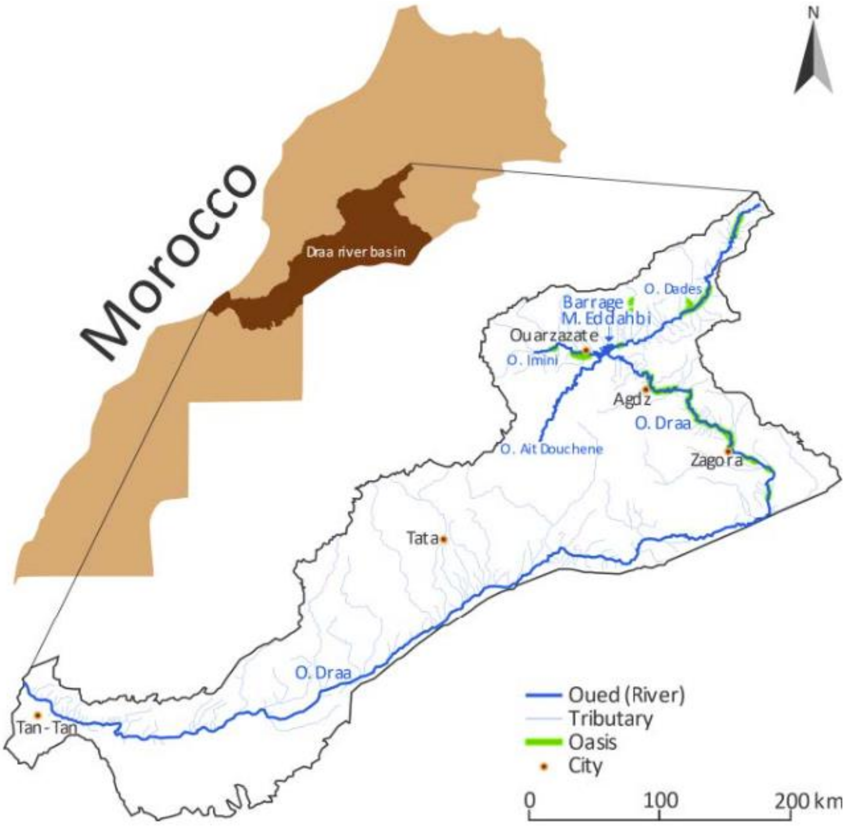

Figure 7. A map indicating the location of the Drâa river basin
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III.2. Water uses, distribution, and management.

The populations living in the Drâa basin, particularly in the upper and middle Drâa sub-basins, are more densely settled along the rivers, where they are directly dependent on the water supply for their domestic and agricultural needs (Diekkrüger et al., 2012; Mahjoubi et al., 2022). Before the construction of the ME dam, water distribution management was different, with upstream areas having privileged access to river water for agricultural use without strict volume restrictions. After the ME dam was built to supply drinking water to the Ouarzazate city and irrigation water to the downstream oases, the regional office of the Ministry of Agriculture, Fisheries, Rural Development, Water and Forests (ORMVA) and the Hydraulic Basin Agency (ABH) regulated the flow of the Drâa using a top-down control system. This involves water releases, known as "lâchers", into a 200km-long valley in the middle of the Drâa, where six oasis belts stretch over 26,000 hectares (ha). These releases are scheduled four times yearly depending on water availability, varying between 30 and 40 million m^3. Furthermore, two new dams have recently been built in the middle Drâa sub-basin, one near Agdz (around 70 km south of Ouarzazate) in 2021 and the other near the Feija plain, as part of the National Drinking Water and Irrigation Plan (Berger et al., 2021). Additionally, five small diversion reservoirs have been constructed at the level of each oasis to control the river flow and recharge the aquifer (Klose et al., 2010; Berger et al., 2021). From there, water is channeled through a system of traditional canals, known as seguia, and distributed to the fields according to established water rights. The water resources of each oasis are controlled by Water Users' Associations (WAUs) and their federation, created by the government in the 1990s (Mahjoubi et al., 2022).

III.3. Agricultural practices and societal needs

Agricultural activities are the main income source for the local population in the Drâa Valley (Heidecke and Heckelei, 2010; HCP, 2015; Berger et al., 2021). Specifically, the Middle Drâa sub-basin is well known for its six oases, where perennial crops such as wheat, barley, maize, alfalfa, vegetables, henna, and date palms are cultivated (Schulz et al., 2008; Heidecke and Heckelei, 2010; HCP, 2015). Alongside traditional agricultural practices, entrepreneurial farming practices have emerged since 2006–2008, involving both investors from outside the region and local people; the latter frequently cultivate watermelons on small parcels (<5 ha) (Berger et al., 2021). These agricultural activities rely heavily on traditional irrigation methods and groundwater pumping (Heidecke and Kuhn, 2006). However, watermelon production in the Middle Drâa Valley relies heavily on pumping groundwater for irrigation. The high-water requirements of this crop pose a potential threat to water resources (Moumane et al., 2021, 2022).

In the Drâa basin, agriculture is crucial to meeting social needs. In addition, other income sources, such as casual labor, commerce, apiculture, and tourism, are becoming increasingly important in terms of their contribution to households' income. Furthermore, migration is a frequently used solution to the negative effects of water scarcity, with many young men seeking alternative income sources in nearby towns (Rössler et al., 2010; Aït Hamza et al., 2010; HCP, 2015; Berger et al., 2021). Overall, the region's livelihoods depend heavily on water supply and security. Thus, water availability and quality may affect their livelihoods and well-being. These may particularly hurt agricultural households, forcing them to find other income sources (Berger et al., 2021).

III.4. Ecosystem services of the Drâa river

The Drâa river provides essential ecosystem services, including provisioning, cultural, recreational, and regulating services. Provisioning services include drinking water, irrigation for oasis maintenance, agricultural production (mainly dates, the main source of income), and domestic water use (cooking, cleaning, washing clothes and carpets), as well as energy production from the upstream dam (ME). Cultural services are characterized by a strong sense of community and identity, traditional water-sharing practices such as the centuries-old Nouba system, the emotional satisfaction provided by irrigation, and the beauty of the palm groves. They also cover recreation, tourism, and the symbolic importance of date palms. Finally, regulating services relate to climate control, as water and vegetation help to create a cooling microclimate that reduces aridity (Mahjoubi, 2024).

III.5. Description of the problems and future challenges

Climate change and the excessive use of water resources in the Drâa catchment have led to a significant drop in groundwater levels (10 meters between 2013 and 2018) (Moumane et al., 2021), accompanied by increasing in salinity soils and water bodies (Johannsen et al., 2016; Speth et al., 2010; Warner et al., 2013). With the recent prolonged droughts, the Drâa basin is also subject to significant intermittency, leading to water scarcity (Moumane et al., 2022). This is actively demonstrated by the disappearance of the temporary Iriki Lake in the lower Central Drâa, which remains dry today (Kingsford, 2006). Additionally, this water scarcity issue intensifies the rising salinization in the catchment (Figure 8) (Cunillera-Montcusí et al., 2022).

Salinization in the upper Drâa basin is mainly due to geological processes, where rocks and soils release ions into the water, as in the case of the El Mellah river (Warner et al., 2013). Salinity levels are increasing considerably in the middle and lower Drâa basins due to reduced rainfall and increased aridity, leading to reduced water dilution and increased evaporation (Beck et al., 2018; Williams, 1999; Warner et al., 2013). This, combined with secondary salinization, such as the over-exploitation of groundwater for irrigation in the middle Drâa, exacerbates the issue (Moumane et al., 2021,2022; Haj-Amor et al., 2016). This increasing salinization affects river biodiversity by reducing species diversity (Cañedo-Argüelles et al., 2013; Schröder et al., 2015) and threatens human well-being by compromising drinking water quality and agricultural productivity. With water-dependent agriculture and pastoralism forming the backbone of the region's economy, the livelihoods of local communities are under pressure in the short and medium term (Johannsen et al., 2016), and water availability can become a source of conflict (Karmaoui et al., 2014; Schilling et al., 2020). As a result, the preservation of this unique and diverse ecosystem is under serious threat (Karmaoui et al., 2014).

Given the current extreme aridity and growing water scarcity in the region, efficient and sustainable management of water resources will undoubtedly be a major challenge. This means taking into account the consequences of dam projects, managing water flows, and developing strategies to minimize the impact of salinization on the environment and local communities (Berger et al., 2021). Overall, balancing the needs of nature conservation and human well-being in the face of rising salinity, water scarcity, and climate change will be a crucial challenge for the future of the Drâa river basin.

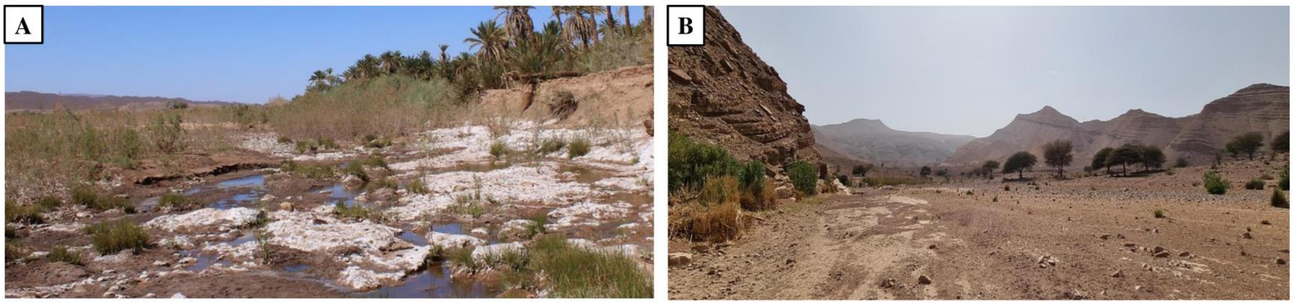

Figure 8. Photos of the main problems in the Drâa basin.
A: Freshwater salinization. **B**: Water scarcity.

IV. Conclusion, research gaps, and challenging prospects

Drylands are expected to increase further (desertification phenomenon) due to climate change. Some of the world's most important rivers, the IRES, drain these drylands. These rivers experience a period of intermittence, exacerbating water scarcity and posing major problems for preserving local livelihoods and biodiversity protection. In addition, freshwater salinization in dry and arid regions, whether natural (primary salinization) and/or anthropogenic (secondary salinization), impacts ecological and social processes, ecosystem services as salinized water becomes unusable for habitat for aquatic organisms, irrigation, and drinking water supply. In arid regions, large dams store water, but they also create new issues with distributive justice, conflicts, water quality, and species protection. It is therefore critical to understand the ecological processes of the aquatic ecosystems under management, define realistic protection objectives, and consider society's multiple demands for use.

Despite their territorial importance and great functional and biological diversity, IRES have received significantly less research than temperate rivers worldwide. Moreover, although scientists are increasingly concerned about freshwater salinization, knowledge of its impact on IRES in arid and semi-arid zones, particularly in North Africa, remains limited. This gap is particularly evident at the least studied trophic levels, notably primary producers, which are represented mainly by benthic microalgae. No comprehensive study has examined the growing problem of salinization from such a system-integrating perspective in North Africa, including Morocco. SaliDrâa Juj, a Moroccan-German research project, addresses this need by identifying appropriate water management objectives in the Drâa river basin that align with ecological and social needs. In this context, the Drâa valley and its catchment area serve as a model region, providing a representative example for many arid catchment areas in North Africa and other regions facing similar challenges. Therefore, this socio-ecological project aims to transform individual and institutional management of water resources under changing environmental conditions for long-term, sustainable conservation of biodiversity and ecosystem services in arid regions, using the Drâa River basin as a study case. A transdisciplinary approach is needed to solve the complex challenges associated with salinization, involving cooperation between different scientific disciplines and the participation of social actors and stakeholders, including water and environmental authorities. This leads to the following five work packages and specific objectives of the project, which are also illustrated in figure **9**:

1- Investigate water-related ecosystem services within socio-ecological supply systems to develop a conceptual framework that promotes transdisciplinary integration and contributes to the concept's development (**WP1**).

2- A comprehensive study of aquatic and semi-aquatic biodiversity and the functions of river ecosystems for the first time, including studying human influence on biodiversity and ecosystem functions, and developing appropriate assessment methods for ecological and water quality (**WP2**).

3- Identify, assess, and prioritize the ecosystem services associated with water from the point of view of the various stakeholders, while studying the systemic relationship between these services and ecological parameters (**WP3**).

4- Study current water and land management practices, and the resulting conflicts, at local and supra-regional levels (**WP4**).

5- Develop and test, as part of a participatory process involving all stakeholders, options for social and institutional transformation to ensure sustainable adaptation to the problem situation described (**WP5**).

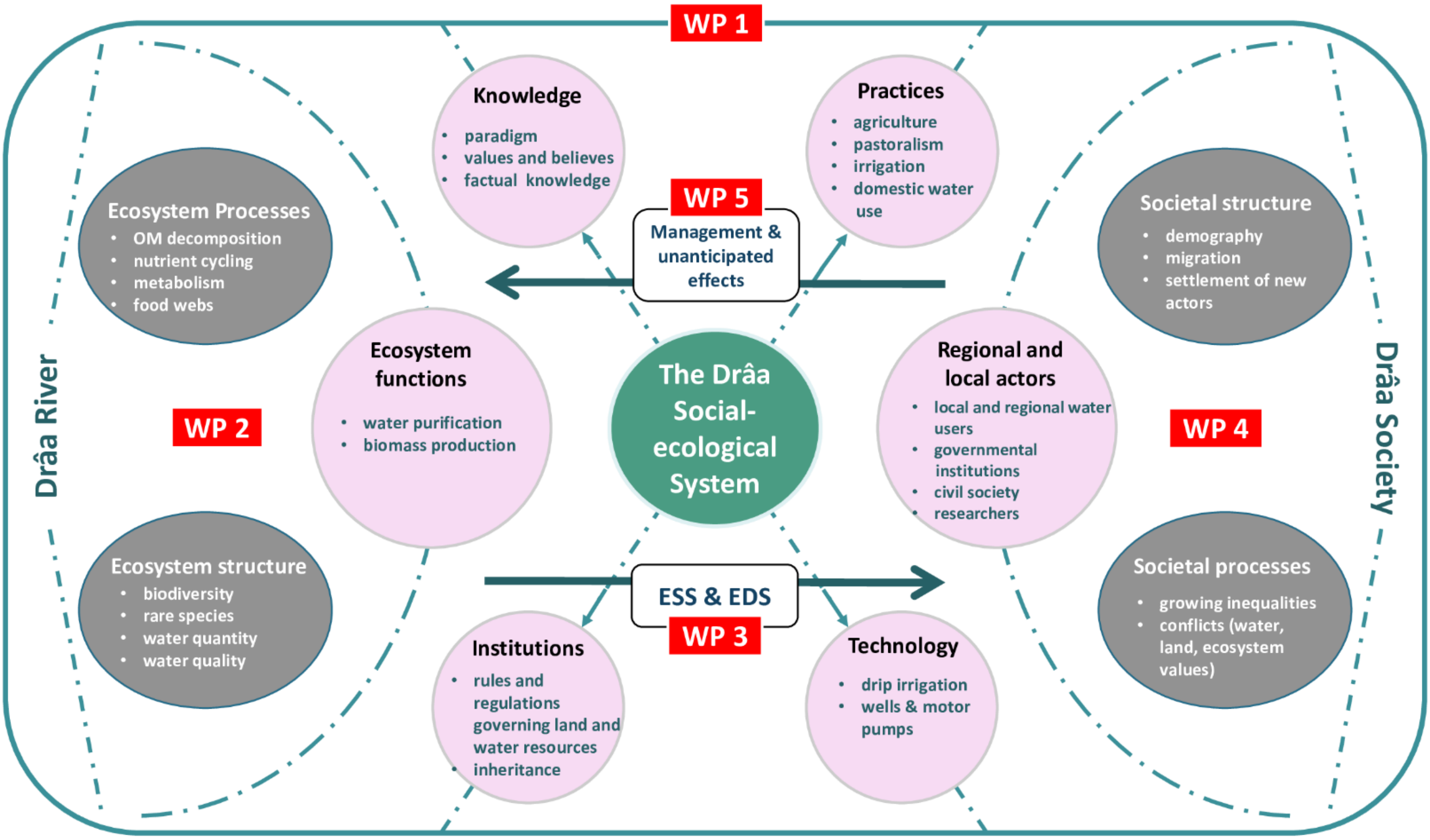

Figure 9. Project structure with transdisciplinary research process description, including specific objectives (WP).
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Among the aquatic organisms targeted by **WP2** are benthic microalgae, the main primary producers of Drâa river. More information is needed on the impact of freshwater salinization on benthic microalgae communities, especially in arid and desert rivers. Indeed, a better understanding of the relationship between increasing salinity levels and changes in the composition and structure of benthic microalgae can provide insights into potential ecological impacts on higher trophic levels and overall aquatic ecosystem functioning. To assess the impact of increasing salinity on the composition, diversity, and structure of benthic microalgae communities in the Drâa river, it is essential to carry out, for the first time, morphological characterization of benthic microalgae. In addition, there is a lack of studies using molecular approaches to investigate benthic microalgae in arid and desert rivers and the impact of freshwater salinization. Consequently, this study suggests, for the first time in Drââ river, to combine new technologies, such as DNA metabarcoding, with the morphological approach. This enables a more comprehensive understanding of microalgal diversity and ecology, providing integrative taxonomic information as well as genetic and ecological data, which is likely to stimulate interdisciplinary research in aquatic ecosystem ecology.

Although diatoms are excellent bioindicators of water quality and ecological status due to their ability to respond rapidly to environmental changes, they are often unsuitable for intermittent river ecosystems (IRES), as they do not consider the specific dynamics of these ecosystems. Furthermore, diatom indices for assessing salinity in IRES remain largely unexploited in scientific literature. Therefore, it is critical to fill this knowledge gap in order to develop assessment methods specific to these ecosystems, enabling a better understanding of their ecological status and responses to environmental pressures, particularly salinization.

Diatom-based indices using morphological and DNA metabarcoding approaches to assess the water quality and ecological status of intermittent rivers provide a comprehensive overview. Combining these approaches improves accuracy, detects cryptic taxa, and increases sensitivity to environmental change. It also facilitates long-term, large-scale monitoring. Integration of these methods fills knowledge gaps, particularly in under-explored regions such as Morocco, and supports interdisciplinary research and effective water management policies.

To understand the consequences and impact of freshwater salinization on ecosystem functioning, it is essential to examine the effects of salinity on benthic microalgae, which are the basis of the food web and the first to be affected by this issue. However, there is a lack of information on the effects of salinization on benthic microalgae physiology and metabolism, as well as their potential impacts at the ecosystem level. Filling this gap is critical for developing future scenarios about the risks of freshwater salinization and effectively managing this problem. An important part of this effort is determining the salinity thresholds of benthic algal communities: the salinity ranges where community composition changes dramatically, as well as the salinity thresholds of isolated microalgae strains in areas affected by freshwater salinization.

Chapter II. Water salinity impacts on the composition, structure and diversity of benthic microalgal communities in the arid Drâa river basin (Southeastern Morocco) using morphological versus molecular metabarcoding

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This chapter was submitted as a paper:

Lazrak, K., Nothof, M., Filker, S., El Bouaidi,W. Berger, E., Loudiki,M. Water salinity impacts on the composition, structure and diversity of benthic microalgal communities in the arid Drâa river (Southeastern Morocco) using morphological and molecular metabarcoding. Science of The Total Environment.

I. Introduction

Intermittent rivers and ephemeral streams (IRES) are watercourses that cease to flow, partially or totally, at a specific point in time and space (Leigh et al., 2016; Colls et al., 2019; Datry et al., 2017). They currently account for 60% of all river systems (Messager et al., 2021). Over the coming years, however, their number is likely to increase, particularly in arid and semi-arid zones, due to dry climatic conditions, human activities, and rising water demand (Datry et al., 2014, 2017; Costigan et al., 2017; Stubbington et al., 2017, 2018; Messager et al., 2021). These factors also exacerbate the adverse effects of increased flow intermittence on IRES biota by inducing significant physicochemical changes (such as increased salinity) as well as hydro-morphological alterations (i.e., habitat fragmentation) (Datry et al., 2021; Medeiros et al., 2020; Song et al., 2020). IRES are more common in the arid regions of the Mediterranean and North Africa and are commonly called “Wadis” (Bonada and Resh, 2013). Despite their remarkable diversity and dynamics, these ecosystems are the most threatened by rising temperatures and aridity caused by climate change and increased water demand. They often receive less attention than perennial streams, resulting in inadequate assessment and protection (Acuña et al., 2014; Messager et al., 2021).

The Drâa river in southeastern Morocco is considered one of the ten driest rivers in the world (Revenga et al., 1998), receiving water only during heavy rainfall, snowmelt, or when the El Mansour Eddahbi dam releases water that regulates the middle Drâa. This river suffers from water scarcity, with human activities lowering the water table and increasing the salinity of soils and aquatic ecosystems (Johannsen et al., 2016; Berger et al., 2021). Salinity in the upper Drâa basin is generally low, with rivers often exhibiting freshwater, except for the El Mellah river, which displays high salinity due to geological factors known as primary salinization (Warner et al., 2013). Further downstream, in the middle and lower Drâa basins, salinity levels increase due to reduced precipitation and a drier climate, surrounded by anthropogenic activities that lead to secondary salinization (Warner et al., 2013; Moumane et al., 2021). This secondary salinization stems from land-use changes in the watershed, including extensive irrigation practices in the major oases of the middle Drâa region. It is also caused by changes in the hydrological cycle, primarily resulting from the construction of the large El Mansour Eddahbi dam between the upper and middle Drâa, so that the middle Drâa only receives water during controlled releases from this dam (Karmaoui, 2014; Berger et al., 2019, 2021). Rising salinity and diminishing water resources in this river endanger the entire food web, instigating cascading effects across various levels. This alters the biota, their functions, and the services they offer, which ultimately jeopardizes human welfare (Lazrak et al., 2022; Berger et al., 2021; Johannsen et al., 2016; Kaczmarek et al., 2023; Mahjoubi and Frör, 2024).

As primary producers, benthic microalgal communities play an essential role in the food web of lotic ecosystems, particularly in the IRES (McIntosh et al., 2017). They contribute to oxygen and energy production via photosynthesis and are essential to aquatic ecosystems' nitrogen cycle and energy dynamics (Battin et al., 2016; Lowe and LaLiberte, 2017). Thus, benthic microalgae absorb nutrients and pollutants, contributing to water self-purification (Sabater et al., 2007; Wu, 2018; Battin et al., 2016). They also create biofilms that provide food and shelter for many organisms, stabilizing river ecosystems (Majdi et al., 2012). Consequently, any alterations in their composition and structure will ultimately impact the aquatic ecosystem (Campeau et al., 1994; B-Béres et al., 2019). Increasing river salinity significantly affects the benthic microalgal structure, composition, and function. It changes taxon dominance and reduces algal species richness, abundance, and diversity (Lazrak et al., 2022, 2024, 2025; Forst et al., 2023). Additionally, increased salinity reduces algal cell density, decreasing photosynthetic capacity due to osmotic stress, which inhibits chlorophyll synthesis and activates its degradation by the chlorophyllase enzyme, ultimately hindering growth (Ji et al., 2018; Vendrell-Puigmitja et al., 2021, 2022; Lazrak et al., 2024, 2025). Along with salinity, hydrological fluctuations in IRES also significantly impact microalgal growth, biomass composition, and structure throughout the year. These include shifting from species adapted to flowing water to those suited for still water, eventually transitioning to aerophilous species during the lentification and riverbed drying (Datry et al., 2017).

Although researchers are increasingly concerned about freshwater salinization, little is known about its impact on IRES in arid and semi-arid regions, particularly in North Africa. This knowledge gap is particularly marked in benthic algae, the least studied trophic level (Cunillera-Montcusí et al., 2022). The Drâa basin is an example of the many arid IRES in North Africa facing similar challenges, with limited studies on benthic algae compared to the other aquatic organisms (e.g., macroinvertebrates) (Lazrak et al., 2022). In this respect, this study aims to investigate the composition, structure, diversity and biomass of benthic microalgal communities in this intermittent river as a function of water salinity (fresh, brackish, and saline waters) during hydrological periods (wet and dry (low water level) using morphological and molecular identification, i.e. DNA metabarcoding. Compared to freshwater environments, we hypothesized that brackish and saline water habitats harbor lower diversity and biomass of benthic microalgal communities during wet and dry periods. Furthermore, brackish and saline water habitats favor microalgae more adapted to saline conditions, while oligohaline species thrive in freshwater environments. This knowledge enables us to enhance our understanding of the impact of freshwater salinization on primary producers, particularly the benthic microalgae of the IRES, in both their wet and dry phases.

II. Materials and Methods

II.1. Study area and sampling sites

The study was conducted in Morocco's longest river, the Drâa basin (southeastern Morocco) (115,000 km^2), which spans from the High Atlas to the Atlantic Ocean in the west and the Sahara Desert in the south (Carrillo-Rivera et al., 2013). The Drâa basin comprises three distinct sub-basins: upper Drâa, middle Drâa, and lower Drâa. The upper Drâa, which is part of the Ouarzazate province, is formed by the confluence of tributaries from the High Atlas (Dades, Imini, Iriri, and El Malleh) and the Anti-Atlas Mountains (Ait Douchen). This water flows into the El Mansour Eddahbi dam (ME) (Warner et al., 2013). The latter flows towards the middle Drâa in the province of Zagora, towards the M'hamid oasis, and finally towards the Atlantic Ocean, known as the lower Drâa (Schulz et al., 2008). The Drâa basin experiences low annual rainfall, ranging from 50 to 800 mm (Karmaoui et al., 2015), but is often disrupted by severe flooding during dry spells (Berger et al., 2021). Most rivers in the basin are dry and temporary, relying on precipitation, snowmelt, or controlled water releases from dams, with only a few permanent rivers (Warner et al., 2013; Berger et al., 2021). Except for the Iriri stream, which is regulated by the Sultan Moulay Ali Cherif dam, the upper Drâa maintains its natural hydrological regime unchanged; however, the middle Drâa sub-basin is controlled by the ME dam. In the Drâa region, communities heavily rely on river water for their livelihoods, particularly in the densely populated upper and middle sub-basins, where water quality and availability are crucial for meeting their domestic and agricultural needs (Diekkrüger et al., 2012; Mahjoubi et al., 2022).

Nineteen sites along the Drâa basin were sampled in October 2020 and 2021 (dry period), as well as in April 2021 and March 2022 (wet period). Twelve of these sites were in tributaries of the upper Drâa, which originate in the Haut-Atlas and Anti-Atlas Mountains. Three sites were selected in the middle of Drâa. Four sites were in the lower Drâa sub-basin, located in a tributary of the Anti-Atlas **(Fig.10).** These sites were sampled at different frequencies based on water availability.: Only one site on the upper Drâa (upstream of Dades affluent) was sampled during the two wet periods. In the middle Drâa, one site was assessed three times (once during the dry periods and twice during the wet ones), another was evaluated once during the wet period, and the third site was sampled twice in each period. Consequently, 32 sites were investigated in dry periods and 36 during wet periods.

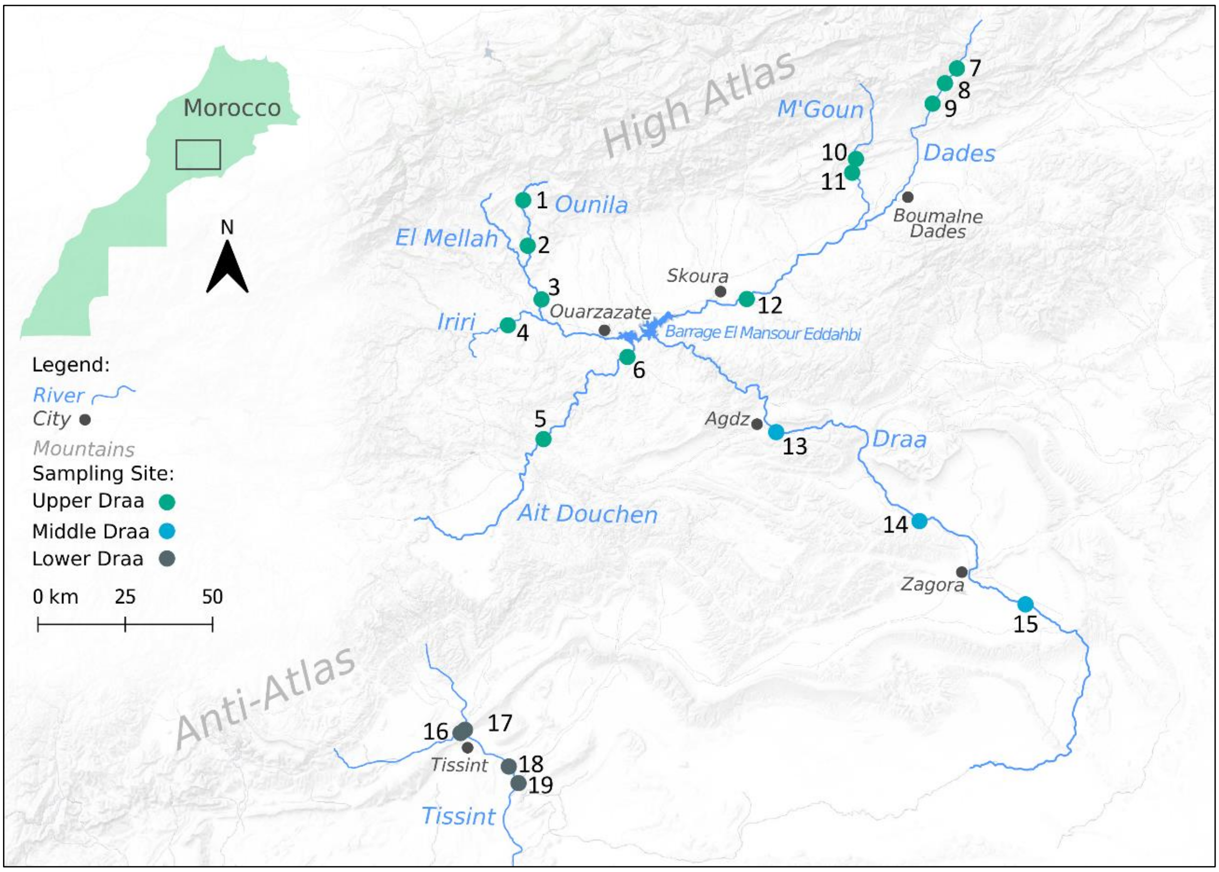

Figure. 10: Drâa basin map illustrating sampling sites
(terrain-base map: © EOX)

II.2. Hydrological and physicochemical parameters

During the study periods, a multi-parameter device (WTW MultiLine® Multi 3510 IDS) was used to measure salinity (g/L), electrical conductivity (µS/cm), water temperature (°C), pH, and dissolved oxygen (mg/L) in situ at each sampling site. Water samples were gathered simultaneously at each site using pre-rinse PVC bottles (1 L) and transferred to the laboratory in a cooling container. Following the analytical methodology of Rodier et al. (2009), water samples were assessed for ammonium (NH_4+), nitrate (NO_3^-), orthophosphate (PO_4^{3-}), chloride (Cl^-), sulfate (SO_4^{2-}), total alkalinity (HCO_3), and total hardness ($CaCO_3$).

Flow velocity was assessed only in October 2021 and March 2022 using a hydrological impeller (SEBA Hydrometrie), then integrated with the cross-sectional area to determine the flow rate.

II.3. Benthic microalgae sampling

At each sampling site, cobbles and cobble-sized stones/rocks were randomly sampled at different locations over a 100-meter section to cover all microhabitats in riffles and pools. Before sampling, all substrates were lightly shaken in river water to remove non-epilithic microalgae and loose sediments. Epilithic biofilm was obtained at each site by scraping a defined substrate surface (at least 10 × 10 cm) using a sterile toothbrush. Next, 2 mL of the periphytic suspensions collected from each site were meticulously pipetted into labeled tubes containing preservation buffer (RNAlater, Qiagen, Hilden, Germany) and stored in a portable freezer (G21 6390525-40L Compressor Car Refrigerator, Germany) at -20 °C for DNA metabarcoding processing. Each sampling site underwent this process three times. The remaining biofilm suspensions from each site were reserved for morphological analysis. All samples were meticulously divided into specific quantities, transferred to appropriately labeled plastic bottles, and preserved in Lugol's solution, except for sub-samples designated for chlorophyll-a analysis. Subsequently, all samples were transported to the laboratory for further processing.

II.4. Biofilm algal biomass measurement

II.4.1. Chlorophyll *a*

Chlorophyll-*a* concentration was determined using the Millerioux method (1975). We extracted three algal biofilm replicates with 90% acetone for 24 hours in the dark. The absorbance of the extracts was measured at 630, 664, and 750 nm with a UV-vis spectrophotometer (Hach Lange, DR 2800, Loveland, CO, USA). The concentration of Chl-*a* was calculated using the formula provided by Jeffrey and Humphrey (1975).

II.4.2. Dry weight

Biofilm sample underwent centrifugation at 6000 rpm for 15 min. Afterwards, the sample was dried in an oven set to 105°C until a constant weight (W1), known as the dry weight (DW), was obtained using a pre-weighed porcelain capsule (W0). The DW was calculated by subtracting W0 from W1, yielding results in mg DW/cm^2. The dried biofilm was then calcined for 5 hours at 550°C in a Nabertherm® muffle furnace (Nabertherm®, Germany). The ash-free dry weight (AFDW) was calculated by deducting W1 from the weight of the residue post-calcination at 550°C (W2).

II.4.3. In situ microalgal biomass determination

Microalgal biofilm biomass was measured only during the last two dry and wet periods (October 2021 and March 2022) using a BenthoTorch (BBE Moldaenke GmbH, Schwentinental, Germany), which provides in situ measurements of the microalgae communities composition by detecting the fluorescence of photosynthetic pigments specific to each group. Three replicate measurements were taken at each sampling site for the biomass of the major photosynthetic groups (cyanobacteria, green algae, and diatoms) in the biofilm communities, quantified in µg chl-*a*.cm^{-2}.

II.5. Microalgal community analysis

II.5.1. Morphological analysis

Biofilm samples were processed according to the method described by Franchini (2013) to identify benthic diatoms morphologically. We treated the samples with 30% hydrochloric acid to dissolve calcium carbonates and eliminate any organic matter. Cleaned diatom frustules were suspended on glass slides using Naphrax® (IR = 1.7). A Motic BA210 optical microscope (Motic® in China) was used to observe the diatom slides at ×1000 magnification. Diatom species identification was based on Krammer and Lange-Bertalot (1986, 1988, 1991a, and 1991b). We updated the nomenclature during identification using Algaebase (Guiry and Guiry, 2023). At least 400 diatom valves were counted for each diatom sample, and the results were expressed in relative abundance as a percentage. The biofilm suspension was examined directly using an optical microscope to identify cyanobacteria and green algae. Cyanobacteria identification taxa were performed based on Komárek and Anagnostidis (1998, 2005), Anagnostidis and Komárek (1989), Komárek (2013), and Komárek et al. (2014). Green algae identification was carried out according to Bourrelly (1972) and Komárek and Fott (1983). A minimum of 400 units (colonies, filaments, or single cells) were counted for each cyanobacterial and green algae taxon using a hemocytometric plate. Only taxa from each algal group with a relative abundance exceeding 1% were incorporated into the statistical analysis.

II.5.2. DNA Metabarcoding analysis

II.5.2.1. DNA extraction, polymerase chain reaction (PCR), and sequencing

The Qiagen DNeasy PowerBiofilm kit (Hilden, Germany) was used to extract DNA from biofilm samples following the manufacturer's instructions. A NanoDrop2000 spectrophotometer (Thermo Scientific, Wilmington, DE) was used to measure the amount of

DNA. We used approximately 50 ng of DNA per sample as a template to amplify the hypervariable V4 region of the 16S rRNA gene (Walters et al., 2016, primer pair) and the hypervariable V9 region of the 18S rRNA gene (Amaral-Zettler et al., 2009) to identify the prokaryotic and microeukaryotic fractions of the biofilms, respectively. V9 primers were labeled with 6nt-long identifiers for multiplex sequencing. To minimize PCR errors caused by polymerases, the Phusion High-Fidelity DNA Polymerase (New England Biolabs) was used for both PCR processes. Following a 30 s initial denaturation step at 98°C, the PCR method consisted of 26 identical amplification cycles. 10 s of denaturation at 98°C, 30 s of annealing at 63°C, and 30 s of elongation at 72°C made up each cycle. A last elongation session of 10 min at 72 °C marked the end of the procedure. The two target genes underwent triplicate amplification to further reduce PCR errors. For each target gene, the three PCR replicates were combined before preparing the sequencing libraries. The NEB Next® Ultra DNA Library Prep Kit was then used to prepare the sequencing libraries for Illumina (NEB, USA). We evaluated the libraries' quality using an Agilent Bioanalyzer system. The sequencing process was carried out on an Illumina MiSeq platform, resulting in paired-end reads of 150 bp for V9 and 250 bp for V4, performed by StarSEQ GmbH in Germany.

II.5.2.2. Sequence data processing and taxonomic assignment

The V9 raw sequencing reads were demultiplexed using cutadapt version 1.18 (Martin, 2011). Then, both V9 and V4 raw reads underwent further processing with cutadapt to remove excessive primer overhangs, followed by the DADA2 pipeline (Callahan et al., 2016), which applied the following parameters for V9 reads: filterAndTrim with truncLen = 100 and maxEE = 1, and for V4 reads: filterAndTrim with truncLen = 140 and maxEE = 1. Reads were merged using a 20-base-pair overlap, permitting up to 2 mismatches, then analyzed using vsearch v2.13.7 for chimera detection and removal (Rognes et al., 2016). The SINTAX algorithm (Edgar, 2016) was employed against the Greengenes v13.5 database for prokaryotes (McDonald et al., 2012) and the PR2 database for eukaryotes (Guillou et al., 2013) to assign taxonomies to the resulting amplicon sequence variants (ASVs). The ASV numbers, along with the corresponding taxonomic data, were then consolidated into an ASV contingency table. ASVs lacking a taxonomic assignment or represented by only one or two sequences (known as singletons and doubletons) were excluded. A total of 1,944,478 reads were classified in 2,009 cyanobacterial ASVs, while 1,525,848 reads were taxonomically assigned to 1,063 green algal ASVs. Additionally, 2,547,334 reads corresponded to 1,906 diatom ASVs.

II.6. Statistical analysis

All data analyses were performed using RStudio version 4.4.2. A two-way ANOVA followed by Tukey's HSD test was used to compare the biomass of samples from different salinity classes during both the dry and wet seasons, utilizing the 'tidyverse', 'ggpubr', 'ggplot2', and 'multcompView' packages. Pearson correlation coefficients were calculated with the correlation function to evaluate the relationships between environmental variables and biofilm biomass parameters. Similarly, the 'ggplot2' and 'vegan' packages were used to perform statistical analyses on all molecular and morphological data, including alpha (richness, Shannon index, and Simpson index) and beta diversity, as well as taxonomic composition.

Shannon–Weaver diversity (H') index: $H' = -\Sigma P_i log_2 P_i ; \ P_i = {}^{n_i}/_{N}$, where *ni* = number of individuals of species *i*; *N* = total number of individuals in the site.

Simpson Diversity Index (SDI): $\mathrm{SDI} = 1 - \Sigma p^2$, where *p* = the proportion (*n*/*N*) of individuals of one particular species found (*n*) divided by the total number of individuals found (*N*).

Hill numbers with exponents 0, 1, and 2 (alpha diversity) quantified alpha diversity in the sequence data. Only the linear model residuals were analyzed when an index exhibited a linear relationship with the transformed square root of the number of reads per sample, indicating potential sequencing depth bias (see Hiiesalu et al., 2014, for further details). Values were centered between -1 and 1 to facilitate comparison of indices. Morphological data remained untransformed. To assess beta diversity, a non-metric multidimensional scale (NMDS) using Bray-Curtis dissimilarity was applied to evaluate differences in algal composition between different salinity conditions and hydrological periods. This analysis was performed separately on datasets sourced from both morphological and molecular techniques. Environmental factors were incorporated into the NMDS plots using the envfit function, allowing for the evaluation of correlations between environmental variables and algal community composition for each dataset. A Monte Carlo analysis with 10,000 permutations was used to determine the fit of each variable ordination (R^2). A PERMANOVA analysis was conducted with 1,000 permutations using the adonis function from the vegan package to compare differences in taxon composition among groups of samples from varying salinity, hydrological periods, and combinations of both factors. To compare the two methods, Venn diagrams were used to illustrate the overlap of genera, while box plots and heat maps were employed to show the dominant genera.

III. Results

III.1. Hydrological and physicochemical characteristics

Hydrological variables exhibited spatiotemporal changes at the Drâa sites (Table S1, Supplementary material). They were generally higher during the wet period than in the dry season. Flow velocity varied from 0 m/s in the middle Drâa site during both periods and downstream of Ait Douchen Oued during the dry period to 0.64 m/s in the Dades site (upper Drâa) during the wet period. Generally, the upper Drâa sites, particularly the Dades and M'goun sites, showed high flow velocities and rates, followed by the lower Drâa and middle Drâa sites. The ME reservoir controls the middle Drâa sites and the small weirs of the southern oasis, making surface flows and habitats more variable in shape and size, often reducing them to isolated or intermittently connected pools.

The physicochemical parameters of water along the Drâa river exhibited significant spatial and temporal variation, highlighting marked discrepancies among the upper, middle, and lower sections. Specifically, conductivity and salinity demonstrated a continuous increase from the upper to the lower Drâa, attaining peak values in the lower reaches, most notably during dry conditions. They ranged from 568 µS/cm and 0.3 g/L during the wet season at Iriri Oued (upper Drâa) to 14240 µS/cm and 9.5 g/L during the dry period downstream of Tissint Oued (lower Drâa). An exception is found at El Mellah Oued, a tributary in the upper Drâa, which showed notably higher salinity than the other upper freshwater tributaries (Iriri, Dades, M'goun, and Ait Douchen Wadies). Similarly, higher concentrations of major ions (chloride, sulfate, and total hardness) were observed at the lower Drâa and Oued El Mellah sites. On the other hand, their lowest concentrations were found in the upper Drâa, with intermediate levels in the middle Drâa.

Therefore, the salinity of the Drâa river water, as examined in this study, was classified into three categories based on chloride concentration, following the modified Van Dam classification (Van Dam et al., 1994): (i) freshwater (<500 mg/L), (ii) brackish water (500 to 5000 mg/L), and (iii) saline water (>5000 mg/L). A total of 35 freshwater, 19 brackish, and 14 saltwater sites were assessed during all study periods. Of the freshwater sites, 16 were sampled under dry periods, and 19 were sampled under wet conditions. Brackish water sites included 9 sites sampled under dry conditions and 10 during wet periods. Saltwater sites were evenly distributed, with 7 samples in each condition.

The pH of the Drâa river water was alkaline, ranging from 7.2 to 9.5. Ammonium, nitrate, and phosphorus concentrations were relatively low at the Drâa river sites, with their highest values of 0.43, 2.88, and 1.74 mg/L downstream of Ait Douchen Oued, the Tissint Oued site (confluence), and the upstream M'goun site, respectively.

III.2. Biofilm biomass

The biofilm biomass in terms of chlorophyll, dry weight (DW), ash-free dry weight (AFDW), and biomass of the main photosynthetic microalgal groups (cyanobacteria, green algae, and diatoms) across two hydrological periods (dry and wet) and three salinity classes (freshwater, brackish and saline water) are illustrated in Fig 11. Biofilm and microalgae biomass values were generally higher during dry periods than during wet ones. The chlorophyll *a* level and dry weight (DW) showed no significant effect of periods and salinity classes. However, freshwater sites during the dry periods showed slightly greater variability in chlorophyll *a* level (Figs. 11A and B). During the dry periods, AFDW levels were highest in freshwater sites (median=1.75 mg.cm^{-2}), then in brackish and saltwater sites (median=1.13 mg.cm^{-2}), without significant difference (Fig. 11C). Conversely, during wet periods, AFDW values at freshwater sites significantly decreased to median = 0.392 mg.cm^{-2}.

The cyanobacteria community was higher in brackish water sites during dry periods (1.83 µg chl *a*.cm^{-2}), followed by saltwater sites (1.34 µg chl a cm^{-2}) and freshwater sites (1.22 µg chl *a*.cm^{-2}), without significant difference (Fig. 11D). However, during wet periods, it significantly decreased in freshwater sites (0.405 µg chl *a*.cm^{-2}) compared to the highest recorded values observed in brackish water sites during dry periods. Green algae and diatom biomass showed no significant differences between periods or among salinity classes; however, freshwater sites during the dry season exhibited high variability (Fig. 11E and F).

Based on the Pearson correlation analysis, a positive correlation was found between chl-*a*, AFDW, and the biomass values of cyanobacteria and diatoms (Fig. S2, Supplementary material).

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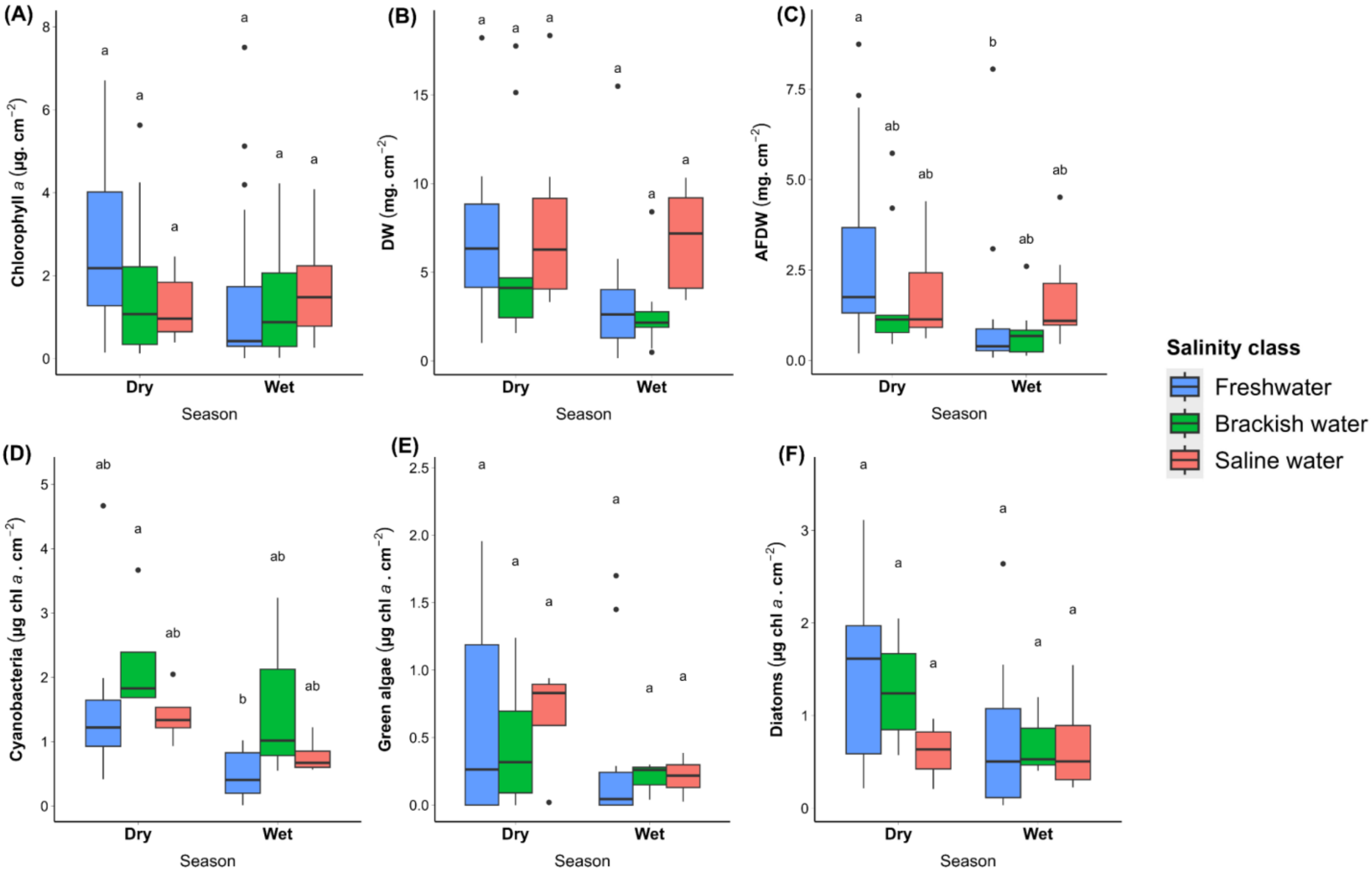

Figure. 11. Box plots of biofilm biomass as chlorophyll a (A), dry weight (DW) (B), ash-free dry weight (AFDW) (C), and biomass of the main microalgal groups (cyanobacteria (D), green algae (E) and diatoms (F)) of samples from different salinity classes during dry and wet periods.

III.3. Microalgal diversity

Figure 12 presents the alpha diversity results obtained from morphological and molecular analyses using the Shannon index. Morphological analysis revealed that diatom communities had a higher Shannon index (>2.2) compared to cyanobacteria and green algae (Fig. 12A). The Shannon index was generally higher during dry periods for microalgae, particularly cyanobacteria and green algae, except at saltwater sites where cyanobacteria showed slightly higher values during wet periods. Diatoms exhibited nearly identical Shannon Index values between the two periods, with high variability during the wet season. Under dry conditions, freshwater sites had the highest Shannon index values for cyanobacteria at 1.59, followed closely by brackish sites (1.54) and saltwater sites (1.17). In contrast, diatoms exhibited higher values in brackish water (2.73) compared to freshwater (2.49 and 2.51) and saltwater (2.31 and

2.4). However, these differences between different periods or among salinity classes were not statistically significant.

The Shannon index of green algae recorded high values of 1.45 in freshwater sites, 1.40 in brackish habitats, and 0.89 in saline waters during dry periods, with no significant difference. Following the same pattern, these values decreased under wet conditions, reaching a significant low of 0.66 in saline water sites (Fig. 12A). The richness and Simpson index for all microalgal groups (cyanobacteria, green algae, and diatoms) showed a trend that closely resembled their Shannon index (Fig. S2, Supplementary material).

In the molecular analysis, alpha diversity for green algae and diatoms was generally consistent with the morphological observations. During the dry periods, cyanobacterial diversity was slightly higher in brackish environments, followed by freshwater and saline sites. Conversely, during the wet periods, it decreased, with freshwater sites exhibiting the highest levels, followed by brackish and saltwater habitats. However, these differences in cyanobacterial diversity, as well as those of diatoms, were not statistically significant among periods or between salinity classes (Fig. 12B and Fig. S3, supplementary material).

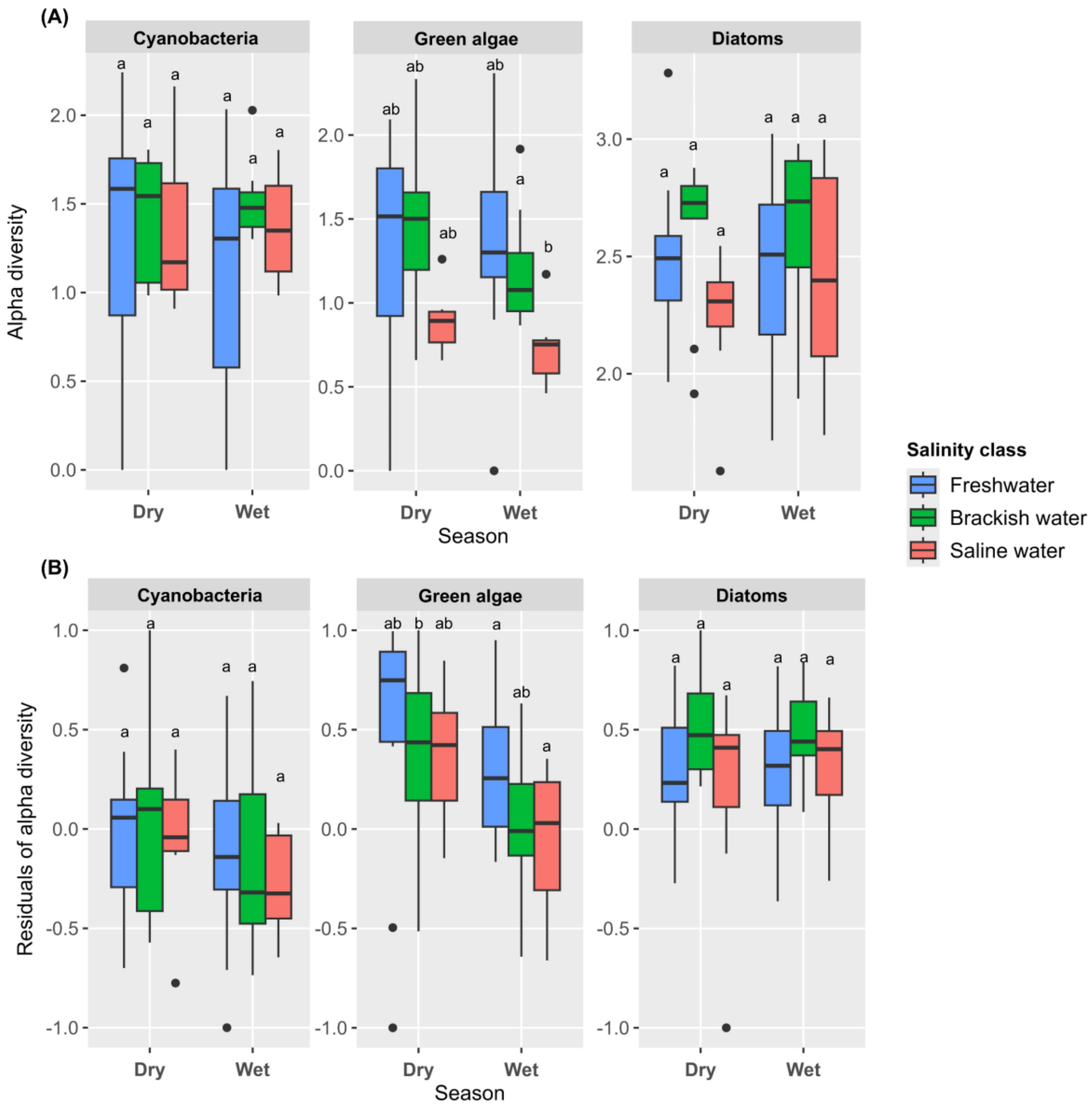

Figure. 12: Box plots of alpha diversity based on the Shannon index for microalgal biofilm communities (cyanobacteria, green algae, and diatoms) of samples from different salinity classes during the dry and wet periods using morphological (A) and DNA metabarcoding methods (B).
The supplementary material contains data on the richness and Simpson indices.

Non-metric multidimensional scaling (NMDS) based on the Bray-Curtis (BC) dissimilarity of all biofilm samples revealed a distinct and nearly identical pattern in the composition of benthic microalgal communities (cyanobacteria, green algae, and diatoms)) for the morphological and metabarcoding data (Figure 13). The NMDS analyses revealed that the Drâa river's benthic microalgae community was divided into three distinct clusters: freshwater, brackish water, and saline water sites. The PERMANOVA results from the Adonis test indicated that salinity was the primary factor impacting all microalgae groups, especially cyanobacteria and diatoms, in both morphological and molecular analyses ($p = 0.001$).

Although the hydrological periods significantly affected green algae and diatoms, they had no significant impact on cyanobacteria. However, examining the interaction between salinity classes and periods revealed significant variations in the assemblages of all benthic microalgal groups, especially diatoms ($R^2 = 0.3071$ and 0.20262, $p = 0.001$) and cyanobacteria ($R^2 = 0.28927$ and 0.1746, $p = 0.001$) (Tables S11 and S12, Supplementary material). Salinity, conductivity, chloride, and orthophosphate were the most significant environmental factors ($p < 0.001$) influencing the structure of all microalgal community groups (cyanobacteria, green algae, and diatoms), as determined by both methods. Additionally, other frequently significant variables include temperature and hardness, particularly for diatoms in both methods. The molecular analysis revealed a wider range of significant parameters than the morphological analysis, highlighting the importance of altitude, alkalinity, sulfate, flow velocity, and flow rate as factors affecting microalgal composition (Tables S5 to S10, Supplementary material). Because of the strong correlation among conductivity, salinity, sulfate, and hardness, only salinity was included in all NMDS analysis plots (Fig. S4, Supplementary material). Overall, the NMDS analysis, employing both morphological and molecular methodologies, revealed that the relationship between salinity classes and fluctuations in hydrological periods had a significant influence on microalgal community composition. Specifically, salinity emerged as the primary environmental variable affecting their assemblages in the Drâa river.

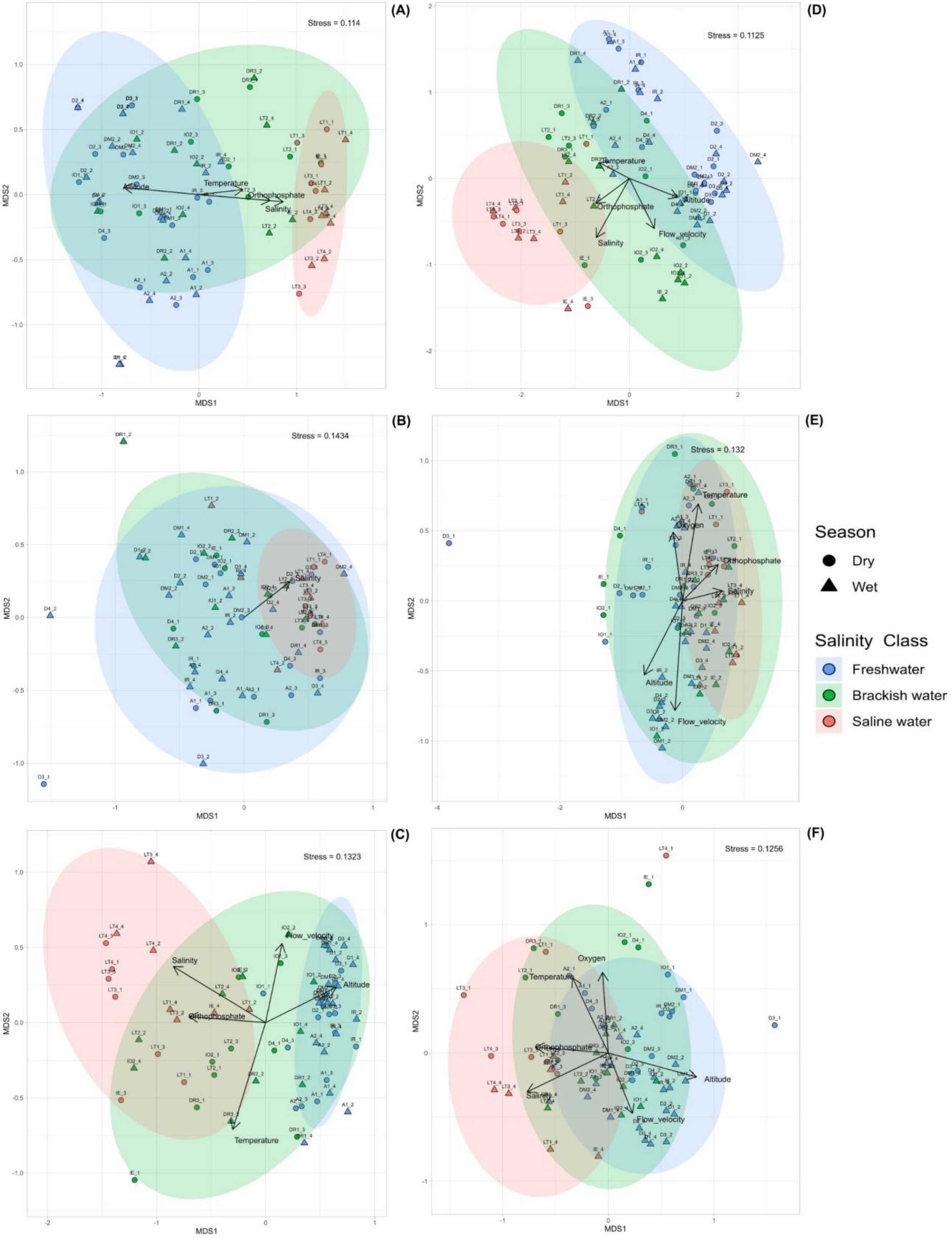

Figure. 13: NMDS based on Bray-Curtis dissimilarity (beta diversity) of microalgal biofilm communities (cyanobacteria, green algae, and diatoms) of samples from different salinity classes during dry and wet periods based on morphological (A, B, and C) and DNA metabarcoding (D, E, and F) analyses with 95% confidence interval ellipses.
The supplementary material provides results concerning the relationship between environmental parameters and NMDS axes (Tables S5 to S10).

III.4. Taxonomic composition

The morphological analysis identified a total of 29 genera and 48 species of cyanobacteria, 29 genera and 40 species of green algae, and 58 genera and 159 species of diatoms (Tables S2, 3, and 4, Figures S5, 6, and 7, Supplementary material). Hydrological periods change in cyanobacteria composition were more pronounced in saline waters than in freshwater and brackish environments. However, variations in salinity significantly affected their composition (Fig. 14A and B). In the freshwater sites, the cyanobacterial community was predominantly represented by the genus *Leptolyngbya* (*Leptolyngbya sp.3*), with 29.4% during the dry periods and 30.24% in the wet one, followed by the genus *Aphanocapsa* (*Aphanocapsa sp.1*) with 12.58% and 17.19%, respectively. Other genera were also found, such as *Synechocystis* (*Synechocystis sp.*) at 10.8% and 9.17%, and *Limnothrix* (*Limnothrix sp.2*) at 5.6% and 1.72% during both dry and wet conditions.

The genus *Leptolyngbya*, which includes *Leptolyngbya sp. 1, sp. 2,* and *sp. 3*, remained dominant in brackish water, representing 34.15% during dry conditions and 25.6% in wet periods. Similarly, the genus *Aphanocapsa* (*Aphanocapsa sp. 1*) was observed in brackish water sites under both conditions, but its relative abundance was lower than in freshwater sites (9% and 7.46%). With a relative abundance almost identical to that of the freshwater sites, the genera *Limnothrix (Limnothrix sp.2*) and *Synechocystis* (*Synechocystis sp.*) were also represented in the brackish environment.

The composition of cyanobacterial communities in saltwater sites exhibited significant differences compared to other salinity classes. *Synechococcus* was dominant in saline environments, reaching its maximum abundance of 27.25% during dry periods but decreasing to 20.75% during wet periods. *Leptolyngbya* (*Leptolyngbya sp. 1*) remained dominant in both periods. Conversely, *Lyngbya* was only found during wet periods, with an abundance of 13.25%. Additionally, *Arthrospira* (*Arthrospira sp.*) was also observed (12.25%) during the wet period.

The composition of the green algae community generally showed that salinity levels and periods variations affected their distribution (Figs. 14C and D). The most abundant genus, *Chlorochytrium* (*Chlorochytrium sp.*), had the highest relative abundance (>30%) across all salinity classes and in both periods. The genus *Cladophora*, represented by *C. glomerata*, was found in freshwater sites during both periods and brackish areas during wet periods, but it was absent in saline waters. Conversely, *Cladophora sp.* demonstrated its highest relative abundance in saline water sites during the wet season (31.21%). It was also present in brackish and freshwater habitats, showing a slight increase during the wet period. Similarly, the genus *Aphanochaete* (*A. repens*) was most abundant during wet periods, exhibiting a high prevalence in brackish water (26.20%), while its abundance decreased significantly in the dry season. Conversely, the occurrence of the genus *Chlorella* (*Chlorella sp.*) was low across all salinity classes and hydrological periods, except for saltwater sites during dry periods, where its presence peaked at 12.23%. Only freshwater sites showed a very low abundance of the genus *Ulothrix*, with a maximum of 2.5% during wet periods.

The diatom community distribution revealed more significant variation across salinity classes and hydrological periods compared to other algal groups (Figs. 14E and F). *Achnanthidium* (*A. minutissimum* and *A. saprophilum*) predominated in freshwater sites, especially during the wet periods, representing 15.47% and 6.12%, respectively. Other genera showing higher abundance in freshwater sites, particularly under wet conditions, included *Gomphonema* (21.45% comprising *G. angustum*, *G. productum*, and *G. parvulum*) and *Cymbella* (11.08% of *C. affinis*). Nevertheless, they were less common in brackish water sites. Although they were present in all salinity classes, *Navicula* and *Nitzschia* exhibited increased tolerance to high salinity and were more prevalent in brackish and saline waters. These include *Navicula recens*, the most prevalent species in saline conditions, while *Nitzschia frustulum* showed moderate abundance in both brackish and saline waters, reaching their highest levels during dry seasons (7.13% and 5.49%, respectively). Additionally, in saline water sites, the genera *Halamphora* (*H. coffeaeformis*, 9.17%) and *Podosira* (*P. dubia*, 9.99%) were more abundant, particularly during the dry periods.

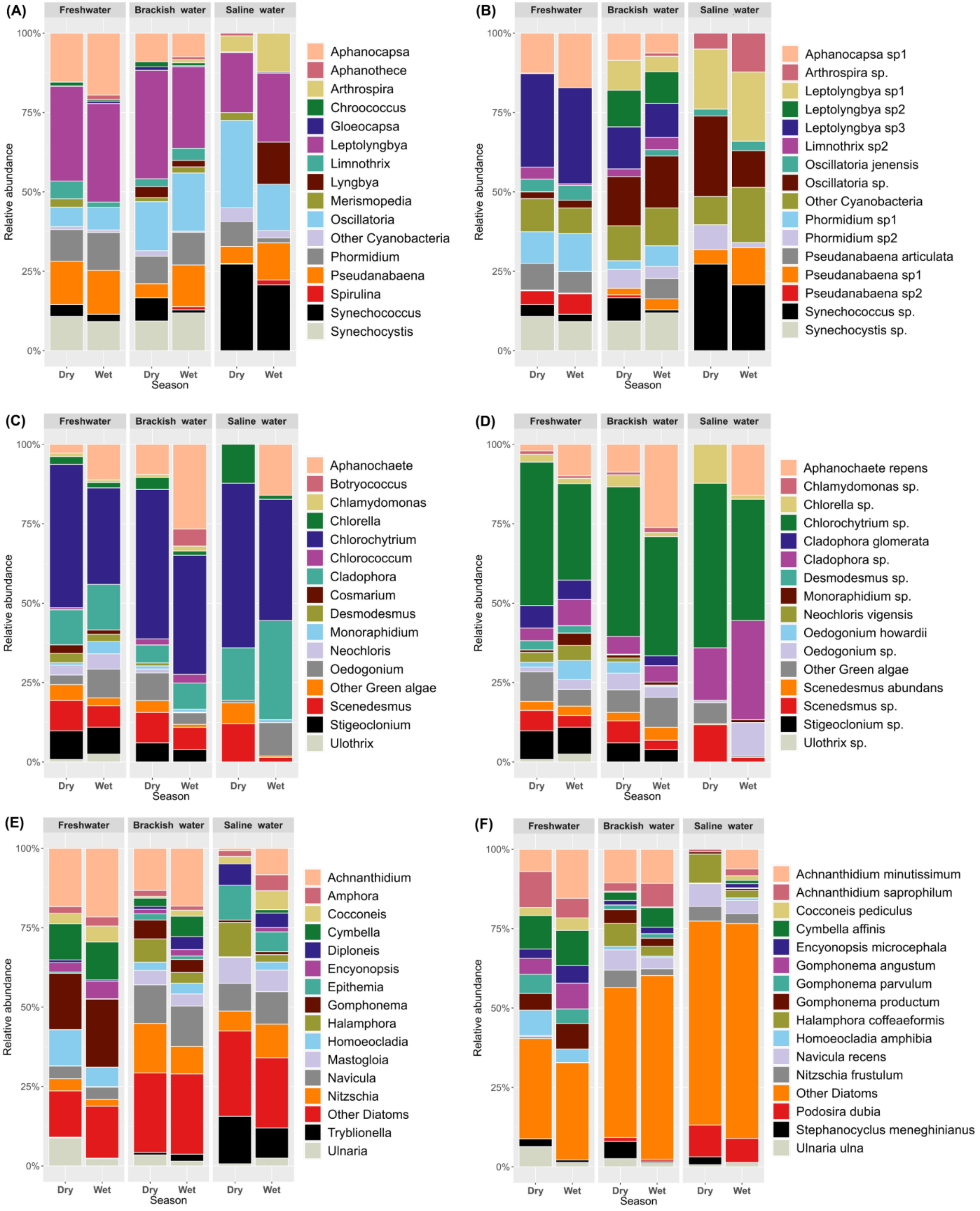

Figure. 14: Taxonomic composition of dominant benthic microalgal communities in freshwater, brackish, and saline water habitats during both seasons (dry and wet) by genus and species levels for cyanobacteria (A and B), green algae (C and D), and diatoms (E and F) using morphological analysis.

Similar to the morphological analysis, the composition of the cyanobacterial community was more influenced by salinity than by hydrological periods, as determined using the DNA metabarcoding method (Fig. 15A). The genus *Leptolyngbya* had the highest relative abundance of cyanobacterial ASVs in freshwater and brackish water samples compared to those in saline water sites, in both periods. Only freshwater environments showed the presence of *Calothrix*, with its maximum abundance of 6.16% during the wet periods. Conversely, *Rivularia* (~4.5%) and *Halomicronema* (~2.5%) were only found in saltwater during both periods, as well as in brackish water, albeit at very low levels (~1.5%). The genus *Synechococcus* exhibited a clear preference for saline conditions, particularly during the dry periods, with a relative abundance of 14.05%.

For green algae, their distribution varied with salinity and hydrological periods (Fig. 15B). The genus *Chlorochytrium* dominated all salinity classes, with a relative abundance exceeding 22.5%, although its presence diminished in saltwater sites during wet periods, dropping to 10.48%. Other genera were found in all salinity classes, including *Cladophora* and *Aphanochaete*. However, their relative abundance was greater during wet periods, with *Cladophora* peaking at saltwater sites (17.8%) and *Aphanochaete* at brackish water sites (7.90%), followed by freshwater sites (5.07%) and saline water (4.45%). The genus *Jaoa* was abundant in freshwater sites during the wet periods (8.42%), while *Uronema* mainly occurred in brackish water environments during dry conditions (4.12%).

The molecular method also revealed that the distribution of the diatom community varied significantly with salinity levels and across hydrological periods (Fig. 15C). *Gomphonema* was the most abundant genus in both freshwater hydrological periods (9.14 and 10.13%), followed by *Cymbella* (7.94 and 7.19%). In saline and brackish waters, *Navicula* was abundant, particularly during the wet periods (8.33 and 7.10%, respectively). *Podosira* exhibited high abundance in both saltwater periods (14.49% and 12.99%). Similarly, during dry periods, *Chaetoceros* was abundant in saltwater sites (7.9%), followed by brackish sites corresponding to 3.8%, showing its high adaptation to saline and dry conditions.

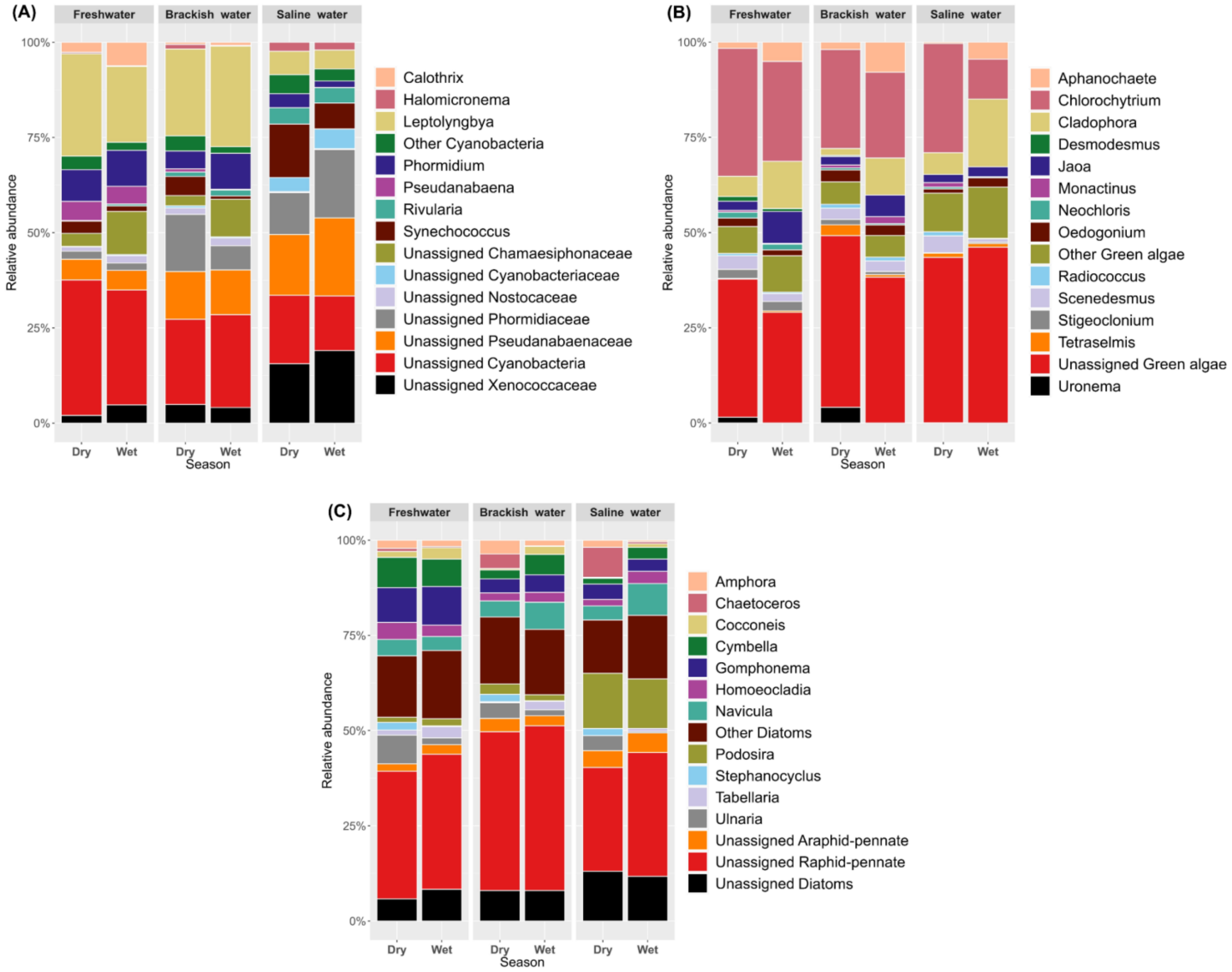

Figure. 15: Taxonomic composition of dominant benthic microalgal communities in freshwater, brackish, and saline water habitats during both seasons (dry and wet) by genus level for cyanobacteria (A), green algae (B), and diatoms (C) using DNA metabarcoding analysis.

III.5. Comparison of two approaches

Morphological identification revealed more taxa and diversity in the cyanobacteria and diatom communities compared to molecular methods (16S rDNA and 18S rDNA). Conversely, the molecular approach identified a higher number of green algae than microscopic observations (Fig.16). Specifically, the latter method identified 29cyanobacteria (Fig.16A), 29 green algae (Fig. 16B), and 58 diatom genera (Fig. 16C). In contrast, the molecular method detected 24 cyanobacteria, 74 green algae, and 47 diatom genera. Both methods also revealed several shared genera, which included 6 cyanobacteria, 23 green algae, and 31 diatom genera.

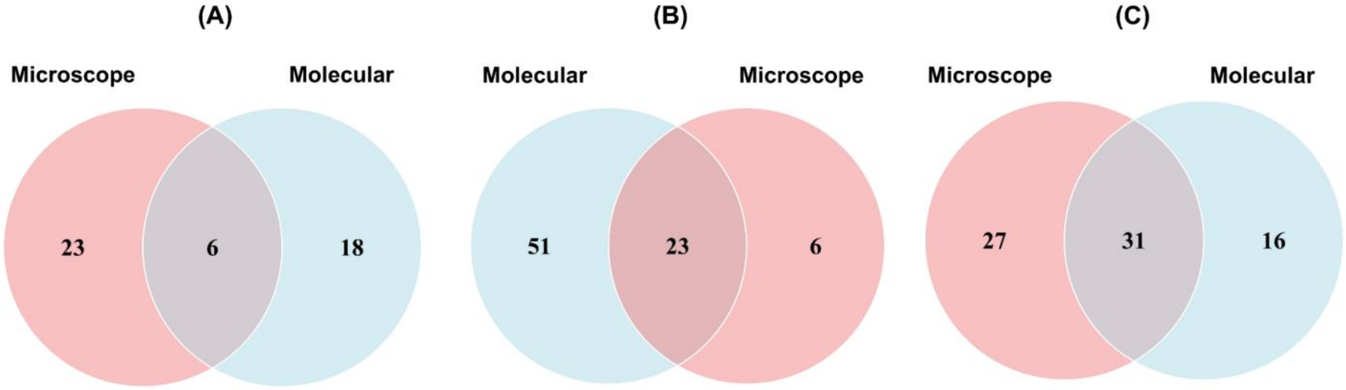

Figure. 16. Venn diagrams illustrating taxonomic similarity between morphological and molecular approaches at the genus level in microalgal biofilm communities (A) cyanobacteria, (B) green algae, and (C) diatoms.

Molecular analysis showed near concordance with microscopic identification for most of the dominant genera of cyanobacteria (Table S13), green algae (Table S14), and diatoms (Table S15), with relative abundances exceeding 5%. However, some genera were only detected by the molecular method or, conversely, by light microscopy (Figs. 17A and B). Both methods primarily identified cyanobacterial genera, including *Leptolyngbya*, *Phormidium, Pseudanabaena*, and *Synechococcus* (Fig. 17A). Specifically, the abundance of *Leptolyngbya* was 24.24% in microscopic identification and 47.44% in molecular analysis, while that of *Synechococcus* was 6.78% and 4.73%, respectively. Only microscopic observations identified the abundant genera *Aphanocapsa* (11.38%), *Oscillatoria* (13.05%), and *Synechocystis* (7.68%). The most abundant genera of green algae found by both microscopic and molecular methods included *Aphanochaete* (7.9% and 8.63%), *Chlorochytrium* (38.36% and 41.62%), *Cladophora* (12.45% and 17.68%), *Oedogonium* (6.34% and 2.36%), *Scenedesmus* (9.28% and 8.66%), and Stigeoclonium (6% and 1.28%) (Fig. 17B). The dominant *Jaoa* genus (6.5%) was only identified using the molecular method. Diatom genera such as *Cymbella* (8.32%, 11.22%), *Gomphonema* (14.20%, 20.25%), *Homoecladia* (7.94%, 12.4%), *Navicula* (6.02%, 6%), and *Ulnaria* (4.43%, 13.1%) were mainly identified by both morphological and molecular methods, respectively (Fig. 17C).

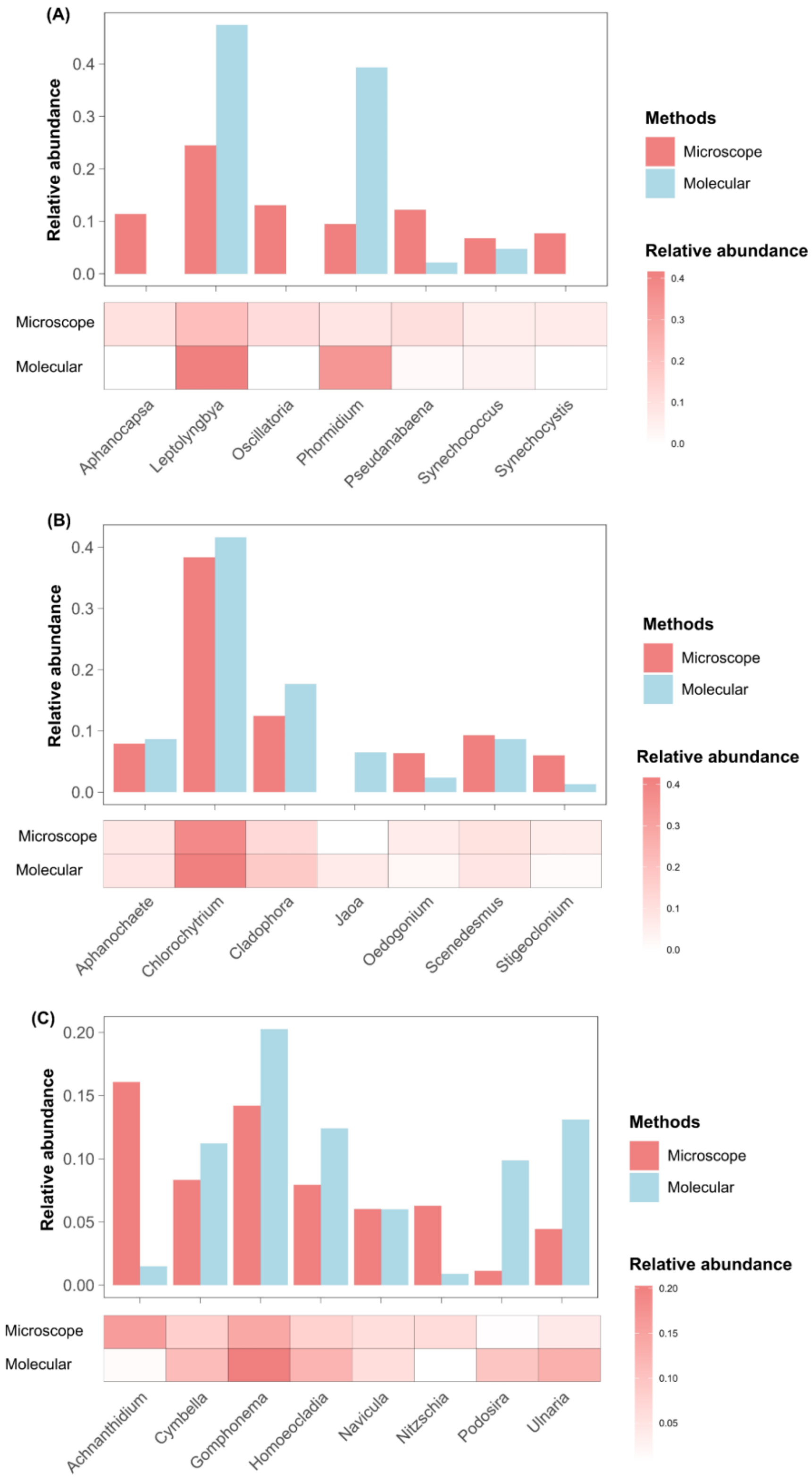

Figure. 17. Comparisons of the dominant genera of (A) cyanobacteria, (B) green algae, and (C) diatoms using morphological and molecular approaches.
Taxa with a relative abundance higher than 5% were considered dominant taxa. The distribution of these dominant taxa is presented in Tables S13, S14, and S15 of the Supplementary material.

IV. Discussion

North Africa's arid and semi-arid regions are characterized by wadies, which play a crucial role in supporting ecosystems, agriculture, and human livelihoods despite limited freshwater availability. Nevertheless, climate change, over-extraction of water, ineffective irrigation methods, and upstream dam construction are negatively impacting both water quality and supply. This deterioration increases salinity, rendering the water less appropriate for drinking, farming, and wildlife (Bonada and Resh, 2013; Kaczmarek et al., 2021; Berger et al., 2019, 2021; Williams, 1999, 2001). Although water salinity levels are expected to increase in the coming year, especially in IRES, the ecological processes and functions of these systems have received less attention compared to permanent water bodies (Datry et al., 2014, 2017a; Messager et al., 2021). Therefore, it is essential to understand the biodiversity within these functional ecosystems, starting with primary producers, mainly benthic algae, and to manage these systems effectively in light of their unique environmental conditions (Datry et al., 2021).

The Drâa river in southeastern Morocco is among the numerous arid regions in North Africa experiencing severe water stress. The dry climate, geological features, and human activities have led to significant physicochemical changes, primarily increased salinity levels and irregular flow patterns (Berger et al., 2021). The physicochemical results of this study indicated that the water's salinity and ionic composition changed over time and space along the Drâa river (upper, middle, and lower subbasins). Salinity and conductivity increased steadily from the upper to the lower Drâa, reaching their highest levels in the lower reaches during periods of low flow or dry conditions. Their values varied from 0.3 g/L and 568 µS/cm during the wet periods at Iriri Oued (upper Drâa) to 9.5 g/L and 14240 µS/cm in the dry season downstream of Tissint Oued. El Mellah Oued, a tributary of the upper Drâa, had higher salinity levels than the other freshwater tributaries of the upper Drâa. Similarly, the lower Drâa had the highest concentrations of chloride, sulfate, and total hardness. In contrast, sites on the upper Drâa recorded their lowest concentrations, while the middle Drâa showed intermediate values. From the ME dam to the downstream area, previous studies revealed a significant increase in salinity along the Drâa river course as it passes through six major oases (Warner et al., 2013; Lazrak et al., 2022). Specifically, it increased progressively from upstream to downstream, rising from 1.5 g/L at the ME dam outlet to 3 g/L in the mid-valley and reaching up to 7 g/L downstream of Zagora city (Busche, 2008). In the upper Drâa, El Malleh Oued has very high salinity, mainly due to geological reasons, which is reflected in high levels of Cl^- and SO_4^{2-}. In the lower Drâa, Tissint Oued also has very high salinity (primary salinization), with salt levels over 11 g/L (more than 16,000 µS/cm conductivity).

Reduced precipitation and increased aridity are driving rises in salinity levels in the middle and lower Drâa basins, which lead to reduced water dilution and increased evaporation (Beck et al., 2018; Williams, 1999; Warner et al., 2013). As a result, during the dry season, the highest amounts of salinity, conductivity, and major ions found in this study are attributed to reduced water flow and increased evaporation, which increases the concentration of dissolved salts compared to wet conditions. Additionally, the drying up of temporary streams in the summer and the changes in natural water flow caused by the ME dam between the upper and middle Drâa rivers put more stress on the watercourse.

Human activities (secondary salinization) further complicate the situation in the Drâa river basin (Warner et al., 2013; Kaczmarek et al., 2023). These activities can significantly raise phosphorus, nitrogen, and other ion levels in rivers. Indeed, the highest levels of ammonium, nitrate, and phosphorus concentrations were observed in the studied sites, especially in some upper Drâa sites (Ait Douchen and M'goun Oued) and lower Drâa (Tissint Oued). Several factors contribute to this issue, including the excessive use of fertilizers in agriculture, which leads to nitrate buildup in the soil and water, thereby contributing to secondary salinization (Lou et al., 2020). Furthermore, chemical contamination from activities such as washing carpets in rivers can increase phosphorus concentration because of the introduction of detergents, cleaning agents, and various other chemicals (Kundu et al., 2015). In addition to various factors contributing to secondary salinization in this arid region, the method of irrigation and elevated groundwater levels exacerbate the issues, particularly when cultivating water-intensive crops such as watermelon. Overall, secondary salinization in the Drâa river is caused by changes in land use, particularly farming methods; unnatural alterations in river flow due to the ME dam; excessive groundwater extraction for irrigation; and the dumping of household waste (Moumane et al., 2021, 2022; Haj-Amor et al., 2016; Berger et al., 2019).

From primary producers to higher consumers, river salinity has a significant impact on all aquatic species along the trophic web. This results in reduced biodiversity, increased stress and mortality rates, and disruption of ecosystem activities and services (Berger et al., 2019; Cunillera-Montcusí et al., 2022). Salinity significantly affects benthic microalgae, thereby affecting their biomass, diversity, composition, and function (Vendrell-Puigmitja et al., 2021, 2022; Lazrak et al., 2024, 2025). Additionally, changes in hydrological conditions influence salinity concentrations, which typically increase during dry phases, impacting microalgal communities. However, this study revealed that during dry periods, all freshwater, brackish, and saltwater sites exhibited higher biofilm biomass value levels compared to wet conditions. This trend was most evident in the freshwater sites, as indicated by significant changes in ash-free dry weight (AFDW). In particular, this decrease in AFDW was associated with the freshwater sites of the upper Drâa, Dades, and M'goun Wadies. These sites exhibited significant flow rates during the studied wet periods, resulting in the scouring and abrasion of the substrate surface. This disturbance in water movement led to a significant decrease or total loss of microalgal biomass (Cao et al., 2022). In contrast, decreased water flow during dry conditions promotes a significant rise in microalgal growth and biomass (Garcia et al., 2015; Neif et al., 2017). Green algae and diatoms biomass in this study revealed no significant effects of salinity, in contrast to the cyanobacterial community, which showed significant differences between its lowest biomass in freshwater sites (0.405 μg chl a cm^{-2}) during wet conditions and its highest values in brackish water sites during dry periods. This can be explained by the fact that certain species of cyanobacteria are able to tolerate and adapt to higher salinity levels without exceeding their threshold, thus increasing the biomass. This is due to their ability to generate specific metabolites and variations in genotypic composition as salinity levels rise (Chakraborty et al., 2011; Lopes and Vasconcelos, 2011; Singh et al., 2022; Reignier et al., 2024). This was also observed in some species of green algae, which can withstand salt conditions by adjusting their osmolyte concentration, modifying their morphology, and developing advantageous mutations (Costelloe et al., 2005; Silva et al., 2000; Shetty et al., 2019). Additionally, long dry periods in IRES favor the growth of cyanobacteria than green algae and diatoms (Robson et al., 2008; Sabater et al., 2017).

Alpha diversity based on the Shannon index using morphological analysis in this study showed that diatom communities had a higher Shannon index (>2.2) than cyanobacteria and green algae. Indeed, of all groups of benthic microalgae, diatoms are the most widespread, abundant, and diverse (Lowe and LaLiberte, 2017; Hu et al., 2024). No significant effect was observed between hydrological periods and salinity on the alpha diversity of cyanobacteria and diatoms. Nevertheless, green algal diversity decreased significantly in saline sites compared to brackish sites during the wet periods. The diversity of each microalgal group under varying salinity conditions depends on the different species-specific responses to salt stress, with some preferring or tolerating, while others diminish or even disappear under high salinity conditions (Berger et al., 2019; Cunillera-Montcusí et al., 2022; Vendrell-Puigmitja et al., 2022; Lazrak et al., 2025). Using molecular analysis, alpha diversity for green algae and diatoms was generally consistent with morphological observations, also without significant differences. However, variation in the pattern of cyanobacterial diversity was observed compared to the morphological analysis, but without significant differences. This could be due to the different taxonomic compositions of the two methods.

Beta diversity based on non-metric multidimensional scaling (NMDS) using Bray-Curtis dissimilarity (both methods) revealed differences in the composition of the benthic microalgal community, divided into three distinct clusters: freshwater, brackish, and saltwater sites. The most significant environmental factors ($p < 0.001$) influencing the structure of all microalgal community groups (cyanobacteria, green algae, and diatoms) were salinity, conductivity, chloride, and orthophosphate. Similarly, the PERMANOVA results of the Adonis test confirmed that salinity was the main factor influencing all groups of microalgae, particularly cyanobacteria and diatoms ($p = 0.001$). These findings align with previous studies indicating that variation in salinity differentiates the composition of biofilm microalgae, including diatoms (Venâncio et al., 2019; Vendrell-Puigmitja et al., 2021, 2022; Lazrak et al., 2022; Frost et al., 2023). Specifically, a study using a microcosm experiment on freshwater biofilm from Iriri Oued of the Drâa river exposed to increasing salinity (1, 10, and 30 g/L) revealed that the beta diversity based on the unweighted pair group method with arithmetic mean (UPGMA) of Bray-Curtis dissimilarity index analysis showed discrete clusters with similarities between communities grew under high salt stress (10 and 30 g/L) and those that thrived under low salinity with control conditions (Lazrak et al., 2024). Furthermore, a previous study (Lazrak et al., 2025) found that salinity had the most significant effect on the composition of benthic microalgae as determined by NMDS analysis during the biofilm transfer experiment between three sites in the Drâa river with different salinity levels.

In terms of hydrological periods, PERMANOVA results showed that they had a significant impact on green algae and diatoms but not on cyanobacteria. Indeed, diatoms are the most affected by hydrological variations, and many of their species are typically less resistant to dry periods and desiccation (Sabater et al., 2017). Piano et al. (2017) revealed that 51% of the diatom community was reduced during the drought season in the study of the water scarcity impacts on benthic algae in five Mediterranean rivers in Liguria, Italy. However, cyanobacteria and green algae preferred warm, isolated waters, typical of dry periods. Even in extreme drought, cyanobacteria can thrive (Costa et al., 2019). The interaction between salinity and hydrological periods in this study revealed significant variations in the assemblages of all groups of benthic microalgae, particularly diatoms and cyanobacteria. This suggests that the interaction between salinity and hydrological variations influences especially benthic diatoms, as they were affected by both factors and their interactions. Thus, salinity impacts more benthic diatoms in the Drâa rivers, and hydrological changes can either amplify or diminish these effects.

Taxonomic composition results based on morphological and metabarcoding data revealed significant changes in the composition and structure of microalgae in the Drâa rivers under different salinity conditions and during two hydrological periods. In the cyanobacteria community, the *Synechococcus* abundance was highest for both methods in saltwater sites and peaked during dry periods. This genus is often found in highly saline water conditions (Lazrak et al., 2024, 2025; Xia et al., 2017) at higher temperatures, generally typical of dry periods (Mackey et al., 2017; Celussi et al., 2019). The green alga genus *Chlorella* (*Chlorella sp.*) showed high abundance in saltwater sites during dry periods in this study. Most of their species showed high salinity and temperature tolerance (Alyabyeve et al.,2007; Ma et al.,2022; Krimech et al., 2022). Similarly, some diatoms taxa, namely *Navicula* and *Nitzschia*, were more prevalent in brackish and saline waters, as most of their species are halotolerant, including *Nitzschia frustulum* (Cañedo-Argüelles et al., 2013; Trobajo et al., 2011). Conversely, *Achnanthidium* (*A. minutissimum*), *Gomphonema* (*G. angustum*, *G. productum*, and *G. parvulum*), and *Cymbella* (*C. affinis*) were predominated in freshwater sites, especially during the wet periods. These oligohaline species are usually considered freshwater ecosystem indicators (Stenger-Kovács et al., 2023; Taukulis, 2007). Overall, these findings indicate that changes in salinity and hydrological periods can contribute to shifts in the composition of benthic algal communities. More specifically, these changes favor species resistant to harsh environmental conditions, such as high salinity and drought.

Although both methods provide almost identical information, the morphological method identified more cyanobacteria and diatom taxa than the molecular identification, while the latter identified more green algae than the traditional approach. The main reason for these discrepancies is the lack of molecular reference databases, leading to misidentification and non-identification of taxa (Bailet et al.,2019; Gelis et al., 2024; Pérez-Burillo et al., 2022; Stuart et al., 2024). Another cause is primer bias, which is frequently considered a significant source of variation, and the efficiency of PCR primers differs between species (Pawlowski et al., 2018; Kermarrec et al., 2013). More specifically, some primers preferentially amplify one taxon over another (Bailet et al.,2019). Additionally, the amplification of green algae in samples using the 18S marker resulted in a low proportion of diatom reads per sample. This could have hindered the amplification and sequencing of certain non-dominant diatom taxa in these samples (Bailet et al., 2019). Therefore, it is preferable to reduce the number of samples multiplexed during sequencing, thus obtaining more reads per sample that can be attributed to diatom taxa (Zimmermann et al. 2015). Taxonomic differences may also result from morphological identification, leading to the misidentification of small species (Bailet et al., 2019). In addition, rare species and those morphologically similar but may exhibit significant genetic variation, known as "cryptic species," can also be overlooked (Wang et al., 2024; Rimet et al., 2018; Zimmermann et al., 2015).

Overall, this study showed a progressive increase in salinity from the upper Drâa (upstream) to the lower Drâa (downstream), which was classified as fresh, brackish, and salt water. The highest salinity concentrations were observed during the dry period, underlining the influence of this period on the increase in salinity levels. Biofilm biomass was generally lower during wet than dry periods, particularly in freshwater sites. This difference was significant in the AFDW, highlighting the effects of scouring and abrasion on the substrate surface during high-flow events, particularly noted in the upper Drâa. Green algae and diatoms biomass showed no significant effects of salinity. In contrast, the cyanobacteria community prefers brackish water sites during dry periods. Alpha diversity, assessed using both methods, showed no significant effects of hydrological periods and salinity. However, beta diversity analysis, revealed that both salinity and hydrological periods significantly affected microalgae community composition, especially diatoms in the Drâa river, with salinity being identified as the primary environmental factor influencing their assemblages. This effect was reflected in changes in community composition, with halophilic and euryhaline taxa predominating in brackish and saline waters, while oligohaline taxa dominated in freshwater. Although morphological and molecular methods yielded different taxonomic compositions in this study, they provided almost identical information, making their complementary use to assess benthic algal communities more effective.

V. Conclusion

This study highlighted the increased salinity in the Drâa river, which is amplified during dry periods due to reduced water flow and increased evaporation. This salinity is caused by geological factors and amplified by the arid climate and human activities, particularly in the middle and lower Drâa. Biofilm biomass was lower during wet periods, showing significant differences in AFDW due to scouring and abrasion from high-flow events, especially in the upper Drâa. The biomass of green algae and diatoms showed no significant effects of salinity. In contrast, the cyanobacteria community showed a preference for brackish water sites during dry periods, probably due to their capacity to withstand abiotic stress factors such as salinity and drought, without exceeding their tolerance thresholds. Morphological and DNA metabarcoding analyses used in this study yielded different taxonomy compositions, but both revealed that salinity and hydrological periods, in the Drâa river significantly affected microalgae community composition, especially diatoms, with salinity being identified as the primary environmental factor influencing their assemblages. This observation indicates that diatoms are more sensitive to environmental disturbances and that two stress factors could sometimes interact synergistically. The variation in salinity over different hydrological periods was responsible for the different structure and composition of microalgae species, with some species showing a higher adaptability to specific environmental conditions. This information provides additional data and baseline information on salinity impacts during dry and wet periods on microalgae assemblages in an intermittent desert river in southern Morocco. Further studies are needed to determine the effect of salinity with other hydrological conditions that may occur in the Drâa river in terms of aquatic phases. Furthermore, more studies are needed to better understand the freshwater salinization in the Drâa river and its effects on microalgae, given that salinity will continue to increase in the coming years. Additionally, it would also be interesting to assess the impact of salinity on benthic microalgae, which constitute the base of the food web along with other higher trophic levels in the Drâa river.

Chapter III. Water quality assessment and diatom-based biomonitoring of the ecological status of the Drâa river basin

I. Introduction

The degradation of freshwater ecosystems, especially in arid regions' intermittent rivers and ephemeral streams, has become a significant issue in recent years. Climate change and human actions are exacerbating environmental and ecological challenges, compromising the quantity and quality of these essential freshwater resources (Magand et al., 2020; Datry et al., 2023). As water quality significantly impacts aquatic and terrestrial organisms, protecting these resources is essential to maintaining the ecosystems' health and human well-being. New methods, such as bioassessments, are being developed and used to monitor and assess the health of freshwater ecosystems to mitigate their decline worldwide (Tan et al., 2017; Atıcı et al., 2018; Zhang et al., 2019). Several countries have applied these methods, resulting in national and international water policies with strict environmental objectives. These include the US Clean Water Act (CWA), the European Water Framework Directive (WFD), and the South African National Water Act (NWA) (Pawlowski et al., 2018; González-Paz et al., 2022).

As water quality can change rapidly and chemical analyses alone are insufficient, it is recommended that physical, chemical, and biological analyses be integrated to assess the ecological status of water bodies. Biological methods offer a more accurate representation of environmental conditions (Lavoie et al., 2006; Jakovljević et al., 2016; Masouras et al., 2021). The most prevalent bioindicators utilized in ecological monitoring are diatoms, a diverse group of photoautotrophic benthic algae. Their rapid responsiveness to environmental fluctuations, widespread distribution, ease of sampling, considerable diversity, and well-established ecological preferences render them particularly suitable for biomonitoring initiatives (Seckbach and Kociolek, 2011; Karacaoğlu and Dalkıran, 2017; Pinheiro et al., 2020). Nevertheless, morphological and taxonomic identification of diatoms presents difficulties that require specialized expertise. Recent research efforts have increasingly focused on developing complementary and alternative methodologies for ecological assessments involving diatoms through the application of molecular techniques (e.g., DNA metabarcoding) (Mann et al., 2010; Kermarrec et al., 2014; Visco et al., 2015; Bailet et al., 2019; Mortágua et al., 2019). This method, which is less time-intensive and less reliant on taxonomic specialists, identifies species from environmental samples by analyzing their DNA through common genetic markers (barcodes) and comparing the sequences with reference databases (Zimmermann et al., 2015; Keck et al., 2018).

In south-west Morocco flows the Drâa river, the country's longest and one of the ten driest rivers in the world (Carrillo-Rivera et al., 2013; Revenga et al., 1998). Most of their rivers are intermittent and ephemeral streams (IRES), which receive their flow mostly from reservoir releases, heavy rainfall, or snowfall (Warner et al., 2013; Berger et al., 2021). The river is facing a major water shortage, exacerbated by human activities that are lowering the water table and degrading water quality, notably by increasing salinity levels (Johannsen et al., 2016; Berger et al., 2021; Kaczmarek et al., 2023). The population in the Drâa basin, primarily located along the riverbanks, depends on these water sources for their daily needs and agricultural activities (Diekkrüger et al., 2012; Mahjoubi et al., 2022). It is, therefore, essential to assess the state of river water quality in this region, as its supply and quality play a key role in people's livelihoods and well-being (Berger et al., 2021).

Only a few studies have used diatoms as biological indicators to assess the quality of Moroccan river ecosystems, including the Drâa river (Fawzi et al., 2001, 2002; Benhassane et al., 2020). Furthermore, how the structure and composition of diatom communities respond to hydrological changes in the IRES, such as those occurring in the Drâa river, is poorly understood. Filling these gaps is essential for developing assessments of the IRES ecosystem (Delgado et al., 2012; Wu et al., 2019; Falasco et al., 2021). In this context, the aim of this study was to assess the water quality of the Drâa river using the physicochemical parameters index (WI) and biological markers based on the diatom index (IPS) using morphological and DNA metabarcoding methods. We compare these different analytical approaches and evaluate the effectiveness of diatom metabarcoding as an operational tool for assessing the ecological quality of intermittent rivers. We hypothesized the following: i) Salinity significantly influences the water quality index of the Drâa river, with pronounced effects observed in the lower Drâa. ii) Water quality in the upper Drâa sites is higher than in the middle and lower Drâa. iii) The water quality index obtained by physicochemical analysis reflects the biological index obtained by both methods, with a lower diatom index correlating to poorer river water quality.

II. Materials and methods

II.1. Study area and sampling sites

The study was conducted in the Drâa basin (southeast Morocco). In October 2020 and 2021 (“low-flow and drought conditions”) and in April 2021 and March 2022 (“wet period”), river water and benthic diatoms were sampled at nineteen sites in the Drâa basin. These sites were divided between the upper, middle, and lower Drâa, as described in section II-1 of Chapter II.

II.2. Physico-chemical parameters

Salinity (g/L), electrical conductivity (µS/cm), water temperature (°C), pH, and dissolved oxygen (mg/L) were measured in situ at each sampling site. The concentrations of ammonium (NH_4^+), nitrate (NO_3^-), orthophosphate (PO_4^{3-}), chloride (Cl^-), sulfate (SO_4^{2-}), total alkalinity (HCO_3^-), and total hardness ($CaCO_3$) in water samples were assessed as previously described in Chapter II (Section II.2).

II.3. Water quality index

This study employed the weighted index (WI), a mathematical formula that calculates the average of combined values for physicochemical parameters, including pH, temperature (T°), electrical conductivity (EC), and chemical oxygen demand (COD). The system set up by the Moroccan Directorate of Water Resources and Planning was utilized (Qalmoun et al., 2022). This system calculates a weighted quality index based on the following equation (Mourhir et al., 2014):

$$\mathrm{WI} = \mathrm{li} + [(\mathrm{ls} - \mathrm{li}) / (\mathrm{bs} - \mathrm{bi})] \times (\mathrm{bs} - \mathrm{pa})$$

li: lower index

ls: upper index.

bi: lower bound.

bs: upper bound.

pa: analyzed indicator value.

Water quality assessment ratings for the grid were converted into unitless values, ranging from 0, indicating bad quality, to 100, reflecting high quality. Each parameter's index (WI) was calculated using weighted parameter concentrations, allowing for a clear classification between bad and high quality based on the quality grid (Table 4). The adjustment index is derived by averaging the weighted limit values of the parameters that define the selected modification. Each quality class is defined by specific threshold values that the various modification parameters must not surpass. This index evaluates overall quality by measuring the minimum alteration index (Mourhir et al., 2014; Qalmoun et al., 2022).

Table 4: Extract from Moroccan surface water quality guidelines.

Parameters	**Unites**	**High**	**Good**	**Moderate**	**Poor**	**Bad**
Indices		100-80	80-60	60-40	40-20	20-00
Temperature						
Temperature	C	< 20	20-25	25-30	30-35	>35
pH						
pH		6.5-8.5	6.5-8.5	8.5-9.2	<6.5ou>9.2	<6.5ou>9.2
Organic and Oxidizable Matter						
DO	mg/l	>7	5-7	3-7	1-3	<1
NH_4^+	mg/l	<30	30-35	35-40	40-80	>80
Nitrates						
NO_3^-	mg/l	<10	10-25	25-50	>50	-
Phosphorus Matter						
PO_4^{3-}	mg/l	<0.2	0.2-0.5	0.5-1	45778	>5
Mineralization						
CE	µs/cm	<750	750-1300	1300-2700	2700-3000	>3000
Cl^-	mg/l	1-200	200-300	300-750	750-1000	1000-7000
SO_4^{2-}	mg/l	1-200	100-200	200-250	250-400	400-3000

II.4. Biological quality

II.4.1. Diatom sampling

Benthic diatoms were sampled at each site, with a detailed description of sampling methods for morphological and molecular analyses, as well as their conservation, provided in Chapter II (section II.3)

II.4.2. Morphological analysis

Diatom sample treatment, preparation of cleaned frustums on a glass slide, observation, identification, and counting were conducted as outlined in Chapter II (Section II.5.1).

II.4.3. DNA metabarcoding analysis

DNA extraction, polymerase chain reaction (PCR), sequencing, sequence data processing, and taxonomic assignment were carried out as previously described in Chapter II (Section II.5.2).

II.4.4. IPS diatom index

The Specific Polluo Sensitivity Index, one of the most widely used and accurate compared to other indices (Descy and Coste, 1991), was applied in this study. IPS values of both methods were calculated using Omnidia software version 6.1 (Lecointe et al.,1993). More specifically, the IPS score of each sampling site is the average of diatom taxa *i* sensitivity values (S_i), weighted by their relative abundance in the sample (A_i) and their indicator value (V_i), as indicated in the following equation:

$$IPS=\sum(Ai\cdot Si\cdot Vi)/\sum(Ai\cdot Vi)$$

IPS scores were converted into assessments of water quality, with IPS ≥ 17 classified as "High"; IPS (13–17) as "Good"; IPS (9–13) as "Moderate"; IPS (5–9) as "Poor"; and IPS (1–5) as "Bad." (Cemagref, 1982).

II.5. Data analysis

All statistical analyses were conducted using RStudio version 4.4.2. A two-way ANOVA followed by Tukey's HSD test was used to compare the means of each index for the upper, middle, and lower Drâa during both the dry and wet periods, using the 'tidyverse', 'ggpubr', 'ggplot2', and 'multcompView' packages. The three indices (WI and IPS derived from both methods) were compared using the Pearson correlation. To further investigate the relationship between the two IPS results of the methods, a chi-square test was conducted using a contingency table for the five quality classes.

III. Results

III.1. Water quality index

The weighted index (WI) was determined mainly by pH, organic and oxidizable matter, or phosphorus matter in the upper Drâa, whereas in the middle and lower Drâa, it was mainly derived from mineralization (Table S16, supplementary material). Based on WI, the river water quality in the upper Drâa sites ranged from good to poor during dry periods (Figs 18A and C). Ounila upstream and two sites at Ait Douchen Oued displayed moderate water quality, whereas Ounila downstream and El Mellah Oued showed poor quality. Site 4 in Iriri Oued consistently showed good water quality. Dades Oued's upstream site (site 7) was dry, the second Dades site (site 8) ranged from good to moderate quality, and the third one (site 9) maintained good quality. In M'goun Oued, the upstream (site 10) and downstream Dades (site 12) varied from poor to moderate, but the downstream M’goun site always showed good quality. In the middle of Drâa, the first site (site 13) varied from dry to moderate water quality; site 14 was consistently dry, and site 15 fluctuated between poor and dry. All three Tissint Oued sites in the lower Drâa showed bad quality, except for site 17, which consistently reported poor quality.

During wet periods, the water quality at the upper Drâa sites varied from high to poor (Figs 18B and D). Both upstream and downstream Ounila Oued sites exhibited moderate quality, whereas El Mellah Oued consistently showed poor quality. Iriri Oued ranged from good to high. Ait Douchen, Dades, and M’goun Wadies generally had good quality, though some sites were moderate. In the middle Drâa, all sites initially had moderate quality; however, site 13 deteriorated to poor, and the others dried out. In the lower Drâa, the two Tissint Oued sites initially reported poor quality, with one worsening to very poor in the second period, while the other sites remained very poor.

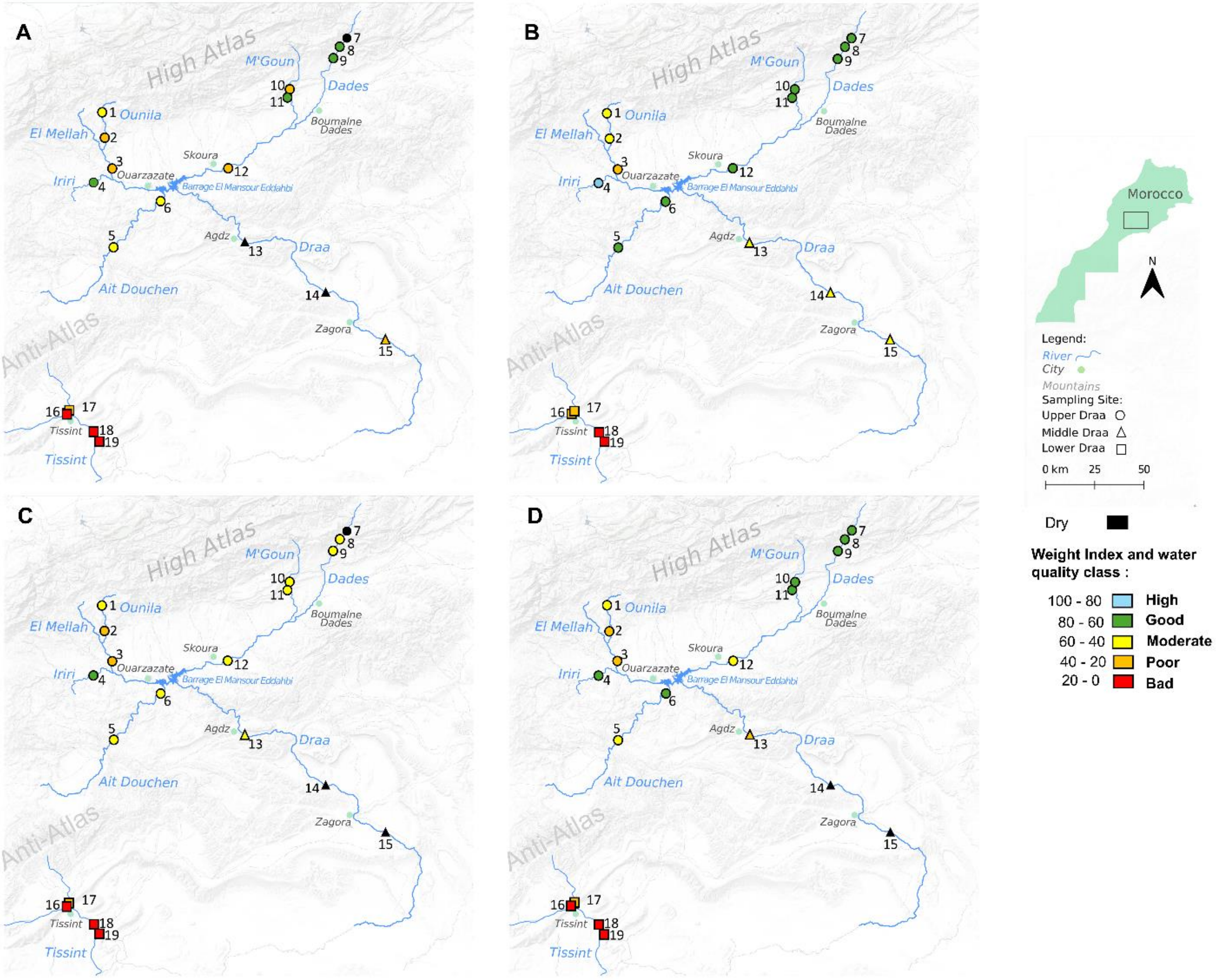

Figure. 18: Spatiotemporal variation in the weight index (WI) values of the Drâa river during dry (A and C) and wet periods (B and D).

Overall, the mean weighted index values for the upper (51.58 and 59.5) and middle (36.64 and 48.31) Drâa were significantly higher than those for the lower Drâa (16.16 and 19.19) (Fig. 19). During the dry period, the weight index values for the upper (51.58), middle (36.64), and lower (16.16) Drâa were lower than those for the wet periods (59.5, 48.31, and 19.19, respectively), although no significant difference was observed between them. Based on these values, water quality in the upper Drâa was generally moderate in both periods, while in the middle Drâa, it ranged from poor during dry periods to moderate during wet periods. In the lower Drâa, however, water was bad in both periods.

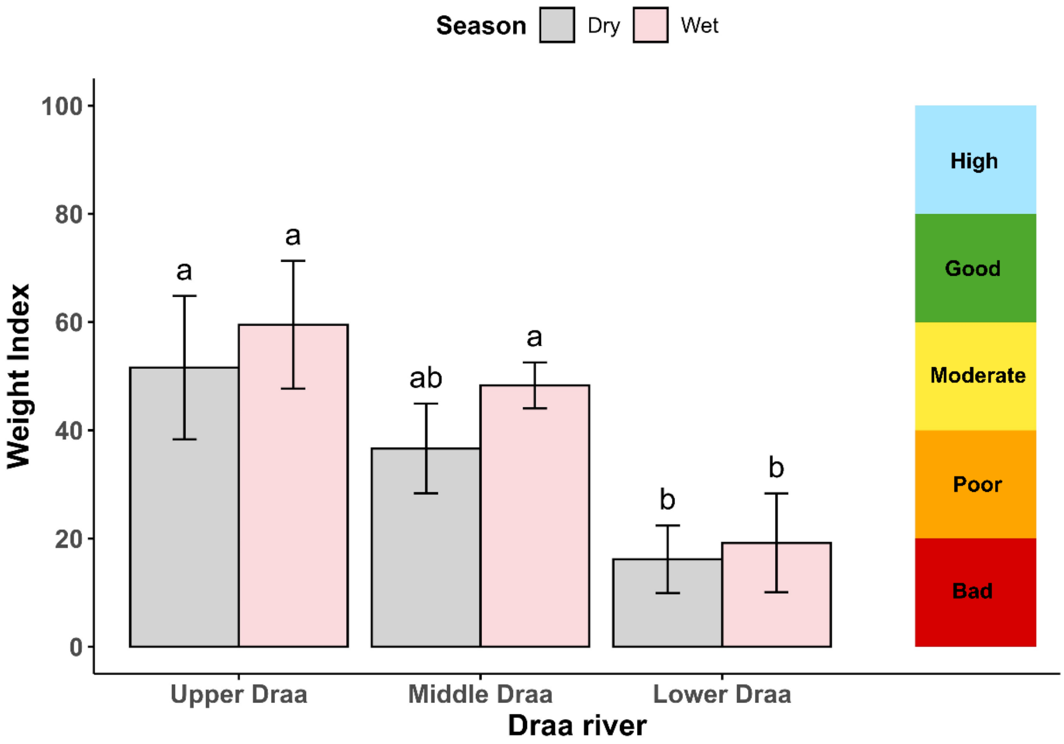

Figure. 19: Mean weight index (WI) values in dry and wet periods for the three Drâa sub-basins (upper, middle, and lower Drâa).

III.2. Biological quality

III.2.1. IPS diatom index based on morphological analysis

The ecological quality, as indicated by the IPS values obtained from the morphological analysis (IPS morpho) (Table S17, supplementary material), ranged from poor to good in the upper Drâa during the dry periods (Figs.20 A and C). Ounila upstream exhibited moderate to good quality, whereas downstream and El Mellah Oued displayed poor to moderate quality. The Iriri Oued site consistently demonstrated good water quality. Dades Oued sites maintained good ecological quality, but its downstream section (site 12) was classified as moderate. In

M’goun Oued, sites 10 and 11 showed variations from moderate to good quality. At the Ait Douchen sites, the upstream site (site 5) varied from poor to moderate, whereas the downstream site (site 6) consistently maintained a moderate quality. In the middle Drâa, site 13 showed a range of conditions from drought to moderate ecological quality. In contrast, site 14 consistently maintained dry conditions, whereas site 15 exhibited variability, fluctuating between moderate ecological quality and arid conditions. In the lower Drâa, the two confluence sites (16 and 17) exhibited different ecological quality levels: one ranged from poor to good, while the other was moderate. Furthermore, the remaining two sites in lower Drâa exhibited poor quality, with one showing variations from low to moderate.

During wet periods, the ecological quality at the upper Drâa sites varied from high to moderate (Figs 20B and D). The Ounila upstream site (1) was moderate, while the downstream site and El Mellah Oued ranged from good to moderate. Iriri Oued had good ecological quality. Ait Douchen Oued was moderate, whereas its downstream ranged from moderate to good. Sites in M'goun Oued were generally of good quality, although one site (9) was of high ecological quality. In the middle of the Drâa, all the sites were initially of moderate quality, whereas the two sites had become dry. In the lower Drâa, the two Tissint Oued sites, located at the confluence, initially had moderate quality but improved to good in the second period. In contrast, the other two sites ranged from poor to moderate quality.

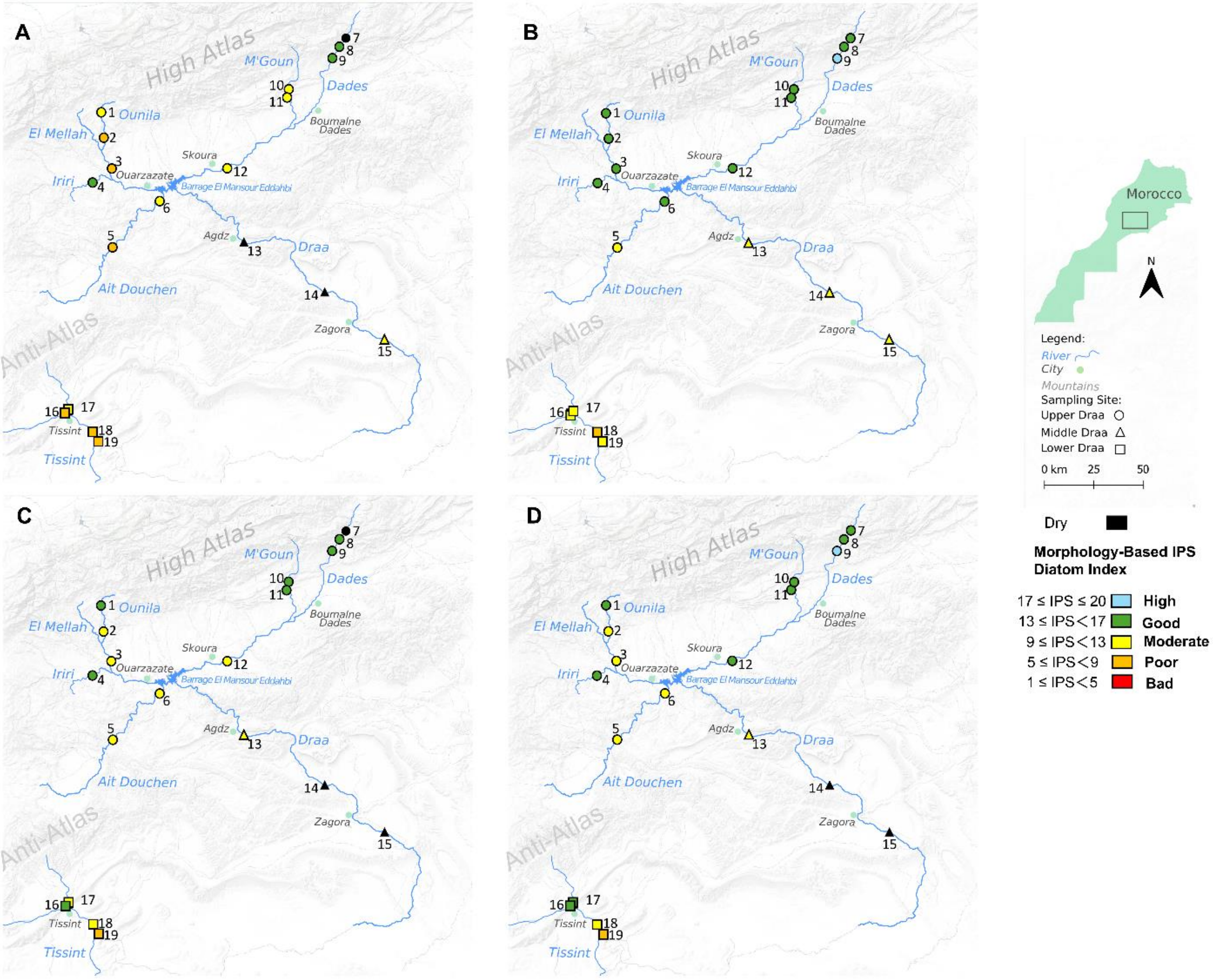

Figure. 20: Spatiotemporal variation in the specific pollution sensitivity index (IPS) obtained from morphological analysis of the Drâa river in dry (A and C) and wet periods (B and D).

The mean IPS morpho for the lower Drâa (9.14 and 10.8) were slightly lower compared to those of the middle Drâa (11.9 and 11.6) and upper (11.9 and 14.2), with the significantly highest value noted in the upper Drâa during wet periods compared to lower Drâa (Fig. 21). Similar to the WI index, the IPS morpho values indicated that during the dry period, those for the Drâa river were slightly lower than those during the wet periods, particularly in the upper and lower Drâa, with no significant difference between them. Ecological quality in the upper Drâa varied from good to moderate during both dry and wet periods. In contrast, the middle and lower Drâa exhibited moderate ecological quality in both periods, with the lower Drâa showing the lowest value in the moderate class during the dry periods.

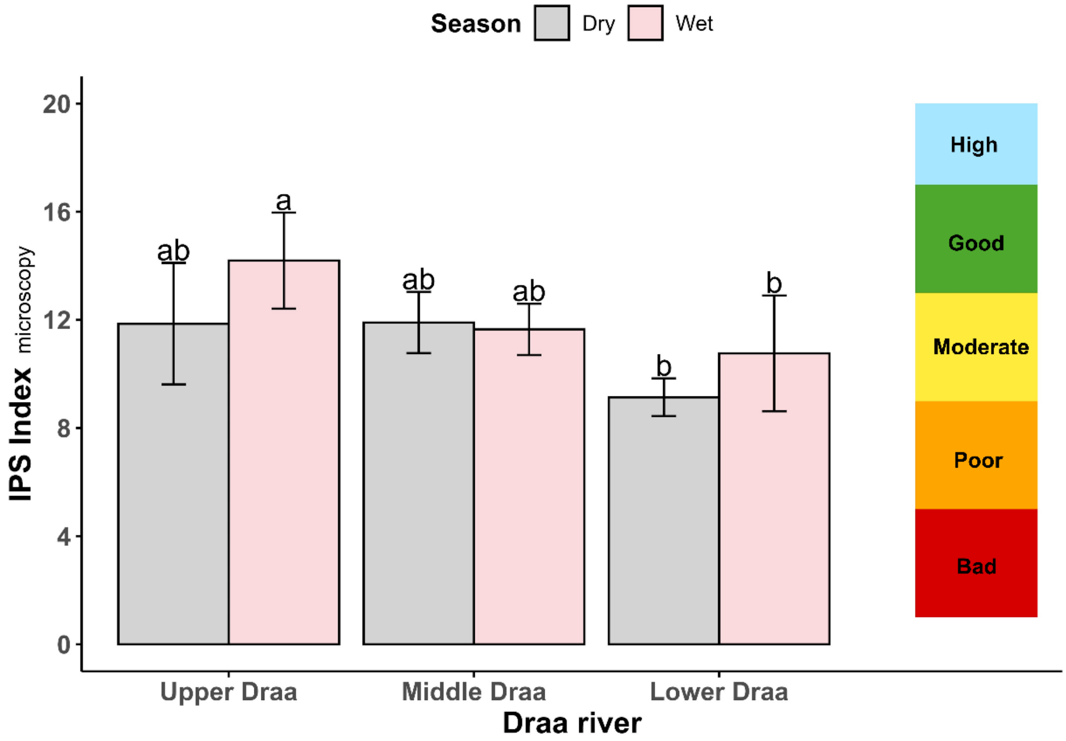

Figure. 21: Mean specific pollution sensitivity index (IPS) values obtained from morphological analysis in dry and wet periods for the three Drâa sub-basins (upper, middle, and lower Drâa).

III.2.2. IPS diatom index based on molecular analysis

According to the IPS values derived from DNA metabarcoding (IPS meta) (Table S17, supplementary material), ecological quality at most sites in the upper Drâa showed similar ecological classes, as indicated by the IPS morpho values (Fig. 22A and C). Except for the downstream section of the Dades, which showed poor quality during the second dry period. In the middle Drâa, the first site (15) showed poor quality. The other sites, on the other hand, had the same ecological class as determined by the morphological method, which is also moderate. In the lower Drâa, the Tissint Oued site, located at the confluence, initially had good ecological quality, but this declined to a moderate level during the second period. In contrast, the other three Tissint Oued sites maintained moderate ecological quality throughout the dry periods.

The ecological quality of the upper Drâa sites during the wet period ranged from good to moderate (Figs. 22B and C). Similar to the IPS morpho results, Ounila, El Mellah, Ait Douchen, and Iriri Wadies ranged from good to moderate. The Dades and M’goun Wadies sites displayed good quality during the first wet period. However, during the second period, both the downstream sites of Dades and M’goun deteriorated to a moderate. In the middle Drâa, the first two upstream sites (13 and 14) were initially considered of good quality, while the third site

(15) was classified as moderate during the first wet periods. However, site 13 deteriorated to a moderate quality, and the other sites experienced desiccation during the second period. The lower Drâa sites typically exhibited moderate quality, except for the confluence site (17), which showed good quality during the initial wet period. However, its quality diminished to a moderate during the second wet period. Two other sites displayed good and high quality (sites 18 and 16), while one maintained a moderate quality (site 18).

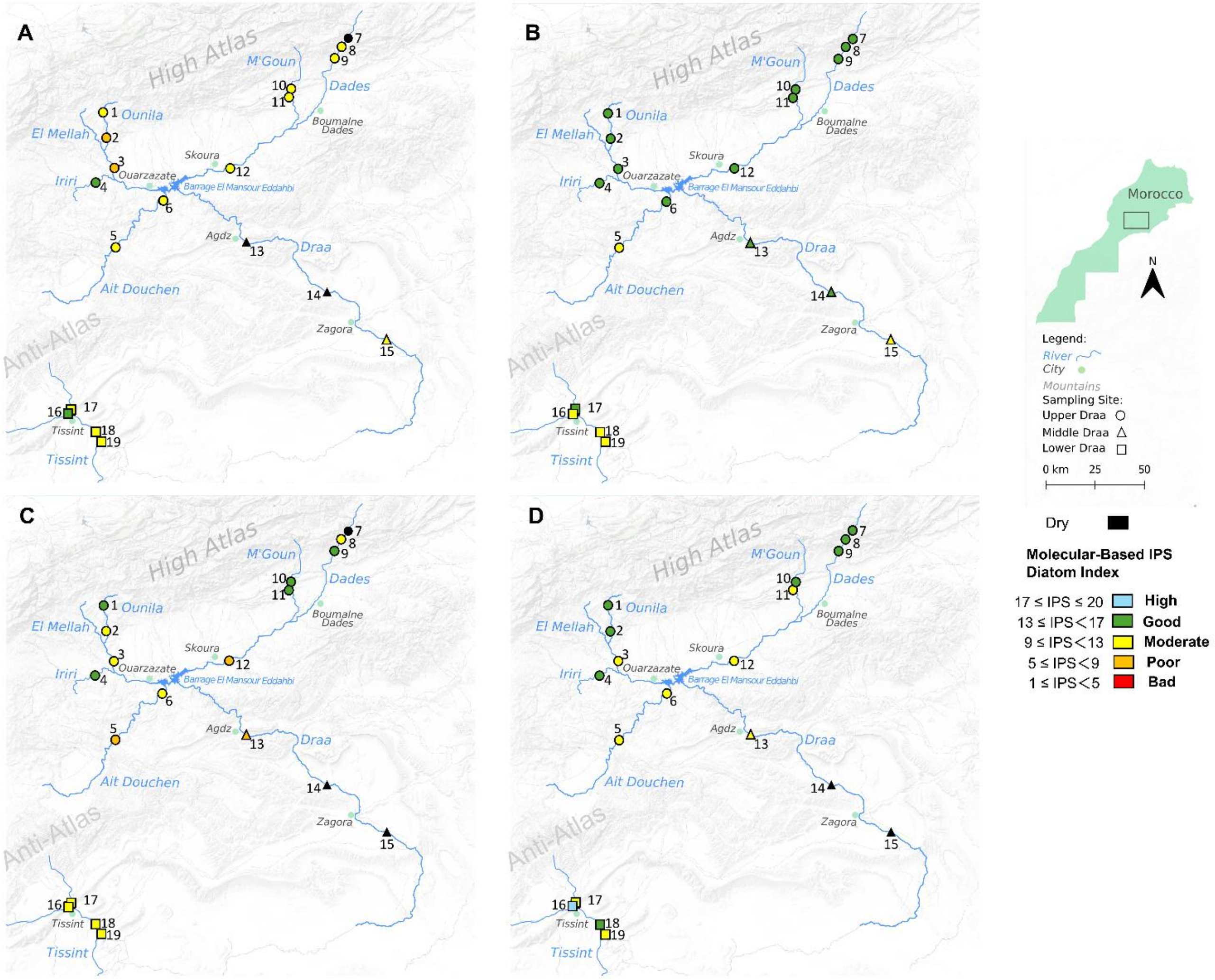

Figure. 22: Spatiotemporal variation in the specific pollution sensitivity index (IPS) obtained from DNA metabarcoding analysis of the Drâa river in dry (A and C) and wet periods (B and D).

Mean IPS meta values were generally slightly higher in the upper Drâa (13.8) compared to the middle and lower Drâa (11.8 and 12.5, respectively) during wet periods (Fig.23). However, molecular methods revealed a decline in IPS values during dry periods compared to wet ones in the Drâa river, with a significant reduction observed in the upper Drâa. Using the molecular method to assess IPS, water quality in the upper Drâa ranged from moderate to good during dry and wet periods, respectively. In contrast, the middle Drâa exhibited poor to moderate quality during the same periods. The lower Drâa maintained a moderate quality in both periods.

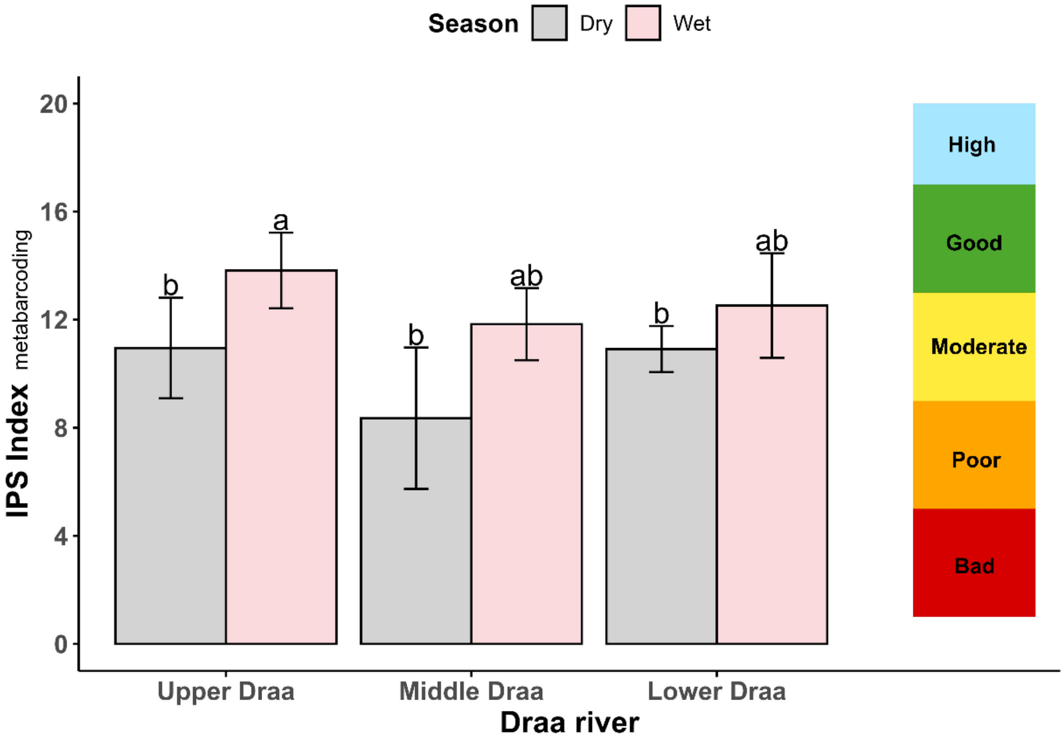

Figure.23: Mean specific pollution sensitivity index (IPS) values obtained from DNA metabarcoding analysis in dry and wet periods for the three Drâa sub-basins (upper, middle, and lower Drâa).

III.3. Comparison between methods

Figure 24 illustrates the relationship between the values of the three indices, WI, IPS morpho, and IPS meta as determined by Pearson's correlation. The WI index, based on physical and chemical measurements, exhibited a positive correlation with the IPS morpho ($r = 0.69$, $p < 0.001$) compared to the IPS meta ($r = 0.38$, $p < 0.01$). Furthermore, the IPS values derived from both methods were positively correlated ($r = 0.62$, $p < 0.001$).

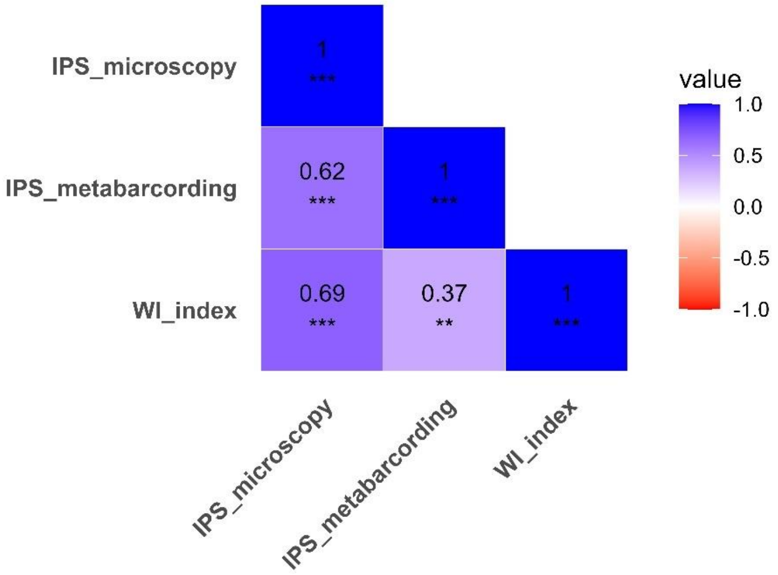

Figure.24: Pearson correlation between the three indices, WI and IPS, obtained from morphological and molecular analyses.
(*** $p < 0.001$), (**$p < 0.01$), and (* $p < 0.05$).

In addition to the positive relationship between the IPS values from both methods, the chi-square test on the contingency table of quality classes also showed a significant correlation ($\chi2 = 27.43$, $p < 0.05$). However, differences in quality classes were observed between the two methods (Fig.25). A total of 68 microscopy samples from 19 sites were classified into four quality categories: 9 as poor, 29 as moderate, 28 as good, and 2 as high quality. Similarly, IPS meta values data also fall into four categories, with 8, 32, 19, 26, and 1 samples classified as poor, moderate, good, and high, respectively. The morphological and molecular data yielded the same quality class for 41 samples (60.3%). 26 samples (39.23%) showed a single quality class difference, while 1 sample (1.47%) showed two class differences.

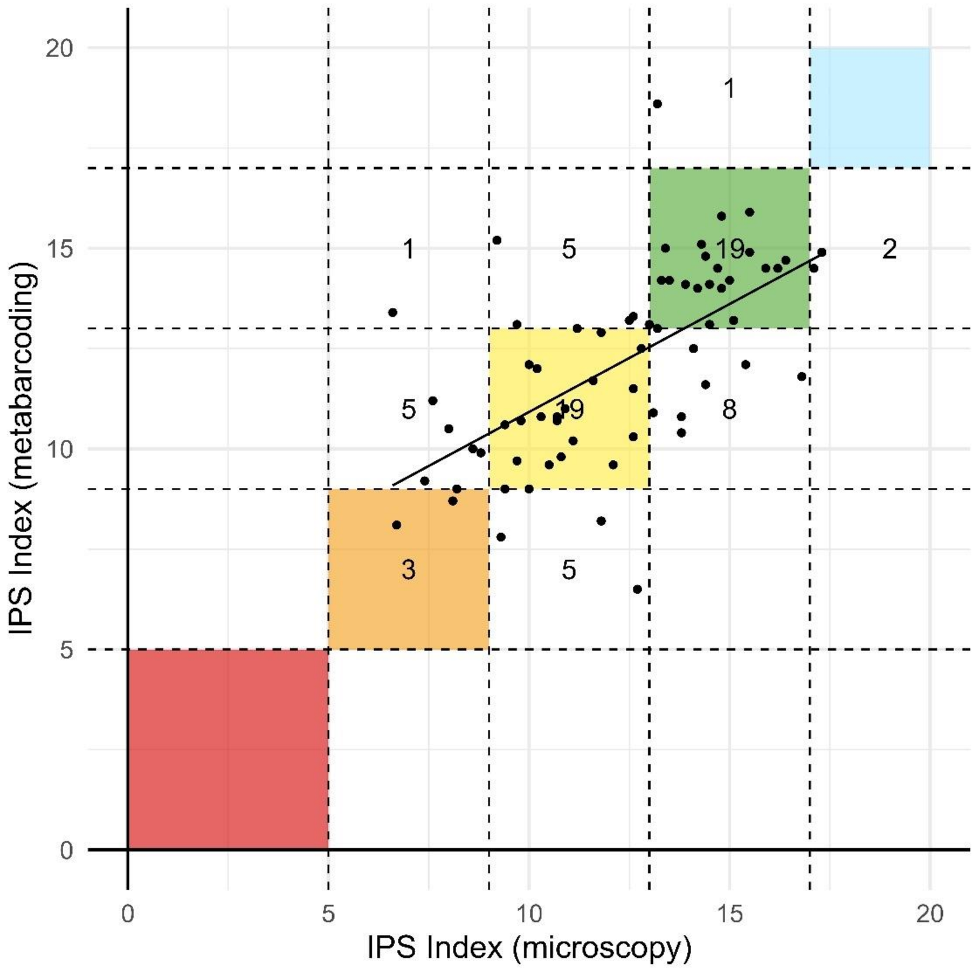

Figure.25. Correlation of IPS values between microscopy and metabarcoding data (Pearson's **r = 0.62**, **p< 0.001**). Colors represent quality classes, while digits denote the number of samples in each class.

IV. Discussion

Arid and semi-arid rivers, most common in North Africa, provide habitats and water for many animals and plants in a hostile desert environment. However, an arid climate, combined with anthropogenic pressures on these rivers, harm water quality, impacting ecosystems and ecological processes that benefit humans (Karaouzas et al.,2018a; Johannsen et al.,2016; Karmaoui et al., 2015). Because the physicochemical properties of water significantly impact aquatic life, assessing these properties is a crucial aspect of managing aquatic ecosystems (Matita et al., 2024; Ali et al., 2025).

Our study showed that water quality in Drâa rivers, assessed using a weighted index (WI) based on physicochemical parameters, was significantly affected by mineralization in the middle and lower Drâa. In contrast, the upper Drâa is more affected by other factors such as phosphorus content. Indeed, the sites with the lowest WI values corresponded to higher levels of conductivity and chloride, particularly in the El Mellah Oued (upper Drâa), middle, and lower Drâa. More specifically, the mean WI value was significantly higher in the upper Drâa, followed by the middle, and then the lower Drâa. This variation was higher during wet periods than during dry ones, but without any significant differences. In general, water quality in the upper Drâa was moderate throughout both periods. The middle Drâa fluctuated between poor during dry periods and moderate during wet conditions. Conversely, poor water quality was observed in the lower Drâa during both periods. A previous study also used physicochemical parameters to assess water quality in the Drâa, revealing that salinity, particularly in terms of conductivity and chloride levels, had a significant impact on water quality. High levels of these parameters corresponded to lower water quality indices in the lower Drâa, with concentrations often exceeding the maximum permitted limits for irrigation and human consumption. Additionally, residents' surveys revealed that the perceived quality of water resources was significantly correlated with their assessments of river water quality, particularly in terms of salinity levels. Most respondents in the lower Drâa, in particular, reported a decline in water quality, describing it as saline (Kaczmarek et al., 2023). In the upper Drâa, 92% of farmers rated the water quality as good for agriculture, whereas only 67% and 35% in the northern and southern middle Drâa, respectively, considered it suitable for farming (Berger et al., 2021). Overall, salinity levels in the Drâa river increased progressively from the upper Drâa to the lower Drâa (Warner et al., 2013; Lazrak et al., 2022), particularly at Tissint Oued, which exhibits very high salt concentrations (over 11 g/L). This is primarily due to geological factors (primary salinization) (Clavero et al., 2017), which are exacerbated by anthropogenic pressures (secondary salinization) and arid climate conditions.

In the Drâa river, higher WI values during dry periods than during wet periods can be attributed to lower dilution and higher evaporation rates, resulting in increased ions and pollution (Sheldon et al., 2010; Mosley, 2015; Schliemann et al., 2021). In addition, in the middle and lower Drâa, irrigation relies mainly on groundwater, as river water is only available during dam releases or rainfall (Berger et al., 2021). This groundwater is generally saline, i.e., it has high ionic concentrations that can affect water quality (Warner et al., 2013; Sheldon et al., 2010; Madlala et al., 2019).

As water quality fluctuates greatly, biological approaches provide a more accurate picture of ecological conditions, making them effective for assessing the ecological status of aquatic ecosystems (Lavoie et al., 2006; Jakovljević et al., 2016; Masouras et al., 2021). Diatoms are the most widely used bioindicators in ecological monitoring (Seckbach and Kociolek, 2011;

Karacaoğlu and Dalkıran, 2017; Pinheiro et al., 2020). However, little research has been conducted on using diatoms to assess the quality of Moroccan lotic ecosystems (Fauzi et al., 2001, 2002; Benhassane et al., 2020). The Specific Pollution Sensitivity Index (IPS) is a diatom-based water quality index that is widely used in intermittent and ephemeral streams, particularly in Mediterranean regions (Lai et al., 2014; Martín et al., 2010; Karaouzas et al., 2018a; Novais et al., 2020; Kiss et al., 2024). In addition, molecular techniques, such as DNA metabarcoding, have recently been designed for diatom-based ecological monitoring, including the IPS index (Kulaš et al., 2022; Pissaridou et al., 2021; Pérez-Burillo et al., 2020).

As with the WI, the mean IPS morpho and meta values in this study revealed that the upper and middle Drâa were higher than those of the lower Drâa. Additionally, their values were generally higher during wet periods than during dry ones. Overall, the upper Drâa was assessed as having good to moderate quality, while the middle and lower Drâa were considered moderate, according to the IPS morpho. Similarly, using metabarcoding analysis, the upper Drâa showed moderate to good quality, the middle Drâa poor to moderate quality, and the lower Drâa moderate quality in both periods. The middle and lower Drâa are undergoing significant human-induced modifications, notably due to the dam construction (ME), which alters river flow and reduces water quantity and quality. Furthermore, changes in land use for agricultural practices, including watermelon cultivation and groundwater extraction, have a profound impact on both water availability and quality (Schulz et al., 2007; Heidecke and Heckelei, 2010; HCP, 2015; Berger et al., 2021; Moumane et al., 2021, 2022). Rising aridity and salinity in the coming decades, coupled with the development of dams (Hssaisoune et al., 2020; Terink et al., 2013; Zarfl et al., 2015), are likely to further degrade water resources in this region. Indeed, in the middle Drâa, due to the severe drought that prevails in this area during the sampling periods, amplified by anthropogenic activities, the three sites in this sub-basin were monitored at different frequencies based on water availability: one site was assessed three times (once during the dry period and twice during the wet one), another once during the wet period, and the third site was investigated twice per period. Therefore, the overall quality results for this sub-basin should be interpreted with caution.

Comparison between WI and IPS using both methods revealed that WI had a positive correlation with the IPS morpho ($r = 0.69$, $p < 0.001$) compared to the IPS meta ($r = 0.38$, $p < 0.01$). However, the IPS morpho showed moderate quality in the lower Drâa, while WI indicated bad quality. This could be explained by the fact that the IPS index is less sensitive to salinity compared to pollution, as sites in the lower Drâa were saline. Conversely, a study of

Cyprus diatom diversity (one of the driest Mediterranean regions) in relation to environmental and anthropogenic influences for ecological assessment, using the IPS index, revealed that a site located near the seashore and subject to wave spray, characterized by the presence of halophilic and euryhaline diatoms, was deemed a bad quality indicator, which was not the case for this site (Pissaridou et al., 2021). Therefore, a high conductivity level is considered a pollution stress factor by IPS (Pérez-Burillo et al., 2020). Another reason could be that the WI provides a complete picture of the current state of the river water by examining its physicochemical characteristics, enabling the identification of pollution incidents in real time. It reacts well to point source pollution (Viso and Blanco, 2023). Conversely, the diatom-based IPS index is considered a good representation of a river's past condition (one month before) rather than its current situation (Taylor et al., 2007; Viso and Blanco, 2023). Therefore, the IPS reflects the global state of water quality in the month prior to diatom sampling. To assess the efficiency of the diatom IPS in representing water quality, the epilithic community should be allowed to develop for at least one month (Taylor et al., 2007; Lavoie et al., 2009; Viso and Blanco, 2023).

IPS values derived from both methods showed a positive correlation ($r = 0.62$, $p < 0.001$). Indeed, the ecological status assessments based on these two approaches were identical for more than half the samples (60.3%). Despite this, significant discrepancies were observed due to variations in community composition and taxon abundance, as reflected by the differences in quality classes between these methods. Previous studies have revealed this significant relationship, albeit with varying correlations among different ecoregions (Bailet et al., 2019; Duleba et al., 2021; Tapolczai et al., 2024). The main reason for discrepancies between the two methods can be attributed to the fact that reference molecular databases are incomplete, as previous studies on barcoding have primarily focused on specific river types and geographical regions (Pawlowski et al., 2018; Pissaridou et al., 2021). This was observed in some sites, particularly in the lower Drâa, which showed high or good quality using the DNA metabarcoding approach due to detecting a lower number of diatom taxa. Both approaches have their limitations, as morphological analysis can often underestimate small diatom species with low sensitivity values, which has a negative influence on IPS scores estimated using DNA metabarcoding. Some valves of these small diatom species (e.g., *Fistulifera saprophila*) are well known to be susceptible to dissolution during laboratory processing, which may explain their neglect in morphological identification (Kelly et al. 2020; Pérez-Burillo et al., 2020; Pissaridou et al., 2021). This could be explained by the fact that the IPS estimated by the molecular approach in this study classified five sites as poor and eight as moderate, whereas they were moderate and good according to the morphological analysis.

Overall, the study of water quality using physicochemical parameters and the ecological status (IPS index) by two methodologies revealed that the upper Drâa was less impacted than the middle and lower Drâa. This was more marked during dry periods than during wet periods. In addition, a significant correlation was observed between the water quality index (WI) and the diatom index (IPS) using a morphological analysis. However, the quality of the water quality index was poorer than that of the diatom index obtained in the lower Drâa. The diatom index IPS derived from both methods was significantly correlated with similar ecological quality classes for more than half of the samples, despite the differences between the two methods.

V. Conclusion

The findings of this study highlight that the water quality index in the lower and middle Drâa was determined by mineralization (conductivity and chloride), while the upper Drâa was influenced by other factors, including phosphorus matter. This latter area experienced less pollution than the middle and lower Drâa, which suffer from anthropogenic pressures. The water quality index (WI) in this study was significantly correlated with the IPS morpho. Indeed, ecological assessments obtained from the IPS morpho showed quality classifications nearly identical to the WI values in the upper Drâa. However, the lower Drâa displayed lower water quality according to the WI values than the IPS diatom index, indicating a strong response to point source pollution. The diatom index IPS reliably indicates a river's past conditions by integrating the river changes over a long period. This confirms the effectiveness of benthic diatoms as valuable bioindicators for Drâa river water quality and ecological status assessments. Using physico-chemical parameters and the IPS diatom index, we can thoroughly assess water quality in the Drâa river, reflecting both current and long-term (nearly one month) conditions.

For the first time, DNA metabarcoding has been compared with morphological approaches for the ecological assessment of river biomonitoring in Morocco. Regarding the diatom IPS indices derived from the two methods, although they produced different taxonomic compositions, the IPS scores revealed a significant positive correlation. These findings provide a better understanding of the differences between water quality assessments using usual chemical analysis and those based on the diatom index (both methods). They can be used to enhance the efficacy of water quality assessment and the ecological status of the Drâa river.

Future studies could examine how water quality based on physicochemical parameters (including uninvestigated variables) with different diatom indices, using both methods, contributes to assessing the Drâa river and other rivers in Morocco. Furthermore, additional studies are required to enhance DNA reference databases for diatom species that are predominantly present and endemic to North Africa, including Morocco. Finally, it would also be important to adapt a diatom index for biomonitoring the ecological status of the Drâa arid river and other similar rivers in Morocco.

Chapter IV. Assessment of the short-term salinity effect on algal biofilm through field transfer in the Drâa river (South-eastern Morocco) using metabarcoding and morphological analyses

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This chapter was published as a paper:

Lazrak, K., Tazart, Z., Nothof, M., Filker, S., Hakkoum, Z., Kaczmarek, N., Berger.E, Mouhri.K; Loudiki, M. (2025). Assessment of the short-term salinity effect on algal biofilm through field transfer in the Drâa river (Southeastern Morocco) using metabarcoding and morphological analyses. Environmental Monitoring and Assessment, 197(4), 1-22. (Q2, IF: 2.9)

I. Introduction

Freshwater salinization (FS) is a global issue that seriously impacts the functioning of aquatic ecosystems, the diversity of organisms, and the benefits supplied by ecosystems to human well-being (Iglesias, 2020; Kaushal et al.,2021; Cunillera-Montcusí et al., 2022). Many rivers and streams have experienced a significant increase in salinity during the last century, particularly permanent and intermittent rivers in dry and semi-arid areas (Cañedo-Argüelles et al., 2013; Kaushal et al., 2021; Berger et al., 2019). This rise in salinity levels is the result of a combination of natural processes known as primary salinization (e.g., rock weathering and seawater intrusion) and human activities defined as secondary salinization (e.g., intensive irrigation, agriculture, and water abstraction), which are aggravated by climate change (e.g., falling rainfall, raising temperatures, and the evaporation process) (IPCC, 2013; Warner et al., 2013; Cañedo-Argüelles et al., 2019; Kaushal et al., 2021). Additionally, water scarcity intensifies the freshwater salinization in arid and semi-arid rivers, amplifying the severity of this issue in these geographical areas (Jeppesen et al., 2020; Cunillera-Montcusí et al., 2022).

The increasing salinization of freshwater ecosystems is affecting their aquatic communities (Cañedo-Argüelles et al., 2017). It changes their composition and structure by reducing species richness, as many species have difficulty surviving beyond a specific salinity threshold (Cañedo-Argüelles et al., 2019; Berger et al., 2019). Within these freshwater communities, microalgae biofilms are primary producers in the food web and, are the first to be affected by FS. They are considered key entry elements of the aquatic ecosystem, and any alteration in their composition and structure has repercussions on the higher trophic levels (Campeau et al., 1994; Cunillera-Montcusí et al., 2022). Benthic microalgae, the main photosynthetic components of biofilms, are rich in species capable of developing in a variety of environmental conditions and have a remarkable ability to react quickly to any changes in their environment (Besemer, 2015; Moreno Osorio et al., 2021). Furthermore, microalgae biofilm communities play an important role in the functioning of aquatic ecosystems, contributing significantly to the self-purification of water through their capability to absorb pollutants and nutrients (Sabater et al., 2007; Wu et al., 2018; Battin et al., 2016). Because of these characteristics, many national and international organizations have suggested that biofilms, and diatoms in particular, are bioindicators of water quality and are used as a suitable method for biomonitoring the ecological status of watercourses (Stevenson et al., 2010).

In rivers, it has been reported that high salinity concentrations negatively affect the composition and structure of benthic microalgae (Vendrell-Puigmitja et al., 2021, 2022; Lazrak et al., 2022, 2024). More specifically, these include a decrease in growth rate, algal cell density, photosynthetic activity, and distortion of diatom frustule (Trobajo et al., 2011, Entrekin et al., 2019; Vendrell-Puigmitja et al., 2021; Noune et al., 2023). At the cellular level, microalgae display a remarkable ability to trigger a range of processes, including morphological modifications, genetic adaptability, changes in physiological exclusion mechanisms, and internal detoxification (Wang et al., 2018; Shetty et al., 2019; Farkas et al., 2023). In addition, at the community level, microalgal biofilms change their composition in favor of species more tolerant of salt stress. This can lead to structural changes by reducing species richness and diversity (Vendrell-Puigmitja et al., 2021, 2022; Lazrak et al., 2022, 2024).

Despite growing scientific concern about freshwater salinization, knowledge of how salinity affects intermittent rivers and ephemeral streams in arid and semi-arid zones, particularly in North Africa, remains limited (Jeppesen et al., 2020; Cunillera-Montcusí et al., 2022). This gap is particularly evident at the least studied trophic levels, notably primary producers represented mainly by benthic algae (Cunillera-Montcusí et al., 2022). Numerous experiments conducted under both natural and laboratory conditions have explored the impacts of long- and short-term environmental changes, including salinization, on benthic algal communities, particularly diatoms (Noune et al., 2023; Vendrell-Puigmitja et al., 2021, 2022; Lazrak et al., 2022, 2024). However, to date, only a limited number of transfer experiments have investigated the effects of alterations in water quality on these communities, focusing on pollution, particularly metallic pollution, while the impact of salinity is still relatively unexplored (Gold et al., 2002; Duong et al., 2012).

In this context, the main objective of this study was to investigate the responses of benthic algal communities to salinity changes by transferring algal biofilms developed on artificial substrates from two less-salty sites (both freshwater) to the saltiest one and *vice versa*. Furthermore, this research aimed to understand how algal biofilms respond to these new conditions, specifically the salinity variations. We postulate that rising salinity can significantly affect benthic microalgae communities by reducing their biomass and diversity, altering their composition, and favoring salt-tolerant species. By better understanding these alterations, it is possible to predict future responses of river ecosystems to changes in salinity, whether anthropogenic or induced by climate change. This is essential for protecting and managing freshwater ecosystems against salinity-related stresses, especially in arid and dryland areas.

II. Material and Methods

II.1. Study area and site selection

The study area is located in the Drâa river basin (southern Morocco), the longest river in Morocco, stretching from the High Atlas Mountains to the Atlantic Ocean in the west and to the Sahara Desert in the south, with a gradual increase in aridity from north to south-east (Carrillo-Rivera et al., 2013, Warner et al., 2013). The Drâa river basin covers several southern high Atlas affluents, including M'Goun, Dades, Oued Iriri, Imini, and El Malleh Oueds, as well as other tributaries originating from anti-Atlas such as Oued Ait Douchen. These confluences define the upper Drâa sub-basin, which flows into the El Mansour Eddahbi dam (ME) (Warner et al., 2013). Downstream, the Drâa river flows southeastwards as the middle Drâa before turning and flowing into the Atlantic Ocean as the lower Drâa. These two sub-basins generally remain dry for most of the year, receiving flows only during flood periods or when the water ME reservoir is released. Due to the extended drought episodes in the region, these hydrological events have become rare or even exceptional (e.g. floods and flooding on 8 and 9 September 2024). This means that most rivers in the Drâa basin are intermittent and ephemeral streams (IRES). In addition, the Drâa river is experiencing a gradual increase in water salinity from upstream to downstream (Warner et al., 2013; Johannsen et al., 2016; Lazrak et al., 2022). This is mainly due to the rivers' natural salinity, which results from the dry climate and geological processes (primary salinization). Furthermore, human activities such as agriculture and intensive irrigation have accentuated water salinization (secondary or anthropogenic salinization), particularly in the middle and lower Drâa (Warner et al., 2013; Moumane et al., 2021).

In this area, three sites with different salinity levels were selected on three perennial tributaries, two of which are located in the upper Drâa and the third in the lower Drâa (Fig. 26). The less salty site (0.2 g/L) is situated in the Iriri Oued (IR); the moderate one (0.6 g/L) is downstream of the Ait Douchen Oued (AR). The choice of this site is due to the sensitivity of microalgae, particularly diatoms, to even low salinity levels (e.g., 0.1 g/L, and 0.4 g/L), affecting their composition, biomass, and diversity (Rovira et al., 2012; Ziemann et al., 2001). The saltiest (7 g/L) is situated in the Tissint Oued (TS), where salinity can exceed 11 g/L (Clavero et al., 2017). This salinity is due to geological causes (primary salinization) amplified by arid climate and human activities (secondary salinization).

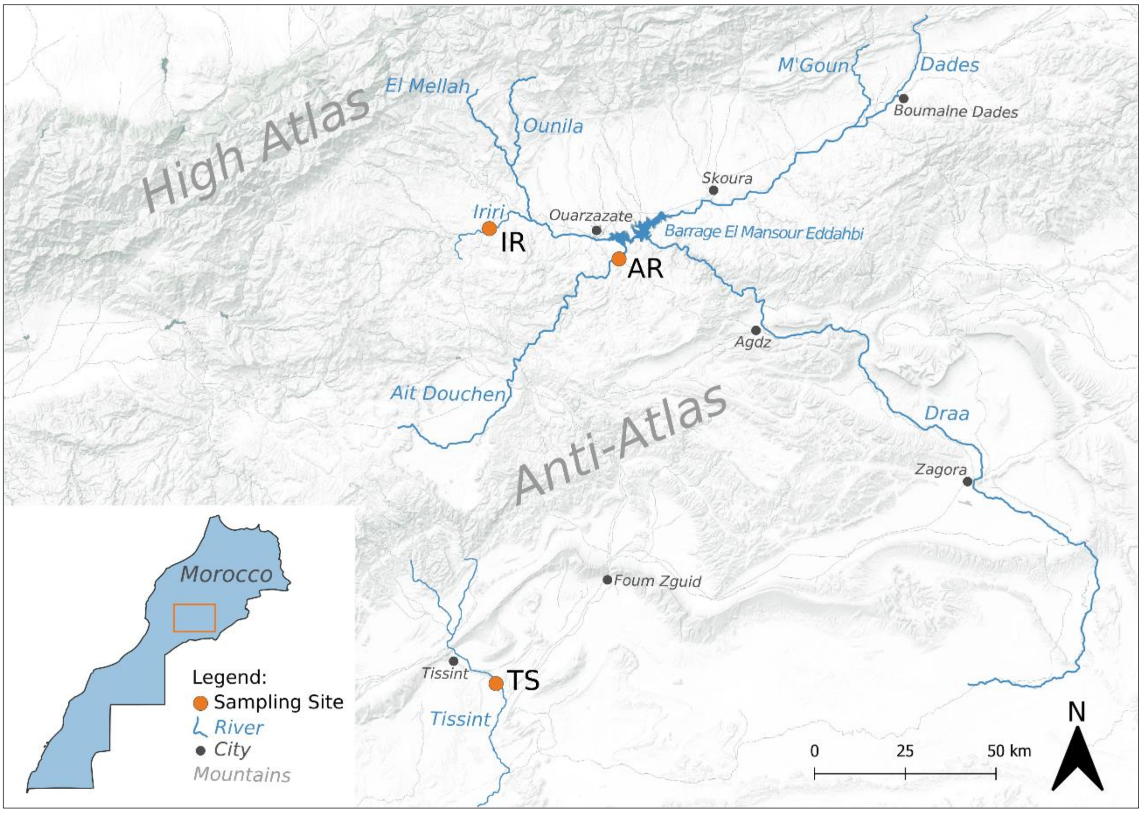

Figure 26. Map of the Drâa river basin showing the three sampling sites. IR: Iriri Oued ; AR: Ait Douchen Oued ; TS : Tissint Oued.

II.2. Water physicochemical characteristics

Salinity (g/L), electrical conductivity (µS/cm), water temperature (°C), pH, and dissolved oxygen (mg/L) were measured in situ twice, separated by four weeks in the watercourses during the experimental period (from 29 May to 24 July 2021), using a Hanna HI98194 multi-parameter device (Hanna Instruments, USA). Water samples were collected concurrently from each site using pre-rinse PVC bottles (1 L) and immediately transported to the laboratory in coolers. Following the analytical methods described by Rodier et al (2009), the water samples were subjected to measurements of several parameters, including ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), orthophosphate (PO_4^{3-}), chloride (Cl^-), sulfate (SO_4^{2-}), total alkalinity (HCO_3^-) and total hardness ($CaCO_3$).

II.3. Experimental design and sampling of biofilm field-transfer

At each study site, 15 plastic bags (26 x 27 cm) containing pieces of tile were used as artificial substrates and placed separately in the center of the riverbed to allow biofilm colonization (Fig. 27). Plastic bags were used due to their multiple advantages such as resistance, uniform surface, preventing the heterogeneity of natural substrates, low selectivity, reproducibility, and ability to serve as a habitat for biofilm communities. Their smooth texture ensures homogeneous algal colonization and facilitates handling, sampling, and recovery of the biofilm. The time required for complete biofilm colonization of the substrate generally ranges from two to four weeks (Hoagland et al., 1982; Tien et al., 2009; Duong et al., 2012). The response time of algal assemblages to changes in water quality generally ranges between one and five weeks (Lavoie et al., 2008). After four weeks (W4), three replicates of the 15 plastic bags colonized by biofilm were collected from each site (biofilm before transfer). Additionally, three colonized plastic bags from the non-saline site (IR) were transferred to the moderate saline site (AR), and three were moved to the saline site (TS). Similarly, three colonized plastic bags from the AR were relocated to the IR, and three were transferred to the TS. For the saline site (TS), three colonized plastic bags were moved to the AR, and three were relocated to the IR. These transferred substrates were incubated for another four weeks (W8) before being collected (biofilm after transfer). All artificial substrates were maintained during transport, immersed in their original river water, and carefully stored in a cooling container. On the other hand, under the laboratory conditions, the three replicates of each biofilm sample were analyzed in terms of biomass (chlorophyll and dry weight) as well as microalgal community analysis (morphological and molecular). For the molecular analysis, DNA was extracted, amplified, and pooled from the three replicates of each biofilm sample to obtain a single sample for analysis.

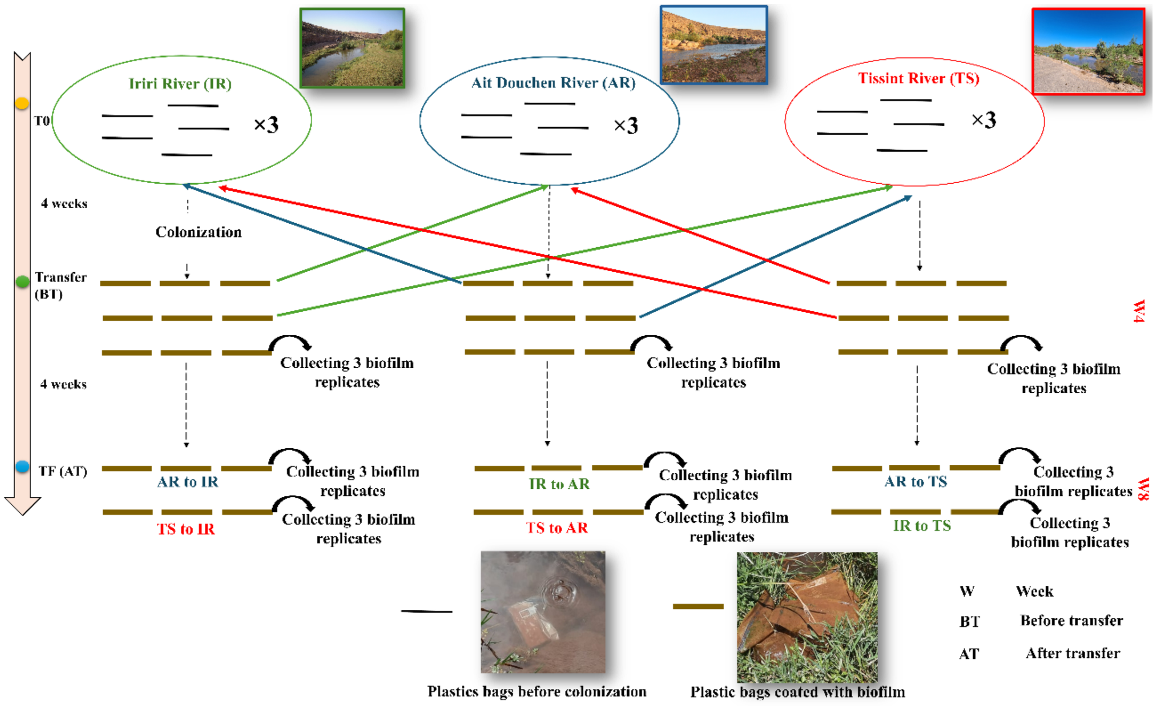

Figure 27. Experimental design of the field transfer experiment.
(T0): initial time before the colonization at the start of the experiment, (TF): final time at the end of the experiment.

II.4. Biofilm algal biomass measurement

II.4.1. Chlorophyll *a*

Chlorophyll-a content was measured following Millerioux's (1975) method. Three samples of algal biofilm, each covering a surface area of 10 cm^2, were extracted with 90% acetone for 24 hours in the dark, as detailed in Chapter II (Section II.4.1).

II.4.2. Dry weight

A 20 cm^2 surface of the biofilm-covered artificial substrate was removed using a razor. Three replicates of biofilm samples were dried and calcined as described in Chapter II (Section II.4.2). Results were expressed in mg $DW.cm^{-2}$ and mg $AFDW.cm^{-2}$.

II.4.3. In Situ microalgal biomass determination

Microalgal biofilm biomass was measured using BenthoTorch®, developed by BBE Moldaenke GmbH (Schwentinental, Germany). For each biofilm sample, three replicate measurements were taken for the biomass of the main photosynthetic groups (cyanobacteria, green algae, and diatoms) within the biofilm communities expressed in µg $chl\text{-}a.cm^{-2}$.

II.5. Microalgal community analysis

II.5.1. Morphological analysis

Diatom sample treatment, preparation of cleaned frustums on a glass slide, observation, identification, and counting of the three microalgae groups (cyanobacteria, green algae, and diatoms) were conducted as outlined in Chapter II (Section II.5.1).

II.5.2. DNA metabarcoding analysis

DNA extraction, polymerase chain reaction (PCR), sequencing, sequence data processing, and taxonomic assignment were carried out as described in Chapter II (Section II.5.2). 14,128 reads were taxonomically assigned to 415 ASVs for cyanobacteria, and 10,830 reads were classified in 277 ASVs for green algae. For diatoms, 46,397 reads were taxonomically assigned to 793 ASVs.

II.6. Statistical analysis

Water physicochemical parameters and algal biomass were investigated in triplicate, and results are presented as mean ± standard deviation (SD). One-way and two-way analyses of variance (ANOVA) were used to evaluate significant differences in biofilm biomass and to identify the factors determining its spatial variability (environmental parameters). The mean values were compared using Tukey's honest significance test at a significance level of $p < 0.05$. The data analyses were performed using R Studio version 4.0.5. Similarly, the 'ggplot2' and 'vegan' packages were used to perform all molecular and morphological data statistical analyses (alpha and beta diversity and taxonomic composition) in R Studio version 4.0.5. Hill numbers with exponents 0, 1, and 2 (richness, Shannon index, and Simpson index) were used to quantify alpha diversity in the sequence data. Only linear model residuals were analyzed if a calculated index showed a linear relationship with the transformed square root of the number of reads per sample, indicating a sequencing depth bias (see Hiiesalu et al. (2014) for more information). Values were centered on -1 to 1 to compare indices. Morphological data was not transformed. For beta diversity, non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity was used to analyze differences in algal composition among biofilm samples. This analysis was applied separately to datasets obtained from both morphological and molecular methods. Environmental factors were fitted to the NMDS plots using the envfit function, enabling the assessment of correlations between environmental variables and algal community composition for each dataset. A 10,000-permutation Monte Carlo analysis was used to determine each variable's fit (R2) to ordination.

III. Results

III.1. Water physicochemical characteristics

During field-transfer experiments corresponding to the dry season, the hydrological regime was globally stable at all three sites, with low velocity and flow. However, this was accompanied by a slight increase in water level during the transfer period (W8), as indicated by the Normal Difference Water Index (NDWI) (Fig.S8, Supplementary material). The pH was slightly increased at the three sites, reaching more alkaline values, and water temperature remained stable between 23 and 28 °C (Table 5). The nitrate, nitrite, ammonium, and phosphorus contents were rather low, suggesting that the water was oligotrophic, unpolluted, and well-oxygenated. Conductivity, salinity, and chloride levels showed similar patterns in all sites during the experimental period. They were significantly lower at the IR site (conductivity less than 600 $\mu S.cm^{-1}$) compared to the AR site (conductivity more than twice as high, 1338 $\mu S.cm^{-1}$) and site TS (conductivity more than 20 times higher, 12280 $\mu S.cm^{-1}$). In addition, high sulfate and total hardness concentrations were measured at the salted site TS.

Table 5. Physicochemical parameters of the three study sites (IR: Irriri Oued, AR: Ait Douchen Oued, and TS: Tissint Oued) during the field-transfer experiments.

Sites	**IR**		**AR**		**TS**	
	Before transfer	**After transfer**	**Before transfer**	**After transfer**	**Before transfer**	**After transfer**
pH	7.7± 0.005^{c}	8.2± 0.1^{b}	8.2± 0.20^{b}	8.6± 0.26 ^{a}	8.21± 0.1^{b}	8.71± 0.11 ^{a}
Temperature (˚C)	28±0.5^{a}	26±0.5^{b}	25±1^{c}	23±0^{d}	25±0.5^{c}	28±0^{a}
Conductivity (µS.cm^{-1})	587.66±6.5^{e}	500±14.04^{f}	1338±2.64^{c}	1255±42.02^{d}	12280±14.01^{a}	11000±38.62^{b}
Salinity (g/L)	0.27±0.02^{d}	0.23±0.07^{d}	0.67±0.02^{c}	0.65±0.07^{c}	7.04±0.07^{a}	6.7±0.08^{b}
O$_2$ (mg/L)	8.8±0.07^{b}	10.78±0.08^{a}	7.6±0.08^{c}	9.65±0.07^{a}	7.5±0.07^{c}	9.2±0.07^{a}
NO$_3^-$ (mg/L)	0.2376±0. 01^{a}	0.0475±0. 007^{b}	0.212±0. 035^{a}	0.022±0. 005^{b}	0.065±0.04^{b}	0.047.5±0. 07^{b}
NO$_2^-$ (mg/L)	0.21±0.01^{a}	0.0406±0.01^{c}	0.0317±0. 01^{cd}	0.02±0. 01^{d}	0.061±0. 008^{b}	0.0372±0. 008^{cd}
NH$_4^+$ (mg/L)	0.8225±0.05^{a}	0.1303±0. 002^{c}	0.4532±0.07^{b}	0.066.3±0. 003^{c}	0.7326±0.12^{a}	0.523±0.03^{b}
PO$_4^{3-}$ (mg/L)	0.01±0. 001^{c}	0.01364±0. 003^{bc}	0.0207±0.01^{b}	0.0064±0. 002^{d}	0.1452±0. 006^{a}	0.1232±0. 003^{a}

Cl$^-$ (mg/L)	191.7±7.1^{e}	142±0^{f}	568±28.4^{c}	390.5±7.1^{d}	8903.4±42.6^{b}	10160.1±8^{a}
SO$_4^-$ (mg/L)	19.35±4.71^{d}	40±2.6^{c}	48.77±10^{c}	81.06±3.05^{b}	346.52±15.3^{a}	338.1±468^{a}
sHCO$_3^-$ (mg/L)	78±8^{f}	142±2^{e}	170±8^{c}	280±0^{b}	164±12^{d}	294±0^{a}
CaCO$_3$ (mg/L)	568±0^{c}	258±0^{f}	360±4^{e}	464±6^{d}	1522±8^{a}	882±4^{b}

Values are expressed as Mean ± ER (n=3). Means with different letters differ significantly according to the Tukey test at $p < 0.05$.

III.2. Biofilm biomass

Before transfer (W4), the algal biofilm of IR and AR sites exhibited significantly higher chlorophyll *a* content (0.40 and 0.58 µg.cm^{-2}, respectively) than those in the saline TS site (0.17 µg.cm^{-2}) (>2-fold) (Table 6). After transfer (W8), when algal biofilm was moved from the IR to TS sites, the chlorophyll *a* dropped significantly to 0.22 µg.cm^{-2} at the AR site and 0.19 µg.cm^{-2} at the TS site. In contrast, the biofilm transfer from AR and TS to IR freshwater site resulted in a significant increase in chlorophyll-*a* level (1.79 µg.cm^{-2} and 0.93 µg.cm^{-2}, respectively). Still, the chlorophyll *a* content decreased significantly in AR biofilm (0.06 µg.cm^{-2}) transferred to the saline TS site while it increased significantly (1.27 µg.cm^{-2}) in the opposite transfer (from TS to AR site).

The biofilm dry weight (DW) showed no significant differences between sites before and after transfer. However, before the transfer, the Ash-free dry weight (AFDW) was significantly higher in IR site (2.836 mg.cm^{-2}) compared to other sites AR (1.874 mg.cm^{-2}) and TS (1.77 mg.cm^{-2}). After transfer, the AFDW was significantly reduced when moving from IR and AR to the TS site, reaching 1 and 0.452 mg.cm^{-2}, respectively. Conversely, it increases significantly upon transfer from the TS saltwater to AR (2.09 mg.cm^{-2}) and IR (2.678 mg.cm^{-2}) freshwater sites.

Before transfer (W4), the biomass of three algal groups (cyanobacteria, green algae, and diatoms) estimated by BenthoTorch® was significantly higher in the IR and AR sites compared to the saline TS site. The cyanobacteria was the dominant group in the three sites with respective values of 1.2±0.37, 1.323±0.9, and 0.326 µg chl-*a*.cm^{-2}. After the transfer (W8), the cyanobacteria biomass in the biofilm moved from IR and AR freshwater sites to the TS saltwater site decreased significantly to 0.055 and 0.12 µg chl-*a*.cm^{-2}, respectively. In contrast, the transferred biofilm from AR or TS to IR site showed a significant increase in biomass, reaching 3.16 µg chl-*a*.cm^{-2} and 1.685 µg chl-*a*.cm^{-2} respectively. The cyanobacteria biomass also increased in the transferred biofilm from TS to AR site (1.223 µg chl-*a*.cm^{-2}). The green algae biomass was also significantly reduced in the moved biofilms from IR and AR to TS site, but no significant effect was observed in the opposite transfer. The diatom biomass followed a similar pattern to that of cyanobacteria, with higher values in IR (0.52 µg chl-*a*.cm^{-2}) and AR (0.413 µg chl-*a*.cm^{-2}) compared to the TS site (0.06 µg chl-*a*.cm^{-2}). The IR and AR biofilms transferred to the TS site resulted in a significant reduction in diatom biomass (0.015 µg chl-*a*.cm^{-2} and 0.065 µg chl-*a*.cm^{-2}, respectively). Conversely, reverse transfer showed a significant increase in diatom biomass.

The Pearson correlation analysis showed a positive correlation between chlorophyll *a* content and ash-free dry weight, as well as cyanobacteria and diatom biomass (Fig. S9, Supplementary material).

Table 6. Biofilm biomass expressed as chlorophyll a (Chl-*a)*, dry weight (DW), ash-free dry weight (AFDW), and algal biomass (cyanobacteria, green algae, and diatoms) in the IR, AR, and TS sites at week 4 (W4) (before transfer), and week 8 (W8) (after transfer). (IR: Iriri Oued, AR: Ait Douchen Oued, and TS: Tissint Oued).

Samples	**Weeks**	**Chl-*a* (µg.cm^{-2})**	**Biofilm DW (mg.cm^{-2})**	**AFDW (mg. cm^{-2})**	**Cyanobacteria (µg chl-*a*.cm^{-2})**	**Green algae (µg chl-*a*.cm^{-2})**	**Diatoms (µg (µg chl-*a*. cm^{-2})**
IR	**W4**	0.40 ± 0.11^{de}	7.56 ± 3.05^{a}	2.836 ± 1.83^{c}	1.2 ± 0.37^{bc}	0.26 ± 0^{a}	0.52 ± 0.28^{ade}
IR to AR	**W8**	0.22 ± 0.01^{ef}	12.578 ± 3.58^{a}	1.874 ± 0.12^{ab}	0.975 ± 0.36^{bcd}	0.01 ± 0^{b}	0.45 ± 0.01^{ad}
IR to TS		0.16 ± 0.02^{f}	12.74 ± 0.32^{a}	1 ± 0.4^{ad}	0.055 ± 0.01^{d}	0.15 ± 0.08^{bc}	0.015 ± 0.05^{c}
AR	**W4**	0.58 ± 0.15^{d}	8.23 ± 2.15^{a}	1.74 ± 1.08^{ab}	1.323 ± 0.9^{b}	0.36 ± 0.06^{a}	0.413 ± 0.23^{a}
AR to IR	**W8**	1.79 ± 0.03^{a}	9.721 ± 2.01^{a}	3.006 ± 0.59^{c}	3.16 ± 2.53^{a}	0.04 ± 0.03^{b}	0.985 ± 0.2^{b}
AR to TS		0.06 ± 0.04^{f}	7.837 ± 0.2^{a}	0.452 ± 0.23^{d}	0.12 ± 0.8^{d}	0.095 ± 0.04^{b}	0.065 ± 0.04^{c}
TS	**W4**	0.17 ± 0.03^{f}	12.607 ± 2.33^{a}	1.77 ± 0.35^{ab}	0.326 ± 0.14^{cd}	0.134 ± 0.09^{bc}	0.06 ± 0.03^{c}
TS to AR	**W8**	1.27 ± 0.02^{b}	13.9 ± 3.84^{a}	2.09 ± 0.29^{c}	1.223 ± 0.21^{b}	0.27 ± 0.13^{ac}	0.726 ± 0.1^{bde}
TS to IR		0.93 ± 0.02^{c}	13.77 ± 1.69^{a}	2.678 ± 0.17^{c}	1.685 ± 0.67^{b}	0.11 ± 0.09^{b}	0.75 ± 0.1^{be}

Values are expressed as Mean ± ER (n=3). Means with different letters differ significantly according to the Tukey test at $p < 0.05$.

1.1. Microalgal diversity

The results of alpha diversity based on the Shannon index using morphological and molecular analyses are illustrated in Figure 28. For morphological analysis, the Shannon index of the cyanobacterial community was higher before transfer in AR (2.25) and IR biofilms (1.66) compared to the TS site (1.33) (Fig. 28A). Biofilm transfer from IR and AR freshwater sites to TS saltwater caused a reduction of the cyanobacteria Shannon index to 1.04 and 1.90, respectively. In contrast, a significant increase was observed in biofilms moved from TS to AR (2.27) and IR (2.18) sites. For green algae, before the transfer, both sites IR (2.68) and AR (2.71) showed a higher Shannon index than the TS site (1.96) (Fig. 28A). This index increased after biofilm transfer from IR and AR to the TS site but decreased in the opposite transfer, falling to 1.72 and 2.18, respectively. Before transfer, the Shannon diversity index for diatoms was generally higher (> 2.5) than cyanobacteria and green algae (Fig. 28A). After transfer, the biofilm moved from freshwater to the saltwater site showed a slight decrease in the Shannon diversity index of diatoms, while the reverse transfer led to a slight increase. The richness and Simpson index showed a roughly similar trend to the Shannon index (Fig. S10, Supplementary material). For molecular analysis, the alpha diversity of cyanobacteria, green algae, and diatoms decreased as the biofilm moved from the IR and AR freshwater sites to the TS salt site, but increased when moving in the opposite way (Fig. 28B and Fig. S11, Supplementary material).

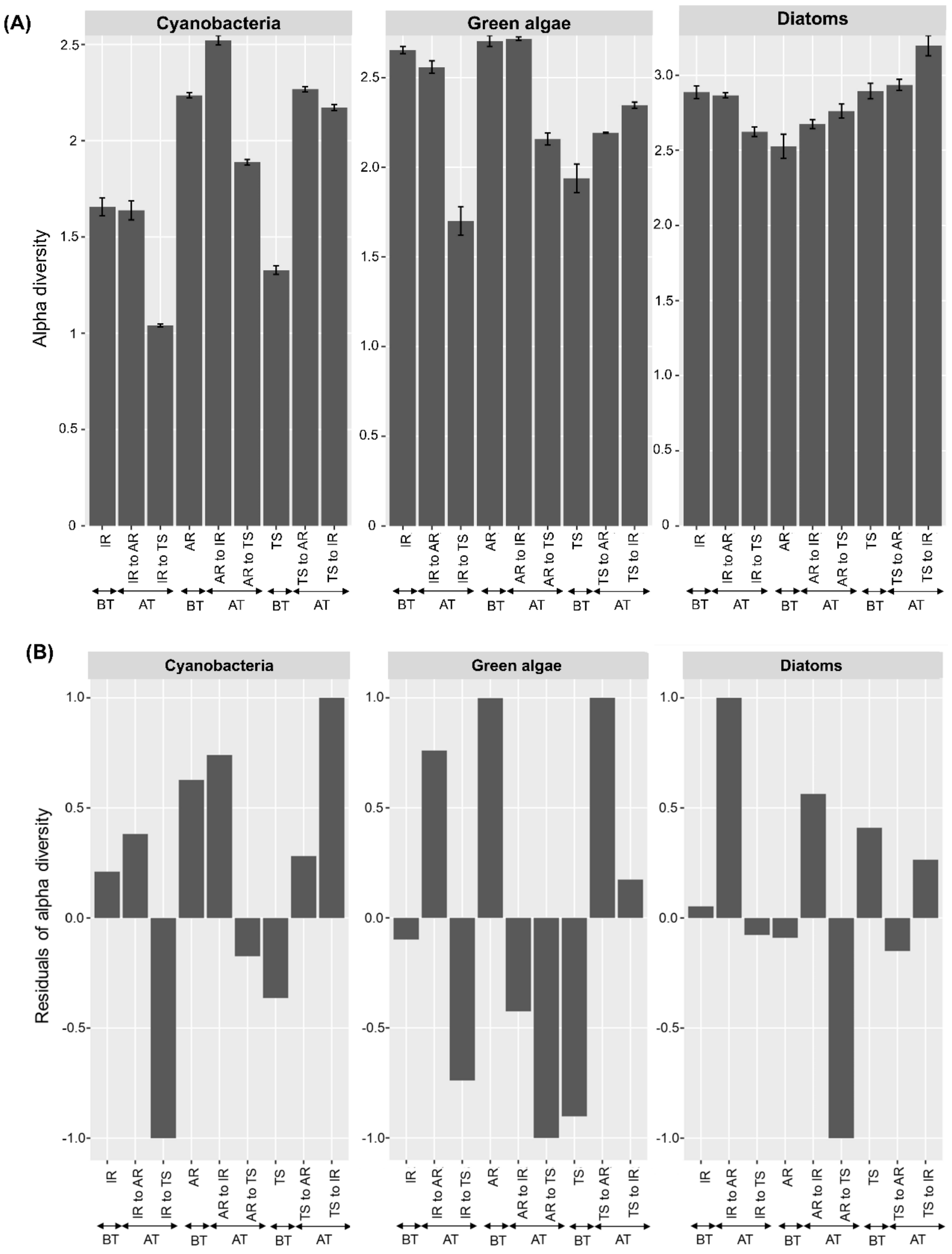

Figure 28. Alpha diversity based on the Shannon index for microalgal biofilm communities (cyanobacteria, green algae, and diatoms) using morphological (A) (mean value, n=3) and DNA metabarcoding methods (B).

Data on richness and Simpson indices are available in the Supplementary material. BT: before transfer, AT: after transfer.

Beta-diversity results using non-metric multi-dimensional scaling (NMDS) of the Bray–Curtis (BC) dissimilarity were compiled in Fig. 29. For both methods, the non-metric multidimensional scaling (NMDS) of the Bray–Curtis (BC) dissimilarity of all biofilm samples revealed that the composition of benthic microalgal communities (cyanobacteria, green algae, and diatoms) showed a clear and almost similar pattern in all the panels of Fig. 29. Before the transfer (W4), the microalgal community composition of the TS salt site was more distinct than that of IR and AR freshwater sites. NMDS revealed a general clustering of biofilm from IR to AR and from AR to IR. However, the biofilm that moved from IR and AR to the TS site formed a cluster with the TS biofilm samples. Conductivity, salinity, chloride, and sulfate showed a significant correlation ($p < 0.01$) with MDS axes based on both morphological and molecular data for all microalgae groups (Tables S21 to S26, Supplementary material). Due to high correlations between these environmental parameters, only salinity was retained in the NMDS analysis plots (Fig. S12, Supplementary material). Using morphological and molecular methods, NMDS showed that biofilm from the TS site and all biofilms transferred to this site (IR to TS and AR to TS) were positioned differently from other samples. Salinity clearly showed the greatest effect on the composition of benthic microalgae, namely, cyanobacteria, green algae, and diatoms.

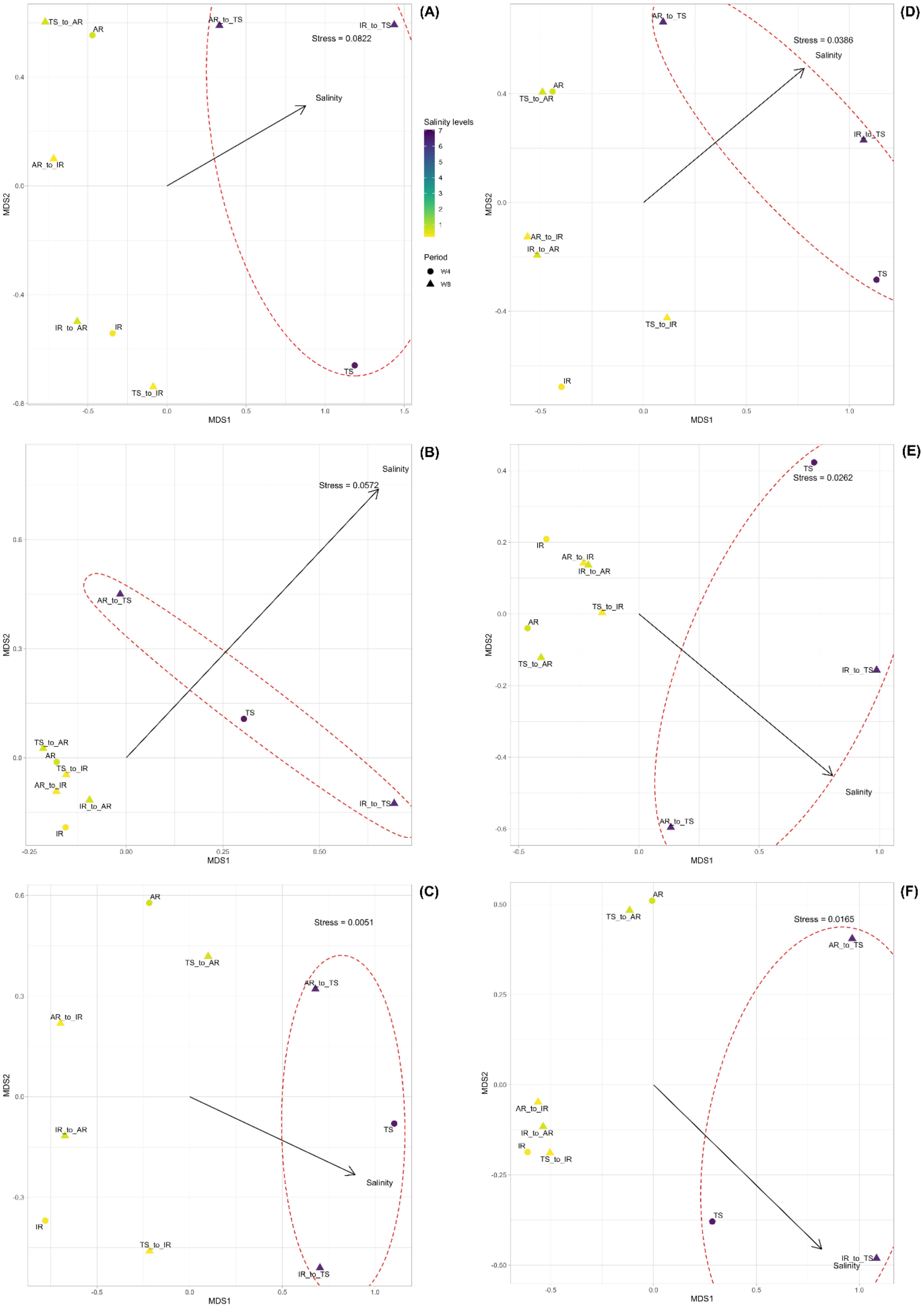

Figure 29. Beta diversity (NMDS based on Bray-Curtis dissimilarity) of microalgal biofilm communities (cyanobacteria, green algae, and diatoms) according to morphological (A, B, and C) (mean value, n=3) and DNA metabarcoding (D, E, and F) methods. Correlation results of environmental parameters with NMDS axes are shown in the Supplementary material (Tables S21 to S26).

III.3. Taxonomic composition

The morphological analysis identified 18 genera and 31 species of cyanobacteria, 20 genera and 29 species of green algae, and 30 genera and 56 species of diatoms (Tables S18, 19, and 20, Supplementary material). *Leptolyngbya* (*Leptolyngbya sp1* and *Leptolyngbya sp2*) was the dominant genus (65.66%) of the cyanobacterial community at IR before the transfer (BT), followed by *Pseudanabaena* (16.33%), including (*P. articulata* and *P. acicularis*) (Figs. 30A and B). The biofilm of the AR site was dominated by *Leptolyngbya sp1* (28.44%), *Aphanocapsa* (13.47%), *Komvophoron* (11.37%), and *Pseudanabaena* (10.77%). After transfer, the biofilm composition of the cyanobacterial community remained practically unchanged. However, the genus *Synechococcus* (*Synechococcus sp.*) showed a significant increase in abundance when the biofilm moved from the IR and AR to the TS site (44.44% and 8%, respectively) while displaying a decrease in the opposite transfer. Meanwhile, the cyanobacterial communities that moved from AR to TS site showed an increase in the relative abundance of *Aphanocapsa* and *Aphanothece* (21.62% and 18.91%, respectively).

For green algae*, Chlorochytrium sp.* dominated all biofilms with a relative abundance higher than 11.5% (Figs. 30C and D). The *Aphanochaete* (*A. elegans* and *A. repens*) and *Oedogonium* were the most dominant genera in IR and AR sites before transfer. These genera remained predominant after transfer between IR and AR sites, or vice versa. In contrast, when biofilms were moved from the IR to the TS site, the *Stigeoclonium* and Chlorella (C. sorokiniana) showed an increase in their relative abundance of 23.76% and 13.86%, respectively, while the *Chaetopeltis* (*C. orbicularis*) disappeared completely. In addition, the relative abundance of the *Stigeoclonium*, *Scenedemus* (*S. abundans*), and *Desmodesmus* genera increased when biofilm was transferred from AR to TS site, while the *Stauridium* and *Chaetopeltis*, represented by *S. tetras* and *C. orbicularis*, disappeared. When the biofilm was transferred from TS to AR and IR sites, the *Chlorella* (*C. sorokiniana*) genus decreased.

The diatoms analysis before transfer showed that each site has a specific community composition compared to other algal groups (Figs 30E and F). At the IR site, *Achnanthidium* (23%, represented only by *A. minutissimum*), *Cymbella* (17%, including 11.2% *C. affinis*), and *Gomphonema* (14.74%, including 3.24% *G.parvulum*) genera dominated the diatom community. *Epithemia* (*E. gibba* 16.28%, *E. sorex* 15.42%) and *Mastogloia* (*M. smithii* 21.71%) were the most abundant genera at AR site. The TS diatom community was dominated by *Halamphora* (*H. coffeaeformis* 8.74%), *Planothidium* (*P. delicatulum* 14.2%), *Tryblionella* (*T. compressa* 8.7%), and *Navicula* (9.56%). When the biofilm of IR was transferred to AR site, the diatom community structure remained unchanged. However, reverse transfer revealed an increase in abundance of *Achnanthidium* (*A. minutissimum*), *Cymbella* (*C. affinis*), *Gomphonema* (*G. parvulum*), and *Cocconeis* (*C. pediculus*) genera, while *Mastogloia* disappeared. On the other hand, the relative abundance of *C. affinis* (3.88%) and *C. pediculus* (2.88%) decreased, and *A. minutissimum*, *G. parvulum*, and *U. ulna* disappeared completely when biofilm was transferred from IR to TS site, Similarly, biofilm transfer from AR to TS site revealed an increase in *Navicula cryptocephala*, a decrease in *Epithemia*, and the absence of A. *minutissimum* and *Ulnaria ulna*. Meanwhile, the transfer of biofilm to AR and IR freshwater sites resulted in a decrease in the dominant taxa at the TS site.

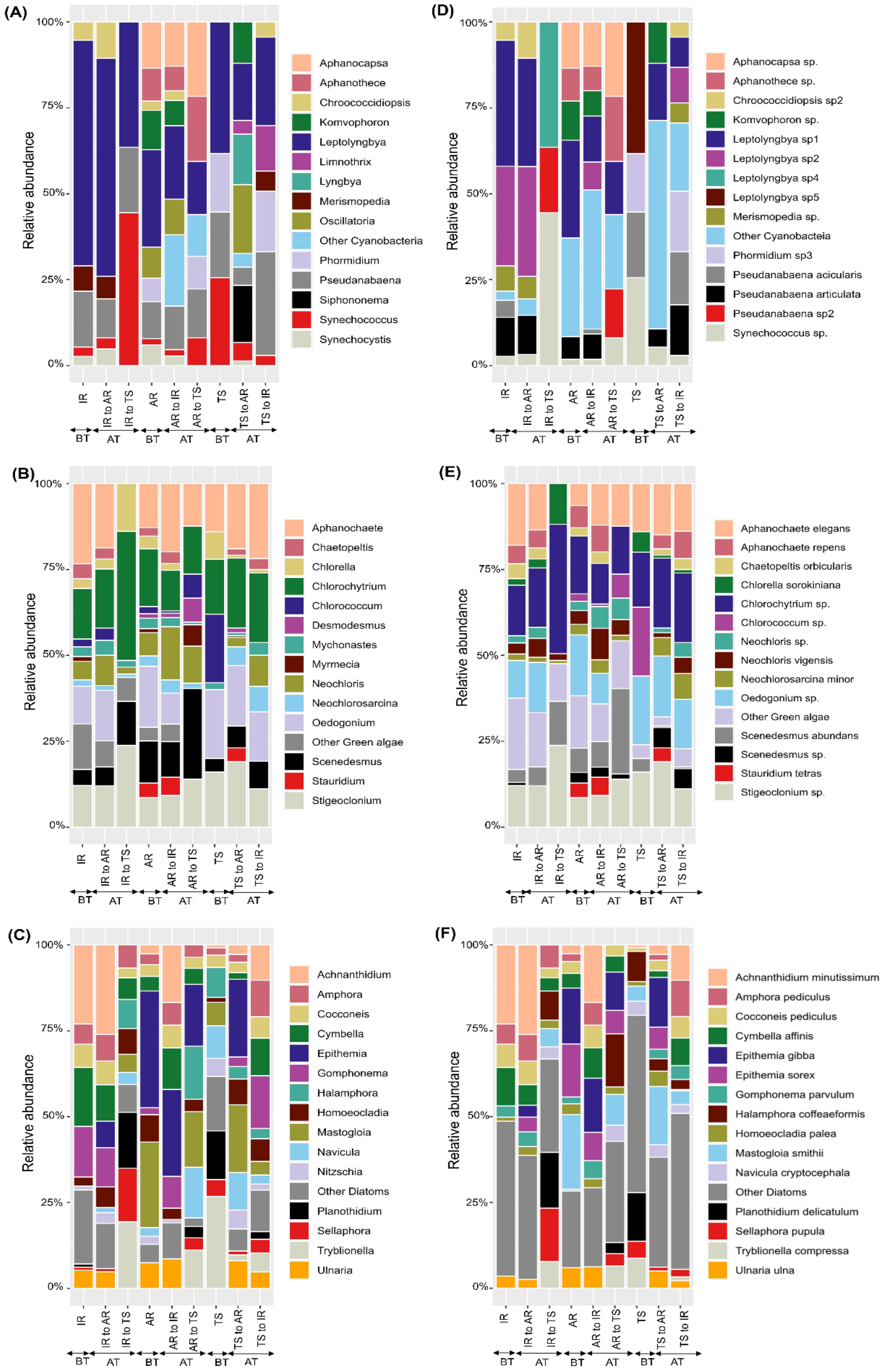

Figure 30. Taxonomic composition (mean value, n= 3) of dominant benthic microalgal communities by genus and species for cyanobacteria (A and D), green algae (B and E), and diatoms (C and F) using morphological method.
BT: before transfer, AT: after transfer.

Based on the relative abundance of cyanobacteria ASVs, *Leptolyngbya* genus had the highest relative abundance in most samples (except those transferred from the IR site to the TS site and those from the TS site) (Fig. 31A). The biofilm transfer from IR and AR to TS site led to an increase in *Synechococcus*, while reverse transfer showed its reduction (from 19% to 6% and 4.2%). The sequence assigned to the *Halomicronema* genus increased in the moved biofilm from AR to IR, but decreased when transferred to the TS site. Conversely, the *Hydrocoleum* genus declined in the transferred biofilm from TS to IR and AR sites.

For green algae, the *Chlorochytrium* genus was dominant in all samples with a relative abundance of over 17% (Fig. 31B). The *Oedogonium* genus decreased (from 6.84% to 5.5% and from 10% to 4%, respectively) in the biofilm moved from AR and IR sites to the TS site while increasing in the opposite transfer. In addition, *Chaetopeltis*, *Fritschiella*, and *Jaoa* genera disappeared when biofilms were moved from the IR to the TS site.

The molecular analysis also revealed significant changes in the composition of diatom communities after the transfer (Fig. 31C). When algal biofilms moved from the IR and/or AR sites to the TS site, *Cymbella*, *Gomphonema*, *Cocconeis*, *Achnanthidium*, and *Epithemia* genera decreased, but they increased when they moved in the opposite direction. Moreover, biofilm communities transferred from IR and/or AR to the TS site showed a slight increase in *Navicula* and *Nitzschia* genera. In contrast, the genus *Sellaphora* decreased in communities transferred from TS to IR and AR sites. Simultaneously, the *Halamphora* genus increased in all biofilm communities transferred to the TS site, with a decrease in the reverse transfer.

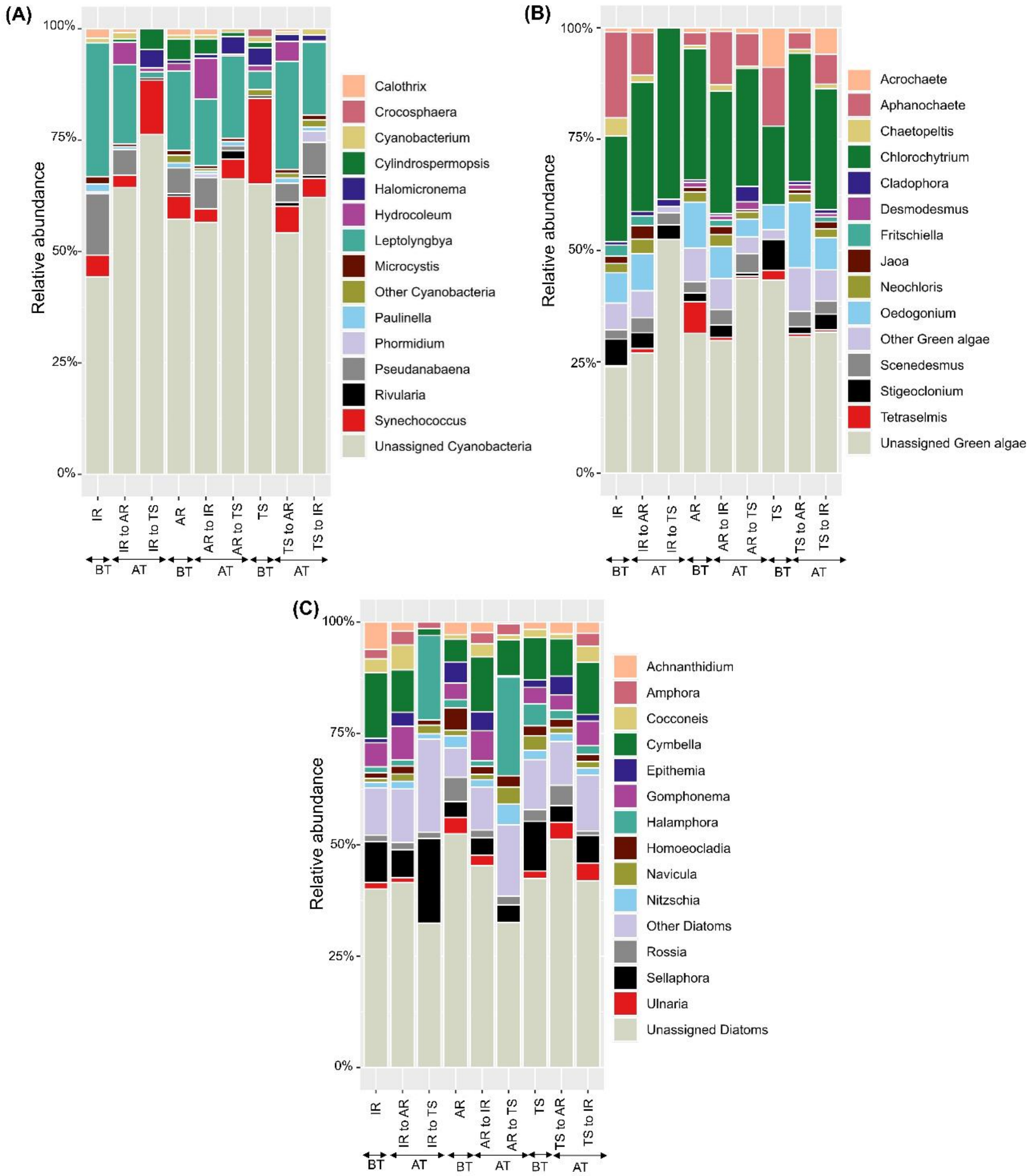

Figure 31. Taxonomic composition of dominant benthic microalgal communities by genus for cyanobacteria (A), green algae (B), and diatoms (C) using DNA metabarcording. BT: before transfer, AT: after transfer.

IV. Discussion

Various stressors, including salinization, have a significant impact on the composition and structure of benthic algal communities in aquatic ecosystems (Vendrell-Puigmitja et al., 2021, 2022; Frost et al., 2023; Noune et al., 2023; Lazrak et al., 2024). Although freshwater salinization has increased over the last century in many rivers (Berger et al., 2019; Cañedo-Argüelles et al., 2019; Kaushal et al., 2021), studies of its effects and ecological impacts are still limited, especially on benthic algae. In this context, our field-transfer experiment aimed to investigate the responses of benthic algal communities to variations in salinity in the Drâa river using plastic bags as artificial substrates. Plastic bags and low-density polyethylene (LDPE) were commonly used in previous experiments (Lazrak et al., 2024; Smith et al., 2021; Vincent et al., 2022) as biofilm colonization substrates due to their facility of handling, low selectivity, reproducibility, and availability. Even though their unrealistic roughness, several studies indicated that plastic supports have not significantly influenced biofilm attachment, development, and composition compared to natural substrates (Coons et al., 2021; Smith et al., 2021; Vincent et al., 2022). Consequently, site characteristics and environmental factors (water chemistry, nutrient availability, and flow conditions) primarily determine the composition of the biofilm community rather than substrate type. The purpose of this study was to transfer algal biofilm communities into sites with varying salinity and to monitor their composition and structural changes using morphological and molecular analyses. Unlike laboratory experiments, field experiments retain the complexity of ecosystems due to the interactions between abiotic and biotic factors (Gold et al., 2002). However, in this study, the three sites were almost identical regarding abiotic conditions, except for differences in salinity.

The field-transfer experiment results showed that the moved benthic microalgae from freshwater to a saltwater site of the Drâa river negatively impacted the biomass, diversity, and composition of the transferred biofilms. Chlorophyll concentrations ranged from 0.17 to 0.58 $\mu g.cm^{-2}$ before transfer, representing relatively low values. Mediterranean rivers, especially in arid and semiarid areas, generally show lower benthic algal biomass (e.g., chlorophyll content) than temperate rivers (Timoner et al., 2014; Dodds et al., 2002; Toskey et al., 2024). Compared to the salty site (TS: Oued Tissint), the freshwater sites (IR: Irriri and AR: Ait Douchen Oueds) exhibited higher chlorophyll-a, ash-free dry weight, cyanobacteria, and diatom biomass. The biofilm biomass significantly decreased when transferred from IR and AR to TS, while it increased in the opposite transfer. This indicated that high saline conditions further exacerbated the low algal biomass by restricting algal growth. This finding aligns with other studies showing that salt conditions ranging from 4 to 10 g/L can reduce algal biomass (Kefford et al., 2011; Rotter et al., 2013). Rovira et al. (2012) found that diatoms react to low Cl^- levels below 0.1 g/L. A previous study (Lazrak et al., 2024) found that exposure to high salt levels (e.g. 10 g/L) significantly reduced the biomass of cyanobacteria, diatoms, and chlorophyll-*a* content in freshwater biofilm. Conversely, no alteration in diatom cell density was observed when different salinity concentrations from ~0.1 to 5.7 g/L NaCl (225 to 10.073 $\mu S.cm^{-1}$ conductivity) were investigated for their effects on freshwater diatom valve shape. However, total chlorophyll-*a* content decreased significantly as salinity increased (Noune et al., 2023). This decrease in pigments can be attributed to oxidative stress, associated with increased chlorophyllase activity and the acceleration of its decomposition (Ji et al., 2018). It may also be due to disrupting ion homeostasis and carbon-concentrating mechanisms (CCMs), resulting in reduced Rubisco activity and insufficient CO_2 absorption (Giordano et al., 2005). Low chlorophyll content reduces light uptake, thus limiting the availability of energy (ATP and NADPH) required by Rubisco to fix CO_2 (Sudhir and Murthy, 2004). Simultaneously, reduced Rubisco activity impairs the Calvin cycle, which in turn further reduces CO_2 uptake and, thus, overall photosynthetic efficiency (Rezayian et al., 2019; Hounslow et al., 2021). In aquatic environments with periodic alternations between fresh and brackish water phases, such as the shallow Mediterranean lake of Vransko (Croatia), higher salt conditions, frequently associated with low algal biomass, favor the dominance of brackish water-adapted taxa (Stanković et al., 2024). Similarly, in Morbihan (France), cyanobacteria biomass decreased with increasing salinity as water changed from freshwater (Pen Mur reservoir) to brackish water (estuary) to marine water (outlet) (Bormans et al., 2019).

In the reverse transfer, an increase in biomass could result from the fact that certain species of benthic microalgae are more favored by a reduction in salinity. On the other hand, a study on benthic diatom communities in brackish systems revealed that reducing salinity led to a drop in biomass but increased the brackish taxa diversity (Virta and Teittinen, 2022). The findings demonstrated reduced green algae biomass as they transitioned from the IR and AR freshwater sites to the TS saltwater site. In contrast, the opposite transfer showed no significant effect. Green algae, essential primary producers in saline habitats, can resist and respond to environmental factors, particularly salt stress. It enables them to adjust their osmolyte concentration, modify their morphology, and develop advantageous mutations that enhance their adaptive capacity (Silva et al., 2000; Costelloe et al., 2005). This was also observed in certain cyanobacteria (e.g., *Microcystis*), which produced specific metabolites (such as toxins and compatible solutes), as well as changes in genotypic composition as salinity levels increased (up to 20) (Reignier et al., 2024).

The algal diversity decreased as the biofilm was transferred from less saline sites, IR and AR, to saltier TS using morphological and molecular methods. Conversely, it increased in the reverse transfer. The microalgal diversity decreases with increasing salinity, and some taxa disappear when they exceed a specific salinity threshold (Cañedo-Argüelles et al., 2019; Lazrak et al., 2022, 2024; Stenger-Kovács et al., 2023). For instance, salinity in the Wupper River (Germany) should not exceed 0.4 g/L chloride to preserve diatom diversity (Ziemann et al., 2001). While Cañedo-Argüelles et al. (2017) suggest that a salinity level of 3 g/L could be a critical threshold for identifying significant impacts of freshwater salinization on biofilm, as substantial reductions in species richness and diversity. Additionally, increasing salinity ~ 0.4, 0.5, 0.65, 0.74, 0.9, and 1g/L NaCl (882, 1092, 1307, 1487, 1734, and 1958 µS.cm^{-1} with NaCl) to assess diatom salinity thresholds for water quality standards in South Australia revealed a decrease in diatom species richness and diversity with significantly altered composition between ~ 0.74 and 0.9 g/L NaCl (1490 and 1730 µS.cm^{-1}) (Frost et al., 2023). Conversely, reducing salinity in the Mediterranean coastal lagoon mainly increased microalgal abundance and decreased their diversity, introducing several rare species (Ligorini et al., 2023). Salt tolerance differs among species, and the exact threshold at which biofilm communities can tolerate salinity levels remains unknown. Some species decline or even disappear with increasing salinity, while others are known to tolerate elevated salt levels (Berger et al., 2019; Cunillera-Montcusí et al., 2022; Vendrell-Puigmitja et al., 2022).

Increasing salinity in freshwater ecosystems changes the composition and structure of biofilm communities (Vendrell-Puigmitja et al., 2021, 2022; Frost et al., 2023; Lazrak et al., 2024). The present study highlights these shifts through morphological and sequencing data results. In most samples, the genus *Leptolyngbya* dominated the cyanobacterial community for both methods. Furthermore, the *Synechococcus* abundance increased in the transferred cyanobacteria communities from freshwater sites to saltwater site but decreased inversely. This genus is commonly found in high-salinity waters (Xia et al., 2017; Lazrak et al., 2024). The green alga *Chlorochytrium* dominated all biofilm samples (more than 11.5% and 17%). When the biofilm was transferred from less salty sites, IR and AR, to the saltiest site, TS, *Chaetopeltis* taxa, particularly *C. orbicularis*, a salt-sensitive species, disappeared (Grimmett and Lebkuecher, 2017). Similarly, the transfer of diatom communities to the saltwater site reduced or eliminated the abundance of certain diatom taxa, notably *Cymbella affinis, Gomphonema parvulum, Cocconeis pediculus*, and *Achnanthidium minutissimum*. These oligohaline species are commonly regarded as indicators of freshwater ecosystems (Stenger-Kovács et al., 2023; Taukulis et al., 2007; Jahn et al., 2009). In contrast, the genera *Navicula* and *Halamphora*, especially represented by *N. cryptocephala* and *H. coffeaeformis*, are halophilic taxa that thrive in salty aquatic environments (Leland et al., 2001; Taukulis and John et al., 2006). Their abundance declined when the biofilm was moved from salty to freshwater conditions. These findings suggest that increasing salinity could be responsible for the reduction or disappearance of oligohaline taxa, replaced by salt-tolerant or halophilic ones. Other studies have also recorded this shift in benthic algal community composition, which increasingly favors species that can tolerate high salt concentrations along the salinity gradient (Vendrell-Puigmitja et al., 2022; Lazrak et al., 2024).

This study's morphological and molecular methods provided nearly identical information, serving as complementary approaches. DNA metabarcoding provided greater taxonomic accuracy for microalgae than the traditional morphological approach (MacKeigan et al., 2022; Kutty et al., 2022; Wang et al., 2024). This is because of the increased precision of taxon sampling due to the higher sequence number recovered compared to the limited number of species accounted for in the morphological method (Stoeck et al., 2009). Consequently, molecular identification significantly increases the detection of rare species (Zimmermann et al., 2015; Wang et al., 2024). Another cause is disguised variety, where morphologically seemingly identical species can exhibit considerable genetic variation ("cryptic species") (Zimmermann et al., 2015; Rimet et al., 2018). However, using DNA metabarcoding instead of the conventional approach poses a significant problem: the absence of specific dominant taxa that are only visible under the microscope. This is mainly due to the lack of completeness of the reference library (Pérez-Burillo et al., 2022; Stuart et al., 2024; Gelis et al., 2024). Therefore, it's more efficient to use both approaches to complement each other (Zimmermann et al., 2015).

Overall, while field experiments capture the complex interactions of environmental factors, isolating the effect of a single parameter, such as salinity, can be challenging. Nonetheless, this field-transfer experiment showed that the ecophysiology of biofilms changes as salinity increases, with a particular drop in chlorophyll concentration. This can naturally decrease primary production and photosynthesis efficiency, which can significantly impact the functioning of freshwater ecosystems. Salinization significantly affects the biomass of the microalgal group's diatoms, green algae, and cyanobacteria. Furthermore, an increase in salt content reduces the diversity of the benthic microalgal community, affecting its species composition. Indeed, some species become dominant, while others decline or disappear, leaving only a limited number of salt-tolerant species. On the other hand, reduced salinity is likely to increase algal biomass and diversity. This could be explained by the preference of some microalgae species for lower salinity conditions in salty environments. In this context, in-depth studies of the impact of several different salinity gradients on transferred benthic microalgae, with post-transfer monitoring, would be crucial. Furthermore, additional research is necessary to comprehend the risks associated with freshwater salinization. These studies should assess the impact of salinization on the physiological and biochemical responses of algae biofilm, which are essential components of the food web, as well as on higher trophic levels. They should also examine the consequences for ecosystem services related to the biofilm-driven processes.

V. Conclusion

The algal biofilm responses to changes in the salinity of the Drâa river were examined by moving initial benthic algal communities, using artificial substrates, from freshwater sites to a saltiest site and vice versa. The field transfer of biofilms from freshwater to a salty site significantly reduced the biomass and diversity of algal communities. In contrast, reverse transfer increased biomass and diversity. Shifts in salinity led to changes in the structure and composition of microalgae; some species were replaced by others better adapted to the new conditions. These results suggest that increased salinity in freshwater ecosystems may harm benthic microalgae (biomass and diversity), while reduced salinity in brackish environments may favor their development. The findings provide a better understanding of the effects of salinity variations on benthic algal communities. They can be used to improve the effectiveness of assessing the risks of increased river salinization and promoting salinity reduction. In addition, more research is needed to study the different salinity gradients on transferred benthic microalgae while closely monitoring their post-transfer evolution. Furthermore, examining the long-term impacts of salt changes on microalgal diversity, ecosystem functioning, and water quality management is essential.

Chapter V. Salt stress responses of microalgae biofilm communities under controlled microcosm conditions

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This chapter was published as a paper:

Lazrak, K., Nothof, M., Tazart, Z., Filker, S., Berger, E., Mouhri, K., & Loudiki, M. (2024). Salt stress responses of microalgae biofilm communities under controlled microcosm conditions. Algal Research, 78, 103430. (Q1, IF: 5.6)

I. Introduction

Biofilms, also known as periphyton, are assemblages of diverse benthic microbial communities such as bacteria, microalgae, fungi, and microfauna, embedded in an extracellular polymeric substance (EPS) (Flemming and Wingender, 2010; Strieth et al., 2018). These compounds are synthesized by microalgae and other microorganisms as a response to physiological pressures occurring in the environment (Romaní et al., 2008). Extracellular polymeric substances (EPS) protect microorganism cells from the external environment and facilitate their adhesion to a surface. EPS contain proteins, polysaccharides, phospholipids, and nucleic acids (Sheng et al., 2010). Microalgae are an important photosynthetic attribute of biofilms, which are rich in species that can be found in a variety of environmental conditions and can respond rapidly to environmental changes (Besemer, 2015; Moreno Osorio et al., 2021). Moreover, algal biofilm communities, as primary producers in river food webs, play a critical role in ecosystem functioning and contribute to pollutant and nutrient uptake that lead to water self-purification (Hall and Meyer, 1998; Power and Dietrich, 2002; Allan et al., 2021; Battin et al., 2016). Additionally, benthic microalgae, especially diatoms, are used as bioindicators of water quality and are suitable for ecological status bioassessment of aquatic ecosystems, as defined by the Water Framework Directive (WFD, 2000).

In freshwater ecosystems, algal biofilm structure, function, and metabolism react to a wide range of environmental changes, including salinization (Ponsatı´ et al., 2016; Pereda et al., 2019; Pu et al., 2019; Martínez-Santos et al., 2021; Vendrell-Puigmitja et al., 2021). Indeed, high salt concentrations or high osmotic pressure may result in a shift in dominant taxa that favor salt-tolerant species in biofilm assemblages (Nuy et al., 2018) as well as a decrease in algal growth, photosynthetic activity, and cell density (Entrekin et al., 2019; Vendrell-Puigmitja et al., 2021). Microalgae under salt stress lead to excessive production of reactive oxygen species (ROS), which are products of altered cellular metabolism produced in the living cell and include highly reactive and unstable oxygen molecules such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), and hydroxyl radicals (^-OH) (Yun et al., 2019; Ma et al., 2020b). However, high ROS accumulation causes ionic disruption, plasmolysis, altered cell water potential, water inflow restriction, and oxidative damage (Almeida et al., 2017; Verslues, 2016). As an antioxidant defense system, microalgae can develop diverse physiological, metabolic, and molecular mechanisms to protect themselves (Wang et al., 2018). These include carbohydrates and lipids accumulation as osmoprotectants, which act as storage molecules to regulate osmotic pressure and maintain algal cells survival (Anand et al., 2019). The cells also accumulate ROS-scavenging enzymes including, catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), and markers of oxidative stress, such as proline, phenolic compounds, and malondialdehyde (MDA) (Pancha et al., 2015; Ismaiel and Piercey-Normore, 2023).

The increasing salinization of freshwater is a concern as it could result in increased stress or mortality of organisms, reduced diversity and/or functionality of freshwater ecosystems, as well as a loss of the services and benefits that they provide to human societies (Cunillera-Montcusí et al., 2022). Over the last century, the salinity of many streams and rivers has dramatically increased, especially in perennial and intermittent dryland rivers (Cañedo-Argüelles et al., 2013; Berger et al., 2019). Increases originate from geologic sources, as they are often naturally saline, and from anthropogenic forms (secondary salinization) related to human land use in the catchment, including artificial flow changes (e.g., water abstraction and dams for irrigation), land use changes in the catchment (e.g., agriculture), domestic and industrial effluents, and are amplified by climate change impacts (e.g., decreased precipitation, increased temperature, evapotranspiration, and climate variability) (Bailey et al., 2006, Cañedo-Argüelles et al., 2013, Kaushal et al.,2021).

Despite their important role in ecosystem functioning as primary producers, the impact of freshwater salinization on benthic microalgal communities is poorly explored compared to aquatic invertebrates (Cunillera-Montcusí et al., 2022). To date, experimental studies on the tolerance of microalgae to salt stress have been more focused on isolated strains (e.g., *Nannochloropsis sp*., *Chlamydomonas reinhardtii*, *Microcystis aeruginosa,* and *Amphora sp.*) (Martínez-Roldán et al., 2014; Fal et al., 2022; Georges des Aulnois et al., 2019; Indrayani et al., 2020) in laboratory batch cultures. Conversely, very few experiments have used whole natural biofilms in microcosm conditions (Vendrell-Puigmitja et al., 2021 and 2022). Therefore, further research is required to investigate the impact of freshwater salinization on algal biofilm communities as the basis of stream food webs and determine its indirect and direct effects on higher trophic levels. In this respect, the present study aims to investigate the physiological and biochemical changes induced by salinity stress in natural algal biofilms sampled in the Drâa River (Southern Morocco) using a controlled microcosm experiment. By understanding the effects of salinity on the composition of biofilm communities, particularly the impact on algal (green alga and diatoms) and cyanobacterial biomass (assessed either by cell count or via Chl-

a content and Benthotorch measurement), we can gain insights into the potential consequences of future river salinization. Our hypothesis is that increasing salinity can significantly affect the composition of the biofilm community, decreasing algal and cyanobacterial biomass. Furthermore, we predict that microalgal cells will undergo physiological and biochemical changes in response to salt stress. By elucidating these responses, we can provide a better understanding of the ecological implications of salinization in freshwater environments and predict changes in their algal biodiversity.

II. Materials and Methods

II.1. Biofilm sampling

The algal biofilm was collected on an artificial substrate (plastic bags) in Oued Iriri, an upstream tributary of the Drâa River, located in the Ouarzazate area (N: 30°93’75.7”, W: 007°21’06.3”; south of Morocco). At the time of sampling, the water physicochemical characteristics were: Conductivity= 587µS, Salinity = 0.2 g/L, pH= 7.7, DO=7.6 mg/L, Turbidity=8.3, Current velocity= 6,58 cm/s, NO_3^- = 0.24 mg/L, NO_2^-= 0.29 mg/L, NH_4^+ = 0.82 mg/L, and PO_4^{3-} = 0.01 mg/L. Six plastic bags (26 x 27 cm) containing tile pieces were deposited in the river bed and incubated in natural conditions to allow algal biofilm colonization. After 4 weeks, the plastic bags coated with biofilm were retrieved from the stream and stored in a portable fridge freezer (G21 6390525- 40L Compressor car Refrigerator, Germany) at 4°C in the dark. 30 L of river water was also sampled, filtered, and stored at 4°C for the laboratory microcosm experiment.

II.2. Experimental setup and design

In the laboratory, the plastic bags colonized by biofilm were cut into small pieces (70 cm^2). Four replicates were immediately incubated in 15 sterile microcosms (2500 ml plastic box) filled with 2000 ml of natural water. After two days of acclimatization of the biofilm to the experimental conditions of the culture chamber, different concentrations of sodium chloride were added successively every two days in each microcosm to obtain three treatments with different final concentrations of sodium chloride, i.e., 1, 10, and 30 g/L, as described in the experimental design presented in Figure 32. Two controls were used: natural water without salt addition (CRW) and natural water enriched with the nutrient “VS medium” (CRW+N) (Gargiulo et al.,2001). The latter was used as a comparison to the salt treatments and the control

(CRW) to ensure that there was no nutrient depletion in the NaCl-treated cultures during the experiment since the water was not renewed. The choice of the three concentrations tested was based on the salinity values found in the Drâa river basin which ranged from 1,5 g/L (at the Upper Drâa) to more than 11 g/L (Lower Drâa, Tissint affluent) (Warner et al., 2013; Clavero et al., 2017). As a result of climate change intensity and human impact, much higher concentrations (up to 30 g/L) could be expected in the future and could occur in some parts of the lower Drâa. Three replicates per treatment were incubated for 14 days under controlled conditions of the culture chamber (temperature 26 ± 2 ◦C, light intensity 63 µmol $m^{-2}.s^{-1}$, light/dark cycle 15 h/9 h). Biofilm samples were collected from each microcosm at two time points: the initial time (T0), marking the start of the experiment before salt addition, and the final time (TF), corresponding to the end of the experiment. These biofilm samples were used for subsequent measurements and analysis.

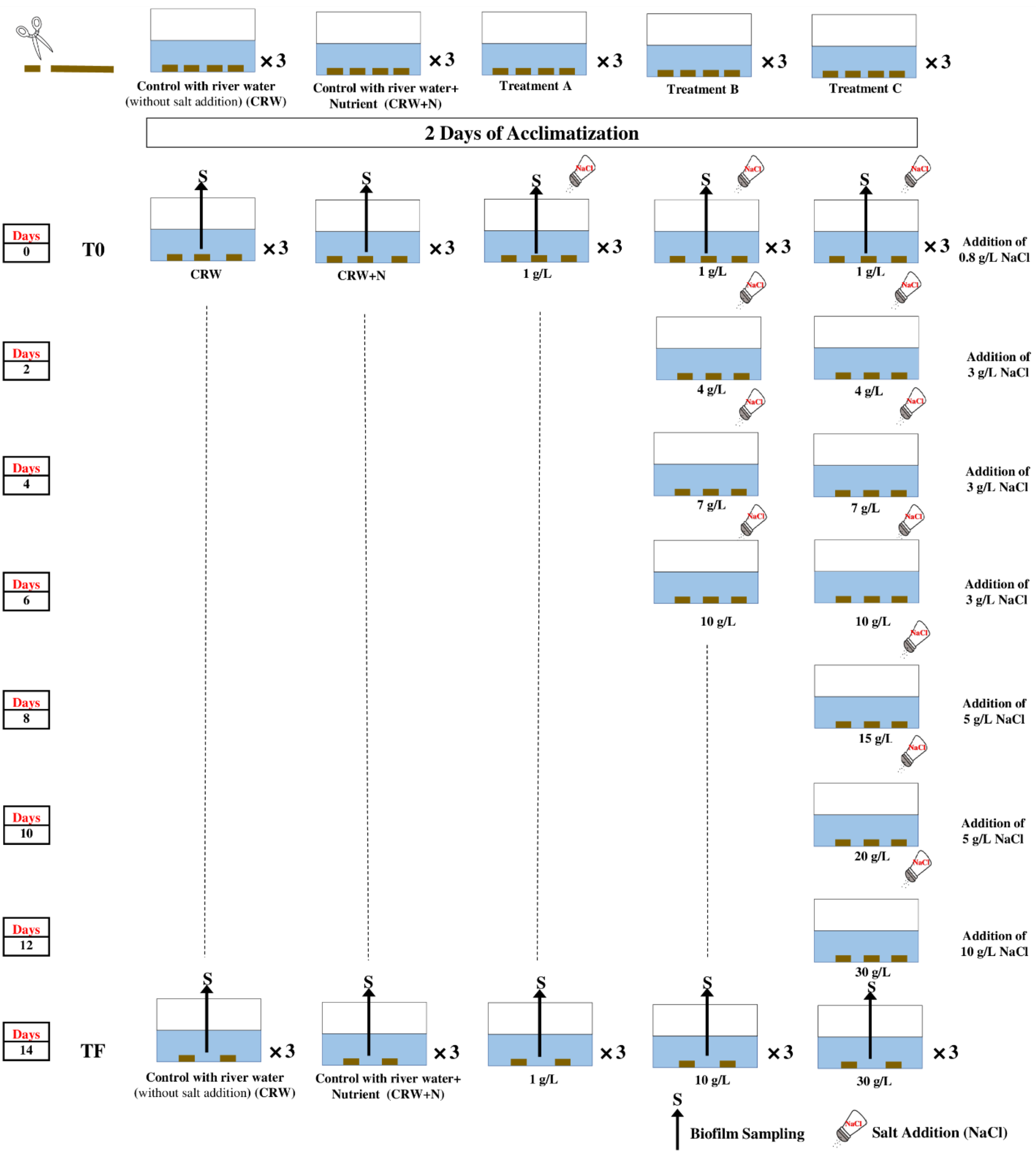

Figure 32. Experimental design, sampling, and salt addition time during the microcosm experiment.
(T0): initial time before the addition of salt at the start of the experiment, (TF): final time at the end of the experiment.

II.3. Biomass and productivity of microalgae biofilm

Biofilm algal species, i.e., green algae, cyanobacteria, and diatoms, were counted in each treatment using a hemocytometer under a light microscope (Motic® BA210, China; 400x magnification). The results were expressed as cell.cm^{-2}. The biomass of these major biofilm algal groups was also measured using a BenthoTorch®, developed by BBE Moldaenke GmbH (Schwentinental, Germany). Three measurements were carried out for each treatment and were expressed as chlorophyll *a* per unit area (µg chl-*a*.cm^{-2}). Based on the biomass results from the BenthoTorch®, biofilm productivity was determined and expressed as biomass produced per unit time (ng chl-*a*.cm^{-2}.d^{-1}).

II.4. Photosynthetic pigments

Carotenoid and chlorophyll-*a* (Chl-*a*) concentrations from 10 cm^2 of a biofilm-covered artificial substrate were determined using spectrophotometric methods according to the protocol of Millerioux (1975). The absorbance measurements were recorded by a UV-vis spectrophotometer (Hach Lange, DR 2800, Loveland, CO, USA) at 750, 665, 662, 645, 630, and 470 nm. The concentrations of the carotenoids and Chl-*a* were calculated according to Jeffrey and Humphrey (1975) and Lichtenthaler and Wellburn (1983).

II.5. DNA Metabarcoding of the biofilm communities

DNA extraction, polymerase chain reaction (PCR), sequencing, sequence data processing, and taxonomic assignment were conducted as previously described in Chapter II (section II.5.2).

II.6. Biochemical analysis of biofilm

II.6.1. Protein quantification

The total soluble protein was calculated using the Bradford method (1976) with bovine serum albumin (BSA) as the reference standard. First, biofilm samples were disrupted by sonication (SONIFER® B-12 Cell Disruptor) for 5 min and homogenized in cold 80% hydrated ethanol, then the homogenate was centrifuged (Eppendorf®, Centrifuge 5804R, Germany) at 4000 g for 25 min at 4°C. The pellet was resuspended in 0.1 M phosphate buffer (pH 6.5) containing 1% (w/v) polyvinylpyrrolidone (PVP). 2 mL of Bradford reagent was added to 100 µL of extract, and the mixture was incubated at room temperature in the dark for 20 min. 100 µL of the phosphate buffer and 2 mL of Bradford reagent were used as a blank. Absorbance was read at 595 nm, and results were expressed in mg of protein.cm^{-2} of biofilm.

II.6.2. Soluble carbohydrates determination

The total soluble sugars were calculated using the Kochert method (1978). 10 mL of 70% ethanol was added to the biofilm sample to extract carbohydrates. 1 mL of the extract was diluted to 2 mL with deionized water and thoroughly mixed with 1 mL of phenol solution (5%). The absorbance of the sample was measured at 485 nm after adding 5 mL of concentrated sulfuric acid (80%). The sugar concentration was calculated by comparing it to the standard curve, and the results were expressed as mg carbohydrates.cm^{-2} of biofilm.

II.6.3. Phenolic compounds

The total polyphenols in the biofilm were analyzed using the Folin-Ciocalteu method, as demonstrated by Li et al. (2008). The biofilm samples were homogenized in 5 mL of 80% ethanol and centrifuged for 10 min at 10000 g. 1 mL of Folin-Ciocalteu Ciocalteu and 5 mL of distilled water were added to the supernatant and mixed before incubating at room temperature in the dark for 5 min. Following that, 2 mL of sodium carbonate (20% w/v) was added and incubated at room temperature for 1 hour in the dark. The solution's absorbance was measured against a blank at 650 nm. The total phenolic content of biofilm samples was expressed in mg eq. of gallic acid equivalent (GAE) using gallic acid as a standard.

II.7. Oxidative and antioxidant activities measurement of biofilm

The biofilm samples were homogenized by sonication for 5 min in 2 mL of buffer containing 50 mM potassium phosphate buffer (pH 7.0), 2 mM EDTA, 5 mM β-mercaptoethanol, 1% polyvinyl pyrrolidone, 1% glycerol, 1 mM polymethyl sulfonyl fluoride (PMSF), and 50 mM NaCl, followed by centrifugation at 12000 g for 10 min at 4 °C. The extracts were used for enzyme activity assays.

II.7.1. Stress biomarkers determination: H_2O_2, MDA, and proline content

To determine H_2O_2 content, biofilm samples were mixed with a 0.1% w/v TCA solution. The homogenate was centrifuged at 11200 g for 10 min. 0.5 mL supernatant was mixed with 0.5 mL 10 mM phosphate buffer (pH 7.0) and 1 mL of 1M KI. Absorbance was measured at 390 nm. H_2O_2 content was expressed as µM H_2O_2. cm^{-2} of biofilm (Velikova et al., 2000).

To measure lipid peroxidation, the biofilm was homogenized in 2 mL of 80:20 (v/v) ethanol and water. After being heated at 95 °C for 25 min, 1 mL of supernatant was combined with 1 mL of 0.65% TBA made in a 20% TCA solution. After the mixture cooled to room temperature, it was centrifuged at 11000 g for 10 min. The absorbances of the supernatants were measured at 450, 532, and 600 nm. The MDA content (µM. cm^{-2} of biofilm) was calculated according to Chokshi et al. (2017a).

The proline content of a biofilm surface was measured using a method described by Bates et al. (1973). 2 mL of DMSO was used to permeabilize the biofilm cells for 1 hour at 40 ºC. Following a 30-second vortexing of the cell extracts, 2 mL of 3% sulfosalicylic acid was added to each tube. After 10 min, the extracts were centrifuged at 4000 g for 20 min to separate them from the cell debris. After being pipetted into 2 mL, the proline-containing supernatant was treated with acid ninhydrin at 90°C for one hour. The colored complex was then extracted into toluene after the reaction was stopped in an ice bath. At 520 nm, the absorbance was measured. Proline was dissolved in 3% sulfosalicylic acid to cover the concentration range of 0.1-20 g/ mL in order to create the proline standard curve.

Each analysis was carried out in triplicates.

II.7.2. Scavenging enzymes assays: SOD, CAT and POD activities

Superoxidase dismutase (SOD) activity was measured according to the method of Beauchamp and Fridovich, (1971) at 560 nm. One unit of SOD per mg of soluble protein was identified as the amount that causes 50 % inhibition of the photochemical reduction of NBT.

Catalase activity (CAT) was determined by measuring the absorption of H_2O_2 decrease at 240 nm. One unit of CAT (U) activity was defined as the number of enzymes that use up 1 mM of H_2O_2 per minute per mg of soluble protein (Rao et al., 1996).

Peroxidase (POD) activity measurement according to Zhou et al. (2016). In the reaction system, 1 mL 0.3% H2O2, 0.95 mL 0.2% guaiacol, 1 mL PBS (pH 7.0), and 0.05 mL enzyme solution were added. The increase rate of absorbance at 470 nm was measured, and an increase of 0.01 per minute was defined as a unit of energy.

All antioxidant enzyme activities were expressed in U. cm^{-2} of the biofilm.

II.8. Nitrate reductase activity

The Hageman and Hucklesby method (1971) was used to measure nitrate reductase activity. With complete vortexing, frozen biofilm material (-80 °C) was added to 1 mL of digest buffer (100 mM Tris/HCl, pH 8.0, 1 mM EDTA, 10 mM cysteine), which was then centrifuged at 4 °C for 10 min at 20,000 g. The supernatant was pipetted into a fresh reaction vessel to keep it cold. Two reaction tubes were made for each sample using the following methodology: 600 µL of reaction buffer (100 mM KNO_3, 1 mM $NADH^+ H^+$, 1.08 mM K_2HPO_4, 1.47 mM Na_2HPO_4) and 400 µL of protein extract were combined (batch reaction). To determine the initial NO_2^- concentration in the biofilm samples, 200 µL of the batch cap (dehydrated zinc acetate; 1 M Zn $(CH3COO)_2.2H2O$) was immediately added to one of the tubes (the reference tube). After that, this reference tube was incubated for 30 min on ice. Before adding the batch cap (zinc acetate, 200 µL) to stop the reaction, the second tube containing the proprietary reaction buffer was also incubated for 30 min at 30°C. After that, the reaction mixtures were centrifuged at 20000 g for 2 min at 20 °C. Aliquots of 500 µL of supernatant from each of these two tubes were pipetted into empty tubes, following the addition of 500 µL of sulfanilamide (25 HCl; 10 mg. L^{-1}) and 500 µL of naphthyl reagent (N-(1-naphthyl) ethylene diamine dihydrochloride; 0.2 mg. L^{-1}) and incubated for 15 min at room temperature for color development. Finally, absorbance was measured at 540 nm. The biofilm produced µM NO_2^- cm^{-2}. h^{-1}, which was expressed as nitrate reductase activity.

II.9. Statistical analysis

GraphPad Prism V. 9 was used for statistical analysis of the biofilm biomass, biochemical parameters, physiological feedback, antioxidant activities, non-enzymatic salt stress markers, osmoprotection indicators, and nitrogen assimilation results. All experiments were performed in triplicate, and the mean values ± SD were used in the calculations.

All statistical analyses of the sequence data, i.e., the ASV tables, were conducted in R studio version 4.0.5 using the packages ‘vegan’ and ‘ggolot2’. For alpha diversity, indices in the form of Hills`s numbers with exponent 0, 1, and 2 (i.e., richness, Shannon index, and inverse Simpson index) were calculated. In case a calculated index revealed a linear correlation with the square root transformed read counts per sample, pointing to a sequencing depth bias, only the residuals of the linear model were analyzed (see Hiiesalu et al., 2014 for more information). Values were centered-scaled on a -1 to 1 range to allow for comparability between the indices. Beta diversity analyses were based on Bray-Curtis dissimilarity indices of Hellinger-transformed ASV matrices. An Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis was used to cluster the samples and visualize shifts in the community structures.

Normalization of all data, with the exception of sequence data, was carried out using Excel 2019 software to ensure comparability and enable cluster analysis and heatmap visualization. The normalization methods, including Z-score normalization, were applied to transform the variables measured on different scales into comparable values. This preprocessing step is crucial for clustering and heatmap visualization based on distance measures. The selected normalization method ensured that all variables were on a common scale, allowing for meaningful comparisons and reducing the bias introduced by varying measurement units. After data normalization, clustering analysis and heatmap visualization were conducted using RStudio software (version 4.2.1). Hierarchical cluster analysis (HCA) helped to identify patterns and groupings within the dataset, while the heatmap visualization provided a visual representation of the relationships between variables and salinity levels.

III. Results

III.1. The effect of salinity on microalgae biofilm biomass, productivity, and photosynthetic pigments.

The results of the salinity effect on microalgal biofilm biomass are presented in Figure 33. Cyanobacteria were the dominant algal group in all treatments at T0 (Figure 33A). The abundance of this prokaryote group was highest in the treatment of 1 g/L NaCl with 376.64 x 10^5 cells.cm^{-2} compared to the control treatments (CRW) and (CRW+N) at the end of the experiment (TF), whereas it was significantly reduced ($p < 0.05$) to 52.95 x 10^5 cells.cm^{-2} and 55.52 x 10^5 cells.cm^2 in biofilms exposed to the highest salinity concentrations of 10 and 30 g/L, respectively. Similarly, diatoms were significantly more abundant in the biofilm treated with 1 g/L NaCl (36.57 x 10^5 cells.cm^{-2}) than in the controls microcosms (CRW; 16.53 x 10^5 cells.cm^{-2}) and (CRW+N; 20 x 10^5 cells.cm^{-2}), while they were significantly decreased in the treatments with high salt concentrations of 10 and 30 g/L with 12.19 x 10^5 and 8.21 x 10^5 cells.cm^{-2}, respectively (Figure 33 C). In contrast, the salt treatments did not clearly affect the green algae (Figure 33B).

Simultaneously, benthic algal biomass and community composition results, as measured via BenthoTorch (BT), revealed that the biofilm treated with 1 g/L NaCl contained significantly more cyanobacteria and diatoms ($p < 0.05$) than the controls (CRW) and (CRW+N). On the other hand, microcosms treated with 10 g/L and 30 g/L NaCl showed a significant decrease in cyanobacteria and diatoms ($p<0.05$) (Figures 33D and F). However, green algae were less affected by changes in salinity (Fig. 33E). In comparison with the results of microscope counts, BenthoTorch measurements were roughly similar in this study.

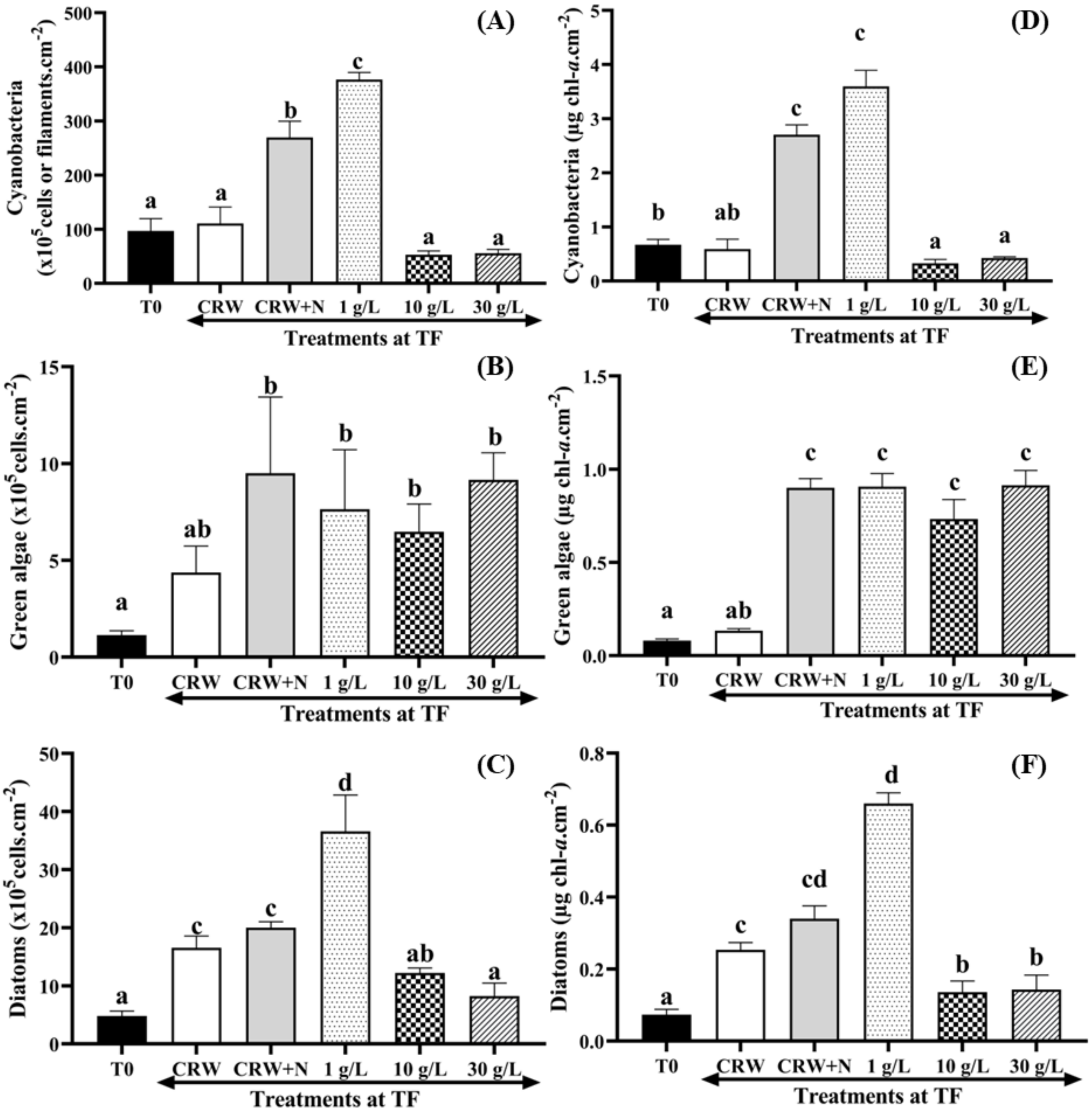

Figure 33. Salinity effects on the biomass of microalgal biofilm communities (cyanobacteria, green algae, and diatoms) exposed to different concentrations of NaCl using a hematocytometer (A, B, and C) and BenthoTorch® (D, E, and F).

All values are means and standard errors. According to the one-way ANOVA test, the ANOVA is significant at the level ($p \leq 0.05$). Same superscripts indicate no significant difference between them ($p > 0.05$).

Cyanobacterial productivity varied significantly, from 3.10 ng chl-*a*.cm^{-2}.d^{-1} in the control cultures (CRW) to 145.24 ng chl-*a*.cm^{-2}.d^{-1} in the CRW+N treatment (Figure 34A). The exposure of biofilm to 1 g/L NaCl showed the highest productivity, with 209.05 ng chl-*a*.cm^{-2}.d^{-1}. However, higher salinity levels (10 and 30 g/L NaCl) resulted in a complete absence of detectable cyanobacterial productivity. Green algae productivity was 3.81 ng chl-*a*.cm^{-2}.d^{-1} in the CRW treatment and increased significantly to 59.05 ng chl-*a*.cm^{-2}.d^{-1} in the 1 g/L NaCl treatment, 46.67 and 59.52 ng chl-*a*.cm^{-2}.d^{-1} in the 10 and 30 g/L NaCl treatments, respectively (Figure 34B). Diatom productivity increased from 12.86 ng chl-*a*.cm^{-2}.d^{-1} under normal conditions (CRW treatment) to 41.90 ng chl-*a*.cm^{-2}.d^{-1} in the biofilm exposed to 1 g/L NaCl. In contrast, their productivity was significantly reduced with increasing salt concentrations, falling to 4.48 ng chl-*a*.cm^{-2}.d^{-1} in treatments with 10 and 30 g/L NaCl, respectively (Figure 34C).

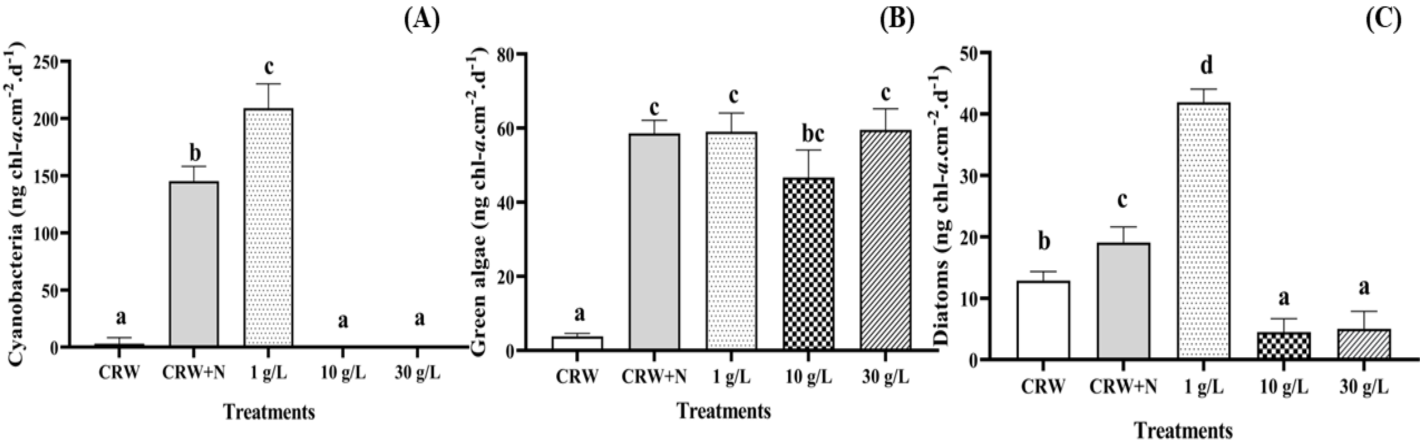

Figure 34. Salinity effects on the productivity of microalgal biofilm communities (A = cyanobacteria, B = green algae, and C = diatoms).
All values are means and standard errors. According to the one-way ANOVA test, the ANOVA is significant at the level ($p \leq 0.05$). Same superscripts indicate no significant difference between them ($p > 0.05$).

Chlorophyll *a* was around 1.32 µg. cm^{-2} in the biofilm at T0 and increased slightly and significantly in the controls (CRW and CRW+N) as well as in the 1 g/L NaCl treatment, with the highest value reaching 2.43 µg. cm^{-2} (Figure 35A). In contrast, it decreased significantly in the 10 and 30 g/L NaCl-treated biofilms with 1.2 and 0.38 µg. cm^{-2}, respectively. Carotenoids followed almost the same pattern as chlorophyll *a,* increasing significantly from 0.12 µg. cm^{-2} in the biofilm at T0 to around 0.33 ~0.35 µg. cm^{-2} in the controls (CRW and CRW+N), with no significant difference between them (Figure 35B). In addition, biofilm treated with 1 g/L NaCl showed a marked increase in carotenoids of up to 0.85 µg. cm^{-2}. On the other hand, carotenoids decreased to 0.53 µg. cm^{-2} in biofilm treated with 10 g/L NaCl, but still higher than in controls, then decreased to 0.21 µg.cm^{-2} in biofilms exposed to 30 g/L NaCl.

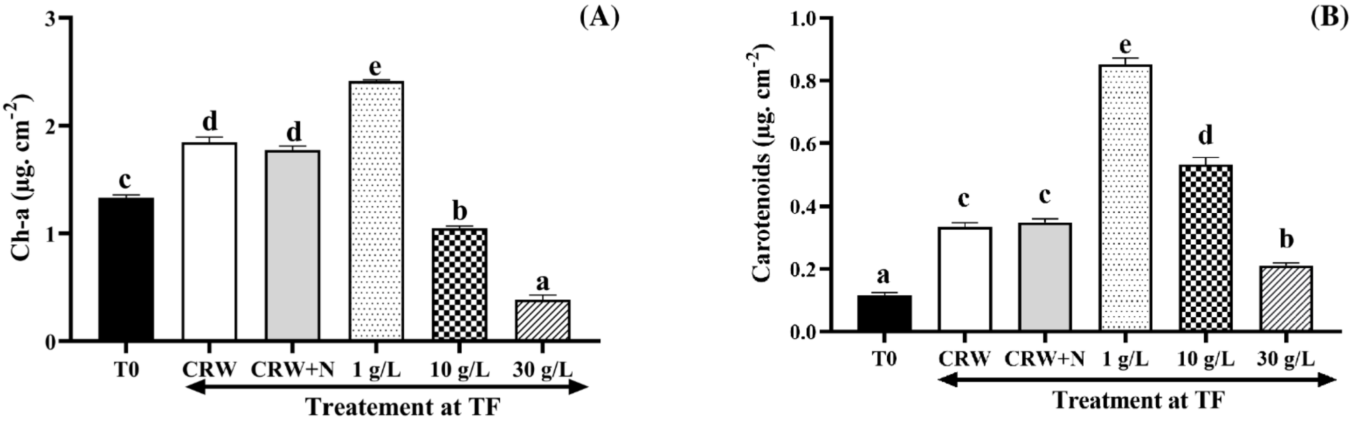

Figure 35. Salinity effects on photosynthetic pigments chlorophyll *a* (A), and carotenoids (B) of the biofilm exposed to different concentrations of NaCl.
All values are means and standard errors. According to the one-way ANOVA test, the ANOVA is significant at the level ($p \leq 0.05$). Same superscripts indicate no significant difference between them ($p > 0.05$).

III.2. Changes in biofilm community structure and composition under increasing salinity.

Shannon-based alpha diversity of the total microeukaryote community of the biofilm appeared to be highest at sampling point T0 (Figure 36A). At the end of the experiment, alpha diversity was lower in both controls than in sample T0. In the salt-treated biofilms, the diversity of microeukaryotes decreased with increasing NaCl concentrations. Green algae alpha diversity was low at timepoint T0, in the control samples, and at a NaCl concentration of 1 g/L but peaked at a NaCl concentration of 10 g/L. Diatom alpha diversity increased from timepoint T0 to the CRW control samples but started to decrease with increasing salinity concentrations. At T0, the total prokaryote alpha diversity was highest and showed low values in the control samples (Figure 36B). The alpha diversity of the communities treated with 1 g/L and 10 g/L NaCl was similarly low and even lower in communities treated with 30 g/L NaCl. Cyanobacteria alpha diversity increased in comparison between the T0 community and the control communities.

Relative to the diversity of the T0 community, diversity was increased in the samples treated with 1 g/L and 10 g/L NaCl, however, as salinity increased, diversity decreased.

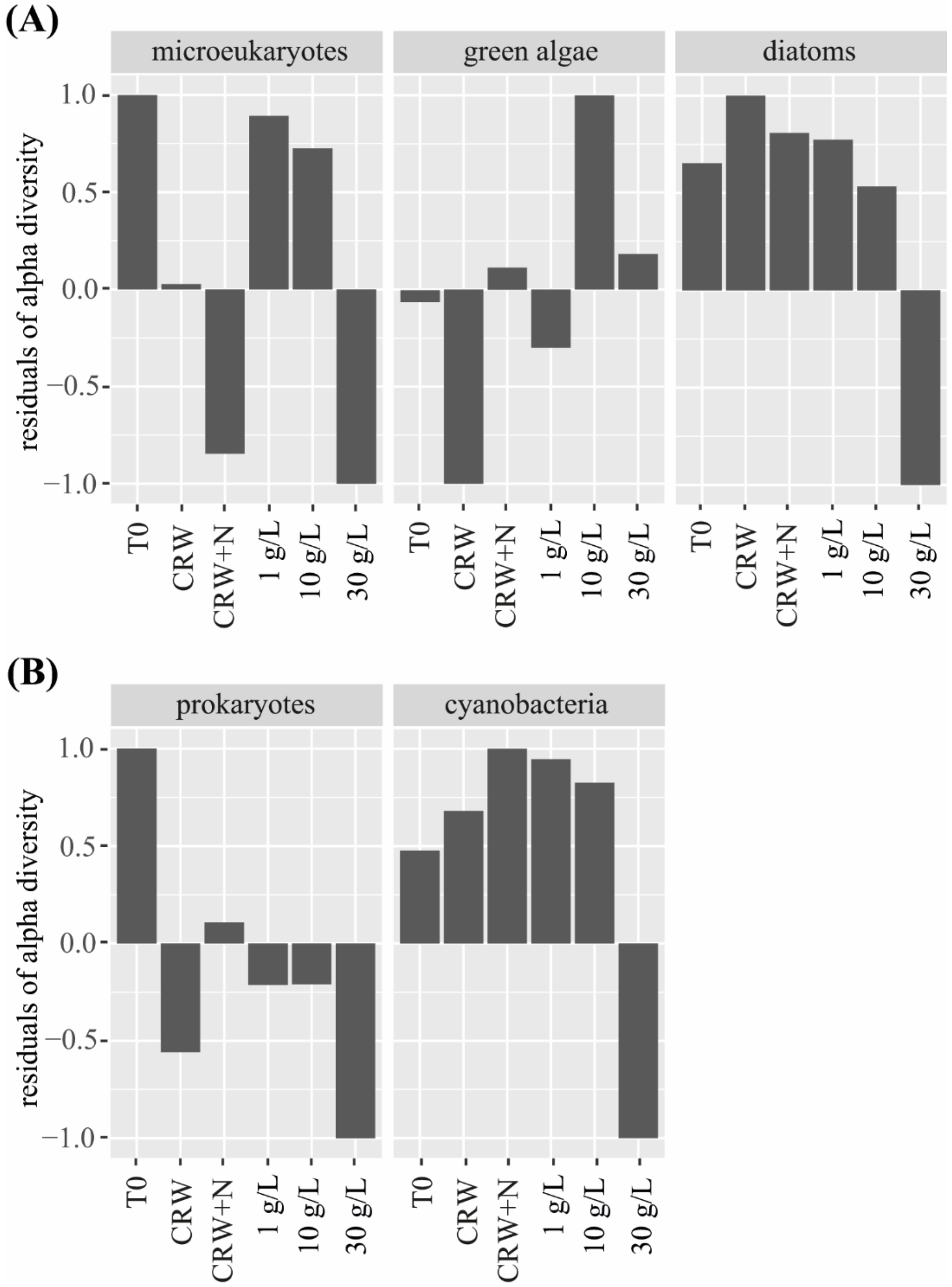

Figure 36. Alpha diversity based on the Shannon index for total microeukaryotes, green algae, and diatoms (A), as well as total prokaryotes and cyanobacteria (B). Data for richness and Simpson indices are provided in Figures S13 and 14, Supplemental material.

Partitioning of microeukaryotic diversity in the biofilm showed two discrete clusters (Figure 37A). The first one united the communities of timepoint T0 with those grown under 10 g/L and 30 g/L NaCl, whereas the latter two appeared more like each other. The second cluster comprised similar control communities as well as the community grown at 1 g/L NaCl. For the prokaryotic counterpart of the biofilm, UPGMA analysis also revealed high similarity between communities grown at 10 and 30 g/L NaCl as well as communities maintained under control conditions and at 1 g/L NaCl (Figure 37B). The sample of timepoint T0 clustered apart from the other ones.

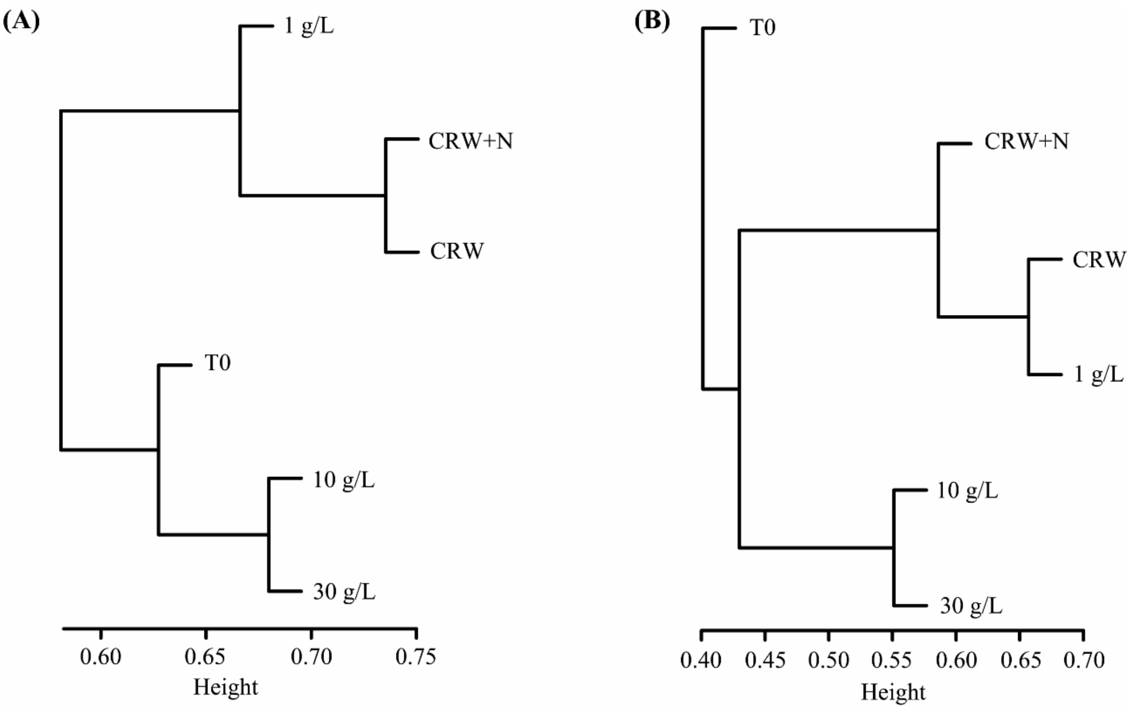

Figure 37. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis of Bray–Curtis dissimilarity indices of the Hellinger-transformed eukaryotic (A) and prokaryotic (B) ASV tables.

The microeukaryotic fraction of the biofilm community at T0 was dominated by Ochrophyta, Chlorophyta, Ciliophora, and Cercozoa (Figure 38A). The phyla Discoba, Haptophyta, Lobosa, Mesomycetozoa, and Sagenista contributed only a little to the overall relative abundance. After 14 days, microeukaryotic communities changed in the control samples: Ochrophytes and Chlorophytes still constituted the most abundant phyla; however, the relative abundances of haptophytes, Lobosa, and Mesomycetozoa increased in CRW, and those of Lobosa, Cercozoa, and Mesomycetozoa in CRW+N. The communities in the NaCl-treated samples continued to be dominated by Ochrophytes, Chlorophytes, and Haptophyta. The Conosa, and Lobosa, however, decreased in relative abundance with increasing salinity (Figure 7A). Within the green algae, most of the samples (CRW, CRW+ N, 1g/L, 30 g/L) were dominated by the genus *Oedogonium,* with more than 10% relative abundance (Figure 38B). Only the T0 sample had a higher proportion of *Chlamydomonas* ASVs (14%) and the 10 g/L samples had a higher proportion of *Chloroparvula* (15%). With increasing salinity, the relative abundances of the genera *Desmodesmus*, *Chlorochytrium,* and *Characiopodium* decreased, whereas those of the genera *Chlamydomonas*, *Jaoa,* and *Stigeoclonium* increased (Figure 38B). Diatom genera appeared to be relatively even represented in terms of relative abundance in the T0 sample (Figure 38C). With increasing NaCl concentration, the relative ASV abundances of the genera *Cocconeis*, *Cymbella,* and *Sellophora* decreased. *Navicula*, *Nitzschia,* and *Fistulifera* abundances, however, increased at the highest salinity (30 g/L). The prokaryotic counterpart of the biofilm consortium was dominated by Proteobacteria (up to 58%), Cyanobacteria (20%), and Planctomycetes (ca. 14%) in all samples. Cyanobacteria's relative abundance, however, decreased with increasing salt concentration (from 20% to 10%, Figure 38D). Most of the cyanobacteria were represented by the genera *Leptolyngbya* (up to 20%) and *Pseudanabaena,* (8 %, Figure 38E). *Leptolyngbya* and *Microcystis* decreased in relative abundance with increasing salinity, whereas the genera *Cyanobacterium*, *Pseudanabaena,* and *Synechococcus* showed an increase in their contribution to the community composition.

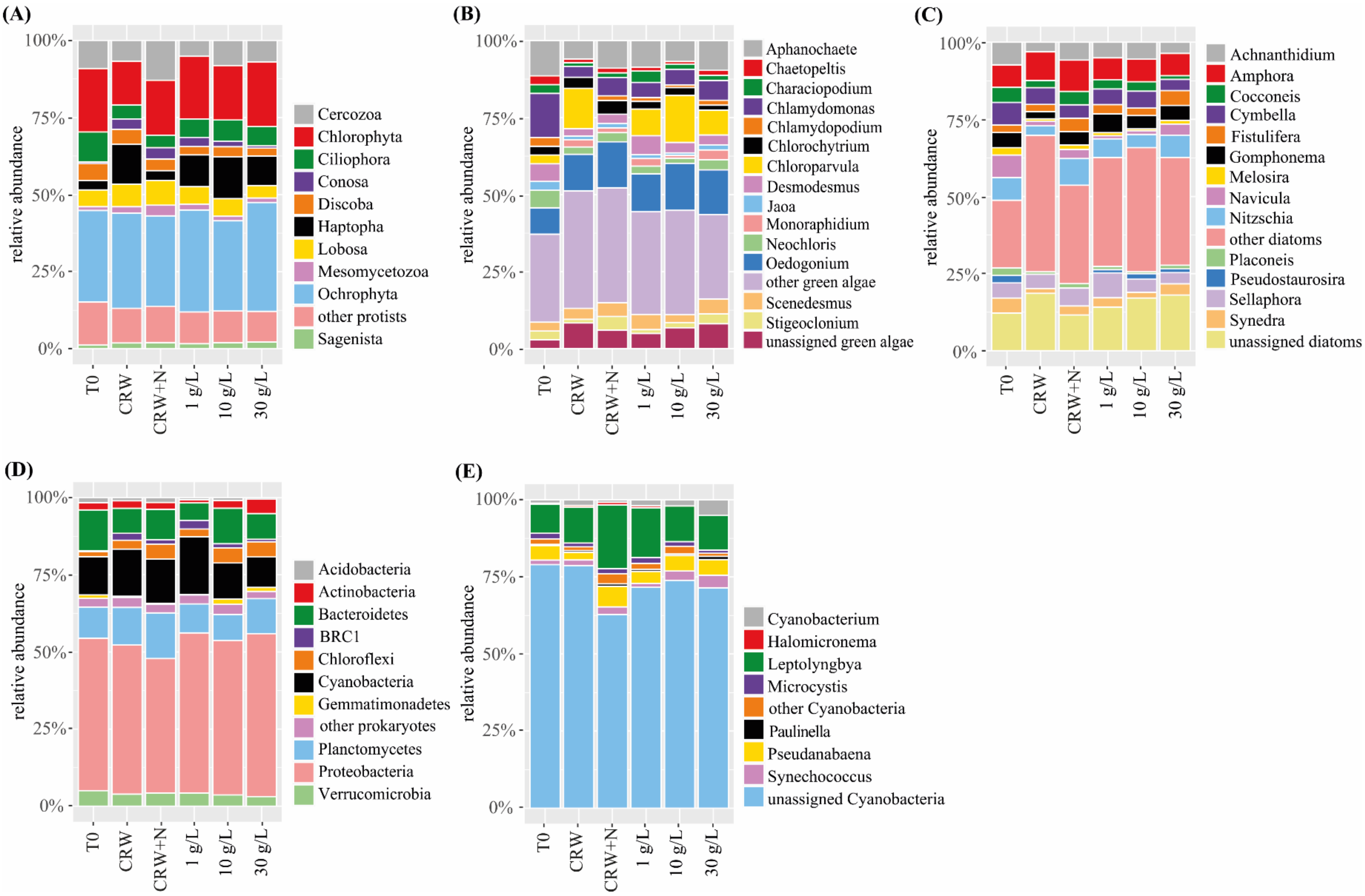

Figure 38. Taxonomic composition of the biofilm samples, separated into total eukaryotic (A, phylum-level), green algae (B, genus-level), diatom (C, genus-level), total prokaryotic (D, phylum-level), and cyanobacteria (E, genus-level) community members.

III.3. Biochemical composition changes of biofilm to salinity stress

The content of proteins and carbohydrates under different NaCl concentrations was measured. The results showed a significant decrease in protein content for the NaCl-treated biofilms compared to the controls (Figure 39A), with 55.15 mg. cm^{-2} at T0, a significant increase to 76.99 and 88.59 mg. cm^{-2} in the controls CRW and CRW+N, respectively, and then decreased progressively to 45.37 mg. cm^{-2} (1 g/L NaCl treatments), 22.41 mg. cm^{-2} (10 g/L NaCl), and 16.98 mg. cm^{-2} (30 g/L NaCl). A significant increase in carbohydrate content was observed in biofilms exposed to 1 g/L NaCl compared to CRW and CRW+N controls, representing the highest value of 324.18 mg. cm^{-2} (Figure 39B), while biofilms treated with 10 g/L NaCl recorded almost the same carbohydrate content value as the CRW control, corresponding to 125.46 and 135.95 mg. cm^{-2}, respectively. In contrast, treatments with 30 g/L NaCl induced a decrease in carbohydrate content corresponding to 35.29 mg. cm^{-2}.

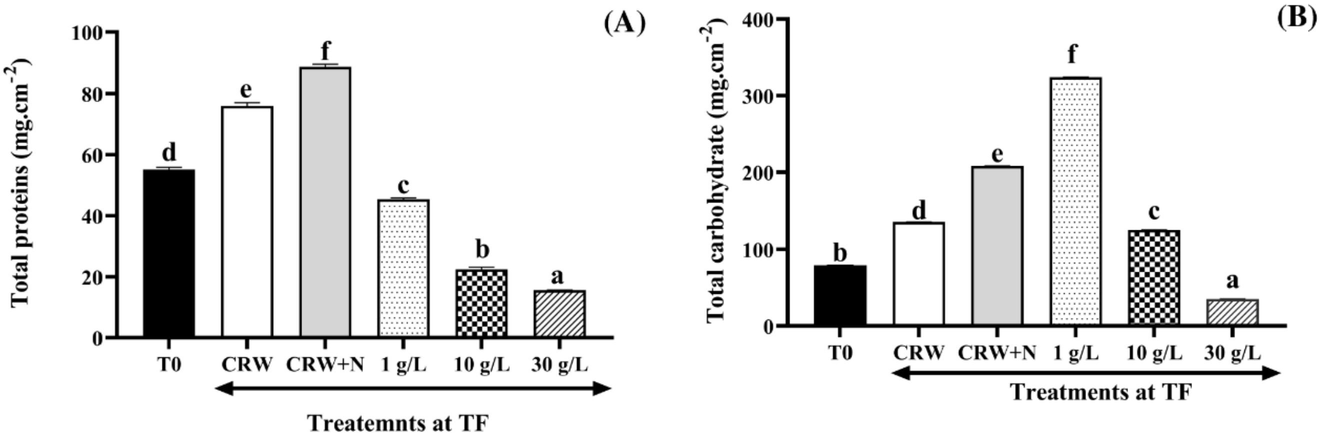

Figure 39. Salinity effect on biochemical composition of the biofilm exposed to different concentrations of NaCl: total proteins (A) and total carbohydrates (B).
All values are means and standard errors. According to the one-way ANOVA test, the ANOVA is significant at the level ($p \leq 0.05$). Same superscripts indicate no significant difference between them ($p > 0.05$).

III.4. ROS content and antioxidant system response in biofilm under salinity stress.

H_2O_2 concentrations and MDA content showed a significant increase in NaCl-treated biofilms compared to CRW and CRW+N controls (Figures 40A and B). The highest values of H_2O_2 and MDA were observed in biofilm exposed to 30 g/L NaCl, corresponding to 344.66 $\mu M.cm^{-2}$ H_2O_2 and 5.97 $\mu M.cm^{-2}$ MDA content, followed by 10 g/L NaCl conditions (164.55 $\mu M.cm^{-2}$ H_2O_2 and 3.52 $\mu M.cm^{-2}$ MDA), and then 1 g/L NaCl-treated biofilm with 89 $\mu M.cm^{-2}$ H_2O_2 and 1 $\mu M.cm^{-2}$ MDA content. However, their contents in the biofilm at T0 and the controls (CRW and CRW+N) at the end of the experiment were almost the same and were very low or even zero, mainly in the MDA content, without any significant difference. Similarly, proline and polyphenol contents as non- enzymatic scavengers also increased remarkably and significantly in the biofilm exposed to 30 g/L NaCl compared to the other NaCl treatments (1 and 10 g/L) and controls (CRW and CRW +N), as shown in Figures 40C and D. At the end of the experiment, proline accumulation was significantly lower in the CRW controls (4.05 $\mu g.cm^{-2}$) similar to the biofilm at T0 (3.95 $\mu g.cm^{-2}$). A slight increase to 6.6 and 7.42 $\mu g.cm^{-2}$ was measured in the CRW+N controls and biofilm under 1g/L NaCl conditions, respectively, which again was quite similar and without significant differences between them. While 10 g/L salt treatment showed a significantly high increase in proline accumulation to 19.08 $\mu g.cm^{-2}$ and an even greater rise to 32.13 $\mu g.cm^{-2}$ in 30 g/L NaCl-treated cultures. In parallel with the proline results, polyphenol accumulation was almost zero in the cultures at T0 and the CRW controls at the end of the experiment, whereas it started to accumulate with very low concentrations in the CRW+N controls (4.6 mg.cm^{-2} of GAE) and 1 g/L NaCl-treated biofilms (4.08 mg.cm^{-2} of GAE) and increased significantly to (10.64 mg.cm^{-2} of GAE) in the 10 g/L salt treatments as well as more than 7-fold (74.38 mg.cm^{-2} of GAE) in the 30 g/L NaCl.

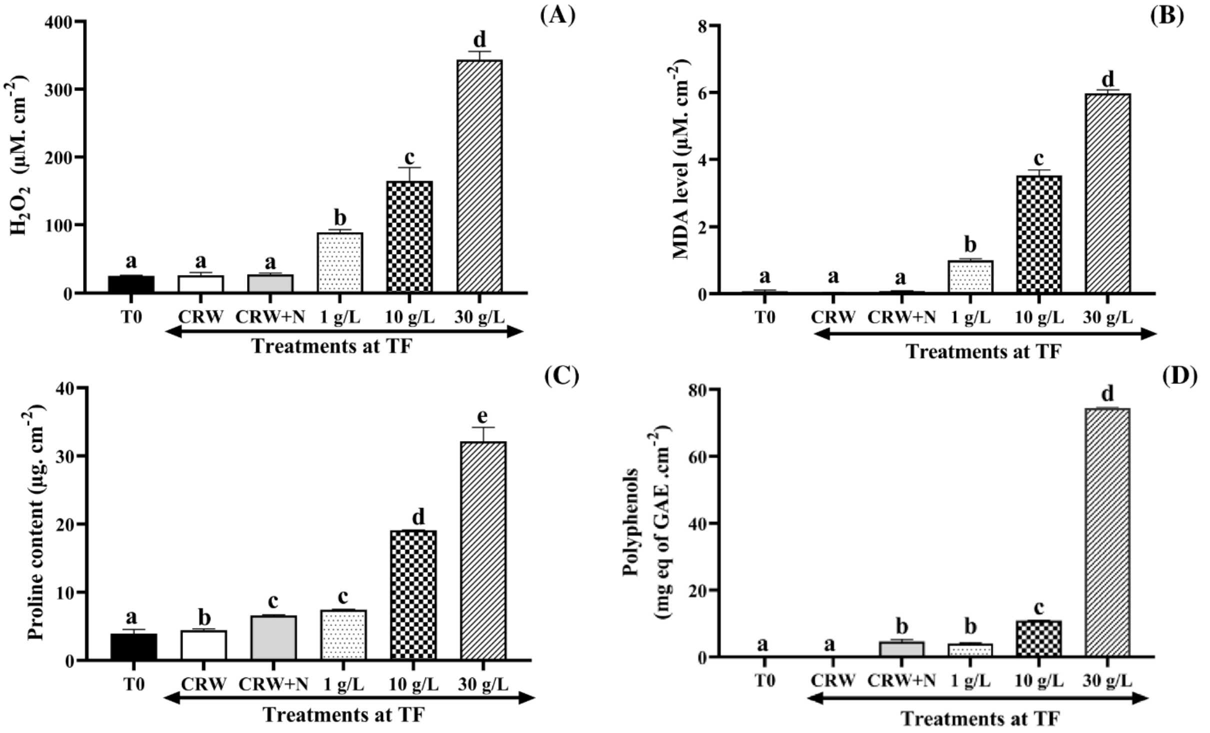

Figure 40. Accumulation of H_2O_2 (A), MDA level (B), proline (C), and polyphenols (D) in biofilms under salinity stress.
All values are means and standard errors. According to the one-way ANOVA test, the ANOVA is significant at the level ($p \leq 0.05$). Same superscripts indicate no significant difference between them ($p > 0.05$).

The activities of the enzymes SOD, CAT, and POD under the different salinity conditions followed the same pattern (Figure 41), which was significantly very high in the 30 g/L NaCl-treated biofilms (14.79 U. cm^{-2}, 16.98 U. cm^{-2} and 8.03 U. cm^{-2} in SOD, CAT, and POD respectively), followed by the 10 g/L salt treatment (10.52 U. cm^{-2} in SOD, 12 U. cm^{-2} in CAT, and POD with 2.87 U. cm^{-2} (more than 2-fold lower the value in biofilm treated with 30 g/L NaCl) and then 1 g/L NaCl-treatment with 7.26 U. cm^{-2} in SOD, 3.70 U. cm^{-2} in CAT, and almost none in POD (0.31 U. cm^{-2}). The biofilm at T0 and the controls (CRW) and (CRW+N) at the end of the experiment were very low or almost zero in the CAT, and POD activities without significant differences (Figure 41B and C) except for SOD activity, which was 5.38 U.

cm^{-2} in the CRW+N control without significant differences with 1 g/L NaCl-treated cultures, thereafter the CRW control corresponded to 1.45 U. cm^{-2} (Figure 41A).

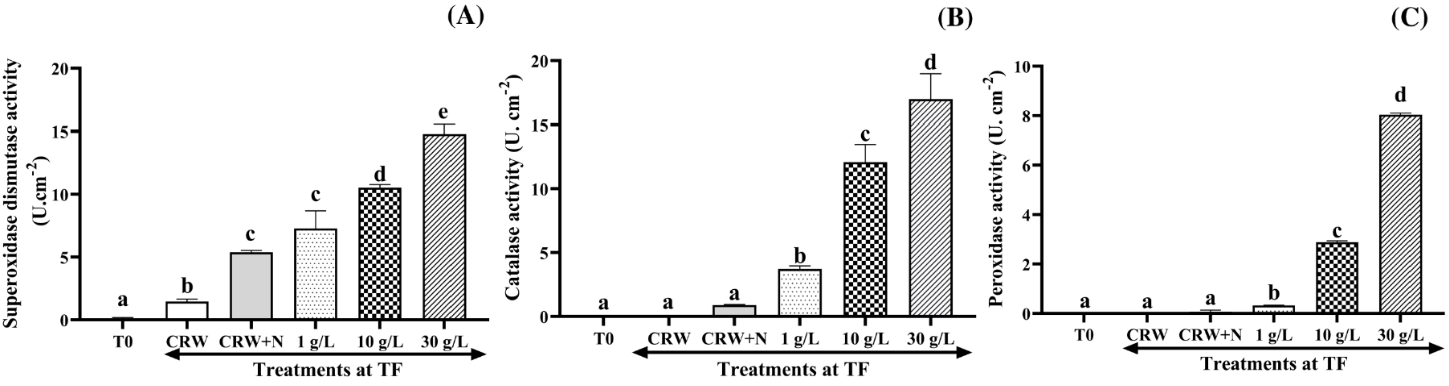

Figure 41. Antioxidant enzyme responses in biofilm exposed to different concentrations of NaCl: superoxidase dismutase (A), catalase (B), and peroxidase (C).
All values are means and standard errors. According to the one-way ANOVA test, the ANOVA is significant at the level ($p \leq 0.05$). Same superscripts indicate no significant difference between them ($p > 0.05$).

III.5. Salinity effect on nitrate reductase activity in biofilm.

Different salt treatments appeared to affect nitrate reductase (NR) activity (Figure 42). Slightly significant reductions were detected in nitrate reductase activity between cultures at T0 and controls (CRW and CRW+N). A significant decrease in NR activity of NaCl-treated cultures was found compared to control biofilms. This decrease was 55.56, 34.54, and 15.36 µM NO_2^-.cm^{-2}.h^{-1}in the 1, 10, and 30 g/L NaCl-treated cultures, respectively. This data suggests a negative effect of salt on NR activity.

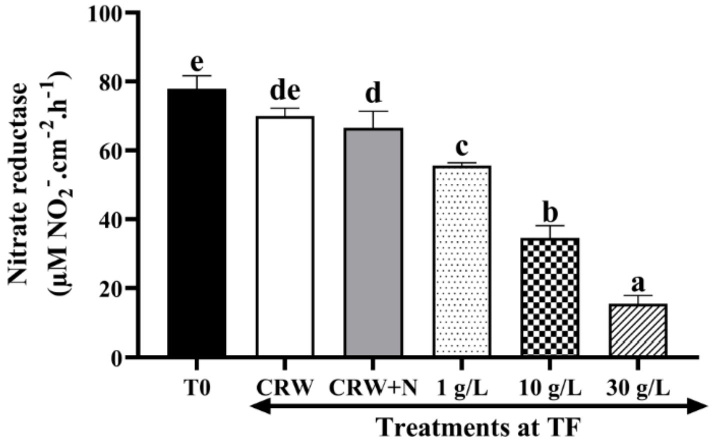

Figure 42. Salinity effects on nitrate reductase activity in biofilm treated with different concentrations of NaCl.
All values are means and standard errors. According to the one-way ANOVA test, the ANOVA is significant at the level ($p \leq 0.05$). Same superscripts indicate no significant difference between them ($p > 0.05$).

III.6. Overall biofilm responses

A heatmap analysis was used to show the distinct effects of salinity on microalgal biofilm communities in relation to their physiological, biochemical, and antioxidant responses (Figure 43). This analysis revealed clusters formed based on similarities in response to salinity. Within these clusters, subsets of parameters and salinity levels consistently exhibited similar patterns. This clustering provides valuable insights into the relationships and dependencies within the dataset.

The biofilm treated with 1 g/L NaCl was clustered with the control (CRW and CRW+N), which had a marked positive effect on diatoms and cyanobacteria relative to the control microcosms, as shown by the intensity of the blue color in the heatmap. Furthermore, it suggests that diatoms and cyanobacteria in this biofilm tolerated or preferred the 1 g/L NaCl concentration.

However, the second cluster of the heat map unites the biofilm samples exposed to high salinity concentrations of 10 and 30 g/L, highlighting a negative effect on diatom and cyanobacterial communities, as demonstrated by the intensity of the orange color. The heatmap shows that as salt concentration increases, stress response markers, including proline, H_2O_2, MDA levels, CAT, SOD, and POD activities, progressively increase (blue color).

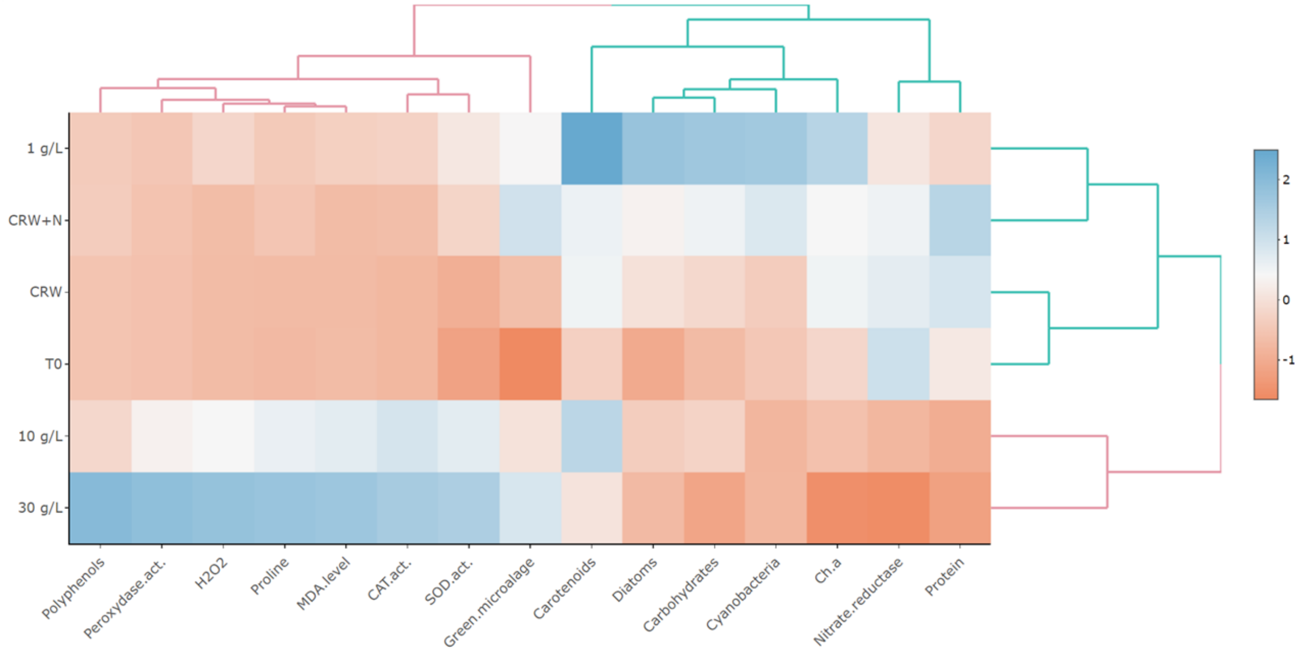

Figure 43. Heat Map analysis representing the effect of salinity on physiological, biochemical, and antioxidant responses of biofilm communities.

IV. Discussion

Our microcosm study aimed at investigating the response of biofilm communities to different salinity levels using several biofilm response characteristics, such as biofilm biomass, composition changes, biochemical parameters, physiological feedback, antioxidant activities, non-enzymatic salt stress markers, osmoprotection indicators, and nitrogen assimilation as descriptors. Based on the results, we further derive suggestions for future trends in freshwater salinization.

Overall, the experimental data revealed that higher salinity results in physiological and biochemical changes, including shifts in biofilm composition and a significant reduction in its productivity. Freshwater salinization resulting from an increase in salinity had a negative impact on the growth, biomass production, and photosynthetic pigment of the biofilm communities. These changes were linked to a rise in reactive oxygen species (ROS) generation (H_2O_2) and antioxidant enzyme activities and a reduction in biofilm biochemical composition (e.g., protein content). Freshwater salinization also impacts biofilm composition, structure, and functional parameters (Vendrell-Puigmitja et al., 2022). Our results highlighted the impact of salinity on algal biofilm communities and their response to changing environmental conditions. The biofilm community of Drâa River exposed to the highest concentrations of NaCl (10 and

30 g/L), showed a significant decrease in the cell density of cyanobacteria and diatoms compared to controls, but the low salt concentration (1 g/L) enhanced them. Cañedo-Argüelles et al. (2017) found that cyanobacteria and diatom densities can quickly increase in salt concentrations equally low as 1.61 ± 0.08 g/L. In fact, diatoms respond to Cl^- variations as low as 100 mg/L (Rovira et al., 2012). Schulz (2016) reported a reduction in the number and diversity of diatoms by monitoring a salt gradient (1,286 - 31 mg Cl^-/L) along the Wipper River (central Germany). However, the different salt levels obtained by adding sea salt (NaCl), ranging from 225 to 10 073 $\mu S.cm^{-1}$ of conductivity, used to assess the salinity effect on the valve morphology of freshwater diatoms, did not reduce their cell density (Noune et al.,2023). On the other hand, our results showed no clear effect of salt exposure on green algae cell density. Indeed, green algae are often the primary producers in saline ecosystems (Costelloe et al., 2005). Due to their high plasticity and resilience to most abiotic stimuli, including salt stress, they undergo severe short-term alterations in osmolyte concentrations and morphology and a long-term accumulation of beneficial mutations (Silva et al., 2000). For instance, the lichenized green microalga *Trebouxia sp.* TR9 displays a remarkable resistance to a wide range of salinities, from high (1 M of NaCl= 58,5 g/L of NaCl) to extreme concentrations of NaCl (5 M of NaCl, close to saturation point), suggesting the development of alternative molecular pathways to cope with highly saline environments (Hinojosa-Vidal et al., 2018). The cell count results using light microscopy showed a close similarity to measurements obtained using the BenthoTorch (BT) method. This observation contrasts with a previous study by Kahlert and McKie (2014), which examined the biomass and composition of benthic algae in freshwater by comparing the BT measurements with the biovolume method.

Furthermore, in freshwater ecosystems, microalgae richness declines along a salinity gradient, and many species disappear when a particular salinity threshold is exceeded (Cañedo-Argüelles et al., 2019; Lazrak et al., 2022). Freshwater microorganisms respond differently to salt stress, and increasing salinity is expected to alter freshwater communities' composition (Berger et al., 2019). Previous works indicate that a salinity level of around 3 g/L may be the threshold for detecting severe effects of freshwater salinization on biofilm communities (i.e., dramatic decreases in surviving taxa and/or abundance) (Cañedo-Argüelles et al., 2017). While other studies of freshwater salinization caused by effluent from an abandoned potash mine (Menteroda, Germany) on biofilm collected from Riera Major, Viladrau, Spain, revealed a threshold response, with more radical changes in biofilm structure and function occurring at salinity concentrations of 15 g/L (a decrease in diatom growth rate related to a reduction in cell size, diversity, and species richness) (Vendrell-Puigmitja et al., 2021, 2022). In this study, Shannon-based alpha diversity and cell densities of diatom and cyanobacterial communities decreased with increasing salt concentrations, reaching their lowest value at a salt concentration of 30 g/L. Since the tolerance of various species to salt is very diverse, the threshold of salt tolerance that biofilm communities can sustain is currently unknown. Others react to increased salinity with different degrees of sensitivity, which may sometimes result in population decreases or even extinction, while many species are well-known for their salt tolerance (Berger et al., 2019; Cunillera-Montcusí et al., 2022; Piscart et al., 2006; Waterkeyn et al., 2008).

Sequencing data results (DNA metabarcoding) highlight significant shifts in biofilm composition and structure. The genus *Chloroparvula* within the green algal community was dominant, representing 15% of the biofilm subjected to a concentration of 10 g/L. In addition, the abundance of the ASVs *Chlamydomonas* and *Stigeoclonium* showed an increase with increasing NaCl concentrations. These green genera appear to thrive in water with higher salinity levels (Cerfonteyn et al., 2023; Bazzani et al., 2021; Vendrell-Puigmitja et al., 2022). Similarly, under higher salinity conditions (30 g/L), some diatom species belonging to genera, namely *Navicula*, *Nitzschia*, and *Fistulifera*, showed an increase in abundance. These halophilic species are generally found in saline aquatic environments (Lange-Bertalot, 2013; Cañedo-Argüelles et al., 2017; Liu et al., 2016). Conversely, *Cymbella* and *Cocconeis* are widely regarded as freshwater indicators (Wang et al., 2005) decrease in the biofilm exposed to high salt concentrations. The abundance of cyanobacteria, specifically *Pseudanabaena* and *Synechococcus* genera showed an increase in high salinity concentrations. Indeed, some species of these genera are considered salt-tolerance taxa (Cegłowska et al., 2020; Xia et. al,2017). This might indicate that the increase in salinity could be sufficient to reduce or eliminate oligohaline taxa, which would then be replaced by halophilic or salt-tolerant taxa. This has already been observed by Vendrell-Puigmitja et al. (2022) and Cañedo-Argüelles et al. (2017) on the shift in the composition of benthic algal communities, favoring salt-tolerant species along the salinity gradient.

In terms of biofilm photosynthetic pigments, chlorophyll *a* concentration decreased in biofilm exposed to high salt concentrations (10 and 30 g/L NaCl) compared to biofilm treated with low salt concentrations (1g/L) and controls. Our findings are congruent with other studies, which have revealed that algal biomass can decrease at salinity levels between 4 and 10 g/L (Kefford et al., 2011; Rotter et al., 2013). Vendrell-Puigmitja et al., (2022) reported that chlorophyll *a* decreased in the 30 and 100 g/L treatments. This could demonstrate that a high salt concentration may have prevented biofilm biomass growth, probably due to the osmotic stress process, which inhibits chlorophyll synthesis and activates its degradation by the chlorophyllase enzyme. Decreased chlorophyll generally indicates activation of the photoprotection mechanism that decreases light absorption to reduce chlorophyll through delayed synthesis or rapid degradation (Taïbi et al., 2016). This was also demonstrated by the high concentration of carotenoids found in the treated biofilm at 10 g/L compared to the controls. As their production is associated with cell disruption and signaling, particularly to neutralize ROS's effect (Zuluaga et al., 2017), it is considered a potent antioxidant that can assist O_2^- dismutase and scavenge H_2O_2 (Mathesius, 2018).

Salt stress impacts biofilm microalgae's biomass, growth, and productivity by modifying their biochemical composition, namely lipids, proteins, and carbohydrate levels (Hounslow et al., 2021; Cochero et al., 2017; Sabater et al., 2016; Kebeish et al., 2014). Our findings showed that salt stress significantly decreased protein content compared to the control. On the contrary, carbohydrate content increased significantly in biofilms treated with 1 g/L NaCl compared to the control, followed by cultures treated with 10 g/L, which recorded almost the same value as the CRW control. Previous studies showed that the release of extracellular polymeric substances, such as total carbohydrate and protein concentration, increased in response to salinity stress (Decho, 2000; Steele et al., 2014). However, it was also revealed that microalgae respond to unfavorable conditions, such as high salinity, by stimulating the accumulation of carbohydrates and lipids rather than proteins (Mirizadeh et al., 2020; Hang et al., 2020). They utilized carbohydrates as osmoprotectant molecules to maintain homeostasis and ensure osmotic adjustment under salt stress (Wang et al., 2018; Tietel et al., 2019). Furthermore, the presence of carbohydrates facilitates cell adaptation to saline conditions (Pandit et al., 2017).

Under these stressful conditions, biofilm microalgae produce ROS, which was investigated by measuring H_2O_2 in this study. The findings revealed that H_2O_2 levels in the biofilm significantly increased with salt concentration, underscoring the fact that high salinity causes oxidative stress, ultimately producing reactive oxygen species. Biofilm cells can cope with oxidative stress and exploit it by using reactive oxygen species (ROS) as a signal or cue to prepare for adaptation to a changing environment (Čáp et al., 2012). However, ROS production can affect microalgal metabolism by decreasing nutrient uptake and CO_2 flux while increasing NADPH synthesis (Srivastava et al., 2017). In addition, ROS also cause lipid peroxidation in the thylakoid membrane, photoreduction, and triplet Chl production (Srivastava et al., 2017; Ji et al., 2018; Singh et al., 2018). Another stress biomarker often used in microalgae is lipid peroxidation, quantified by the concentration of MDA in cells, which is the main lipid peroxidation product generated when free radicals oxidize polyunsaturated fatty acids (Pancha et al., 2015; Wang et al., 2020; Meng et al., 2020). In parallel with H_2O_2 concentration, MDA content in this study significantly increased with rising salt concentrations, reflecting ROS-mediated lipid accumulation.

To mitigate adverse effects, microalgae can activate various defense mechanisms against stress conditions, mainly involving osmoprotectants and non-enzymatic and enzymatic antioxidant defense compounds (Wang et al., 2020). As a non-enzymatic scavenger, proline is an essential amino acid with many functions under stress. It acts as a ROS scavenger, osmolyte, and molecular chaperone, stabilizing protein structure (Tietel et al., 2019; Zalutskaya et al., 2020). Similar to proline, polyphenols are strong ROS scavengers that maintain cell membrane integrity, osmotic adjustment, and free radical scavenging to reduce salt stress (Kumar et al., 2020). In this study, the biofilm treated with 30 g/L showed a highly significant accumulation of proline, especially polyphenols, compared to biofilms subjected to 1, 10 g/L salt stress and controls. This was already observed in previous studies showing a high accumulation of proline and polyphenols in microalgae species exposed to salt stress (Chokshi et al., 2017b; Fal et al., 2022).

Regarding scavenging enzyme activities, SOD, CAT, and POD were measured to assess the antioxidant defense system of biofilm subjected to salinity conditions in this study. Enzymatic antioxidants provide a first line of defense, converting reactive oxygen products into H_2O_2, which is then converted into H_2O (Ighodaro and Akinloye, 2018). SOD is a potent antioxidant that converts harmful O_2^- into less harmful H_2O_2 (Gill and Tuteja, 2010; Ighodaro and Akinloye, 2018). The latter, produced by SOD, is then decomposed into H_2O and O_2 by an efficient antioxidant, CAT, and into two molecules of water by enzyme's peroxidase and ascorbate peroxidase, which use ascorbic acid as an electron donor (Gill and Tuteja, 2010; Nimse et Pal, 2015; Wang et al., 2020; Ighodaro and Akinloye, 2018). Therefore, CAT, SOD, and POD form a complete chain of antioxidant enzymes (Chokshi et al., 2017b). Our results generally showed a significant increase in these antioxidant enzymes with increasing NaCl concentrations compared to the unstressed biofilm. This could indicate an enhanced antioxidant defense response to counteract ROS production under stressful conditions. This was also observed for other stressors, for instance, by Bonet et al. (2012) in biofilms subjected to Zn pollution; CAT showed a clear increase after several weeks of exposure. On the other hand, Hou et al. (2016) reported that microorganisms in biofilm exposed to Zn pollution increased CAT and SOD activities compared to controls; thereafter, gradual decreases in these enzymes were observed over time, revealing an incomplete chain of defense against the onset of oxidative damage in organisms. These results show that changes in low-molecular compounds and antioxidant enzyme activities vary in response to microalgae species, developmental stage, metabolic state, salt concentration applied, and the duration of exposure to stress (Fal et al., 2022).

Overall, this experimental study showed that an increase in salt concentration has a significant influence on the algal biofilm composition and causes a decline in the diversity of periphytic communities. Salinization reduces the biofilm biomass mainly of two algal groups of cyanobacteria and diatoms, leading to a decrease in alpha diversity and alterations in their productivity. In addition, increasing salinity induces biofilm physiological changes, notably a reduction in chlorophyll *a* content, which can obviously lead to a decrease in photosynthesis efficiency and primary production. Conversely, the carotenoid content showed a substantial increase, even at high salinity (10 g/L), highlighting that this pigment synthesis could be one of the algal biofilm defense mechanisms activated in response to salt stress. Our findings also revealed that high salinity level causes biochemical changes in algal biofilm, such as increased carbohydrate content and decreased protein content. Metabolically, there was an increase in H_2O_2 content, oxidative damage, and lipid peroxidation, as shown by increased levels of MDA and the activity of antioxidant enzymes that eliminate ROS, highlighting the possible long-term impacts of freshwater salinization on primary producers. In this respect, it would be crucial to research the detailed impacts of several different salinity gradients on the diversity of microalgae and other microbial components of the biofilm in order to identify the salt tolerance threshold of biofilm communities. To quantify the overall effects of freshwater salinization on biofilm, assessing their impact on ecosystem services linked to biofilm-induced processes is preferable. In addition, to obtain a global view of freshwater salinization risks, more studies are needed to assess the impact of salinization on biofilm as the basis of the food web, with other higher trophic levels.

V. Conclusion

This microcosm experimental study highlighted that freshwater salinization leads to adverse changes in the composition and productivity of the algal biofilm communities. Cyanobacteria, diatoms, and green algae responded differently to salt concentrations. Cyanobacteria and diatoms responded more strongly and seemed to be more sensitive to salt stress than green algae, which showed a relative resilience that could be explained by their ability to resist abiotic disturbances such as salinity. Whereas biomass production of the biofilm was enhanced by low NaCl concentrations (1 g/L), the photosynthetic pigment content decreased at high concentrations, and the biochemical components (i.e., protein and carbohydrate) were altered under salt stress. In NaCl-treated biofilms, H_2O_2, measured as ROS and MDA levels, increased significantly, indicating salt stress-induced oxidative damage. The biofilm sets up defense mechanisms by accumulating osmoprotective compounds, notably proline, and polyphenols, and enhancing antioxidant enzymes such as SOD, CAT, and POD. Our findings indicate that biofilm communities respond to salt stress with physiological and biochemical modifications for their survival, but only up to a certain threshold of their resistance. This observation suggests that low salinity concentrations may promote biofilm development, while higher concentrations may have a detrimental effect. This information helps us understand how freshwater salinization affects benthic algal communities and may be used to improve current tools to assess the risks of increasing salinization in rivers. Furthermore, further studies are needed to determine a more precise threshold for salinity between 1 to 10 g/L and 10 to 30 g/L. Finally, it would also be important to investigate the long-term impacts of salt stress on biofilm communities, ecosystem functioning, and water quality management.

Chapter VI. Salt stress responses of two diatom strains isolated from different saline habitats under laboratory batch cultures

I. Introduction

Diatoms are a diverse group of unicellular microalgae that are covered in silica and inhabit all ecosystems, ranging from aquatic environments to terrestrial soils. They frequently dominate the algal community in periphyton (Potapova and Charles, 2003; Allan et al., 2021). These microorganisms play a crucial ecological role in lotic ecosystems, primarily serving as primary producers that provide a vital energy source, facilitate nutrient cycling, and deliver oxygen to aerobic life forms (Stevenson et al., 1996; Pan et al., 1999; Lowe and LaLiberte, 2017). Diatoms have short generation cycles, exhibit specific growth preferences, and respond rapidly to any environmental change, including salinity, which influences their distribution in different habitat types and makes them excellent indicators of ecological change (Smol and Stoermer, 2010; Hering et al., 2006; Rimet, 2012; Bagmet et al., 2017; Godhe and Rynearson, 2017).

Over the past century, a combination of natural and human factors has led to an increase in river salinity, particularly in arid and semi-arid regions (Stenger-Kovacs et al., 2023; Cunillera-Montcusí et al., 2022). The increasing salinity in rivers has significant ecological effects on diatoms, notably influencing their growth, morphological characteristics, and community structure (Balycheva et al., 2023; Frost et al., 2023). These changes are also associated with physiological and metabolic modifications in their cells (Shetty et al., 2019; Lazrak et al., 2024; Fal et al., 2022). Due to cellular metabolic changes, high salt concentrations can lead to the production of excess reactive oxygen species (ROS). They disrupt the ionic balance, induce plasmolysis, alter cell water potential, inhibit water uptake, and cause oxidative stress (Almeida et al., 2017; Verslues, 2016; Yun et al., 2019; Ma et al., 2020). Cellular signaling pathways typically utilize reactive oxygen species (ROS) as secondary messengers to trigger responses to external stressors, including salt. However, their excess damages proteins, lipids, and nucleic acids in microalgae. They affect various metabolic and physiological functions, including photosynthesis, respiration, osmoregulation, and nutrient uptake (Čáp et al., 2012; Ma et al., 2020; Kuo et al., 2020; Drira et al., 2021; Figler et al., 2019). Diatoms exhibit exceptional acclimatization mechanisms that mitigate adverse effects, enabling them to regulate turgor pressure and maintain ionic homeostasis while synthesizing osmoprotectant-appropriate solutes through diverse biochemical pathways and synergistic processes (Stenger-Kovács et al., 2023a). In contrast to freshwater diatom strains, saltwater diatom strains demonstrate pronounced physiological plasticity in response to increasing salinity (Prelle et al., 2021; Fu et al., 2022; Stenger-Kovács et al., 2023a).

Every diatom species has a salinity tolerance threshold beyond which it cannot survive or develop, determined by its physiological adaptations. Some species thrive preferentially in freshwater environments, others in brackish or saline environments (Van Dam et al., 1994; Snoeijs and Weckström, 2010). Although research has investigated the salinity impact on diatom assemblages in many ecosystems (Potapova and Charles, 2003; Schröder et al., 2015; Lazrak et al., 2022; Frost et al., 2023; Stenger-Kovács et al., 2023b), studies examining the responses of isolated diatom strains from distinct habitats remain relatively rare. In this context, the main objective of this study was to investigate how increasing salinity affects the growth, biochemical composition, and metabolomic profiles of the diatom species *Homoeocladia palea* (freshwater strain) and *Nitzschia frustulum* (saltwater strain). Thus, the aim was also to determine their tolerance limits and predict the potential impact on benthos evolution in response to increasing salinity. Additionally, we assessed the impact of salt on conductivity, chloride concentration, and nutrient uptake (nitrate and orthophosphate) in these diatom isolates. We hypothesized that diatom species typically found in saltwater habitats would exhibit greater salt tolerance than those typically found in freshwater habitats. Furthermore, we expect saltwater diatoms to respond to higher salinity levels by increasing their ability to remove ions. Investigating how these isolated strains physiologically respond to variations in salinity may yield valuable insights into their potential resilience under shifting environmental conditions. Understanding these responses is crucial for predicting how microalgae will respond to changes in salinity driven by climate change, natural salinization, or human activities.

II. Materials and Methods

II.1. Isolation of diatom strains

Two diatom strains, *Homoeocladia palea* and *Nitzschia frustulum*, were isolated from distinct locations with different salinities in the Drâa basin (Southeastern Morocco). *Homoeocladia palea* (freshwater strain) was from the Dades river (upper Drâa tributary) (~ 0.5 g/L salinity), and *Nitzschia frustulum* (saltwater strain) was from the Tissint river (lower Drâa tributary) (~ 7.5 g/L salinity). The two diatom strains were isolated from several dilutions of the microorganism mixture (biofilms). Then, the successive subculturing technique was used to isolate the two diatom strains. This technique involves performing several sub-cultures on a solid WC medium using the quadrangular streak method. After subculturing each isolated colony of the diatom strain on a solid medium, a pure culture of a single diatom strain is obtained and processed in a liquid WC medium.

II.2. Diatoms culture conditions

Specific culturing conditions were maintained to ensure optimal growth of these diatom strains. The cultures were kept at a constant temperature of 26±2°C, with a consistent light intensity of 4000 $lx.m^{-2}s^{-1}$. Additionally, a 15-hour light cycle was followed by a 9-hour dark cycle. These controlled conditions were established to ensure the reliability and consistency of experimental results for *Homoeocladia palea* and *Nitzschia frustulum*.

II.3. Experimental Design

The experiment was performed using WC medium cultures in 500 ml Erlenmeyer flasks with a final volume of 400 ml. To ensure statistical robustness, each diatom was maintained in triplicate. Each replicate is inoculated with a diatom strain (*H. palea* or *N. frustulum*) at an initial density of 2 to 3 x 10^5 cells. mL^{-1}. Salt (3 $g.L^{-1}$ NaCl) was added incrementally every other day for 12 days to obtain the final salinity concentration (3, 9, 15, and 21 $g.L^{-1}$ NaCl), plus the control group with no added salt. In the experiment, “negative control” compositions (nutrient solutions plus salt, without algae) were used to determine the accurate initial conductivity and chloride concentration and to adjust results derived from algae cultures for variations in algae-free compositions. Three replicates of each diatom strain treatment were incubated for 16 days under controlled conditions.

II.4. Growth and photosynthetic pigments measurement

The growth of both diatom species (*H. palea* and *N. frustulum*) was evaluated daily by counting using a hemocytometer under a light microscope (Motic BA210, China; 400× magnification).

At the end of the experiment (16 days), the amounts of photosynthetic pigments, chlorophyll *a*, and carotenoids in *H. palea* and *N. frustulum* were measured using the method previously described in Chapter V (section II.4).

II.5. Oxidative and antioxidant activities measurement

II.5.1. Stress biomarkers (MDA and proline) determination

At the end of the experiment, the methodology for determining MDA and proline levels in the diatom culture samples was previously detailed in Chapter V, Section II.7.1.

II.5.2. SOD and CAT enzyme assays

At the end of the experiment, the methodology for determining superoxide dismutase and catalase activities in the diatom culture samples was previously detailed in Chapter V, section II.7.2.

II.6. Measurement of conductivity, chloride, and nutrient (nitrate and orthophosphate) content changes

The conductivity, chloride, and nutrient levels (nitrate and orthophosphate) of the diatom cultures were assessed at the beginning and end of the experiment. Negative control compositions were used to measure baseline values. The reduction in conductivity and chloride content at the end of the experiment (16th day) was quantified as a percentage, with the initial values taken as a 100% baseline. To assess variations in nutrient composition, a volume of cell-free samples was taken at the beginning and on day 16 of the experiments. The difference between day 0 and day 16 was calculated to determine the reduction in nitrate and phosphate levels. These values were corrected based on the changes observed in the compositions of the negative control, thus enabling the separation of the reduction attributed only to algae. The initial values were used as a 100% baseline to determine the percentage reduction in nitrate and phosphate levels by day 16. Supernatant conductivity was determined using a multiparameter device (WTW MultiLine® Multi 3510 IDS). Precipitation titration was used to measure the chloride content of the supernatants, and spectrophotometric methods were applied to determine nitrate and orthophosphate concentrations, following the analytical techniques described by Rodier et al. (2009).

II.7. Statistical analysis

All experiments were performed in triplicate, and data were expressed as mean values ± standard error (SE). Statistical analyses were carried out using GraphPad Prism version 9.

To evaluate the impact of salinity on the growth of diatoms, specifically *Homoeocladia palea* and *Nitzschia frustulum*, a repeated measures ANOVA was conducted, followed by

Tukey’s post-hoc multiple comparisons test. Significant differences among the treatment groups were denoted by asterisks ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

The study examined photosynthetic pigments, particularly chlorophyll a, and carotenoids, alongside stress markers such as malondialdehyde (MDA) and proline concentrations, as well as the activities of antioxidant enzymes, including catalase and superoxide dismutase. These analyses were performed using one-way ANOVA, followed by Tukey’s post hoc test, to determine significant differences among the groups. Statistically significant differences among treatments were denoted by distinct letters ($*p < 0.05$).

For the removal of conductivity, chloride, and nutrients (nitrate and orthophosphate), statistical differences between treated cultures of each species (indicated in the columns) were also determined using a one-way ANOVA followed by Tukey's test, with significance indicated by asterisks ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$). Furthermore, significant variations between the two diatom strains subjected to the same treatment (displayed within rows) were indicated by different letters ($*p < 0.05$). Additionally, paired t-tests were used to compare the values for conductivity, chloride concentration, and nutrient levels (nitrate and orthophosphate) at both the beginning and end of the experiment. Throughout all statistical analyses, results were deemed statistically significant when $p < 0.05$.

III. Results

III.1. Salinity effect on diatoms' growth and photosynthetic pigments

The results of the salinity effect on *H. palea* and *N. frustulum* growth are presented in Figure 1. Over 16 days, increasing salinity significantly reduced *H. palea* growth compared to controls, except for a 3 $g.L^{-1}$ NaCl concentration (Fig. 44). Under conditions of 3 g/L NaCl, the growth of *H. palea* increased significantly, reaching its maximum value of 22.7 $\times 10^6$ cells/mL on day 10 and stabilizing at around 19.66 $\times 10^6$ cells/mL at the end of the experiment (day 16) compared with the control, whose value was around 18 $\times 10^6$ cells/mL on day 16. However, raising salinity to 9, 15, and 21 g/L significantly decreased its growth, with 16, 10.06, and 7.33 $\times 10^6$ cells/mL, respectively, at the end of the experiment (Fig. 44a). In contrast to *H. palea*, *N. frustulum* growth showed a significant increase with increasing salinity, reaching a maximum growth rate under saline conditions of 21 *g* L^{-1} NaCl (Figure 44b). Indeed, at the end of the experiment, its growth had reached 27.5 $\times 10^6$, 30.65 $\times 10^6$, and 38.66 $\times 10^6$ cells/mL under salinity conditions of 3, 9, and 15 g/L NaCl, respectively, peaking at 50.7 $\times 10^6$ cells/mL at a salinity of 21 g/L NaCl, compared to the control group, corresponding to 23.58 $\times 10^6$ cells/mL.

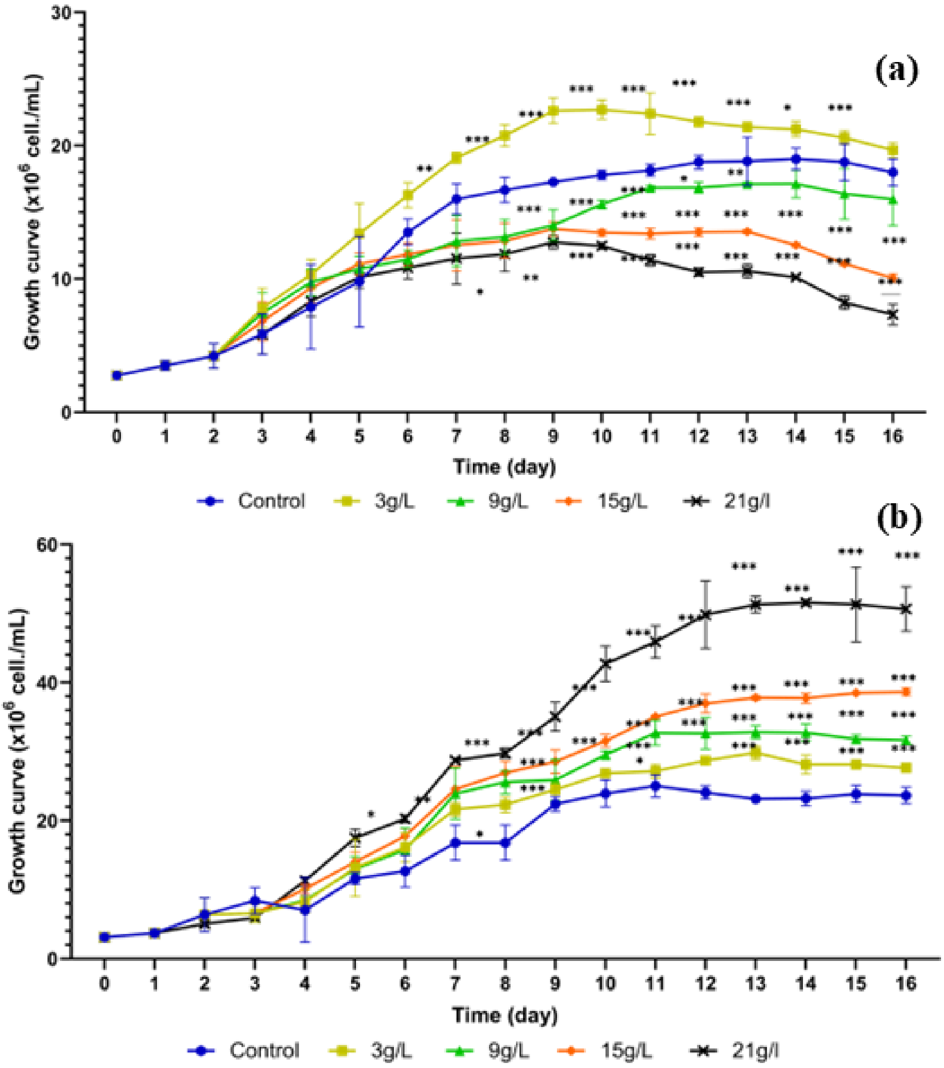

Figure 44: Effect of salinity on the growth of *Homoeocladia palea* (a) and *Nitzschia frustulum* (b).

Data are presented as means and standard errors of three repetitions. According to Tukey's t-test, asterisks mean that the difference is statistically significant (*p<0.05, **p<0.01, ***p<0.001).

Figure 45 illustrates the impact of different salinity levels (3, 9, 15, and 21 g/L of NaCl) on chlorophyll *a* (b) and carotenoid (a) levels in two species, *H. palea* and *N. frustulum*. Compared with the control group, *H. palea* chlorophyll *a* level decreased slightly and significantly in the 15 and 21 g/L high-salt treatments, from 2.56 to 2.3 and 1.9 µg.mL^{-1}, respectively (Fig. 45b). The Saline conditions (9, 15, and 21 g/L) significantly increased chlorophyll *a* levels in *N.frustulum*, with the 21 g/L NaCl treatment achieving the highest value of 6.44 µg.mL^{-1}. The carotenoid content of *H. palea* in treatments at all salinity levels increased significantly compared with the control, from 0.5 µg.mL^{-1} to 1.31, 1.14, 1.25, and 1.23 µg.mL-1 (under salinity conditions of 3, 9, 15, and 21g/L, respectively) (Fig. 45a). However, no significant differences were observed between these different NaCl treatment conditions. Similarly, the carotenoid content of *N. frustulum* was around 0.2 µg.mL^{-1} in the control group but increased significantly in the salt treatments, reaching its highest levels of 1.9 and 1.81 µg. mL^{-1} in 9 and 15 g/L NaCl. However, it decreased slightly under the highest salt conditions (21 g/L) to a concentration of 1.28 µg. mL^{-1}.

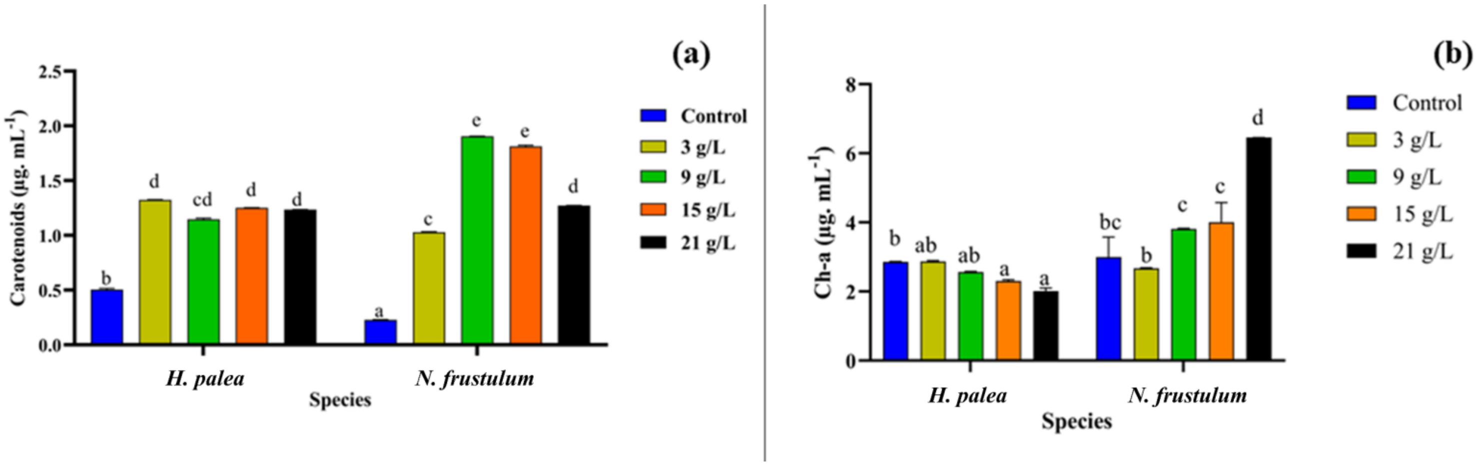

Figure 45: Effect of salinity on diatoms' pigments, chlorophyll *a* (b) and carotenoids (a). Data are presented as means and standard errors of three repetitions. According to Tukey's t-test, different letters indicate a significant difference at (*$p < 0.05$).

III.2. The antioxidant system responses of diatom species to salinity stress

Both diatom species exhibited a significant increase in MDA content when subjected to high-salinity conditions, compared to control groups. However, *H. palea* showed higher MDA production than *N. frustulum* (Fig. 46b). The highest value of the MDA level was observed in *H. palea* under 21 g/L NaCl conditions corresponding to 2.35 µM.mL^{-1}, followed by 0.82, 0.93, and 0.98 µM.mL^{-1} for 3, 9, and 15 g/L NaCl concentrations, respectively (Fig. 46b).On the other hand, the MDA level in *N. frustulum* under high salt conditions (21 g/L) was 0.72 µM.mL^{1} compared to 0.5 µM.mL^{1} (3 g/L), 0.44 µM.maL^{-1} (9 g/L), and 0.41 µM.mL^{1} (15 g/L

). Similarly, proline content, as a non-enzymatic scavenger, increased with increasing salinity in both species compared to the control groups, with the highest levels recorded under 21 g/L NaCl conditions (Fig. 46a). It increased significantly in *H. palea* from 21.87 $\mu g.mL^{-1}$ in the control group to 42.33, 46.06, and 51.68 $\mu g.mL^{-1}$ in the 3, 9, and 15 g/L salt treatments, respectively, reaching its maximum value of 103.57 $\mu g.mL^{-1}$ under the highest salt conditions of 21 g/L. For *N. frustulum*, the proline content increased significantly from 24.74 in the control condition to 56.37, 61.28, 80.22, and 96.26 $\mu g.mL^{-1}$ in the 3, 9, 15, and 21 g/L salt treatments.

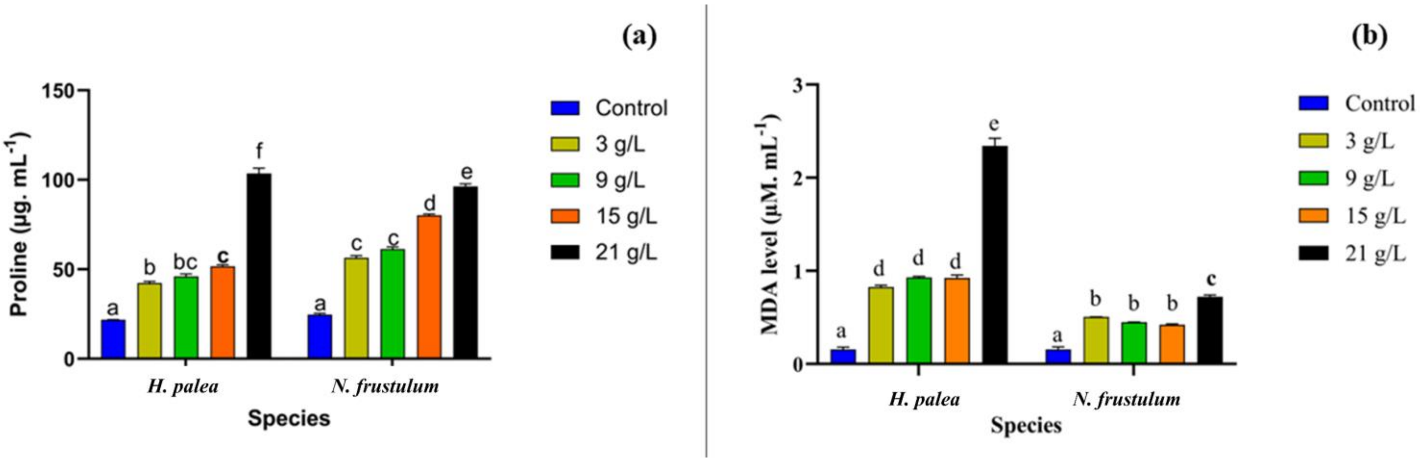

Figure 46: Effect of salinity on diatoms stress markers MDA level (a) and proline(b). Data are presented as means and standard errors of three repetitions. According to Tukey's t-test, different letters indicate a significant difference at (*p < 0.05).

Both catalase (CAT) and superoxide dismutase (SOD) activities increased under salinity stress in both species (Fig. 47). For catalase activity, *H. palea* showed a gradual rise from the control group of 2.66 $U.mL^{1}$ to a peak of 5.6 $U.mL^{1}$ at 15 g/L NaCl-treated conditions, followed by a slight, non-significant decline to 5.4 $U.mL^{1}$ at 21 g/L of salt treatment (Fig. 47a). In contrast, *N. frustulum* showed significantly higher catalase activity in all saline treatments, reaching a maximum of ~8.5 U.mL-^{1} at all salinity conditions tested (3-21 g/L). SOD activity showed a similar trend to CAT, which increased in *H. palea* from 4.63 U.mL ^{1} under control conditions to a peak of 8.93 U.mL ^{1} at 21 g/L NaCl treatment (Fig. 47b). However, *N. frustulum* showed a more pronounced increase, reaching 12.05 and 12.2 $U.mL^{1}$ under conditions of 15 and 21 g/L NaCl, respectively, indicating a stronger antioxidant defense system under salt stress.

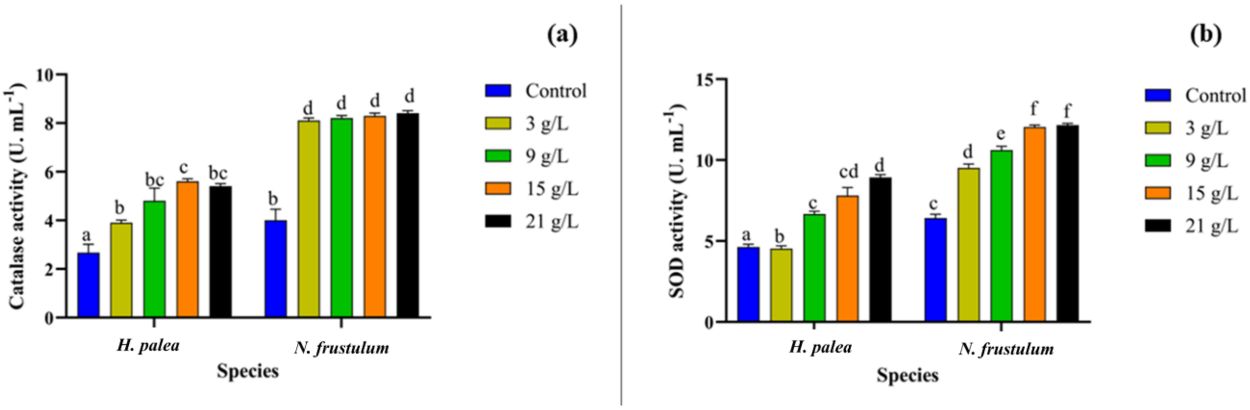

Figure 47: Effect of salinity on diatoms' stress markers, catalase (a) and superoxide dismutase (b) activities.
Data are presented as means and standard errors of three repetitions. According to Tukey's t-test, different letters indicate a significant difference at (*$p < 0.05$).

III.3. Conductivity, chloride, and nutrient content (nitrate and orthophosphate) removal

Conductivity and chloride decreased significantly ($p < 0.05$) from the beginning to the end of the experiment (16th day) in the control group and in certain treated salt cultures of *N. frustulum* compared to *H. palea*. The reduction in conductivity and chloride ranged from 9.6% and 3.7% in the control group to 24.33% and 27.06 %, respectively, in the 21 g/L salt treatment, indicating a significant increase with rising salinity (Table 2).

Table 7: Levels of conductivity and chloride reduction (%) in different treated cultures (control, 3, 9,15, and 21 g/L) of the diatom strains studied.

EC	*H. palea*	*N. frustulum*	Cl^-	*H. palea*	*N. frustulum*
Control	5.2±3.5 ^{a}	9.6±1.8 ^{a}	**Control**	8.01±2.8 ^{a}	3.7±2.8 ^{a}
3	11.02±6.1	-	**3**	15±4.7	-
9	6.2±4.2	-	**9**	4.8±3.1	-
15	-	17.09±4.7 *	**15**	-	13±3.4*
21	-	24.33±1,5*	**21**	-	27.06±6.02**

Significant differences between treated cultures of a species (columns; $p < 0.05$) are indicated by asterisks. Significant variations between diatom strains of the same treatment (rows; $p < 0.05$) are indicated by different letters.

Nitrate and orthophosphate decreased significantly ($p < 0.05$) from the beginning to the end of the experiment (16th day) in the control group and in treated salt cultures in *H. palea* and *N. frustulum* (Table 2). *N. frustulum* showed an exceptionally high nitrate removal capacity across all salinity levels, increasing from 19.02 % in the control group to 95–99.9% under salt stress conditions (3–21 g/L), with highly significant statistical differences ($p < 0.001$). In contrast, *H. palea* exhibited significantly lower NO_3^- removal efficiency, ranging from 29.05% at 9 g/L to a maximum of 38.4 % at 15 g/L under salt conditions. Orthophosphate removal rates remained high consistently across both species and all treatments, showing no statistically significant differences between the two species ($p > 0.05$). *H. palea* consistently achieved removal efficiencies exceeding 86%, whereas *N. frustulum* showed values between 87.23% and 93.58%.

Table 8: Levels of nitrate (NO_3^-) and orthophosphate (PO_4^{3-}) removal (%) in different treated cultures (control, 3, 9,15, and 21 g/L) of the diatom strains studied.

NO_3^-	*H. palea*	*N. frustulum*	PO_4^{3-}	*H. palea*	*N. frustulum*
Control	3,2±0,5^{a}	19,02±2,4^{b}	**Control**	95,2±2,68 ^{a}	87,7±1,6 ^{a}
3	-	95±3***	**3**	94,4±1,8 ^{a}	93,58±2,83 ^{a}
9	29,05±1^{a,***}	98±2^{***,b}	**9**	89,1±4,64 ^{a}	89,85±2,5 ^{a}
15	38,4±2,4 ^{a,***}	99,9±0,1^{***,b}	**15**	86,2±2,1 ^{a}	87,23±1,2 ^{a}
21	25,3±1,6 ^{a,***}	99,9±0,1^{***,b}	**21**	87,3±2,7 ^{a}	89,13±3,82 ^{a}

Significant differences between treated cultures of a species (columns; $p < 0.05$) are indicated by asterisks. Significant variations between diatom strains of the same treatment (rows; $p < 0.05$) are indicated by different letters.

IV. Discussion

In recent years, increasing river salinity, especially in arid and semi-arid regions, has gained widespread recognition as a significant environmental issue affecting all aquatic ecosystems' organisms (Cunillera-Montcusí et al., 2022; Cañedo-Argüelles et al., 2013). Although scientists are increasingly concerned about this issue, its effects on primary producers, notably benthic algae, including diatoms, remain poorly studied (Cunillera-Montcusí et al., 2022). Diatoms are the dominant benthic algae essential to aquatic food webs and nutrient cycling. Therefore, studying their salinity, tolerance, and behavior is crucial. Based on the findings of this study, we provide an overview of how two diatom strains, *H. palea* and *N. frustulum*, from different environments respond and adapt to increasingly saline water conditions.

Salinity tolerance differs significantly among microalgae: Freshwater diatoms could benefit from low salt levels that stimulate their metabolism, but high concentrations can inhibit growth and cause mortality. On the other hand, halophilic diatoms possess mechanisms that allow them to withstand and flourish in high salinity conditions (Stenger-Kovács et al., 2023a). The growth of freshwater *H. palea* in this study increased slightly and significantly at 3 g/L NaCl compared with the control. Still, it decreased with rising salinity (9, 15, and 21 g/L) at the end of the experiment. In contrast, *N. frustulum* growth significantly enhanced as the salinity level increased, reaching its highest level under salinity conditions of 21 g/L NaCl. Our findings are congruent with a study on the growth of *H. palea* (0, 6, 9, 16, and 22 g/L) and *N. frustulum* (9.5, 16, 22, 27, and 32 g/L) at different salinity levels, isolated from the fresh and brackish waters of the Ebro river in Catalonia (Spain), which showed that *H. palea* grows less when the salt level is 16 g/L or higher. On the other hand, *N. frustulum* tolerates a wide range of salinities, showing considerable growth even at 32 g/L (Trobajo et al., 2011) and continuing to grow at 75 g/L salinity (Clavero et al., 2000). Additionally, this species has been reported as an indicator of high salinity, ranging from 0.96 to 19.2 g/L (~1500 to 30000 µS cm-1) (Blinn, 1993). Therefore, it can adapt to a continuous increase in salt conditions, which is ecologically beneficial in environments likely to experience rising salinity levels. Diatoms from an oligotrophic section of the Spring Creek stream showed great productivity and abundance of *H. palea* when the NaCl was increased from 0.25 g/L (~400 µS/cm conductivity) to 2.18 g/L (~3500 µS/cm conductivity) (Cohen, 2010). Bagmet et al. (2017) revealed that the isolated *H. palea* thrives optimally at salinity levels ranging from 1 to 6 g/L, with a notable decline in growth and reproduction as salinity approaches 17 g/L.

Salt stress strongly affects the physiological and biochemical mechanisms underlying the growth and development of microalgae (Zhang et al., 2018; Ismaiel et al., 2018; Lazrak et al., 2024). These include photosynthesis, respiration, osmoregulation, and nutrient uptake (Čáp et al., 2012; Ma et al., 2020; Kuo et al., 2020; Drira et al., 2021). Indeed, osmotic stress from high salt concentrations lowers chlorophyll synthesis and activates chlorophyllase, promoting Chl degradation (Ji et al., 2018). In this study, the 15 and 21 g/L high-salt treatments' *H. palea* chlorophyll *a* declined significantly, from 2.56 to 2.3 and 1.9 µg/mL, respectively, compared with the control group. However, the saline conditions (9, 15, and 21 g/L) significantly raised the *N. frustulum* chlorophyll *a*, with the NaCl 21 g/L treatment yielding the highest value. A previous study revealed that the growth and physiological responses of *H. palea* to different levels of salt and alkaline stress reached their highest chlorophyll a content at a salinity of 6 g/L (60 mM, composed of 20 mM NaCl and 40 mM $NaHCO_3$), in contrast to treatment at a salinity of 9 g/L (Wang et al., 2024). However, *Nitzschia frustulum* prefers high temperature and salinity (Lengyel et al., 2015), suggesting this species maintains efficient photosynthetic performance even under high salt stress, a trait beneficial for survival in fluctuating environments. In response to salt stress, diatoms can enhance carotenoid production to regulate their antioxidants and prevent cell damage caused by ROS (Stenger-Kovács et al., 2023a). In this study, the highest levels of carotenoids were found in both species under increased salt conditions, with *N. frustulum* displaying a greater concentration than *H. palea*.

Under stress conditions, ROS lead to lipid peroxidation in the thylakoid membrane, photoreduction, and Chl triplet generation. Lipid peroxidation is a biomarker of stress in microalgae, usually quantified by measuring the concentration of malondialdehyde (MDA) in cells. This is the main product of lipid peroxidation, which occurs when free radicals oxidize polyunsaturated fatty acids (Pancha et al., 2015; Wang et al., 2020). This was observed in this study, which showed that both diatom species showed a significant increase in MDA content under high salinity conditions compared with control groups. However, *H. palea* generated more MDA than *N. frustulum*, indicating that the latter was less affected by salt stress than *H. palea*. To mitigate detrimental effects, microalgae employ various adaptive strategies and mechanisms to safeguard themselves against salinity stress, such as activating the antioxidant system (Shetty et al., 2019; Verma et al., 2019; Fal et al., 2022).

As part of the antioxidant defense under stressful conditions, microalgae accumulate non-enzymatic ROS scavengers, including proline, betaine-glycine, carbohydrates, and others, which also function as osmoprotective molecules. They also produce ROS-scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), and glutathione reductase (GR) (Pancha et al., 2015; Ismaiel et al., 2018; Drira et al., 2021; Fal et al., 2022). Our study revealed a significant increase in proline levels, which act as non-enzymatic ROS scavengers, in both species. However, *N. frustulum* accumulated more proline at high salinity than *H. palea*, reinforcing its adaptive superiority. The antioxidant enzyme response observed in this study, in particular the activities of superoxide dismutase (SOD) and catalase (CAT), revealed that both species implement defense mechanisms to reduce oxidative damage. *N. frustulum* exhibited elevated activities of superoxide dismutase (SOD) and catalase (CAT) at increased salinity levels, suggesting the presence of a robust antioxidant defense mechanism. This adaptation indicates a strategy for synthesizing co-solutes and osmoregulatory metabolites to mitigate potential damage (Lengyel et al., 2015).

Adapting algae to elevated salt concentrations involves the uptake and export of ions, such as Na+ and K+, facilitated by a redox-driven Na+ pump (Apte and Thomas, 1983; Wiangnon et al., 2007; Farghl et al., 2019). Salt tolerance mechanisms may vary between halophilic and halotolerant taxa. However, species with greater tolerance may exhibit enhanced plasticity in maintaining ion balance, with advanced ion exchange processes becoming more significant than ion accumulation. Furthermore, it was also reported that the ability to reduce conductivity was not definitively correlated with salt tolerance and chloride removal (Farghl et al., 2015, 2019). In this study, conductivity and chloride reduction in *N. frustulum* ranged from 9.6% and 3.7% in the control group to 24.33% and 27.06 % in the 21 g/L salt treatment. This indicates that although *N. frustulum* can thrive and perform metabolic functions in high salt conditions, its ability to eliminate or store chloride ions and reduce overall salinity is low. This also suggests that microalgae are limited in sequestering chloride ions because their main adaptation strategies are centered around sustaining internal ionic balance, rather than removing ions from their surroundings. Consequently, the process of salinity removal appears to be significantly influenced by various environmental factors and is notably dependent on the specific strain involved (Farghl et al., 2019).

N. frustulum was expected to have a greater ability to remove nutrients even at high salt concentrations than *H. plaea*. Indeed, our results demonstrate that *N. frustulum* exhibited greater nutrient removal efficiency under saline conditions, particularly for nitrate and orthophosphate. Nitrate removal by *N. frustulum* approached nearly 100% under high salinity, while *H. palea* showed a significantly lower removal rate. Additionally, the salt concentration significantly influenced nitrate removal, which increased with higher salinity. This may be explained by the production of nitrogen-containing osmolytes in microalgae, such as glycine, betaine, and proline (von Alvensleben et al., 2016; Farghl et al., 2019), but confirmation of this hypothesis requires further research (Farghl et al., 2019). Regarding phosphate removal, it remained high (>86%) under all conditions, indicating that both diatoms can effectively assimilate phosphorus at varying salinity levels. In fact, phosphorus elimination can be improved with an appropriate nitrogen/phosphorus ratio in the culture medium and is not influenced by salt concentration research (Farghl et al., 2019). Indeed, it has been reported that maximum removal of both nutrients occurs when the N:P ratio is between 7 and 10 (Alketife et al., 2107), and that P removal decreases by 15-16%, compared to 100% removal from the medium with an N:P ratio of 1.88 (Arora et al., 2019). In our study, the N:P ratio of the medium was 6.2.

Overall, this study found that *N. frustulum* from saltwater habitats showed greater salt tolerance than *H. palea* in freshwater conditions of the Drâa river. This was indicated by higher growth and chlorophyll a levels at increased salt concentrations in *N. frustulum* compared to *H. palea*. In addition, salt stress induced ROS generation and caused oxidative damage and lipid peroxidation in both diatom strains, with increased MDA levels, especially in *H. palea*. To mitigate this damage, a higher proline content was observed, and the biofilm demonstrated antioxidant defense mechanisms through increased activity of ROS scavenger enzymes, specifically SOD and CAT, with the highest levels observed in *N. frustulum* compared to *H. palea*. In terms of ion removal capacity, *N. frustulum* sequestered fewer chloride ions as its adaptation focuses on maintaining internal ionic balance rather than removing surrounding ions.

Nevertheless, it showed higher nitrate removal efficiency under saline conditions than *H. palea*, although both diatom strains displayed significant phosphate removal under all conditions.

V. Conclusion

This experimental study showed that increasing salinity impacts the growth and physiology of diatom strains, with an increase in halotolerant *N. frustulum* and a decrease in freshwater *H. palea*. This was coupled by salt stress-induced oxidative damage and lipid peroxidation in both diatom strains, with increased MDA levels, especially in freshwater *H. palea* compared to *N.frsutulum*. In this study, diatom strains trigger defense mechanisms by accumulating osmoprotective compounds, mainly proline, and boosting antioxidant enzymes such as SOD and CAT. Furthermore, *N. frustulum* showed more significant physiological plasticity in response to elevated salinity compared to *H. palea*. This suggests that *N. frustulum* is able to withstand a range of salinities and occupy a variety of salinity-related ecological niches, giving it a distinct advantage over other diatom species. However, with increasing salinity, *H. palea* can decrease or disappear at certain thresholds. Consequently, increasing freshwater salinization can lead to a reduction or disappearance of some microalgae, while others can thrive up to certain salinity thresholds. *N. frustulum* sequestered fewer chloride ions, concentrating on internal ion balance rather than removal of surrounding ions. However, nitrate removal efficiency under saline conditions was superior to that of *H. palea*, while both diatom strains achieved significant phosphate removal under all conditions. This highlights the significant role of microalgae, including diatoms, in pollutant and nutrient uptake, contributing to water self-purification. Furthermore, additional studies are needed to conduct field investigations to confirm laboratory results in natural ecosystems exhibiting varying salinity levels. Additionally, performing transcriptomic and proteomic analyses will clarify the essential genes and proteins linked to halotolerance. Lastly, it is essential to investigate the complexities of the mechanisms responsible for ion removal, especially nitrate and phosphate.

General conclusions and perspectives

IRES are more frequent in arid and semi-arid zones, especially in North Africa, and are expected to increase due to climate change, human activities, and the growing water demand. These intermittent rivers suffer from water scarcity and increased salinity, both natural and man-made, making the water unsuitable for aquatic life, agriculture, and human use, ultimately compromising local livelihoods and species conservation. Despite their importance and functional diversity, IRES are among the most threatened ecosystems and often receive less attention than perennial streams, resulting in inadequate assessment and management protection. Moreover, while freshwater salinization is a growing concern, our understanding of its effects on IRES in arid regions, particularly in North Africa, remains limited. This gap is most evident at the least studied trophic levels, particularly primary producers, which are mainly benthic algae. The Drâa river (Southeastern Morocco) is among those in the arid regions of North Africa that face such challenges. To this end, the main objective of this thesis is to investigate the structure, composition, and dynamics of benthic microalgae in the arid Drâa river. This involves examining the effects of salinization and assessing the ecological status and water quality.

This aim was assessed using fieldwork and laboratory approaches. Fieldwork involved investigating microalgal communities along salinity gradients during different hydrological periods using morphological and DNA metabarcoding analyses, examining water quality through physicochemical parameters and diatom index based on microscopic and molecular data, and studying community responses to salinity via a biofilm transfer experiment. In the laboratory, we assessed the salinity impacts on natural biofilms from the Drâa river through a microcosm experiment. We also evaluated the responses of two diatom strains from different saline environments to increasing salt under controlled culture conditions.

Our results revealed that the salinity in the Drâa river increased during dry periods compared to the wet ones due to reduced water flow and heightened evaporation, particularly in the middle and lower Drâa. The morphological and DNA metabarcoding characterization of microalgae community composition indicates that salinity and hydrological conditions significantly influence their assemblages, particularly diatoms, with salinity being the primary factor affecting them. This indicates that diatoms exhibit heightened sensitivity to environmental disturbances, with two stress factors- salinity and dry periods- potentially interacting synergistically. This information provides data on the effects of salinity on microalgae assemblages in an intermittent desert river in southern Morocco during dry and wet periods.

Examining water quality index through physicochemical parameters revealed that mineralization (conductivity and chloride) influences the lower and middle Drâa, while the upper Drâa was affected by factors like phosphorus. This area experiences less pollution than the middle and lower Drâa, which face anthropogenic pressures. IPS diatom index assessments matched the water quality index (WI) in the upper Drâa, while the lower Drâa exhibited poorer water quality, showing a strong response to point source pollution. The IPS diatom index provides reliable information about the Drâa river's historical conditions by encompassing long-term shifts. This underscores the significance of benthic diatoms as bioindicators for assessing the water quality and ecological status of the Drâa river. Therefore, using physico-chemical parameters and the IPS diatom index can provide a complete assessment of water quality in the Drâa river, capturing both current and long-term (nearly one month) conditions. Additionally, the diatom IPS index obtained from the morphology and DNA metabarcoding methods showed a significant correlation, which can be used to improve the assessment of water quality and ecological status using both methods for the Drâa river.

The transfer experiment demonstrated that increasing salinity in the Drâa river negatively affects benthic microalgae, both in terms of biomass and diversity, while lower salinity in brackish areas can benefit their growth. This understanding can help address the risks associated with rising salinization of the Drâa river, predict the consequences on aquatic ecosystem services and water quality, and promote the consideration of reducing anthropogenic stressors that contribute to the secondary effects of salinization.

To confirm these findings, the freshwater natural biofilms of the Drâa river were exposed to varying salinity levels (1, 10, 30 g/L NaCl) through a microcosm experiment. The freshwater biofilm communities exhibit physiological and biochemical changes in response to salt stress for survival, albeit only within a certain resistance threshold. Low salinity levels in the Drâa river may enhance freshwater biofilm development, while higher levels could negatively impact it. Specifically, the effect of increasing salinity was evaluated on two diatom species sourced from distinct salt habitats in the Drâa river. The findings revealed that the halotolerant diatom strains exhibit greater physiological plasticity when facing increased salinity than the freshwater diatom strain. This indicates that with rising salinity in the Drâa river, freshwater diatoms might decline or vanish beyond specific thresholds. Meanwhile, halotolerant diatoms can tolerate various salinities.

In summary, this thesis emphasizes that salinity significantly affects the composition and structure of the microalgal community in the Drâa river. As the region confronts rising water scarcity and salinization, the implications for primary producers may ripple through the entire food web, impacting local populations. Protecting the area's freshwater ecosystems from additional stressors, such as secondary salinization and overexploitation, is crucial. Furthermore, it is vital to prevent saline rivers from exacerbating salinity levels, preserving natural water flows, and maintaining habitat connectivity. Encouraging sustainable irrigation practices and water-efficient crops can alleviate some pressure on the region's water resources.

Nevertheless, in light of these thesis findings, we estimate that further studies will need to be conducted to investigate, namely:

1) Assessing the impact of salinity on the entire trophic web, from the microalgae communities to the higher trophic levels (local population), would provide valuable insights into the potential cascading effects of salinity on the Drâa river.
2) Investigating the impact of multiple environmental stressors (e.g., temperature, oxygen) on benthic microalgae in the Drâa river
3) Further research is necessary to improve DNA reference databases for microalgae species, including diatoms that are mainly found and endemic to North Africa, particularly Morocco.
4) Adapting a diatom index to monitor the ecological condition of Morocco's arid Drâa river and similar intermittent, saline rivers, and explore new assessment methods such as DNA metabarcoding.
5) Further research is needed to determine the salinity threshold for freshwater biofilm in the Drâa river to support effective ecosystem management.
6) It would be interesting to conduct transcriptomic and proteomic analyses to understand the essential genes and proteins associated with halotolerance compared to the freshwater strain.
7) Valorization through the use of halotolerant microalgal species, applicable in biomass production for aquaculture and various biotechnological applications.
8) Future policies and action plans should take into account the growing impact of salinity and water scarcity in the Drâa river on all components of the aquatic ecosystem, including local populations, in order to improve environmental and social well-being.

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Supplementary material

Supplementary material. Chapter 2

Table S1. Physico-chemical and hydrological parameters of sampled sites in the Drâa river according to different salinity conditions and hydrological periods.

EC: Conductivity. DO: Dissolved oxygen. PO_4^{3-}: Orthophosphate. NH_4^+: Ammonium. NO_3^-: Nitrate.SO_4^{2-}: Sulfate. Cl^-: Chloride. HCO_3: Total alkalinity. $CaCO_3$: Total hardness. FV: Flow velocity. FR: Flow rate. NA: Not assessed. Grey highlighted cells indicate dry rivers.

N°	Sub-basins	Site name	Code	X	Y	Altitude (m)	Period	Season	Salinity class	EC (µS/cm)	Temperature (°C)	Salinity (g/L)	DO	pH	PO43- (mg/L)	NH4+ (mg/L)	NO3- (mg/L)	SO42- (mg/L)	Cl- (mg/L)	HCO3 (mg/L)	CaCO3 (mg/L)	FV (m/s)	FR (m³/s)
1	upper Drâa	Ounila Upstream	IO1_1	-7.1542015	31.2617547	1747	October_2020	Dry	Freshwater	1849	17,36	0,94	5,00	7,92	0,94	0,14	1,24	177	305	292	369	NA	NA
2		Ounila Downstream	IO2_1	-7.140803	31.146719	1412	October_2020	Dry	Brackish_water	3032	24,38	1,54	4,14	7,59	0,08	0,07	0,16	174	859	318	405	NA	NA
3		El Mellah	IE_1	-7.10006	31.011	1231	October_2020	Dry	Brackish_water	10634	23,80	5,62	3,91	7,56	0,14	0,03	0,21	77	4791	272	982	NA	NA
4		Iriri	IR_1	-7.19923	30.94573	1252	October_2020	Dry	Freshwater	663	18,03	0,35	3,26	7,91	0,06	0,23	0,71	10	158	246	301	NA	NA
5		Ait douchen	A1_1	-7.094214	30.657043	1332	October_2020	Dry	Freshwater	1018	22,60	0,53	2,77	7,88	0,06	0,03	2,31	44	202	296	167	NA	NA
6		Tarmigte	A2_1	-6.84642778	30.8652278	1114	October_2020	Dry	Freshwater	1416	26,99	0,78	2,13	8,27	0,05	0,05	0,07	54	297	384	512	NA	NA
7		Dades Upstream	D1_1	-5.873425	31.593432	1801	October_2020	Dry															
8		Dades Gorges	D2_1	-5.908688	31.556222	1753	October_2020	Dry	Freshwater	1312	16,88	0,70	3,47	7,97	0,04	0,05	1,17	65	220	290	337	NA	NA
9		Dades Middle	D3_1	-5.94536	31.50473	1657	October_2020	Dry	Freshwater	1127	21,10	0,58	3,95	8,09	0,05	0,08	0,82	52	192	220	281	NA	NA
10		M'goun Upstream	DM1_1	-6.171667	31.365555	1547	October_2020	Dry	Freshwater	1102	11,98	0,57	2,43	8,22	1,74	0,03	0,84	113	178	210	276	NA	NA
11		M'goun Downstream	DM2_1	-6.182975	31.330998	1511	October_2020	Dry	Freshwater	1110	15,68	0,57	6,39	8,10	0,04	0,03	0,80	111	171	196	265	NA	NA
12		Dades Downstream	D4_1	-6.49404	31.01195	1188	October_2020	Dry	Brackish_water	6016	26,74	3,26	3,72	7,37	0,06	0,04	0,96	344	1803	314	762	NA	NA
13	middle Drâa	Kasbah Tamnogalt	DR1_1	-6.407056	30.674778	904	October_2020	Dry															
14		Taghzout	DR2_1	-5.984194444	30.4486944	781	October_2020	Dry															
15		Tamgroute	DR3_1	-5.671111	30.236556	1332	October_2020	Dry	Brackish_water	3828	22,96	1,96	3,34	8,31	0,04	0,02	0,03	227	944	110	315	NA	NA
16	lower Drâa	Akka Nait Sidi salty	LT1_1	-7.33102	29.911138	702	October_2020	Dry	Saline_water	9547	29,23	5,20	3,70	7,53	0,08	0,12	0,70	263	7657	672	1109	NA	NA
17		Akka Nait Sidi less salty	LT2_1	-7.330219444	29.9098167	562	October_2020	Dry	Brackish_water	5654	27,52	3,01	3,55	7,64	0,11	0,01	1,62	257	2491	788	867	NA	NA
18		Tissint Upstream	LT3_1	-7.196667	29.822222	487	October_2020	Dry	Saline_water	11567	24,97	6,43	3,00	8,00	0,23	0,00	0,13	347	9906	464	1302	NA	NA
19		Tissint Downstream	LT4_1	-7.168112	29.779739	483	October_2020	Dry	Saline_water	13227	26,41	7,51	2,78	8,10	1,06	0,00	0,30	367	11162	428	1409	NA	NA
1	upper Drâa	Ounila Upstream	IO1_2	-7.1542015	31.2617547	1747	April_2021	Wet	Brackish_water	1418	15,92	0,87	5,93	7,30	0,22	0,03	0,25	198	802	112	162	NA	NA
2		Ounila Downstream	IO2_2	-7.140803	31.146719	1412	April_2021	Wet	Brackish_water	1624	16,10	1,00	4,54	7,55	0,03	0,04	0,20	156	944	122	190	NA	NA
3		El Mellah	IE_2	-7.10006	31.011	1231	April_2021	Wet	Brackish_water	5223	25,57	2,77	4,18	7,27	0,07	0,06	0,79	87	1023	148	498	NA	NA
4		Iriri	IR_2	-7.19923	30.94573	1252	April_2021	Wet	Freshwater	568	19,13	0,31	5,81	7,35	0,04	0,05	0,13	6	119	242	98	NA	NA
5		Ait douchen	A1_2	-7.094214	30.657043	1332	April_2021	Wet	Freshwater	854	18,69	0,45	4,38	7,46	0,01	0,10	2,00	28	274	174	126	NA	NA
6		Tarmigte	A2_2	-6.84642778	30.8652278	1114	April_2021	Wet	Freshwater	1079	24,90	0,53	6,54	7,71	0,01	0,43	0,09	46	338	198	134	NA	NA
7		Dades Upstream	D1_2	-5.873425	31.593432	1801	April_2021	Wet	Freshwater	644	13,83	0,40	6,57	7,26	0,12	0,03	0,92	34	170	98	101	NA	NA
8		Dades Gorges	D2_2	-5.908688	31.556222	1753	April_2021	Wet	Freshwater	806	15,03	0,50	5,93	7,85	0,25	0,03	0,41	78	197	102	117	NA	NA
9		Dades Middle	D3_2	-5.94536	31.50473	1657	April_2021	Wet	Freshwater	835	15,30	0,51	5,70	7,70	0,15	0,04	0,41	51	167	113	158	NA	NA
10		M'goun Upstream	DM1_2	-6.171667	31.365555	1547	April_2021	Wet	Freshwater	626	18,78	0,35	6,21	7,22	0,37	0,06	0,30	95	120	206	123	NA	NA
11		M'goun Downstream	DM2_2	-6.182975	31.330998	1511	April_2021	Wet	Freshwater	774	21,07	0,41	6,07	7,35	0,12	0,03	0,28	111	106	209	109	NA	NA
12		Dades Downstream	D4_2	-6.49404	31.01195	1188	April_2021	Wet	Freshwater	705	13,24	0,45	5,72	7,31	0,02	0,06	0,05	280	128	208	134	NA	NA
13	middle Drâa	Kasbah Tamnogalt	DR1_2	-6.407056	30.674778	904	April_2021	Wet	Brackish_water	1241	19,30	0,70	5,94	7,37	0,13	0,11	0,67	27	1143	128	362	NA	NA
14		Taghzout	DR2_2	-5.984194444	30.4486944	781	April_2021	Wet	Brackish_water	2435	24,83	1,25	6,35	7,42	0,10	0,02	0,46	30	1207	196	328	NA	NA
15		Tamgroute	DR3_2	-5.671111	30.236556	1332	April_2021	Wet	Brackish_water	1961	24,61	1,00	6,66	7,43	0,02	0,09	0,04	202	972	128	252	NA	NA
16	lower Drâa	Akka Nait Sidi salty	LT1_2	-7.33102	29.911138	702	April_2021	Wet	Saline_water	9101	23,76	5,22	6,31	7,37	0,02	0,27	0,70	219	8031	412	470	NA	NA
17		Akka Nait Sidi less salty	LT2_2	-7.330219444	29.9098167	562	April_2021	Wet	Brackish_water	5414	25,74	2,87	6,29	7,39	0,01	0,13	0,83	199	2020	104	534	NA	NA
18		Tissint Upstream	LT3_2	-7.196667	29.822222	487	April_2021	Wet	Saline_water	11537	27,36	6,24	7,01	7,38	0,07	0,11	0,17	307	10860	454	782	NA	NA
19		Tissint Downstream	LT4_2	-7.168112	29.779739	483	April_2021	Wet	Saline_water	13310	28,94	7,05	7,24	7,33	0,02	0,06	0,39	379	12080	276	848	NA	NA
1	upper Drâa	Ounila Upstream	IO1_3	-7.1542015	31.2617547	1747	October_2021	Dry	Brackish_water	2810	21,60	1,57	7,99	7,10	0,40	0,12	1,75	202	534	309	517	0,30	0,07
2		Ounila Downstream	IO2_3	-7.140803	31.146719	1412	October_2021	Dry	Brackish_water	3820	20,70	2,22	8,10	7,90	0,42	0,14	0,21	213	938	338	541	0,44	0,14
3		El Mellah	IE_3	-7.10006	31.011	1231	October_2021	Dry	Saline_water	11520	25,10	6,54	6,83	7,70	0,70	0,03	0,81	133	5694	398	1254	0,23	0,01
4		Iriri	IR_3	-7.19923	30.94573	1252	October_2021	Dry	Freshwater	649	19,80	0,35	8,44	7,80	0,40	0,38	0,19	19	148	187	311	0,07	0,03
5		Ait douchen	A1_3	-7.094214	30.657043	1332	October_2021	Dry	Freshwater	1134	22,40	0,59	5,60	7,74	0,61	0,10	2,05	50	187	327	178	0,00	0,00
6		Tarmigte	A2_3	-6.84642778	30.8652278	1114	October_2021	Dry	Freshwater	1239	25,50	0,61	14,02	7,53	0,44	0,03	0,10	50	372	289	428	0,01	0,01
7		Dades Upstream	D1_3	-5.873425	31.593432	1801	October_2021	Dry															
8		Dades Gorges	D2_3	-5.908688	31.556222	1753	October_2021	Dry	Freshwater	1163	17,50	0,68	10,60	8,52	0,40	0,06	1,98	81	218	286	247	0,09	0,04
9		Dades Middle	D3_3	-5.94536	31.50473	1657	October_2021	Dry	Freshwater	1077	16,50	0,65	9,09	8,50	0,47	0,03	0,87	67	142	201	332	0,34	0,02
10		M'goun Upstream	DM1_3	-6.171667	31.365555	1547	October_2021	Dry	Freshwater	1030	19,50	0,57	8,76	8,40	0,53	0,02	0,91	102	111	171	223	0,47	0,45
11		M'goun Downstream	DM2_3	-6.182975	31.330998	1511	October_2021	Dry	Freshwater	1087	19,90	0,60	8,70	8,16	0,44	0,03	0,87	113	128	180	229	0,18	0,04
12		Dades Downstream	D4_3	-6.49404	31.01195	1188	October_2021	Dry	Freshwater	1792	21,30	0,98	15,83	8,01	0,82	0,05	0,94	302	198	191	202	0,02	0,01
13	middle Drâa	Kasbah Tamnogalt	DR1_3	-6.407056	30.674778	904	October_2021	Dry	Brackish_water	2500	25,20	1,28	17,23	8,06	0,33	0,12	1,29	176	1151	276	497	0,00	0,00
14		Taghzout	DR2_3	-5.984194444	30.4486944	781	October_2021	Dry															
15		Tamgroute	DR3_3	-5.671111	30.236556	1332	October_2021	Dry															
16	lower Drâa	Akka Nait Sidi salty	LT1_3	-7.33102	29.911138	702	October_2021	Dry	Saline_water	10220	23,60	5,94	9,60	7,70	0,95	0,02	2,26	292	9002	702	1189	0,12	0,02
17		Akka Nait Sidi less salty	LT2_3	-7.330219444	29.9098167	562	October_2021	Dry	Brackish_water	5680	25,60	3,03	12,40	7,30	1,03	0,10	2,89	261	2443	627	883	0,17	0,02
18		Tissint Upstream	LT3_3	-7.196667	29.822222	487	October_2021	Dry	Saline_water	11210	24,30	6,46	11,16	8,20	0,82	0,02	0,16	339	9856	461	1285	0,17	0,04
19		Tissint Downstream	LT4_3	-7.168112	29.779739	483	October_2021	Dry	Saline_water	14240	18,80	9,49	8,72	8,90	0,80	0,30	0,34	396	12154	425	1472	0,08	0,04

1	upper Drâa	Ounila Upstream	IO1_4	-7.1542015	31.2617547	1747	March_2022	Wet	Brackish_water	2150	18,50	1,27	6,05	8,30	0,36	0,02	0,09	188	1082	262	267	0,33	0,05
2		Ounila Downstream	IO2_4	-7.140803	31.146719	1412	March_2022	Wet	Brackish_water	3080	18,30	1,87	6,10	8,30	0,42	0,01	0,01	212	1204	132	305	0,30	0,06
3		El Mellah	IE_4	-7.10006	31.011	1231	March_2022	Wet	Saline_water	10570	17,10	7,17	5,94	8,10	0,62	0,03	0,02	123	5027	341	906	0,17	0,01
4		Iriri	IR_4	-7.19923	30.94573	1252	March_2022	Wet	Freshwater	613	16,10	0,36	6,64	8,50	0,38	0,03	0,03	14	129	78	93	0,07	0,02
5		Ait douchen	A1_4	-7.094214	30.657043	1332	March_2022	Wet	Freshwater	1137	20,10	0,63	5,00	7,80	0,52	0,21	1,07	38	327	108	158	0,02	0,01
6		Tarmigte	A2_4	-6.84642778	30.8652278	1114	March_2022	Wet	Freshwater	1175	20,70	0,64	7,15	8,40	0,30	0,02	0,15	49	341	171	151	0,08	0,01
7		Dades Upstream	D1_4	-5.873425	31.593432	1801	March_2022	Wet	Freshwater	670	10,70	0,46	6,54	8,51	0,31	0,02	0,53	32	181	111	109	0,37	0,80
8		Dades Gorges	D2_4	-5.908688	31.556222	1753	March_2022	Wet	Freshwater	792	14,20	0,50	6,10	8,40	0,30	0,04	0,36	66	142	103	109	0,15	0,13
9		Dades Middle	D3_4	-5.94536	31.50473	1657	March_2022	Wet	Freshwater	779	13,10	0,50	6,18	8,50	0,34	0,05	0,41	44	112	91	141	0,64	0,09
10		M'goun Upstream	DM1_4	-6.171667	31.365555	1547	March_2022	Wet	Freshwater	883	15,10	0,54	6,00	8,40	0,37	0,06	0,89	103	171	212	158	0,55	0,54
11		M'goun Downstream	DM2_4	-6.182975	31.330998	1511	March_2022	Wet	Freshwater	861	15,20	0,53	6,64	8,50	0,35	0,07	0,57	111	163	198	147	0,56	0,35
12		Dades Downstream	D4_4	-6.49404	31.01195	1188	March_2022	Wet	Freshwater	1182	13,20	0,77	6,04	8,40	0,40	0,07	0,35	301	365	247	287	0,52	0,49
13	middle Drâa	Kasbah Tamnogalt	DR1_4	-6.407056	30.674778	904	March_2022	Wet	Freshwater	260	23,00	0,13	6,14	9,50	0,27	0,03	0,50	219	19	71	40	0,00	0,00
14		Taghzout	DR2_4	-5.984194444	30.4486944	781	March_2022	Wet															
15		Tamgroute	DR3_4	-5.671111	30.236556	1332	March_2022	Wet															
16	lower Drâa	Akka Nait Sidi salty	LT1_4	-7.33102	29.911138	702	March_2022	Wet	Saline_water	10210	23,90	5,90	6,37	7,90	0,90	0,13	0,81	290	8274	423	489	0,13	0,05
17		Akka Nait Sidi less salty	LT2_4	-7.330219444	29.9098167	562	March_2022	Wet	Brackish_water	5620	20,90	3,33	6,84	7,80	0,92	0,04	1,31	201	3001	120	411	0,14	0,02
18		Tissint Upstream	LT3_4	-7.196667	29.822222	487	March_2022	Wet	Saline_water	11970	15,10	8,62	6,10	8,50	0,86	0,03	0,06	348	1093	443	703	0,12	0,04
19		Tissint Downstream	LT4_4	-7.168112	29.779739	483	March_2022	Wet	Saline_water	13680	21,70	8,49	5,88	8,60	0,72	0,01	0,42	372	12673	487	896	0,16	0,03

Table S2. Morphologically identified cyanobacteria during all periods studied.
The meaning of the code sites is given in Table S1.

Taxons	IO1	IO2	IR	IE	A1	A2	D1	D2	D3	DM1	DM2	D4	DR1	DR2	DR3	LT1	LT2	LT3	LT4
O/Synechococcales																			
F/Synechococcaceae																			
G/*Synechococcus*																			
Synechococcus sp. Nägeli	x	x		x	x					x								x	x
G/*Cyanobium*																			
Cyanobium sp. R.Rippka & G.Cohen-Bazire																		x	
F/Merismopediaceae																			
G/*Aphanocapsa*																			
Aphanocapsa sp1 Nägeli	x	x		x		x	x			x		x		x			x		
Aphanocapsa sp2 Nägeli			x										x						
G/*Synechocystis*																			
Synechocystis sp. Sauvageau	x	x	x		x	x			x	x	x		x	x	x		x		
G/*Merismopedia*																			
Merismopedia sp1 Meyen	x																		
Merismopedia sp2 Meyen	x															x	x		
Merismopedia sp3 Meyen											x	x						x	
F/Pseudanabaenaceae																			
G/*Jaaginema*																			
Jaaginema sp. Anagnostidis & Komárek																		x	
G/*Pseudanabaena*																			
Pseudanabaena articulata Sjuka	x	x	x		x	x				x	x	x							
Pseudanabaena limnetica (Lemmermann) Komárek	x	x											x			x			
Pseudanabaena minima (G.S.An) Anagnostidis					x					x								x	
Pseudanabaena sp1 Lauterborn				x	x												x	x	x

Pseudanabaena sp2 Lauterborn		x	x		x	x				x	x								
G/*Limnothrix*																			
Limnothrix pseudospirulina (Pascher) Anagnostidis			x																
Limnothrix sp1 M.-E.Meffert					x														
Limnothrix sp2 M.-E.Meffert	x										x								
F/Leptolyngbyaceae																			
G/*Leptolyngbya*																			
Leptolyngbya sp1 Anagnostidis & Komárek				x	x											x	x	x	x
Leptolyngbya sp2 Anagnostidis & Komárek		x													x				
Leptolyngbya sp3 Anagnostidis & Komárek	x	x			x			x	x	x	x	x	x						
Leptolyngbya subcapitata (Petersen) Anagnostidis						x													
F/Schizotrichaceae																			
G/*Schizothrix*																			
Schizothrix sp. Kützing ex Gomont																x			
O/Oscillatoriales																			
F/Oscillatoriaceae																			
G/*Lyngbya*																			
Lyngbya sp1 C.Agardh ex Gomont																			x
Lyngbya sp2 C.Agardh ex Gomont															x	x			x
G/*Oscillatoria*																			
Oscillatoria jenensis G.Schmid					x	x								x		x			
Oscillatoria sp. Vaucher ex Gomont			x	x									x		x	x	x	x	x
G/*Phormidium*																			
Phormidium sp1 Kützing ex Gomont			x		x	x		x				x	x	x					
Phormidium sp2 Kützing ex Gomont																x	x	x	
F/Borziaceae																			
G/*Borzia*																			
Borzia sp.															x				

F/Coleofasciculaceae																		
G/*Geitlerinema*																		
Geitlerinema splendidum (Gomont) Anagnostidis															x			
F/Gomontiellaceae																		
G/*Gomontiella*																		
Gomontiella sp. Teodoresco				x														
G/*Hormoscilla*																		
Hormoscilla sp. Anagnostidis & Komárek															x			
F/Cyanothecaceae																		
G/*Cyanothece*																		
Cyanothece sp. Komárek							x		x								x	
F/Microcoleaceae																		
G/*Arthrospira*																		
Arthrospira sp. Stizenberger ex Gomont			x													x	x	x
O/Chroococcales																		
F/Aphanothecaceae																		
G/*Aphanothece*																		
Aphanothece sp. Nägeli					x	x										x	x	x
G/*Gloeothece*																		
Gloeothece sp1 Nägeli																	x	
Gloeothece sp2 Nägeli																	x	
F/Chroococcaceae																		
G/*Chroococcus*																		
Chroococcus sp1 Nägeli	x	x			x			x	x			x	x	x	x			
Chroococcus sp2 Nägeli				x								x						
G/*Gloeocapsa*																		
Gloeocapsa sp1 Kützing				x											x	x		x
Gloeocapsa sp2 Kützing				x														

G/*Gloeocapsopsis*																			
Gloeocapsopsis sp. Geitler ex Komárek					x														
G/*Geminocystis*																			
Geminocystis sp. Korelusová, Kaštovský & Komárek										x			x						x
O/Spirulinales																			
F/Spirulinaceae																			
G/*Spirulina*																			
Spirulina subtilissima Kützing ex Gomont	x	x		x						x								x	
O/Nostocales																			
F/Nostocaceae																			
G/*Nostoc*																			
Nostoc sp. Vaucher ex Bornet & Flahault						x													
O/Chroococcidiopsidales																			
F/Chroococcidiopsidaceae																			
G/*Chroococcidiopsis*																			
Chroococcidiopsis sp1 Geitler		x																x	
Chroococcidiopsis sp2 Geitler		x				x											x		
O/Pleurocapsales																			
F/Hyellaceae																			
G/*Chroococcopsis*																			
Chroococcopsis sp.						x						x	x					x	

Table S3. Morphologically identified green algae during all periods studied.
The meaning of the code sites is given in Table S1.

Taxa	**IO1**	**IO2**	**IR**	**IE**	**A1**	**A2**	**D1**	**D2**	**D3**	**DM 1**	**DM 2**	**D4**	**DR1**	**DR2**	**DR3**	**LT1**	**LT2**	**LT3**	**LT4**
O/Chaetophorales																			
F/Aphanochaetaceae																			
G/Aphanochaete																			
Aphanochaete elegans Tupa	x		x					x		x	x								
Aphanochaete repens A.Braun	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x		
F/Fritschiellaceae																			
G/Stigeoclonium																			
Stigeoclonium sp. Kützing	x	x	x	x	x	x	x	x		x	x	x	x						
O/Trebouxiales																			
F/Botryococcaceae																			
G/Botryococcus																			
Botryococcus braunii Kützing																	x		x
F/Trebouxiaceae																			
G/Myrmecia																			
Myrmecia bisecta Reisigl			x																
Myrmecia sp. Printz			x					x									x		
G/Trebouxia																			
Trebouxia sp. Puymaly			x																
O/Chaetopeltidales																			
F/Chaetopeltidaceae																			
G/Chaetopeltis																			
Chaetopeltis orbicularis Berthold			x	x				x					x						
O/Chlamydomonadales																			
F/Chlamydomonadaceae																			
G/Chlamydomonas																			

Chlamydomonas sp. Ehrenberg	x				x	x	x			x		x	x	x					
F/Chlorochytriaceae																			
G/Chlorochytrium																			
Chlorochytrium sp. Cohn	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
F/Chlorococcaceae																			
G/Chlorococcum																			
Chlorococcum sp. Meneghini		x			x		x			x		x	x				x		x
F/Haematococcaceae																			
G/Chlorogonium																			
Chlorogonium elongatum (P.A.Dangeard) Francé			x																
F/Chlorosarcinaceae																			
G/Neochlorosarcina																			
Neochlorosarcina minor (Gerneck) V.M.Andreeva		x															×		
O/Chlorellales																			
F/Chlorellaceae																			
G/Chlorella																			
Chlorella sp. Beyerinck [Beijerinck]		x			x	x	x			x		x	x		x	x	x	x	
G/Closteriopsis																			
Closteriopsis acicularis (Chodat) J.H.Belcher & Swale																	x		
G/Nannochloris																			
Nannochloris sp. Naumann	x									x								x	
G/Neochloris																			
Neochloris sp. Starr					x														
Neochloris vigensis P.A.Archibald	x	x	x	x	x	x		x		x	x	x							
O/Cladophorales																			

F/Cladophoraceae																			
G/Cladophora																			
Cladophora glomerata (Linnaeus) Kützing	x		x	x	x	x		x		x	x		x						
Cladophora sp. Kützing	x	x	x	x				x		x	x	x	x	x		x	x	x	x
O/Desmidiales																			
F/Closteriaceae																			
G/Closterium																			
Closterium sp. Nitzsch ex Ralfs																	x		
F/Desmidiaceae																			
G/Cosmarium																			
Cosmarium formosulum Hoff													x	x					
Cosmarium sp. Corda ex Ralfs			x					x					x						
O/Sphaeropleales																			
F/Scenedesmaceae																			
G/Desmodesmus																			
Desmodesmus sp. (Chodat) S.S.An, T.Friedl & E.Hegewald		x		x	x	x		x		x	x		x	x					
Desmodesmus communis (E.Hegewald) E.Hegewald																	x		x
G/Scenedesmus																			
Scenedesmus armatus (Chodat) Chodat			x	x															
Scenedsmus sp. Meyen	x		x	x	x	x		x		x	x	x	x	x		x	x	x	x
Scenedesmus abundans (O.Kirchner) Chodat	x	x	x		x	x	x	x		x	x	x					x		
G/Tetradesmus																			
Tetradesmus sp. G.M.Smith			x	x									x						
F/Selenastraceae																			
G/Monoraphidium																			
Monoraphidium sp. Komárková-Legnerová	x	x	x	x	x		x	x	x	x	x	x	x						

F/Mychonastaceae																	
G/Mychonastes																	
Mychonastes jurisii (Hindák) Krienitz, C.Bock, Dadheech & Proschold	x		x	x	x	x								x		x	x
F/Hydrodictyaceae																	
G/Pediastrum																	
Pediastrum boryanum (Turpin) Meneghini				x													
Pediastrum duplex Meyen			x		x												
Pediastrum sp. Meyen					x												
G/Stauridium																	
Stauridium tetras (Ehrenberg) E.Hegewald			x														
F/Radiococcaceae																	
G/Radiococcus																	
Radiococcus sp. Schmidle			x	x							x	x	x				
O/Zygnematales																	
F/Zygnemataceae																	
G/Mougeotia																	
Mougeotia sp. C.Agardh				x													
O/Oedogoniales																	
F/Oedogoniaceae																	
G/Oedogonium																	
Oedogonium howardii G.S.West	x		x	x	x	x	x	x		x	x	x	x				
Oedogonium sp. Link ex Hirn			x	x	x	x	x	x			x	x	x		x		
O/Ulotrichales																	
F/Ulotrichaceae																	
G/Ulothrix																	
Ulothrix sp. Kützing			x	x	x	x											

Table S4. Morphologically identified diatoms during all periods studied.
The meaning of the code sites is given in Table S1.

Taxon	**IO1**	**IO2**	**IR**	**IE**	**A1**	**A2**	**D1**	**D2**	**D3**	**DM1**	**DM2**	**D4**	**DR1**	**DR2**	**DR3**	**LT1**	**LT2**	**LT3**	**LT4**
G/*Achnanthidium*																			
Achnanthidium minutissimum (Kützing) Czarnecki	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x		
Achnanthidium saprophilum (H.Kobayashi & Mayama) Round & Bukhtiyarova	x		x	x	x	x		x	x	x	x	x				x	x		
G/*Amphipleura*																			
Amphipleura pellucida (Kützing) Kützing							x		x										
G/*Amphora*																			
Amphora advena VanLandingham																x	x		
Amphora capitellata F.Meister															x	x			
Amphora castellata Giffen																	x		
Amphora inariensis Krammer			x		x			x				x	x						
Amphora pediculus (Kützing) Grunow	x		x		x	x		x	x		x	x	x				x		
Amphora sp. Ehrenberg ex Kützing																x		x	x
Amphora libyca Ehrenberg	x				x		x	x				x	x		x				
G/*Aulacoseira*																			
Aulacoseira granulata (Ehrenberg) Simonsen			x						x			x			x			x	x
G/*Bacillaria*																			
Bacillaria paxillifera (O.F.Müller) T.Marsson				x											x		x		
G/*Brachysira*																			
Brachysira vitrea (Grunow) R.Ross					x	x	x	x	x	x	x	x	x				x		
Brachysira aponina Kützing		x		x			x											x	x
G/*Caloneis*																			
Caloneis amphisbaena (Bory) Cleve						x							x	x					
Caloneis silicula (Ehrenberg) Cleve		x			x								x				x		

Caloneis westii (W.Smith) Hendey																			x
Caloneis absoluta E. Manguin																x	x		
Caloneis macedonica Hustedt						x													
G/*Campylodiscus*																			
Campylodiscus clypeus (Ehrenberg) Ehrenberg ex Kützing																	x		
G/*Cocconeis*																			
Cocconeis pediculus Ehrenberg	x		x		x	x	x	x	x	x	x	x	x					x	x
Cocconeis placentula Ehrenberg	x		x		x		x	x		x	x					x	x		
Cocconeis stauroneiformis H.Okuno																		x	x
G/*Conticribra*																			
Conticribra weissflogii (Grunow) Stachura-Suchoples & D.M.Williams		x		x								x							
G/*Craticula*																			
Craticula halophila (Grunow) D.G.Mann						x			x										
Craticula riparia (Hustedt) Lange-Bertalot												x							
Craticula cuspidata (Kützing) D.G.Mann														x					
G/*Ctenophora*																			
Ctenophora pulchella (Kützing) D.M.Williams & Round	x	x																	
G/*Cyclotella*																			
Cyclotella distinguenda Hustedt							x	x											
G/*Cymbopleura*																			
Cymbopleura amphicephala (Nägeli ex Kützing) Krammer	x	x				x	x	x	x	x	x	x		x					
Cymatopleura elliptica W.Smith								x											
Cymatopleura solea (Brébisson) W.Smith						x						x	x						
G/*Cymbella*																			

Cymbella affinis Kützing	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Cymbella cymbiformis C.Agardh			x		x	x												
Cymbella helevtica Kützing		x								x	x							
Cymbella tumida (Brébisson) Van Heurcka			x			x					x		x					
G/*Diatoma*																		
Diatoma moniliformis (Kützing) D.M.Williams	x	x	x			x	x	x	x	x	x	x			x			
Diatoma vulgaris bory			x					x			x	x						
G/*Diploneis*																		
Diploneis oblongella (Nägeli ex Kützing) A.Cleve								x	x	x	x	x				x	x	x
Diploneis elliptica (Kützing) Cleve	x	x		x									x	x	x	x	x	x
G/*Dorofeyukea*																		
Dorofeyukea rostellata (Hustedt) Kulikovskiy & Kociolek			x								x	x						
G/*Encyonema*																		
Encyonema caespitosum Kützing	x		x				x	x	x	x		x						
Encyonema minutum (Hilse) D.G.Mann			x										x	x		x	x	
G/*Encyonopsis*																		
Encyonopsis microcephala (Grunow) Krammer	x	x	x		x	x	x	x	x	x	x	x	x			x	x	
G/*Entomoneis*																		
Entomoneis paludosa (W.Smith) Reimer	x	x		x									x				x	
Entomoneis punctulata (Grunow) K.Osada & H.Kobayasi				x									x					
G/*Eolimna*																		
Eolimna minima (Grunow) Lange-Bertalot					x						x							
G/*Epithemia*																		

Epithemia sorex Kützing			x		x	x													
Epithemia musculus Kützing		x		x														x	x
Rhopalodia gibba (Ehrenberg) Kützing		x	x			x									x				
G/*Fallacia*																			
Fallacia pygmaea (Kützing) Stickle & D.G.Mann						x							x						
G/ *Fragilaria*																			
Fragilaria vaucheriae (Kützing) J.B.Petersen			x										x	x					
G/*Fragilariforma*																			
Fragilariforma virescens (Ralfs) D.M.Williams & Round	x	x								x	x	x							
G/*Frustulia*																			
Frustulia vulgaris (Thwaites) De Toni	x						x		x		x								
G/*Gogorevia*																			
Gogorevia exilis (Kützing) Kulikovskiy & Kociolek																	x		
G/*Gomphonella*																			
Gomphonella olivacea (Hornemann) Rabenhorst	x	x	x		x	x						x							
Gomphonella calcarea (Cleve) R.Jahn & N.Abarca			x		x	x	x												
G/Gomphonema																			
Gomphonema affine Kützing	x	x				x		x		x	x								
Gomphonema angustum C.Agardh		x	x		x	x	x	x	x	x	x	x							
Gomphonema gracile Ehrenberg						x						x							
Gomphonema micropus Kützing								x											
Gomphonema parvulum (Kützing) Kützing	x	x	x		x	x	x	x	x	x	x	x		x	x	x			

Gomphonema productum (Grunow) Lange-Bertalot & E.Reichardt	x	x	x		x	x	x	x	x	x	x	x	x		x	x	x	x	x
Gomphonema rosenstockianum Lange-Bertalot & Reichardt						x			x										
Gomphonema tergestinum (Grunow) Fricke	x			x		x	x					x							
Gomphonema truncatum var. Turgidum (Ehrenberg) R.M.Patrick			x		x	x		x		x					x				
Gomphonema intricatum kützing															x				
Gomphonema italicum Kützing					x														
G/Grunowia																			
Grunowia tabellaria (Grunow) Rabenhorst			x					x	x		x	x							
G/*Halamphora*																			
Halamphora coffeaeformis (C.Agardh) Mereschkowsky		x		x												x	x	x	x
Halamphora veneta (Kützing) Levkov					x										x	x			
G/*Hantzschia*																			
Hantzschia amphioxys (Ehrenberg) Grunow															x				
Hantzschia virgata (Roper) Grunow															x				
G/*Haslea*																			
Haslea spicula (Hickie) Bukhtiyarova		x		x											x	x		x	x
Haslea stundlii (Hustedt) Blanco, Borrego-Ramos & Olenici		x		x												x		x	x
G/*Hippodonta*																			
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski																	x		
Hippodonta hungarica (Grunow) Lange-Bertalot, Metzeltin & Witkowski					x												x		
G/*Homoeocladia*																			

Homoeocladia amphibia (Grunow) Kuntze		x	x		x	x		x	x	x	x	x	x	x	x	x	x	x	
Homoeocladia inconspicua (Grunow) Kuntze	x		x			x			x	x	x		x		x				
Homoeocladia palea (Kützing) Kuntze	x	x	x		x	x	x	x	x	x	x	x	x			x	x	x	
Homoeocladia filiformis W.Smith		x				x	x					x							
G/*Luticola*																			
Luticola goeppertiana (Bleisch) D.G.Mann ex Rarick, S.Wu, S.S.Lee & Edlund			x																
G/*Mastogloia*																			
Mastogloia elliptica (C.Agardh) Cleve		x		x		x									x			x	x
Mastogloia smithii Thwaites ex W.Smith		x		x	x	x									x			x	x
Mastogloia braunii Grunow		x		x														x	x
G/*Melosira*																			
Melosira varians C.Agardh			x		x	x		x	x				x	x	x				
G/*Navicula*																			
Navicula arvensis var. dubia Lange-Bertalot																	x		
Navicula cryptocephala Kützing		x			x	x							x				x		
Navicula cryptotenella Lange-Bertalot						x		x		x									
Navicula cryptotenelloides Lange-Bertalot			x		x	x	x	x	x	x	x	x	x	x					
Navicula gregaria Donkin	x			x	x	x			x	x			x	x			x	x	
Navicula menisculus Schumann					x						x	x						x	
Navicula phyllepta Kützing	x	x		x		x					x	x	x	x	x	x	x	x	
Navicula radiosa Kützing	x	x		x	x	x		x						x	x	x	x	x	x
Navicula recens (Lange-Bertalot) Lange-Bertalot	x	x		x	x	x					x	x	x	x	x	x	x	x	x
Navicula salinicola Hustedt		x																	
Navicula tripunctata (O.F.Müller) Bor	x	x		x				x					x	x				x	
Navicula veneta Kützing		x										x	x				x		
Navicula viridula (Kützing) Ehrenberg																	x		

Navicula capitatoradiata H.Germain ex Gasse				x			x	x	x		x	x		x					
Navicula rostellata Kützing			x		x	x				x			x						
G/*Navicymbula*																			
Navicymbula pusilla (Grunow) Krammer		x		x		x								x		x			
G/*Neidium*																			
Neidium affine (Ehrenberg) Pfitzer						x			x										
G/*Nitzschia*																			
Nitzschia clausii Hantzsch						x							x	x	x		x	x	
Nitzschia denticula Grunow	x				x	x	x	x		x	x	x	x		x	x	x		
Nitzschia dissipata (Kützing) Rabenhorst	x	x	x		x	x	x	x	x	x	x		x						
Nitzschia dubia W.Smith				x															
Nitzschia fonticola (Grunow) Grunow		x		x	x	x		x			x	x	x					x	x
Nitzschia frustulum (Kützing) Grunow	x	x		x								x			x	x	x	x	x
Nitzschia homburgiensis Lange-Bertalot															x				
Nitzschia hybrida Grunow																	x		
Nitzschia microcephala Grunow	x	x			x	x		x		x	x	x	x		x	x	x	x	
Nitzschia paleacea (Grunow) Grunow	x	x		x				x	x						x				
Nitzschia scalpelliformis Grunow				x														x	x
Nitzschia bergii A.Cleve						x			x										
Nitzschia capitellata Hustedt	x	x			x	x						x							
Nitzschia communis Rabenhorst					x	x													
Nitzschia elegantula Grunow		x		x											x	x	x	x	
Nitzschia intermedia Hantzsch ex Cleve & Grunow									x										
Nitzschia lacunarum Hustedt		x		x											x	x			
Nitzschia linearis W.Smith								x		x		x							
Nitzschia reversa W.Smith				x															
Nitzschia sigma (Kützing) W.Smith		x																	

Nitzschia thermaloides Hustedt		x		x									x						
Nitzschia vitrea G.Norman		x																	
G/*Pantocsekiella*																			
Pantocsekiella ocellata (Pantocsek) K.T.Kiss & Ács			x																
G/*Phaeodactylum*																			
Phaeodactylum tricornutum Bohlin																	x		
G/*Planothidium*																			
Planothidium delicatulum (Kützing) Round & Bukhtiyarova																	x	x	x
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	x	x	x		x	x		x				x				x			
G/*Pleurosigma*																			
Pleurosigma intermedium W.Smith		x				x										x	x	x	x
G/*Podosira*																			
Podosira dubia (Kützing) Grunow	x														x	x	x	x	x
G/*Pseudostaurosira*																			
Pseudostaurosira brevistriata (Grunow) D.M.Williams & Round			x		x										x				
Pseudostaurosira elliptica (Schumann) Edlund, Morales & Spaulding						x									x				
G/*Reimeria*																			
Reimeria sinuata (W.Gregory) Kociolek & Stoermer									x	x	x								
G/*Rhopalodia*																			
Rhopalodia acuminata Krammer																			x
Rhopalodia gibberula (Ehrenberg) O.Müller						x													
G/*Sellaphora*																			

Sellaphora bacillum (Ehrenberg) D.G.Mann		x		x	x	x					x	x				x			
Sellaphora pupula (Kützing) Mereschkovsky					x	x						x	x				x		
Sellaphora sp. Mereschowsky							x												
Sellaphora seminulum (Grunow) D.G.Mann	x									x									
Sellaphora stauroneioides (Lange-Bertalot) Veselá & J.R.Johansen									x										
G/*Stephanocyclus*																			
Stephanocyclus meneghinianus (Kützing) Kulikovskiy, Genkal & Kociolek	x	x	x	x		x				x	x	x	x		x		x	x	x
G/*Stephanodiscus*																			
Stephanodiscus astraea (Kützing) Grunow						x						x					x	x	
G/*Surirella*																			
Surirella angusta Kützing						x			x			x							
Surirella brebissonii Krammer & Lange-Bertalot														x					
Surirella brebissonii var. kuetzingii Krammer & Lange-Bertalot	x	x			x	x	x	x	x			x	x						
Surirella minuta Brébisson ex Kützing	x																		
G/*Tabularia*																			
Tabularia fasciculata (C.Agardh) D.M.Williams & Round	x	x											x		x		x	x	x
G/*Thalassiora*																			
Thalassiosira pseudonana Hasle & Heimdal		x				x							x		x				x
G/*Tryblionella*																			
Tryblionella angustatula (Lange-Bertalot) Cantonati & Lange-Bertalot												x							
Tryblionella gracilis W.Smith						x							x						
Tryblionella calida (Grunow) D.G.Mann		x																	

Tryblionella chutteri var. aestuarii Martinez-Goss																x		x	x
Tryblionella victoriae Grunow																x	x	x	x
Tryblionella apiculata W.Gregory	x	x		x				x				x		x		x	x	x	x
Tryblionella compressa (Bailey) Poulin																		x	x
Tryblionella hungarica (Grunow) Frenguelli		x				x		x						x					
G/*Ulnaria*																			
Ulnaria acus (Kützing) Aboal	x	x	x		x	x	x	x	x	x	x	x	x	x	x		x	x	x
Ulnaria ulna (Nitzsch) Compère	x	x	x		x	x		x	x	x	x	x	x	x		x	x	x	x

Table S5. Correlation results between environmental variables and the two axes of the NMDS analysis (beta diversity) for cyanobacteria based on the morphological method.

Parameter	**NMDS1**	**NMDS2**	**r^2**	**Pr(>r)**
Altitude	-0.99740	0.07212	0.6011	0.001 ***
Conductivity	0.99934	-0.03642	0.7719	0.001 ***
Temperature	0.99604	0.08894	0.2034	0.032 *
Salinity	0.99817	-0.06053	0.7497	0.001 ***
Oxygen	-0.99682	0.07963	0.0064	0.913
pH	-0.51490	0.85725	0.0436	0.503
Orthophosphate	0.99826	-0.05900	0.5323	0.001 ***
Ammonium	0.90252	0.43066	0.0153	0.792
Nitrate	0.02468	0.99970	0.0270	0.661
Sulfate	0.97755	0.21069	0.2547	0.008 **
Chloride	0.99782	-0.06604	0.5832	0.001 ***
Alkalinity	0.99433	-0.10632	0.4116	0.001 ***
Hardness	0.99603	-0.08901	0.5784	0.001 ***
Flow velocity	-0.89535	0.44536	0.1254	0.119
Flow rate	-0.56182	-0.82726	0.2212	0.017 *

Table S6. Correlation results between environmental variables and the two axes of the NMDS analysis (beta diversity) for green algae based on morphological method.

Parameter	**NMDS1**	**NMDS2**	**r^2**	**Pr(>r)**
Altitude	-0.87934	0.47620	0.1111	0.168
Conductivity	0.84623	0.53282	0.1960	0.034 *
Temperature	0.64758	-0.76199	0.1670	0.061 .
Salinity	0.82355	0.56724	0.1876	0.041 *
Oxygen	0.65405	-0.75645	0.1558	0.076 .
pH	-0.01874	0.99982	0.0198	0.723
Orthophosphate	0.96748	0.25295	0.1826	0.041 *
Ammonium	0.50429	-0.86354	0.1212	0.131
Nitrate	0.98130	-0.19246	0.0108	0.846
Sulfate	0.95310	0.30265	0.1556	0.077 .
Chloride	0.93805	0.34649	0.1573	0.071 .
Alkalinity	0.89450	0.44706	0.1330	0.113
Hardness	0.96472	0.26329	0.1728	0.058 .
Flow velocity	-0.06377	0.99796	0.1574	0.084 .
Flow rate	-0.56100	0.82782	0.1960	0.044 *

Table S7. Correlation results between environmental variables and the two axes of the NMDS analysis (beta diversity) for diatoms based on morphological method.

Parameter	NMDS1	NMDS2	r^2	Pr(>r)
Altitude	0.94029	0.34037	0.4779	0.001 ***
Conductivity	-0.95518	0.29602	0.8708	0.001 ***
Temperature	-0.38959	0.92099	0.5956	0.001 ***
Salinity	-0.91496	0.40355	0.8542	0.001 ***
Oxygen	-0.07556	-0.99714	0.1097	0.168
pH	0.28141	0.95959	0.0595	0.419
Orthophosphate	-0.99839	0.05671	0.4915	0.001 ***
Ammonium	0.62106	-0.78377	0.0005	0.995
Nitrate	0.11047	-0.99388	0.0406	0.524
Sulfate	-0.93856	0.34512	0.5999	0.001 ***
Chloride	-0.99040	0.13826	0.6937	0.001 ***
Alkalinity	-0.99849	0.05498	0.4558	0.001 ***
Hardness	-0.99986	-0.01669	0.7194	0.001 ***
Flow velocity	0.27551	0.96130	0.3004	0.008 **
Flow rate	0.47246	0.88135	0.2715	0.012 *

Table S8. Correlation results between environmental variables and the two axes of the NMDS analysis (beta diversity) for cyanobacteria based on molecular analysis.

Parameter	NMDS1	NMDS2	r^2	Pr(>r)
Altitude	0.97139	-0.23749	0.8463	0.001 ***
Conductivity	-0.66942	-0.74288	0.8566	0.001 ***
Temperature	-0.95027	0.31142	0.3459	0.002 **
Salinity	-0.66386	-0.74786	0.8391	0.001 ***
Oxygen	-0.52904	0.84860	0.0485	0.487
pH	0.45013	0.89296	0.0133	0.812
Orthophosphate	-0.90610	-0.42307	0.4703	0.001 ***
Ammonium	-0.35668	0.93423	0.1223	0.156
Nitrate	-0.03870	0.99925	0.0543	0.428
Sulfate	-0.75819	-0.65204	0.5177	0.001 ***
Chloride	-0.77262	-0.63487	0.6554	0.001 ***
Alkalinity	-0.71723	-0.69683	0.4791	0.001 ***
Hardness	-0.64704	-0.76246	0.7389	0.001 ***
Flow velocity	0.62159	-0.78334	0.5492	0.001 ***
Flow rate	0.93887	-0.34426	0.2056	0.032 *

Table S9. Correlation results between environmental variables and the two axes of the NMDS analysis (beta diversity) for green algae based on molecular analysis.

Parameter	NMDS1	NMDS2	r^2	Pr(>r)
Altitude	-0.76285	0.64658	0.6680	0.001 ***
Conductivity	0.98953	-0.14432	0.4397	0.001 ***
Temperature	0.35033	-0.93663	0.5432	0.001 ***
Salinity	0.99252	-0.12210	0.4266	0.001 ***
Oxygen	-0.30011	-0.953911	0.2631	0.005 *
pH	-0.28345	0.95391	0.0424	0.535
Orthophosphate	0.91386	-0.40602	0.4074	0.003 **
Ammonium	0.43045	-0.90261	0.0620	0.405
Nitrate	-0.08564	-0.99633	0.0944	0.233
Sulfate	0.97365	-0.22803	0.2098	0.023 *
Chloride	0.98512	-0.17185	0.2931	0.004 **
Alkalinity	0.91483	-0.40383	0.2518	0.018 *
Hardness	0.94226	-0.33488	0.2653	0.011 *
Flow velocity	-0.14545	-0.98795	0.6254	0.001 ***
Flow rate	-0.31170	-0.95018	0.2656	0.008 **

Table S10. Correlation results between environmental variables and the two axes of the NMDS analysis (beta diversity) for diatoms based on molecular analysis.

Parameter	NMDS1	NMDS2	r^2	Pr(>r)
Altitude	0.97566	-0.21930	0.7343	0.001 ***
Conductivity	-0.94402	-0.32990	0.6863	0.001 ***
Temperature	-0.49940	0.86637	0.4567	0.001 ***
Salinity	-0.93025	-0.36693	0.6836	0.001 ***
Oxygen	-0.08746	0.99617	0.3987	0.001 ***
pH	-0.09299	-0.99567	0.0935	0.268
Orthophosphate	-0.99783	0.06589	0.4781	0.001 ***
Ammonium	-0.43860	0.89868	0.0743	0.327
Nitrate	-0.22072	0.97534	0.0899	0.246
Sulfate	-0.99999	0.00489	0.6158	0.001 ***
Chloride	-0.96159	-0.27451	0.5728	0.001 ***
Alkalinity	-0.95862	0.28470	0.4306	0.002 **
Hardness	-0.99588	0.09069	0.5264	0.001 ***
Flow velocity	0.43700	-0.89946	0.2740	0.005 **
Flow rate	0.50135	-0.86524	0.1815	0.049 *

Table S11. Results of PERMANOVA (ADONIS) between salinity classes, season, and the interaction of salinity class and season using morphological analysis.

Microalgae groups	**Cyanobacteria**			**Green algae**			**Diatoms**		
Factor	R^2	F-value	p-value	R^2	F-value	p-value	R^2	F-value	p-value
Salinity class	0.2691	11.966	0.2691	0.08264	2.9276	0.001 ***	0.25106	10.895	0.001 ***
Season	0.00739	0.4914	0.842	0.0364	2.4931	0.017 *	0.02961	2.0139	0.039 *
Salinity class × Season	0.28927	5.0468	0.001 ***	0.12674	1.7997	0.004 **	0.3071	5.4958	0.001 ***

Table S12. Results of PERMANOVA (ADONIS) between salinity classes, season, and the interaction of salinity class and season using molecular analysis.

Microalgae groups	**Cyanobacteria**			**Green algae**			**Diatoms**		
Factor	R^2	F-value	p-value	R^2	F-value	p-value	R^2	F-value	p-value
Salinity class	0.13434	5.0435	0.001 ***	0.09046	3.2325	0.001 ***	0.11106	4.0605	0.001 ***
Season	0.01864	1.2536	0.161	0.06706	4.7441	0.001 ***	0.0632	4.4526	0.001 ***
Salinity class × Season	0.17469	2.6247	0.001 ***	0.18423	2.8004	0.001 ***	0.20262	3.151	0.001 ***

Table S13. Dominant cyanobacteria genera identified by morphological and DNA metabarcoding approaches (>5% relative abundance).

Taxonomy	**Percentage (%)**	
	Microscopic observation	**Molecular method (18S rDNA)**
Order/Synechococcales		
F/Synechococcaceae		
Synechococcus	6.78	4.73
Synechocystis	7.68	
F/Leptolyngbyaceae		
G/*Leptolyngbya*		
Leptolyngbya	24.46	47.44
F/Merismopediaceae		
Aphanocapsa	11.38	
Order/Oscillatoriales		
F/Oscillatoriaceae		
Oscillatoria	13.05	
Phormidium	9.47	39.31
F/Pseudanabaenaceae		
Pseudoanabeana	12.19	2.14
Sum	**85.01 %**	**93.62 %**

Table S14. Dominant green algae genera identified by morphological and DNA metabarcoding approaches (>5% relative abundance).

Taxonomy	**Percentage (%)**	
	Microscopic observation	**Molecular method (18S rDNA)**
Order/Chaetophorales		
F/Aphanochaetaceae		
Aphanochaete	7.9	8.6
F/Fritschiellaceae		
Stigeoclonium	6	1.29
Order/Chlamydomonadales		
F/Chlorochytriaceae		
Chlorochytrium	38.36	41.62
Order/Cladophorales		
F/Cladophoraceae		
Cladophora	12.45	17.68
Order/Ulvales		
F/Jaoaceae		
Jaoa		6.5
Order/Oedogoniales		
F/Oedogoniaceae		
Oedogonium	6.34	2.36
Order/Sphaeropleales		
F/Scenedesmaceae		
Scenedesmus	9.28	8.66
Sum	**80.33 %**	**86.71 %**

Table S15. Dominant diatoms genera identified by morphological and DNA metabarcoding approaches (>5% relative abundance).

Taxonomy	**Percentage (%)**	
	Microscopic observation	**Molecular method (18S rDNA)**
Order/ Achnanthales		
F/ Achnanthidiaceae		
Achnanthidium	16.08 %	1.5 %
Order/ Cymbellales		
F/ Cymbellaceae		
Cymbella	8.32 %	11.22 %
Gomphonema	14.2 %	20.25 %
Order/ Bacillariales		
F/ Bacillariaceae		
Homoeocladia	7.94 %	12.4 %
Nitzschia	6.27 %	0.9 %
Order/ Naviculales		
F/ Naviculaceae		
Navicula	6.02 %	6 %
Order/ Melosirales		
F/ Hyalodiscaceae		
Podosira	1.13 %	9.87 %
Order/Licmophorales		
F/Ulnariaceae		
Ulnaria	4.44 %	13.10 %
Sum	**64.4 %**	**62.14%**

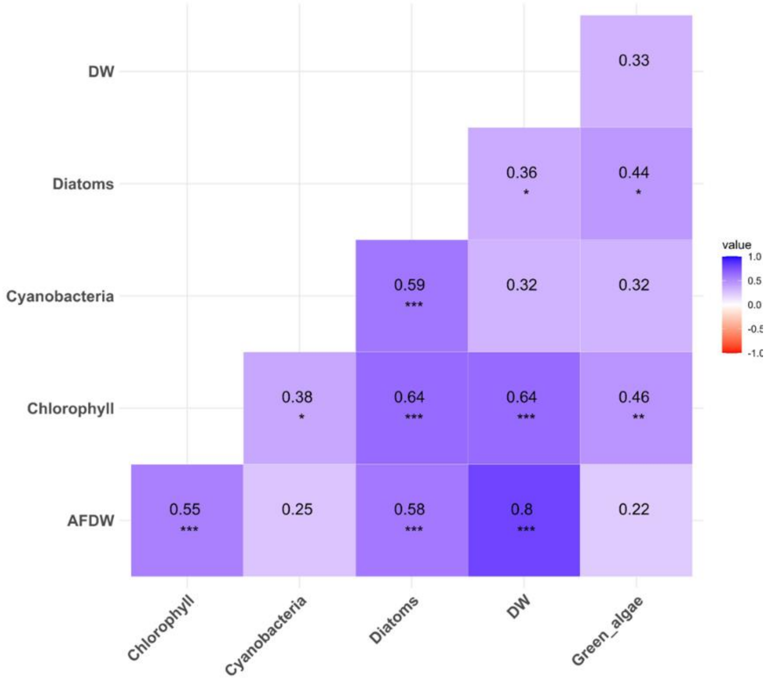


Figure S1. Pearson correlation coefficient values between biofilm biomass parameters. (*** $p < 0.001$), (**$p < 0.01$), and (* $p < 0.05$).

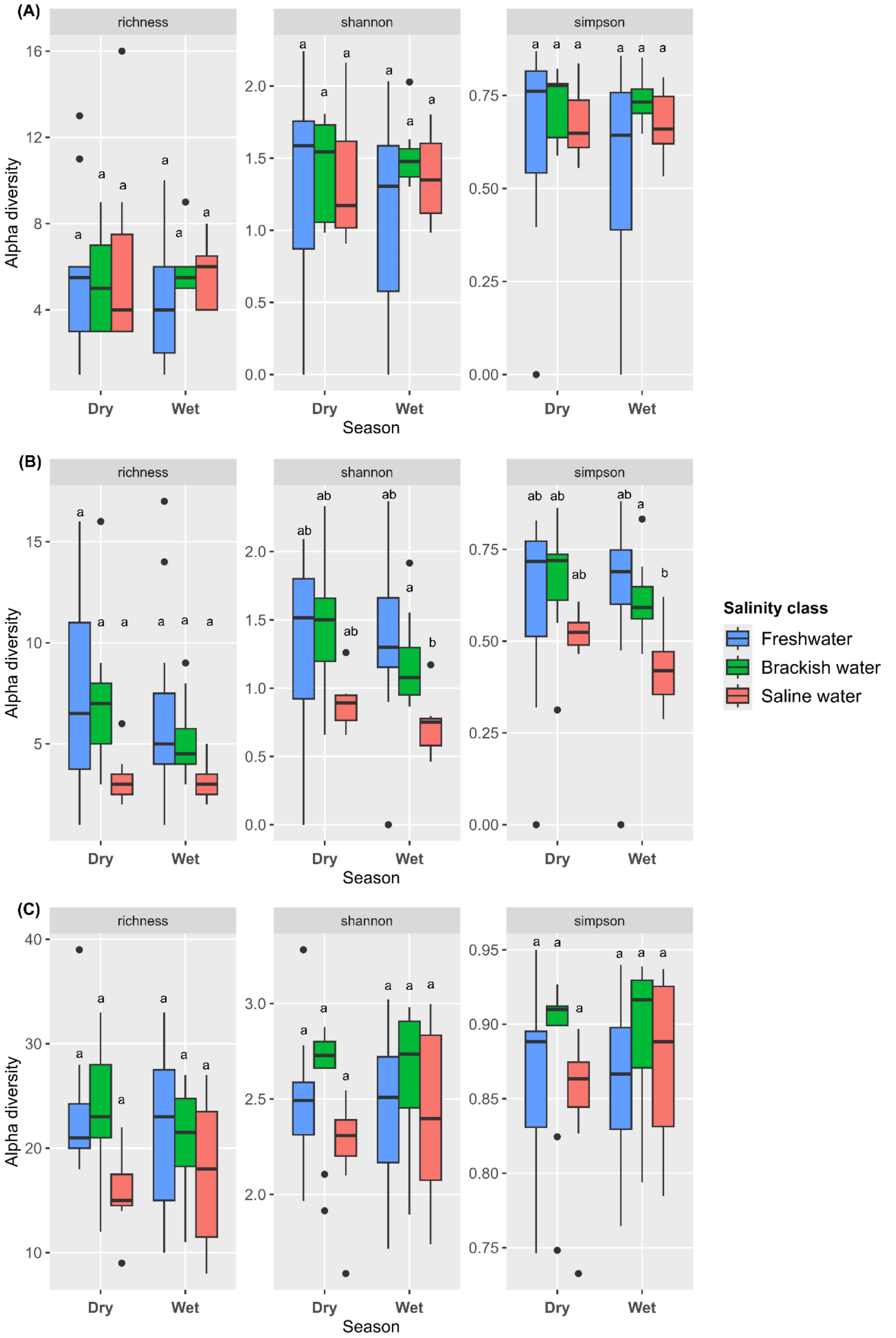


Figure S2. Alpha diversity based on morphological identification (i.e., richness, Shannon index, and Simpson index) for cyanobacteria (A), green algae (B), and diatoms (C) of samples from different salinity classes during the dry and wet periods.

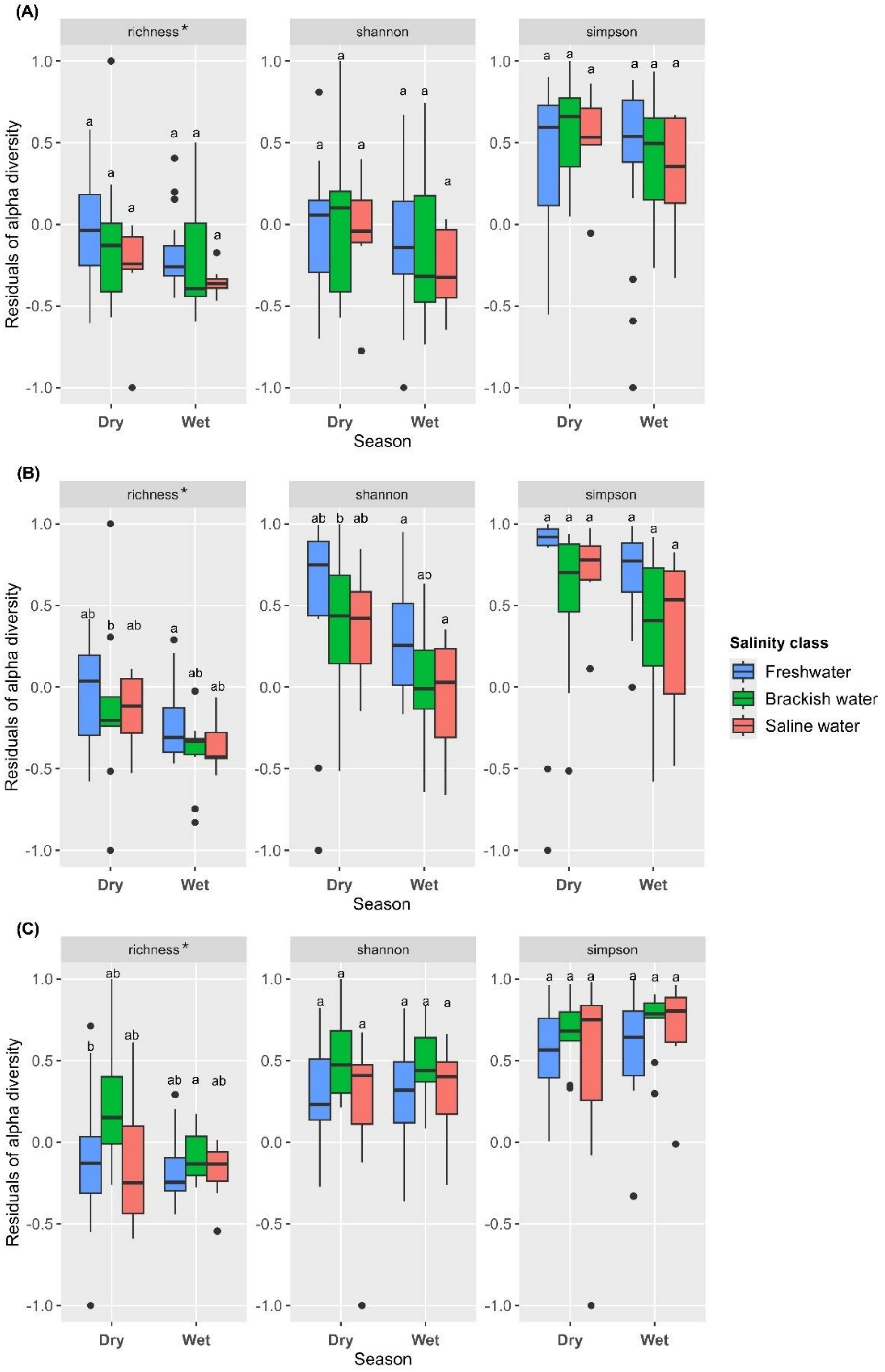


Figure S3. Alpha diversity based on molecular analysis.calculated as Hills numbers with exponent 0, 1, and 2 (i.e, richness, Shannon, and Simpson indexes) for cyanobacteria (A), green algae (B), and diatoms (C) of samples from different salinity classes during the dry and wet periods.

In case a calculated index revealed a linear correlation with the square root transformed read counts per sample pointing to a sequencing depth bias. Only the residuals of the linear model were analyzed (indicated by *). Values were centered-scaled on a -1 to 1 range to allow for comparability between the indices.

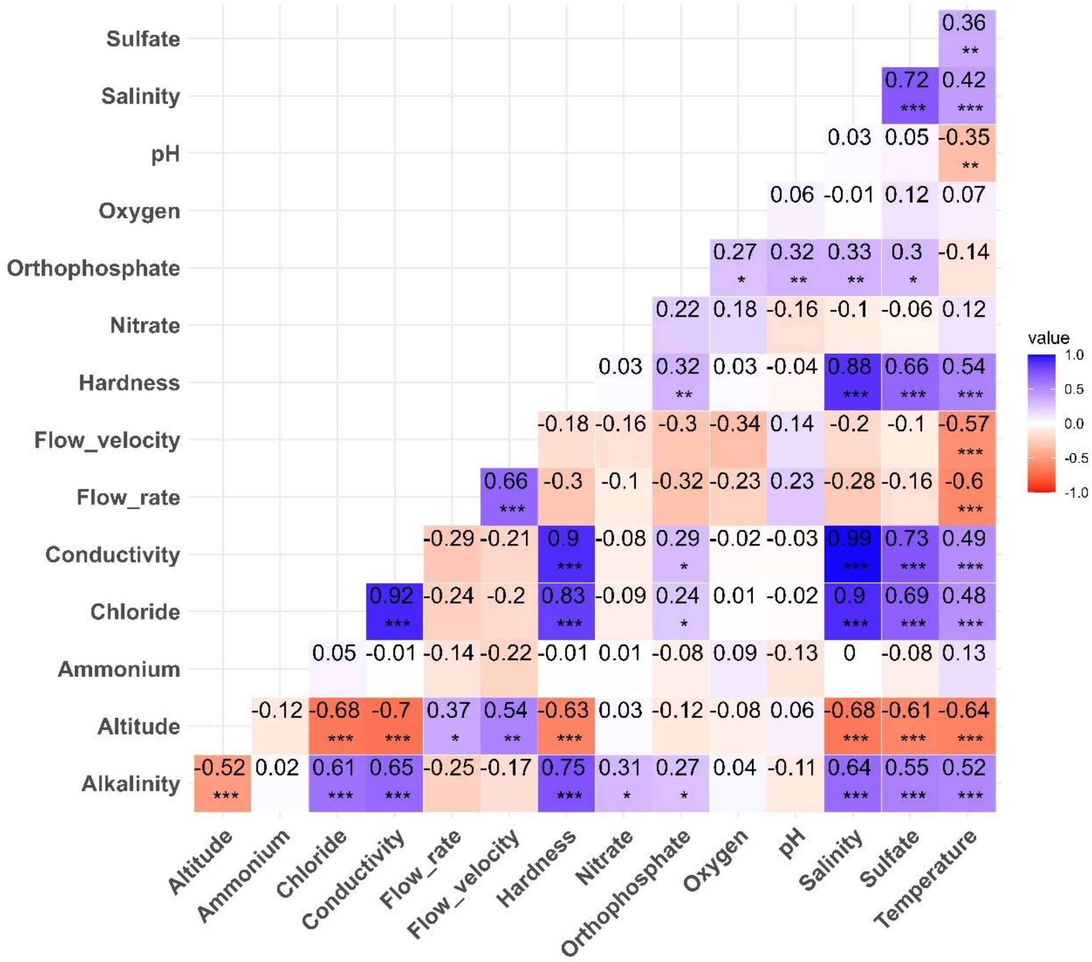


Figure S4. Pearson correlation coefficient values between environmental variables. (*** $p < 0.001$), (**$p < 0.01$), and (* $p < 0.05$).

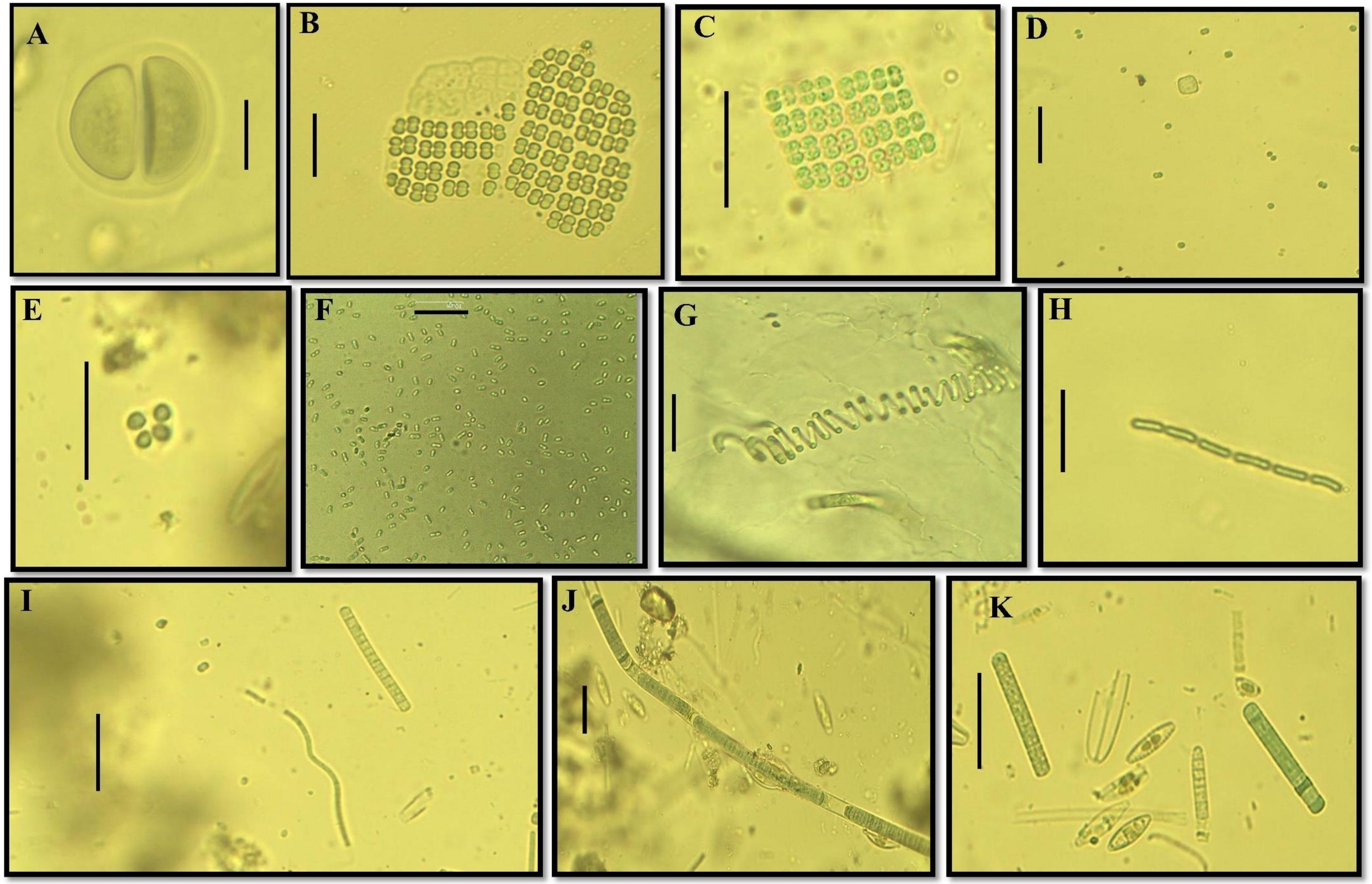


Figure S5: LM photomicrographs of some identified cyanobacteria taxa.

A: *Chroococcus sp.*; B: *Merismopedia* sp1; C: *Merismopedia* sp2; D: *Synechocystis* sp. E: *Gloeocapsa* sp.; F: *Synechococcus* sp.; G: *Arthrospira* sp.;H: *Pseudanabaena limnetica*; I: *Leptolyngbya* sp.; J: *Lyngbya* sp.; K: *Oscillatoria* sp. Scale bar:10 µm

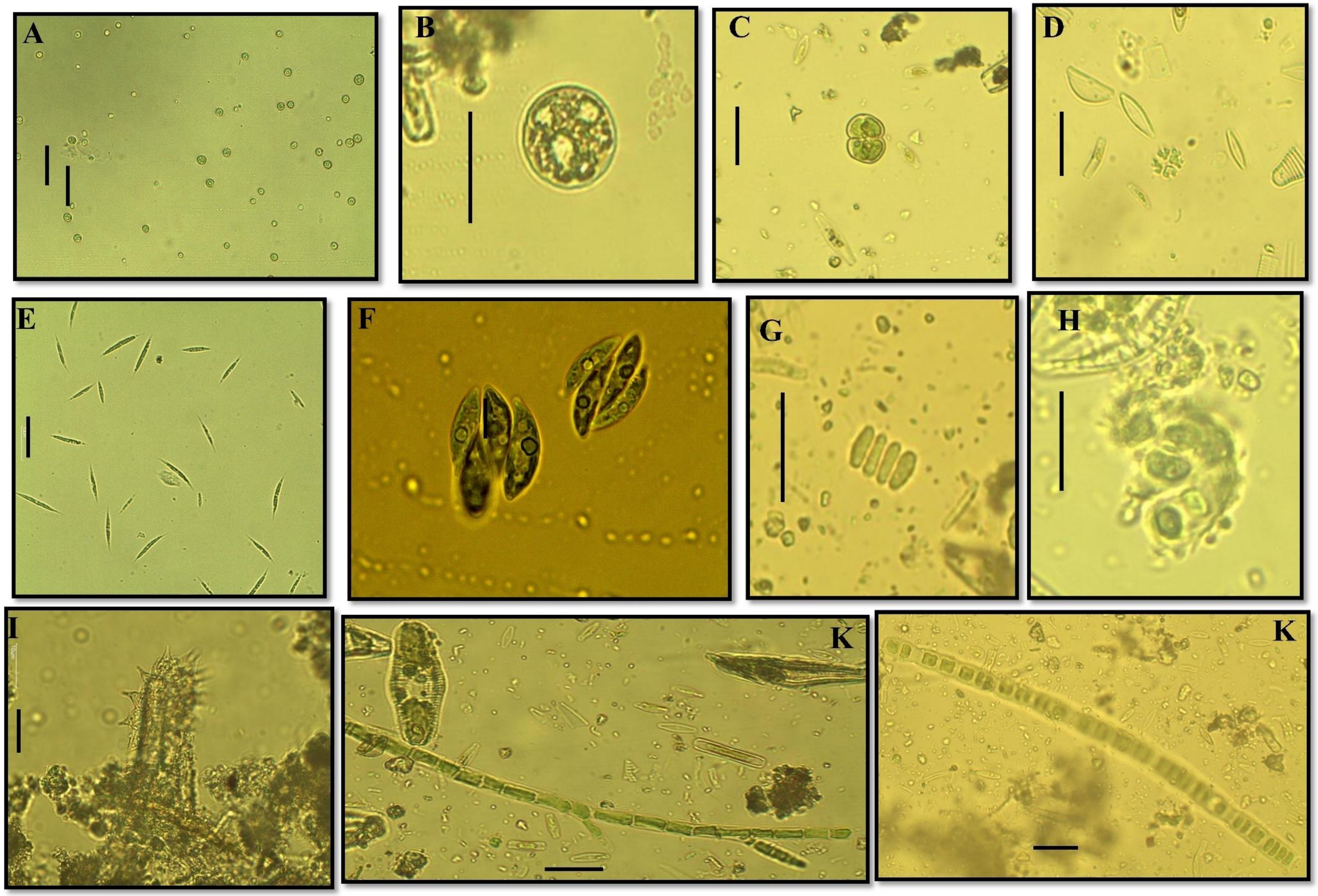


Figure S6: LM photomicrographs of some identified green algae taxa.

A: *Chlorella* sp.; B:*Neochloris vigensis*; C: *Cosmarium* sp.; D: *Stauridium tetras*; E: *Monoraphidium* sp.; F: *Tetradesmus* sp.;G: *Scenedesmus* sp. H: *Neochlorosarcina minor* ; I: *Aphanochaete repens*.; J: *Stigeoclonium* sp.; K: *Ulothrix* sp. Scale bar:10 µm

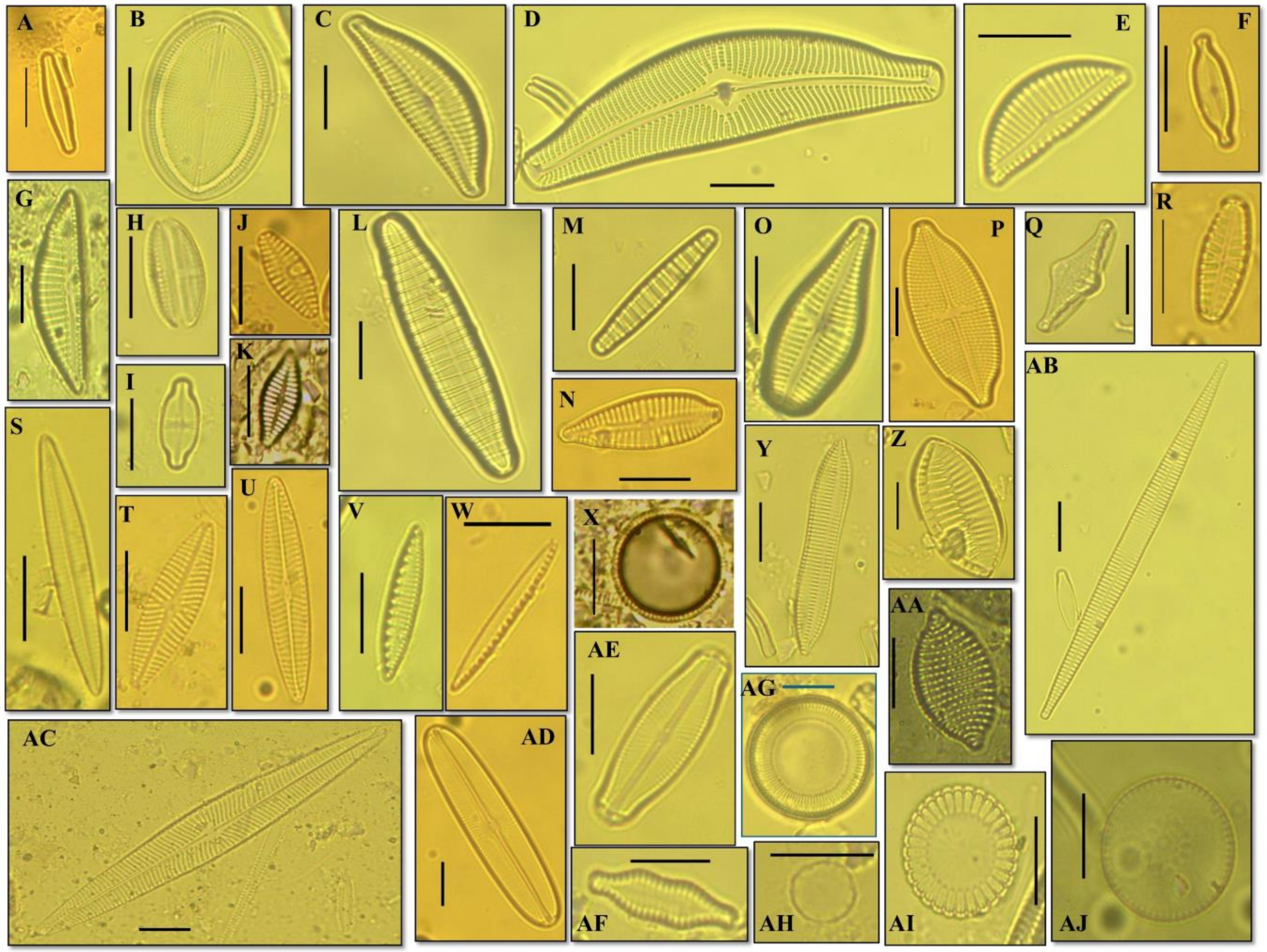


Figure S7: LM photomicrographs of some identified diatom species.

A: *Achnanthidium minutissimum*; B:*Cocconeis placentula*; C: *Cymbella affinis*; D: *Cymbella tumida*; E:*Encyonema caespitosum*; F: *Encyonopsis microcephala*; G:*Encyonema minutum*; H: *Amphora pediculus*; I:*Gogorevia exilis*; J:*Planothidium lanceolatum*; K:*Planothidium delicatulum*; L: *Diatoma vulgaris*; M: *Diatoma moniliformis*; N: *Gomphonema parvulum*; O: Gomphonema truncatum var. turgidum ; P: *Mastogloia smithii*; Q: *Grunowia tabellaria*; R: *Hippodonta hungarica*; S: *Haslea stundlii*, T: *Navicula menisculus*;U: *Navicula recens*; V: *Homoeocladia amphibia*;W: *Nitzschia frustulum*; X: *Podosira dubia*;Y: *Tryblionella apiculata*;Z: *Tryblionella victoriae*; AA: *Tryblionella compressa*; AB: *Ulnaria acus*; AC: *Navicula radiosa*; AD: *Sellaphora bacillum* , AE: *Sellaphora pupula*;AF: *Pseudostaurosira brevistriata*;AG: *Cyclotella distinguenda*; AH:*Thalassiosira pseudonana*;AI: *Stephanocyclus meneghinianus*; AJ: *Conticribra weissflogii*. Scale bar:10

Supplementary material. Chapter 3

Table S16: Spatial and temporal changes in physicochemical quality parameters and weighted index values recorded in the Drâa river. Sitecode_1 and _3: dry periods, Sitecode_2and_4: wet periods.

N°	Sub-basins	Site name	Code	Temperature	pH	Organic and Oxidizable Matter	Nitrates	Phosphorus Matter	Mineralization	WI value
1	upper Drâa	Ounila Upstream	IO1_1	82,64	71,60	59,00	97,52	42,27	58,87	42,27
2		Ounila Downstream	IO2_1	62,48	78,20	67,49	99,67	92,41	38,84	38,84
3		El Mellah	IE_1	64,80	78,80	72,53	99,58	85,61	35,19	35,19
4		Iriri	IR_1	81,97	71,80	65,35	98,58	94,06	88,18	65,35
5		Ait douchen	A1_1	69,60	72,40	58,12	95,39	93,71	80,37	58,12
6		Tarmigte	A2_1	52,03	64,60	58,94	99,87	95,29	69,34	52,03
7		Dades Upstream	D1_1							
8		Dades Gorges	D2_1	83,12	70,60	72,51	97,65	96,30	74,29	70,60
9		Dades Middle	D3_1	75,60	68,20	67,16	98,36	95,07	78,90	67,16
10		M'goun Upstream	DM1_1	88,02	65,60	59,35	98,32	36,28	75,58	36,28
11		M'goun Downstream	DM2_1	84,32	68,00	80,41	98,40	95,95	75,87	68,00
12		Dades Downstream	D4_1	53,03	82,60	72,02	98,09	93,53	20,31	20,31
13	middle Drâa	Kasbah Tamnogalt	DR1_1							
14		Taghzout	DR2_1							
15		Tamgroute	DR3_1	68,16	63,80	75,98	99,95	95,64	30,79	30,79
16	lower Drâa	Akka Nait Sidi salty	LT1_1	43,07	79,40	66,10	98,60	92,08	19,05	19,05
17		Akka Nait Sidi less salty	LT2_1	49,91	77,20	76,11	96,76	89,44	24,07	24,07
18		Tissint Upstream	LT3_1	60,13	70,00	60,00	99,73	78,23	13,06	13,06
19		Tissint Downstream	LT4_1	54,37	68,00	61,10	99,41	39,68	10,61	10,61
1	upper Drâa	Ounila Upstream	IO1_2	84,08	84,07	82,10	99,50	78,94	51,51	51,51
2		Ounila Downstream	IO2_2	83,90	78,93	68,64	99,60	97,16	49,55	49,55
3		El Mellah	IE_2	57,73	84,60	67,98	98,42	93,00	39,52	39,52
4		Iriri	IR_2	80,87	83,00	81,35	99,74	95,80	90,59	80,87
5		Ait douchen	A1_2	81,31	80,80	63,47	96,00	98,88	78,58	63,47

6		Tarmigte	A2_2	60,40	75,73	64,05	99,82	99,47	72,39	60,40
7		Dades Upstream	D1_2	86,17	84,73	79,36	98,15	88,42	86,35	79,36
8		Dades Gorges	D2_2	84,97	73,07	81,99	99,18	76,96	80,88	73,07
9		Dades Middle	D3_2	84,70	75,93	82,89	99,18	84,66	83,37	75,93
10		M'goun Upstream	DM1_2	81,22	85,67	78,17	99,40	68,69	84,13	68,69
11		M'goun Downstream	DM2_2	75,73	82,93	81,67	99,44	87,98	82,10	75,73
12		Dades Downstream	D4_2	86,76	83,87	79,93	99,91	97,82	68,17	68,17
13	middle Drâa	Kasbah Tamnogalt	DR1_2	80,70	82,60	75,11	98,66	87,31	58,85	58,85
14		Taghzout	DR2_2	60,68	81,67	81,14	99,08	90,03	52,50	52,50
15		Tamgroute	DR3_2	61,57	81,40	72,73	99,93	97,76	44,02	44,02
16	lower Drâa	Akka Nait Sidi salty	LT1_2	64,95	82,53	69,23	98,60	98,09	23,87	23,87
17		Akka Nait Sidi less salty	LT2_2	57,05	82,20	72,84	98,33	98,68	31,50	31,50
18		Tissint Upstream	LT3_2	50,55	82,33	89,69	99,66	92,74	14,39	14,39
19		Tissint Downstream	LT4_2	44,23	83,40	93,29	99,22	98,42	9,59	9,59
1	upper Drâa	Ounila Upstream	IO1_3	73,60	88,00	88,07	96,50	66,72	47,15	47,15
2		Ounila Downstream	IO2_3	77,20	72,00	87,52	99,58	65,41	32,83	32,83
3		El Mellah	IE_3	59,60	76,00	77,43	98,37	51,93	30,54	30,54
4		Iriri	IR_3	80,20	74,00	81,04	99,63	66,66	88,02	66,66
5		Ait douchen	A1_3	70,40	75,20	76,99	95,91	55,69	79,08	55,69
6		Tarmigte	A2_3	58,00	79,40	86,97	99,80	64,00	69,69	58,00
7		Dades Upstream	D1_3							
8		Dades Gorges	D2_3	82,50	59,43	88,51	96,04	66,66	75,07	59,43
9		Dades Middle	D3_3	83,50	60,00	93,84	98,26	62,32	80,18	60,00
10		M'goun Upstream	DM1_3	80,50	62,00	95,19	98,17	58,89	79,43	58,89
11		M'goun Downstream	DM2_3	80,10	66,80	94,86	98,26	64,06	77,47	64,06
12		Dades Downstream	D4_3	74,80	69,80	82,38	98,11	47,40	55,41	47,40
13	middle Drâa	Kasbah Tamnogalt	DR1_3	59,20	68,80	74,77	97,43	71,47	42,51	42,51
14		Taghzout	DR2_3							
15		Tamgroute	DR3_3							

16	lower Drâa	Akka Nait Sidi salty	LT1_3	65,60	76,00	94,68	95,49	42,00	16,75	16,75
17		Akka Nait Sidi less salty	LT2_3	57,60	84,00	82,54	94,22	39,83	23,90	23,90
18		Tissint Upstream	LT3_3	62,80	66,00	92,27	99,67	47,23	13,66	13,66
19		Tissint Downstream	LT4_3	81,20	48,57	82,54	99,32	47,83	8,22	8,22
1	upper Drâa	Ounila Upstream	IO1_4	81,50	64,00	82,95	99,81	69,27	43,39	43,39
2		Ounila Downstream	IO2_4	81,70	64,00	83,84	99,98	65,13	31,59	31,59
3		El Mellah	IE_4	82,90	68,00	82,18	99,97	55,34	32,08	32,08
4		Iriri	IR_4	83,90	60,00	78,68	99,95	67,81	89,36	60,00
5		Ait douchen	A1_4	79,60	74,00	57,26	97,85	59,01	72,40	57,26
6		Tarmigte	A2_4	77,20	62,00	97,55	99,70	73,58	70,99	62,00
7		Dades Upstream	D1_4	89,30	60,00	80,26	98,93	72,70	85,91	60,00
8		Dades Gorges	D2_4	85,80	62,00	80,24	99,27	73,27	83,67	62,00
9		Dades Middle	D3_4	86,90	60,00	79,34	99,18	70,37	86,36	60,00
10		M'goun Upstream	DM1_4	84,90	62,00	79,26	98,22	68,43	79,17	62,00
11		M'goun Downstream	DM2_4	84,80	60,00	74,92	98,85	70,01	79,18	60,00
12		Dades Downstream	D4_4	86,80	62,00	78,24	99,30	66,80	51,53	51,53
13	middle Drâa	Kasbah Tamnogalt	DR1_4	68,00	38,00	81,21	98,99	75,21	81,24	38,00
14		Taghzout	DR2_4							
15		Tamgroute	DR3_4							
16	lower Drâa	Akka Nait Sidi salty	LT1_4	64,40	72,00	72,29	98,39	44,15	17,17	17,17
17		Akka Nait Sidi less salty	LT2_4	76,40	74,00	76,37	97,39	43,30	30,65	30,65
18		Tissint Upstream	LT3_4	84,90	60,00	81,06	99,87	45,42	16,99	16,99
19		Tissint Downstream	LT4_4	73,20	57,14	84,36	99,16	51,36	9,36	9,36

Table S17: IPS values obtained from morphological and DNA metabarcoding analysis in the Drâa river.
Sitecode_1 and _3: dry periods, Sitecode_2and_4: wet periods.

N°	Sub-basins	Site name	Code	IPS_microscopy	IPS_metabarcoding
1	upper Drâa	Ounila Upstream	IO1_1	9,70	9,70
2		Ounila Downstream	IO2_1	8,10	8,70
3		El Mellah	IE_1	6,70	8,10
4		Iriri	IR_1	13,50	14,20
5		Ait douchen	A1_1	12,10	9,60
6		Tarmigte	A2_1	8,60	10,00
7		Dades Upstream	D1_1		
8		Dades Gorges	D2_1	13,80	10,80
9		Dades Middle	D3_1	13,80	10,40
10		M'goun Upstream	DM1_1	12,80	12,50
11		M'goun Downstream	DM2_1	12,60	10,30
12		Dades Downstream	D4_1	10,00	9,00
13	middle Drâa	Kasbah Tamnogalt	DR1_1		
14		Taghzout	DR2_1		
15		Tamgroute	DR3_1	11,10	10,20
16	lower Drâa	Akka Nait Sidi salty	LT1_1	6,60	13,40
17		Akka Nait Sidi less salty	LT2_1	9,40	10,60
18		Tissint Upstream	LT3_1	7,40	9,20
19		Tissint Downstream	LT4_1	8,00	10,50
1	upper Drâa	Ounila Upstream	IO1_2	14,30	15,10
2		Ounila Downstream	IO2_2	15,90	14,50
3		El Mellah	IE_2	14,40	14,80
4		Iriri	IR_2	14,80	14,00
5		Ait douchen	A1_2	10,70	10,80
6		Tarmigte	A2_2	13,00	13,10

7		Dades Upstream	D1_2	15,50	15,90
8		Dades Gorges	D2_2	14,70	14,50
9		Dades Middle	D3_2	17,30	14,90
10		M'goun Upstream	DM1_2	13,40	15,00
11		M'goun Downstream	DM2_2	14,80	15,80
12		Dades Downstream	D4_2	13,90	14,10
13	middle Drâa	Kasbah Tamnogalt	DR1_2	12,50	13,20
14		Taghzout	DR2_2	12,60	13,30
15		Tamgroute	DR3_2	10,70	10,70
16	lower Drâa	Akka Nait Sidi salty	LT1_2	11,60	11,70
17		Akka Nait Sidi less salty	LT2_2	11,20	13,00
18		Tissint Upstream	LT3_2	7,60	11,20
19		Tissint Downstream	LT4_2	10,20	12,00
1	upper Drâa	Ounila Upstream	IO1_3	15,10	13,20
2		Ounila Downstream	IO2_3	10,90	11,00
3		El Mellah	IE_3	9,40	9,00
4		Iriri	IR_3	14,50	13,10
5		Ait douchen	A1_3	9,30	7,80
6		Tarmigte	A2_3	10,30	10,80
7		Dades Upstream	D1_3		
8		Dades Gorges	D2_3	15,40	12,10
9		Dades Middle	D3_3	14,20	14,00
10		M'goun Upstream	DM1_3	13,30	14,20
11		M'goun Downstream	DM2_3	15,00	14,20
12		Dades Downstream	D4_3	11,80	8,20
13	middle Drâa	Kasbah Tamnogalt	DR1_3	12,70	6,50
14		Taghzout	DR2_3		
15		Tamgroute	DR3_3		

16	lower Drâa	Akka Nait Sidi salty	LT1_3	13,10	10,90
17		Akka Nait Sidi less salty	LT2_3	9,80	10,70
18		Tissint Upstream	LT3_3	10,00	12,10
19		Tissint Downstream	LT4_3	8,80	9,90
1	upper Drâa	Ounila Upstream	IO1_4	13,20	13,00
2		Ounila Downstream	IO2_4	9,20	15,20
3		El Mellah	IE_4	11,80	12,90
4		Iriri	IR_4	14,50	14,10
5		Ait douchen	A1_4	10,50	9,60
6		Tarmigte	A2_4	12,60	11,50
7		Dades Upstream	D1_4	15,50	14,90
8		Dades Gorges	D2_4	16,40	14,70
9		Dades Middle	D3_4	17,10	14,50
10		M'goun Upstream	DM1_4	16,20	14,50
11		M'goun Downstream	DM2_4	16,80	11,80
12		Dades Downstream	D4_4	14,10	11,80
13	middle Drâa	Kasbah Tamnogalt	DR1_4	10,80	9,80
14		Taghzout	DR2_4		
15		Tamgroute	DR3_4		
16	lower Drâa	Akka Nait Sidi salty	LT1_4	13,20	18,60
17		Akka Nait Sidi less salty	LT2_4	14,40	11,60
18		Tissint Upstream	LT3_4	9,70	13,10
19		Tissint Downstream	LT4_4	8,20	9,00

Supplementary material. Chapter 4

Table S18: Cyanobacteria identified based on morphological identification.
(IR: Iriri Oued, AR: Ait Douchen Oued, and TS: Tissint Oued). (×) presence

Taxa	**IR**	**IR to AR**	**IR to TS**	**AR**	**AR to IR**	**AR to TS**	**TS**	**TS to AR**	**TS to IR**
	Before transfer	After transfer		Before transfer	After transfer		Before transfer	After transfer	
O/ Synechococcales									
F/ Merismopediaceae									
Aphanocapsa sp. Nägeli				×	×	×			
Merismopedia sp. Meyen	×	×							×
Synechocystis sp1 Sauvageau	×	×		×	×				
Synechocystis sp2 Sauvageau				×				×	
F/ Leptolyngbyaceae									
Leptolyngbya sp1 Anagnostidis & Komárek	×	×		×	×	×		×	×
Leptolyngbya sp2 Anagnostidis & Komárek	×	×			×				×
Leptolyngbya sp3 Anagnostidis & Komárek									×
Leptolyngbya sp4 Anagnostidis & Komárek			×						
Leptolyngbya sp5 Anagnostidis & Komárek							×		
F/Pseudanabaenaceae									
Limnothrix sp. M.-E.Meffert								×	×
Pseudanabaena acicularis (Nygaard) Anagnostidis & Komárek	×				×		×		×
Pseudanabaena articulata Sjuka	×	×		×	×			×	×

Pseudanabaena sp1 Lauterborn				×	×				
Pseudanabaena sp2 Lauterborn			×			×			
F/Synechococcaceae									
Synechococcus sp. Nägeli	×	×	×	×	×	×	×	×	×
O/ Chroococcales									
F/ Aphanothecaceae									
Aphanothece sp. Nägeli				×	×	×			
F/Chroococcaceae									
*Chroococcus sp.*Nägeli								×	
Gloeocapsa sp. Kützing						×			
F/ Entophysalidaceae									
*Siphononema sp.*Geitler								×	
O/Chroococcidiopsidales									
F/Chroococcidiopsidaceae									
Chroococcidiopsis sp1 Geitler				×	×				
Chroococcidiopsis sp2 Geitler	×	×							×
O/ Oscillatoriales									
F/Gomontiellaceae									
Gomontiella sp. Teodoresco					×				
Hormoscilla sp. Anagnostidis & Komárek					×				
Komvophoron sp. Anagnostidis & Komárek				×	×			×	
F/Oscillatoriaceae									
*Lyngbya sp.*C.Agardh ex Gomont								×	
Oscillatoria sp1 Vaucher ex Gomont				×	×			×	
Oscillatoria sp2 Vaucher ex Gomont								×	
Oscillatoria sp3 Vaucher ex Gomont				×	×			×	

Phormidium sp1 Kützing ex Gomont						×			
Phormidium sp2 Kützing ex Gomont				×					
Phormidium sp3 Kützing ex Gomont							×		×

Table S19: Green algae identified based on morphological identification.
(IR: Iriri Oued, AR: Ait Douchen Oued, and TS: Tissint Oued). (×) presence.

	IR	**IR to AR**	**IR to TS**	**AR**	**AR to IR**	**AR to TS**	**TS**	**TS to AR**	**TS to IR**
Taxa	Before Transfer	After transfer		Before Transfer	After transfer		Before transfer	After transfer	
O/Chaetopeltidales									
F/Chaetopeltidaceae									
Chaetopeltis orbicularis Berthold	×	×		×	×			×	×
O/Chaetophorales									
F/Aphanochaetaceae									
Aphanochaete elegans Tupa	×	×		×	×	×	×	×	×
Aphanochaete repens A.Braun	×	×		×	×			×	×
F/Fritschiellaceae									
Stigeoclonium sp. Kützing	×	×	×	×	×	×	×	×	×
O/Chlamydomonadales									
F/Chlamydomonadaceae									
Chlamydomonas sp. Ehrenberg	×	×			×				
F/Chlorochytriaceae									
*Chlorochytrium sp.*Cohn	×	×	×	×	×	×	×	×	×
F/Chlorococcaceae									
Chlorococcum infusionum (Schrank) Meneghini	×	×							

*Chlorococcum sp.*Meneghini				×	×	×	×		
F/Chlorosarcinaceae									
Neochlorosarcina minor (Gerneck) V.M.Andreeva	×	×	×	×	×	×		×	×
O/Chlorellales									
F/Chlorellaceae									
Chlorella sorokiniana Shihira & R.W.Krauss	×	×	×				×	×	×
Chlorella sp. Beyerinck	×	×	×	×	×		×		
O/Klebsormidiales									
F/Klebsormidiaceae									
Interfilum sp. Chodat	×				×				
O/Trebouxiales									
F/Trebouxiaceae									
Myrmecia bisecta Reisigl	×								
Myrmecia sp. Printz				×		×		×	
Trebouxia sp. Puymaly	×	×	×						
O/Oedogoniales									
F/Oedogoniaceae									
Oedogonium sp. Link ex Hirn	×	×		×	×		×	×	×
O/Sphaeropleales									
F/Hydrodictyaceae									
Stauridium tetras (Ehrenberg) E.Hegewald				×	×			×	
F/Mychonastaceae									
Mychonastes sp. P.D.Simpson & S.D.Van Valkenburg	×	×	×	×	×	×	×		×
Mychonastes jurisii (Hindák) Krienitz	×	×		×	×			×	×

F/Neochloridaceae									
Neochloris sp. Starr	×	×		×	×	×		×	×
Neochloris vigensis P.A.Archibald	×	×	×	×	×	×		×	×
F/Scenedesmaceae									
Desmodesmus sp.(Chodat) S.S.An, T.Friedl & E.Hegewald				×	×	×		×	
Scenedesmus abundans (O.Kirchner) Chodat	×	×	×	×	×	×	×	×	×
Scenedesmus quadricauda (Turpin) Brébisson				×					×
*Scenedesmus sp.*Meyen	×			×	×	×		×	×
Scenedesmus subspicatus Chodat				×					
F/Selenastraceae									
*Monoraphidium sp.*Komárková-Legnerová	×	×		×	×				
O/Stichococcaceae									
F/Stichococcus									
Stichococcus sp. Nägeli	×	×	×	×	×				
O/Ulotrichales									
F/Ulotrichaceae									
*Ulothrix sp.*Kützing	×	×							

Table S20: Diatom identified based on morphological identification.
(IR: Iriri Oued, AR: Ait Douchen Oued, and TS: Tissint Oued). (×) presence.

Taxa	**IR**	**IR to AR**	**IR to TS**	**AR**	**AR to IR**	**AR to TS**	**TS**	**TS to AR**	**TS to IR**
	Before transfer	After Transfer		Before transfer	After transfer		Before transfer	After transfer	
G/Achnanthidium									
Achnanthidium minutissimum (Kützing) Czarnecki	×	×		×	×		×	×	×
G/Amphora									
Amphora ovalis (Kützing) Kützing				×		×	×	×	
Amphora pediculus (Kützing) Grunow	×	×	×	×	×			×	×
G/Aulacoseira									
Aulacoseira granulata (Ehrenberg) Simonsen							×		
G/Cocconeis									
Cocconeis pediculus Ehrenberg	×	×	×	×	×	×	×	×	×
Cocconeis stauroneiformis H.Okuno							×		
G/Cymbella									
Cymbella affinis Kützing	×	×	×	×	×	×		×	×
Cymbella cymsbiformis C.Agardh	×	×			×				
Cymbella tumida (Brébisson) Van Heurcka	×	×	×		×				×
G/Diatoma									
Diatoma vulgaris bory	×				×				×
G/Diploneis									
Diploneis puella (Schumann) Cleve							×		
G/Encyonema									
Encyonema caespitosum Kützing	×	×			×				
Encyonema minutum (Hilse) D.G.Mann	×								×
G/Encyonopsis									

Encyonopsis microcephala (Grunow) Krammer	×	×		×	×			×	×
G/Eolimna									
Eolimna minima (Grunow) Lange-Bertalot	×	×							
G/Epithemia									
Epithemia gibba (Ehrenberg) Kützing		×		×	×	×		×	
Epithemia sorex Kützing		×		×	×	×		×	
Epithemia turgida (Ehrenberg) Kützing				×	×			×	
G/Frustulia									
Frustulia vulgaris (Thwaites) De Toni							×		
G/Gomphonella									
Gomphonella olivácea (Hornemann) Rabenhorst				×	×				
G/Gomphonema									
Gomphonema angustatum (Kützing) Rabenhorst	×	×							×
Gomphonema affine Kützing	×	×			×				×
Gomphonema intricatum kützing	×	×			×				×
Gomphonema parvulum (Kützing) Kützing	×	×		×	×			×	×
Gomphonema truncatum var. Turgidum (Ehrenberg) R.M.Patrick	×	×							×
G/Grunowia									
Grunowia tabellaria (Grunow) Grunow	×	×							
G/Halamphora									
Halamphora coffeiformis (C.Agardh) Mereschkowsky			×			×	×	×	×
G/Homoeocladia									

Homoeocladia amphibia (Grunow) Kuntze	×	×		×	×	×		×	×
Homoeocladia inconspicua (Grunow) Kuntze	×	×	×						×
Homoeocladia palea (Kützing) Kuntze	×	×	×	×	×	×	×	×	×
G/Mastogloia									
Mastogloia elliptica (C.Agardh) Cleve				×		×	×	×	
Mastogloia smithii Thwaites ex W.Smith			×	×		×	×	×	×
G/Melosira									
Melosira varians C.Agardh	×	×			×				
G/Navicula									
Navicula cryptocephala Kützing			×	×		×	×	×	×
Navicula cryptotenella Lange-Bertalot								×	
Navicula phyllepta Kützing		×		×		×	×	×	
Navicula tripunctata (O.F.Müller) Bory						×			
G/Nitzschia									
Nitzschia frustulum (Kützing) Grunow							×	×	
Nitzschia microcephala Grunow	×	×		×	×			×	×
G/Planothidium									
Planothidium delicatulum (Kützing) Round & Bukhtiyarova			×			×	×		
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	×								×
G/Pleurosigma									
Pleurosigma intermedium W.Smith							×		
G/Podosira									
Podosira dubia (Kützing) Grunow			×			×	×		
G/Pseudostaurosira									
Pseudostaurosira brevistriata (Grunow) D.M.Williams & Round	×	×							×

Pseudostaurosira elliptica (Schumann) Edlund, Morales & Spaulding	×								×
G/Sellaphora									
Sellaphora bacillum (Ehrenberg) D.G.Mann	×	×							×
Sellaphora pupula (Kützing) Mereschkovsky			×			×	×	×	×
G/Stephanocyclus									
Stephanocyclus cryptica Reimann, J.C.Lewin & Guillard							×	×	
Stephanocyclus meneghinianus (Kützing) Kulikovskiy, Genkal & Kociolek	×	×	×	×				×	×
G/Tabularia									
Tabularia fasciculata						×	×	×	×
G/Tryblionella									
Tryblionella apiculata W.Gregory			×			×	×		×
Tryblionella compressa (Bailey) Poulin			×			×	×		×
Tryblionella sp. W.Smith			×			×	×	×	×
Tryblionella victoriae Grunow			×			×	×		
G/Ulnaria									
Ulnaria acus (Kützing) Aboal	×	×		×	×			×	×
Ulnaria ulna (Nitzsch) Compère	×	×		×	×			×	×

Table S21: Correlation results between environmental parameters and axes 1 and 2 of the NMDS analysis (beta diversity) for cyanobacteria using morphological identification.

Parameter	NMDS1	NMDS2	r2	Pr(>r)
Conductivity	0.95661	0.29136	0.8498	0.005 **
Salinity	0.94788	0.31861	0.8558	0.005 **
pH	0.31195	0.95010	0.3818	0.271
Oxygen	-0.95389	-0.30014	0.1606	0.534
Temperature	0.72747	0.68614	0.5435	0.098
Nitrate	-0.87771	-0.47920	0.0315	0.903
Nitrite	-0.03889	-0.99924	0.1664	0.630
Ammonium	0.97507	-0.22191	0.3403	0.259
Orthophosphate	0.68058	0.73267	0.0903	0.749
Chloride	0.92144	0.38852	0.8670	0.008 **
Sulfate	0.93633	0.35113	0.8299	0.009 **
Alkalinity	0.27059	0.96269	0.3854	0.267
Hardness	0.98767	-0.15655	0.6714	0.022 *

Table S22: Correlation results between environmental parameters and axes 1 and 2 of the NMDS analysis (beta diversity) for green algae using morphological identification.

Parameter	NMDS1	NMDS2	r2	Pr(>r)
Conductivity	0.66244	0.74911	0.9621	0.003 **
Salinity	0.66164	0.74982	0.9713	0.003 **
pH	0.46573	0.88493	0.3351	0.277
Oxygen	-0.68805	-0.72567	0.1232	0.689
Temperature	0.69297	0.72097	0.4819	0.126
Nitrate	-0.46967	-0.88284	0.1287	0.663
Nitrite	-0.19243	-0.98131	0.1158	0.489
Ammonium	0.88797	0.45990	0.1983	0.528
Orthophosphate	0.34411	0.93893	0.0492	0.846
Chloride	0.66009	0.75118	0.9793	0.002 **
Sulfate	0.65223	0.75802	0.75802	0.002 **
Alkalinity	0.48347	0.87536	0.2952	0.331
Hardness	0.71656	0.69752	0.6362	0.057

Table S23: Correlation results between environmental parameters and axes 1 and 2 of the NMDS analysis (beta diversity) for diatoms using morphological identification.

Parameter	NMDS1	NMDS2	r2	Pr(>r)
Conductivity	0.97034	-0.24173	0.8600	0.001 ***
Salinity	0.96778	-0.25180	0.8507	0.001 ***
pH	0.81713	0.57645	0.2619	0.372
Oxygen	-0.74240	-0.66996	0.2580	0.401
Temperature	0.70275	-0.71143	0.2714	0.405

Nitrate	-0.95448	0.29826	0.1140	0.657
Nitrite	-0.41140	-0.91145	0.2487	0.466
Ammonium	0.71160	-0.70258	0.2030	0.489
Orthophosphate	0.44825	0.89391	0.1420	0.616
Chloride	0.95958	-0.28142	0.8100	0.010 **
Sulfate	0.97583	-0.21854	0.8564	0.003 **
Alkalinity	0.79593	0.60539	0.2285	0.417
Hardness	0.94774	-0.31905	0.7171	0.009 **

Table S24: Correlation results between environmental parameters and axes 1 and 2 of the NMDS analysis (beta diversity) for cyanobacteria based on molecular analysis.

Parameter	NMDS1	NMDS2	r2	Pr(>r)
Conductivity	0.85699	0.51534	0.8523	0.004 **
Salinity	0.84514	0.53455	0.8520	0.002 **
pH	0.19821	0.98016	0.5870	0.077 .
Oxygen	-0.82383	-0.56684	0.1495	0.608
Temperature	0.66349	0.74819	0.3511	0.263
Nitrate	-0.53315	-0.84602	0.1157	0.730
Nitrite	-0.07925	-0.99685	0.3913	0.188
Ammonium	0.89627	-0.44350	0.2737	0.396
Orthophosphate	0.56767	0.82326	0.1120	0.686
Chloride	0.81404	0.58081	0.8406	0.005 **
Sulfate	0.81597	0.57810	0.8538	0.003 **
Alkalinity	0.15521	0.98788	0.5568	0.102
Hardness	0.99568	0.09281	0.6598	0.024 *

Table S25: Correlation results between environmental parameters and axes 1 and 2 of the NMDS analysis (beta diversity) for green algae based on molecular analysis.

Parameter	NMDS1	NMDS2	r2	Pr(>r)
Conductivity	0.89547	-0.44512	0.8380	0.007 **
Salinity	0.87208	-0.48937	0.8528	0.007 **
pH	0.31458	-0.94923	0.5307	0.112
Oxygen	-0.59412	-0.80438	0.0976	0.768
Temperature	0.43121	-0.90225	0.7259	0.053 .
Nitrate	-0.67268	0.73994	0.1723	0.554
Nitrite	-0.23524	0.97194	0.1407	0.494
Ammonium	0.85978	0.51066	0.1865	0.575
Orthophosphate	0.96343	-0.26797	0.0187	0.958
Chloride	0.80529	-0.59288	0.8870	0.004 **
Sulfate	0.85379	-0.52062	0.8622	0.002 **
Alkalinity	0.27853	-0.96043	0.4971	0.126
Hardness	0.95216	0.30561	0.6264	0.071 .

Table S26: Correlation results between environmental parameters and axes 1 and 2 of the NMDS analysis (beta diversity) for diatoms based on molecular analysis.

Parameter	NMDS1	NMDS2	r2	Pr(>r)
Conductivity	0.86634	-0.49946	0.8561	0.024 *
Salinity	0.87361	-0.48662	0.8759	0.022 *
pH	0.87453	0.48496	0.4285	0.225
Oxygen	-0.92018	-0.39150	0.1366	0.629
Temperature	0.93767	-0.34751	0.6304	0.069 .
Nitrate	-0.69237	0.72154	0.0678	0.883
Nitrite	-0.50288	-0.86435	0.1696	0.611
Ammonium	0.61824	-0.78599	0.2275	0.455
Orthophosphate	0.72653	0.68714	0.0740	0.759
Chloride	0.89158	-0.45287	0.9188	0.015 *
Sulfate	0.88820	-0.45946	0.8706	0.022 *
Alkalinity	0.83620	0.54843	0.4372	0.230
Hardness	0.68466	-0.72886	0.5184	0.099 .

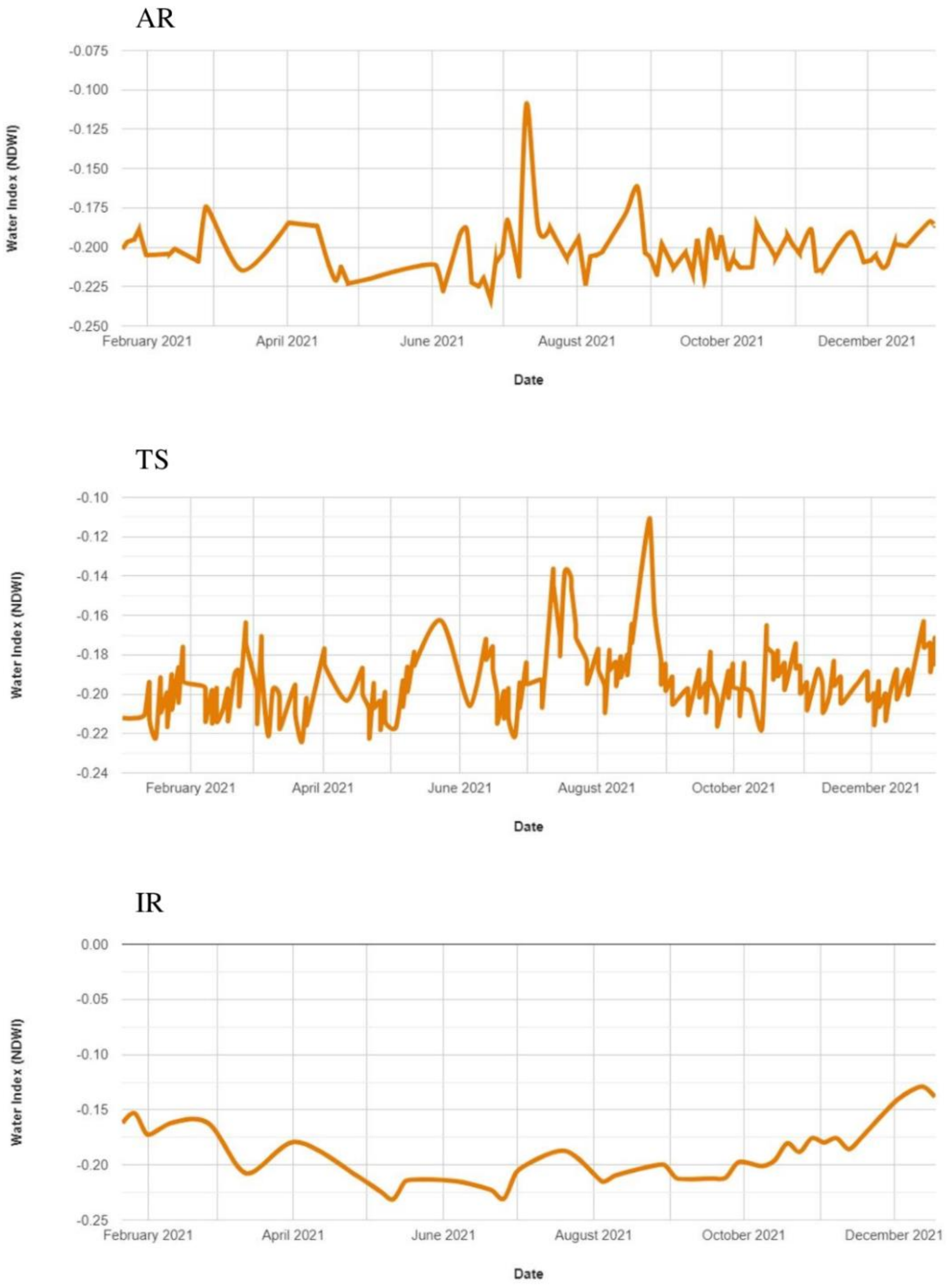


Figure S8: Normalized difference water index (NDWI) using Sentinel imagery from 2021.

Calculation of a 1 km² grid area around each studied site with a maximum cloud cover of 3%.

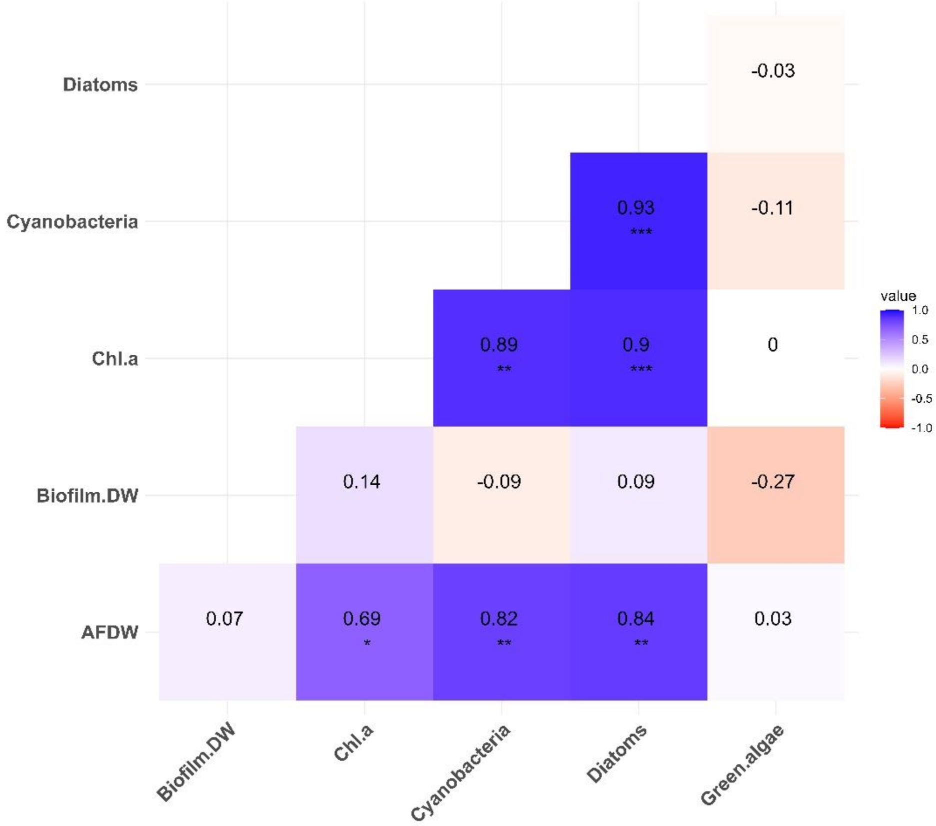
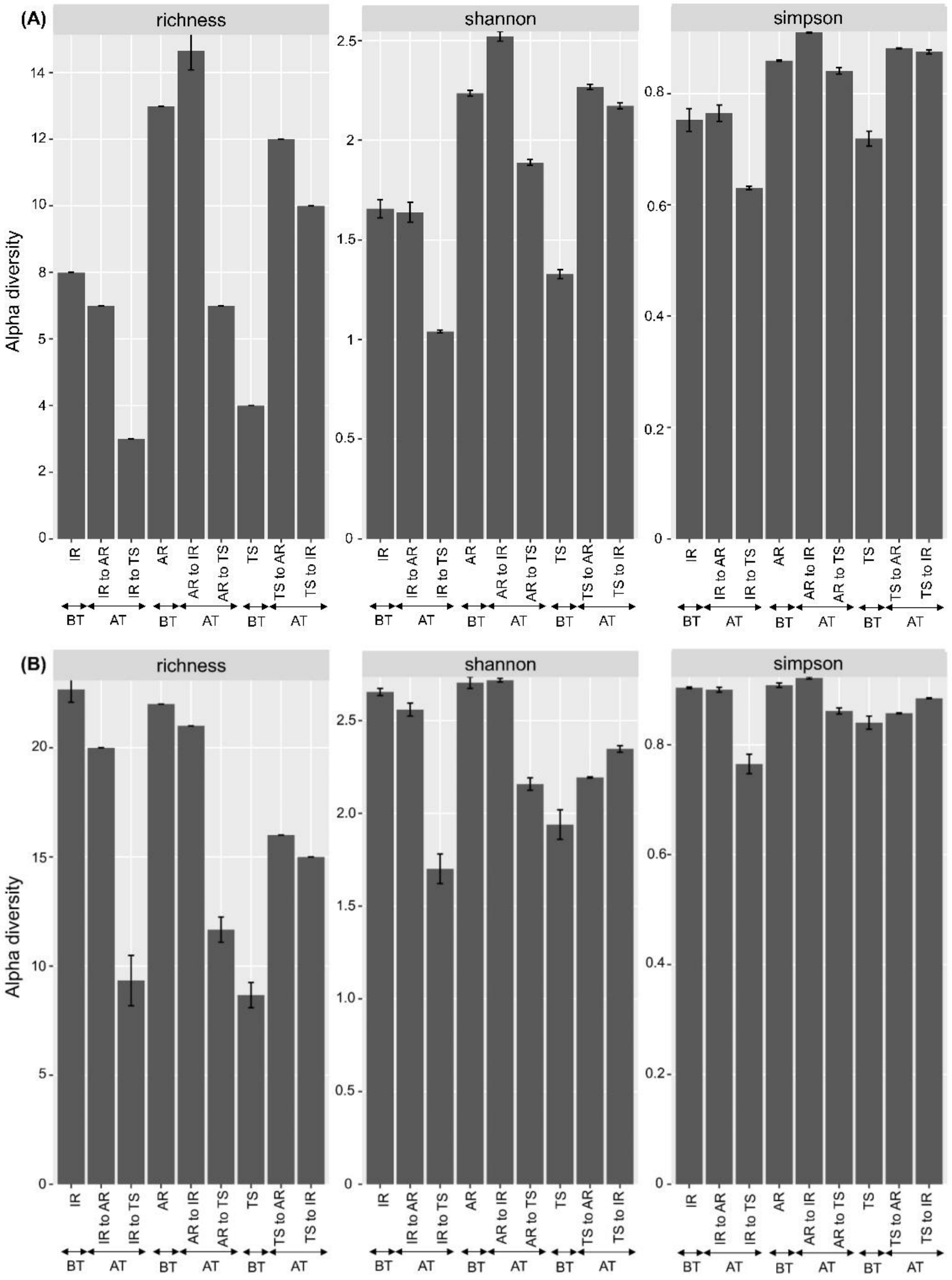


Figure S9: Pearson correlation coefficient values between biofilm biomass parameters. (*** $p < 0.001$), (**$p < 0.01$), and (* $p < 0.05$).


(A)
richness
shannon
simpson
Alpha diversity
0
2
4
6
8
10
12
14
0
0.5
1
1.5
2
2.5
0
0.2
0.4
0.6
0.8
IR
IR to AR
IR to TS
AR
AR to IR
AR to TS
TS
TS to AR
TS to IR
BT
AT
(B)
richness
shannon
simpson
Alpha diversity
0
5
10
15
20
0
0.5
1.0
1.5
2.0
2.5
0
0.2
0.4
0.6
0.8
IR
IR to AR
IR to TS
AR
AR to IR
AR to TS
TS
TS to AR
TS to IR
BT
AT

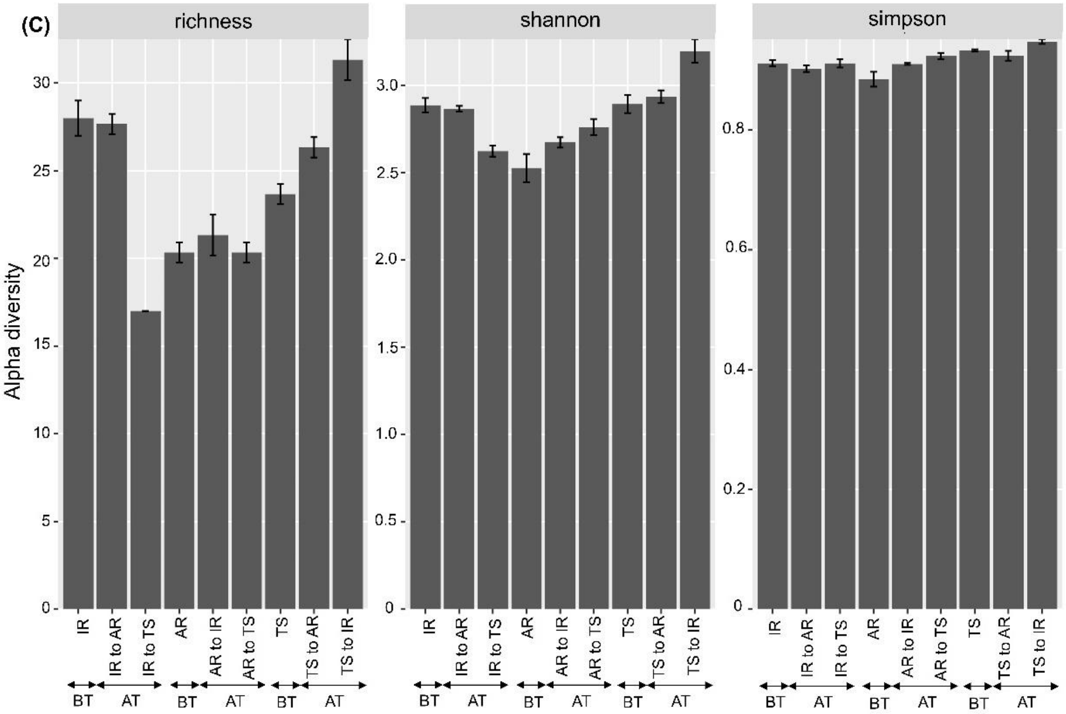
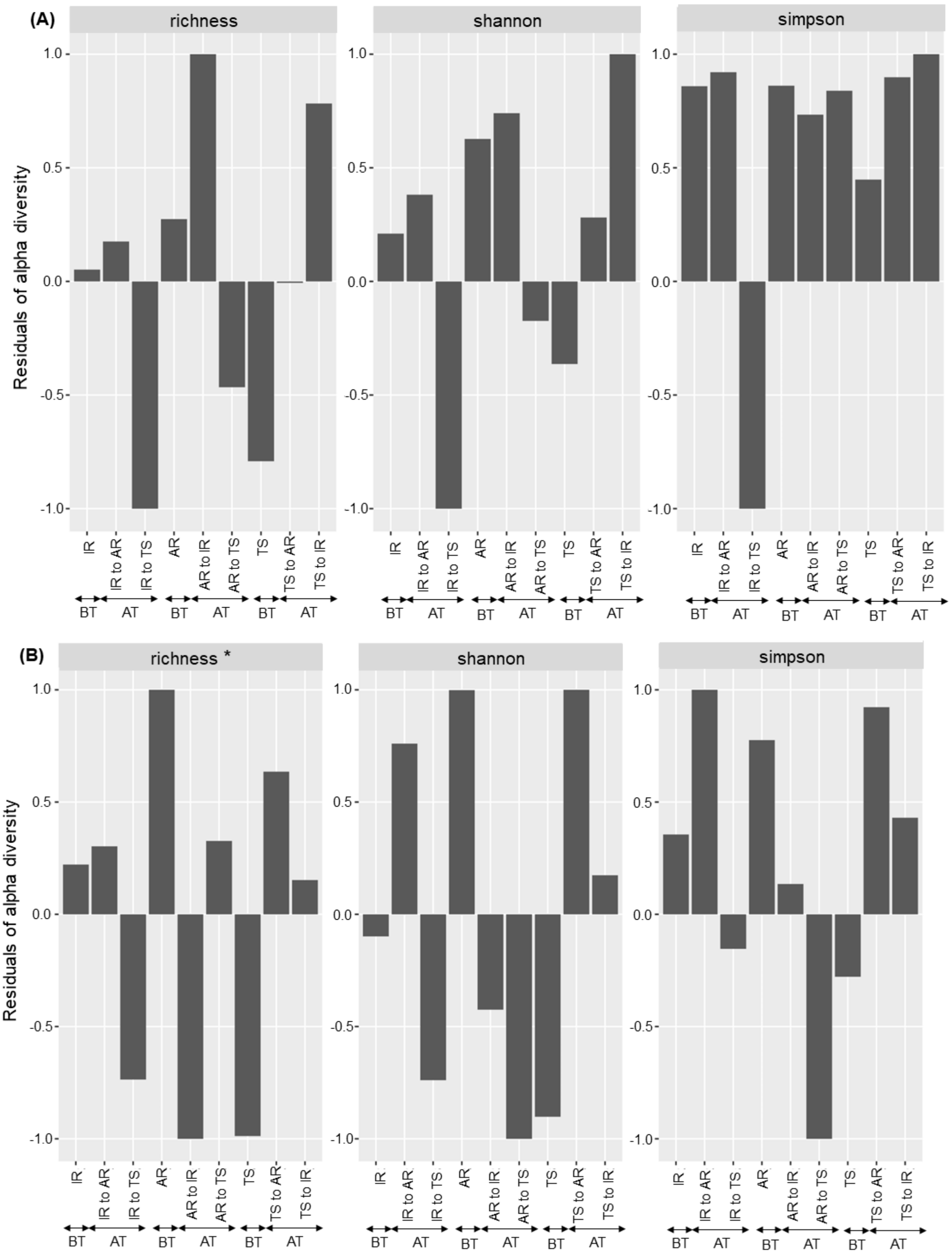


Figure S10: Alpha diversity based on morphological identification (i.e., richness, Shannon index, and Simpson index) for cyanobacteria (A), green algae (B), and diatoms (C).

BT: before transfer. AT: after transfer.


(A)
richness
shannon
simpson
Residuals of alpha diversity
1.0
0.5
0.0
-0.5
-1.0
IR
IR to AR
IR to TS
AR
AR to IR
AR to TS
TS
TS to AR
TS to IR
BT
AT
(B)
richness *
shannon
simpson
Residuals of alpha diversity

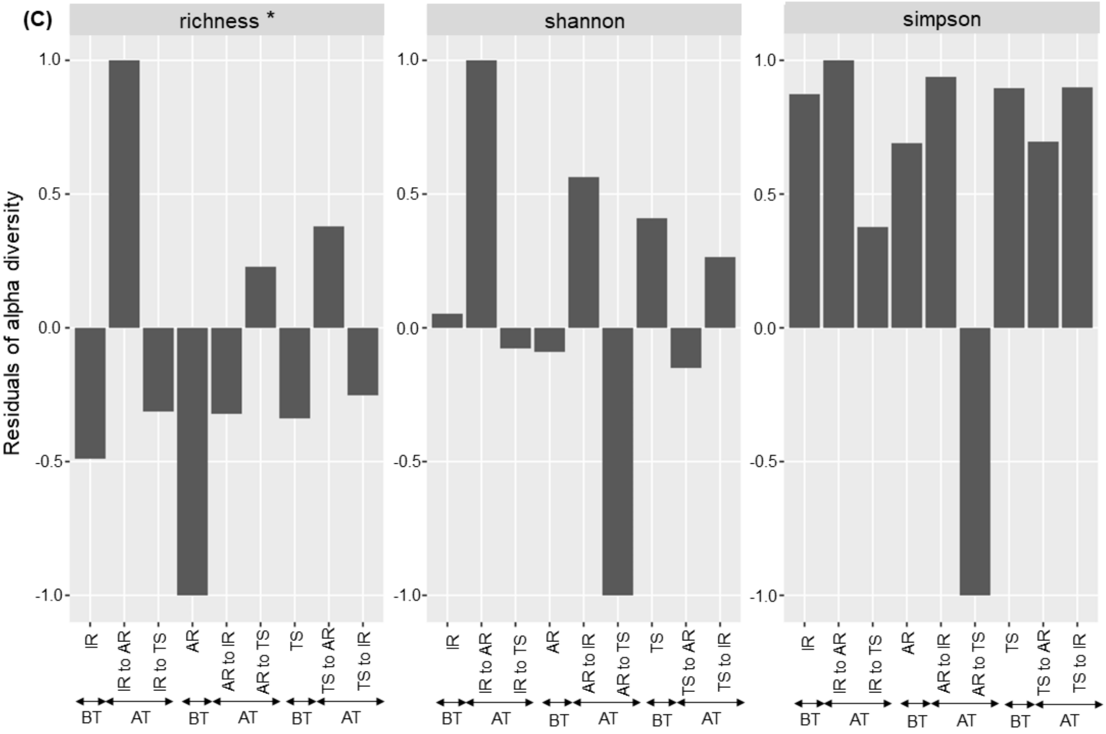


Figure S11: Alpha diversity based on molecular analysis, calculated as Hills numbers with exponent 0, 1, and 2 (i.e., richness, Shannon index, and Simpson index) for cyanobacteria (A), green algae (B), and diatoms (C).

In case a calculated index revealed a linear correlation with the square root transformed read counts per sample pointing to a sequencing depth bias, only the residuals of the linear model were analyzed (indicated by *). Values were centered-scaled on a -1 to 1 range to allow for comparability between the indices. BT: before transfer. AT: after transfer.

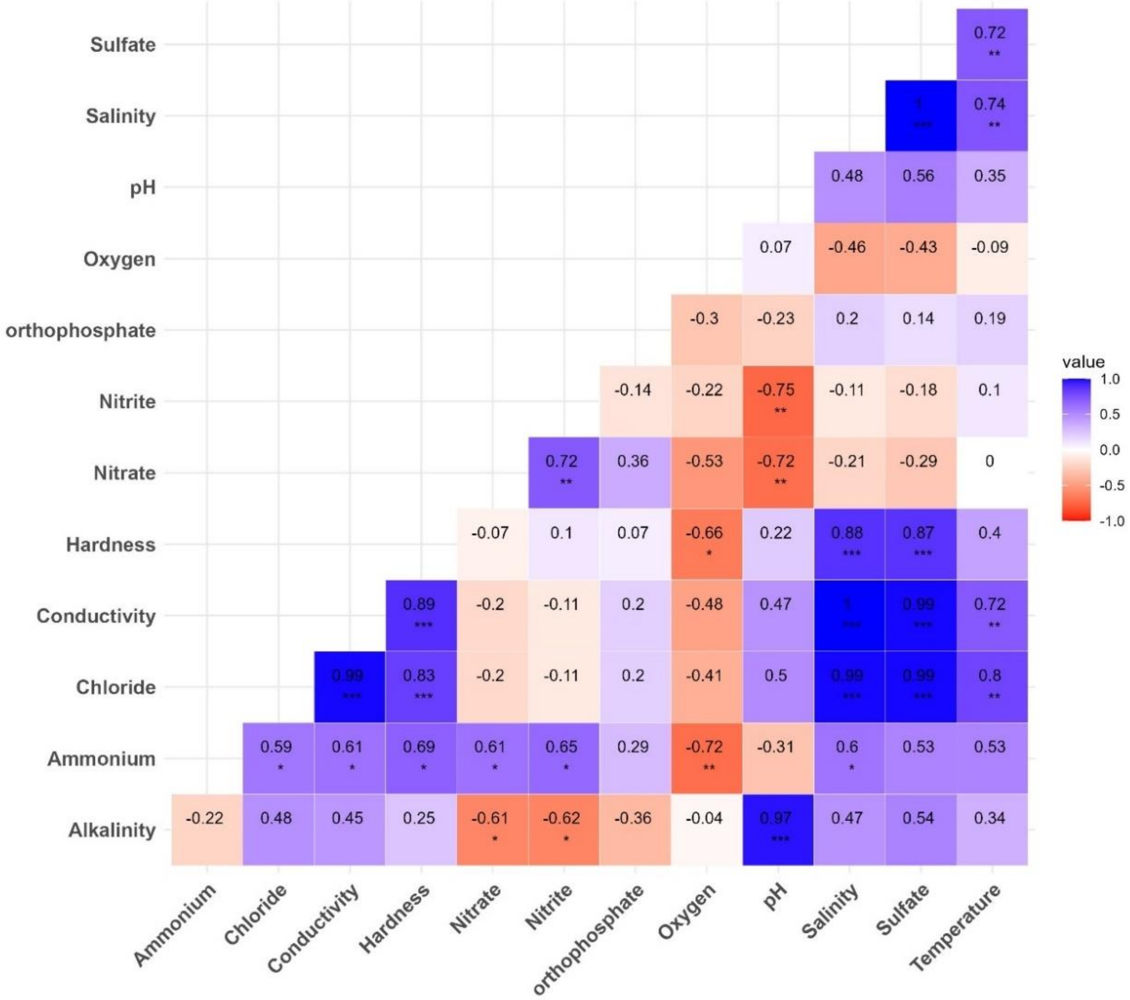
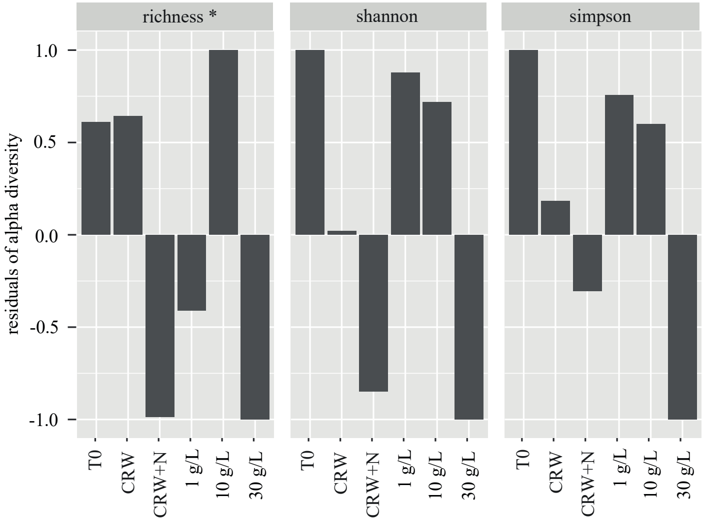
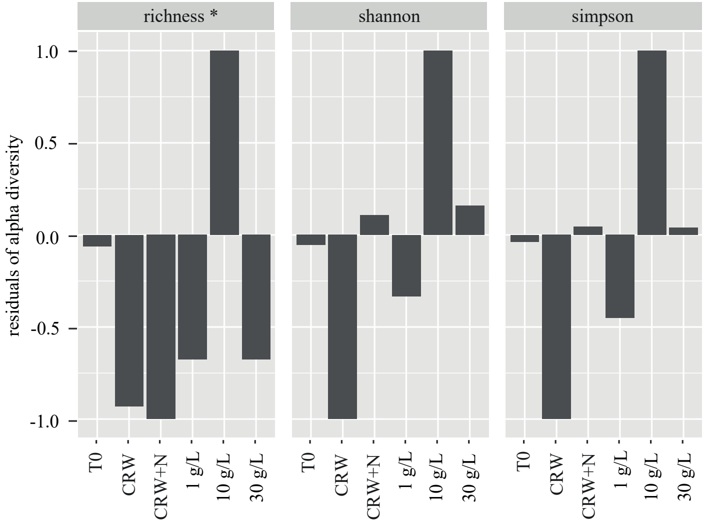


Figure S12: Pearson correlation coefficient values between environmental variables. (*** $p < 0.001$), (**$p < 0.01$), and (* $p < 0.05$).

(A) microeukaryotes



(B) green algae



(C) diatoms

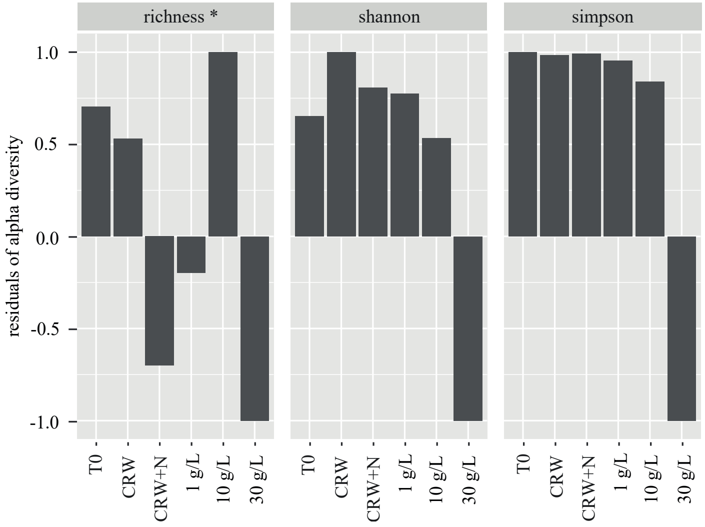


Figure S13. Alpha diversity, calculated as Hills numbers with exponent 0, 1, and 2 (i.e., richness, Shannon index, and inverse Simpson index) for total microeukaryotes (A), green algae (B), and diatoms (C).

In case a calculated index revealed a linear correlation with the square root transformed read counts per sample pointing to a sequencing depth bias, only the residuals of the linear model were analysed (indicated by *). Values were centred-scaled on a -1 to 1 range to allow for comparability between the indices.

the

a -

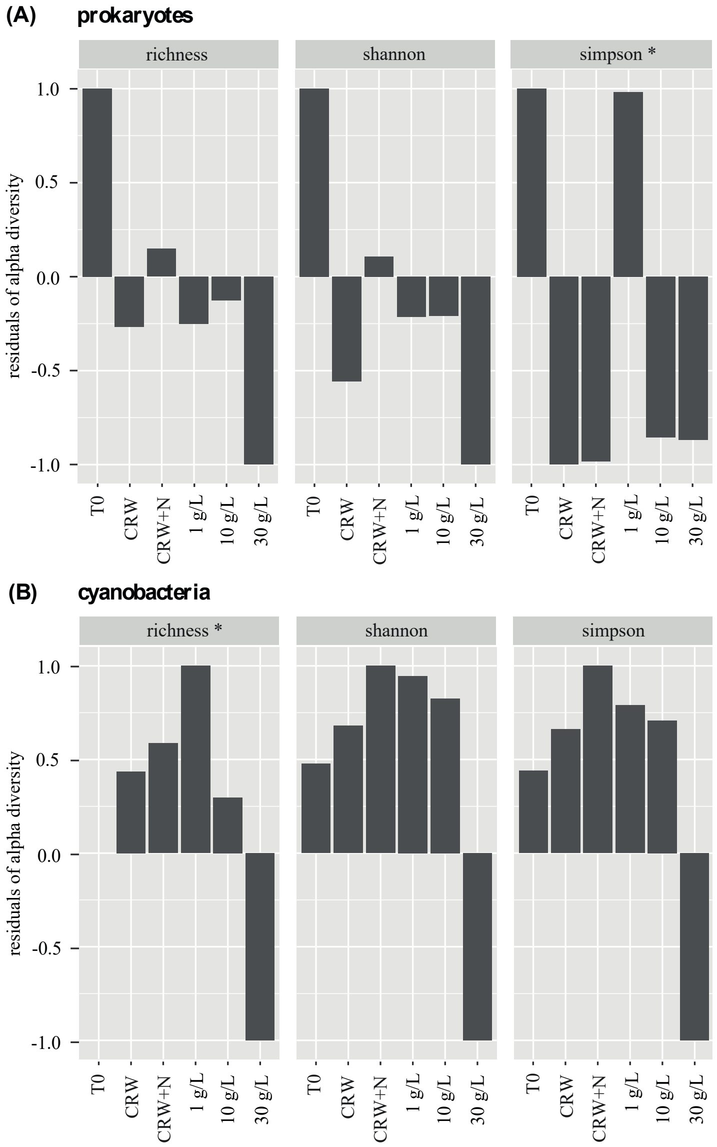


Figure S14. Alpha diversity, calculated as Hills numbers with exponent 0, 1, and 2 (i.e., richness, Shannon index, and inverse Simpson index) for total prokaryotes (A) and cyanobacteria (B).

In case a calculated index revealed a linear correlation with square root transformed read counts per sample pointing to a sequencing depth bias, only the residuals of the linear model were analyzed (indicated by *). Values were centered-scaled on 1 to 1 range to allow for comparability between the indices.

Author Contributions

Article 1:

Lazrak, K., Nothof, M., Filker, S., El Bouaidi,W. , Berger, E., Loudiki,M. Water salinity impacts on the composition, structure and diversity of benthic microalgal communities in the arid Drâa river (Southeastern Morocco) using morphological and molecular metabarcoding. Submitted.

Lazrak, K.: Conceptualization, Methodology, Formal analysis, Investigation, Writing of original draft, and review and editing. **Nothof, M.**: Data curation, Formal analysis, Methodology, Writing – review and editing. **Filker, S**.: Formal analysis, Methodology, Writing – review and editing. **El Bouiadi, W**.: Formal analysis, Investigation. **Berger, E**.: Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing – review and editing. **Loudiki, M**.: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – review and editing.

Article 2

Lazrak, K., Nothof, M., Filker, S., Berger, E., Loudiki,M. Water quality assessment and diatom-based biomonitoring of the ecological status of the Drâa river basin.

Lazrak, K.: Conceptualization, Methodology, Formal analysis, Investigation, Writing of original draft, and review and editing. **Nothof, M.**: Data curation, Formal analysis, Methodology, Writing – review and editing. **Filker, S**.: Formal analysis, Methodology, Writing – review and editing. **Berger, E**.: Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing – review and editing. **Loudiki, M**.: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – review and editing.

Article 3:

Lazrak, K., Tazart, Z., Nothof, M., Filker, S., Hakkoum, Z., Kaczmarek, N., ... & Loudiki, M. (2025). Assessment of the short-term salinity effect on algal biofilm through field transfer in the Drâa river (Southeastern Morocco) using metabarcoding and morphological analyses. Environmental Monitoring and Assessment, *197*(4), 1-22.

Lazrak, K.: Conceptualization, Methodology, Formal analysis, Investigation, Writing of original draft, and review and editing. **Tazart, Z**.: Formal analysis, Data curation, Investigation, Methodology. **Nothof, M**.: Data curation, Formal analysis, Methodology, Writing – review & editing. **Filker, S**.: Formal analysis, Methodology, Writing – review & editing. **Hakkoum, Z.**: Formal analysis, Investigation. **Kaczmarek, N**.: Formal analysis, Investigation. E. B.: Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing– review &

editing., **Mouhri, K**.: Conceptualization, Supervision, Writing – review & editing. **Loudiki, M.**: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – review and editing.

Article 4

Lazrak, K., Nothof, M., Tazart, Z., Filker, S., Berger, E., Mouhri, K., & Loudiki, M. (2024). Salt stress responses of microalgae biofilm communities under controlled microcosm conditions. Algal Research, 103430.

Lazrak, K.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Nothof, M**: Data curation, Formal analysis, Methodology, Writing – review and editing. **Tazart, Z**.: Data curation, Investigation, Methodology. **Filker, S**: Formal analysis, Methodology, Writing – review and editing. Elisabeth Berger: Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing – review and editing. **Mouhri, K**.: Conceptualization, Supervision, Writing – review and editing. **Loudiki, M.**: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – review and editing.

Article 5

Lazrak, K., Tazart, Z., Berger, E., Mouhri, K., & Loudiki, M. Salt stress responses of two diatom strains isolated from different saline habitats under laboratory batch cultures.

Lazrak, K.: Conceptualization, Methodology, Formal analysis, Investigation, Writing of original draft, and review and editing. **Tazart, Z**.: Formal analysis, Data curation, Investigation, Methodology. **Mouhri, K**.: Conceptualization, Supervision, Writing – review and editing. **Loudiki, M.**: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – review and editing.

Curriculum Vitae

Khawla Lazrak

Education

2019-2025: PhD in Environmental Sciences

Faculty of Sciences, Semlalia Marrakech, Cadi Ayyad University, and iES Landau, RPTU Kaiserslautern-Landau, Landau, Germany.

July 2018: Master's degree in Ecology and Management of Continental Aquatic Ecosystems (Faculty of Sciences, Semlalia, Marrakech), Cadi Ayyad University.

Thesis: Structure and diversity of periphytic algal communities in the Drâa river: impact of water salinization

July 2016: Bachelor's degree in animal physiology.

Faculty of Sciences Semlalia Marrakech, Cadi Ayyad University.

Thesis: Comparative study of the analgesic and anti-inflammatory activity of the mixture of Ormenis multicaulis and Carum carvi and their extracts alone

June 2012: High school diploma in Physics and Chemistry (Al Manar School, Marrakech).

Experience

- **2019 - 2020**: Co-supervision of bachelor's project, semester 6 at Cadi Ayyad University, Marrakech. Study of the algal flora of the aquatic basin of the Semlalia Faculty of Sciences.

- **2019 – 2020**: Participation in teaching practical courses on "systematics, evolution, and biogeography" as part of the Master's program in Biodiversity, Climate Change, and Sustainable Development during Semester 1 at the Faculty of Sciences Semlalia Marrakech.

- **2019 – 2020**: Participation in teaching practical classes on "Physico-chemical analysis of water" as part of the "Chemistry and Microbiology of Water" modules of the LP-STADLS professional bachelor's degree, and "Ecological Diagnosis and Analysis Techniques" of the fundamental bachelor's degree in Life Sciences, GEBC course at the Faculty of Sciences Semlalia Marrakech.

- **2018 – 2019:** Co-supervision of bachelor’s project, semester 6 at Cadi Ayyad University, Marrakech. Tolerance and impact of salinity on microalgae communities in rivers.

- **2018 – 2019**: Participation in teaching practical classes on “Algae Biodiversity” as part of the Biodiversity and Secondary Metabolism of Plants module, within the Applied Biology for Plant Production course in Semester 6 of the bachelor's students at the Faculty of Sciences, Semlalia, Marrakech.

Publications

- **Lazrak, K.,** Tazart, Z., Nothof, M., Filker, S., Hakkoum, Z., Kaczmarek, N., Mouhri K. & Loudiki, M. (2025). Assessment of the short-term salinity effect on algal biofilm through field transfer in the Drâa river (Southeastern Morocco) using metabarcoding and morphological analyses. Environmental Monitoring and Assessment, 197(4), 1-22.
- **Lazrak, K.,** Nothof, M., Tazart, Z., Filker, S., Berger, E., Mouhri, K., & Loudiki,M. (2024). Salt stress responses of microalgae biofilm communities under controlled microcosm conditions. Algal Research, 78, 103430.
- **Lazrak, K**., Tazart, Z., Berger, E., Mouhri, K., & Loudiki, M. (2022). Spatial variation in Benthic diatom communities in relation to salinity in the arid Drâa river basin (Southern Morocco). Applied Ecology & Environmental Research, 20(5).
- Tazart, Z., **Lazrak, K**., El Bouaidi, W., Redouane, E. M., Tebaa, L., Douma, M.,
- ... & Loudiki, M. (2024). Scaling up the Natural Mode of Action of Macrophyte Allelochemicals and Their Effect on Toxic Cyanobacteria Using a Nitrogen-Limited Chemostat. Processes, 12(9), 1883.
- Kaczmarek, N., Benlasri, M., Schäfer, R. B., Aabid, A., Nothof, M., **Lazrak, K**., ... & Berger, E. (2024). Macroinvertebrate community responses to salinity around non-saline–saline confluences in the Drâa river basin, Morocco. Hydrobiologia, 851(9), 2189-2204.
- Douma, M., Tazart, Z., Tebaa, L., El Bouaidi, W., Hakkoum, Z., Minaoui, F., **Lazrak, K.,**.. & Loudiki, M. (2021). Algicidal effect of extracts from a green macroalgae (Chara vulgaris) on the growth of the potentially toxic cyanobacterium (Microcystis aeruginosa). Applied Ecology & Environmental Research, 19(6)

Communications

- **Lazrak K.**, Tazart Z., Berger E., Mouhri K. and Loudiki M. International Conference on Water Innovation and Smart Irrigation (ICWAS), 25-27 September 2024, Marrakesh, Morocco. Poster communication.
- **Lazrak K.,** Nothof M., Tazart Z., Filker S., Berger E., El Bouaidi W., Mouhri K. and Loudiki M. Effect of salinity changes on composition and biomass of benthic diatom communities: Biofilm-field transfer experiment in the Drâa river (southeastern Morocco).6th Euro-Mediterranean Conference for Environmental Integration (EMCEI), 15-18 May 2024, Marrakesh, Morocco. Oral communication.
- **Lazrak K.**, Tazart Z., Berger E., Mouhri K. and Loudiki M. Effect of increasing salinity on diatom species in laboratory batch cultures. 3ème Congrès International sur les Algues, 26-28 October 2023. Marrakesh. Morocco. Oral communication.
- **Lazrak K.**, Tazart Z., Berger E., Mouhri K. and Loudiki M. Salinity effect on river biofilm communities: A Microcosm Study. Second Meeting of the Iberian Ecological Society (SIBECOL) XXI conference of the Iberian Association of Limnology (AIL) and the 21st National Congress of the Portuguese Ecological Society (SPECO). Aveiro, Portugal. Communication orale.
- **Lazrak K.**, Berger E., Mouhri K. and Loudiki M. Distribution of benthic diatom communities in the arid Drâa river basin (South of Morocco) in relation to water salinity. Ecole International de Recherche «8ème édition» Biodiversité, Biotechnologies, Durabilité & Innovation, 20-22 December 2021, Agadir, Morocco. Oral communication.
- **Lazrak K.**, Berger E., Mouhri K. and Loudiki M. Salinity impacts on water quality and benthic diatoms communities in the arid Draa river basin (South of Morocco). 9th International Virtual Conference in the Mediterranean Basin (WATMED9), 26-28 May 2021, Marrakesh, Morocco. Oral communication