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Camille COURCOUL

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Influence du régime de perturbations sur la stabilité de communautés microbiennes aquatiques dans un contexte d'intermittence hydrique

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Thèse dirigée par

Joséphine LEFLAIVE et Stéphanie BOULÊTREAU

Jury

Annette BÉRARD, Rapporteure
Helena GUASCH PADRO, Rapporteure
Thibault DATRY, Examinateur
Jean-Luc ROLS, Président du Jury
Joséphine LEFLAIVE, Directrice de thèse
Stéphanie BOULÊTREAU, Co-Directrice de thèse
Martin LAVIALE, Invité

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Table des matières

Table des matières 7
Introduction générale 11
1. Perturbation et Stabilité 14
1.1 Concept de perturbation 14
1.1.1 Définition de la perturbation 14
1.1.2 Propriétés des perturbations 15
1.2 Stabilité des communautés et des écosystèmes 18
1.2.1 Composantes de la stabilité 19
1.2.2 Mesures de stabilité 21
1.2.3 Biodiversité et stabilité 23
1.2.4 Le rôle de l'histoire des communautés 27
1.3 Réponse des communautés aux perturbations multiples successives 27
2. Stabilité des communautés microbiennes 31
2.1 Caractéristiques des communautés microbiennes 31
2.2 Réponse des communautés microbiennes aux perturbations multiples 33
3. Le biofilm phototrophe comme modèle d'étude 35
3.1 Structure et formation 35
3.2 Rôles du biofilm dans l'écosystème 39
3.3 Réponse des biofilms à l'intermittence 41
3.3.1 L'intermittence hydrique 41
3.3.2 L'intermittence : sources de perturbations multiples 42
3.3.3 Réponse des biofilms phototrophes 44
4. Enjeux et objectifs de la thèse 46
Chapitre 1- Importance des caractéristiques des communautés (diversité et histoire thermale) dans la réponse à un stress thermique 53
Disentangling the effects of microalgal diversity and thermal history on freshwater phototrophic biofilms facing heat stress: A thermal dose approach 59
1. Introduction 60
2. Materials & methods 63
3. Results 69
4. Discussion 79
References 84
Supplementary information 90

Chapitre 2- Effet de l'intensité d'un assec sur la stabilité de la qualité de la ressource et conséquences sur la production secondaire.....101

Flow intermittency affects the capacity of phototrophic biofilms to sustain secondary production.....107

1. **Introduction**.....108
2. **Material and methods**.....111
3. **Results**.....117
4. **Discussion**.....129

References.....133

Supplementary information.....138

Chapitre 3- Identification des propriétés d'une séquence de perturbations qui contrôlent la stabilité fonctionnelle des communautés.....143

The time-dependency of interactions between successive disturbances is driven by the nature and intensity of events.....149

1. **Introduction**.....149
2. **Material and Methods**.....152
3. **Results**.....156
4. **Discussion**.....163

References.....165

Supplementary information.....169

Chapitre 4 - Influence de l'histoire d'intermittence hydrique sur la sensibilité des biofilms à un contaminant complexe.....173

The sensitivity of aquatic microbial communities to a complex agricultural contaminant depends on previous drought conditions.....179

1. **Introduction**.....180
2. **Material and methods**.....183
3. **Results**.....189
4. **Discussion**.....195

References.....200

Supplementary information.....205

Chapitre 5- Influence de l'histoire thermale sur la stabilité du biofilm phototrophe dans une rivière intermittente.....213

Thermal history influences the stability of phototrophic biofilms exposed to agricultural run-off in intermittent rivers.....219

1. **Introduction**.....219
2. **Material and methods**.....222

3. Results 230
4. Discussion 239
References 242
Supplementary information 248
Conclusion générale 255
Références bibliographiques 271
Annexe 285

Introduction générale

Introduction générale

L'écologie du stress ou l'écologie des perturbations est une sous-discipline de l'écologie qui vise à mesurer et évaluer l'impact des perturbations naturelles ou d'origines externes sur la structure et le fonctionnement d'un système écologique (Barrett, 1981). Le nombre de perturbations d'origine anthropique se multiplie dans le contexte actuel du changement global. Les effets sur les écosystèmes, tels que ceux liés à l'augmentation générale des températures, l'augmentation des rejets de gaz à effet de serre, l'installation d'espèces invasives, etc., ont d'ailleurs reçu beaucoup d'attention de la part de la communauté scientifique. Les changements observés sont non seulement multiples mais également rapides et simultanés, ce qui se traduit par une complexification de la dynamique spatiale et temporelle des régimes de perturbations (Turner, 2010). Outre la potentielle simultanéité des perturbations, le changement global induit aussi une augmentation de l'intensité, de la fréquence et du nombre d'occurrences des perturbations qui affectent les écosystèmes. Il est donc crucial de comprendre la manière dont les différentes perturbations interagissent entre elles et d'évaluer la capacité des écosystèmes et des communautés à faire face à ces changements de régime de perturbations (Newman, 2019).

Dans ce travail, je m'intéresse aux effets du changement de régime de perturbations sur les communautés microbiennes, celles-ci étant ubiquistes et cruciales pour le fonctionnement des écosystèmes. Contrairement au cas des communautés microbiennes du sol qui ont été assez largement étudiées, la réponse des communautés microbiennes aquatiques aux changements de régimes de perturbations est encore assez mal comprise alors même que les écosystèmes aquatiques d'eaux douces sont les écosystèmes les plus menacés par les activités humaines et le changement global qui en découle (Carpenter et al., 2011; Dudgeon et al., 2006). Les communautés microbiennes sont réputées pour être relativement stables face aux perturbations et aux fluctuations environnementales : après une perturbation, elles sont capables de se maintenir ou de revenir à un état d'équilibre (Botton et al., 2006). Cela est notamment lié à leur grande plasticité et réactivité face aux changements. Malgré cela, l'hétérogénéité spatiale et temporelle induite par le changement global orientent les communautés et les écosystèmes au-delà de leur gamme normale de variabilité (Lear et al., 2008). L'influence des interactions entre perturbations sur la réponse de communautés microbiennes n'est pas facilement prédictible, comme

cela a été montré pour des biofilms de rivière (Romero et al., 2018). Ainsi, la manière dont interagissent les perturbations peut mener à des « surprises écologiques », avec des changements d'états dans les systèmes quand des seuils de perturbation sont franchis (Ives & Carpenter, 2007). Il apparait donc nécessaire d'identifier les propriétés des perturbations et les caractéristiques des communautés qui influencent la stabilité des communautés microbiennes aquatiques.

Dans cette introduction générale, je m'attache dans un premier temps à définir les concepts de perturbation et de stabilité. Dans une deuxième partie, je décris et explique les facteurs qui contrôlent la stabilité des communautés microbiennes en réponse à des perturbations multiples séquentielles. Enfin, après avoir exposé ma problématique générale, je présente mon modèle d'étude qui est le biofilm phototrophe de rivière.

1. Perturbation et Stabilité

1.1 Concept de perturbation

1.1.1 Définition de la perturbation

Les termes de stress et perturbation ont souvent été utilisés de manière synonyme en écologie (Rykiel, 1985). Dans leur ouvrage de 2016, Battisti et ses collaborateurs placent les termes de perturbation et de stress sous le terme général d'interférence, c'est-à-dire d'un facteur qui affecte les populations ou les communautés. En s'appuyant sur les travaux de Grime (1979) spécifiques aux plantes, les auteurs font donc la distinction entre perturbation et stress de manière à l'appliquer à tous les milieux, de la façon suivante : si la population ou la communauté s'écarte de l'état stationnaire, suite à l'événement puis entre dans une phase de croissance exponentielle, on parle de perturbation. A l'inverse, le stress ne fait pas dévier les compartiments considérés de l'état stationnaire mais diminue plutôt leur taux de croissance (Battisti et al., 2016).

Contrairement à la langue française, l'anglais distingue "*disturbance*" et "*perturbation*" et "stress" et "*stressors*". Dans la langue anglaise, les termes "*stressors*" et "*disturbance*" sont employés pour parler de l'évènement (ex : une crue), alors que les termes de "*perturbation*" et "stress" se réfèrent plutôt à la conséquence ou à l'effet induit (Rykiel, 1985), alors qu'en français, le terme "*stress*" désigne à la fois la cause d'un évènement et

sa conséquence (i.e. la réponse de l'organisme). De manière générale, ces concepts impliquent l'existence d'un état d'équilibre du système. Etudier l'effet des perturbations revient ainsi à étudier la stabilité des populations ou des communautés autour de cet équilibre (Pimm, 1984).

La perturbation écologique ("*disturbance*") a été définie de nombreuses manières. L'une des plus utilisée est celle la définissant comment étant un événement discret à la fois dans le temps et l'espace qui compromet la structure d'un écosystème, d'une communauté ou d'une population, et qui modifie la ressource et l'environnement physique (White and Pickett, 1985). Cette définition implique de se situer sur une échelle de temps définie, qui varie cependant en fonction de l'événement considéré, pouvant s'étendre de quelques secondes (ex : une chute d'arbre), à quelques heures (ex : un feu de forêt ou une crue), voire à plusieurs semaines (ex : une sécheresse). De même, en fonction des communautés, ou populations considérées, la temporalité des conséquences de l'événement varie. Par exemple, la chute d'un arbre perturbe une communauté végétale à court terme, alors que les communautés microbiennes du sol sont impactées à plus long terme (Shade et al., 2012). Plus récemment, Plante (2017) a défini une perturbation comme étant un événement ponctuel et imprévisible engendrant une forte diminution de la biomasse et qui altère ainsi la structure des communautés. Bien que cette définition ait été proposée pour les communautés microbiennes, elle est applicable à tout système biologique.

Dans la suite de ma thèse, j'emploie le terme de "perturbation" au sens de "*disturbance*" (événement), et pour parler des conséquences, je parle généralement de "réponse" ou d'"effet" du fait de l'absence de terme dédié en français.

1.1.2 Propriétés des perturbations

Plusieurs propriétés des perturbations sont importantes à considérer quand on étudie la réponse qu'elles induisent chez des communautés. Premièrement, il est essentiel de prendre en compte la nature, c'est-à-dire si la perturbation est d'origine biotique ou abiotique, physique ou chimique. Il est aussi nécessaire de considérer sa forme c'est-à-dire la manière dont la perturbation s'applique, la temporalité et la persistance de l'évènement. Généralement, on distingue des formes de types "pulse", "press" ou "ramp" (Figure 1) (Ives and Carpenter, 2007; Lake, 2000). Une perturbation "pulse" est de courte durée mais intense (ex. une crue), une perturbation "press" est plus ou moins continue et

constante (ex. rejet en sortie de station d'épuration), alors qu'une perturbation "ramp" empire au cours du temps (ex. une sécheresse). Une perturbation peut aussi varier en intensité, cette dernière représentant la force physique exercée sur le système à la fois en termes d'espace et de temps.

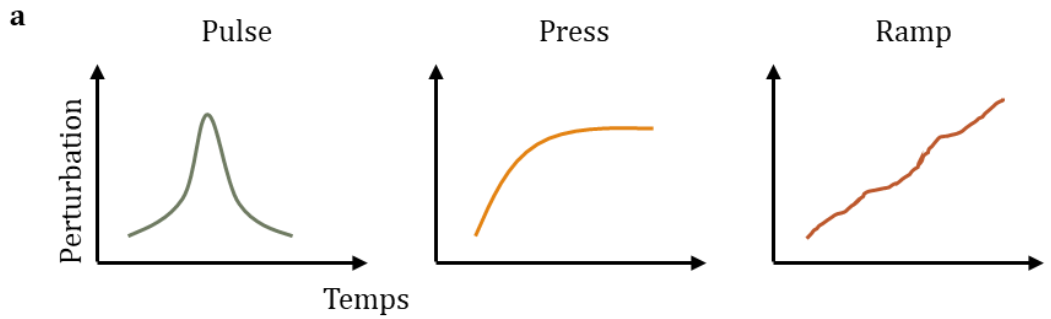


Figure 1 : Schéma des différents types de perturbation, classées selon leur dynamique temporelle.

Les écosystèmes sont généralement soumis à des stress ou perturbations multiples, qui peuvent se produire de manière simultanée ou successive/séquentielle (Figure 2a). Un régime de perturbations se définit par l'ensemble des propriétés individuelles des perturbations (nature, intensité, type), leur nombre, et la manière dont elles s'appliquent les unes par rapport aux autres (simultanément, successivement) dans quel ordre et à quelle fréquence. Les régimes de perturbations influent sur la réponse des communautés (Figure 2b). La fréquence représente l'inverse de l'intervalle de temps écoulé entre deux perturbations et est un paramètre important dans la prédictibilité des événements, par exemple dans la prédiction des épisodes de crues. Dans le cas de perturbations successives, l'intervalle de temps entre deux perturbations est parfois trop court pour permettre une récupération du système (Burton et al., 2020). L'ordre des perturbations de différentes natures affectant un système est aussi une propriété importante à considérer car elle peut influer sur la réponse des systèmes au régime de perturbations (Philippot et al., 2021).

De manière générale, les perturbations cumulées (simultanées ou successives) peuvent interagir entre elles et mener à des "surprises écologiques", c'est-à-dire à des effets différents de que ce qui est attendu par une simple addition des effets. En d'autres termes, dans le cas de perturbations séquentielles induisant chacune un effet négatif, la première perturbation peut altérer la réponse induite par la deuxième (Buma, 2015;

Buma and Wessman, 2011), et ainsi résulter en une augmentation des effets négatifs sur le niveau biologique considéré (= synergie) ou au contraire dans une diminution de l'effet (= antagonisme) (voir partie 1.3). L'étude des effets combinés de perturbations simultanées et des interactions entre des perturbations multiples est déterminante pour comprendre l'effet des régimes de perturbations sur les écosystèmes et les communautés.

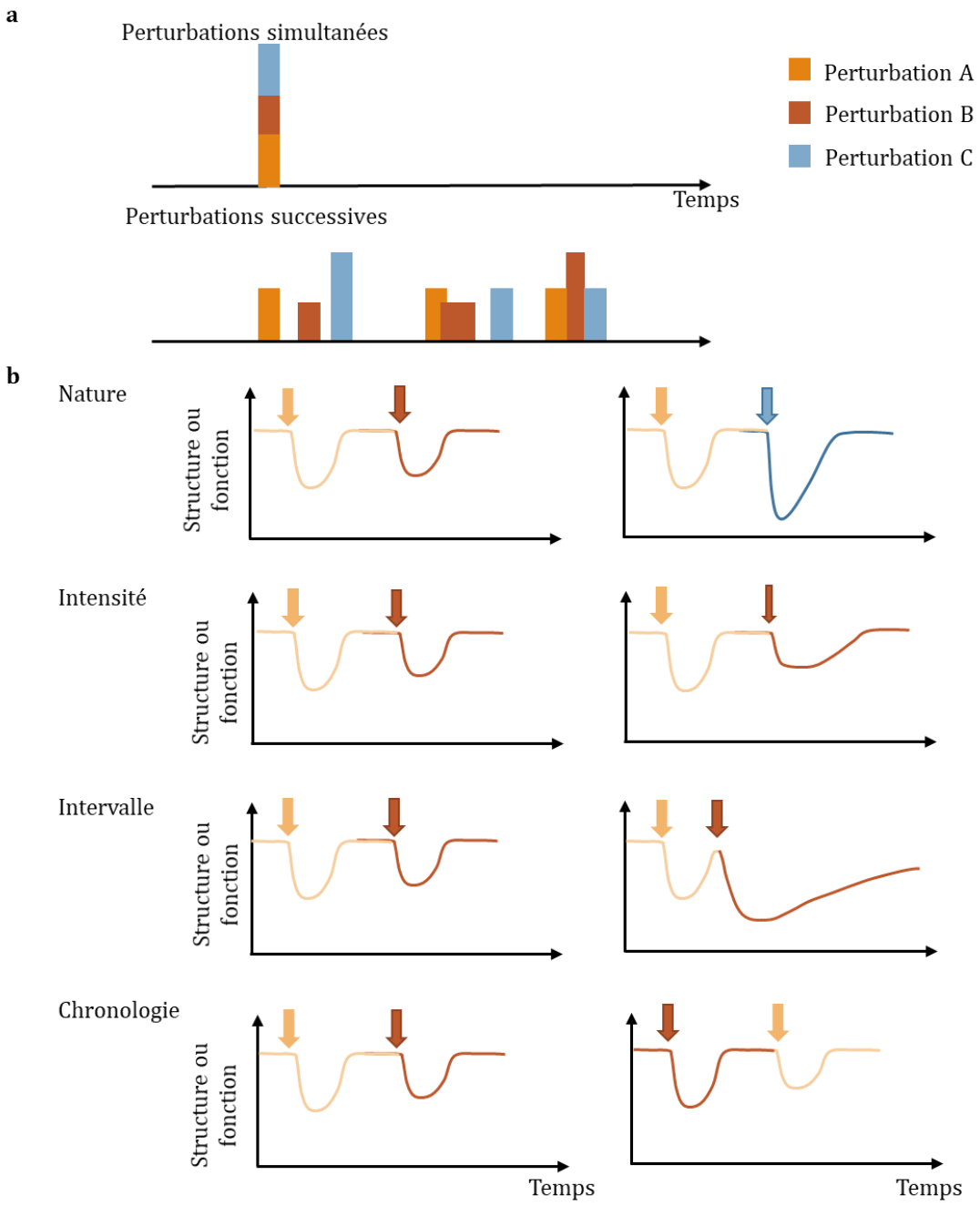


Figure 2 : Caractérisation des perturbations. Schéma décrivant les régimes de perturbations multiples, simultanées ou séquentielles (a) et des différentes propriétés de perturbations séquentielles à considérer (b), *repris de Philippot et al. (2021).*

1.2 Stabilité des communautés et des écosystèmes

La stabilité écologique est un concept général qui revient à étudier la réponse d'un système suite à une perturbation. De fait, considérer la stabilité écologique ne peut se faire qu'en référence à une perturbation (Rykiel, 1985). En effet, la stabilité traduit la capacité d'un système biologique à revenir à un état d'équilibre suite à une perturbation (Holling, 1973). En 1984, Pimm définit un système comme étant stable si et seulement si suite à une perturbation toutes les variables considérées (structurelle ou fonctionnelle) reviennent à leur état de référence, c'est-à-dire autour de leur équilibre initial ou de leur dynamique initiale. Cependant, certains écosystèmes peuvent présenter plusieurs états d'équilibre alternatifs, de sorte que si un seuil de perturbation est dépassé, un nouvel état d'équilibre apparait (Folke et al., 2004). Un exemple emblématique de ces multiples états d'équilibre alternatifs est celui des lacs peu profonds : en fonction de la concentration en nutriments dans l'eau, le système peut être soit "clair" soit "turbide". En effet, la présence de macrophytes favorise un état clair, tandis que l'augmentation en nutriments, en favorisant la croissance d'algues phytoplanctoniques, limite la quantité de lumière disponible pour les macrophytes et fait ainsi basculer le système vers un état turbide (Scheffer et al., 1993). Cette bascule d'un état d'équilibre à un autre est classiquement illustrée sous la forme d'un paysage possédant différents bassins d'attractions, chacun représentant un domaine de stabilité caractérisé par des conditions environnementales qui lui sont propres (Figure 3) (Beisner et al., 2003; Gunderson, 2000; Scheffer et al., 2001). Si la perturbation appliquée dépasse le seuil que le système est capable d'endurer sans changement, il bascule vers un nouvel état de stabilité après être passé par un état instable. Plus la taille du bassin d'attraction est petite, plus le système bascule facilement (Figure 3) (Scheffer and Carpenter, 2003).

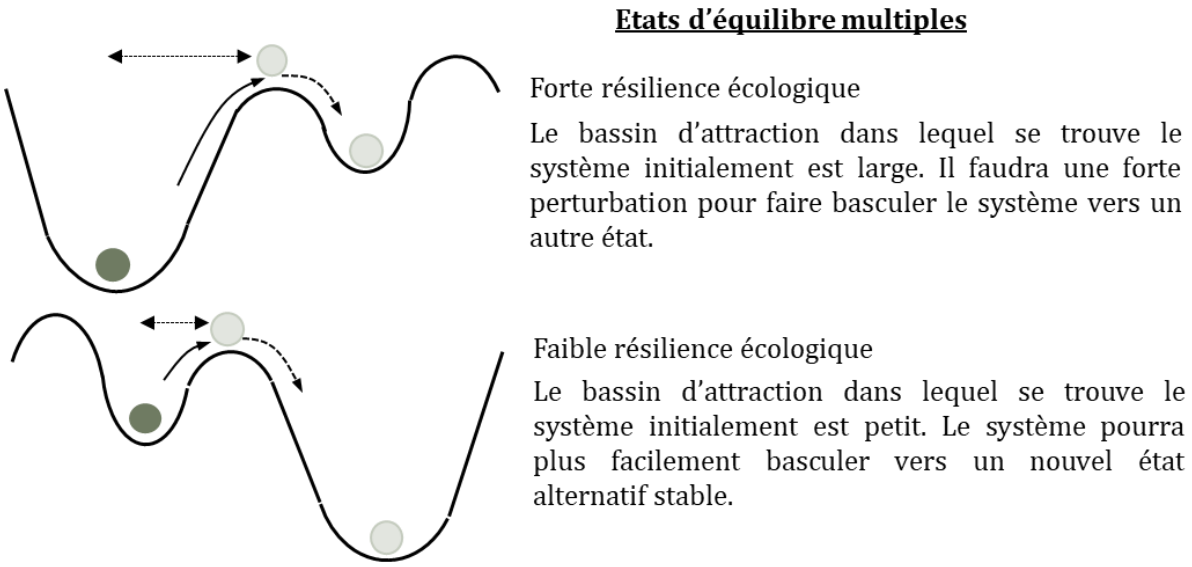


Figure 3 : Description de la stabilité selon la théorie des bassins d'attraction pour les écosystèmes possédant plusieurs états alternatifs stables.

1.2.1 Composantes de la stabilité

La stabilité d'un écosystème est un concept multidimensionnel qui repose notamment sur les capacités de résistance, de récupération et de résilience des communautés à une perturbation. Ces notions sont propres à l'écosystème étudié et étroitement liées l'une à l'autre (Donohue et al., 2016), et l'étude de la stabilité des communautés nécessite l'étude de ces différentes notions (Urrutia-Cordero et al., 2022). La résistance est habituellement définie comme le degré auquel une communauté reste inchangée suite à une perturbation (Konopka et al., 2015; Pimm, 1984). La récupération de la communauté est définie comme étant la capacité du système à récupérer suite à l'impact engendré par la perturbation (Lloret et al., 2011). En revanche, la résilience est plus difficile à définir et diverses propositions coexistent dans la littérature. Traditionnellement, elle a été définie comme la capacité d'une communauté à revenir à son état de référence suite à une perturbation, c'est-à-dire à l'état pré-perturbation (Grimm and Wissel, 1997; Lloret et al., 2011; Pimm, 1984). La résilience dépend donc de la résistance et de la récupération des communautés. Cependant, bien que le concept général de stabilité fasse l'objet d'un consensus, ses composantes sont sujettes à de nombreuses définitions et interprétations en fonction des auteurs et du contexte dans lequel elles s'interprètent (Todman et al., 2016). Dans cette thèse, je me réfère

généralement à trois composantes principales (Figure 4) comme définies dans Lloret et al. (2011). Il s'agit de la résistance (*Rt*), de la résilience (*Rl*), et enfin de la récupération du système (*Rc*, "recovery" en anglais), pour estimer la capacité de récupération du système au cours du temps.

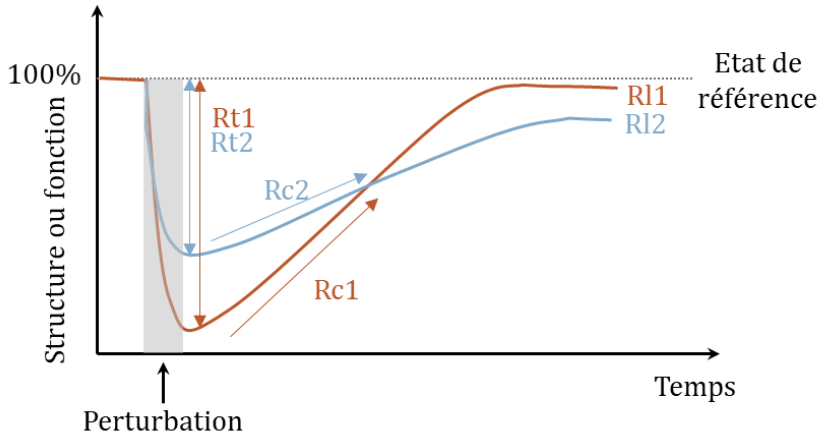


Figure 4 : Représentation graphique des paramètres de stabilité. La stabilité d'une structure ou d'une fonction s'étudie au cours du temps. Deux situations sont représentées. Le système 1 (orange) a une résistance (*Rt*) plus faible que le système 2 (bleu). En revanche, il a une meilleure récupération au cours du temps (*Rc*) et une meilleure résilience au temps *t* (*Rl*).

Dans le cas des écosystèmes présentant plusieurs états alternatifs stables, l'étude des changements de trajectoires présente un intérêt majeur. Ces changements d'états non désirés sont engendrés à la fois par les forces externes au systèmes (i.e. intensité/force/fréquence d'une perturbation) et la capacité interne du système à absorber la perturbation. Quand la résilience diminue, l'écosystème devient vulnérable et peut plus facilement changer d'état (Folke et al., 2004).

Cependant la résilience telle que définie précédemment est difficilement applicable aux systèmes comprenant plusieurs états alternatifs stables (Scheffer et al., 2001). Une deuxième définition a donc été proposée, appelée résilience écologique (ou "*ecological resilience*") et a été définie pour les systèmes qui suite à une perturbation peuvent basculer vers un nouvel état alternatif stable (Holling, 1973). Dans ce cas, la résilience se réfère à la quantité de perturbation que le système peut absorber avant de basculer vers un nouveau domaine de stabilité (Figure 3) (Gunderson, 2000). Pour éviter la confusion

entre ces deux types de résilience, la première définition proposée a été rebaptisée sous le nom de résilience ingénieure (ou "*engineering resilience*") (Holling, 1996). Une définition alternative, plus générale est que la résilience décrit le processus de récupération d'un système après une perturbation (Hodgson et al., 2015).

1.2.2 Mesures de stabilité

Mesurer la stabilité d'une communauté ou d'un écosystème dans son ensemble implique un suivi dynamique, c'est-à-dire, pendant (ou juste après) la perturbation, et lors de la période de récupération suite à la perturbation (Nimmo et al., 2015). De manière générale, la résistance et la résilience sont mesurées par rapport à un état de référence qui peut être soit l'état mesuré avant la perturbation, soit un contrôle non perturbé. Ces différentes mesures peuvent être exprimées en valeurs absolues ou relatives par rapport à l'état de référence (Ingrisch and Bahn, 2018, Nimmo et al., 2015), ou encore normalisées par rapport à l'impact de la perturbation (Ingrisch and Bahn, 2018). De très nombreux indicateurs ont été proposés pour quantifier la résistance et la résilience (voir par exemple : Ingrisch and Bahn, 2018; Isbell et al., 2015; Nimmo et al., 2015; Orwin and Wardle, 2004). Quelques métriques existantes sont rapportées dans la Table 1.

Cependant, dans certains systèmes, tels que les communautés microbiennes, qui sont naturellement extrêmement dynamiques, un retour vers l'état de référence initial est très rarement observé après une perturbation, d'où l'intérêt d'utiliser comme référence un contrôle non perturbé (Shade et al., 2012).

Table 1 : Exemples des métriques existantes pour quantifier la stabilité. Ce tableau illustre quelques métriques utilisées pour quantifier la résistance, la résilience et la récupération. Dans les équations, C0 représente l'état du système avant perturbation (ou du contrôle non perturbé à un instant *t*), D1 représente l'état du système en fin de perturbation et Dx au cours du temps suivant la fin de la perturbation (voir figure 4).

	Equation	Remarque	Référence
Rt	$\frac{D1}{C0}$		Lloret et al., 2011 Nimmo et al., 2015
Rt	$1 - \frac{2\|C0 - D1\|}{(C0 + \|C0 - D1\|)}$	Comprise entre -1 et 1, (1 la résistance maximale)	Orwin & Wardle, 2004 Shade et al., 2012
Rt	$\frac{C0}{\|D1 - C0\|}$		Isbell et al., 2015
Rt	(1) $ln\frac{D1}{C0}$ OU (2) $sim\frac{D1}{C0}$	Les auteurs utilisent (1) pour mesurer la résistance d'une fonction et (2) pour celle de la composition d'une communauté.	Hillebrand et al. 2018
Rl	(1) $ln\frac{Dx}{C0}$ OU (2) $sim\frac{Dx}{C0}$	Les auteurs utilisent (1) pour mesurer la résilience d'une fonction et (2) pour celle de la composition d'une communauté. Dans leurs travaux, les auteurs utilisent le terme de Recovery pour parler de résilience	Hillebrand et al. 2018
Rl	$\frac{Dx - D1}{C0}$		Lloret et al., 2011 Nimmo et al., 2015
Rl	$\frac{2\|C0\|}{(\|C0\| + \|Dx\|)} - 1$	Comprise entre -1 et 1	Orwin & Wardle, 2004
Rl	$-[-100\frac{C0 - D1}{C0} + 100\frac{C0 - D1}{C0}]$		Banning & Murphy, 2008
Rl	$(C0 - \frac{C0}{Dx}) * 100$	Converge vers 0 pour recovery totale	Griffiths et al., 2000
Rc	$ln\frac{Dx}{C0} = i + Rc * t$	Définit comme la résilience dans l'article. Avec i étant l'ordonnée à l'origine et t le temps.	Hillebrand et al. 2018

1.2.3 Biodiversité et stabilité

Le terme biodiversité peut être défini comme « la variété et l'abondance des espèces sur une unité d'étude définie » (Magurran, 2003). La biodiversité est représentée par deux composantes principales qui sont la richesse spécifique, c'est-à-dire le nombre d'espèces représentées sur l'aire étudiée, et l'équitabilité (ou "evenness"), c'est-à-dire la répartition des abondances relatives des espèces (Wittebolle et al., 2009). De nombreux indices existent pour mesurer ces deux composantes tels que l'indice de Shannon (Shannon, 1948), de Gini-Simpson (Simpson, 1949) ou l'indice d'équitabilité de Piélou. Ces mesures ne prennent pas en compte les similarités ou les différences entre les espèces, contrairement aux indices mesurant la diversité phylogénétique. La diversité phylogénétique présente un intérêt important dans l'étude de la stabilité des écosystèmes, puisque plus la distance phylogénétique entre deux espèces est importante, plus les rôles écologiques, et l'utilisation des ressources de ces dernières sont différents (Cadotte et al., 2012). La diversité fonctionnelle est définie comme la distribution des espèces et leur abondance au sein d'une même niche écologique (Mason et al., 2005). Les espèces sont rassemblées en groupes fonctionnels, qui sont déterminés en fonction de leurs traits morphologiques, physiologiques et comportementaux ainsi que de l'influence exercée sur le fonctionnement de l'écosystème (Tilman, 2001). Elle regroupe deux composantes majeures qui sont la richesse fonctionnelle, c'est-à-dire la quantité de niches utilisées par les espèces au sein d'une communauté et l'uniformité fonctionnelle (ou evenness), soit la régularité dans la distribution des abondances au sein des niches. Généralement, plus la distribution des espèces au sein des niches est uniforme, plus la redondance fonctionnelle, c'est-à-dire le nombre d'espèces remplissant la même fonction est importante (Fonseca and Ganade, 2001). De nombreuses études ont montré l'importance de la diversité dans la stabilité des écosystèmes (Folke et al., 2004; Fonseca and Ganade, 2001), que ce soit pour les écosystèmes terrestres (Tilman et al., 2014), les sols (Bardgett and Caruso, 2020; Wittebolle et al., 2009), ou encore les écosystèmes aquatiques (Lundberg and Moberg, 2003).

De nombreux modèles ont tenté d'expliquer la relation existante entre diversité et stabilité comme développé par Peterson et al. (1997). Le modèle « species-diversity » d'abord proposé par Darwin (1859) puis repris par MacArthur (1955), suppose que plus la richesse spécifique est élevée, plus la stabilité fonctionnelle augmente (Figure 5a).

Cependant, cette modélisation est trop simplificatrice puisqu'elle sous-entend que la capacité du système à accueillir de nouvelles espèces est constante. Ainsi, d'autres modèles ont été proposés en tentant de prendre en compte les éléments clés oubliés dans le premier, dont le fait qu'un écosystème ne puisse pas accueillir un nombre infini d'espèces. Il existe notamment le modèle « idiosyncratique » proposé par Lawton (1994), qui prédit que le degré de stabilité d'un écosystème dépend des espèces présentes et que la contribution d'une espèce à une fonction écologique dépend des interactions avec les autres espèces (Figure 5b). Le modèle « rivet » (Ehrlich & Ehrlich, 1981) considère que différentes espèces assurent la même fonction et qu'ainsi la disparition d'une espèce n'engendre pas de perte de fonction grâce à une compensation par les espèces restantes. C'est le principe de redondance fonctionnelle (Figure 5c). Cependant la perte de redondance fonctionnelle engendre une diminution de la stabilité du système face à une perturbation puisque sa capacité de résistance peut être impactée. Enfin, le dernier modèle présenté dans l'article de Peterson et al. (1997) est le modèle « Drivers and Passengers » (Walker, 1995, 1992) qui suggère que toutes les espèces n'ont pas le même impact sur les fonctions écologiques (Figure 5d). Les espèces "drivers" ont le rôle le plus important, tandis que les espèces "passengers" ont moins d'impact. Ainsi, c'est la présence ou l'absence des drivers qui détermine la stabilité fonctionnelle.

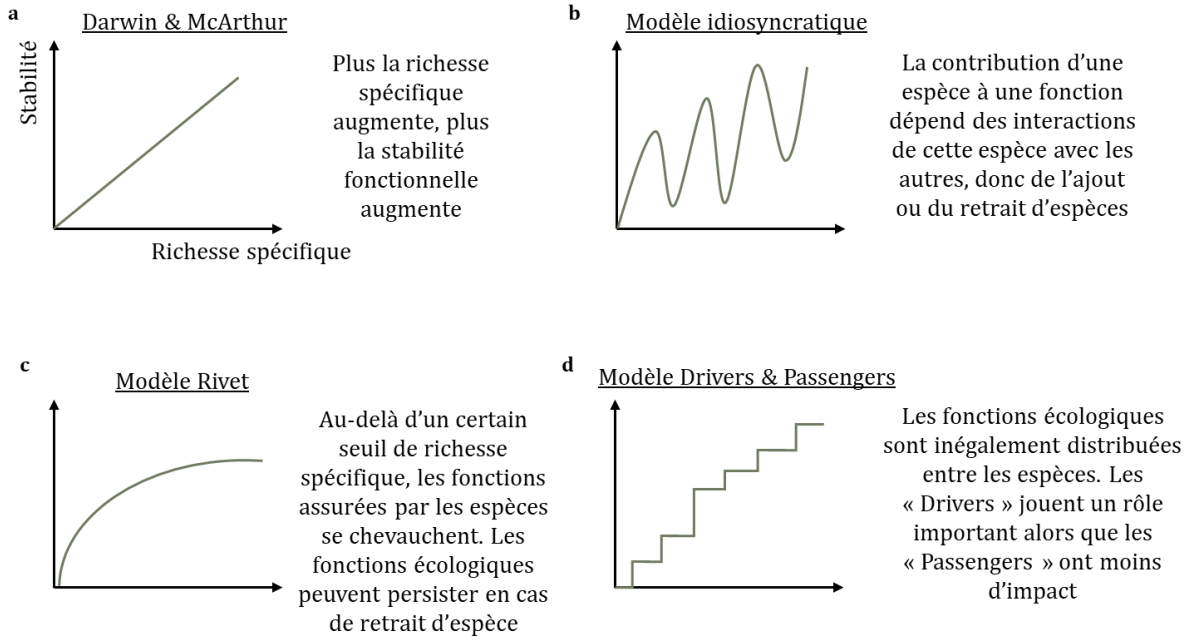


Figure 5 : Illustrations des différents modèles de diversité-stabilité fonctionnelle décrit dans Peterson et al. (1997).

Bien qu'exprimant des idées différentes, tous ces modèles reposent sur l'importance des groupes fonctionnels dans la stabilité écologique et sur l'hypothèse d'assurance (Yachi and Loreau, 1999). Cette hypothèse prédit que la biodiversité assure le maintien fonctionnel des écosystèmes car plus le nombre d'espèces est important, plus le nombre d'espèces capables de maintenir des fonctions là où d'autres échouent est élevé, du fait que les espèces ne répondent pas de manière identique aux changements environnementaux et aux perturbations. Dans une étude sur les communautés végétales, Walker et al. (1999) ont mis en évidence l'importance des espèces rares dans la stabilité fonctionnelle du fait qu'elles remplissent les mêmes fonctions que les espèces dominantes mais se maintiennent mieux face aux perturbations. Quand les espèces dominantes déclinent, l'abondance des espèces rares augmente ce qui permet le maintien de l'exercice des fonctions écologiques au sein de la communauté.

Ainsi, la diversité joue un rôle important dans la stabilité en réponse à une perturbation, mais les perturbations influencent aussi la diversité et peuvent ainsi modifier la structure et potentiellement le fonctionnement des écosystèmes. En 1978, Connell propose la théorie de la perturbation intermédiaire ("Intermediate disturbance hypothesis" (IDH)) qui prédit qu'à un niveau de perturbation intermédiaire, la richesse spécifique devrait être maximale car les espèces colonisatrices et les espèces les plus compétitrices sont capables de coexister. En revanche, des perturbations trop intenses et fréquentes entrainent une disparition des espèces sensibles, une diminution de la capacité de colonisation et donc une richesse spécifique plus faible. A l'inverse, si les perturbations sont rares, seules les espèces les plus compétitrices dominent et la richesse spécifique est également faible. Un niveau intermédiaire de perturbation garantit un compromis entre colonisation et compétition, en permettant la coexistence entre les espèces ayant une forte capacité de dispersion et de colonisation (stratèges r) et les espèces les plus compétitrices (stratèges K) (Roxburgh et al., 2004). La stabilité de l'environnement est ainsi une force motrice de sélection des stratégies mises en place par les espèces pour assurer leur maintien et leur succès au sein d'une communauté et d'un écosystème (Ponge, 2013). Cette théorie a cependant été remise en cause à la fois empiriquement, puisque les observations qui la confirment sont rarement faites dans le milieu naturel, mais aussi théoriquement du fait de l'invalidité des mécanismes sur lesquels est construite l'IDH (Fox, 2013). En effet, les relations diversité-perturbation ("diversity-

disturbance relationship" (DDR)) peuvent prendre différentes formes selon les propriétés de la perturbation utilisées pour construire les modèles, c'est-à-dire sa fréquence et/ou son intensité (Mancuso et al., 2021; Miller et al., 2011). Dans leur article, Mancuso et al. (2021) mettent en avant 5 formes de DDR : négative, neutre, en forme de U, positive et en bosse comme tel qu'attendue avec l'IDH (Figure 6). Toutes ces formes de DDR peuvent être obtenues en utilisant une fréquence de perturbations plus ou moins élevée, et faisant varier la gamme d'intensités considérée.

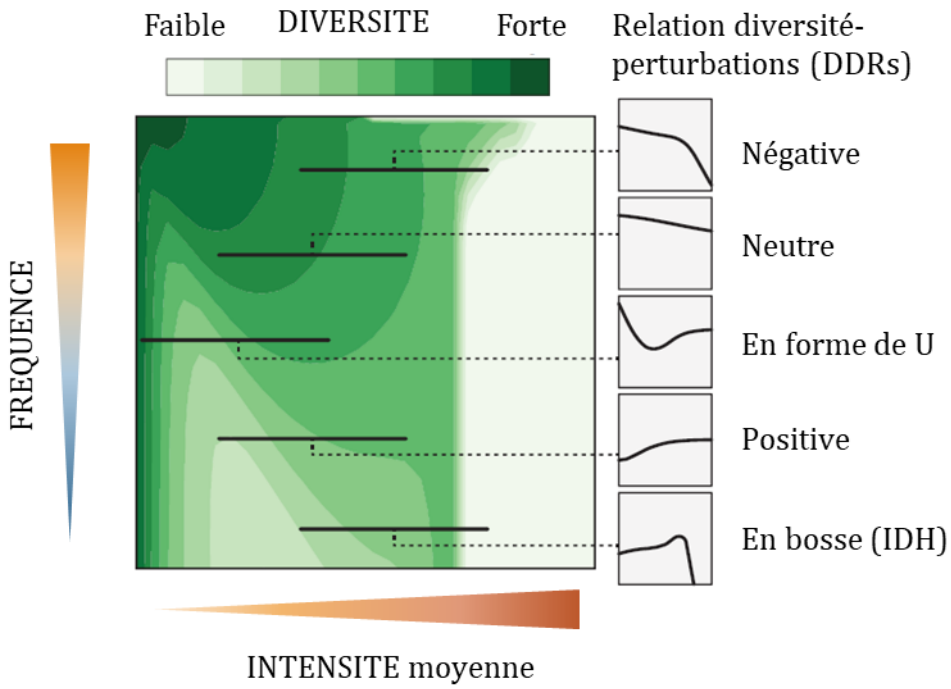


Figure 6 : Différentes formes de relations diversité-perturbations existantes en fonction de la fréquence et de l'intensité des perturbations (*Traduit de Mancuso et al., 2021*). Pour la relation négative, quand la fréquence des perturbations est élevée, plus elles sont intenses, plus la diversité diminue. La relation est neutre si la diversité diminue de manière linéaire avec l'intensité de la perturbation lorsque la fréquence est élevée. Pour des perturbations de fréquence moyenne, contrairement à la forme prédite par l'IDH, la diversité est plus forte pour les faibles et les fortes intensités. Si à de faibles fréquences d'occurrences, la diversité augmente avec l'intensité de la perturbation, alors la relation diversité-perturbations est positive. Et enfin, comme prédit par l'IDH, la diversité est maximale à un niveau de perturbation d'intensité intermédiaire quand elles ne sont pas très fréquentes.

1.2.4 Le rôle de l'histoire des communautés

L'histoire des communautés joue un rôle important dans la stabilité en réponse à une perturbation. La littérature s'appuie sur les termes de mémoire écologique ("*ecological memory*") ou d'effet d'héritage ("*legacy effect*") pour décrire l'effet de l'histoire. Ces termes font référence à la fois aux conditions historiques, telles que les effets qu'une espèce peut continuer à exercer sur les autres après sa disparition de l'écosystème (Cuddington, 2011), mais aussi aux effets des conditions environnementales sur le long terme (Rousk et al., 2013). Par exemple, l'effet d'héritage a été montré dans les successions des communautés végétales : il existe des rétroactions entre les espèces végétales primaires et les communautés microbiennes du sol, qui, en influant sur les patrons de dominance des communautés végétales, influencent la succession secondaire (Kardol et al., 2007). Cependant, ces termes sont aussi employés pour parler de l'historique de perturbation, c'est-à-dire l'effet des perturbations antérieures à moyen terme sur la stabilité face à une nouvelle perturbation (ex : Hughes et al., 2019; Johnstone et al., 2016). Dans le cas d'histoire des perturbations, cette mémoire écologique implique deux types d'héritage : un héritage d'information et un héritage matériel (Johnstone et al., 2016). Dans leur publication, Johnstone et al. (2016) expliquent que les réponses adaptatives à un environnement changeant sont liées aux traits d'histoire de vie des espèces et émergent sur le long terme en réponse à un régime de perturbations plutôt qu'à des événements isolés. Dans ce cas, on parle d'héritage d'information. En revanche, lorsqu'une perturbation ou des fluctuations environnementales induisent des changements biotiques et/ou abiotiques dans l'écosystème, qui sont donc immédiats et dépendants des propriétés de l'événement perturbateur, il s'agit d'héritage matériel. Enfin, le terme de *legacy effect* est aussi employé dans la littérature pour décrire l'influence d'une première perturbation sur la réponse à une seconde dans le cas des perturbations successives (Dal Bello et al., 2017; Jurburg et al., 2017).

1.3 Réponse des communautés aux perturbations multiples successives

Les communautés sont le plus souvent soumises à des perturbations multiples, qui peuvent les affecter de manière simultanée ou séquentielle. Le changement global altère

les régimes naturels de perturbations et impacte donc les propriétés des séquences de perturbations (intensité, fréquence…). Cependant, les effets des perturbations successives sur les communautés sont étudiés (Gunderson et al., 2016). En réponse aux changements dans les régimes de perturbations, ou à l'augmentation de l'occurrence d'événements extrêmes répétés, deux scenarii sont possibles : l'histoire de perturbation peut augmenter la stabilité face à une nouvelle perturbation, ou au contraire, la diminuer en accroissant la vulnérabilité du système. Dans le premier cas, on parle de résistance au stress acquise, de priming ou de co-tolérance positive (Rillig et al., 2015; Vinebrooke et al., 2004) : la première perturbation prépare la communauté à résister au second événement. Un tel phénomène a été mis en évidence pour des perturbations identiques mais aussi pour des perturbations de natures différentes. Par exemple, il a été montré que l'intensité du blanchiment des coraux de la Grande Barrière suite aux vagues de chaleurs de l'année 2017 dépendait des événements de vagues de chaleur subis l'année précédente à l'origine d'un blanchiment sans précédent en 2016 (Hughes et al., 2019). Les auteurs ont montré que les coraux situés dans les zones ayant subi un fort impact par le passé étaient moins affectés que ceux ayant été moins exposés. Dans le cas de perturbations de natures différentes, l'étude de la réponse de communautés de macro-invertébrés aquatiques issus de rivières fréquemment perturbées ou pas, a mis en évidence un effet de pondération de l'histoire sur l'effet de la nouvelle perturbation (Eveleens et al., 2019). En effet, dans les rivières non perturbées par les crues, la sécheresse a des effets très négatifs sur la réponse structurelle, alors que lorsque les communautés sont habituées à subir des crues régulières, l'impact de la sécheresse est moindre.

Les effets des perturbations passées et présentes peuvent également s'additionner ou interagir de manière synergique, de sorte que le système perd en stabilité suite à l'exposition à une nouvelle perturbation. Ce phénomène a été montré sur des forêts dominées par des espèces résistantes au feu. Quand les arbres étaient exposés à une sécheresse avant l'épisode de feu, la mortalité augmentait (van Mantgem et al., 2013). De même, une étude sur les communautés microbiennes du sol a mis en évidence un effet négatif d'une succession de perturbations (stress thermiques) sur la capacité de récupération des communautés (Jurburg et al., 2017). Dans ce cas, la première perturbation (exposition à de fortes températures) affecte fortement la structure des communautés, et ces changements réduisent la capacité du microbiome du sol à résilier lorsque les communautés sont ensuite exposées à de faibles températures.

Les perturbations successives affectant les écosystèmes interagissent entre elles, mais il est compliqué de prédire le sens de ces interactions (Orr et al., 2020). Les effets cumulés peuvent être de trois types : additif, antagoniste ou synergique positif ou négatif (Figure 7a) (Crain et al., 2008; Piggott et al., 2015). Le sens de ces effets dépend de la réponse engendrée par les perturbations prises de manière isolée. Par exemple, dans le cas d'une combinaison de perturbations ayant chacune un effet négatif sur la réponse de la variable considérée, une interaction antagoniste ou synergique positive engendre une réponse moins forte que ce qui est attendu par une simple addition, c'est-à-dire une atténuation des effets, voire une inversion des effets dans le cas de synergie positive, tandis qu'une interaction synergique négative accentue l'effet négatif (Figure 7a.i). Dans le cas où les perturbations isolées ont des effets opposés, la synergie négative résulte en un effet plus négatif qu'attendu par une simple addition (Figure 7a.ii). Enfin, dans le cas où les perturbations isolées ont chacune un effet positif sur la variable considérée, une synergie positive implique un effet plus positif que lorsque chaque perturbation est considérée de manière indépendante, tandis que l'antagonisme et la synergie négative résultent tous les deux en des effets plus négatifs que si les effets s'additionnent (Figure 7a.iii). Plusieurs travaux théoriques ont tenté d'identifier et de décrire ces interactions (Côté et al., 2016; Piggott et al., 2015). Les effets cumulés des facteurs de stress sont influencés par les propriétés du régime de perturbation (Gunderson et al., 2016). En effet, la durée entre deux événements (Dal Bello et al., 2019), la nature, la chronologie des événements, c'est-à-dire l'ordre dans lequel les perturbations se produisent (Calderón et al., 2018), ainsi que l'intensité des perturbations (Renes et al., 2020) influencent l'effet général des perturbations, donc la stabilité des communautés. Particulièrement, la durée entre deux événements peut avoir un fort impact sur la réponse : si les événements sont suffisamment éloignés, ils agissent comme deux événements indépendants (Figure 7b1). S'ils sont trop rapprochés, la réponse des communautés aux deux événements n'est pas indépendante, avec des interactions antagonistes ou synergiques selon l'intervalle de temps (Figure 7b2 et 3). Enfin si les perturbations sont trop rapprochées, voir simultanées, la réponse des communautés est plus forte (Figure 7b4).

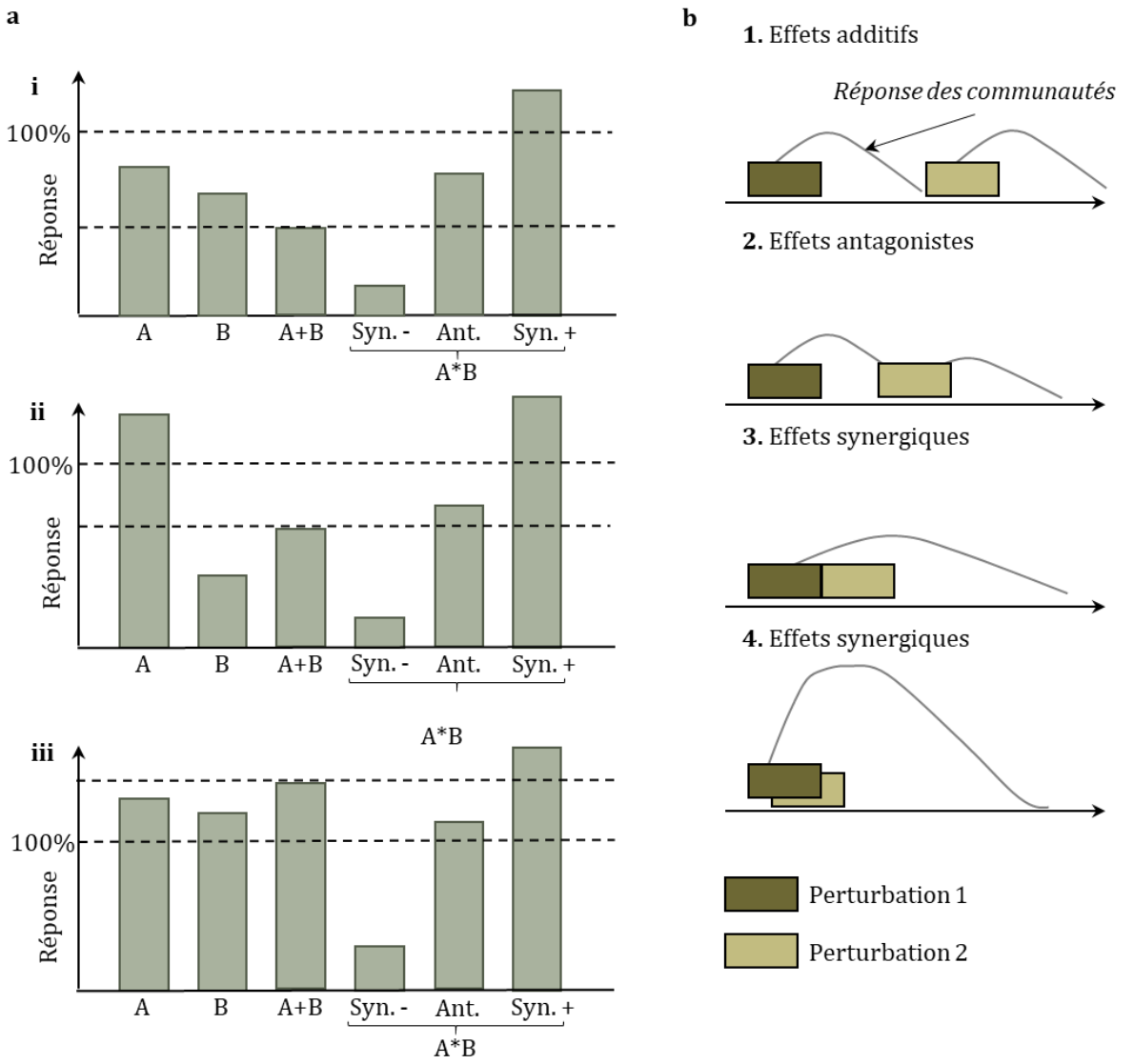


Figure 7 : Interaction entre perturbations. a. Approche conceptuelle des interactions entre perturbations dans le cas de stress ayant un double effet négatif (i), des effets opposés (ii) ou un double effet positif (iii) ; A+B représente un effet additif des perturbations, Syn. des effets synergiques (positif + ou négatif -) et Ant. des effets antagonistes *(Repris de Piggott et al., 2015)*. **b.** Scénarios hypothétiques de la juxtaposition temporelle des effets de deux perturbations (blocs). Les courbes modélisent la réponse des communautés. Dans le cas 1, l'intervalle de temps entre les perturbations est suffisant pour que les perturbations agissent de manière indépendante, les réponses physiologiques ne se superposant pas. Dans le cas 2, il n'y pas encore eu de retour à l'homéostasie, la réponse est ainsi partiellement anticipée, ce qui réduit l'effet. Dans les cas 3 et 4, les perturbations sont très rapprochées ou simultanées et agissent comme une seule perturbation. *(Repris de Gunderson et al., 2016)*.

2. Stabilité des communautés microbiennes

Les communautés microbiennes sont composées d'organismes ubiquistes, peuplant tous les écosystèmes, du corps humain aux océans, en passant par les sols et les eaux douces, et sous tous les climats, même les plus extrêmes (Yin et al., 2019). Les communautés microbiennes jouent un rôle fonctionnel indispensable dans tous les milieux qu'elles colonisent, puisqu'elles sont à l'origine de nombreux processus écosystémiques, tels que la décomposition, la minéralisation, le recyclage des nutriments inorganiques (Allison & Martiny, 2008 ; Bissett et al. 2013) contrôlant ainsi les cycles biogéochimiques (Konopka, 2009). De ce fait, l'étude de leur stabilité face aux perturbations a reçu beaucoup d'attention.

2.1 Caractéristiques des communautés microbiennes

Les communautés microbiennes doivent faire face en permanence à un environnement changeant (Konopka et al., 2014), pourtant leur fonctionnement peut montrer une forte stabilité dans le temps (Fuhrman et al., 2015). Cette stabilité apparente est liée à différents traits que partagent les microorganismes aux différents niveaux d'organisation biologiques que sont l'individu, la population et la communauté (Figure 8) (Philippot et al., 2021; Shade et al., 2012). Les microorganismes montrent généralement un taux de croissance rapide, une forte plasticité et un taux d'évolution rapide au travers de transferts de gènes horizontaux notamment, ainsi qu'une capacité de mise en dormance lorsque les conditions ne sont pas favorables (Allison and Martiny, 2008; Bardgett and Caruso, 2020; Li et al., 2014). De manière générale, la diversité des communautés est aussi un facteur important de la stabilité, notamment pour la stabilité fonctionnelle (Allison and Martiny, 2008; Bardgett and Caruso, 2020; Isbell et al., 2015) puisque en accord avec les modèles de diversité-stabilité, la redondance fonctionnelle joue un rôle important dans la réponse aux perturbations (Wohl et al., 2004), de même que la complémentarité des niches (Konopka et al., 2015). Par exemple, la capacité de résistance à l'invasion par un pathogène dépend de la diversité de la communauté initiale, et du taux d'utilisation des ressources propres à chaque espèce, soit de la compétition pour les ressources entre les espèces (van Elsas et al., 2012). Les interactions interspécifiques au sein de la communauté sont primordiales dans la réponse aux perturbations (Shade et al., 2012) puisqu'elles augmentent la résistance (Madsen et al., 2018). De plus la connectivité entre les communautés, à l'échelle des méta-communautés,

est aussi primordiale dans la stabilité et repose sur les capacités de dispersion et de colonisation propres à chaque espèce (Shade et al., 2012).

Outre ces facteurs de stabilisation propres aux différents niveaux d'organisation biologiques des communautés microbiennes, la forme de la perturbation (i.e. "pulse" ou "press") est aussi un facteur important à considérer lors de l'étude de la stabilité. Il a été montré que bien que les communautés microbiennes soient sensibles aussi bien aux perturbations de type "press" que de type "pulse", elles tendent à être plus résilientes après des perturbations de types "pulse" grâce aux capacités de mise en dormance de certaines espèces, leur taux de dispersion et de turnover (Shade et al., 2012), puisque pour que ces mécanismes soient efficaces, il faut que les conditions reviennent à leur état initial. De manière générale, il est difficile de prédire la stabilité et la réponse des communautés microbiennes aux changements globaux et aux changements dans les régimes de perturbations puisque de nombreux facteurs influencent cette stabilité (Figure 8) (Bissett et al., 2013).

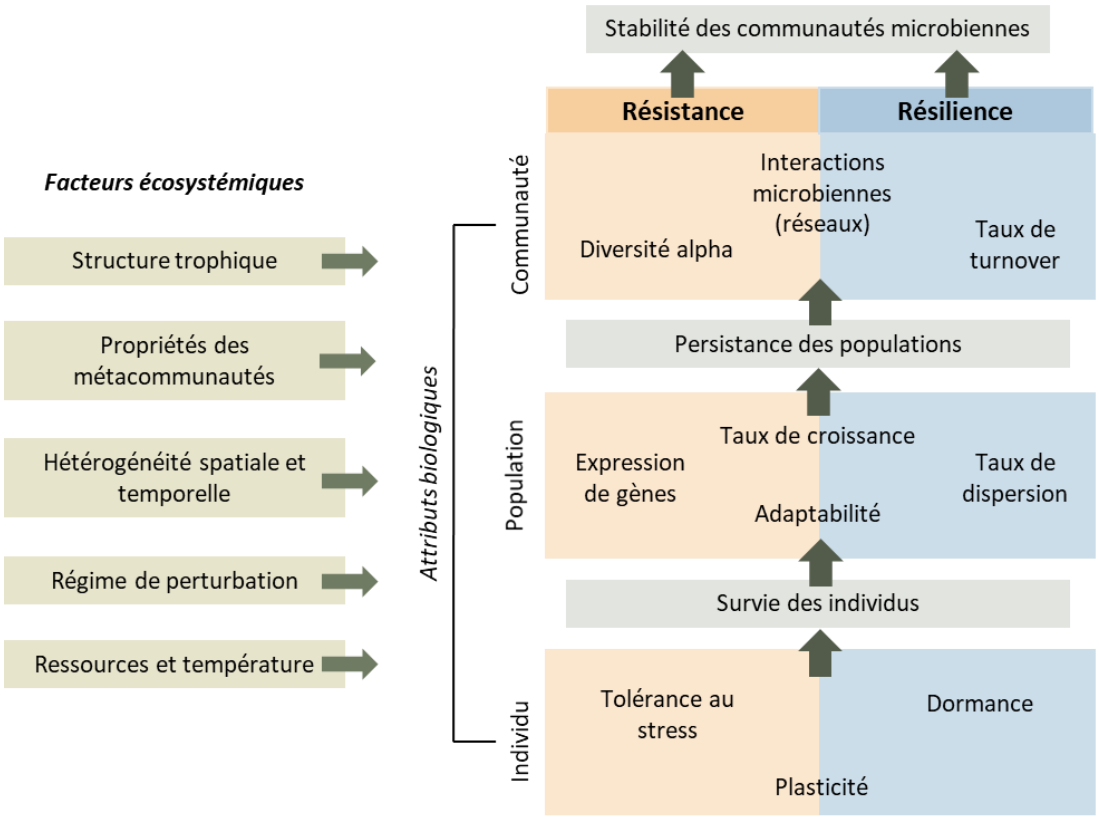


Figure 8 : Schéma des propriétés influençant la stabilité des communautés microbiennes exposées à une perturbation. (*Adapté de Shade et al., 2012*).

2.2 Réponse des communautés microbiennes aux perturbations multiples

La réponse des communautés microbiennes aux perturbations multiples simultanées, et la manière dont interagissent les facteurs de stress ont été assez largement étudiées (Jackson et al., 2016; Rillig et al., 2019; Romero et al., 2018). De manière générale, tous les types de réponses entre les perturbations sont observés (additivité, antagonisme, synergisme), et varient en fonction des natures des perturbations appliquées, ou des variables réponses observées. Par exemple, Jackson et al. (2016) ont mis en évidence que la majorité des combinaisons de facteurs de stress résultent en des interactions antagonistes, tandis que Romero et al. (2018) ont davantage observés des effets additifs. Ces différentes études montrent la difficulté à prédire les effets combinés des perturbations simultanées sur les communautés et l'observation de surprises écologiques. Mais à l'heure actuelle, les réponses des communautés microbiennes dans le cas de perturbations multiples séquentielles restent moins étudiées (Gunderson et al., 2016).

Les études réalisées sur l'effet de perturbations séquentielles montrent que dans le cas de perturbations de *même nature*, la première exposition conditionne la réponse à la seconde grâce aux mécanismes de résistance au stress acquise discutés plus tôt. Par exemple, une étude sur du phytoplancton d'eau douce a montré qu'une première exposition à une acidification prolongée augmente la résistance à un stress d'acidification plus sévère (van Moorsel et al., 2021). De la même manière, des effets de co-tolérance ont régulièrement été observés sur des communautés microbiennes exposées à des contaminants métalliques (Azarbad et al., 2016) : des biofilms phototrophes préalablement exposés à différents éléments trace métalliques ont montré une meilleure tolérance à un second stress de la même nature (Tlili et al., 2011). Ces effets de résistance au stress acquise ou de co-tolérance dépendent toutefois de certaines propriétés de perturbation, et des variables réponses considérées. Par exemple, une répétition de stress identiques peut augmenter la stabilité d'un paramètre (activité enzymatique) mais diminuer celle d'un autre (production de biomasse) (Renes et al., 2020). De plus, il a été montré que l'effet de la répétition de stress anoxiques sur des communautés microbiennes benthiques de milieu marin peut réduire la résilience fonctionnelle, et qu'au-delà d'un seuil, les perturbations initialement ponctuelles ont finalement un effet continu de forme "press"

(Villnäs et al., 2013). Des résultats similaires ont été mis en évidence sur des communautés bactériennes planctoniques exposées à des répétitions de stress salins, où plus le nombre de répétitions des stress est important, plus leur impact augmente et plus la résilience diminue (Berga et al., 2012).

Dans le cas d'une séquence de perturbations de natures différentes, il est plus difficile de prédire la stabilité des communautés microbiennes, puisque la réponse dépend à la fois de la nature des perturbations appliquées de leur chronologie (Calderón et al., 2018; Tobor-Kapłon et al., 2006) et de l'intervalle de temps entre les perturbations (Dal Bello et al., 2019). Il a été montré que l'ordre dans lequel les perturbations se produisent est un aspect important à considérer dans la stabilité des communautés microbiennes, puisque la réponse peut différer en fonction de cette chronologie. Par exemple, Calderón et al. (2018) ont montré que l'effet d'une séquence de perturbations sur les activités liées au cycle de l'azote peut être 3 fois plus important en fonction de l'ordre dans lequel les perturbations de différentes natures (stress thermique (+ ou -) et anoxique) sont appliquées. Ainsi, quand le stress thermique est appliqué avant le stress d'anoxie, la réponse des communautés du sol est moins forte que si le stress d'anoxie est appliqué avant le stress thermique.

De la même manière, des communautés du sol antérieurement exposées à deux histoires de perturbations de différentes natures (contamination au cuivre ou exposition à un pH bas) présentent des différences en terme de stabilité à la suite d'un second stress (thermique ou sécheresse) (Tobor-Kapłon et al., 2006). Par exemple, quand les communautés ont été exposées à un stress d'acidification par le passé, la respiration bactérienne du sol est plus impactée que lorsque la perturbation antérieure est une exposition au cuivre. L'augmentation de la stabilité d'une communauté à un stress à la suite d'un précédent stress de nature différente a été observée à plusieurs reprises. Par exemple, des bactéries planctoniques lacustres exposées à un stress salin ont montré une meilleure résistance fonctionnelle quand elles avaient subi un stress de pH auparavant (Sjöstedt et al., 2018). Dans le sol, le même phénomène a été observé sur la stabilité des communautés bactériennes à une contamination au mercure quand elles ont préalablement été exposées à de fortes températures, à une contamination au cuivre ou à l'atrazine, ce qui laisse supposer une préadaptation des communautés (Bressan et al., 2008).

Les études sur la réponse de communautés microbiennes à des perturbations multiples concernent majoritairement les communautés du sol (Rillig et al., 2019) ou des communautés marines (Crain et al., 2008). Dans leur revue bibliographique réalisée sur des études en milieu marin, Gunderson et al. (2016) ont montré que dans la majorité des cas, les réponses des communautés sont étudiées sous un niveau d'intensité constant. Les études sur les effets des régimes de perturbations, et plus spécialement l'effet de la fréquence des perturbations sont sous représentées (Philippot et al., 2021). Dans leur revue bibliographique, Philippot et ses collaborateurs (2021) mettent aussi en avant la nécessité de mesurer l'évolution de la stabilité au cours du temps afin de pouvoir intégrer au mieux l'effet des perturbations multiples sur les communautés.

Mon travail de thèse a visé à étudier la stabilité des communautés microbiennes aquatiques dans le contexte de changement global, en prenant en compte à la fois les dimensions liées aux caractéristiques des communautés étudiées, mais aussi aux propriétés et à la complexité des régimes de perturbations appliquées. Cette thèse s'articule autour de la question générale suivante :

Comment les propriétés des régimes de perturbations (nature, intensité, fréquence… des événements), les caractéristiques et l'histoire des communautés microbiennes influencent la stabilité de ces dernières ?

Pour aborder cette problématique, j'ai étudié la réponse de biofilms phototrophes de rivières à des perturbations simples ou séquentielles associées au contexte d'intermittence hydrique. Dans la suite de cette introduction, je m'attache donc à caractériser le biofilm phototrophe de rivière, la manière dont il se structure, ses rôles fonctionnels et enfin sa réponse à l'intermittence hydrique en rivière.

3. Le biofilm phototrophe comme modèle d'étude

3.1 Structure et formation

Dans les rivières de tête de bassin, une grande partie de la production primaire est assurée par les biofilms phototrophes (Figure 9). Les films biologiques recouvrent toute surface immergée organique ou inorganique (galets, sédiment, sable, plantes, matière

organique en décomposition, interface eau-air, etc.). De manière générale, quand la lumière atteint ces surfaces, les biofilms sont phototrophes, i.e. contiennent des microorganismes photosynthétiques. Dans ma thèse, je m'intéresse seulement aux biofilms épilithiques, c'est-à-dire qui recouvrent les substrats rocheux en rivière. Ces films biologiques sont constitués d'un assemblage complexe de microorganismes benthiques phototrophes et hétérotrophes tels que des microalgues, des bactéries, des archées ou encore des champignons, mais aussi de protozoaires unicellulaires ainsi que des organismes plus gros représentant la méiofaune (30-500 µm), voire de la macrofaune retrouvée épisodiquement. Cet assemblage s'intègre dans une matrice visqueuse formée de polymères extracellulaires (EPS) qui en plus de permettre l'adhésion au substrat, confère aux biofilms une protection contre l'environnement extérieur et joue un rôle dans le stockage des nutriments (Wolfaardt et al., 1999). Les EPS représentent une large partie du carbone organique total du biofilm, et sont composés entre autres de polysaccharides et de protéines (Bharti et al., 2017).



Figure 9 : photographies de biofilms phototrophes (rivières du sud de la France).

La formation des biofilms comprend plusieurs étapes (Figure 10). Dans un premier temps, des cellules microbiennes planctoniques (eucaryotes ou procaryotes) s'attachent à la surface du substrat. Par la suite, des micro-colonies se forment et des EPS sont synthétisés, rendant l'attachement irréversible. Puis les micro-colonies formées lors de cette étape de consolidation croissent et se développent. C'est l'étape de maturation, au cours de laquelle le biofilm s'épaissit et se structure avec une succession de changements dans la composition de la communauté (Roeselers et al., 2007). L'architecture tri-dimensionnelle du biofilm induit aussi des gradients verticaux en termes de lumière et de ressources (Bharti et al., 2017). Comme pour les biofilms strictement bactériens, il y a donc une stratification des communautés au sein des biofilms et la création de microenvironnements (Costerton et al. 1995). La proximité des microorganismes favorise la complémentarité dans la maximisation de l'utilisation des ressources (Battin et al.,

2016), ainsi que les interactions entres espèces (facilitation, compétition, allélopathie, etc.). Par exemple, les organismes phototrophes exsudent des composés organiques, qui sont alors disponibles pour les hétérotrophes. En retour, le dioxyde de carbone produit par ces hétérotrophes peut être assimilé par les phototrophes. Ces interactions font que les biofilms peuvent devenir auto-suffisants pour le carbone, ce qui les rend moins dépendants des ressources externes (Battin et al., 2003; Haack and McFeters, 1982). Enfin, la phase d'accroissement du biofilm est suivie d'une phase de sénescence induite par différents facteurs, tels qu'un arrachage mécanique lié aux effets abrasifs du courant, ou encore à cause d'un détachement autogène, c'est-à-dire lié à la mortalité des cellules bactériennes et algales en contact avec le substrat (Boulêtreau et al., 2006). Lors de cette étape, les cellules se dispersent pour coloniser de nouveaux substrats à l'aval (Bharti et al., 2017).

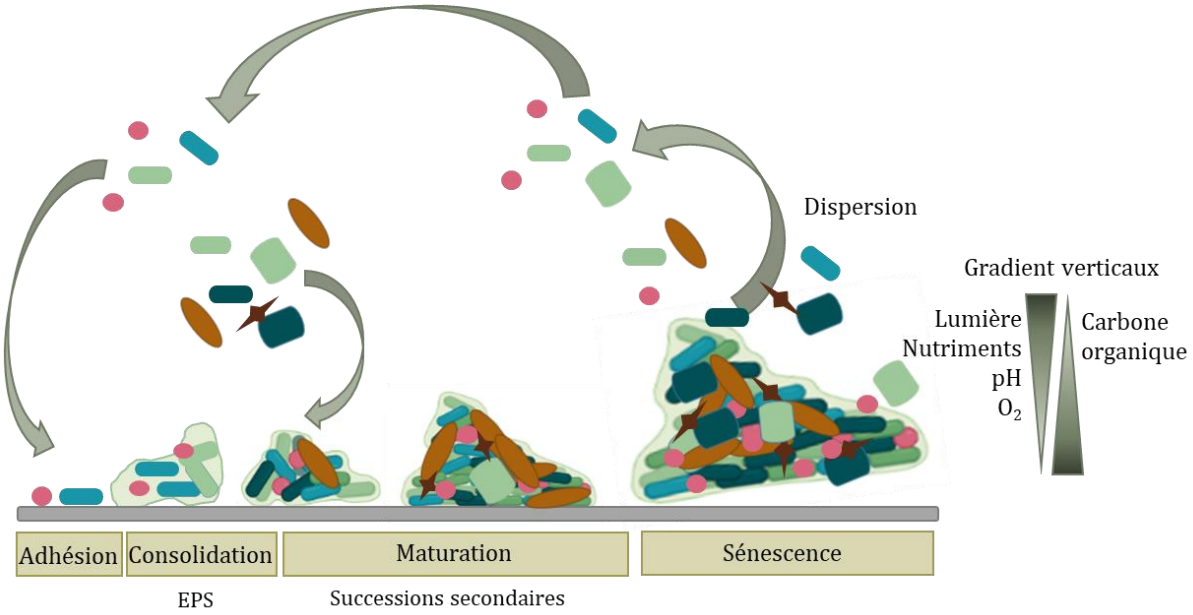


Figure 10 : Formation des biofilms phototrophes. Dans un premier temps, des cellules de la colonne d'eau viennent adhérer au substrat, puis s'y développent en sécrétant des EPS lors de l'étape de consolidation. Ensuite, le biofilm mature, grâce à l'immigration de nouvelles espèces dans l'assemblage et à la multiplication des cellules présentes, induisant ainsi des modifications dans la structure physique et dans le fonctionnement du biofilm. Cette période de croissance est suivie par une étape de sénescence lors de laquelle le biofilm se détache et se disperse.

Les structures physique et biologique des biofilms sont contrôlées par divers processus physiques, chimiques et biologiques des rivières. Ainsi, le régime hydrologique joue une part importante dans la structuration physique des biofilms, qui n'est pas la même selon que l'écoulement est turbulent ou non (Besemer et al., 2007). En effet, les biofilms produits dans des conditions d'écoulement lent sont plus épais, moins adhérents et cohésifs au substrat, et peuvent stocker plus d'éléments nutritifs (Battin et al., 2003) que ceux produits dans des conditions d'écoulement rapide. Les paramètres physico-chimiques tels que le pH, l'intensité lumineuse, la température de l'eau qui influent sur la disponibilité en nutriments et éléments organiques, sont également des facteurs importants de la structuration physique et biologique, de même que les interactions entre espèces et la capacité de dispersion des communautés (Figure 11) (Battin et al., 2016; Biggs, 1996; Mora-Gómez et al., 2016). Ces paramètres sont fortement impactés par les activités humaines et le changement global.

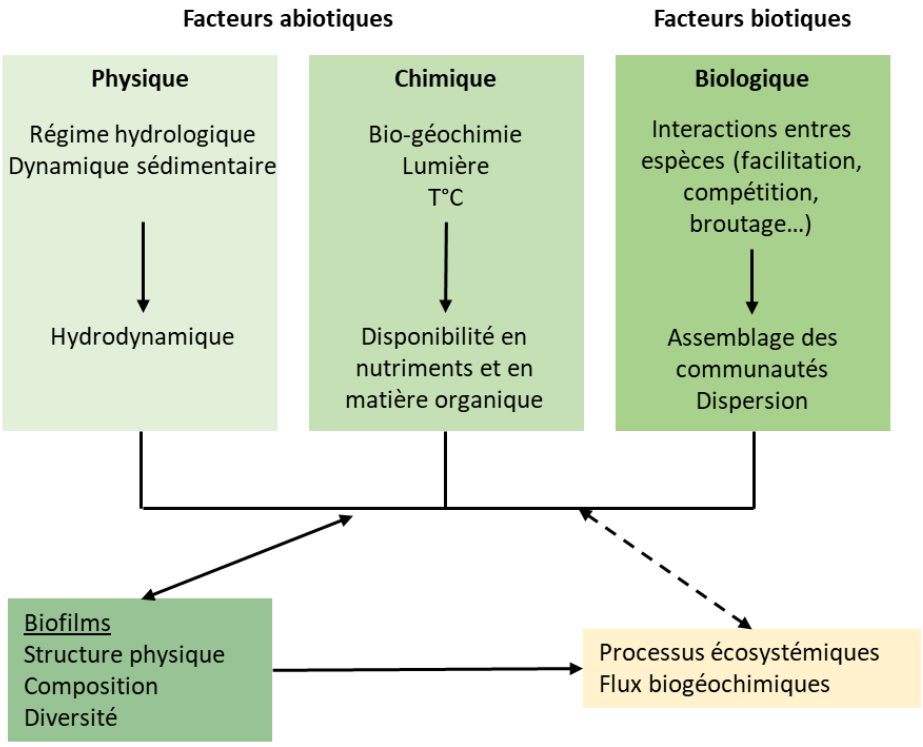


Figure 11 : Facteurs contrôlant la formation, la structure et le fonctionnement des biofilms phototrophes de rivière (*Simplifié à partir de Battin et al., 2016*).

Dans les biofilms, la photosynthèse est assurée par de nombreuses microalgues, les plus fréquentes étant les diatomées et les algues vertes, ainsi que par les cyanobactéries, procaryotes. Pendant la phase de colonisation, les diatomées dominent généralement puis sont progressivement remplacées par des cyanobactéries ou des algues vertes filamenteuses (Besemer et al., 2007; Biggs, 1996). Cependant les successions d'espèces photosynthétiques dépendent aussi de l'environnement local et saisonnier (Griffith and Perry, 1995). Par exemple, les diatomées sont plutôt dominantes en hiver et au début du printemps quand les températures de l'eau sont relativement froides, alors qu'avec le réchauffement progressif des températures elles sont remplacées par les cyanobactéries qui sont elles-mêmes remplacées par des algues vertes filamenteuses en fin d'été (Biggs, 1996). Il a été montré que les groupes algaux ne présentent pas les mêmes optima de températures, et qu'ainsi, les cyanobactéries ont l'optimum le plus élevé, suivis des algues vertes et enfin des diatomées (Nalley et al., 2018), bien que ces optima soient aussi dépendants des espèces considérées. Lorsque les niches thermales des espèces se chevauchent, ce sont les plus compétitrices qui prennent la dominance au sein de la communauté (De Nicola, 1996). En effet, toutes les espèces ne présentent pas les mêmes niches thermales et les variations de températures peuvent mener à de la compétition entre espèces et à terme à des exclusions (McCormick, 1996). De la même manière, la disponibilité en lumière et en nutriments au sein du biofilm joue un rôle dans la succession des espèces puisque la limitation de ces facteurs lors du développement induit aussi de la compétition (McCormick, 1996).

3.2 Rôles du biofilm dans l'écosystème

Les biofilms sont fortement impliqués dans le fonctionnement des écosystèmes (Battin et al., 2003), notamment en participant au recyclage de la matière organique dissoute et en assurant une part significative de la production primaire, occupant ainsi la base des réseaux trophiques (Battin et al., 2016; Lear et al., 2008). Ils participent aussi activement aux cycles biogéochimiques (Battin et al., 2016; Romaní et al., 2016) et jouent un rôle important dans les cycles biogéochimiques du carbone, de l'azote et du phosphore notamment (Leff et al., 2016). En effet, la structure tri-dimensionnelle du biofilm permet de capturer de nombreux éléments nutritifs qui sont ainsi assimilables au sein du biofilm. Dans ces processus, les EPS jouent un rôle important puisqu'en plus de capturer ces

nutriments, ils retiennent les enzymes extracellulaires produites par les microorganismes et qui sont primordiales dans le recyclage des nutriments et de la matière organique (Flemming and Wingender, 2010; Sabater et al., 2002). Les biofilms contribuent aussi de manière significative au cycle de l'azote puisqu'ils se composent de bactéries nitrifiantes qui transforment l'ammoniaque en nitrate et de bactéries dénitrifiantes qui transforment les nitrates et vont relarguer de l'oxide nitrique et des gaz azotés dans l'atmosphère (Battin et al., 2016). Enfin, les biofilms interviennent aussi dans le cycle du phosphore en assimilant le P de la colonne d'eau et en le retenant dans sa biomasse (Dodds, 2003; Liu et al., 2019).

En rivière, en fonction des caractéristiques morphologiques, les biofilms phototrophes sont à la base des réseaux trophiques (Stevenson, 1996). En réalisant la photosynthèse, ces producteurs primaires fournissent de l'oxygène et sont une source de nourriture importante pour les consommateurs (Lamberti, 1996). Cette production primaire est contrôlée par de nombreux facteurs biotiques et abiotiques, tels que la température, la disponibilité en nutriments et en lumière (Ylla et al., 2007), ou encore la densité de consommateurs. L'activité de broutage est un facteur critique de la structuration et la production du biofilm phototrophe. En effet les brouteurs maintiennent les communautés algales à des stades précoces de successions (Mora-Gómez et al., 2016; Thompson et al., 2004). Cependant il a aussi été montré que le broutage peut augmenter la diversité algale dans les biofilms en ouvrant des niches, permettant ainsi différents stades de succession au sein des communautés (Lang et al., 2015). La composition du biofilm pourrait aussi être directement influencée par le comportement sélectif des consommateurs. Par exemple les gastéropodes sont capables de sélectionner les algues plutôt que les bactéries lors du broutage (Lawrence et al., 2002), mais certains herbivores sont aussi capables de sélectionner certaines algues plutôt que d'autres (Mialet et al., 2013).

Les biofilms jouent un rôle essentiel dans le fonctionnement de nombreux écosystèmes d'eau douce. Ce rôle repose sur leur structure biologique et physique, laquelle est contrôlée par différents facteurs qui sont influencés par le changement global et les pressions anthropiques. Il est donc crucial de considérer les réponses de ces communautés microbiennes aux variations environnementales et aux changements dans les régimes de perturbations.

3.3 Réponse des biofilms à l'intermittence

3.3.1 L'intermittence hydrique

L'intermittence de l'écoulement des eaux de surface est un phénomène naturel se produisant partout dans le monde indépendamment du type de climat, et on estime qu'à l'heure actuelle, plus de 50 % des cours d'eau dans le monde sont intermittents (Messager et al., 2021). Un cours d'eau est considéré comme intermittent si son écoulement cesse et que le lit de la rivière s'assèche une partie du temps. Ces cours d'eau sont désignés en fonction de la durée de la période d'assec, et sont généralement regroupés sous l'appellation d'IRES (Intermittent Rivers and Ephemeral Streams Datry et al., 2017a) par opposition au cours d'eau pérennes, c'est-à-dire qui ne connaissent pas d'assèchement. Les causes naturelles de l'intermittences sont multiples, par exemple les conditions climatiques ou les caractéristiques physiques du cours d'eau (Larned et al., 2010). Cependant, avec le changement global, des cours d'eau habituellement pérennes commencent à faire l'expérience de périodes d'assec (voir figure 12) (Datry et al., 2014). En effet, les changements dans les patrons de précipitations, avec l'augmentation des périodes sèches, de l'augmentation globale des températures et de l'étendue des vagues de chaleur jouent un rôle dans ces changements de régimes hydrologiques (Sauquet et al., 2021). De plus, les activités humaines exacerbent les stress hydriques et peuvent mener à l'assèchement, notamment *via* la régulation de l'écoulement des cours d'eaux au niveau des barrages et des prélèvements d'eau de surface ou de nappes souterraines alimentant les cours d'eau pour l'irrigation (Fovet et al., 2021).



Figure 12 : L'Aveyron (août 2022), qui est une rivière pérenne alimentant le Tarn (44.09624, 1.31676, Tarn et Garonne, France). L'été 2022 et ses nombreuses vagues de chaleurs ont fortement contraint la continuité hydrologique dans cette rivière.

3.3.2 L'intermittence : sources de perturbations multiples

L'intermittence s'accompagne de nombreux changements physiques et chimiques dans le cours d'eau (Leigh et al., 2016) qui représentent des perturbations séquentielles pour la biocénose. Lors du processus d'assèchement, l'écoulement de l'eau cesse progressivement, puis le niveau d'eau de la rivière diminue (Figure 13) (Datry et al., 2017b). Cette lentification s'accompagne généralement d'une fragmentation importante de l'habitat, d'une augmentation de la température dans l'eau et d'une forte altération des conditions physico-chimiques avec de fortes modifications de pH ou de salinité par exemple (Gómez et al., 2017; Mosley, 2015). Par la suite, le lit de la rivière s'assèche pendant une période plus ou moins longue, pouvant aller de quelques jours à plusieurs mois (Sabater et al., 2016). Lors de cette étape, des mares résiduelles peuvent persister et servir de refuges pour les organismes vivants dans ces écosystèmes. Enfin, lors du retour de l'écoulement, qui est soudain, contrairement au processus d'assèchement, les organismes sont de nouveau exposés à des changements physico-chimiques de l'eau. Par exemple, une augmentation de la quantité de nitrates dans l'eau a été observée lors du

retour de l'écoulement (Mosley, 2015). De même, en fonction de l'occupation du sol autour du cours d'eau, les contaminants agricoles contenus dans le sol peuvent être lessivés et gagner le cours d'eau par ruissellement, exposant ainsi les communautés à une nouvelle source de perturbation chimique.

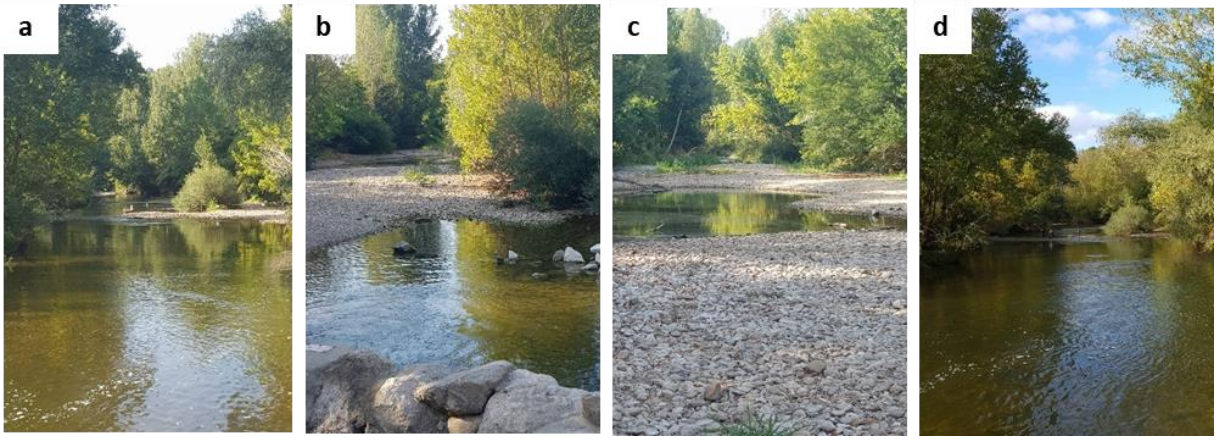


Figure 13 : Photographies illustrant la séquence d'assèchement en rivière. (a) l'écoulement s'arrête et la hauteur d'eau diminue. En conséquence la physico-chimie de l'eau change. Puis le lit de la rivière s'assèche progressivement (b). Des mares résiduelles peuvent subsister et former un refuge pour les organismes (c). Enfin, l'écoulement reprend (d).

Dans les IRES, les périodes d'assec sont saisonnières, alors que pour les cours d'eau habituellement pérennes, l'assèchement représente une perturbation sévère et l'événement met à mal l'intégrité du système en affectant fortement les communautés et les populations (Lake, 2011). Cependant, l'augmentation de l'étendue des périodes sèches qui affectent les IRES à cause du changement global, fait que l'effet de l'asséchement compromet aussi la stabilité des organismes habitant ces écosystèmes (Lake, 2003). Les effets de l'intermittence sont principalement documentés pour les IRES, bien que le phénomène concerne localement la majorité des rivières sujettes à une diminution de la ressource en eau. En effet, les rivières pérennes peuvent faire l'expérience d'assecs partiels lorsqu'elles sont soumises à de fortes fluctuations de débit et de niveaux d'eau, affectant ainsi les communautés de berges, notamment fixées (Lake, 2011; Sarremejane et al., 2022).

3.3.3 Réponse des biofilms phototrophes

Lors de la séquence d'assèchement, la biocénose est fortement impactée. Dans les rivières exposées à l'intermittence, le rôle des communautés benthiques dans la production primaire est d'autant plus crucial que l'importance de la colonne d'eau est fortement réduite. Cependant, un simple arrêt de l'écoulement suffit à fortement diminuer le métabolisme des biofilms. En effet, une étude a montré que lorsque le niveau d'eau diminue lors des périodes de faible écoulement, la production primaire et la respiration diminuent (Arias Font et al., 2021). L'assèchement impacte ensuite fortement les communautés algales microbiennes, à la fois en termes de biomasse (Colls et al., 2019) et de composition (Dahm et al., 2003a). Ces changements dans les communautés dépendent de la capacité des espèces à résister à la dessiccation via l'entrée en dormance par exemple, mais aussi de la capacité à utiliser les refuges disponibles (mares résiduelles par exemple) (Sabater et al., 2016). De manière générale, l'arrêt de l'écoulement et l'assèchement, en agissant sur la composition des communautés, engendrent une diminution de la biodiversité des microorganismes. Le compartiment microbien autotrophe est plus fortement impacté par l'assèchement que le compartiment hétérotrophe, surtout pour les biofilms épilithiques (Timoner et al., 2012), et ce d'autant plus que la durée de l'assec augmente (Colls et al., 2019). La conséquence de cela est que l'assec induit un basculement du système vers un fonctionnement hétérotrophe. Il faut cependant noter que lorsque la période d'assec s'allonge, les effets négatifs s'intensifient et au-delà d'une durée critique, le compartiment hétérotrophe est aussi fortement impacté (Acuña et al., 2015).

Parmi les microorganismes phototrophes, tous ne présentent pas la même sensibilité à l'assèchement. Il a été montré que bien souvent les cyanobactéries et les algues vertes sont plus résistantes que les diatomées, mais ces dernières sont généralement plus résilientes (Acuña et al., 2015; Sabater et al., 2017; Timoner et al., 2014c). De manière générale, la récupération des organismes autotrophes est rapide après la remise en eau. En effet, plusieurs études ont mis en évidence une reprise rapide du métabolisme des biofilms quand les conditions redeviennent favorables (Romani and Sabater, 1997; Skoulikidis et al., 2017; Timoner et al., 2012). Cependant, la majorité des études sur la réponse des biofilms de rivières à l'assèchement étudient des biofilms issus de rivières sujettes à l'intermittence naturellement. Les algues des IRES présentent des

capacités d'adaptation, telles que la capacité de mise en dormance chez certaines espèces, qui une fois que les conditions environnementales sont à nouveau favorables, sont rapidement réactivées (Sabater et al., 2017). Certaines espèces sont aussi capables de synthétiser des pigments protecteurs (Timoner et al., 2014c). En revanche, dans les rivières pérennes, les organismes ne présentent pas de telles adaptations. Dans une étude de 2020, Timoner et al. ont mis en évidence que la structure des biofilms de cours d'eau pérennes est moins stable, c'est-à-dire moins résistante et résiliente, à l'assèchement que celle des cours d'eau intermittents (Timoner et al., 2020).

Quelques études se sont intéressées à la réponse de biofilms exposés à une combinaison d'intermittence et à d'autres perturbations. Par exemple, Colls et al. (2019) ont mis en évidence que la température et l'irradiance moyennes auxquelles sont exposés les biofilms pendant l'assec peuvent fortement impacter la réponse des communautés. Trois études ont démontré une diminution de la récupération des biofilms quand ces derniers sont soumis à l'intermittence puis exposés à des contaminants lors de la remise en eau (Corcoll et al., 2015; Pesce et al., 2016; Proia et al., 2011). L'exposition de biofilms à un stress hydrique puis à un bactéricide, amplifie les effets négatifs du contaminant sur les communautés, induisant une moins bonne récupération après assec (Proia et al., 2013). Il a aussi été montré qu'après un assec, la sensibilité des biofilms à des contaminants pharmaceutiques est accentuée, particulièrement chez les autotrophes, et que d'importants changements dans la composition des communautés se produisent (Corcoll et al., 2015). Enfin, dans une étude où l'effet combiné d'un assec et de l'exposition à un fongicide a été examiné sur des communautés microbiennes impliquées dans la décomposition, une amplification de l'effet du biocide par l'assec a aussi été mise en évidence (Pesce et al., 2016).

Ces différents exemples suggèrent l'existence d'interactions significatives entre l'assèchement et d'autres types de perturbations. Romero et al. (2018) ont étudié de façon systémique, dans un même cadre expérimental, la fréquence de ces interactions par rapport à de simples effets additifs. Ils ont ainsi montré que malgré quelques interactions synergiques ou antagonistes, les effets des perturbations physiques (assec et température) et de la contamination sont majoritairement additifs. La réponse des biofilms à ces combinaisons de perturbations dépend du type de contaminant mais aussi des variables réponses observées. Ainsi, des divergences entre la réponse des autotrophes

et des hétérotrophes peuvent être observées. Dans ce contexte, il est difficile de prédire comment un contaminant complexe composé de multiples polluants induisant des réponses à priori opposées sur les biofilms peut interagir avec d'autres perturbations.

Du fait de l'augmentation de la prévalence de l'intermittence et des fluctuations du débit des cours d'eau pérennes, les biofilms sont soumis à des perturbations multiples, simultanées mais aussi séquentielles, dont la fréquence et l'intensité augmentent. Bien que la réponse des biofilms à l'assèchement ait été abondamment étudiée, l'état actuel des connaissances ne permet pas de prédire la stabilité de ces acteurs majeurs du fonctionnement des rivières face à ces perturbations multiples et à ces séquences complexes.

4. Enjeux et objectifs de la thèse

Mon travail de thèse vise à comprendre comment les caractéristiques, l'histoire des communautés microbiennes aquatiques et les propriétés des régimes de perturbations influencent leur stabilité (Figure 14). Pour répondre à ces questions, j'ai étudié la stabilité structurelle et fonctionnelle - résistance et/ou résilience - de biofilms phototrophes de rivière exposés à des perturbations associées à l'intermittence hydrique. Outre leur rôle fonctionnel primordial, ces communautés des biofilms phototrophes ont l'avantage d'être assez facilement manipulables en laboratoire, qu'elles soient issues du milieu naturel ou produites artificiellement. Je me suis dans un premier temps attachée à comprendre l'effet d'une perturbation unique sur la stabilité des communautés microbiennes à différents niveaux de structure et diversité. J'ai ensuite cherché à comprendre la réponse des communautés face à des perturbations séquentielles plus complexes, typiques notamment d'une situation d'intermittence hydrique. Les 5 chapitres de ma thèse sont organisés en fonction de la complexité de la perturbation appliquée lors des expériences (Table 2).

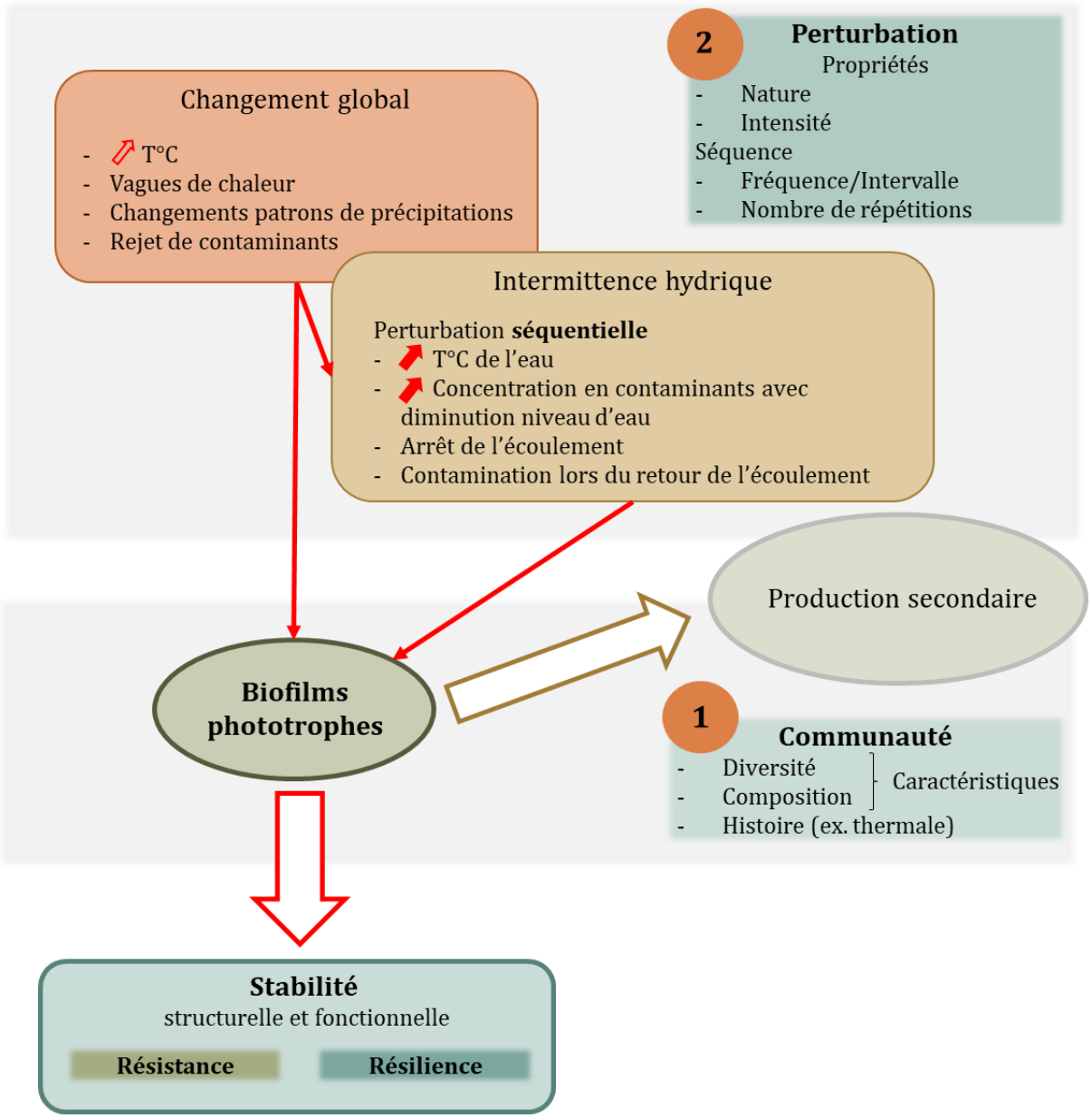


Figure 14 : Schéma d'articulation de la thèse. Ce schéma est centré autour des deux principaux objectifs de ma thèse qui sont : 1. Quelles caractéristiques des communautés microbiennes influencent leur stabilité ? et 2. Quelles propriétés d'une perturbation ou d'une séquence de perturbations jouent sur la stabilité des communautés microbiennes ?

Le **premier chapitre** vise à déterminer et comprendre l'importance de la complexité et de l'histoire thermale des biofilms dans la résistance de leur activité photosynthétique en réponse à un stress thermique sévère typique d'une vague de chaleur. Pour répondre à cette question, des augmentations de température de durées et d'intensités variables (31 à 44 °C x 30 à 180 min) ont été appliquées à des souches algales

cultivées à 20°C en laboratoire, à des communautés synthétiques constituées de 3 souches algales préalablement acclimatées à 15 °C, 20 °C ou 25 °C, et enfin à des biofilms échantillonnés en rivière à différentes saisons. Le concept de dose thermale a été utilisé pour synthétiser l'effet combiné de l'intensité et de la durée du stress et extraire 2 métriques de réponse de l'activité photosynthétique au stress thermique : la tolérance et la réactivité de la réponse de la communauté à la dose thermale.

Dans le **deuxième chapitre**, j'ai évalué dans quelle mesure l'intensité de l'assèchement d'une rivière impacte la qualité nutritionnelle potentielle des biofilms et leur capacité à soutenir la production secondaire après l'assèchement et au cours de la remise en eau. Ainsi, 4 biofilms de compositions spécifiques contrastées ont été produits en canaux au laboratoire, avant de subir un assec court de 3 jours ou un assec plus long de 2 semaines, et d'être remis en eau pendant 2 semaines. L'évaluation de la qualité nutritive des biofilms a été réalisée avant, après perturbation et après récupération, en analysant de nombreux paramètres associés à la qualité biochimique et compositionnelle de la ressource (proportions de pigments spécifiques à chaque groupe algal, les ratios stœchiométriques et les profils d'acides gras). Des consommateurs primaires ont été également nourris avec ces différentes ressources pendant 1 mois, et les traits écologiques des individus ont été mesurés (taux de croissance, taux de consommation, indice de condition, et vitesse de nage). L'ensemble des données a permis de calculer un indice de production secondaire.

Dans le **troisième chapitre**, j'ai analysé comment la nature, l'intensité des perturbations et la fréquence de leurs répétitions modulent la résilience de la production primaire des biofilms. Pour cela, des biofilms synthétiques constitués de 5 souches algales de laboratoire et leur cortèges bactériens ont été exposés à des séquences de perturbations répétées 1 à 8 fois, de durée constante (2 h), de même nature (thermique, chimique ou salin), de même intensité (5 intensités testées et à intervalles de temps réguliers (30 min, 3 h ou 22 h). La production primaire a été mesurée 24 h après la fin de chaque séquence de perturbations et modélisée en fonction du nombre de perturbations pour déterminer à quel scénario de réponse aux perturbations successives (effets additifs, synergiques ou antagonistes) la résilience des biofilms obéit.

Le **quatrième chapitre** quantifie comment la sensibilité de biofilms phototrophes exposés à un contaminant complexe mimant un lessivat agricole (nitrates, cuivre, et

pesticides) lors de leur remise en eau est modulée par l'histoire d'intermittence hydrique antérieure. Du biofilm issu du milieu naturel a été cultivé en laboratoire puis exposé à des conditions croisant 3 types de régime hydrique (simple arrêt de l'écoulement, assec court de 3 jours ou assec long de 3 mois) et 2 températures (22 °C et 32 °C). Lors de la remise en eau, les biofilms ont été exposés à un gradient de 6 concentrations du contaminant. La sensibilité structurelle et fonctionnelle des biofilms au contaminant (pente de la réponse) a été analysée après la fin de la contamination et comparée selon l'histoire d'intermittence.

Enfin, le **cinquième chapitre** de ma thèse vise à comprendre comment l'histoire thermale influence la trajectoire de récupération structurelle et fonctionnelle des biofilms de rivière intermittente après une contamination par un lessivat agricole. Dans cette expérience, la stabilité des communautés a été mesurée (après perturbation et deux fois au cours de la période de récupération) en suivant de nombreux descripteurs de la structure (diversité des procaryotes et eucaryotes du biofilm, composition pigmentaire, ratios stœchiométriques, EPS, ergostérols) et du fonctionnement (diversité fonctionnelle hétérotrophe, production primaire, respiration, activité photosynthétique) des biofilms. Pour chaque concentration en contaminant testée, la comparaison de la réponse entre les biofilms ayant fait l'expérience d'histoires thermales différentes a été étudiée.

Table 2 : Organisation des 5 chapitres de la thèse. Chaque colonne décrit le type de perturbation appliqué, sa nature, les propriétés manipulées, le type d'assemblage microbien sur lequel l'expérience a été réalisée, les composantes de la stabilité étudiées, les métriques mesurées et enfin l'objectif auquel répond ce chapitre dans le schéma d'articulation.

Chapitre	Perturbation	Nature	Propriété manipulée	Type d'assemblage microbien	Composante de la stabilité	Métrique	Objectif
I	Unique	T °C	Intensité Durée	Souches isolées Communautés synthétiques Communautés naturelles	Résistance à 24 h	Fonctions	1
II	Unique	Assec	Intensité	Communautés naturelles cultivées en laboratoire	Résistance et Résilience	Structure et qualité de la ressource	1
III	Séquence de perturbations identiques	T °C Cuivre Salinité	Intensité Fréquence Répétitions	Communautés synthétiques	Résistance à 24 h	Fonctions	2
IV	Séquence de perturbations différentes	T °C Assec Stress chimique	Intensité Durée	Communautés naturelles cultivées en laboratoire	Résistance	Structure et Fonctions	2
V	Séquence de perturbations différentes	T °C et stress chimique après assec	Intensité	Communautés naturelles cultivées en laboratoire	Résistance et Résilience	Structure, Diversité et Fonctions	1-2

Chapitre 1

Importance des caractéristiques des communautés (diversité et histoire thermale) dans la réponse à un stress thermique

Chapitre 1 : Importance des caractéristiques des communautés (diversité et histoire thermale) dans la réponse à un stress thermique

Contexte

Le réchauffement global se traduit par une augmentation de la fréquence et de l'intensité des vagues de chaleur et menace les écosystèmes aquatiques (Seneviratne et al., 2021). Ces événements de vagues de chaleurs s'accompagnent d'une baisse du niveau de l'eau ainsi que d'une augmentation marquée de la température de l'eau suite à la diminution de l'inertie thermale qu'elle engendre. Lors des étiages estivaux, la très forte corrélation entre les températures dans l'air et dans l'eau expose les communautés aquatiques à des stress thermiques aigus (Gómez et al., 2017). L'effet de ces stress thermiques sur les communautés algales a été bien étudié dans les milieux marins et les zones intertidales (e.g. Deldicq et al., 2021; Román et al., 2020; Weitzman et al., 2021). Cependant, au vu de l'important rôle fonctionnel des biofilms phototrophes dans les rivières et de leur exposition croissante aux situations de stress thermique, il est aujourd'hui important d'étudier leur réponse à des fortes variations de température, et d'identifier les facteurs qui contribuent à leur stabilité fonctionnelle.

De manière générale, la réponse des individus à la température dépend de l'espèce considérée (Samuels et al., 2021). Chaque espèce présente une niche thermale pour laquelle les performances (croissance, photosynthèse, etc.) sont maximales à une température dite température optimale. En revanche, il a été montré qu'au-delà de cette niche thermale, de nombreuses réponses physiologiques sont mises en place et peuvent à terme mener à la mort cellulaire (Souffreau et al., 2010).

L'histoire thermale d'une population ou d'une communauté influence sa réponse à un stress thermique. En effet, la température moyenne antérieure conditionne la réponse individuelle des espèces et potentiellement leur sensibilité à un stress thermique en modifiant leurs niches thermales (Luhring and DeLong, 2017; Padfield et al., 2016). De plus, dans le milieu naturel, les changements de température conduisent à des changements dans la composition des communautés via des remplacements d'espèces qui peuvent ainsi conférer une meilleure stabilité face au stress

La diversité d'une communauté impacte aussi sa réponse à un stress thermique. L'hypothèse d'assurance (Yachi et Loreau, 1999) prédit que l'association de plusieurs espèces ayant des niches thermales différentes augmente la niche thermale globale de la communauté et induit ainsi une meilleure stabilité.

Les effets d'un stress thermique dépendant à la fois de la durée et de l'intensité du stress, il est pertinent d'utiliser un paramètre unique résumant ces deux informations. La « **dose thermale** » définie comme le produit du temps et d'une fonction exponentielle de la température, semble appropriée pour étudier la réponse de microalgues à des stress de température (Béchet et al., 2017).

Objectifs et hypothèses

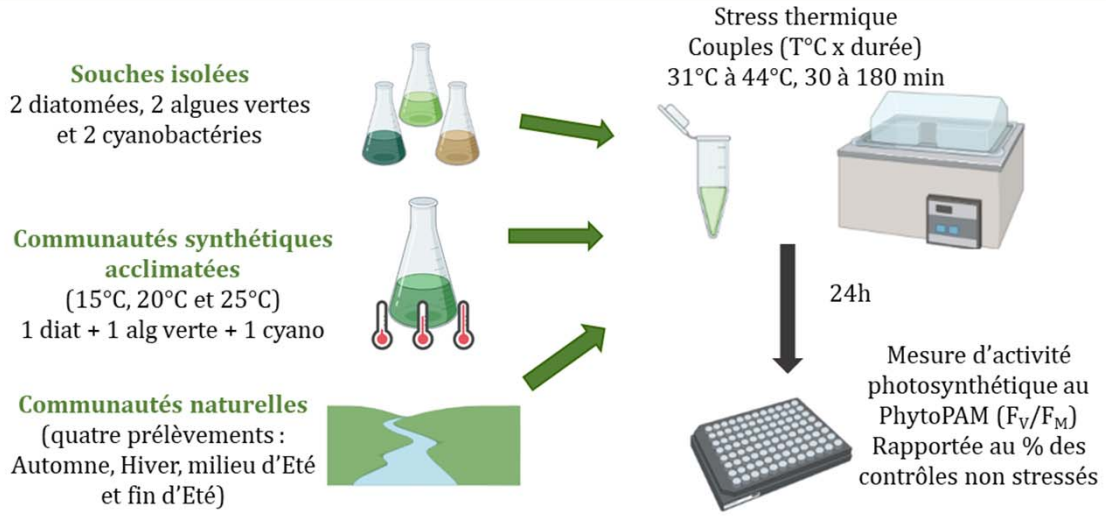
L'objectif de ce chapitre est donc de comprendre comment les biofilms phototrophes de rivière répondent à des stress courts et intenses de température tels qu'ils peuvent se produire en milieu naturel lors des étiages estivaux. Pour se faire, j'ai cherché à comprendre l'importance de la diversité et de l'histoire thermale des communautés dans leur réponse au stress en étudiant la photosynthèse, un processus très sensible à la température (Glatz et al., 1999), chez des algues benthiques.

Mes hypothèses sont :

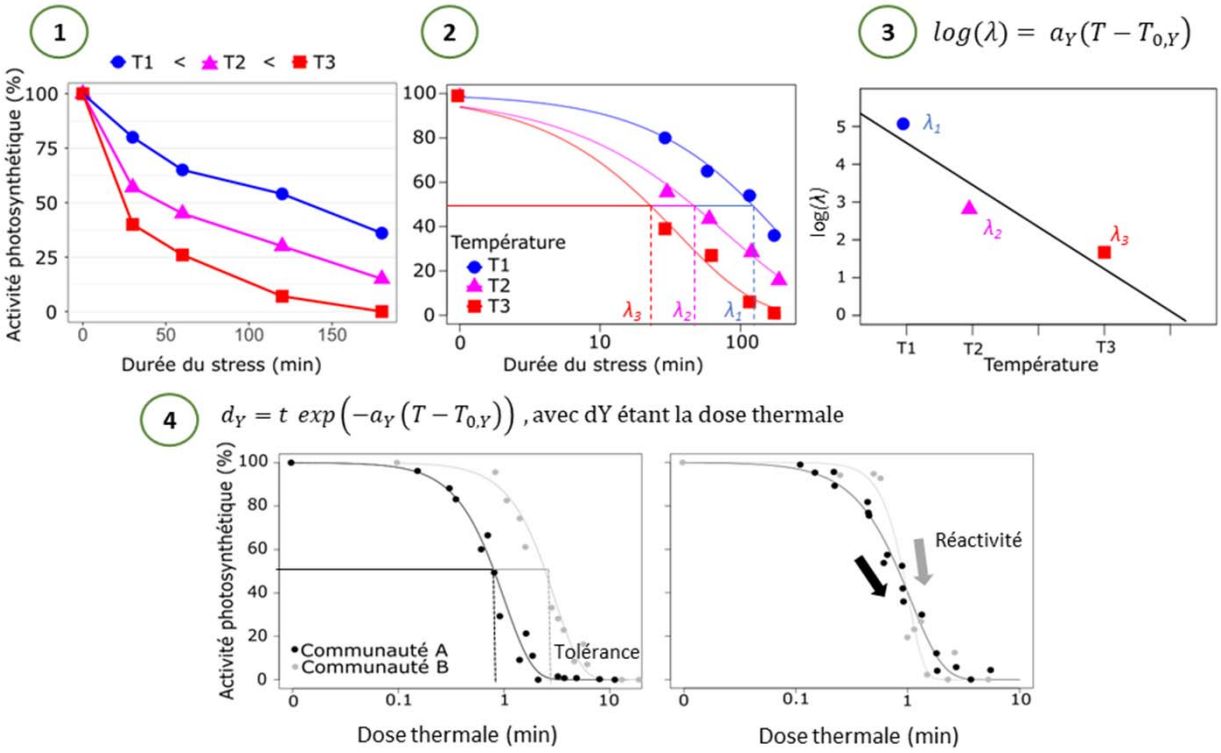
1. La réponse des biofilms dépend de leur histoire thermale : la tolérance au stress augmente avec l'augmentation de la température d'acclimatation.
2. La réponse au stress dépend aussi de la diversité : plus la communauté est diversifiée, moins elle est impactée par le stress thermique.

Matériel et méthodes

Design expérimental



L'approche par dose thermale



Deux paramètres déduits de l'approche par dose thermale : la tolérance et la réactivité de la réponse

La **tolérance** : représente la dose thermale que l'échantillon est capable de tolérer au point d'inflexion

La **réactivité** : représente la force de la réponse, plus la pente est élevée, plus la réponse au stress est intense

Application au milieu naturel

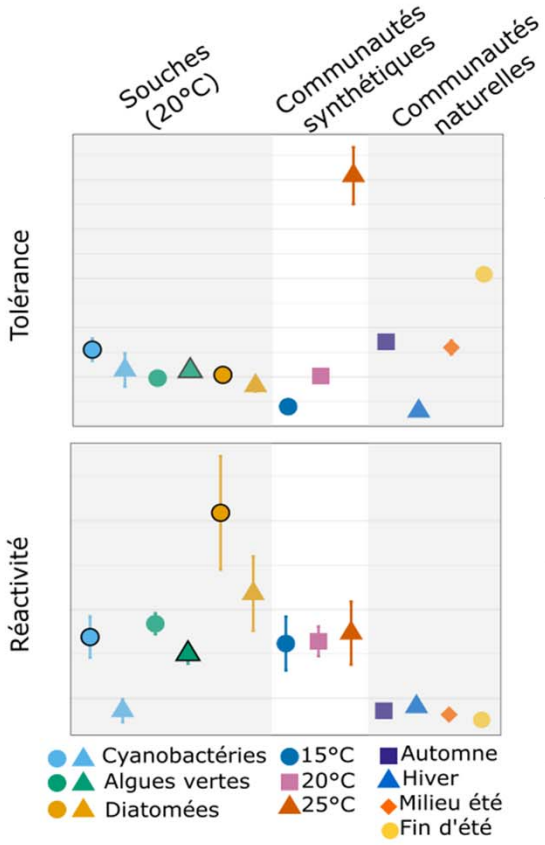
- Mesures des températures dans la rivière Garonne, dans une zone potentiellement déconnectée en été
- Estimation du lien entre productivité primaire brute (PPB, mesure des flux d'O_2) et activité photosynthétique (F_V/F_M)

Résultats

Caractérisation des communautés naturelles

	Automne	Hiver	Milieu d'été	Fin d'été
T°C moyenne sur 27 jours	11,5°C	7,5°C	19,7°C	21,6°C
Min – max T°C	11 – 12,2	7 – 8,4	18,2 – 21,6	19 – 24,8
Richesse spécifique	15	14	9	17
Diversité (Shannon)	2,21	1,61	0,78	2,12
Equitabilité (Pielou)	0,82	0,61	0,36	0,75

Réponse de l'activité photosynthétique au stress thermique

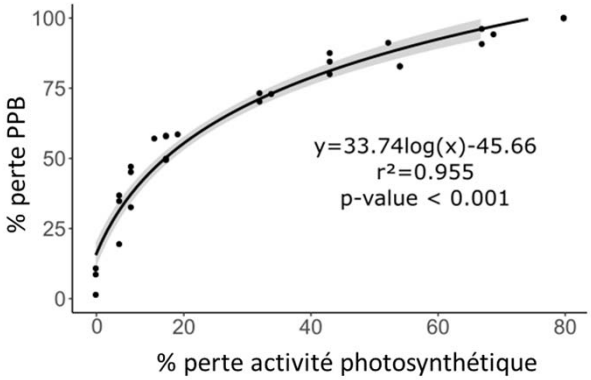


L'histoire thermale joue un rôle important dans la **tolérance** au stress thermique comme on peut le voir pour les communautés synthétiques pré-acclimatées aux 3 différentes températures.
La diversité a aussi un effet sur la tolérance : le biofilm de milieu d'été est moins tolérant que celui de fin d'été malgré des histoires thermales très similaires.

La **réactivité** de la réponse dépend de l'espèce algale. Les diatomées tendent à répondre plus rapidement que les algues vertes et les cyanobactéries.
Il n'y a pas d'effet de la température d'acclimatation sur la réactivité de la réponse.
La diversité joue également un rôle important puisque la réponse des communautés naturelles, plus diversifiées que les communautés synthétiques, répond globalement plus graduellement à l'augmentation de la dose thermale.

Relation entre PPB et activité photosynthétique en réponse au stress thermique

La relation entre la perte d'activité photosynthétique et la perte de PPB n'est pas linéaire. Pour les températures les plus faibles, la PPB diminue beaucoup plus fortement que l'activité photosynthétique → une faible diminution de l'activité photosynthétique peut se traduire par une forte diminution de la productivité primaire.



Conclusions et Perspectives

L'utilisation de la dose thermale pour modéliser la réponse des communautés permet de facilement comparer les effets du stress thermique sur l'activité photosynthétique entre les différents matériels biologiques utilisés.

Avantages de l'approche : Le calcul de la dose thermale, prenant en compte à la fois la température et la durée d'exposition, apporte un meilleur examen de la réponse des communautés qu'une simple approche dose-réponse basée sur les analyses de seuils de type ED50.

L'histoire thermale

L'histoire thermale (i.e. acclimatation) joue un rôle important dans la tolérance au stress thermique

→ Validation de l'hypothèse 1

En plus des effets liés à l'acclimatation, dans le milieu naturel, l'augmentation des températures en été engendre une sélection d'espèces tolérantes aux plus fortes températures. Dans les communautés étudiées, des diatomées du genre *Achnantidium*, *Cocconeis* et *Nitzschia* connues pour être capables de survivre à de fortes températures (Delgado et al., 2020) sont retrouvées en fin d'été et en automne. Cet héritage de la composition des communautés, lié aux températures estivales, pourrait être à l'origine de la similarité de la tolérance observée entre les biofilms d'automne et de milieu d'été.

La diversité algale

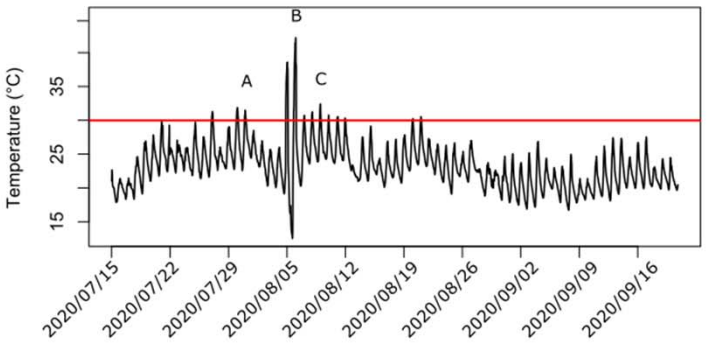
La diversité de la communauté est un facteur important de tolérance au stress thermique qui pourrait expliquer les différences observées entre les biofilms échantillonnés en milieu et en fin d'été, présentant des histoires thermales similaires. La communauté de milieu d'été est moins diversifiée que celle de fin d'été (voir tableau section résultats), et montre une tolérance plus faible.
De manière générale, les communautés naturelles présentent une réactivité au stress thermique moins forte que les souches isolées ou que les communautés synthétiques

→ Validation de l'hypothèse 2

De plus, la variabilité de la réactivité est beaucoup plus faible pour ces communautés, en accord avec l'hypothèse d'assurance (Yachi et Loreau, 1999) et avec les études réalisées sur des communautés microbiennes du sol, elles aussi exposées à des fluctuations de températures en laboratoire (Leary et Petchey, 2009).

Application au milieu naturel

L'étude de la relation entre la PPB et l'activité photosynthétique permet d'anticiper les effets potentiels de pics de chaleurs dans le milieu naturel en été. A partir des relevés de température réalisés en rivière, j'ai mis en évidence, que même des évènements de faible intensité pourraient fortement impacter la productivité primaire.



Chronique de T°C mesurées dans un bras déconnecté du chenal principal de la Garonne en été 2020

Lors des fortes vagues de chaleur, avec la diminution du niveau d'eau, les températures peuvent dépasser des seuils critiques pendant plusieurs jours. Il serait alors intéressant de déterminer si l'effet d'une vague de chaleur est équivalent à la séquence des effets de plusieurs stress courts d'intensité différentes, et donc à l'addition des doses thermales déterminées dans ce travail.

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Disentangling the effects of microalgal diversity and thermal history on freshwater phototrophic biofilms facing heat stress: A thermal dose approach

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Camille Courcoul*, Martin Laviale, Vincent Baillard, Elise Billoir, Joséphine Leflaive, Stéphanie Boulêtreau

[1] - Laboratoire écologie fonctionnelle et environnement, Université de Toulouse, CNRS, INPT, UPS, Toulouse, France

[2] - Université de Lorraine, CNRS, LIEC, F-57000 Metz, France

*camille.courcoul@univ-tlse3.fr

Abstract

1. The increase in heatwave intensity and duration is challenging microalgal photosynthesis in shallow waters. In particular, the extent of photosynthesis impairment or limitation due to short extreme heat stress events occurring during a heatwave remains unknown.
2. In this study, we investigated the importance of microalgal diversity and thermal history in the response of phototrophic biofilms to severe heat stress. We exposed isolated microalgal strains, synthetic communities previously acclimated at three temperatures, and natural phototrophic biofilms sampled at different seasons to several temperature/duration pairs (from 31 to 44 °C for 30, 60, 120 or 180 min). Microalgal photosynthetic activity (F_V/F_M) was measured after each heat stress and was shown to be a function of thermal dose, defined as the product of time and an exponential function of temperature.
3. It appeared that the thermal history plays an important role in tolerance to heat stress, with synthetic communities acclimated at highest temperature being more tolerant. However, the responsiveness (i.e., the strength of the response) to heat stress was driven by the microalgal diversity of the community.

4. *Synthesis*: Our analysis showed that although photosynthetic activity was negatively impacted by heat stress, gross primary productivity was even more strongly and rapidly impacted, highlighting the fact that *in situ,* even moderate heat stress can significantly impair primary productivity in shallow waters.

Keywords: freshwater microalgae, heatwaves, photosynthetic activity, productivity, thermal dose, thermal tolerance

1. Introduction

Many ecosystems are usually exposed to seasonal, as well as daily fluctuations in temperature. In addition to these natural variations, global warming threatens aquatic ecosystems worldwide through the increase in intensity and frequency of heatwaves (Seneviratne et al., 2021). As photosynthesis, in particular photosystem II activity, is one of the most heat-sensitive cellular processes (Glatz et al., 1999), it is necessary to better understand the effects of heat stress on photosynthetic activity and on the resultant primary productivity. To date, most of previous works on the effects of heat stress on phototrophic organisms have been conducted in marine systems. In pelagic systems, heatwaves generally induce shifts in primary producer community composition (Soulie et al., 2022) together with a decline in species richness (Polazzo et al., 2022) which can ultimately severely impair ecosystem functioning (Frölicher and Laufkötter, 2018). In the intertidal zone, which can already experience extreme heat stress during low tide, both macroalgal and microphytobenthic communities' productivity can be limited by high temperatures (Brito et al., 2013; Laviale et al., 2015; Román et al., 2020; Weitzman et al., 2021). In comparison, the effect of heat stress is poorly documented in freshwater ecosystems, especially for benthic microalgae despite their ecological importance.

Phototrophic biofilms represent the major part of primary production in many shallow water bodies and running aquatic ecosystems (Biggs, 1990; Sánchez et al., 2017). Because of climate change, the prevalence of nonperennial rivers and streams is enhanced (Messager et al., 2021), and the probability for benthic communities to face heat stress increases (Datry et al., 2014). During low-flow periods, the water level sharply decreases, reducing the thermal inertia of water. Flow cessation enhances the correlation between water and air temperatures, leading to larger daily variations in temperature with notably

higher water temperatures in the daytime (Morrill et al., 2005). During heatwaves, some unusually high temperatures can be reached (Gómez et al., 2017; Vidal-Abarca et al., 2002), triggering acute heat stress in phototrophic benthic microorganisms. Given the important functional role of phototrophic biofilms and the predicted climate change scenario, the effects of heat stress on these communities have been understudied.

The individual response to a given temperature is usually defined by the organism's thermal niche, which is species- and even strain-dependent (Samuels et al., 2021). The thermal niche is defined by a thermal performance curve characterised by an optimum temperature at which performance is maximal (Romaní et al., 2014). Within the curve's optimal range, if the temperature remains below the thermal optimum, some positive effects (e.g., higher growth rate) may be observed with increasing temperature (Angilletta, 2009). When ambient temperature increases over the thermal optimum, several physiological responses are induced that may lead to cell death, depending on the duration and intensity of the stress (Souffreau et al., 2010a). Thermal tolerance is associated with cellular stress responses, including the modification of gene expression and the synthesis of heat shock proteins (Horváth et al., 2012). The latter can interact with other peptides and proteins to prevent their denaturation by temperature and the consequent inactivation of cellular processes (Vinocur and Altman, 2005).

The response of communities to heat stress strongly depends on their thermal history, i.e., the temperature the community experienced for a given time before the application of the stress. Thermal history can alter thermal niches (Luhring and DeLong, 2017; Padfield et al., 2016) and significantly modify individual tolerance to heat stress. Microalgae previously exposed to temperatures lower than their thermal optimum can enhance their individual tolerance to a new heat stress, as shown for marine taxa (e.g. Magozzi and Calosi, 2015; Sasaki and Dam, 2019). In contrast, when grown above their thermal optimum, marine diatoms have higher mortality rates during heatwaves (Samuels et al., 2021). For various aquatic taxa, growth at high but sublethal temperatures can impair their ability to recover from heat stress (Marbà and Duarte, 2010; Samuels et al., 2021; Siegle et al., 2018). Nevertheless, the tolerance of individuals is not sufficient to infer the response to temperature at the community level. The thermal history induces changes in community composition due to competition and mortality that, in turn, bring changes in tolerance to temperature at the community scale. For instance, in freshwater, cyanobacteria tend to dominate the microalgal community as temperature increases

(Watermann et al., 1999). In communities dominated by diatoms, increasing temperatures reduced species diversity (Delgado et al., 2020). Moreover, community diversity may also affect its response to heat stress. in agreement with the insurance hypothesis (Yachi and Loreau, 1999). The association of various species with slightly different thermal niches could increase the general thermal niche of the community, thus allowing better resistance to a changing environment.

Since the effect of heat stress depends on both the duration of the stress and the temperature applied, understanding this effect requires a parameter that summarises both pieces of information. For this purpose, the 'thermal dose' was defined as the product of time and an exponential function of temperature. It has been used for decades in clinical studies, including the application of hyperthermia in cancer therapy (Gerner, 1987). More recently, it was successfully adapted to model the impact of heat stress on microalgal viability and activity to minimise the risk of culture collapses in large-scale outdoor cultivation systems (Béchet et al., 2017). Microalgal viability and photosynthetic activity were both shown to be functions of the thermal dose. The calculation of a 'dose of heat' allowed us to synthesize heat stress and to predict its effect on organisms.

The objective of our study was to understand how phototrophic biofilms respond to short and intense heat stress, as occurs during low-flow periods. We investigated the effects of thermal history and microalgal diversity on the response of photosynthesis to heat using the thermal dose approach. To this end, we performed several temperature experiments using (1) cultivated benthic microalgal strains, (2) simplified microalgal communities obtained by mixing microalgal strains previously acclimated at contrasting temperatures and (3) natural phototrophic communities sampled during different seasons. Each experiment consisted of exposing microalgal samples to a wide range of temperatures (from 31 to 44 °C) and durations (from 30 to 180 min). We measured the photosynthetic activity and modelled its response to heat as a function of the thermal dose. We hypothesised that (1) the response of phototrophic biofilms is controlled by the thermal history of the community as the tolerance to heat stress increases with acclimation temperature and (2) the community response to heat stress is also driven by its diversity, which increases its ability to mitigate the heat stress effect. Last, we applied the thermal dose models to temperature fluctuations observed in the field to test and discuss the significance of the thermal dose approach for predicting the effects of heat stress events on river primary productivity.

2. Materials & methods

The experiments were conducted on six nonaxenic microalgal strains: two green algae, *Uronema confervicolum* and *Scenedesmus* sp., both isolated from rivers in southwestern France; two diatoms, *Gomphonema parvulum* (SAG-1032-1) and *Nitzschia palea* (CPCC-160); and two cyanobacteria, *Komvophoron* sp. (southwestern France) and *Nostoc* sp. (PCC 7120). All strains were cultivated under controlled conditions (20 °C, light/dark cycle 16 h/8 h, 30 µmol.m^{-2}.s^{-1}). Diatoms and green algae were grown in COMBO medium (Kilham et al., 1998), and cyanobacteria were grown in BG-11 medium (Stanier et al., 1971). All these strains are common benthic species, except S*cenedesmus* sp., a planktonic microalgae that is often found in river biofilms due to sedimentation (Lowe and Gale, 1980). *Scenedesmus* sp., *Gomphonema parvulum* and *Komvophoron* sp. were also acclimated at 15 °C or 25 °C for 5 months in the same light and medium conditions as for strains cultivated at 20 °C. To ensure the good physiological state of the culture, the medium was renewed 3 days before each experiment.

For Experiment 1, the cell density of each microalgal strain suspension was chosen so that the chlorophyll fluorescence signal was high enough to be easily detected by the PHYTO-PAM instrument (Phytoplankton Analyser, Heinz Walz GMBH, Effeltrich, Germany). The chosen concentrations depended on cell biovolume and pigment concentration and covered a range from 5.10^{6} cell.ml^{-1} to 8.10^{7} cell.ml^{-1}. For Experiment 2, synthetic microalgal communities were produced by mixing one strain of each main algal group, i.e., *Scenedesmus* sp., *Gomphonema parvulum* and *Komvophoron* sp. in equivalent biomass proportions. Three synthetic communities were produced with the same microalgae acclimated at either 15°C, 20°C or 25°C. The cell concentrations of strains in the mix were chosen to reach a total biovolume of approximately 1 10^{6} µm^{3}.cell^{-1} (Table S1).

Natural communities

Experiment 3 was performed on natural phototrophic biofilms. Biofilms were sampled four times throughout the year, in November 2019 (autumn), January 2020 (winter), July 2020 (midsummer) and September 2020 (endsummer), on a gravel bank in the river Garonne (43°23'47.5"N 1°17'58.9"E, southwestern France). Biofilms were scrapped from 15 hand-sized pebbles with a toothbrush directly on site and suspended in river water.

Returning to the lab, the 1-l suspension was homogenised in river water with an ultraturrax (T25, Janke-Kunkel) at 1000 g before filtration to 500 µm to remove coarse sediments and macrofauna. For each sampling date, the biofilm was diluted so that the initial F_0, a proxy of algal biomass measured with PHYTO-PAM after dark adaptation, was the same. The diluted suspension was kept overnight at 20 °C in the dark with bubbling before heat stress exposure.

To assess the thermal history of natural communities in the river, water temperature was measured every 5 minutes from November 2019 to September 2020 with one data recorder (TG-4100, TinyTag) positioned at the bottom of the water line. Additional biofilm samples were preserved in formaldehyde (4%) for microscopic analysis to characterise the microalgal composition. After a first observation under a microscope that was completed to identify soft bodied algae, sulfuric acid (98%) was added to eliminate organic matter and to allow for only diatom frustules to remain for diatom species identification. For each dominant species, the average length and width were retrieved from the literature (Table S2), and the theoretical biovolume was estimated using the appropriate geometric formula (Hillebrand et al. 1999). The relative abundance of each dominant species could then be calculated based on biovolume: only species that represented more than 1% in terms of biovolume were considered to calculate diversity indices. The taxonomic diversity dominating each sample was assessed using richness, Shannon diversity and Piélou equitability indices.

Heat stress exposure

Microalgal and biofilm suspensions were exposed to temperatures from 31 to 44 °C, with one-degree increments, for 30, 60, 120 or 180 min. The tested temperature ranges were adjusted for each strain based on preliminary range-finding experiments. A first set of preliminary experiments (data not shown) was performed in order to design an alternative approach to performing each experimental treatment (heat stress) at a similar point in the day/night cycle of our cultures. Thus, the following approach was used: heat stresses were applied between 8 am (2 hrs after the start of the usual light period) and 6 pm, and the temperature conditions were randomly distributed along the day of experiment (for instance stresses at 35, 38 and 41 degrees in the morning and stresses at 36, 37, 39 degrees in the afternoon). Moreover, prior to and during heat stress exposure, each biological material was kept under low light condition for 12 h to ensure circadian

rhythm does not influence the response of microalgae to temperature. Stress exposures were conducted in a water bath in 1.5-ml tubes with 200 µl of biofilm or microalgal suspension in each, in six replicates for natural biofilms (Experiment 3) and four replicates for laboratory cultures (Experiments 1 and 2) since variability between replicates was lesser with single strains and synthetic communities than natural biofilms. For each experiment, unstressed 1.5-ml tubes were used as controls (n= 4 for Experiments 1 and 2, n=6 for Experiment 3). At the end of each exposure, the tubes were placed in a chamber at 20 °C or at temperature acclimation for Experiment 2 under low light intensity (neon tubes, 5 µmol photons.m^{-2}.s^{-1}) for 24 h to recover. The recovery duration was based on Béchet et al. (2017); it allowed optimisation of the numerous measurements.

Photosynthetic activity

The photosynthetic activity was estimated 24 h after heat stress using the pulse amplitude modulated (PAM) fluorescence technique with a PHYTO-PAM instrument (Phytoplankton Analyser, Heinz Walz GMBH, Effeltrich, Germany) following a protocol described by Barthès et al. (2015). For the measurements, microalgal or biofilm suspensions were first transferred from the tubes into black 96-well plates and then dark-acclimated for 20 min. The maximum photochemical quantum yield of photosystem II (F_V/F_M, Kitajima and Butler, 1975) of each sample was estimated by placing the optic fibre above each well. The obtained values were expressed as the percent of the mean value of the corresponding controls.

Thermal dose

To analyse the response of photosynthetic activity for each combination of exposure duration and temperature, we adapted the concept of thermal dose that Béchet et al. (2017) developed to model the viability and photosynthetic activity of *Dunaliella salina* cultures in response to high temperatures (Fig. 1).

For each tested temperature, we first used the Weibull equation to model the response of the photosynthetic activity $Y(t)$ (% of the control) as a function of the exposure duration t (min) using Equation 1, where λ is the abscissa of the inflection point (min) and n is the shape factor (Fig. 1a):

$$Y(t) = Y(0)\, exp\left(-\left(\frac{t}{\lambda}\right)^{n}\right) \quad (1)$$

We assumed a normal distribution of model errors, i.e., discrepancies between the data and the theoretical model. Only temperatures that induce at least a 20% loss in photosynthetic activity were considered to ensure a reliable estimation of parameters; otherwise, the model was unable to estimate λ. Second, the inflexion points λ produced by Equation (1) were expressed as an exponential function of temperature T (°C) using Equation (2), where $T_{0,Y}$ is the temperature (°C) at which photosynthetic yield reaches zero, and a_Y is the exponential coefficient (°C^{-1}) (Fig. 1b):

$$log(\lambda) = a_Y(T - T_{0,Y}) \quad (2)$$

For each experiment and for each biological material, the obtained linear regression was tested through ANCOVA to test whether a_Y or $T_{0,Y}$ could be fixed among communities or strains depending on the experiment for parsimony. For Experiments 2 and 3, the a_Y was thus fixed, as coefficients were not significantly different among communities.

Based on both Equations (1) and (2), the thermal dose d_Y (min) was defined for each combination of temperature and time as follows:

$$d_Y = t\ exp\left(-a_Y\left(T - T_{0,Y}\right)\right) \quad (3)$$

Finally, the photosynthetic activity $Y(t)$ (% of the control) was represented as a function of the thermal dose d_Y(min) by fitting a Weibull model:

$$Y(t) = Y(0)\, exp(-(d_Y)^{n}) \quad (4)$$

For each experiment, as there was no relationship between n and temperature (Equation 1, Table S3), we used for Equation (4) the mean value of the estimations obtained using Equation (1). The obtained parameter λ' (min) is the inflexion point, and b (% min^{-1}) is the slope. In our study, the inflexion point λ' was used as a tolerance parameter, as it represents the thermal dose the algal sample was able to tolerate: the higher the lambda, the higher the thermal dose required to disrupt photosynthetic activity (Fig. 1c). The slope of curve b was used as a responsiveness parameter, as it represents the ability of algae to cope with stress: the higher the slope is, the quicker the response to heat stress is initiated (Fig. 1d).

Data analysis was conducted in R software (v 3.6.1) using the R package *{drc}.* For each parameter, 95% confidence intervals were calculated using the function "*confint.drc*" and were used to test differences in responses to heat stress between biological materials. If confidence intervals did not overlap, differences were considered significant.

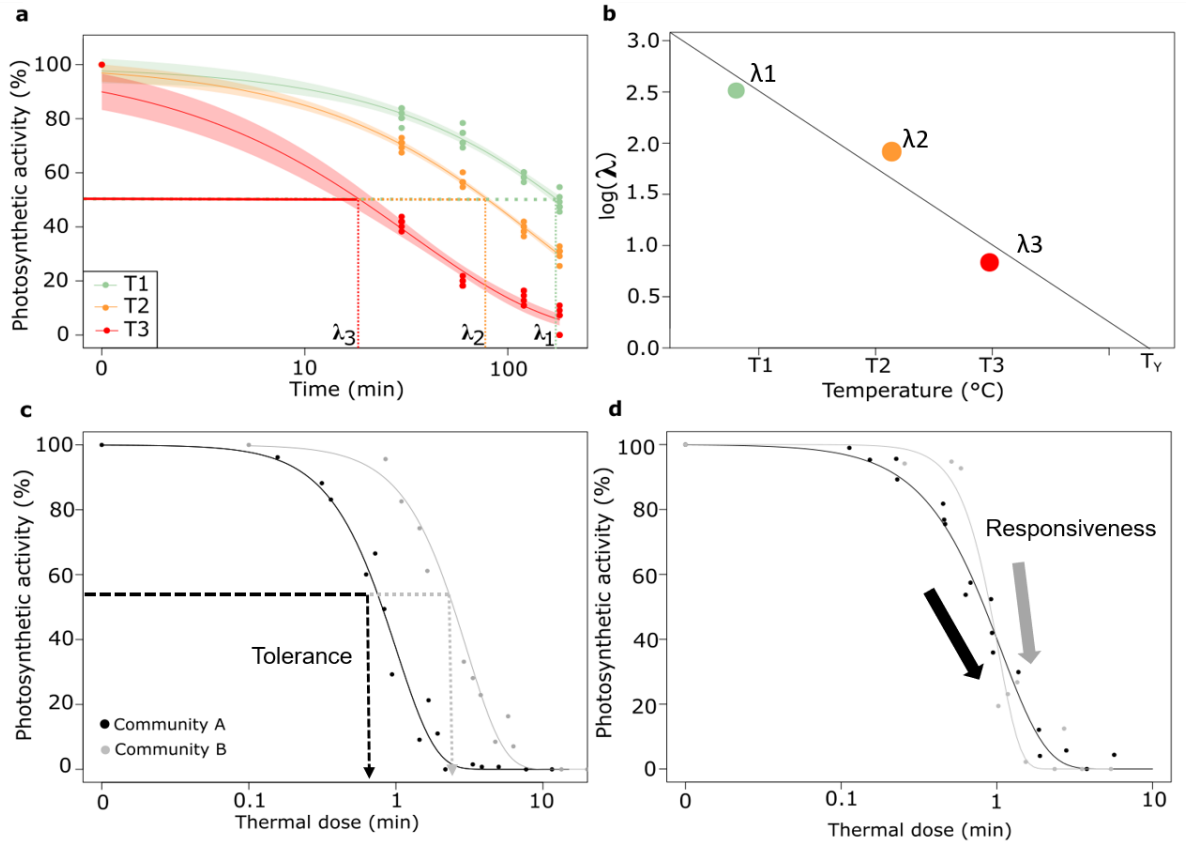

Figure 1: Thermal dose approach adapted from (Béchet et al., 2017). (a) The inflexion point λ was estimated for each tested temperature using the Weibull relationship between the photosynthetic activity and the exposure duration according to Equation (1). (b) The linear regression between log(λ) and temperature allowed us to estimate the exponential coefficient $\boldsymbol{a_Y}$ and the estimated temperature at which the photosynthetic activity reaches zero $T_{0,Y}$ using Equation (2). Both parameters were required for the thermal dose calculation using Equation (3). In plots (c) and (d), the photosynthetic activity was represented as a function of the thermal dose using Equation (4). Curve parameters represent tolerance (inflexion point, λ') and responsiveness *(*slope*, b)* to thermal dose. In (c), communities have the same responsiveness to heat stress, but community A is more tolerant than community B, while in (d), both community tolerances are similar, but community A is less responsive than community B.

Application to field conditions

To test the significance of the thermal dose approach for predicting the effects of heat stress events on *in situ* primary productivity, it was necessary to establish the link between photosynthetic activity and river phototrophic biofilm primary productivity. We performed an additional experiment on a natural phototrophic biofilm to establish the relationship between the loss of photosynthetic activity and the loss of gross primary productivity due to heat.

The biofilm was collected in September 2020, and the suspension was prepared as described before. In this experiment, we assumed that the relationship between productivity and photosynthetic activity does not depend on exposure duration. One 30-min heat stress was applied to phototrophic biofilm suspensions in triplicate for twelve temperatures ranging from 32 °C to 44 °C. For each of the three replicates, one 15-ml tube was filled with 7.2 ml of biofilm suspension and placed in a water bath at an adequate temperature. After 30 min, the tube was removed and placed in a water bath at 20 °C. Unstressed controls were prepared the same way and maintained at 20 °C in triplicate. After homogenisation, 200 µl of each tube was immediately transferred into a black 96-well plate and kept for 20 min in the dark before measuring photosynthetic activity with the PHYTO-PAM instrument. The remaining volume of biofilm suspension was transferred into an 8-ml transparent vial closed with a gas tight lid for the assessment of oxygen production with FireSting oxygen sensors (PyroScience, Germany). Measurements were conducted under low magnetic agitation. Concentrations of dissolved oxygen were monitored every 5 s for 5 min under light (neon lamp, 90 µmol photons.m^{-2}.s^{-1}) to measure the net primary productivity (NPP), followed by 5 more min in the dark to measure oxygen consumption (respiration). The gross primary productivity (GPP) was calculated as the sum of NPP and respiration. Losses of photosynthetic activity and GPP were expressed as the percent of the mean values of the corresponding controls. The relationship between the loss of photosynthetic activity and the loss of GPP was then described through regression of a logarithmic equation.

Then, we used the thermal doses previously calculated on midsummer and endsummer biofilms for every combination of exposure duration and temperature to evaluate the potential impact of the summer temperatures measured in the field in a disconnected branch of the river from mid-July to August 2020. The effective thermal doses inducing 10%, 25%, 50%, 75% and 90% photosynthetic activity loss were estimated using the

function *"ED"* of the package *{drc}*. The thermal dose values were then projected on a heatmap using effective thermal doses as thresholds to visualise the potential loss of photosynthetic activity for different combinations of duration and temperature. This heatmap scale was transformed to visualise the potential loss of GPP using the relationship between photosynthetic activity and GPP previously established. The high summer temperature events measured in the field were positioned on the heatmaps considering the heat stress events independently.

3. Results

Microalgal strains

Green algae had the highest photosynthetic activity, with a mean (± sd) F_V/F_M in controls equal to 0.75 (± 0.01) and 0.70 (± 0.01) for *Scenedesmus* sp. and *U. conferviculum*, respectively. For diatom controls, it was equal to 0.68 (± 0.05) for *G. parvulum* and to 0.70 (± 0.01) for *N. palea.* Cyanobacteria had the lowest photosynthetic activity, with values of 0.33 (± 0.02) for *Komvophoron* sp. and 0.32 (± 0.01) for *Nostoc* sp. The threshold temperature inducing a negative response of photosynthetic activity was variable between species. Most of the strains were impacted by heat stress at a temperature equal or above 39 °C (Fig. S1), while for *Scenedesmus* sp. the threshold temperature was 41 °C. *Nostoc* sp. was impacted by all tested temperatures, but F_V/F_M never reached 0, while 1 h of exposure at 41 °C was enough to fully inhibit photosynthetic activity in *Komvophoron* sp.

The effects of exposure duration and temperature on photosynthetic activity were synthesised using the thermal dose approach (Fig. 2a). The tolerance of microalgal strains to heat stress did not significantly differ between algal groups or species (Fig. 2b). However, diatom tolerance appeared to be more variable than for other strains, as shown by the relatively high 95% confidence interval in *G. parvulum* and *N. palea* in comparison to other species. In contrast to tolerance, responsiveness to heat stress was strain dependent (Fig. 2c). *Nostoc* sp. was less responsive than other species, including the other cyanobacteria species *Komvophoron* sp., while diatoms, and notably *Gomphonema* sp. were more responsive than cyanobacteria and green algae.

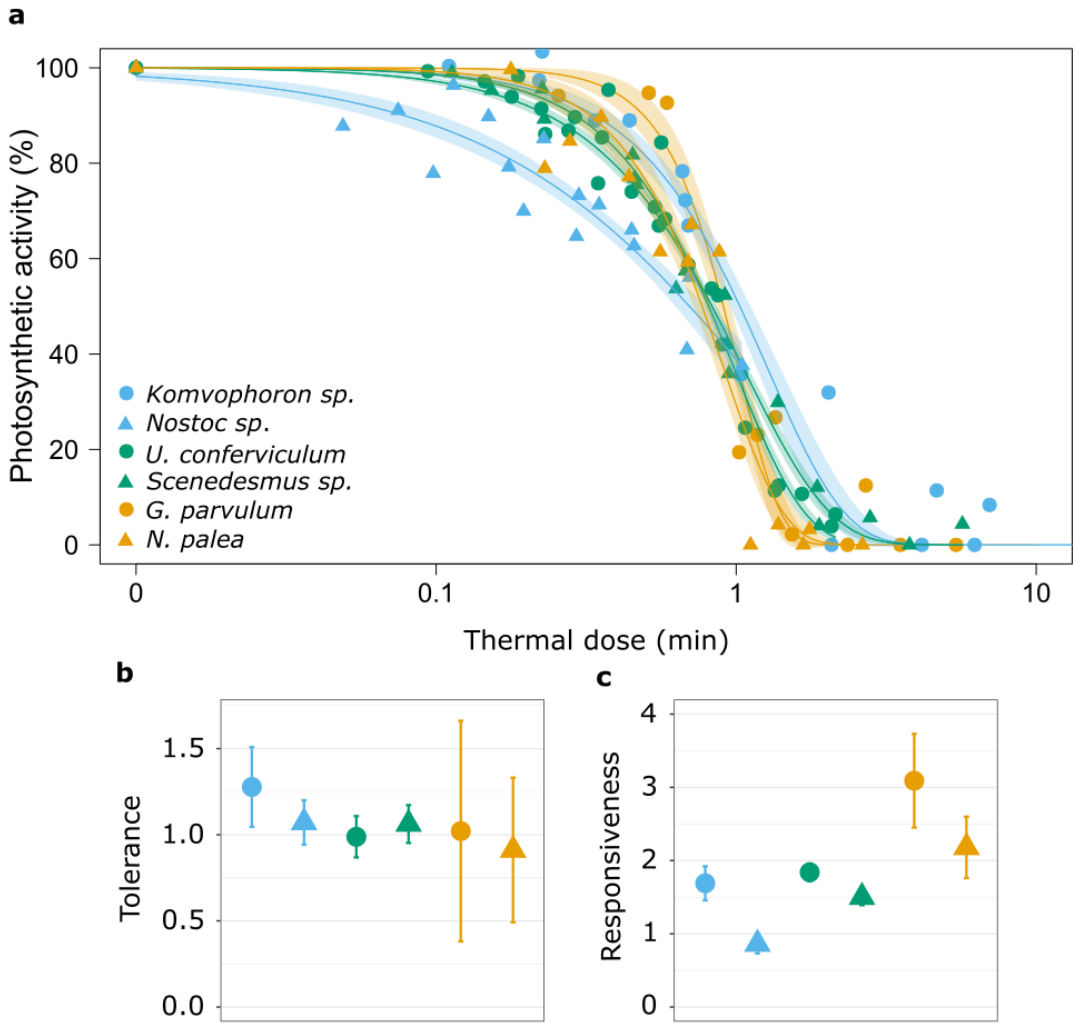

Figure 2: (a) Photosynthetic activity response (% control) of microalgal strains (cyanobacteria, green algae and diatoms) to thermal dose (eq. min), (b) tolerance (λ', inflexion point) to heat stress, and (c) responsiveness (*b*, slope) to heat stress for microalgal strains acclimated at 20 °C for cyanobacteria (blue), green algae (green) and diatoms (brown). Model specific confidence intervals and estimated parameters ± 95% confidence intervals are represented for each microalgal strain (n=4).

Synthetic communities

The mean F_V/F_M (± sd) of the control synthetic communities was 0.63 (± 0.01), 0.66 (± 0.01) and 0.55 (± 0.01) for communities acclimated at 15 °C, 20 °C and 25 °C, respectively. The tested temperatures above 41 °C had a significant effect (Fig. S2). The thermal dose approach showed that tolerance to heat stress increased with the acclimation temperature (Fig. 3a, 3b): the synthetic community was three times more tolerant to heat stress when composed of microalgal strains acclimated at 25 °C than with microalgae acclimated at 20 °C. The synthetic community obtained by mixing microalgae acclimated at 15 °C was less tolerant than that obtained by mixing microalgae acclimated at 20 °C. In contrast to tolerance, the responsiveness of synthetic communities to heat stress did not depend on the acclimation temperatures (Fig. 3c).

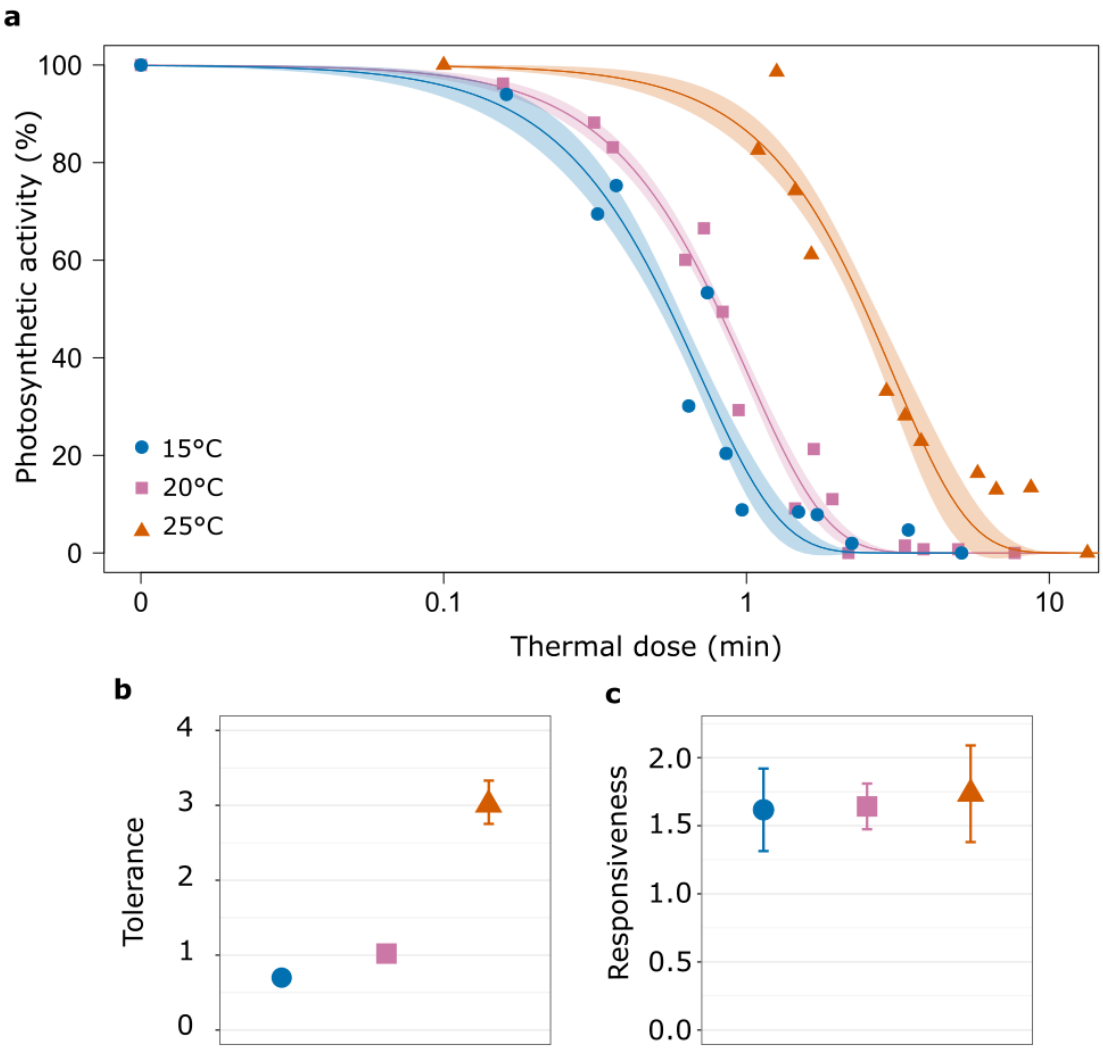

Figure 3: (a) Photosynthetic activity response (% control) of synthetic communities to thermal dose (eq. min) depending on acclimation temperature, (b) tolerance (λ', inflexion point) to heat stress, and (c) responsiveness (b, slope) to heat stress for synthetic communities acclimated at 15 °C (blue), 20 °C (pink) and 25 °C (red). Model specific confidence intervals and estimated parameters ± 95% confidence intervals are represented for each synthetic community (n=4).

Natural communities

Unstressed samples had a mean (± sd) F_V/F_M of 0.55 (± 0.02) in autumn, 0.62 (± 0.02) in winter, 0.37 (± 0.02) in midsummer and 0.52 (± 0.03) in the endsummer. All tested temperatures had a negative effect on the photosynthetic activity of phototrophic biofilms. The response of biofilms was gradual (Fig. S3). Thermal dose analysis highlighted differences in tolerance and responsiveness to heat stress between communities (Fig. 4). Winter biofilms were the least tolerant to heat stress, endsummer biofilms were the most tolerant, and midsummer and autumn biofilms had the same intermediate value of tolerance (Fig. 4b). The endsummer community was less responsive to heat stress than the autumn and winter communities (Fig. 4c).

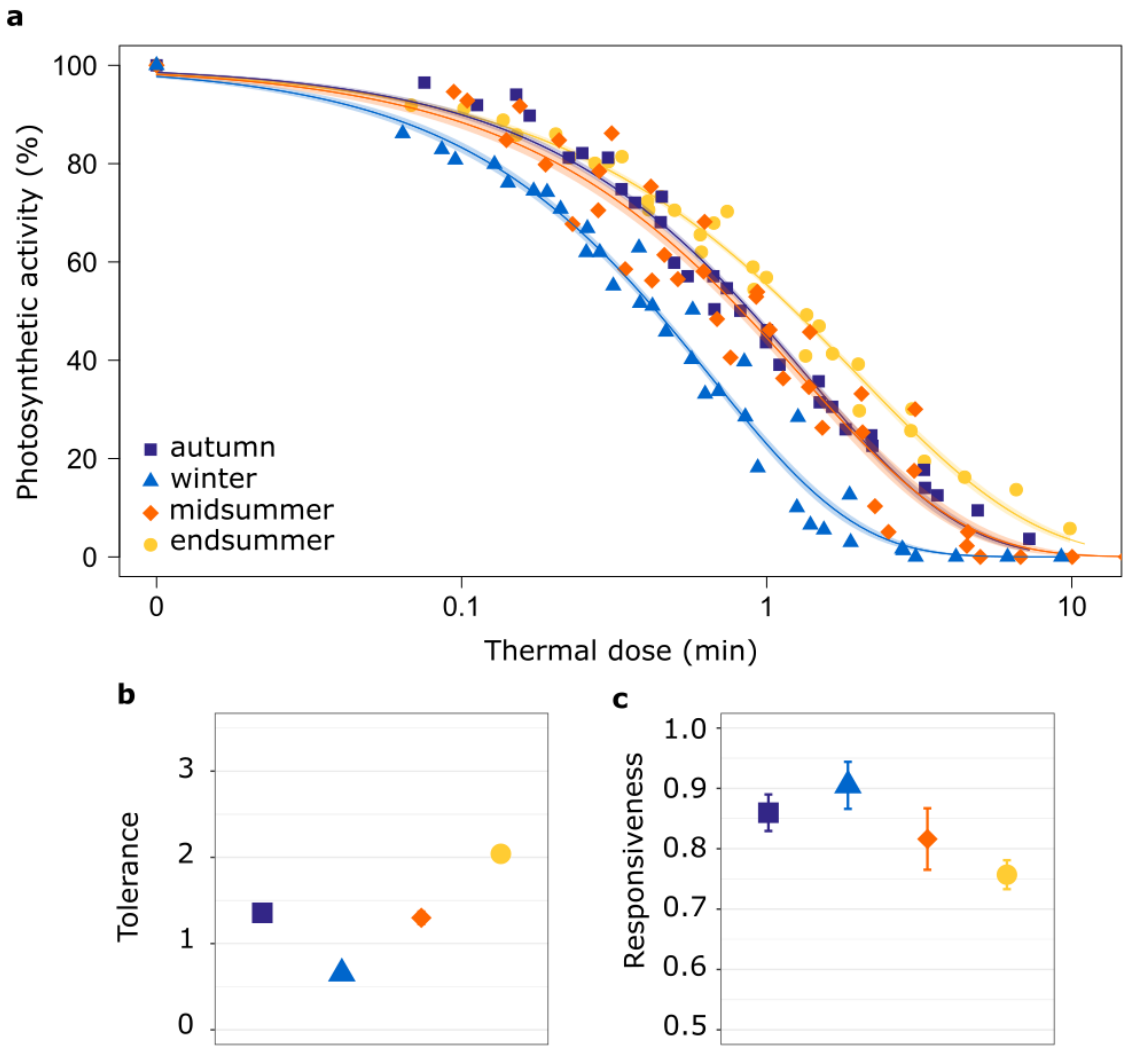

Figure 4: (a) Photosynthetic activity response (% control) of natural biofilms to thermal dose (eq. min) depending on the sampling period, (b) tolerance (λ', inflexion point) to heat stress, and c) responsiveness (b, slope) to heat stress for natural communities in autumn (purple), winter (blue), midsummer (orange) and endsummer (yellow). Model specific confidence intervals and estimated parameters ± 95% confidence intervals are represented for each natural community (n = 6).

The temperatures recorded in the river 27 d before sampling are reported in Table 1a and Figure S4. For autumn and winter, the mean temperature and amplitude were relatively low compared to the two other communities, while for midsummer and endsummer, the temperatures experienced were higher but also more variable, as shown by the larger

thermic amplitude. The midsummer algal community had the lowest species richness, diversity and equitability indices (Table 1b). For autumn biofilms, large (biovolume > 500 μm^3) and very large (> 1000 μm^3) species accounted for 80% of the total biovolume. For winter and endsummer biofilms, very large species represented more than 60% of the total biovolume. In contrast, in the midsummer biofilm, the species contributing more to the total biovolume were small (biovolume <200 μm^3) (Fig. S5). It was dominated at 80% of the total biovolume by the cyanobacteria *Leptolyngbya* sp. (Fig. S6). The other biofilms were mainly composed of diatoms. In winter and endsummer biofilms, the high-sized diatom *Diatoma vulgaris* was the dominant species in biovolume (60% and 40% total biovolume, respectively). In the autumn biofilm, *D. vulgaris* and *Nitzschia* species dominated (30% of the biovolume contribution each) (Fig. S6). In addition, autumn and endsummer biofilms were more diversified, and species were more evenly distributed (Table 1b).

Table 1: (a) *In situ* thermal history in natural biofilms. Mean temperature represents the mean water temperature experienced during the 27 days before biofilm sampling, values in brackets are the mean minimal and the maximal temperatures measured during this period. Mean (min and max) daily temperature amplitudes represent the mean (min and max) of water temperature daily amplitudes over the 27-day period and (b) specific diversity indices of the algal community.

(a)	autumn	winter	midsummer	endsummer
Mean temperature	11.5	7.5	19.7	21.6
(min – max) (°C)	(11.0-12.2)	(7.0-8.4)	(18.2-21.6)	(19.0-24.8)
Mean daily temperature	1.2	1.4	3.4	5.8
amplitude (min – max) (°C)	(0.7 – 3.4)	(0.4 – 4.5)	(0.8 – 8.1)	(2.6 - 9.7)
(b)				
Species richness	15	14	9	17
Diversity (Shannon)	2.21	1.61	0.78	2.12
Equitability (Piélou)	0.82	0.61	0.36	0.75

Comparison between experiments

Communities acclimated at higher temperatures (i.e., synthetic community acclimated at 25 °C and endsummer phototrophic biofilm) were more tolerant than other tested biological materials (Fig. 5a). On the other hand, the responsiveness was more variable and hardly predictable within strains, as it seems to depend on the observed species. Generally, natural communities were less responsive than microalgal strains and synthetic communities (Fig. 5b).

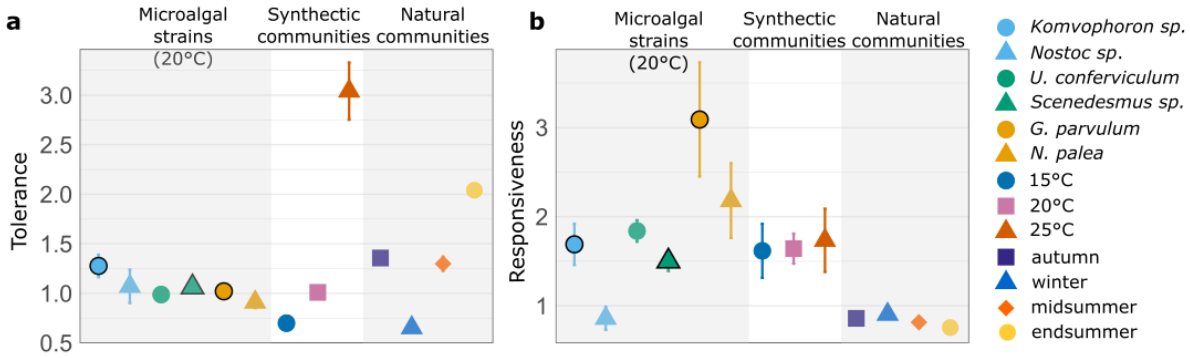

Figure 5: (a) Tolerance (λ', inflexion point) and (b) responsiveness (*b*, slope) to heat stress of microalgal strains (n = 4), synthetic communities (n = 4) and natural communities (n = 6). Each point represents the mean ± 95% confidence interval of parameters for different tested samples in each experiment. Strains used in synthetic communities are represented with black outlines.

Application to field conditions

The last objective of the study was to compare the models of biofilm physiological response to heat stress to *in situ* conditions to provide insights into the potential impact of a summer heat wave in the natural environment. To do this comparison, we performed a new heat stress experiment to relate photosynthetic activity and GPP (Fig. 6). In this experiment, unstressed biofilms had a mean F_V/F_M (± sd) of 0.54 (± 0.01) and a mean GPP (± sd) of 0.015 (± 0.001) mg $O_2.h^{-1}$. After exposure at 44 °C for 30 min, F_V/F_M decreased to 0.11, and GPP decreased to 0 mg $O_2.h^{-1}$. The loss of GPP due to heat stress logarithmically increased with the loss of photosynthetic activity (ANOVA, r^2= 0.955, p value < 0.001, Fig. 6). From 35 to 39 °C, GPP was 3 times more affected by heat stress (loss from 0 to 60%)

than the loss of photosynthetic activity (loss from 0 to 20%). In contrast, from 40 to 44 °C, the GPP loss decreased, while photosynthetic activity was strongly reduced (from 30 to 80% loss).

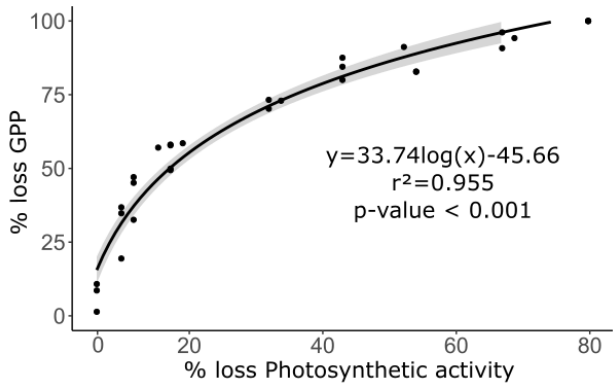

Figure 6: Relationship between the loss of photosynthetic activity (%, F_V/F_M) and the loss of gross primary productivity (%, GPP) of natural biofilms after exposure to one 30-min heat stress at temperatures from 35 to 44 °C.

Then, we used the *in situ* monitoring water temperatures to identify extreme events during which primary productivity can be impacted. These events were then projected on heatmaps constructed for the midsummer and endsummer biofilms (Fig. 7a). For that projection, thermal doses were calculated for combinations of exposure duration and temperatures and traduced into levels of effects from previous results of GPP loss (Fig. 6). The effects of heat stress depended on the biofilm sampling period and on the level of effects measured (i.e., GPP) (Fig. 7a): endsummer biofilms could be affected by heat stress from 33 °C, while exposures of midsummer biofilms at 31 and 32 °C for more than 1 hour could induce losses of GPP.

Finally, we used heatmaps to estimate the effect of temperatures measured *in situ* on natural communities. The water temperature exceeded 30 °C in the afternoon several days in summer (Fig. 7b), especially between the 8th and 12th of August, with events higher than 31 °C for 180 min. Temperature exhibited extreme values on the 6th and 7th of August, reaching 41 °C during more than 2 hours on the 7th. We positioned three heat stress events (i.e., A, B and C) on heatmaps as examples to estimate their potential effect on natural communities in the field. Event A, which corresponded to exposure at 31 °C for 60 min, only impacted the midsummer community by inducing a 30% loss of GPP. Event C

corresponding to exposure at the same temperature for 120 min would induce between 30 and 70% loss of GPP. In contrast, the endsummer community would not be impacted by events A and C, as the water temperature would remain below the critical threshold of 33 °C. For both midsummer and endsummer communities, event B would have induced a complete inhibition of GPP.

a

	midsummer				endsummer			
	30 min	60 min	120 min	180 min	30 min	60 min	120 min	180 min
31°C		0.094 **(A)**	0.188 **(C)**	0.283		**(A)**	**(C)**	
32°C		0.141	0.280	0.421				
33°C	0.104	0.209	0.417	0.626	0.068	0.137	0.274	0.410
34°C	0.155	0.310	0.621	0.931	0.102	0.204	0.407	0.611
35°C	0.231	0.462	0.924	1.386	0.151	0.303	0.606	0.909
36°C	0.344	0.687	1.375	2.062	0.225	0.451	0.901	1.352
37°C	0.511	1.023	2.045	3.068	0.335	0.671	1.341	2.012
38°C	0.761	1.522	3.043	4.565	0.499	0.998	1.996	2.993
39°C	1.132	2.264	4.529	6.793	0.742	1.485	2.969	4.454
40°C	1.685	3.369	9.739	10.108	1.105	2.209	4.418	6.628
41°C	2.507	5.014	10.027 **(B)**	15.041	1.644	3.287	6.575 **(B)**	9.862

% Loss GPP
<30
30-70
70-80
80-95
>95
x

b

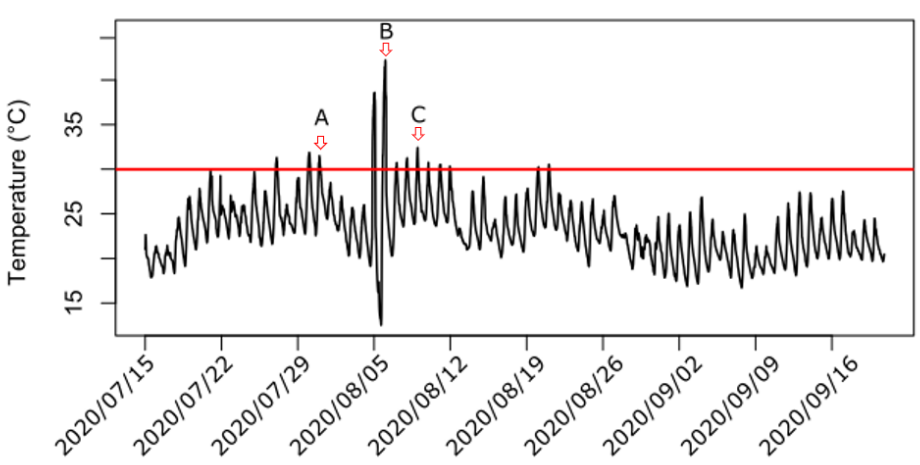

Figure 7: a) Heatmaps for midsummer and endsummer biofilms transformed into levels of effects depending on the threshold calculated from photosynthetic activity and primary production losses. Values inside boxes are thermal doses calculated from models (in min). Empty boxes indicate that heat stress has no effect. b) Measured temperature in the field from mid-July to August 2020. The red line corresponds to a threshold of 30 °C, and A, B and C correspond to the chosen peaks, with: 60 min at 31°C, B: 120 min at 41°C and C: 120 min at 31°C. The data logger was positioned in a branch of the Garonne River, temporally disconnected from the main stream during the low water period.

4. Discussion

We used the thermal dose approach to investigate the relative contribution of thermal history and diversity to the response of benthic freshwater microalgae to extreme heat stress. The thermal dose approach allows us to clearly and easily compare the response of photosynthetic activity between experiments thanks to the two metrics calculated using the Weibull model. We compared Weibull with logistic model, and selected the Weibull model because it fitted our data the best (lowest AIC). In addition, it is commonly used for dose–response representations in ecotoxicological studies, as it was found to improve the fit compared to other models (Forfait-Dubuc et al., 2012; Ritz, 2010). It has been widely used in food research for modelling microbial heat inactivation as a nonlinear response (Smelt and Brul, 2014; van Boekel, 2002). The thermal dose we used in our study enables a full and more refined examination of community responses compared to a simple threshold analysis of ED50.

Effect of thermal history

The temperature at which microalgal strains grow controlled the tolerance of their photosynthetic activity to heat stress, confirming our first hypothesis. Indeed, all our microalgal strains, which were cultivated in the same lab conditions for one decade prior, showed the same tolerance to heat stress, while the synthetic communities acclimated at different temperatures exhibited significant differences in tolerance. The higher the acclimation temperature is, the better the synthetic microalgal communities tolerate high heat doses. Such increased tolerance has been observed in marine copepods: a 4 °C increase during development enables better thermal tolerance (Sasaki and Dam, 2019). Similarly, phytoplankton communities acclimated to thermal conditions above their optimum exhibited a better tolerance to heat stress (Baker and Geider, 2021). In our study, the relationship between acclimation temperature and tolerance of photosynthetic activity of synthetic microalgal communities was nonlinear, with an increase in tolerance four times higher when acclimation temperature increased from 20 °C to 25 °C than from 15 °C to 20 °C. For two species belonging to the genera *Scenedesmus* and *Gomphonema*, 25 °C was the optimal growth temperature (Guedes et al., 2011; Rose, 2008), and it is likely that species grown near their optimal temperature could exhibit a better tolerance to heat stress. Such an increase in tolerance might be due to changes in cell physiology owing to

growth temperature. For instance, it has been shown that marine diatoms grown at 35 °C accumulate more saturated and monounsaturated fatty acids at the cost of polyunsaturated fatty acids than diatoms grown at lower temperatures (Scholz and Liebezeit, 2013). A similar phenomenon was observed in natural communities, with an increase in the concentration of polyunsaturated fatty acids in cooler autumn (Whorley and Wehr, 2018). The decrease in unsaturated fatty acids has been considered responsible for the high-temperature tolerance of photosynthesis in a mutant of *Chlamydomonas reinhardtii* (Sato et al., 1996). Fatty acid profiles seem to be important to tolerate heat stress since a thermotolerant diatom, *Chaetoceros muelleri*, displayed greater changes in fatty acid composition than *Phaedoctylum tricornutum*, which is thermosensitive (Rousch et al., 2003). Such biochemical changes could explain the observed increased tolerance to heat stress in the experiment where synthetic communities were grown at 25 °C.

The comparison between the results observed for natural communities sampled in winter and sampled in summer also tends to highlight the effect of thermal history on biofilm tolerance to heat stress. In the natural environment, thermal conditions combined with other factors, such as irradiance, can induce the selection of more thermophilic species. For instance, some species from the genera *Achnantidium*, *Cocconeis* and *Nitzschia* are able to survive at very high temperatures (Delgado et al., 2020) and were present in summer and autumn but not in winter in our samples (see Fig. S6). Another explanation for the better tolerance of summer biofilms to heat stress could be the high amplitude in temperature experienced by the biofilms before the experiment (see Fig. S4). It has been shown that regular temperature fluctuations can have positive effects, such as increased growth rates (Gill et al., 2022). Similarly, in another experiment conducted on the marine diatom species *Thalassiosira pseudonana*, it was shown that growth in a fluctuating environment enhanced the tolerance to heat stress through increased genetic divergence over generations (approximately 1 year) (Schaum et al., 2018). In addition, an experiment conducted on two seagrass species pointed out that previous exposure to thermal stress enhanced the ability to cope with heat stress compared to unstressed plants, notably through the reduced expression of genes related to stress (Nguyen et al., 2020).

Effect of microalgal diversity

In addition to thermal history, microalgal assemblage diversity is likely an important factor for explaining the heat stress tolerance of freshwater biofilms, as biofilms sampled in midsummer and at the end of summer displayed slight differences in their tolerance while experiencing very similar thermal conditions. Microalgal species richness and diversity might explain such differences in community response to heat stress. The midsummer biofilm was less diversified than the endsummer biofilm, which exhibited the highest microalgal richness and diversity. In addition, endsummer biofilm showed the lowest evenness. When communities are dominated by a single or low number of species, their tolerance/resistance to environmental stress is reduced, as has been shown in denitrifying bacterial communities exposed to salinity stress (Wittebolle et al., 2009), while a higher diversity induces better resistance to environmental stresses, as shown in a meta-analysis conducted on microbial communities and plants (Hong et al., 2022). While tolerance to heat stress did not significantly differ between the studied microalgal strains, the responsiveness of photosynthesis varied between microalgal species. Differences in responsiveness to heat stress were overall significant when comparing responses of laboratory assemblages with natural ones. Whatever the sampling period, complex microalgal communities were less responsive to increasing heat stress than microalgal strains and synthetic communities, validating our second hypothesis. The reduced variability in responsiveness for natural communities supports the insurance hypothesis. In a study where the diversity level of ciliates and bacteria was manipulated, the authors showed that differences in species response to environmental changes helped stabilise the effects of temperature fluctuations (Leary and Petchey, 2009). To explain the link between diversity and stability, Loreau and de Mazancourt (2013) notably argued that the response of species to stressors is asynchronous and that species do not respond at the same speed to stress. Such mechanisms might explain the observed difference in responsiveness between synthetic and natural communities and the buffering effect of diversity observed in our study (Fig. 5b). This is consistent with another study carried out on phytoplankton communities exposed to heat stress (+4 °C for 7 h per day), which demonstrated that natural communities were better adapted to the disturbance than artificial communities (Striebel et al., 2016). In an experiment carried out on phytoplankton where the species richness was manipulated and exposed to different heat stress (+4 °C), the authors highlighted the importance of complementarity between

species to maintain growth and phosphorus content (Schabhüttl et al., 2013). Similar results have been observed in other microbial communities living in soil ecosystems: they showed enhanced stability after exposure to heat stress due to increased diversity (Tardy et al., 2014). These results support the importance of diversity in communities to reduce the responsiveness of photosynthesis to heat stress.

Field application of thermal dose

The objective of this study was to apply the thermal dose model to data collected in the field to predict the response of phototrophic biofilms *in situ.* On some days in summer, the temperature measured in the river exceeded 30 °C for several hours (Fig. 7b). Despite the relatively low number of such critical days, our analysis highlighted the fact that a short extreme event, for instance, one-hour exposure at 35 °C, could decrease the photosynthetic activity by 30% 24 hours after the stress. The effects of such short extreme events on benthic primary productivity were all more significant, as the relationship between photosynthetic activity and gross primary productivity was nonlinear (Fig. 6). A 30% loss in photosynthetic activity would correspond to at least a 70% loss of gross primary productivity. In addition, the response of biofilm depended on the community composition, with the midsummer biofilm being less tolerant to heat stress than the endsummer biofilm. Contrary to the photosynthetic activity that can be maintained at a quite high level thanks to a few more resistant species, GPP depends on biofilm biomass, with lower biomass implying lower productivity and therefore a stronger decrease due to heat stress.

The application of the thermal dose approach to natural communities and heatmap construction provided interesting information regarding the potential impact of extreme heat temperature events in the field. Some additional experiments should be conducted to decipher the potential additive properties of thermal doses. In the field, the temperature increase occurs more gradually than what was experimentally tested, generating a succession of several heat stresses, such as those applied in this study (e.g., 1 h at 31 °C + 2 h at 32 °C + 1 h at 33 °C). When facing a succession of such events during summer heat waves, phototrophic biofilms may acclimate and ultimately maintain a certain level of primary productivity. Several studies have pointed out the enhanced stability of microbial communities after repeated disturbances, such as heat stress (Renes

et al., 2020; Samuels et al., 2021) or acidification stress (van Moorsel et al., 2021). However, other studies have underscored opposite results, with a decreasing stability of communities because of stress repetition (e.g., Jurburg et al., 2017). Prior exposure to high temperatures can increase the sensitivity of organisms to heat stress. For instance, in a study conducted on marine diatoms grown at different temperatures and exposed to different heat wave intensities, it was shown that when grown at suboptimal temperatures, the survival of algae decreased after a heatwave (Samuels et al., 2021). Similarly, the thermal tolerance and survival of copepods was reduced when organisms were previously exposed to prior sublethal temperatures (Siegle et al., 2018). The response of aquatic microbial communities to repeated heat stress could depend on the intensity, frequency and duration between repetitions (Dal Bello et al., 2019) but also on the predictability of temperature fluctuations (Gill et al., 2022).

Conclusion

Our work provides clear insight into the effects of short heat stress on the tolerance and responsiveness of microalgae at various levels of diversity. We found that the tolerance of communities to stress depended on thermal history or temperature acclimation, while the responsiveness of the photosynthetic activity of the whole community rested on its diversity because natural communities were less responsive than synthetic communities. In addition, we found that a slight decline in photosynthetic activity could have a major impact on oxygen productivity and that during low flow periods in intermittent rivers or in shallow waters, when the thermal inertia is low, primary productivity can be strongly reduced. This may threaten the whole ecosystem as trophic chains would be impacted. The thermal dose approach appears to be a relevant tool to understand and compare the effect of heat stress and could be useful to predict the impact of a summer heat wave in the field, even if it needs further investigation.

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Conflict of interest

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

Authors' contribution

M. Laviale, J. Leflaive and S. Boulêtreau conceived the ideas. The methodology was designed with the help of C. Courcoul. C. Courcoul, J. Leflaive and S. Boulêtreau proceeded the laboratory work. C. Courcoul analysed the data with the help of V. Baillard, E. Billoir and M. Laviale. C. Courcoul led the writing with the help of J. Leflaive and S. Boulêtreau. All authors contributed to the drafts and gave final approval for publication.

Data availability statement

The data are available from Figshare doi: 10.6084/m9.figshare.20485311. (Courcoul, 2022)

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Supplementary information

Table S1: List of the used isolated strains and their related information

	Species	Strain	Origin	Cultivation medium	Cell concentration (cell.ml^{-1})	Biovolume in synthetic com ($\mu m^3.ml^{-1}$)
Green algae	*Uronema conferviculum*	uro co 01 t	SW rivers (France)	COMBO (Kilham et al., 1998)	*	
	Scenedesmus sp.	sce sp1 01 c	SW rivers (France)	COMBO	1.4 10^7	1.33e^9
Diatoms	*Gomphonema parvulum*	SAG 1032-1	USA, Connecticut	COMBO	3.9 10^6	1.02e^9
	Nitzschia palea	CPCC-160	Cedar Creek, Piscataway, NJ, USA	COMBO	5.0 10^6	
Cyanobacteria	*Komvophoron sp.*	kom sp3 01 g	SW rivers France	BG-11 (Stanier et al., 1971)	8.0 10^7	1.11e^9
	Nostoc sp.	PCC 7120	Source unknown	BG-11	*	

*cell concentration based on F0 measured with PhytoPAM. The F0 was comprised between 150 and 200 at a gain of 23 for *U. conferviculum,* and 19 for *Nostoc sp*.
SAG: Sammlung von Algenkulturen Gottingen, Germany
CPCC: Canadian Phycological Culture Centre
PCC: Pasteur Culture Collection

Table S2: Theoretical biovolume of dominant species described in natural communities. Mean values of length and width were used to calculate the theoretical biovolume.

Taxa	Length (µm)	Width (µm)	Shape (from Hillebrand et al., 1999)	Theoretical biovolume (µm3)	Reference (for length and width values)
Achnanthidium delmontii	7.3-21.4	3.3-5.1	Elliptic prism	70	Pérès et al., 2012
Achnanthidium druartii	14-25	4-5.5	Elliptic prism	70	Rimet et al., 2010
Ceratoneis arcus	15-150	6.0-8.0		720	Germain, 1981
Cocconeis euglypta	28	16	Elliptic prism	1480	Romero and Jahn, 2013
Cocconeis pediculus	17-41.5	14-23	Elliptic prism	740	Jahn et al., 2009
Cymbella excisa	24-34	6.1-7.1	Cymbelloid	400	Krammer, 2002
Diatoma erhenbergii	30-120	4.5-9	Elliptic prism	7200	Patrick and Reimer, 1966
Diatoma vulgaris	15-60	8.0-12.0	Elliptic prism	3600	Williams 1985
Encyonema silesiacum	19.6-48.7	6.0-11	Cymbelloid	800	Patrick and Reimer, 1975
Encyonema ventricosum	9.0-21.0	4.5-5.9	Cymbelloid	210	Krammer, 1997
Gomphonema olivaceum	13-40	6.0-9.0	Gomphonemoid	630	Germain, 1981
Melosira varians	4 - 14	8 - 35	Cylinder	3700	Crawford, 1978
Navicula capitatoradiata	35-40	9.0-10.0	Elliptic prism	520	Germain, 1981
Navicula cryptonella	14 - 40	5 - 7	Elliptic prism	195	Lange-Bertalot, 1993
Nitzschia dissipata	12.5 - 72	3.0- 8	Prism	625	Laviale (personal communication)
Nitzschia tabellaria	9.0-21.0	4.5-8.0	Prism	150	Cleve and Grunow, 1880
Ulnaria ulna	50-250	2.0-9.0	Elliptic prism	1200	Lange-Bertalot, 1980
Leptolyngbya sp.	0.5-3.2		Cylinder	16	Anagnostidis and Komárek, 1988

Anagnostidis, K., Komárek, J., 1988. Modern approach to the classification system of cyanophytes. 3 - Oscillatoriales. Algological Studies/Archiv für Hydrobiologie, Supplement Volumes 327–472.
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Jahn, R., Kusber, W.-H., Romero, O.E., 2009. Cocconeis pediculus EHRENBERG and C. placentula EHRENBERG var. placentula (Bacillariophyta): Typification and taxonomy. Fottea 9, 275–288. https://doi.org/10.5507/fot.2009.027
Patrick, R., Reimer, C.W., 1975. The diatoms of the United States. Vol. 2 Part 1. Acad. Nat. Sci. Phil. Monograph 13.
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Table S3: Parameters estimated with thermal dose model

Experiment	Biological material	a_Y	$T_{0,Y}$	*n* (± sd)
1: Isolated strains	*Komvophoron* sp.	-1.121	44.30	1.43 (± 0.78)
	Nostoc sp.	-0.424	55.13	1.22 (± 0.49)
	U. conferviculum	-0.436	54.24	2.04 (± 0,72)
	Scenedesumus sp.	-0.708	48.88	1.67 (± 0.55)
	G. parvulum	-0.833	44.72	4.36 (± 3.63)
	N. palea	-0.453	50.24	2.77 (± 0.68)
2: Synthetic communities	15°C	-0.85	47.26	1.35 (± 0.67)
	20°C		47.29	1.78 (± 0.76)
	25°C		46.63	1.80 (± 1.02)
3: Natural communities	autumn	-0.397	47.86	0.87 (± 0.23)
	winter		48.27	0.96 (± 0.28)
	midsummer		47.25	0.81 (± 0.31)
	endsummer		48.31	0.87 (± 0.17)

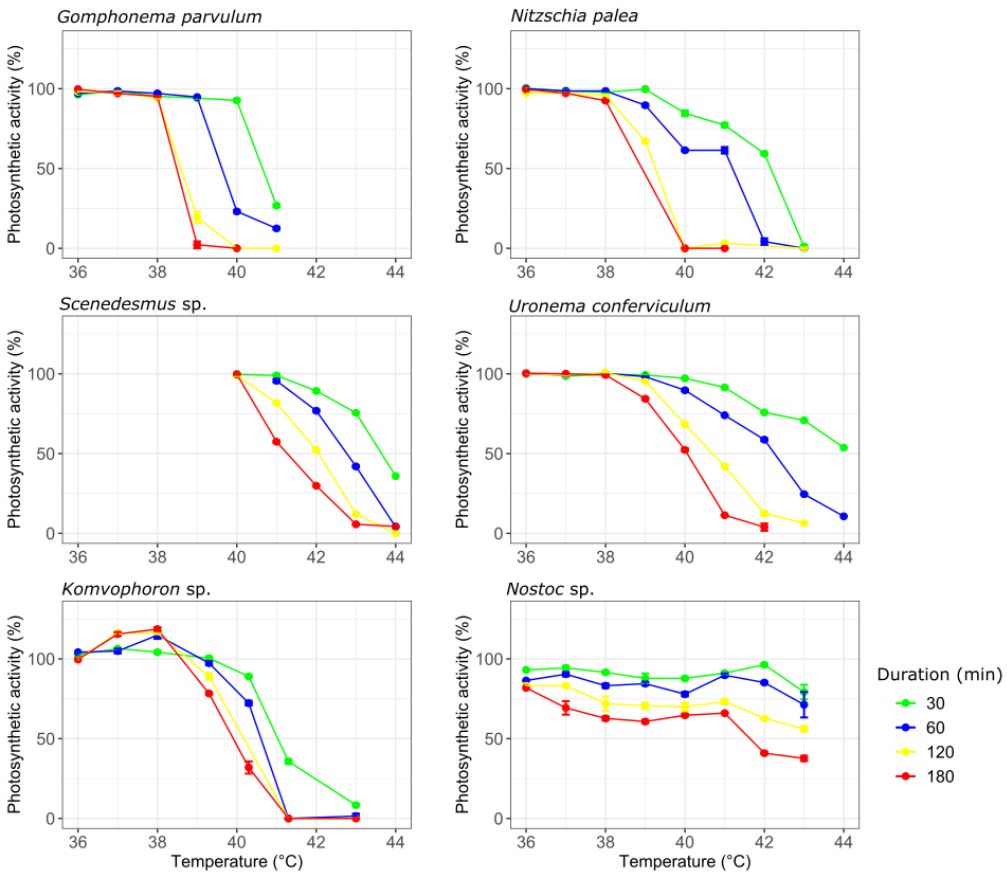


Figure S1: Photosynthetic activity (in % control) as a function of temperature (°C) for each duration (from 30 to 180 min) and for each isolated strain (mean ± se). Diatoms: *G.parvulum* and *N.palea*, green algae: *Scenedesmus* sp. and *U.conferviculum,* cyanobacteria: *Komvophoron* sp. and *Nostoc* sp..

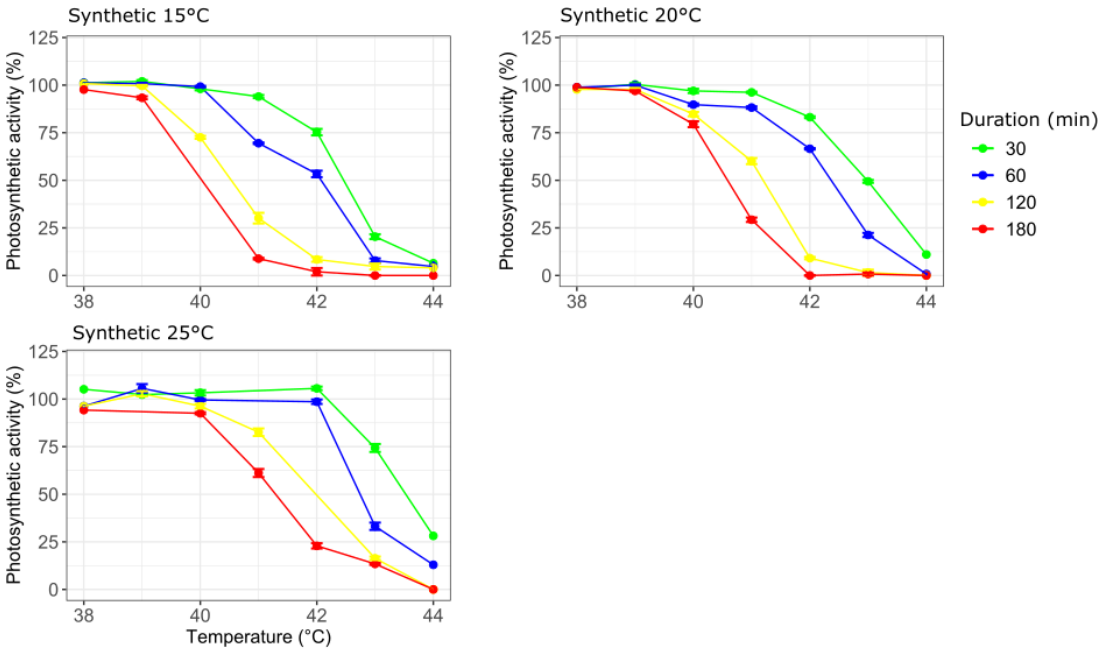


Figure S2: Photosynthetic activity (in % control) as a function of temperature (°C) for each duration (from 30 to 180 min) and for each synthetic community acclimated at various temperatures (mean ± se).

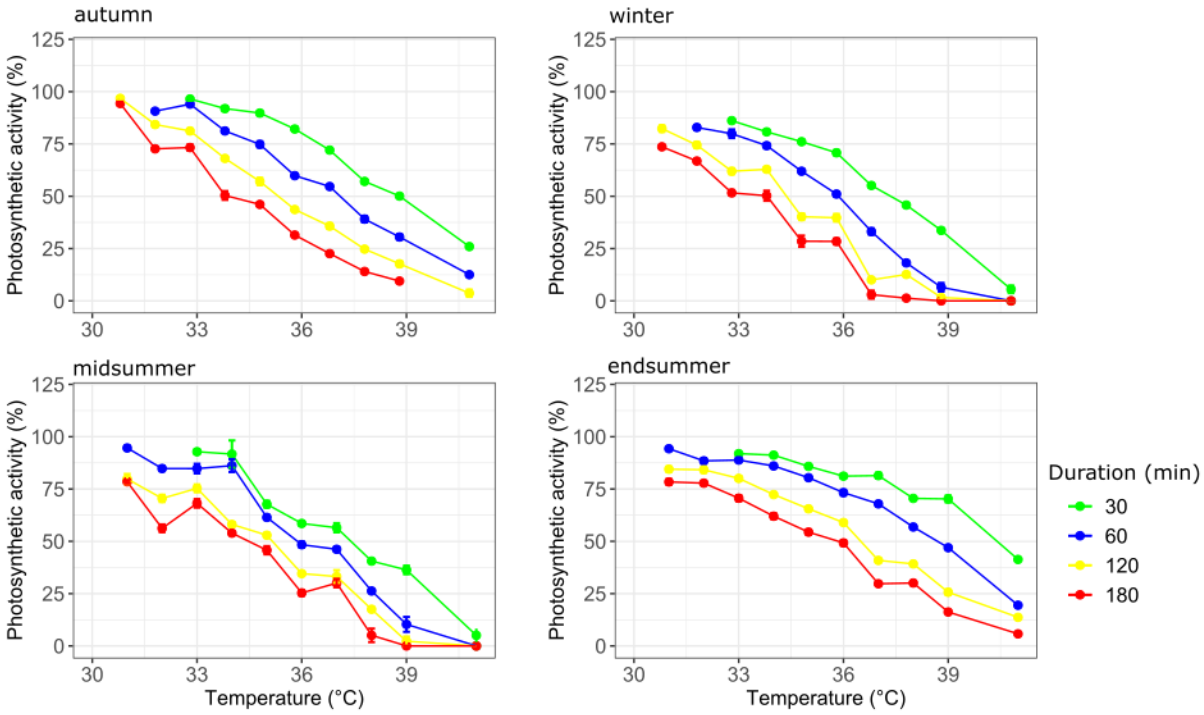


Figure S3: Photosynthetic activity (in % control) as a function of temperature (°C) for each duration (from 30 to 180 min) and for natural communities collected at different periods of the year (mean ± se).

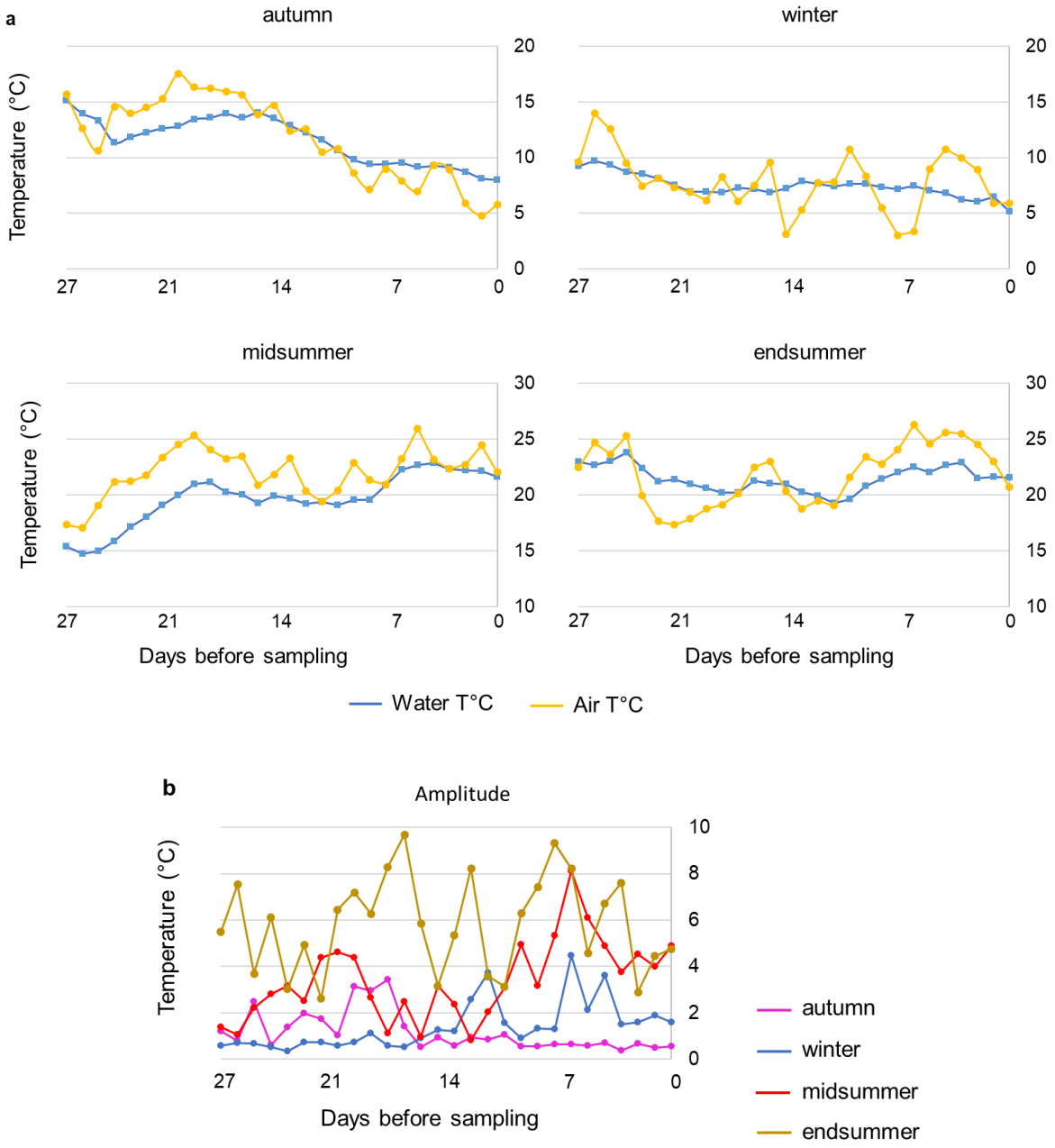


Figure S4 a. Mean daily temperature in water (blue) and air (yellow) during 27 d before each natural community sampling. Data for air temperatures have been measured in the nearest weather station located in Blagnac (43°62N 1°38 E, Haute-Garonne, France) b. Water temperature amplitude calculated as the difference between the maximal and the minimal temperature measured per day, for each natural community on the 27 d before sampling.

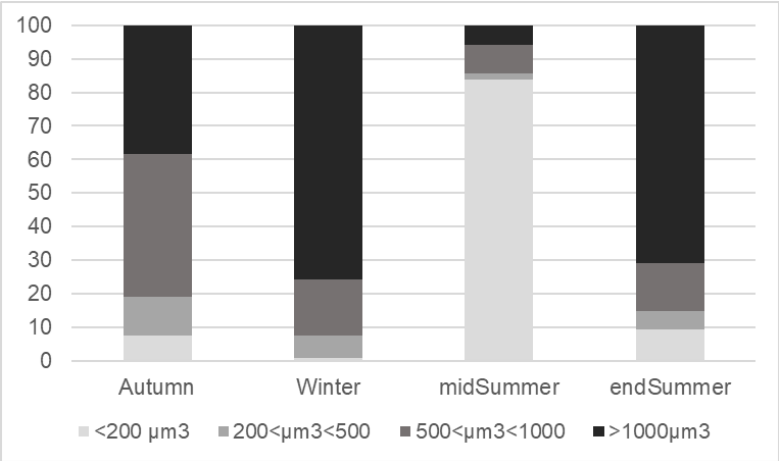


Figure S5: Relative contibution of identified algal species to total biovolume for each sampled natural community (in %). The four size classes represent the summed relative contribution of small species < 200 µm^3, species between 200 and 500 µm^3, largest species between 500 and 1000 µm^3 and very large species > 1000 µm^3.

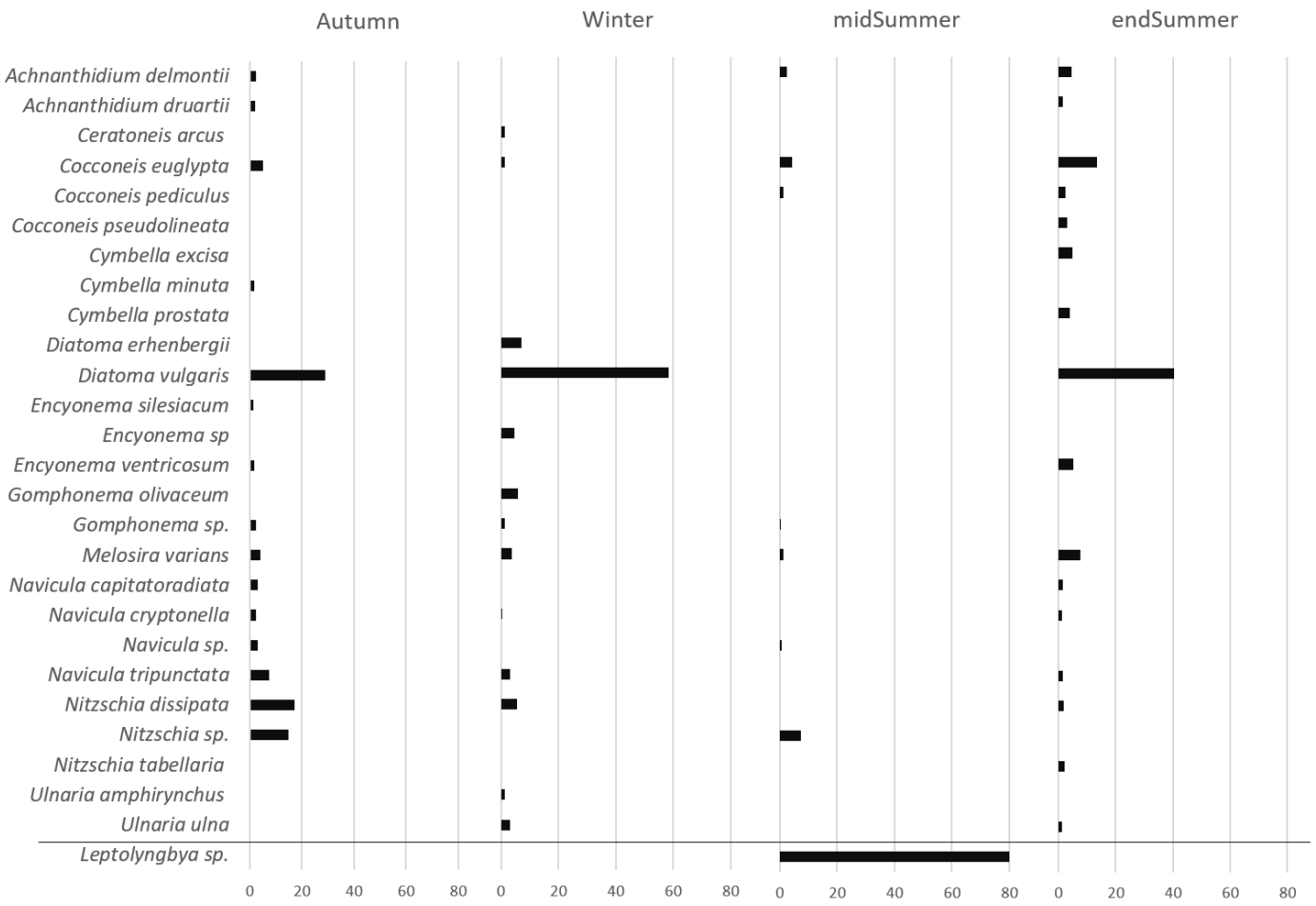


Figure S6: Algal species relative abundance (in % of total biovolume) of each natural community collected at different periods of the year (Experiment 3). All species are diatoms excepted *Leptolyngbia sp.* which is a cyanobacteria

Chapitre 2

Effet de l'intensité d'un assec sur la stabilité de la qualité de la ressource et conséquences sur la production secondaire

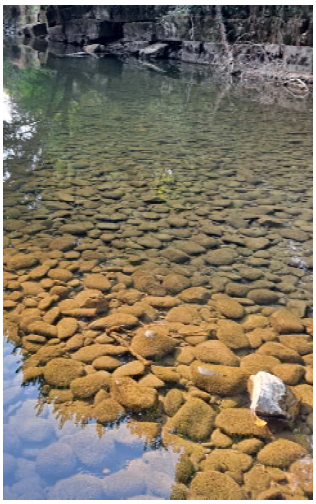
Chapitre 2 : Effet de l'intensité d'un assec sur la stabilité de la qualité de la ressource et conséquences sur la production secondaire

Contexte

L'intermittence hydrique affecte l'ensemble de l'écosystème et modifie les interactions entre les différents niveaux trophiques (Ledger et al., 2013). Les communautés de consommateurs sont impactées directement via la réduction de leur habitat, induisant une perte de diversité et d'abondance, mais également par les changements dans la composition et la disponibilité de leurs ressources (Lake, 2003).

La qualité d'une ressource dépend de sa composition en éléments nutritifs (Masclaux et al., 2009). De manière générale, les consommateurs sélectionnent les ressources de bonne qualité (Marcarelli et al., 2011). La composition générale, y compris la stœchiométrie et les profils en acides gras déterminent la qualité de la ressource (Burian et al., 2018; Danger et al., 2022).

Les biofilms phototrophes sont à la base des réseaux trophiques dans de nombreux écosystèmes aquatiques (Battin et al., 2016). Leur importance a été largement démontrée, et plus spécialement dans les rivières intermittentes dans lesquelles les consommateurs assimilent plus de biomasse algale et moins de ressource allochtone (Siebers et al., 2019). De par sa composition biochimique, cette ressource est essentielle, même en faible quantité (Crenier et al., 2017).



Biofilm de rivière. *J. Leflaive*

Cependant tous les groupes d'algues ne représentent pas le même intérêt en tant que ressource. Par exemple, contrairement aux eucaryotes, les cyanobactéries sont considérées come une ressource de faible qualité, car elles ne contiennent pas d'acides gras insaturés de longue chaine ni de stérols (Martin-Creuzburg et al., 2005, 2006).

La réponse des biofilms à l'assèchement a été largement étudiée. Juste après l'assec, les effets sont généralement négatifs sur le compartiment algal alors que les hétérotrophes sont moins impactés (Timoner et al., 2012) et le contenu en acides gras est fortement réduit (Sanpera-Calbet et al., 2017). Ces effets empirent quand la durée d'assec augmente (Acuña et al., 2015).
Les microorganismes phototrophes réagissent différemment à l'assèchement. Les algues vertes et les cyanobactéries sont généralement plus résistantes que les diatomées, qui sont en revanche plus résilientes (Acuña et al., 2015; Sabater et al., 2017; Timoner et al., 2014).

Objectifs et hypothèses

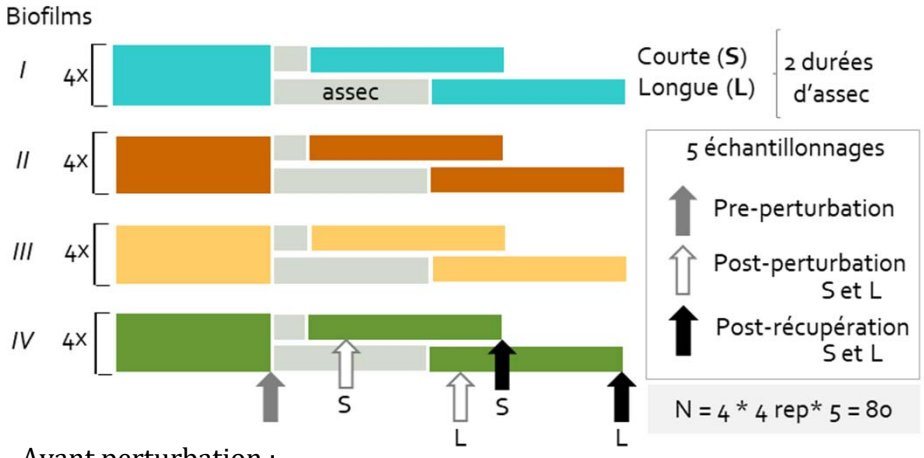
L'objectif de cette étude est de comprendre comment l'assèchement affecte la qualité nutritionnelle des biofilms et leur capacité à soutenir la production secondaire. J'ai cherché à comprendre dans quelle mesure les réponses post-assec et après récupération dépendent de la durée de l'assec et de la composition initiale des biofilms, en exposant des biofilms de compositions algales différentes à deux durées d'assec, puis en nourrissant des consommateurs avec ces ressources.

Mes hypothèses sont :

1. L'assec diminue la qualité nutritionnelle des biofilms, donc leur capacité à soutenir la production secondaire. Plus l'assec est long, plus cette diminution de qualité est importante. Les effets dépendent aussi de la composition du biofilm, les diatomées étant moins résistantes à l'assec.
2. La remise en eau favorise le retour des diatomées connues pour leur meilleure résilience à l'assec. En conséquence, la qualité de la ressource et la production secondaire augmentent après la phase de récupération, du fait d'une meilleure qualité nutritionnelle des diatomées.

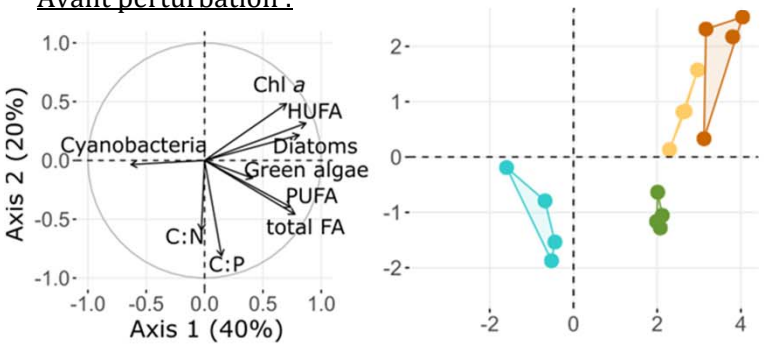
Matériel et méthodes

1. Les biofilms



Mesures :
- Biomasse algale ($mg.mgC^{-1}$)
- Composition pigmentaire
- Stœchiométrie
- Quantification des acides gras ($mg.mgC^{-1}$)

Avant perturbation :



L'ACP montre qu'avant assec, les 4 biofilms produits sont différents en termes de composition pigmentaire, élémentaire et biochimique.

Lors de chaque prélèvement, une fois les différentes analyses réalisées, le biofilm restant a été lyophilisé afin de préparer des pellets pour nourrir les gammares.

2. Les gammares

Les pellets ont été préparées comme décrit dans Crenier et al., (2017), pour nourrir des gammares (*Gammarus fossarum*) sélectionnés en fonction de leur taille. Pour chaque échantillon de biofilm (n=80), 3 individus ont été nourris (n=240). Dans chaque pellet, la quantité de carbone était constante. Les individus ont été nourris pendant 29 jours.



Paramètres mesurés : Survie, taux de croissance, état physiologique, taux de consommation et vitesse médiane de déplacement

3. La production secondaire

La production secondaire potentielle a été estimée à partir de la quantité de carbone consommée par les gammares et à partir de la quantité de ressource disponible par unité de surface.

$$Production\ secondaire(mg.cm^{-2}.j^{-1}) = \frac{(masse\ finale\ gammares - masse\ initiale\ gammares)(mg) * biofilm\ total\ C\ (mgC.cm^{-2})}{carbone\ consommé\ (mgC) * durée\ de\ l'expérience\ (day)}$$

4. Analyses des données

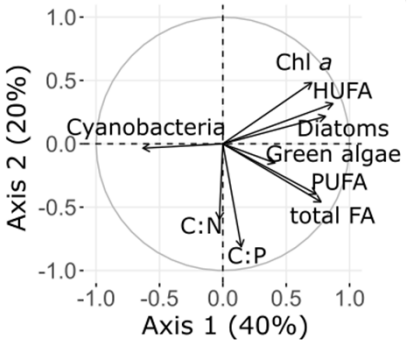
Les trajectoires d'évolution de la qualité nutritionnelle des biofilms (i.e. principaux groupes algaux, rapports stœchiométriques, biomasse vivante et contenus en acides gras) ont été étudiées en analyse multivariée (ACP). La même analyse a été réalisée sur les traits écologiques des gammares. Les effets de la durée d'assec et de la date d'échantillonnage ont été testés en ANOVA sur mesures répétées sur les coordonnées des 1ers axes de chaque ACP.

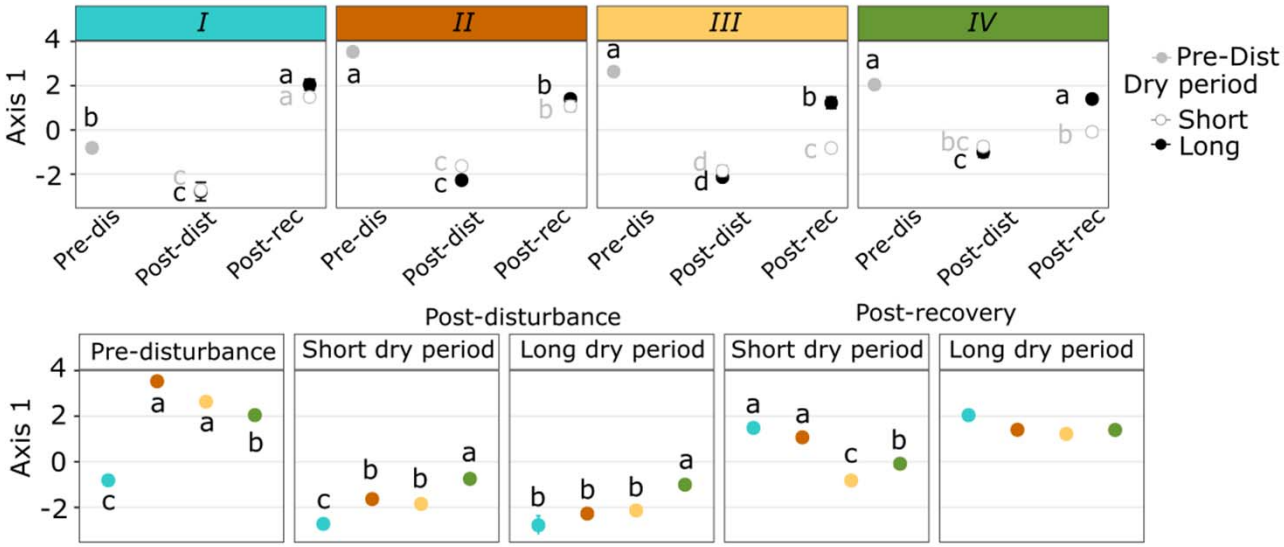
Enfin, les changements liés à l'assec sur la quantité de ressource disponible et sur la production secondaire ont été quantifiés en standardisant par les valeurs mesurées *Pre-disturbance* pour chaque biofilm.

Résultats

Effet de l'assec sur la qualité de la ressource

Les biofilms de bonne qualité, riches en chlorophylle *a*, diatomées et acides gras, sont sur la droite du plan de l'ACP réalisée sur les descripteurs du biofilm. L'axe 1 résume bien la qualité de la ressource.
Avant assec (i.e. *Pre-dist*) le biofilm *I* est de moins bonne qualité.
Il y a un effet négatif de l'assec sur la qualité de tous les biofilms, peu importe la durée *(Post-dist*).
Au cours de la période de récupération *(Post-rec*), la qualité augmente fortement pour le biofilm *I,* elle reste plus faible qu'avant perturbation pour les biofilms *II* et *III* tandis que pour le *IV*, cela dépend de la durée d'assec.
Après un assec long, la qualité des biofilms s'homogénéise.





Effet de la ressource sur la réponse des gammares

Le taux de croissance et l'indice de condition des gammares sont positivement corrélés avec les contenus en chlorophylle *a*, les proportions de diatomées et d'algues vertes et le contenu en acides gras, et négativement corrélés avec la proportion de cyanobactéries.

Les axes 1 des biofilms et des gammares sont corrélés ($p < 0{,}001$), ce qui confirme que les descripteurs utilisés pour décrire la qualité potentielle des biofilms sont pertinents car corrélés à la réponse physiologique et comportementale des gammares.

Effets sur la production secondaire

Avant perturbation, la production secondaire est significativement plus importante pour le biofilm IV. Malgré la bonne qualité potentielle des biofilms II et III avant perturbation, la production secondaire potentielle est la même que pour le biofilm I.
En fin de perturbation, l'assec ne diminue pas significativement la production secondaire, bien que celle-ci tende à s'homogénéiser entre les différents biofilms après l'assec long.
Après récupération, la production secondaire potentielle a tendance à augmenter dans tous les biofilms, mais après récupération suivant l'assec long, elle est significativement plus importante pour les biofilms III et IV. Les augmentations observées sur la qualité potentielle des biofilms ne se traduisent pas nécessairement au niveau de la production secondaire potentielle.

Conclusions et Perspectives

Réponse des biofilms à l'assec

L'assec diminue fortement la qualité nutritionnelle potentielle des biofilms. Comme attendu, il a un impact très fort sur la proportion de diatomées, puisqu'aucun pigment spécifique de ce groupe algal n'est présent après la perturbation. La quantité d'acides gras polyinsaturés (PUFA et HUFA) est quasiment nulle après assec. Des résultats similaires ont été observés dans des biofilms de rivières méditerranéennes exposées à l'assec (Sanpera-Calbet et al., 2017).

De manière générale, la durée de l'assec n'a pas d'effet sur la qualité nutritionnelle potentielle. En revanche, après un assec long, la quantité de biofilm disponible diminue plus fortement qu'après un assec court. Ce résultat est en accord avec de précédentes études qui montrent que plus l'assec dure dans le temps, plus la biomasse algale diminue (Acuña et al., 2015).

→ Validation partielle de l'hypothèse 1

Récupération des biofilms après l'assec et effets sur la production secondaire

Les résultats mettent en évidence un effet de la durée de l'assec après la période de récupération pour certains biofilms. Cela est sans doute lié au fait que la résilience des diatomées est meilleure après un assec long, la compétition avec les cyanobactéries étant réduite : les cyanobactéries qui bien que peu affectées par un assec court, sont elles aussi plus impactées après un assec long (Barthes et al., 2015).

Les trajectoires de récupération des biofilms dépendent de la composition initiale des biofilms. Pour le biofilm *I* la qualité potentielle a fortement augmenté alors que pour les autres biofilms, les effets sont plus mitigés.

→ Validation partielle de l'hypothèse 2

Effets sur la production secondaire

Dans cette étude, les effets nets de l'assec sur la qualité nutritionnelle potentielle des biofilms se traduisent par de plus faibles effets sur la réponse des gammares ou sur la production secondaire.

Malgré la diminution de la qualité potentielle des biofilms après l'assec, la réponse des gammares et la production secondaire ne changent pas de manière significative. Cela est probablement dû aux faits que la biomasse totale ne diminue pas et que lorsque la quantité de biomasse est limitante, l'importance de la qualité de la ressource diminue (Hartwich et al., 2012; Sterner, 1997).

Après la phase de récupération, bien que la qualité nutritionnelle change pour certains biofilms, les effets sur la production secondaire sont plus limités. Par exemple pour le biofilm *I*, la biomasse disponible étant trop faible malgré l'augmentation de la qualité potentielle, sa capacité à soutenir la production secondaire reste faible aussi.

Ces résultats mettent bien en évidence l'importance de considérer à la fois la qualité d'une ressource et sa quantité pour comprendre les effets de l'intermittence hydrique sur la production secondaire.

Il serait intéressant de savoir dans quelle mesure les réponses mesurées persistent dans le temps. Dans cette étude, la résilience a été évaluée après seulement deux semaines de récupération. Les biofilms phototrophes étant des systèmes très dynamiques, il est possible que les changements observés sur la qualité potentielle des biofilms s'amplifient à plus long terme et ainsi affectent leur capacité à soutenir la production secondaire.

Présentation orale au congrès SFE²-GBO, Metz 2022

Publication à soumettre dans *Freshwater Biology*

Flow intermittency affects the capacity of phototrophic biofilms to sustain secondary production

Camille Courcoul[1*], Stéphanie Boulêtreau[1], Alexandre Bec[2], Michael Danger[3,4], Vincent Felten[3,4], Cédric Pradalier[5], Mathilde Roche-Bril[3], Joséphine Leflaive[1]

[1] Functional Ecology and Environment Laboratory, University of Toulouse, CNRS UMR 5245, UPS, INPT, Toulouse, France

[2] Université Clermont Auvergne, CNRS, LMGE, Clermont-Ferrand, France

[3] Université de Lorraine, CNRS, LIEC, Metz, France

[4] LTER- "Zone Atelier Moselle", Metz, France

[5] GeorgiaTech Lorraine- International Research Lab Georgia Tech - CNRS IRL 2958, France

Abstract

1. In streams, phototrophic biofilms are a good quality resource for consumers and are essential to support secondary production. In the current context of global change, flow intermittency phenomenon increases. Few information exists on the effect of drying and rewetting events on biofilms nutritional quality and its consequences on consumers. This study aims at understanding how river intermittency affects the nutritional quality of phototrophic biofilms and their capacity to support secondary production after drying and during rewetting.
2. We first hypothesized that dry period negatively affects biofilms' nutritional quality and that the impact is stronger for long dry period duration. Our second hypothesis was that rewetting favours after sufficient time the recovery of better-quality algae, thus increasing nutritional quality and the secondary production.
3. Four phototrophic biofilms of different nutritional quality were grown in flowing water, and then exposed to a short (3 days) or a long dry period (14 days). Biofilms

were samples 3 days and 18 days after rewetting (post-disturbance and post-recovery) to assess changes in their nutritional quality compared to pre-disturbance through pigment, fatty acid and stoichiometric analyses. Gammarids (*Gammarus fossarum*) were fed with all these biofilms for 29 days to measure individual growth, feeding and activity and a secondary production index.

4. We found that dry period drastically reduced the nutritional quality of biofilms with few effects of the dry period duration. During recovery, nutritional quality indicators were generally improved with rewetting duration without necessarily translating to significant responses of consumers' performance traits and increases in secondary production.
5. Our study highlights the need to consider both the quality and the quantity when studying changes in resources and its effect on food web, since secondary production appeared to strongly depends on both.

Keywords: freshwater periphyton, primary consumers, dry period, nutritional quality, intermittent rivers

1. Introduction

Flow intermittency is a general phenomenon occurring within every climate worldwide, and rivers that cease to flow for at least one day per year account for more than a half of the global network (Messager et al., 2021). Due to global change, there is not only an increase in dry period duration in intermittent rivers and ephemeral streams (IRES, Datry et al., 2017a), but more and more streams or rivers that were perennials so far start also to experience dry phases. Water intermittency affects the whole biota, resulting in an alteration of the trophic structure of the ecosystem because of modified interactions between trophic levels (Ledger et al., 2013). It impacts consumers directly through both habitat fragmentation and loss of species richness and abundance (Acuña et al., 2005; Bogan et al., 2015; Boulton and Lake, 2008). In addition, consumers are indirectly affected through changes in food resources composition and availability mediated by drought (Lake, 2003). Resource composition is an important property of food-quality as it defines its palatability or its toxicity (Masclaux et al., 2009), and generally, aquatic consumers select high-quality resource (Marcarelli et al., 2011). Food quality relies on several parameters, including the stochiometric composition described for instance by the C:N

and C:P ratios, and the biochemical composition, notably the fatty acids profiles (Burian et al., 2018; Danger et al., 2022; Ruiz et al., 2021). Considering the qualitative and quantitative modifications of resources is crucial to fully predict the effects of river intermittency on food web structure and secondary production.

Phototrophic biofilms are major primary producers in many flowing ecosystems where they occupy the basis of the food web (Battin et al., 2016). They are mainly represented by diatoms, green algae and cyanobacteria, which are embedded in an extracellular polymeric matrix with other heterotrophic microorganisms like bacteria or fungi. The importance of this algal autochthonous resource in food web integrity has been demonstrated in several studies (Allan and Castillo, 2007; Crenier et al., 2017; Kühmayer et al., 2020), and especially in intermittent streams, where it has been shown that consumers assimilate more algal biomass and less allochthonous matter than in perennial systems (Siebers et al., 2019). Algal food sources, even in low quantity, are essential to ensure secondary production (Crenier et al., 2017; Labed-Veydert et al., 2022; Twining et al., 2017). Indeed, stream biofilms manifest a lower C:N ratio than leaf litter (Lau et al., 2009), which increases their digestibility and their quality (Allan and Castillo, 2007). In addition, algae exhibit larger essential polyunsaturated fatty acids (PUFA) contents than allochthonous matter (Descroix et al., 2010; Labed-Veydert et al., 2021; Torres-Ruiz et al., 2007). PUFAs are nearly exclusively synthesized by phototrophic primary producers, and consumers must take them up from their food source (Brett and Müller-Navarra, 1997). Algal composition also drives resource quality for consumers, as all algal groups do not represent the same resource interest. Several studies have highlighted the lower nutritional quality of cyanobacteria compared to eukaryotes in pelagic ecosystems (e.g. Gergs et al., 2014). This is because cyanobacteria, like other prokaryotic organisms, lack sterols (Martin-Creuzburg et al., 2006, 2005) and n-3 series PUFA (Bec et al., 2006; Guo et al., 2016; Müller-Navarra et al., 2000), and are known to be less palatable for consumers than diatoms and green algae (Robson et al., 2008).

The response of river phototrophic biofilms to desiccation has been widely studied. In terms of biomass, the autotrophic compartment is generally more negatively impacted by desiccation than the heterotrophic compartment (Timoner et al., 2012), and the more the disturbance lasts, the more autotrophic processes are impacted (Acuña et al., 2015). Responses differ between algal groups, as generally cyanobacteria and green algae are

more resistant to desiccation than diatoms, but less resilient (Acuña et al., 2015; Sabater et al., 2017; Timoner et al., 2014c). Longer dry periods can favor cyanobacteria (Robson et al., 2008) although the opposite trend was observed in Barthès et al., (2015) where cyanobacteria that dominated before disturbance were replaced by diatoms when the dry period lasted at least 4 weeks, in a laboratory experiment. These modifications of biofilm composition are expected to have consequences for consumers. It was shown that the biofilm total fatty acid content decreased in a Mediterranean stream after a drought (Sanpera-Calbet et al., 2017), but the cascading effects were not assessed on consumers. One work showed that the consumption rate of *Physella acuta* decreased when fed with resources exposed to water intermittency (Mas-Martí et al., 2017). As a whole, these results suggest the responses of the nutritional quality of phototrophic biofilms in intermittent rivers largely depend on specific and biochemical composition of algal communities. However, few information exists to predict how the nutritional quality of phototrophic biofilms changes along drying and rewetting events and how these changes affect the consumers.

In this study, we aim at understanding how river intermittency affects the nutritional quality of phototrophic biofilms and their capacity to support secondary production. We investigated in what extent their response to drying and their recovery from drought depend on the disturbance intensity and on the biofilm initial composition, by exposing four biofilms contrasting in their algal composition or stoichiometry to two dry period durations followed by rewetting. The potential nutritional quality of each biofilm was evaluated through pigment, stoichiometric and biochemical composition before drying, and 3 and 18 days after rewetting. The capacity of biofilms to sustain the secondary production was assessed by feeding metazoan consumers with each produced biofilm and by measuring their ecological traits. We first hypothesize that drying reduces biofilm capacity to sustain secondary production by decreasing its nutritional quality. More specifically, this reduction increases with the dry period duration due to algal mortality, and is more limited in green algae- and/or cyanobacteria- dominated biofilms than in diatom biofilms. Our second hypothesis is that biofilms sampled 18 days after rewetting are more diatom-dominated and thus, are more prone to sustain secondary production than biofilms sampled 3 days after rewetting, thanks to better potential nutritional quality of diatom assemblage.

2. Material and methods

Biofilm production

Biofilm inoculation

In order to grow contrasted resources in terms of biochemistry and stoichiometry, different *inocula* were used for biofilm cultures along with different culture conditions (i.e. depletion or not in phosphorus). To do so, we collected phototrophic biofilms from two sites of the Garonne River (South-West France), where hydrological and nutrient conditions favored different communities: Aouach (43.396635, 1.300394, South-West France), dominated by diatoms and Toulouse (43.561740, 1.437750, South-West France), with more green algae. We collected immerged pebbles on the riverbank and scrapped biofilm with a toothbrush directly in river water. Back to the lab, the suspensions were homogenized with an ultra-turrax (T25, Janke-Kunkel) and then filtrated on a 500-µm thieve to remove large mineral particles and macrofauna. To modify the stoichiometry of the biofilm, Aouach *inoculum* was cultivated in standard and P-depleted conditions. To ensure the presence of cyanobacteria in at least one biofilm, a part of the initial *inoculum* from Aouach was additionally filtrated on a 40-µm thieve to eliminate large diatoms and then enriched with laboratory cultures of several cyanobacteria (*Komvophoron* sp, *Phormidium autumnale* and *Leptolyngbya* sp., grown in BG11 culture medium (Stanier et al., 1971). This way we obtained four different biofilms: Aouach *inoculum* enriched with cyanobacteria (Biofilm *I*), Aouach *inoculum* P-depleted (Biofilm *II*), Aouach *inoculum* (Biofilm *III*), and Toulouse *inoculum* (Biofilm *IV*).

Biofilm culture and sampling

The biofilm culture device consisted of 32 channels (8 per biofilm type, l=50 cm, L= 8 cm, h= 10 cm) placed in a controlled environment (22°C, light intensity: 88 µmol. m^{-2}. s^{-1} (±7) for 16 hours/day). Ceramic coupons (5.76 cm^2, 54 per channel) were placed at the bottom as growth substrate. During biofilm growth (14 days) the channels were connected two by two (S and L dry period, 4 replicates) by sharing the water tank (see Fig. 1). Water circulation was maintained by aquarium pumps (Tetra WP-300, 300 l. h^{-1}). We used Volvic® water as culture medium. Evaporation was compensated three times a week with

demineralized water at the same time than nutrient addition. The same quantity of nitrogen (1.4 mg. l^{-1} $N\text{-}NO_4$) and phosphorus (200 µg. l^{-1} $P\text{-}PO_4$) was added in each channel, except for the P-depleted condition (biofilms *II*) where the quantity of phosphorus was 10 times lower (20 µg. l^{-1}). In the channels hosting biofilms *II* and *III*, silica (21.3 mg. l^{-1} SiO_2) was also added to favor diatom growth.

After the growth step, a first sampling was done to characterize each biofilm before drying (pre-disturbance, Fig. 1) by collecting 11 coupons in each channel (22 coupons per replicate). Biofilm was gently removed from coupons with a scraper and resuspended in Volvic® water (4 ml per coupon). After this sampling, channel pairs were disconnected and dried-out by removing the water with a vacuum pump. One channel of each pair underwent a short dry period (3 days, S), while the other one underwent a longer dry period (14 days, L). After each dry period, flow resumed in the same condition than during growth phase, but independently in each channel. Biofilms were sampled during rewetting 3 days after flow resumption (post-disturbance) and 18 days after flow resumption (recovery). For each sampling, 22 ceramic coupons were retrieved from each channel, and treated as for the pre-disturbance sampling. Corresponding sampled biofilms were respectively named *pre-disturbance biofilms*, *post-disturbance biofilms* and *post-recovery biofilms* in the following text.

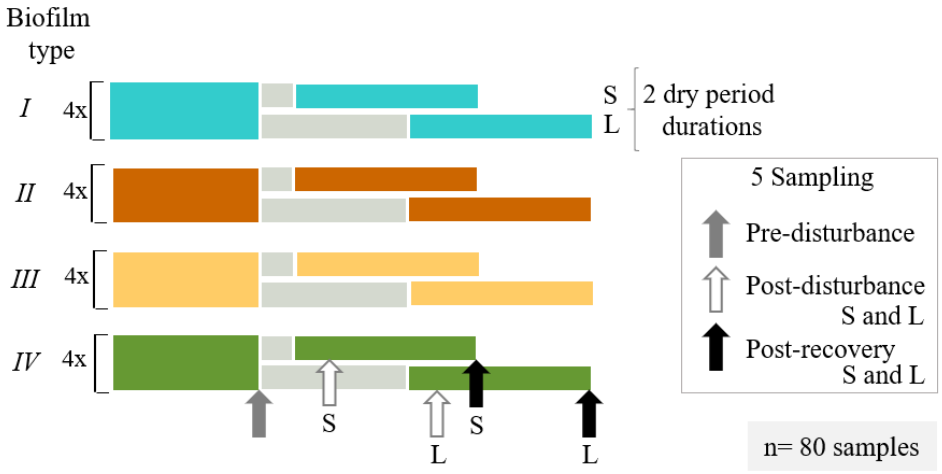


Figure 1: Experimental design: 4 types of biofilms were grown in a total of 32 channels, with 4 replicates per biofilm type and dry period duration. After 2 weeks in standard flow condition, biofilms were sampled (grey arrow) and all the channels were disconnected and dried out for 3 days (Short dry period: S) or 14 days (Long dry period: L). After

rewetting, two samplings were conducted, after 3 days (white arrows) and 18 days (black arrows).

Biofilm sample analysis

Biomass, algal pigment composition, stoichiometry and fatty acids composition were measured on each biofilm sample. Each variable was normalized by biofilm carbon weight to assess the biofilm nutritional quality, and was expressed per coupon surface unit when considering the quantity of biofilm available for consumers.

Pigment composition

1.5 ml of each biofilm suspension were centrifuged (10 000 g for 5 min at 4°C) in microtube, the supernatant was removed and biofilm was kept at -80°C until being freeze-dried right before pigment extraction. Pigments were extracted in 2 ml of 2% ammonium acetate 1 M buffered methanol (Capdeville et al., 2019). The tubes were then sonicated for 3 min and incubated at -20°C for 15 min. After another 3 min sonication, tubes were centrifuged (10 000 g for 5 min at 4°C). The supernatant was gently pipetted out and stored at 4°C. Another extraction step was performed on the samples and the supernatants were pooled, except when the supernatant was not colored. Pigment analyses were performed using HPLC (LC1200 series, Agilent Technologies, United States), as described in (Majdi et al., 2011), from 1 ml of each extract pre-filtered in a glass vial with a 0.2 µm PTFE membrane syringe filter (VWR International, United States). Pigment concentration was quantified by comparing the peak area of the chromatogram with those of standards with the ChemStation software (Agilent Technologies, Unites States). To obtain a proportion of each main primary producers, specific pigments of green algae, diatoms or cyanobacteria were summed as described in (Allen et al., 2021) and expressed in proportion of the total pigment content (%). The quantity of chlorophyll *a* in samples was used as an indicator of the live algal biomass.

Stoichiometric composition

One ml of biofilm suspension was oven dried at 60°C for 24h in pre-weighed microtubes. Nitrogen and carbon content were quantified by elemental analysis with a CHN analyzer (Flash 2000, Thermo Scientific). Phosphorus quantification was done with a

spectrophotometer at 880 nm (Uvi Light XT5, SECOCAM, Alès, France), after a pre-treatment described in (Courcoul et al., 2022).

Fatty acid composition

One ml of biofilm suspension was freeze-dried and used to assess the fatty acid (FA) composition. To ensure enough quantity of carbon and for logistic reasons, the 4 replicates from each treatment were pooled. FA were extracted twice following the method developed by (Folch et al., 1957) to break protein-lipid bonds to keep only the lipidic phase. Biofilms were suspended in clean glassware and in a chloroform: methanol (2:1) mix with two standards (20 µl of 13:0 and 23:0 at 2 mg. ml^{-1}). Then, FA were methylated to obtain FA methyl-esters (FAME) through an esterification reaction in an acid medium (4% H_2SO_4 in methanol at 75°C for 2 h) of FA chains. FAME were then diluted in 200 µl of hexane in dark glass vials before quantification. FAME were separated and analyzed using an Agilent 6850 gas chromatograph equipped with a J&WDB-WAX capillary column (Agilent, 30 m × 0.25 mm ID) as described in Gerphagnon et al. (2019). Peak areas were expressed as FA quantity according to internal standards and identified using the FA retention time of an external standard Supelco 37 Component FAME Mix (Merck).

In addition to the total FA content, the quantity of PUFA (poly-unsaturated FA) was calculated by summing unsaturated FA with more than 18C. Similarly, the quantity of HUFA (highly-unsaturated FA) was calculated by summing 20:4(n-3), 20:5(n-3) and 22:6(n-3) (Bec et al., 2010).

Feeding experiment

The remaining volume of biofilm suspension was freeze-dried, weighed and kept at -20°C until the preparation of pellets to feed crustacean amphipods *Gammarus fossarum,* which are generalist consumers.

Selection of individuals

Gammarus fossarum individuals were collected in a second-order stream in Vosges (eastern France). The feeding experiment was conducted in river water collected during individuals catching. Back to the lab, organisms were acclimated in the dark, at 5°C with

oxygen supply for 5 days. During acclimation, gammarids were fed with alder leaves (*Alnus glutinosa*) collected on the riparian zone of the same stream. Gammarid individuals were sorted to keep only juveniles with similar length (4.8 mm ± 0.3).

Agarose pellet preparation

The agarose pellets were prepared as described by Crenier et al. (2017) at a carbon concentration of 2%. 50% of C came from agarose, the remaining 50% from biofilm was based on the biofilm C content previously quantified. The biofilm-agarose mixture was homogenized at 38°C to avoid jellification and then poured into Plexiglas molds (39.27 mm^3) until solidification. Pellets were stored at -20°C in Petri dishes until use.

Gammarid feeding

3 gammarids were fed with each biofilm sample (n = 80). 240 gammarids were individually disposed in Petri dishes containing 30 ml of river water, and kept at 12°C in the dark during the 29 days of the experiment. Once a week, water and biofilm pellets were renewed. Each time, the remaining pellets were stored in microtubes and kept at -20°C to later be weighted and calculate the consumed biofilm.

Ecological trait analysis

In addition to counting gammarid survivors every day, growth rate, condition index, feeding rate and median locomotion speed were measured on individuals that survived along the feeding experiment.

Growth traits

The weight growth rate (in day^{-1}) was calculated as follows:

$$Growth\ rate = \frac{(final\ body\ dry\ weight\ (mg) - initial\ body\ dry\ weight\ (mg))}{initial\ body\ dry\ weight\ (mg) * experiment\ duration(day)}$$

The final dry weight (in mg) of individuals was measured at the end of the experiment, on freeze-dried individuals, using a precision balance (RADWAG, UYA 2.4.Y). The initial dry weight was estimated from the initial body size using the following allometric relationship:

$$Initial\ dry\ weight = 0.0609 * initial\ size^2 - 0.2897 * initial\ size + 0.4291$$

The length was measured between the antennule basis and the telson using the VHX-6000_950F software, on pictures made with a digital microscope (VHX 6000, Kayence). The formula described in Danger et al. (2013) was used to take the curved body shape into consideration and deduce the length in a linear state:

$$linear\ state\ =\ curved\ state\ *\ 0.88$$

The Fulton condition factor (g. cm^{-3}) of gammarids was calculated from final weight and length (Ricker, 1975) to relate the physiological state of individuals:

$$condition\ index\ =\frac{(\ body\ dry\ weight\ (mg))}{body\ length\ (mm)^{\ 3}}$$

The factor is comprised between 0 and 1, with 0 representing a bad physiological condition.

Feeding rate

The feeding rate (in mg biofilm. mg $gammarid^{-1}$. day^{-1}) was calculated as follows:

$$Feeding\ rate\ =\ \frac{consumed\ biofilm\ weight\ (mg)}{final\ body\ dry\ weight\ (mg)\ *\ experiment\ duration\ (day)}$$

Median speed

We measured the median locomotion speed (in cm. sec^{-1}) as a good proxy of individual gammarid activity. Each gammarid was placed in Petri dishes wrapped in black tape to facilitate tracking into a closed and opaque small chamber (116 * 50 * 50 cm) in which light can be easily controlled (Led, 250 µmol. m^{-2}. s^{-1}) and equipped with a camera (Canon EOS M50). Locomotion of each individual was tracked during 8 min.

Potential secondary production

The potential secondary production was calculated from previous parameters as follows:

$$Secondary\ production\ (mg.cm^{-2}.day^{-1})$$
$$=\frac{(final\ body\ dry\ weight\ -initial\ body\ dry\ weight)(mg)\ *\ biofilm\ total\ C\ (mgC.cm^{-2})}{consumed\ biofilm\ carbon(mgC)\ *\ \ experiment\ duration\ (day)}$$

This parameter represents the weight of gammarids biofilm biomass is able to daily support.

Statistical analyses

All statistical analyses were performed on R software (version 3.6.1, R development Core Team). The normality of the variables was systematically tested, and variables were transformed when necessary. Global Pearson correlations between biofilm descriptors and gammarid response variables were tested (*Hmisc* package). PCA analyses were conducted on biofilm quality descriptors and on gammarid response variables in order to highlight differences between biofilms and sampling times. The coordinates of PCAs first axis were extracted and used as summary variables. To compare the evolution of potential quality between biofilms, and the response of gammarids over time, repeated measures ANOVA were conducted within dry period duration and sampling time (i.e. pre-disturbance, post-disturbance and post-recovery), and appropriate post-hoc analyses were conducted if significant. Finally, the relative changes of biofilm variables expressed by surface unit was calculated by standardizing the post-disturbance and recovery values by their respective pre-disturbance state (i.e. for each channel). Post-hoc analyses were conducted to highlight significant differences in proportion of changes between post-disturbance and post-recovery for the interesting variables.

3. Results

Pearson correlation analysis highlights biofilm autotrophy (Chl *a*) positively correlates with total FA, PUFA, HUFA contents and diatom proportion, but negatively with cyanobacteria proportion (Table 1). Total FA and PUFA contents were also positively correlated with C:P ratio and diatom proportion, while HUFA content was negatively correlated with the C:N ratio and cyanobacteria proportion, but positively correlated with other algal groups, especially diatoms. The correlation matrix also points out the negative relationship between cyanobacteria proportion with other main algal groups and the live biomass (i.e. Chl *a* and total FA content).

Table 1: Pearson correlation coefficients (r) between biofilm quality descriptors. Only significant correlations are presented. The asterisks represent the level of significance: 0.05 > * > 0.01 > ** > 0.001 >***.

	Chl *a*	Tot FA	PUFA	HUFA	Green Algae	Diat	Cyano	C:N	C:P
Chl *a* (mg. mgC^{-1})	1								
Total FA (mg. mgC^{-1})	0.38 ***	1							
PUFA (mg. mgC^{-1})	0.41 ***	0.83 ***	1						
HUFA (mg. mgC^{-1})	0.68 ***	0.46 ***	0.45 ***	1					
Green Algae (Proportion)				0.32 **	1				
Diatoms (Proportion)	0.59 ***	0.56 ***	0.43 ***	0.79 ***		1			
Cyanobacteria (Proportion)	-0.28 **	-0.28 **		-0.60 ***	-0.6 ***	-0.42 ***	1		
C:N	-0.34 ***			-0.34 ***				1	
C:P		0.42 ***	0.41 ***						1

Gammarid growth rate was positively correlated with Chl *a*, diatom and green algae proportions, total FA, PUFA, HUFA contents and C:N ratio (Table 2). The same significant correlations were observed for the condition index of gammarids, except with C:P. As expected, both gammarid growth rate and condition index were negatively correlated with the cyanobacteria proportion. The feeding rate was positively correlated with Chl *a*, but negatively with green algae proportion. Gammarid median speed increased when C:N ratio and total FA and PUFA contents in biofilms increased.

Table 2: Pearson correlation coefficients (r) between biofilm quality descriptors (rows) and gammarid response variables (columns). Only significant correlations are presented. The asterisks represent the level of significance: 0.05 > * > 0.01 > ** > 0.001 >***.

		Gammarids			
		Growth rate (mg. day^{-1})	Condition index (mg. cm^{-3})	Feeding rate (mg. mg^{-1}. day^{-1})	Median speed (cm. sec^{-1})
Biofilms	Chl *a* (mg. mgC^{-1})	0.33**	0.33**	0.44***	
	Total FA (mg. mgC^{-1})	0.54***	0.54		0.44***
	PUFA (mg. mgC^{-1})	0.4***	0.42		0.27*
	HUFA (mg. mgC^{-1})	0.28*	0.33		
	Green algae (Proportion)	0.22*	0.31**	-0.24*	
	Diatoms (Proportion)	0.40***	0.42***		
	Cyanobacteria (Proportion)	-0.26*	-0.38**		
	C:N	0.27*		-0.29*	0.32**
	C:P			-0.36**	

Pre-disturbance biofilms

The use of different *inocula* and culture conditions allowed the production of contrasted biofilms, as shown by the proportions of the three main algal groups (cyanobacteria, green algae and diatoms) (Fig. 2a). After growth, biofilm *I* was dominated by cyanobacteria-specific pigments at more than 54% and green algae-specific pigments at 37%. In biofilm *II*, 66% of pigments were diatom-specific. Biofilm *III* was dominated by diatoms (45%) and cyanobacteria (36%), while biofilm *IV* showed a dominance of both green algae (40%) and diatoms (40%). The pigment composition was more variable in biofilms *I* than in other biofilms.

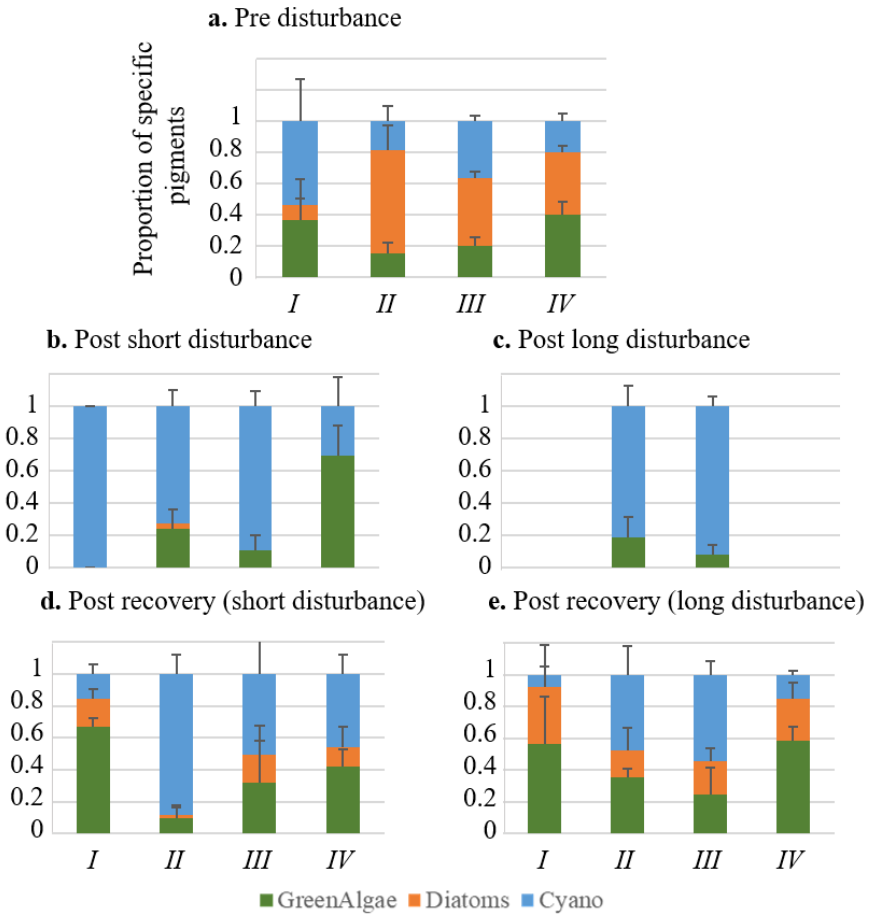


Figure 2: Proportions of each main algal group within biofilms (mean ± SD, n = 4), estimated through pigment analysis, at pre-disturbance sampling (a), after a short dry period (left) and a long dry period (right) at post-disturbance (b and c) and at post-recovery (d et e) samplings. For c, there are no values for biofilms *I* and *IV*, because no specific pigment was detected in the samples.

The PCA analysis clustered biofilms into 3 groups on the two first principal components (Fig. 3a): biofilms *I*, biofilms *II-III*, and biofilms *IV*. Biofilms *I* were separated from the others along the first axis for their higher proportion of cyanobacteria. Biofilms *II* and *III* differed from the two others along the second axis, which was mainly negatively correlated with C:P and C:N ratios. The PCA analysis performed on gammarid data distinguished biofilm *I* from the others on the first axis, and indicated that gammarids fed with biofilm *I* had lower growth rate and condition index and swam slower than gammarids fed with other biofilm types (Fig. 4a). The correlation circle suggested that "good nutritional quality" biofilms were on the left of the plan, growth, physiological and

behavioral traits representative of "healthy" individuals being positive in this part of the plan (Fig. 4 circle). The first axis of this PCA, which separates biofilms according to their nutritional quality for gammarids, was negatively correlated to the first axis of the PCA analysis performed on biofilm data (r^2 = -0.49, $p < 0.001$) and not to the second one. This places “good nutritional quality" biofilms on the right half of the PCA plan, which corresponds to biofilms with a large proportion of diatoms and a large quantity of fatty acids, HUFA and PUFA (Fig. 3, circle).

Dry weight and Chl *a* biomasses per surface unit were the lowest in biofilm *I*, while biofilm *III* exhibited the highest biomasses (Table 3). Biofilm *IV* was enriched in FA and had the highest potential secondary production compared to the other biofilms.

Biofilm responses to disturbance

Dry period and the following recovery period dramatically changed biofilm algal group proportion compared to pre-disturbance state (Fig. 2a, 2b, 2c). Diatom specific pigments collapsed in post-disturbance biofilms, independently from the dry period duration, while the proportion of cyanobacteria-specific pigments drastically increased in all biofilms except in the biofilm *IV* exposed to a short dry period where green algae became dominant. In post-recovery biofilms, diatom specific pigments were once again well-represented, without reaching pre-disturbance proportion in biofilms *II*, *III* and *IV* (Fig. 2d, 2e). Post-recovery biofilms exposed to a short dry period had a mean diatom proportion (8 ± 9%) significantly lower (t-test, $p = 0.028$) than post-recovery biofilms exposed to a long dry period (18 ± 14%). The proportion of cyanobacteria also differed and tented to be higher (t-test, $p = 0.077$) in recovery biofilms exposed to a short dry period (37 ± 20%) than in those exposed to a long dry period (25 ± 18%).

Drying modified algal, elemental and biochemical composition of biofilms, and reduced their potential nutritional quality, as all the post-disturbance biofilms moved towards the left side of the plan, independently from the dry period duration (Fig. 3b, 3c). This is confirmed by the analysis of first axis coordinates (Fig. 3f). At post-disturbance, biofilms *IV* had the best nutritional quality compared to other biofilms (Fig. 3g). Post-recovery state depended on the biofilm type and, in some cases, on the dry period duration. Between pre-disturbance and post-recovery samplings, biofilms *I* moved towards the

upper right panel, which suggests an increase in potential nutritional quality. Biofilms *II* and *III* moved mainly along the axis 2, indicating an increase in C:P and C:N ratios. Biofilms *IV* moved along the first PCA axis (short dry period) or returned to the same place on the PCA plan (long dry period) (Fig. 3f). Post-recovery biofilms *I* and *II* were a better resource than post-recovery biofilms *III* and *IV* after the short dry period, while post-recovery biofilms had a similar nutritional quality in all biofilms after the long dry period (Fig. 3g).

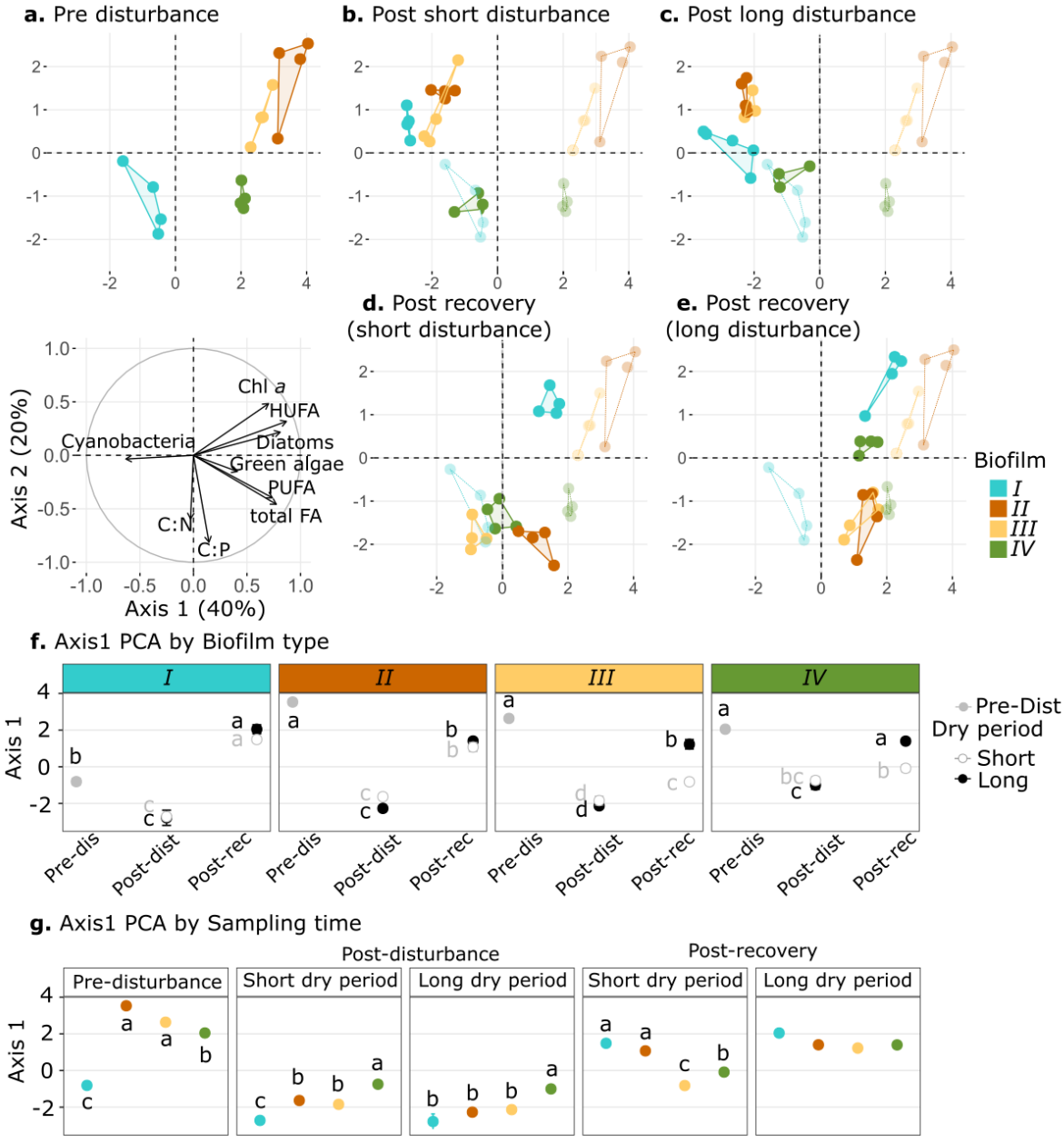


Figure 3: PCA performed on biofilm quality descriptors. Points are grouped by biofilm type (n=4). Each panel (a, b, c, d, e) represents the points corresponding to one sampling time and dry period duration on the same PCA plan. Pre-disturbance composition has been reported in transparency on each panel to make comparisons easier. f. represents the PCA first axis values (mean ± SE, n=4) at each sampling time and reports the statistical results of the multiple comparisons performed between treatments for each biofilm type. g. represents the PCA first axis values (mean ± SE, n=4) for each biofilm type and reports the statistical results of the multiple comparisons performed between biofilms for each sampling time.

Gammarid response to disturbance

Some mortality occurred for gammarids fed with each biofilm sample (Fig. S1). The biofilm *I* was the one with the highest mortality rate as 21 out of 60 individuals died over time, especially when gammarids were fed with biofilm sampled at post-disturbance following the long dry period, as only one third of individuals survived (p=0.006). There was no other significant difference in gammarids' survival.

Drying decreased biofilm nutritional quality for gammarids in biofilms *II* and *III*, with post-disturbance points tending to move towards the right part of the PCA plan (Fig. 4b, 4c). This is confirmed by the significant increase in axis 1 coordinates for biofilms *III* (Fig. 4f). There was no visible effect of the dry period duration on gammarid response. Gammarids fed with all post-recovery biofilms exhibited similar responses, suggesting the previous differences between biofilms disappeared (Fig. 4d and 4e). For biofilms *II* and *III*, the displacement of points along the second axis between post-disturbance and post-recovery states indicated a reduction in gammarid feeding rate after rewetting. Biofilm *I* quality tended to increase during rewetting especially when previously exposed to a short dry period (Fig. 4d, Fig. 4f). Comparisons between biofilm types showed that at post-short disturbance, biofilms *IV* were the best resource for gammarids, while after the long dry period the effects were the same than biofilms *II* and *III* (Fig. 4g). At post-recovery following the short dry period, there was no significant differences on gammarids between all biofilm types, but after the long dry period, the biofilms *III* were significantly a better resource than biofilms *I* for gammarids (Fig. 4g).

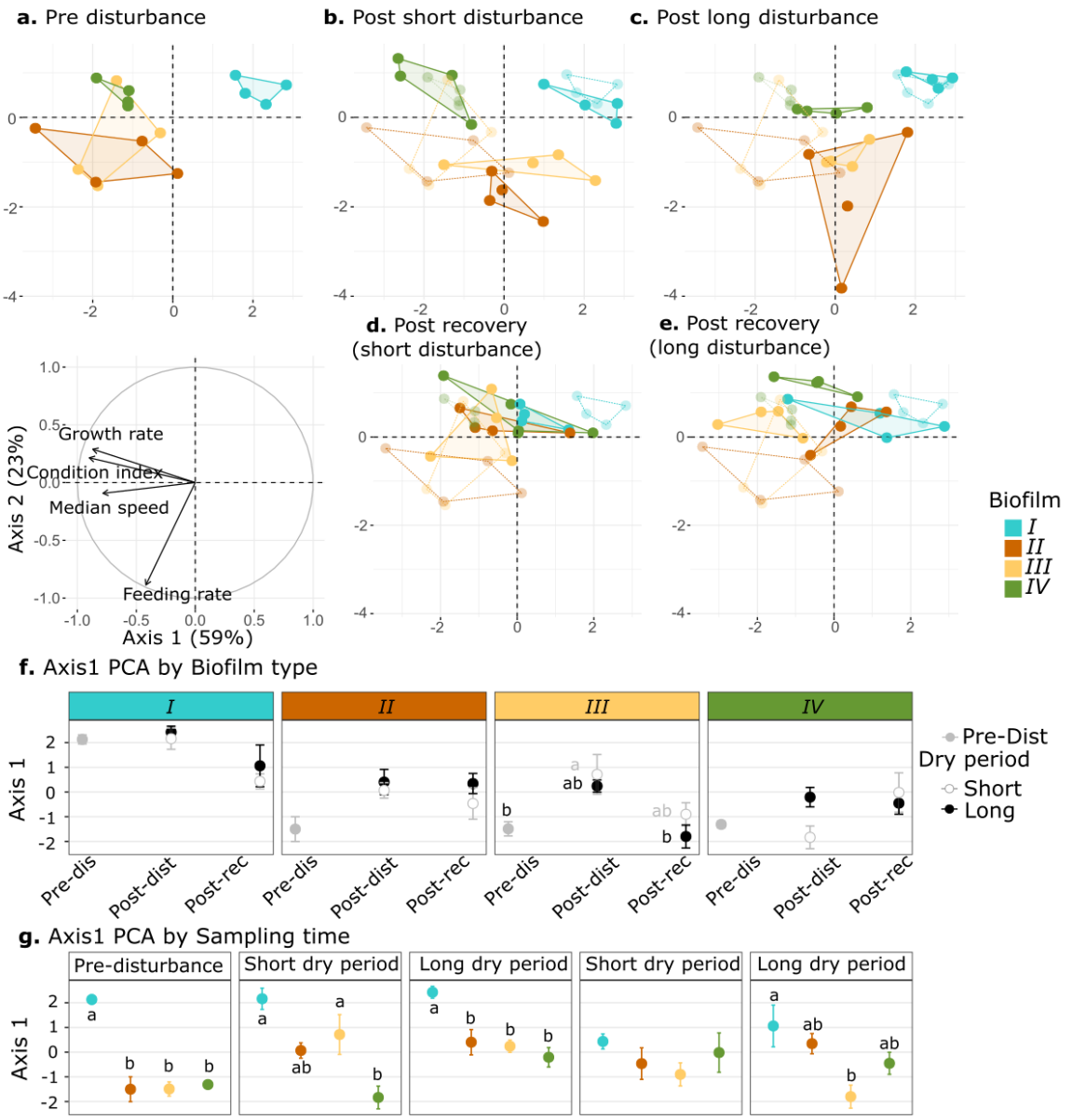


Figure 4: PCA performed on gammarid response variables. Points are grouped according to the biofilm type the individuals were fed with (n=4). Each panel (a, b, c, d, e) represents the points corresponding to one sampling time and dry period duration on the same PCA plan. The response of gammarids fed with pre-disturbance biofilms has been reported in transparency on each panel. f. represents the PCA first axis values (mean ± SE, n=4) at each sampling time and reports the statistical results of the multiple comparisons performed between treatments for each biofilm type. g. represents the PCA first axis values (mean ± SE, n=4) for each biofilm type and reports the statistical results of the multiple comparisons performed between biofilms for each sampling time.

Potential secondary production

In addition to considering the composition of biofilms and the response of gammarids fed with a standardized quantity of biofilm, we tested the effects of the disturbance on the quantity of the biofilm resource available using variables expressed per surface unit (Fig. S2). In order to compare changes over time and between biofilm types, these values were standardized by their respective pre-disturbance biofilm (Fig. 5 and Fig. S3). Biofilm total biomass estimated by total carbon content or dry weight was not significantly affected by the dry period but it significantly increased in every biofilm after rewetting (Table 3). The relative increase to pre-disturbance was higher for biofilm *II* than biofilms *I* and *IV* for both dry period durations (ANOVA, $p < 0.01$, Fig. S3).

The live biomass tended to decrease in every post-disturbance biofilm after a short dry period and significantly decreased after a long dry period, except for biofilm *I* where pre-disturbance chlorophyll *a* content was already low (Table 3). After rewetting, it tended to increase compared to pre-disturbance in all biofilms but results were only significant for biofilms *I* (ANOVA, $p = 0.003$) and *IV* (ANOVA, $p < 0.001$, Fig. 5b).

The absolute content in HUFA per surface unit drastically decreased in every biofilm after drying, but strongly increased after rewetting, no matter the dry period duration (Table 3, Fig. 5c). The increase in HUFA in post-recovery biofilms appeared to depend on both dry period duration and pre-disturbance biofilm type with a strongest increase in HUFA in biofilms *I* and *II* after the short dry period than the long one, while the strongest increase in HUFA was observed after the long dry period in biofilms *III* and *IV* (Fig. 5c).

Based on previous results, we estimated the potential of a given surface of biofilm to sustain secondary production. The potential secondary production was highly correlated with biofilm total carbon ($r = 0.81$) as expected, but also on live biomass estimated through the quantity of chl *a* and total fatty acids per surface unit ($r = 0.60$ and 0.71, respectively) and variables of "good" quality (PUFA, $r = 0.72$ and HUFA, $r = 0.38$). The correlations with C:N and C:P ratios were also positive ($r = 0.34$ and 0.30, respectively).

At post-disturbance, secondary production was not significantly impacted by the short dry period (Table 3, Fig. 5d). The potential secondary production tended to globally increase after rewetting in all biofilms while this increase in relation to pre-disturbance was only significant following a long dry period for biofilm *III* due to high heterogeneity

of available biomass between replicates (Fig. 5d, Table 3, Fig. S4). There was no difference in secondary production between post-recovery biofilms that have experienced a short dry period (ANOVA, p = 0.36 mean = 0.11 ± 0.10 mg. cm^{-2}. day^{-1}) while secondary production of post-recovery biofilms *III* and *IV* mean = 0.23 ± 0.05 and 0.25 ± 0.10 mg. cm^{-2}. day^{-1}) was higher than ones of recovering biofilms *I* and *II* (mean = 0.04 ± 0.1 and 0.08 ± 0.03 mg. cm^{-2}. day^{-1}) after a long dry period (ANOVA, p = 0.04, Fig. S4).

Table 3: Biofilm descriptors (mean ± SD, n=4, except for PUFA, n = 1), expressed per surface unit. Multiple comparisons between biofilms have been tested before disturbance, and multiple comparisons between sampling times have been conducted for each biofilm. Different letters represent significantly different values.

		Dry Weight	Carbon content	Chlorophyll *a*	HUFA	C:N	C:P	Secondary production
		$mg.cm^{-2}$	$mg.cm^{-2}$	$mg.m^{-2}$	$\mu g.cm^{-2}$			$mg.cm^{-2}.\ d^{-1}$
Pre-disturbance	I	1.25 (± 0.22) ^{c}	0.45 (± 0.06) ^{b}	0.03 (± 0.01) ^{c}	8.52	18.22 (± 2.14) ^{a}	224.12 (± 48.59) ^{a}	0.03 (± 0.02) ^{b}
	II	2.74 (± 0.43) ^{b}	0.41 (± 0.19) ^{b}	0.31 (± 0.07) ^{a}	10.14	12.49 (± 0.32) ^{c}	171.94 (± 65.54) ^{ab}	0.03 (± 0.01) ^{b}
	III	3.16 (± 0.25) ^{a}	0.53 (± 0.11) ^{b}	0.31 (± 0.10) ^{a}	18.20	16.37 (± 0.90) ^{b}	119.47 (± 19.53) ^{b}	0.05 (± 0.02) ^{b}
	IV	2.49 (± 0.18) ^{b}	0.78 (± 0.05) ^{a}	0.14 (± 0.03) ^{b}	12.90	17.75 (± 1.15) ^{a}	185.31 (± 20.59) ^{ab}	0.09 (± 0.02) ^{a}
I	Pre-dist	1.25 (±0.18) ^{b}	0.45 (±0.06) ^{b}	0.03 (± 0.01) ^{b}	8.52	18.22 (± 2.14) ^{a}	224.12 (± 48.59) ^{a}	0.03 (± 0.02) ^{ab}
	Post-dist S	0.90 (±0.32) ^{b}	0.31 (±0.14) ^{b}	0.02 (± 0.02) ^{b}	2.30	12.03 (± 1.75) ^{b}	148.61 (± 35.30) ^{b}	0.01 (± 0.01) ^{b}
	Post-dist L	0.88 (±0.15) ^{b}	0.31 (±0.06) ^{b}	0.00 (± 0.00) ^{b}	0.38	14.41 (± 3.43) ^{ab}	169.29 (± 51.86) ^{ab}	0.02 (± 0.02) ^{b}
	Post-recov S	2.73 (±0.15) ^{a}	1.06 (±0.08) ^{a}	0.55 (± 0.11) ^{a}	30.02	11.72 (± 1.72) ^{b}	139.18 (± 15.81) ^{b}	0.08 (± 0.04) ^{a}
	Post-recov L	2.59 (±0.56) ^{a}	0.94 (±0.21) ^{a}	0.40 (± 0.26) ^{a}	25.42	10.75 (± 1.26) ^{b}	107.55 (± 13.78) ^{b}	0.04 (± 0.04) ^{ab}
II	Pre-dist	2.74 (±0.43) ^{d}	0.41 (±0.19) ^{c}	0.31 (± 0.07) ^{bc}	10.14	12.49 (± 0.32) ^{a}	171.94 (± 65.54) ^{b}	0.03 (± 0.01) ^{b}
	Post-dist S	3.87 (±0.52) ^{c}	0.52 (±0.08) ^{c}	0.17 (± 0.07) ^{cd}	3.30	10.18 (± 0.09) ^{c}	128.04 (± 14.54) ^{b}	0.02 (± 0.01) ^{b}
	Post-dist L	2.71 (±0.26) ^{d}	0.28 (±0.08) ^{c}	0.02 (± 0.01) ^{d}	0.78	11.51 (± 0.99) ^{b}	101.16 (± 33.94) ^{b}	0.01 (± 0.01) ^{b}
	Post-recov S	6.72 (±0.25) ^{a}	1.61 (±0.16) ^{a}	0.48 (± 0.07) ^{a}	69.41	11.17 (± 0.35) ^{bc}	335.45 (± 28.83) ^{a}	0.13 (± 0.10) ^{a}
	Post-recov L	5.41 (±0.76) ^{b}	1.17 (±0.33) ^{b}	0.41 (± 0.13) ^{ab}	51.39	11.43 (± 0.40) ^{b}	271.83 (± 66.06) ^{a}	0.08 (± 0.03) ^{a}
III	Pre-dist	3.16 (±0.25) ^{b}	0.53 (±0.11) ^{b}	0.31 (± 0.10) ^{ab}	18.20	16.37 (± 0.90) ^{b}	119.47 (± 19.53) ^{b}	0.05 (± 0.02) ^{b}
	Post-dist S	3.21 (±0.29) ^{b}	0.64 (±0.27) ^{b}	0.14 (± 0.05) ^{bc}	6.16	12.77 (± 1.19) ^{c}	128.57 (± 54.24) ^{b}	0.02 (± 0.01) ^{b}
	Post-dist L	3.42 (±0.42) ^{b}	0.53 (±0.04) ^{b}	0.10 (± 0.03) ^{c}	4.17	11.88 (± 1.05) ^{c}	111.87 (± 18.23) ^{b}	0.02 (± 0.01) ^{b}
	Post-recov S	7.10 (±0.35) ^{a}	1.86 (±0.20) ^{a}	0.34 (± 0.06) ^{ab}	58.52	19.94 (± 0.78) ^{a}	227.65 (± 31.76) ^{a}	0.21 (± 0.13) ^{a}
	Post-recov L	7.78 (±0.93) ^{a}	1.93 (±0.30) ^{a}	0.50 (± 0.24) ^{a}	99.24	16.67 (± 0.72) ^{b}	171.37 (± 27.13) ^{ab}	0.23 (± 0.05) ^{a}
IV	Pre-dist	2.49 (±0.22) ^{b}	0.78 (±0.05) ^{b}	0.14 (± 0.03) ^{b}	19.20	17.75 (± 1.15) ^{a}	185.31 (± 20.59) ^{ab}	0.09 (± 0.02) ^{ab}
	Post-dist S	2.48 (±0.18) ^{b}	0.73 (±0.08) ^{b}	0.04 (± 0.02) ^{bc}	6.36	19.69 (± 1.20) ^{a}	192.18 (± 10.61) ^{a}	0.11 (± 0.04) ^{ab}
	Post-dist L	1.87 (±0.33) ^{b}	0.53 (±0.05) ^{b}	0.01 (± 0.01) ^{c}	2.17	17.21 (± 1.09) ^{a}	190.84 (± 30.69) ^{a}	0.04 (± 0.01) ^{b}
	Post-recov S	4.06 (±0.82) ^{a}	1.48 (±0.31) ^{a}	0.13 (± 0.07) ^{bc}	36.93	17.39 (± 1.40) ^{a}	206.30 (± 26.15) ^{a}	0.14 (± 0.14) ^{ab}
	Post-recov L	4.77 (±0.76) ^{a}	1.73 (±0.28) ^{a}	0.58 (± 0.14) ^{a}	58.45	13.63 (± 1.50) ^{b}	142.38 (± 8.69) ^{b}	0.25 (± 0.10) ^{a}

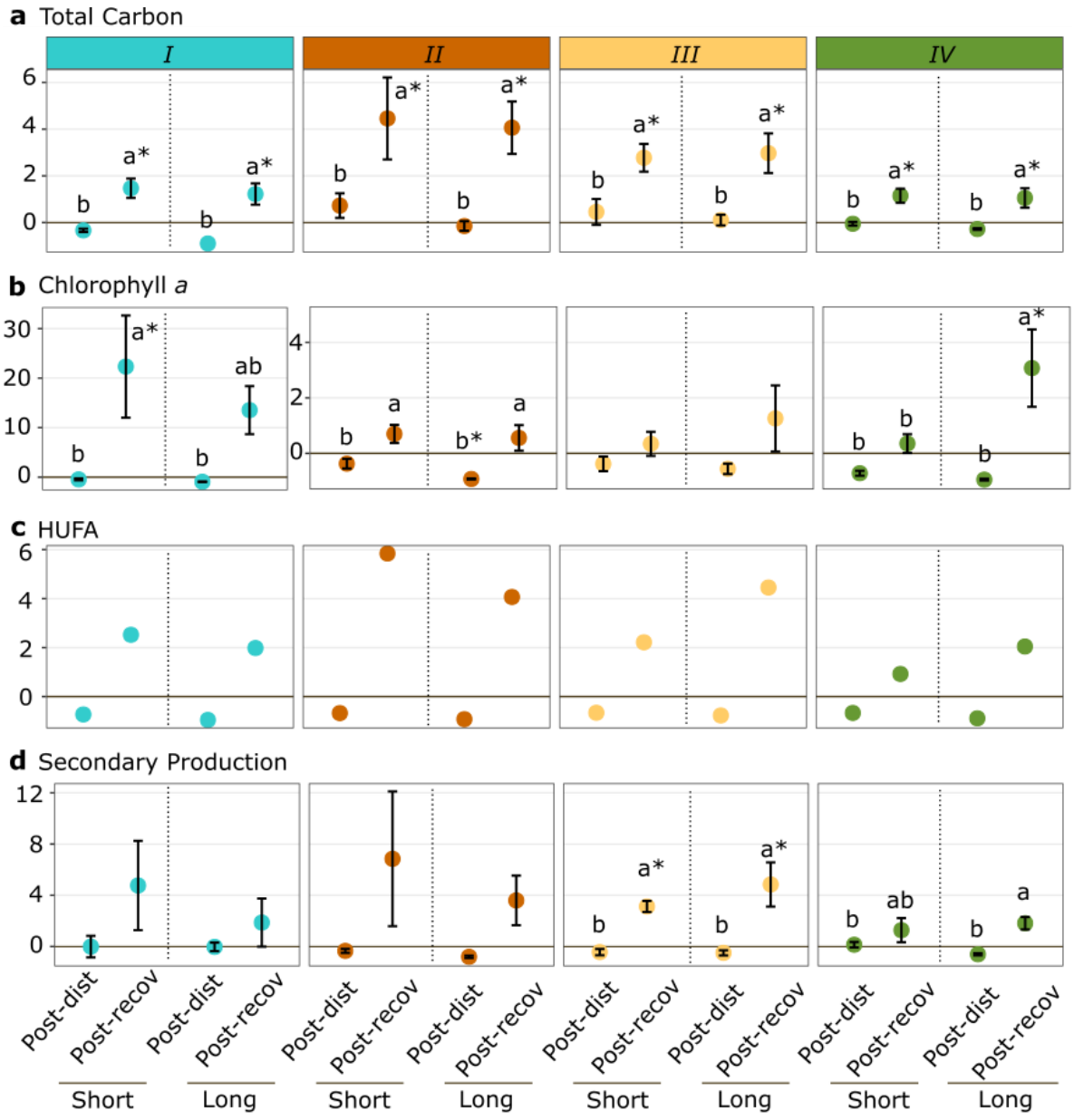


Figure 5: Relative changes compared to pre-disturbance in total carbon (mg. cm^{-2}; a), chlorophyll a (mg. cm^{-2}; b), HUFA (mg. cm^{-2}; c) and potential secondary production (mg gammarids. cm^{-2}. day^{-1}; d) between sampling time and dry period duration for each biofilm type. For more clarity, short and dry period have been separated on each plot (short and long dry periods are respectively on the left and on the right), but multiple comparisons were conducted between each sample. For each biofilm type, different letters, means significant differences, and asterisks represent significant changes in relation to pre-disturbance. For changes in chl *a* content (b), the axis unit differs between biofilm *I* and other biofilms.

4. Discussion

In this study, we investigated how dry period affects biofilms' capacity to sustain secondary production in river ecosystems by comparing biofilms with contrasted algal compositions and by considering both their qualitative and quantitative dimensions.

In agreement with our first hypothesis, the overall quality of biofilms significantly decreased during the dry period. As expected, the diatom specific pigments were not found in post-disturbance biofilms where only cyanobacteria and green algae specific pigments remained, but in very low quantities. This is in line with several studies arguing that organisms belonging to those groups have the ability to set up desiccation resistance mechanisms (Potts, 1999; Romaní et al., 2013). Particularly, in our study, PUFA and HUFA were almost undetectable in post-drying biofilms. Similar results were observed on biofilms from Mediterranean streams subjected to drought (Sanpera-Calbet et al., 2017). While drying decreased the overall quality of biofilms, it did not change the relative qualities between biofilms, with diatom and green alga-dominated biofilms *IV* remaining significantly more qualitative while cyanobacterial biofilm *I* less qualitative than the other ones. However, rewetting significantly changed initial differences in nutritional quality between biofilms. More specifically, biofilm *I* that was initially the least good resource became the better one after a short dry period. Such an increase in biofilm quality was partly due to the decrease in cyanobacteria specific pigments in biofilms, their replacement by recovering diatom specific pigments, associated to an increase in HUFA and PUFA. Therefore, our results highlight that initial algal biofilm composition is crucial in shaping drying effect on biofilm nutritional quality and confirm that the dominance of diatoms, as a high-quality food source compared to cyanobacteria (Martin-Creuzburg and von Elert, 2009), drives the biofilm nutritional quality during river intermittency. Our results also seem to marginalize the role of C:P and C:N ratios in biofilm nutritional quality for gammarids, compared to fatty acid composition. Indeed, the second axis of the PCA analysis performed on biofilm data, mainly associated with these ratios, was not correlated to gammarids response. Stoichiometric ratios could be a better predictor when studying allochthonous versus autochthonous resource, as generally, microalgae exhibit lower C:N ratios than terrestrial organic matter, with generally no significant differences between different streams primary producers (Lau et al., 2009). However, the importance

of algal FA is crucial as they are critical for growth of consumers as it was shown on several macroinvertebrate consumers (Torres-Ruiz et al., 2007).

Desiccation is a disturbance that severely impacts ecosystems (Sarremejane et al., 2022). Some studies have highlighted the negative impact of a short dry period on cell viability, especially on diatoms (Souffreau et al., 2010, Barthès et al., 2015b). In our study, even a short dry period (3 days) was sufficient to strongly decrease biofilm nutritional quality, certainly because of diatom vanishing. On the opposite, the dry period duration negatively impacted biofilm variables expressed per surface unit, corroborating the hypothesis that dry period duration magnifies the negative impact of the disturbance on resource availability. This is especially true on the algal biomass that was more strongly affected by drying after the longest dry period in biofilms *II, III* and *IV* and this is consistent with results from other studies, where it was shown that the longer the dry period duration, the higher the decrease in chlorophyll *a* (Acuña et al., 2015; Angradi and Kubly, 1993). In the present study, the effects of the dry period duration on the biofilm nutritional quality were more noticeable in post-recovery biofilms, and highlight a better resilience in nutritional quality of biofilms *III* and *IV* after the long dry period than the short one. This was partially linked to a better recovery of diatoms after the longest dry period, consistently with the results of Barthes et al (2015a). These authors suggested the low recovery of diatoms for short dry periods to be the result of direct or indirect negative interactions with cyanobacteria, which were more resistant. For longer dry periods, the cyanobacteria were more affected by dryness which may have reduce their negative effect on diatoms, allowing a better recovery of the latter.

Gammarid growth and physiological state depended on biofilm live biomass and nutritional quality, as the more chlorophyll *a* and fatty acids were available in pellets, the more gammarid individuals grew effectively. Similar results were observed on *Chironomus* growth along a concentration of resource with different quality (Goedkoop et al., 2007). In addition, gammarid feeding rate was linked to algal biomass as the more the chlorophyll *a* content increased in pellets, the more gammarids consumed them. Moreover, when the biofilm quality tended to decrease, biofilm consumption decreased as evidenced by the negative relationship between gammarid feeding rate and biofilm C:N and C:P ratios. Such results are opposed to what could have been expected, as usually,

when food quality decreased, consumers enhance their feeding rate for compensatory feeding as shown on caddisfly larvae, another detritivores (Flores et al., 2014).

Despite a consistent response between gammarid ecological traits and biofilm nutritional quality indicators, the effects of river intermittency that our results revealed on biofilm nutritional quality did not translate into so strong effects on gammarid response and estimated secondary production. There were notably few effects of the dry period on the post-disturbance secondary productions while live biomass and HUFA contents were largely decreased by drying. We believe this was because of the insignificant decrease in Carbon content on which secondary production was calculated. Secondary production systematically increased after rewetting, probably driven by important biomass biofilm accrual. Such a significant biomass accrual after flow resumption has been already observed in natural environments (Robson, 2000). After rewetting, and following the long dry period, the secondary production of biofilms *I* and *II* was significantly lesser than the ones of biofilms *III* and *IV*, but such differences cannot be explained by consistent differences in variables we considered as representative of a good quality resource as HUFA. Biofilm *II* biomass, fatty acid content and C:N ratio that turned out to be positively correlated with secondary production were in the same range than in biofilms *III* and *IV*, while biofilm *I* C:P ratio was lower. Thus, low secondary production in biofilm *II* might be related to a phosphorus limitation. It was shown that when fed with P-limited resource, the growth of *Daphnia,* a pelagic filter-feeder, was negatively impacted despite HUFA availability (Ruiz et al., 2021). Several other studies on pelagic ecosystems have highlighted the negative effect of P limitation on *Daphnia* growth and reproduction (Boersma, 2000; Ravet and Brett, 2006). Studies have highlighted that the importance of food quality decreases at low quantity (Hartwich et al., 2012; Sterner, 1997). The quantity of HUFA strongly increased during recovery of biofilm *I*, but it might be not only due to a large variability between replicates but also to a limited biomass availability compared to other biofilm types. Such results emphasize the importance to consider both quantitative and qualitative aspects when studying the ability of a resource to sustain secondary production.

In addition, it would be interesting to assess in what extent these differences persist over time, as the dry period disturbance has pushed biofilms on a new dynamic trajectory. Biofilms did not return to their pre-disturbance state after more than two weeks, and the

new community assembly exhibits different properties. Following the principle of ecological resilience (Holling, 1973), the communities that shifted to an alternative steady state would continue to develop and thus increase their capacity to sustain secondary production. It would have been interesting to assess taxonomic changes over time in biofilms, as even if pigment analyses provide useful information about the main algal group representation in biofilms, it was not sufficient to clearly explain the effects on resource quality.

Conclusion

Our study assesses the effects of dry period on aquatic benthic resources, depending on both the composition of these resources before the disturbance and the intensity of the dry period. We show that desiccation significantly reduced the biofilm nutritional quality indicators and that despite a magnification of some effect after the longer dry period, even a short drying had strong negative impacts on these parameters. During rewetting, biofilm compositions changed in very diverse ways, depending on their initial composition. Finally, our results also showed increases in biofilm biomasses after rewetting compared to biomasses observed before drought. Such increases might have compensated for reduced biofilm quality generally observed after drought, highlighting the need to consider both the quality and the quantity when assessing the ability of a resource to sustain secondary production.

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Author Contribution Statement

Conceptualisation: CC, SB, MD, VF, JL. Developing methods: CC, SB, AB, MD, VF, MRB, JL. Data analysis: CC. Preparation of figures and tables: CC. Conducting the research, data interpretation, writing: CC, SB, AB, MD, VF, MRB, JL.

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Supplementary information

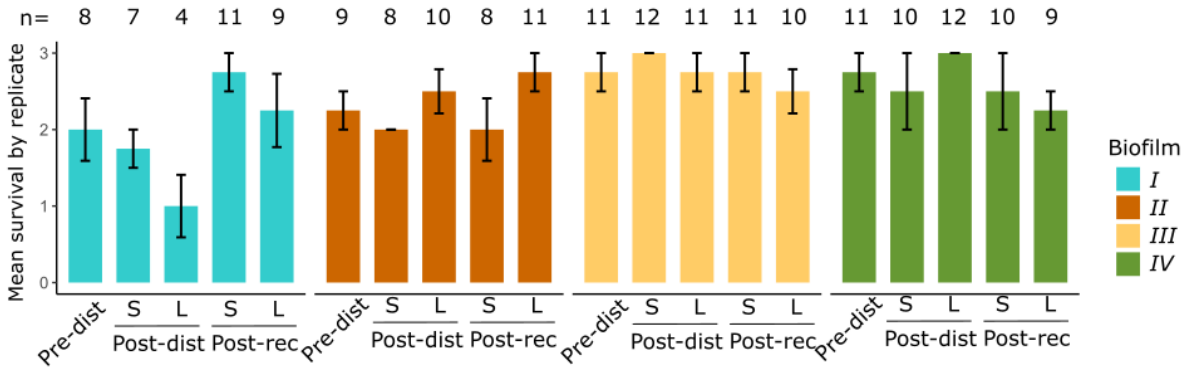


Figure S1: Gammarid survival per replicate (mean ± SE n=4) for each biofilm type and sampling time (S for short dry period and L for long dry period). Values on the top of bars represent the number of survivors at the end of the feeding experiment and on which ecological trait measurements were conducted.

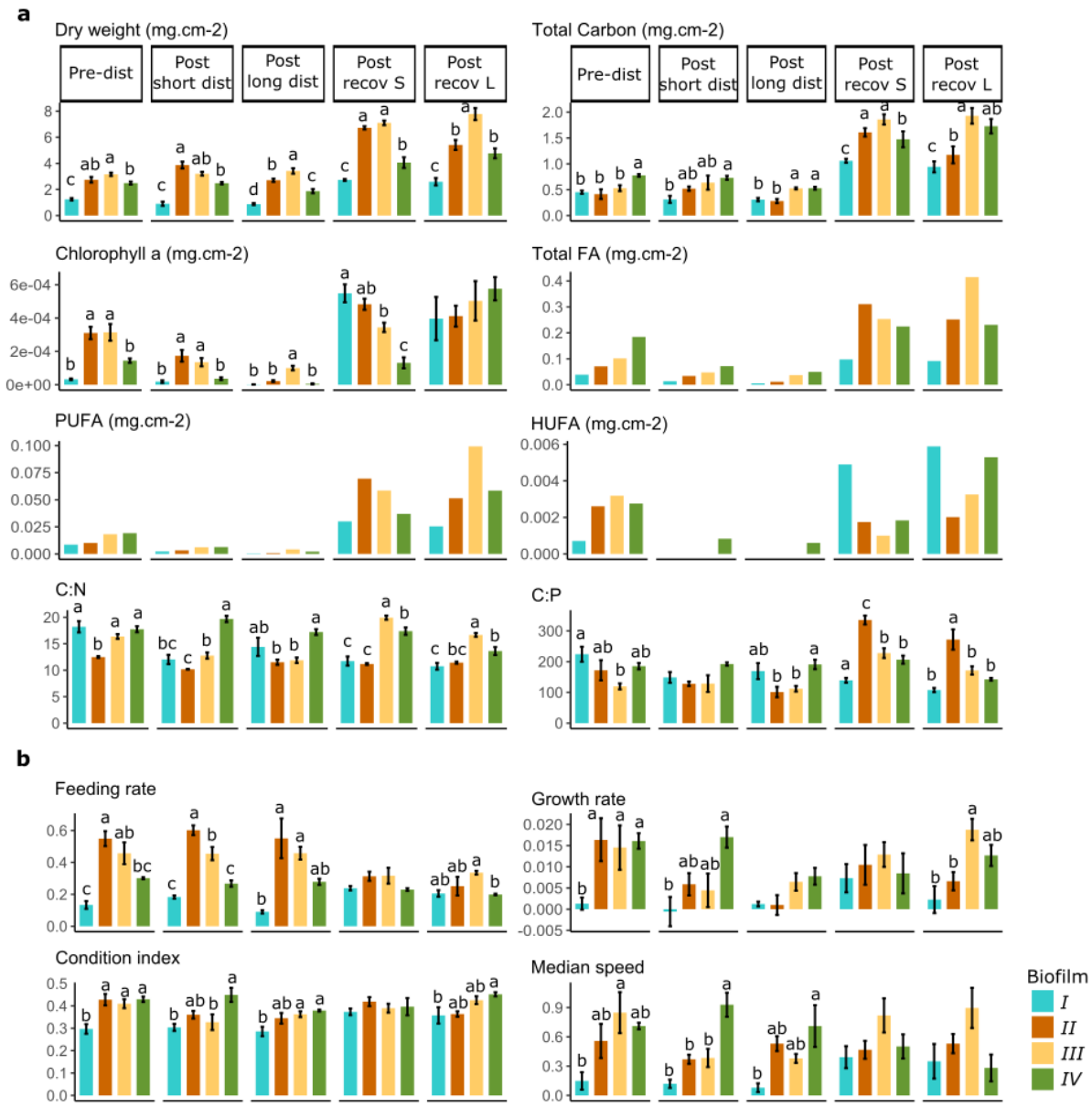


Figure S2: Changes in biofilms' quality variables (a) and gammarids' response variables (b) between biofilm types at each sampling time (mean ± SE, n=4).

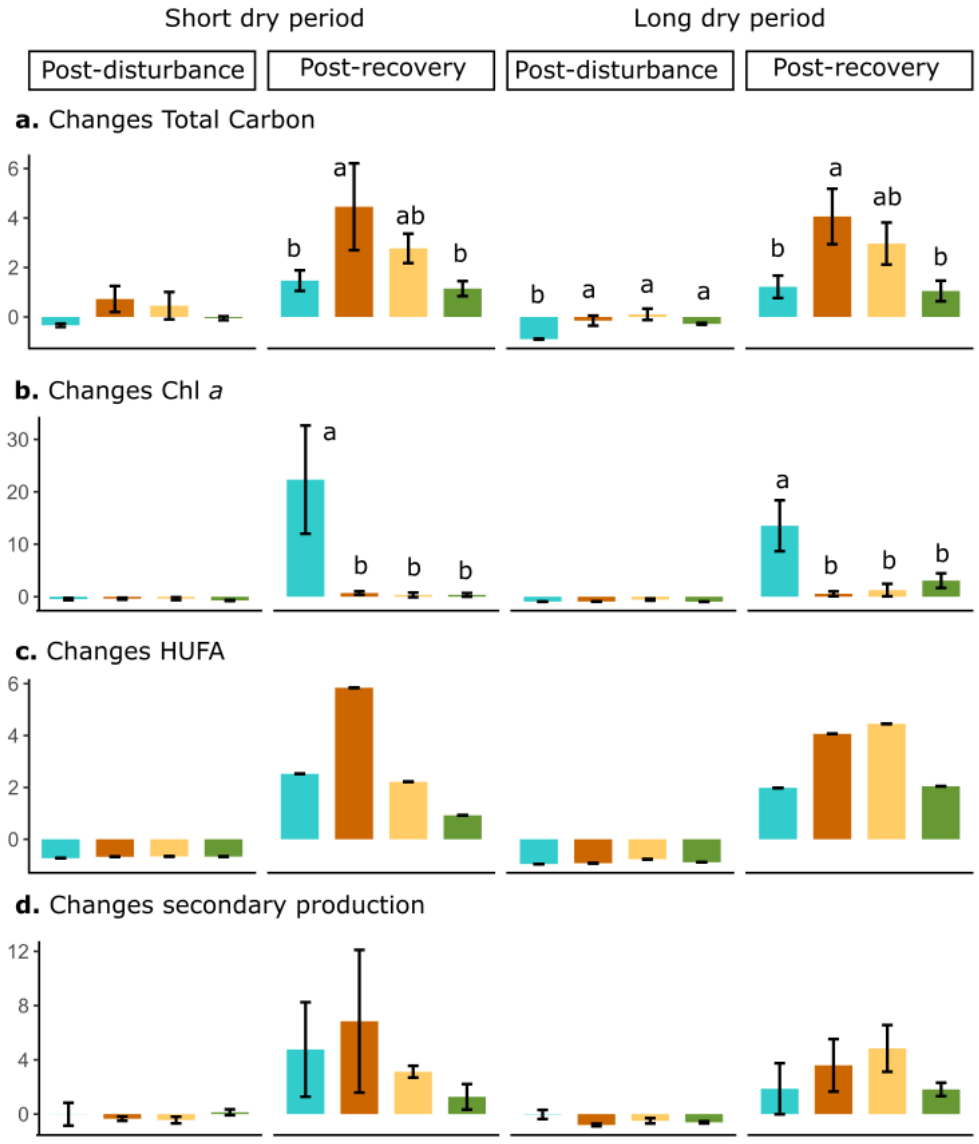


Figure S3: Relative changes compared to pre-disturbance in total carbon (mg. cm^{-2}; a), chlorophyll a (mg. cm^{-2}; b), HUFA (mg. cm^{-2}; c) and potential secondary production (mg gammarids. cm^{-2}. day^{-1}; d) between biofilm types at each sampling time (mean change ± SE, n=4). Multiple comparisons between biofilm types have been carried out for each sampling time.

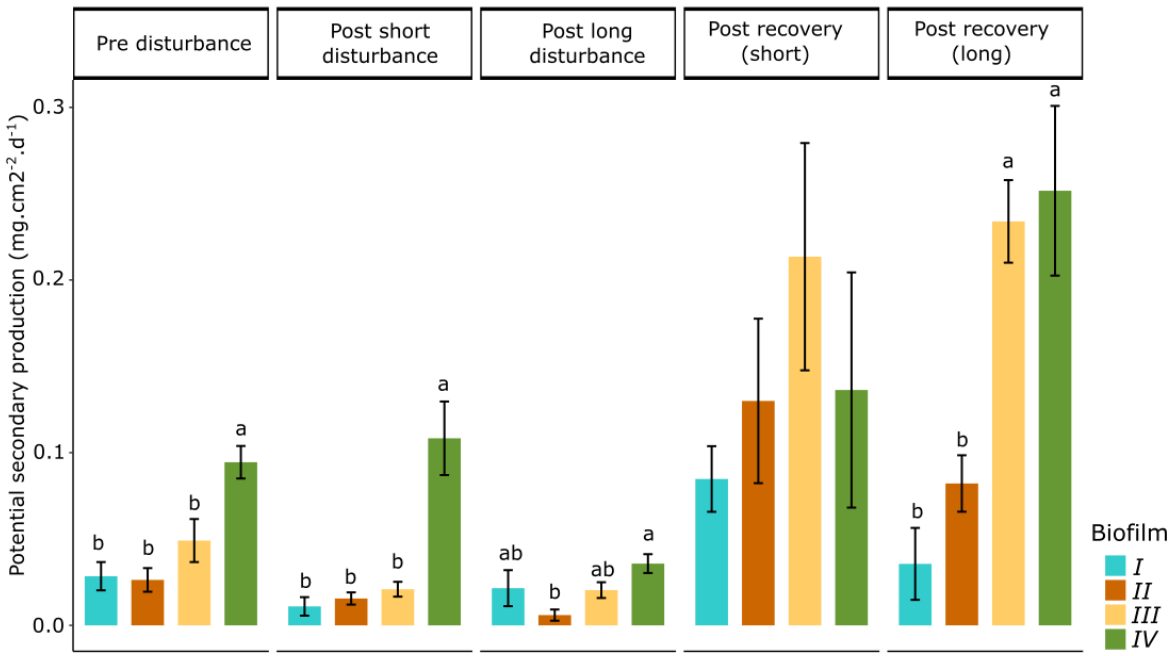


Figure S4: Evolution of potential secondary production (in mg gammarids .cm^{-2}. day^{-1}) in biofilms at each sampling time (mean ± SE, n=4). Multiple comparisons between biofilm types have been carried out for each sampling time.

Chapitre 3

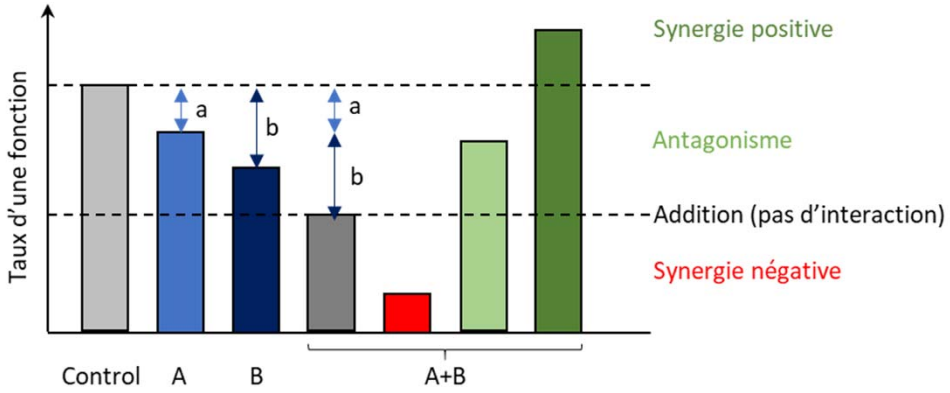
Identification des propriétés d'une séquence de perturbations qui contrôlent la stabilité fonctionnelle des communautés

Chapitre 3 : Identification des propriétés d'une séquence de perturbations qui contrôlent la stabilité fonctionnelle des communautés

Contexte

La majorité des expériences qui testent l'effet des perturbations sur la stabilité des communautés microbiennes considèrent une perturbation unique et concernent les communautés de sol (Philippot et al., 2021, Rillig et al., 2019, Shade et al., 2012). Pourtant, dans le milieu naturel, toutes les communautés microbiennes sont généralement exposées à des perturbations multiples, simultanées ou séquentielles. Or, il est difficile de prédire comment les communautés vont répondre à des multiples perturbations à partir de résultats issus de la réponse à une seule perturbation (Rillig et al., 2019).

Il a été montré que des perturbations multiples simultanées pouvaient soit avoir des effets additifs soit interagir de différentes manières, induisant alors des effets plus forts (synergie négative) ou plus faibles (antagonisme), voire même opposés (synergie positive) (Piggott et al., 2015).



Lorsque l'on considère des perturbations séquentielles, la stabilité des communautés est conditionnée par différentes propriétés de la séquence (Philippot et al., 2021) :

- L'intervalle de temps entre les deux perturbations
- La nature des perturbations (ex : stress physique vs. chimique)
- L'intensité des perturbations
- Le nombre total d'événements (i.e. le nombre de répétitions)
- La chronologie des événements

A l'heure actuelle, il n'existe pas de critère permettant de prédire ou d'anticiper la réponse des communautés microbiennes à des répétitions de perturbations, alors que ces séquences se produisent fréquemment dans le milieu naturel (ex : vagues de chaleurs en été avec pics journaliers de températures).

Objectifs et hypothèses

Dans ce chapitre, l'objectif est de tester de manière systématique l'effet de l'intervalle de temps sur les interactions entre des perturbations successives (Gunderson et al., 2016), et d'identifier dans quelle mesure cet effet est modulé par l'intensité et la nature des perturbations. Pour se faire, des biofilms synthétiques de laboratoire ont été exposés à des séquences de perturbations identiques, de différentes natures, intensités, et intervalles de temps.

Mes hypothèses sont :

1. L'augmentation de l'intervalle de temps entre deux répétitions favorise les effets additifs entre les événements (pas d'interaction).
2. Pour un même intervalle de temps, l'augmentation de l'intensité de la perturbation favorise les interactions.
3. La réponse dépend de la nature de la perturbation.

Matériel et méthodes

1. Les communautés microbiennes

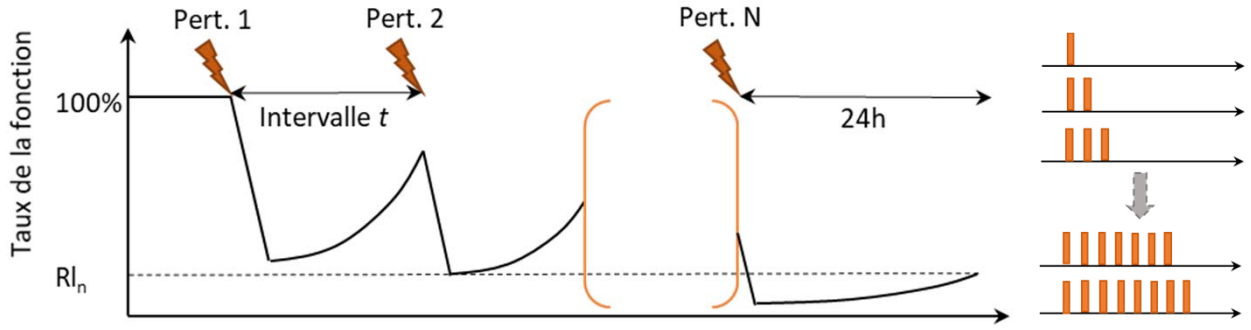
Pour ces expériences, des communautés synthétiques on été générées à partir de 5 espèces de diatomées cultivées de façon non-axénique au laboratoire (*Eolimna subminuscule*, *Fistulifera saprophila*, *Gomphonema parvulum, Navicula pelliculosa* et *Nitzschia palea*). Le but est d'obtenir un biofilm phototrophe de compositions fixée et répétable.

2. Les séquences de perturbations

Pour répondre aux objectifs, la réponse des communautés à des séquences de perturbations identiques a été testée pour 3 natures de perturbations (stress salin, stress chimique (cuivre) et stress thermique), appliquées à 5 intensités et selon 3 intervalles de temps (30 min, 3h et 22h).

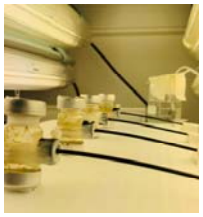
	Salin	**Cuivre**	**Thermique**
Intensité	5, 10, 15, 20 et 25 $g.L^{-1}$	2, 5, 10, 15, 20 $mg.L^{-1}$	35, 37, 39, 40 et 41°C

Chaque perturbation a été répétée entre 2 et 8 fois (n). L'effet sur la fonction a été mesuré 24h après chaque fin de perturbation (Rl_n).



3. Mesures de production primaire brute

La fonction suivie était la production primaire brute (PPB). La concentration d'oxygène produite a été mesurée à l'aide de capteurs via une fibre optique. Dans un premier temps, les mesures ont été réalisées dans des conditions lumineuses pour mesurer la production nette d'O_2, puis à l'obscurité pour mesurer la consommation d'O_2. La production brute a alors été calculée en soustrayant la respiration (consommation) à la production nette.



4. Analyses des données

Pour déterminer les effets entre les événements de la séquence sur l'état de la fonction, la récupération fonctionnelle a été modélisée ainsi :

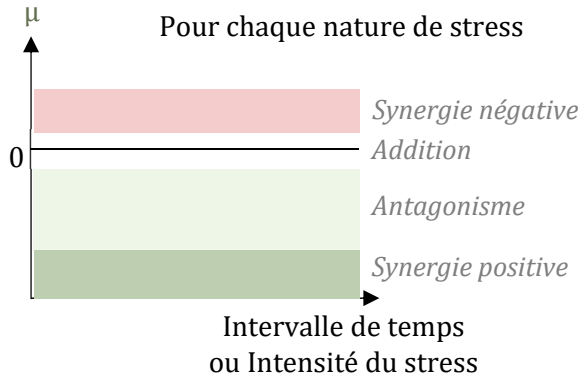
$$Rl_{n+1} = (100 - \Delta_1)(100 - (\Delta_1 + \mu))^n$$

Avec, n = le nombre de répétitions,

Δ_1 = 100 – Rl1 : la perte de PPB par rapport au contrôle mesurée 24h après la fin de la 1ère perturbation

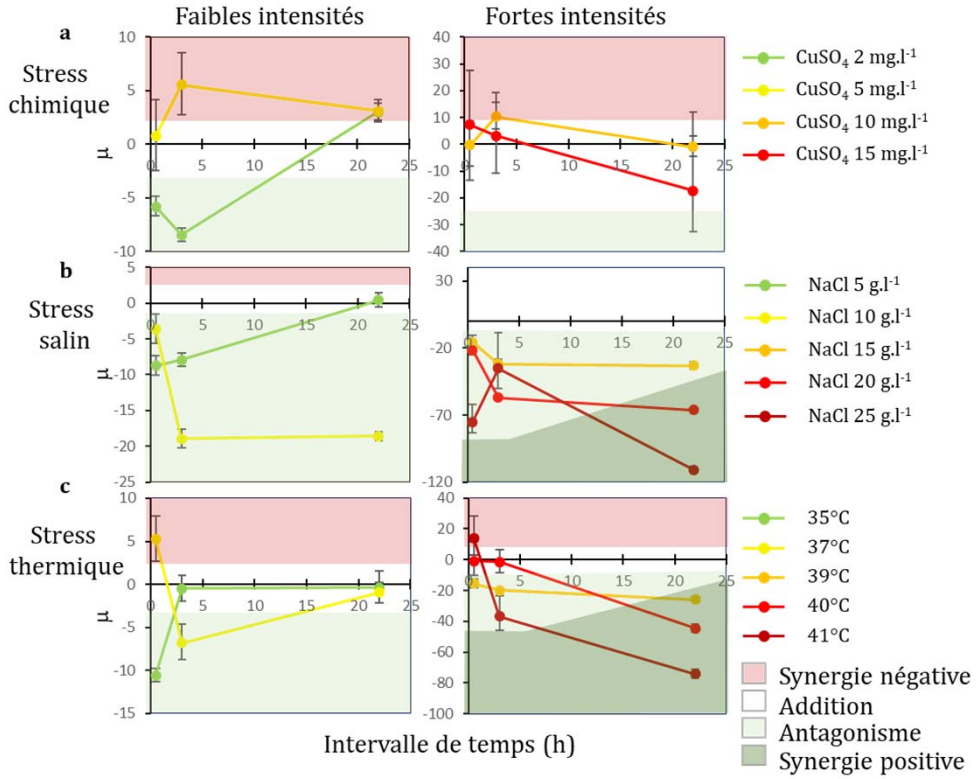
μ : paramètre ajusté mathématiquement à chaque séquence pour décrire au mieux (AIC) l'évolution de l'effet des perturbations successives

- si μ =0, addition des effets
- si μ > 0, amplification des effets (synergie négative)
- si $\Delta_1 < \mu < 0$, atténuation des effets (antagonisme)
- si $\mu < -\Delta_1$: inversion des effets (synergie positive)



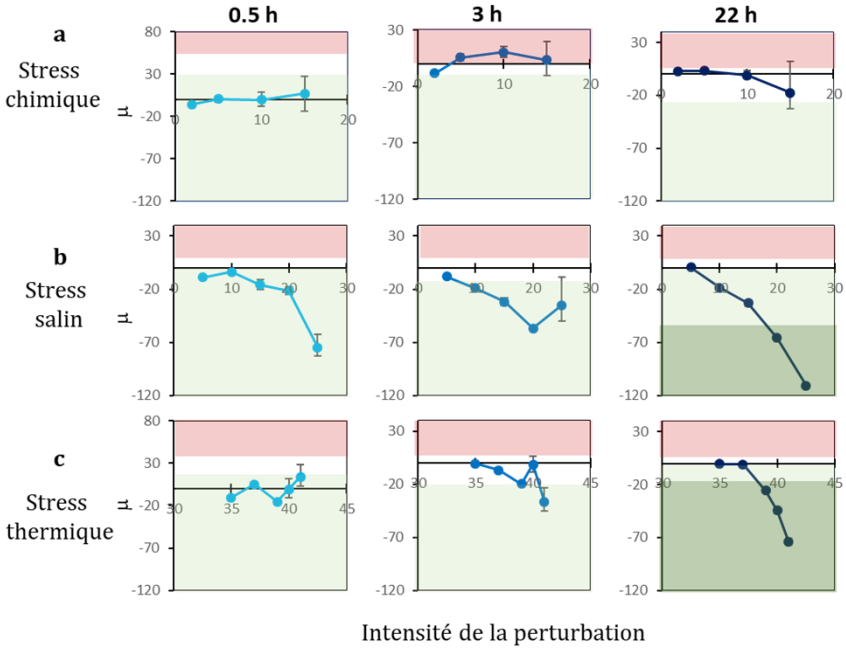
Résultats

μ en fonction de l'intervalle de temps



Aucune tendance claire n'est observée pour le stress chimique (cuivre). Pour les stress salins et thermiques, deux types de réponses sont observés en fonction de l'intensité de la perturbation. Pour les faibles intensités, le paramètre μ tend vers zéro à mesure que l'intervalle de temps augmente, ce qui met en évidence l'absence d'interactions (effets additifs). Pour les fortes concentrations, c'est l'inverse qui est observé : quand l'intervalle de temps augmente, le paramètre μ aussi.

μ en fonction de l'intensité de la perturbation



Il y a peu d'effet de l'intensité du stress chimique sur μ, peu importe l'intervalle de temps.

Pour les stress salins et thermiques, l'augmentation de l'intensité des perturbations successives augmente la valeur de μ, tout particulièrement pour l'intervalle de temps le plus long.

Conclusions et Perspectives

Le modèle développé dans cette étude permet d'identifier les effets entre les perturbations au sein d'une séquence de perturbations identiques.

Avantages de l'approche : à partir des Rl_n mesurés pour chaque répétitions de perturbations (n = 2 à 8), et de la perte du taux de fonction entrainé par le premier stress, un seul paramètre μ est estimé pour décrire ces effets.

Influence de l'intervalle de temps entre les répétitions

Les résultats mettent en évidence que l'augmentation de l'intervalle de temps favorise les effets additifs entre les répétitions, seulement pour les perturbations de faibles intensités, alors que pour les fortes intensités, au contraire, des interactions positives (antagonismes ou synergies positive) sont observées. Cependant, cela dépend de la nature de la perturbation

→ Validation partielle de l'hypothèse 1

Il est possible que pour les intensités les plus fortes, l'intervalle de temps ne soit pas suffisamment long pour observer une indépendance des réponses induites par chaque perturbation.

Influence de l'intensité de la perturbation

L'augmentation de l'intensité des perturbations successives induit une augmentation des interactions entre les répétitions pour les intervalles de temps les plus longs, pour les stress salins et thermiques.

→ Validation partielle de l'hypothèse 2

Peu d'interactions sont mises en évidence pour le stress chimique, avec majoritairement des effets additifs des stress, contrairement aux deux autres natures de stress.

Influence de la nature de la perturbation

De manière générale, les résultats observés dépendent fortement de la nature des stress appliqués.

→ Validation de l'hypothèse 3

Les résultats montrent que dans l'ensemble, des effets de synergies positives et d'antagonismes sont davantage observés. Ces résultats sont en accord avec l'hypothèse de Vinebrooke et al. (2004). C'est-à-dire que dans cette étude les tolérances des espèces à chaque perturbation sont corrélées puisque les perturbations appliquées sont de même nature.

Par la suite, il sera intéressant de chercher à savoir si la réponse des biofilms à une séquence de perturbations identiques peut être prédite à partir de la réponse à une seule perturbation.

The time-dependency of interactions between successive disturbances is driven by the nature and intensity of events

Camille Courcoul[1*], Stéphanie Boulêtreau[1], Jessica Ferriol[1], Vincent Baillard[2], Martin Laviale[2], Elise Billoir[2], Joséphine Leflaive[1]

[1] - Laboratoire écologie fonctionnelle et environnement, Université de Toulouse, CNRS, INPT, UPS, Toulouse, France

[2] - Université de Lorraine, CNRS, LIEC, F-57000 Metz, France

*camille.courcoul@univ-tlse3.fr

1. Introduction

Microorganisms are everywhere and their essential role in the provision of ecosystem goods and services is irrefutable. Microbial communities are permanently exposed to natural disturbances whose intensity and frequency are expected to increase with global change (Hoegh-Guldberg et al., 2018). Understanding the stability of communities exposed to these disturbances is essential to predict the future of the ecosystem services they provide. When disturbances are strong enough, a transition of the system from a stable state to an alternative stable state can be induced, which is referred as 'ecological resilience' (Holling, 1973). In other cases, the community structure or functioning returns partially or totally to its initial state, which corresponds to 'engineering resilience' (Holling, 1996). This idea of variables returning to their initial values fits with the concept of ecological stability defined by Pimm (1984). The key components of stability include resistance (ability to withstand a disturbance, measured as the difference compared to an undisturbed control), speed of recovery and resilience (maximum degree of recovery after a period of time) (Lloret et al., 2011). These dimensions of stability can be either correlated or decorrelated, according to whether they refer to the structure or to the functioning of a community (Hillebrand et al., 2018).

Although many studies considered the stability of microbial communities facing disturbances, several biases prevent from drawing a global picture. In most cases, only one dimension of stability (resistance) is considered, giving a partial understanding of the

system response to a disturbance (Donohue et al., 2016). The majority of the experiments used soil communities and applied a single disturbance (Philippot et al., 2021; Rillig et al., 2019; Shade et al., 2012). However, disturbances are rarely isolated and, natural environments are almost always exposed to multiple human-derived stressors (Folt et al., 1999), either simultaneously or sequentially. It was experimentally demonstrated, on soil communities, that the response to multiple disturbances cannot be easily predicted from the response to a single disturbance (Rillig et al. 2019) due to complex synergistic or antagonistic interactions between the disturbances, that induce a deviation from simple addition of the individual effects. Although the terms 'synergisms' and 'antagonisms' are abundantly used in literature, their definition may be delicate especially when the disturbances induce opposite effects (detrimental and beneficial). Piggott et al. (2015) proposed a directional classification system of the interactions between simultaneous stressors by considering that synergisms refers to 'more-than' predicted additive effect and antagonisms to 'less-than' predicted additive effect (Fig. 1). Several meta-analyses focusing on different organisms and environments showed that synergistic effects of multiple simultaneous disturbances are more frequent than antagonistic or additive effects (e.g. Crain et al., 2008; Holmstrup et al., 2010). However, the reported synergies are not always properly or formally tested, which may lead to over-emphasizing this phenomenon (Côté et al., 2016). Recently, Romero et al. (2018) assessed the potential interactions of multiple combinations of 4 different co-occurring stressors applied on phototrophic biofilms and observed that only 14.5 % of the effects were interactive.

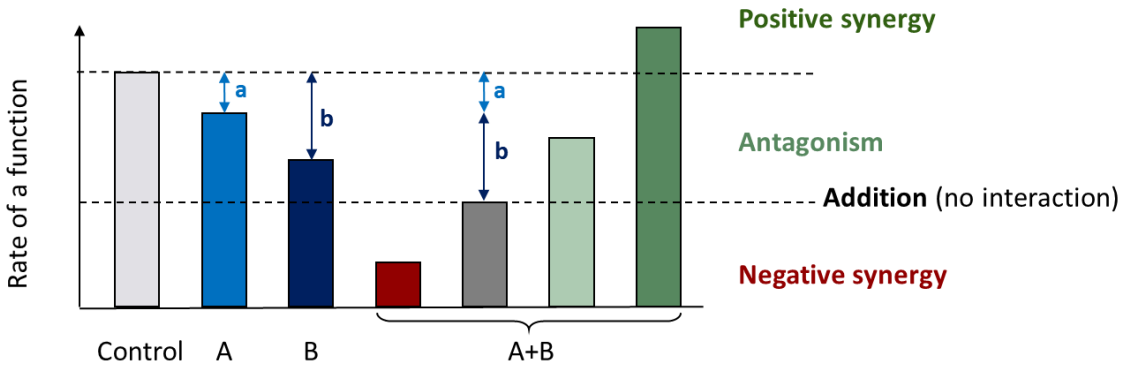


Figure 1: Illustration of the potential interactions between two combined stressors, A and B, inducing both individually a negative effect on the rate of a function (after Piggott et al, 2015).

The importance of sequence in exposure to disturbances has received less attention. Yet, the definitions of synergisms and antagonisms proposed by Piggott et al. (2015) could also be applied to successive disturbances. In that case, the outcome of the succession of disturbances is expected to depend on the time interval between the 2 disturbances, compared to the time required for the physiological adjustments of the organisms and the return to homeostasis (Gunderson et al., 2016). When there is no overlap in the 2 successive physiological responses, there should be no interaction between the disturbances (additive effects). When the time interval decreases and the response to the first disturbance is still on-going when the second one is applied, co-tolerance may occur thanks to priming (Rillig et al., 2015), leading to antagonistic effects. Very short time-intervals are more expected to induce synergistic effects, the second disturbance increasing the intensity or duration of the first one's effects. The threshold between short- and long-time intervals should depend on the duration of the physiological response, which is partly linked to the intensity of the disturbance. The time-dependency of successive multiple disturbance effects is well illustrated by the in-situ experiment of Dal Bello et al. (2019): when the recovery time for rocky intertidal biofilms exposed to a sediment deposition disturbance was not long enough, a short heat stress was sufficient to induce a shift toward a new stable state while no shift occurred for a longer time interval.

In addition to the temporality of the successive disturbances, several other characteristics of the disturbance sequence may regulate the potential interactions between disturbances and therefore the stability of microbial communities. The nature of the disturbance (physical or chemical, abiotic or biotic stressors) is one of the critical parameters, since it notably conditions species tolerance to the disturbances. Species tolerance was suggested to control the potential interactions between successive disturbances (Vinebrooke et al., 2004). Positive co-tolerance is expected to decrease the effects of the second disturbance, while negative co-tolerance should induce synergistic effects. Examples corresponding to both possibilities were reported in the literature. Sjöstedt et al. (2018) observed a higher stability of bacterioplankton production to pH disturbance after a previous exposure to a salt disturbance. In contrast, soil communities, which were very resistant to an isolated cold shock, were strongly impacted when it was preceded by a heat shock (Jurburg et al., 2017). Calderón et al. (2018) observed either a

decrease or an increase of the resilience of soil communities exposed to the repetition of the same disturbance, depending on the nature of the disturbance (freeze-thaw, anoxia or heat). The same experiment emphasized the importance of the chronology on the resilience of communities when disturbances of different natures were combined. Besides the influence of the nature and the chronology of disturbances, increasing the number of successive disturbances seems to decrease the stability of microbial communities (Berga et al., 2012; Villnäs et al., 2013).

All the previous results point to the difficulty of predicting the stability of microbial communities facing successive disturbances, especially because we are not able to anticipate how each disturbance interacts with the others. It is therefore necessary to explore more systematically the importance of the inherent characteristics of a disturbance sequence, i.e. time interval, nature, chronology, intensity and number of disturbances, for microbial stability (Philippot et al., 2021). Our objective here was to test experimentally the model of time-dependency proposed by Gunderson et al. (2016) and to demonstrate that the effect of time interval on the interactions between successive disturbances depends on the intensity and the nature of the disturbances. For this purpose, we exposed simplified phototrophic biofilms to 1 to 8 successive disturbances of the same nature and, we measured their functional resilience after each disturbance. The experiment was reproduced for 3 natures of disturbance (heat, salinity and chemical stressors), 5 intensities of disturbance and 3 time intervals between disturbance events. The hypotheses tested were (1) an increase of the time interval favors additive effects between the successive disturbances, (2) for the same time interval, an increase in the intensity of the disturbance favors synergisms or antagonisms, and (3) the transition from additive to synergistic or antagonistic effects depends on the nature of the disturbance.

2. Material and Methods

Organisms and culture conditions

The biofilms used in this experiment contained 5 non-axenic diatom strains: *Eolimna subminuscule*, *Fistulifera saprophila*, both isolated from rivers in the southwestern France, *Gomphonema parvulum* (SAG-1032-1), *Navicula pelliculosa* (CCMP 543) and *Nitzschia palea* (CPCC-160). All strains were first cultivated under controlled conditions (18°C,

light/dark cycle 16 h/8 h, 25 µmol photon.m^{-2}.s^{-1}) in COMBO medium (Kilham et al., 1998). All these strains are common freshwater benthic species. The medium of the stock cultures was renewed twice a week to maintain algal exponential growth. To grow the artificial biofilms, an initial algal mix at 5.10^6 cell.ml^{-1} was prepared, with the same initial biomass for each algal strain (see Table S1). In 12-well plates, each well was inoculated with 2 ml of this mix, and biofilms were cultivated at the same temperature and light condition than stock cultures. The plates were inoculated 3 d before the beginning of the stress experiment to allow the establishment of the biofilm.

Experimental design

The tested disturbance sequences were composed of *n* 2-h identical disturbances, with $1 \leq n \leq 8$, interspaced by a time interval *t*, with *t* = 0.5, 3 or 22 h (Fig. 2). The total duration of the sequence was comprised between 20 h and 8 d. The gross primary production (see next paragraph) was measured 24 h after the end of the last disturbance of the sequence to assess the functional resilience. The duration of the recovery period was chosen to be consistent with the duration of the disturbance.

The disturbances sequences were applied to 3 different natures of disturbance and at 4 to 5 levels of intensities for each disturbance sequence. These intensities were chosen after preliminary experiments to cover a wide range of responses after one disturbance (see Fig. 3). For heat stress, the plates were incubated for 2 h in a water bath at 35°C, 37°C, 39°C, 40°C or 41°C and put back at 18°C and light conditions (25 µmol photons.m^{-2}.s^{-1}) after disturbance. For salt and chemical stress, the culture medium was replaced, after gently removing the water without disturbing the biofilm, by COMBO medium containing NaCl at 5, 10, 15, 20 or 25 g.l^{-1} and $CuSO_4$ at 2, 5, 10, 15 and 20 mg.l^{-1}, respectively. After a 2-h incubation at 18°C and light conditions (25 µmol photons.m^{-2}.s^{-1}), the culture medium was replaced by fresh COMBO medium. The same manipulations were applied to undisturbed biofilms (controls), but with standard COMBO medium. For the biofilms exposed to heat stress, the culture medium was renewed once a day, in heated and control wells. All incubations of disturbed biofilms were made in triplicate with one corresponding control.

For each stress and intensity, the gross primary production was measured 1, 3, 6 and 22 h after the first disturbance in order to determine the dynamics of biofilm response after

a single disturbance (Fig. 2A). For each disturbance sequence of *n* events, the gross primary production of control and disturbed biofilms was measured 24 h after the end of the last disturbance, giving the resilience of the biofilm (Rl_n) (Fig. 2B).

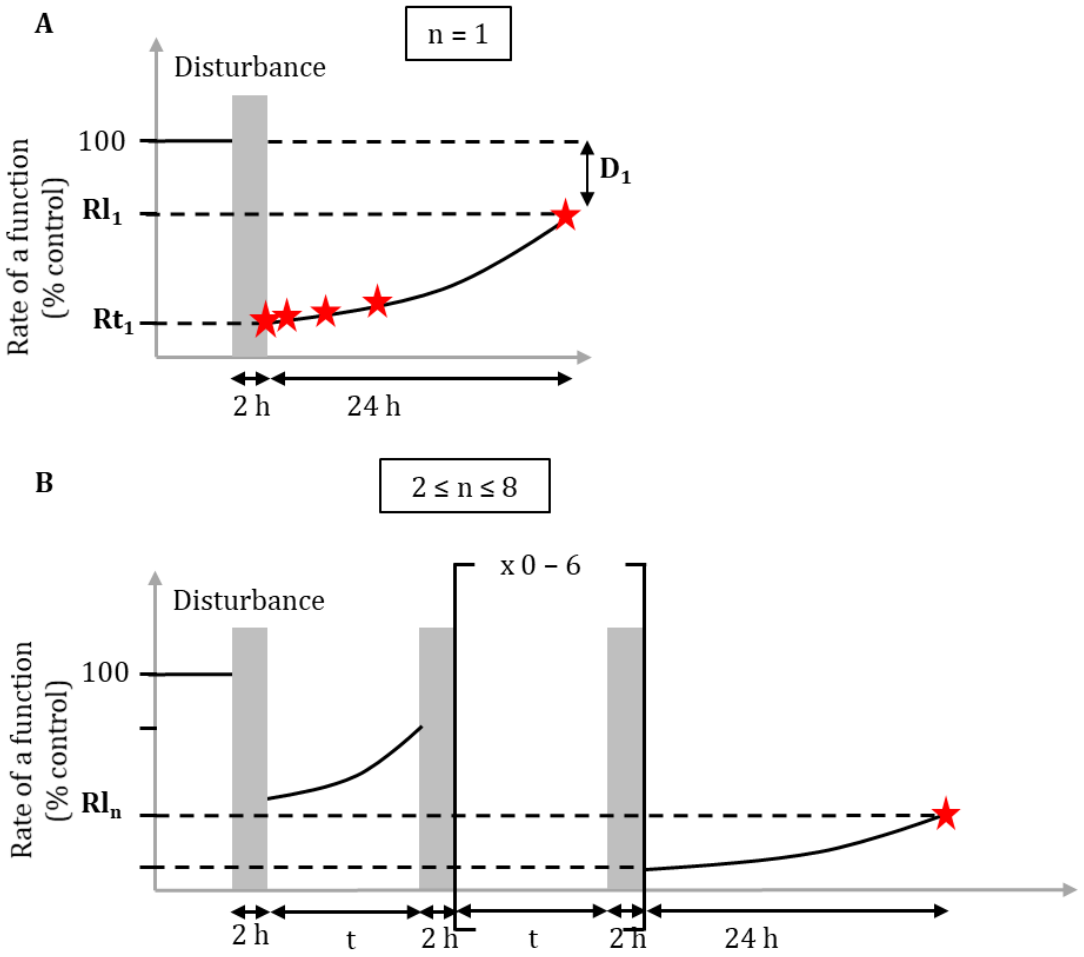


Figure 2: Illustration of the experimental design, for n=1 (A) and 2 to 8 (B) 2-h successive disturbances (grey area) spaced by t hours (0.5, 3 or 22 h). In each disturbance sequence of *n* events, the gross primary production of control and disturbed biofilms was measured 24 h after the end of the last disturbance, giving the resilience of the biofilm (Rl_n) (red stars). The gross primary production of control and disturbed biofilms was also measured at the end of the first disturbance (Rt_1) and 1, 3, 6 and 22 h after (A).

Gross primary production measurement

For each measurement, the biofilm was carefully scraped to obtain a 2 ml algal suspension. Gross primary production was assessed by measuring net primary production and respiration, through the changes in oxygen concentration over time using FireSting oxygen sensors (PyroScience). Four samples were measured simultaneously: 1

control and 3 disturbed biofilms. Oxygen measurements were performed under agitation in 8-ml transparent vials containing the 2-ml biofilm suspension and 5 ml of fresh COMBO medium, and closed with a gas tight lid. Dissolved oxygen concentration within each vial was monitored every 5 s over 10 min using oxygen logger software. Net primary production ($mgO2.h^{-1}$) was determined by measuring dissolved oxygen changes over a 5 min period in light condition (neon lamps, 90 µmol $photons.m^{-2}.s^{-1}$). The biofilm respiration was then determined as the change in oxygen concentration over a 5 min incubation in the dark. The gross primary production was calculated by summing net primary production and respiration. A change in gross primary production could be due to a change of the biofilm physiological state but also of its biomass, both being affected by the disturbance. Since the biomass is expected to increase during the longest disturbance sequences, at least in the control wells, for each sampling, the results were expressed as percentage of the control biofilm measured at the same time.

Data analyses

Data analyses were performed using R software (version 3.6.1, R development Core Team). Changes in gross primary production relative to control were analysed between disturbance intensities, or time intervals between successive disturbances using ANOVA. If data normality was not verified, we used the Kruskal-Wallis non-parametric test.

In order to analyze the effects of the successive disturbances on the functional resilience, we modeled the functional resilience Rl after each sequence of n disturbances as follows:

$$Rl_{n+1} = (100 - \Delta_1)(100 - (\Delta_1 + \mu))^n$$

where n represents the number of disturbances ($1 \leq n \leq 7$) and Δ_1 the loss of gross primary production compared to the control after the recovery time following the first disturbance ($\Delta_1 = 100 - Rl_1$). For each nature of disturbance, level of intensity and time interval, the model was fitted to data (including the complete sequence of 8 disturbance events) using nonlinear regression. The parameter μ was estimated according to the least square criterion. μ value was interpreted as follows:

If μ is not different from 0, then all disturbances of the sequence were considered to have the same effect and there is no interaction.

If μ is positive, then the effect of the successive disturbances increased over the sequence. This corresponds to the negative synergisms as defined by Piggott et al. (2015).

If $\Delta_1 < \mu < 0$, then the effect of the successive disturbances decreased over the sequence, as expected for antagonistic interactions.

Finally, if $\mu < \Delta_1$, the effects of the successive disturbances can be reversed, as in the case of positive synergistic interactions.

3. Results

Response of gross primary production to one single disturbance

The range of stress intensities that was chosen covered a wide range of impacts on the biofilms (Fig. 3). The 2-h exposure to one single disturbance induced a decrease in gross primary production between 2% and 91% compared to the controls, with a mean for overall intensities of 37%, 65% and 54% for the chemical, salt and heat stress, respectively (Fig. 3). After 24 h, the gross primary production reached between 10% and 96% of the controls. When considering all the intensities, 24 h after exposure to the chemical stress, the percent of the controls tended to remain the same or to slightly decrease, for the highest intensities. On the opposite, it tended to increase for the salt and heat stresses, at most intensities, indicating a recovery of the gross primary production (Fig. 3).

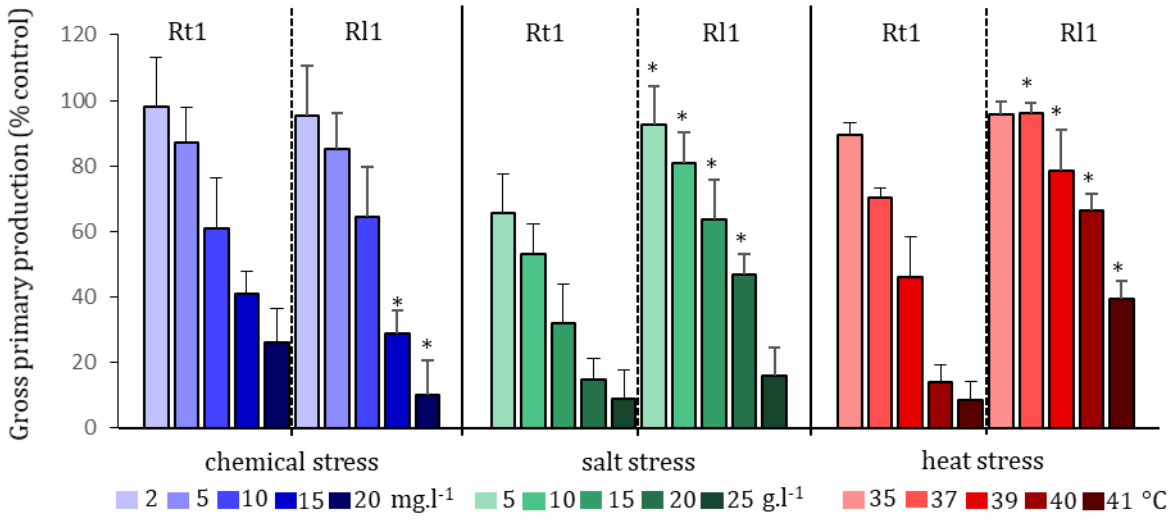


Figure 3: Biofilm gross primary production after a single disturbance, expressed as percent of the gross primary production in the control (mean ± SD, n = 3). For each nature of disturbance (chemical, salt and heat stress), the left and the right panels represent the function measured at the end of the disturbance (Rt_1) and after 24 h (Rl_1) in function to the disturbance intensity. The darker colors correspond to the higher disturbance intensity. Asterisks mean in which intensity Rl_1 is significantly different from Rt_1.

The response of the gross primary production after one single disturbance was followed during 24 h (Fig. 4), for the lowest and the highest intensities of each nature of disturbance. It showed very contrasted dynamics depending on the applied stress. For the low intensity, the biofilms fully recovered within 6 h whatever the stress, but we observed a transitory positive effect of the heat stress on the gross primary production after the heat stress. After a high-intensity stress decreasing gross primary production by more than 60%, there was no recovery for the chemical stress and only a partial recovery for the two others. The recovery took at least 6 h for the salt stress, but only 1 h for the heat stress.

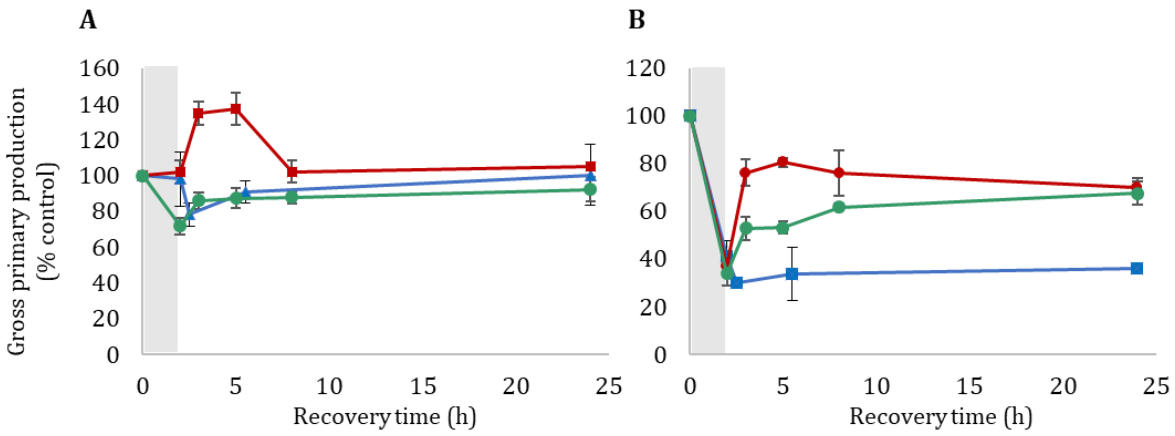


Figure 4: Temporal dynamics of biofilm gross primary production (percent of control, mean ± SD, n = 3) after one single 2-hour disturbance (grey area), either a chemical (blue), salt (green) or heat (red) stress at (A) low intensities ($CuSO_4$ 2 mg.l^{-1}, NaCl 5 g.l^{-1}, 35°C) and (B) high intensities ($CuSO_4$ 15 mg.l^{-1}, NaCl 20 g.l^{-1}, 39°C).

Resilience of gross primary production to successive disturbances

The figure 5 shows an example of the dynamics of gross primary production during the complete sequence of 8 disturbances, for the 3 different natures of stress at intermediate to high intensity, for the 22h time interval. For the chemical stress ($CuSO_4$ 10 mg.l^{-1}), the gross primary production progressively decreased during the disturbance sequence, to reach less than 10% of the control. For the salt stress (NaCl 20 g.l^{-1}), despite a strong loss of 85% at the end of the first disturbance, the gross primary production tended to increase so that it reached near 60% of the control after the 8th disturbance. For the heat stress

(40°C), the gross primary production first decreased by 85%, then increased up to 127 % after the 5th disturbance before to decrease during the 3 last disturbances down to 83% of the control.

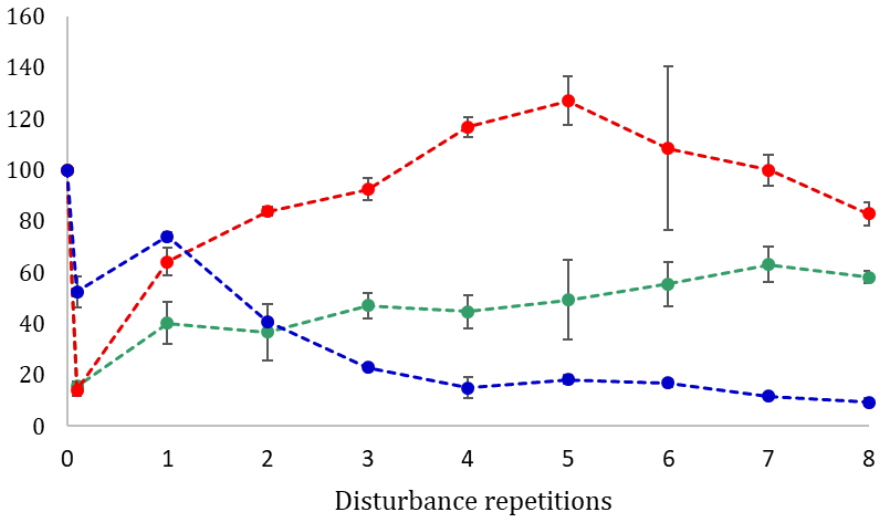


Figure 5: Recovery dynamics of biofilm gross primary production (percent of control, mean ± SD, n=3) after 1 to 8 disturbances, either a chemical (blue, $CuSO_4$ 10 mg.l^{-1}), salt (green, NaCl 20 g.l^{-1}) or heat (red, 40°C) stress, for the time interval 22-h.

Those dynamics depend on the intensity of the disturbance, and also on the time interval between the disturbances, as illustrated by the Rl values for chemical stress for the time interval 22-h and salt stress at 10 and 20 g.l^{-1}for the 3 time intervals (Fig. S1). At the end of the complete sequence of 8 successive disturbances, the resilience of the gross primary production depended on the disturbance nature ($p < 0.001$, Fig. S2A). When considering all the time intervals and stress intensities, it was significantly lower for chemical stress (28% ± 37%) than for salt stress (46% ± 28%) and heat stress (58% ± 32%). The time interval also had a clear effect on the ability of biofilms to recover at the end of the disturbance sequence, the recovery being higher for the 22-h interval (62% ± 33%) than for 3-h (39% ± 30%) and 0.5-h (30% ± 34%) intervals ($p < 0.001$, Fig. S2B). When the impact of a single disturbance was similar, for instance for CuSO4 15 mg.ml^{-1}, NaCl 15 g.l^{-1} and 39°C, the same observations could be done: the resilience was the lowest for chemical stress and the highest for heat stress, and it increased with the time interval (Fig. 6).

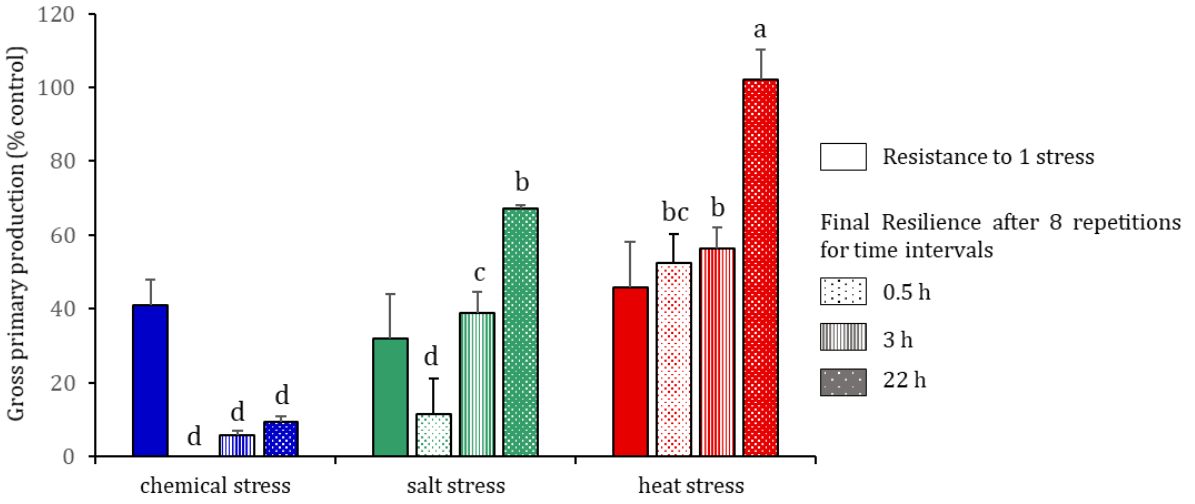


Figure 6: Gross primary production (percent of control, mean ± SD, n = 3) measured immediately at the end of the first one disturbance (Rt_1, full bars) and 24 hours after the 8th disturbance, for sequences with 0.5, 3 and 22-h time intervals, for chemical (blue, $CuSO_4$ 15 $mg.l^{-1}$), salt (green, NaCl 15 $g.l^{-1}$) and heat (red, 39°C) stresses.

Modeling the resilience in function of the number of disturbances

The model $Rl_{n+1} = f(n)$ describes very well the data for all the sequences of disturbances (Fig. S3). The value of the parameter μ indicates if the successive disturbances interact, and the direction and intensity of the potential interaction (Table 1). The higher the absolute value of μ, the higher the intensity of the interaction. When the gross primary production reached 0% of the control from the second disturbance, the model was not able to accurately discriminate the different situations. Those cases, corresponding to $CuSO_4$ 20 $mg.l^{-1}$, were removed from the analyses.

The figure 7 shows the parameter μ in function of the time interval for each nature and intensity of disturbance. No clear tendency could be seen for the chemical stress, for which the interactions between successive disturbances remained low. For both salt and heat stresses, the parameter μ showed 2 opposite trends depending on the disturbance intensity. For low intensities, μ values decreased or increased towards 0 as the time interval increased, suggesting less interactions between disturbances. For high intensities, on the contrary, the absolute value of μ strongly increased with the time interval.

At each time interval, the intensity of the disturbances had little effect on the interactions of successive chemical stresses (Fig. 8). For both salt and heat stresses, the increase of the intensity of the successive disturbances induced an increase in the absolute value of μ, especially for the longest time interval. We observed positive synergism for the high intensities and the 22-h time interval.

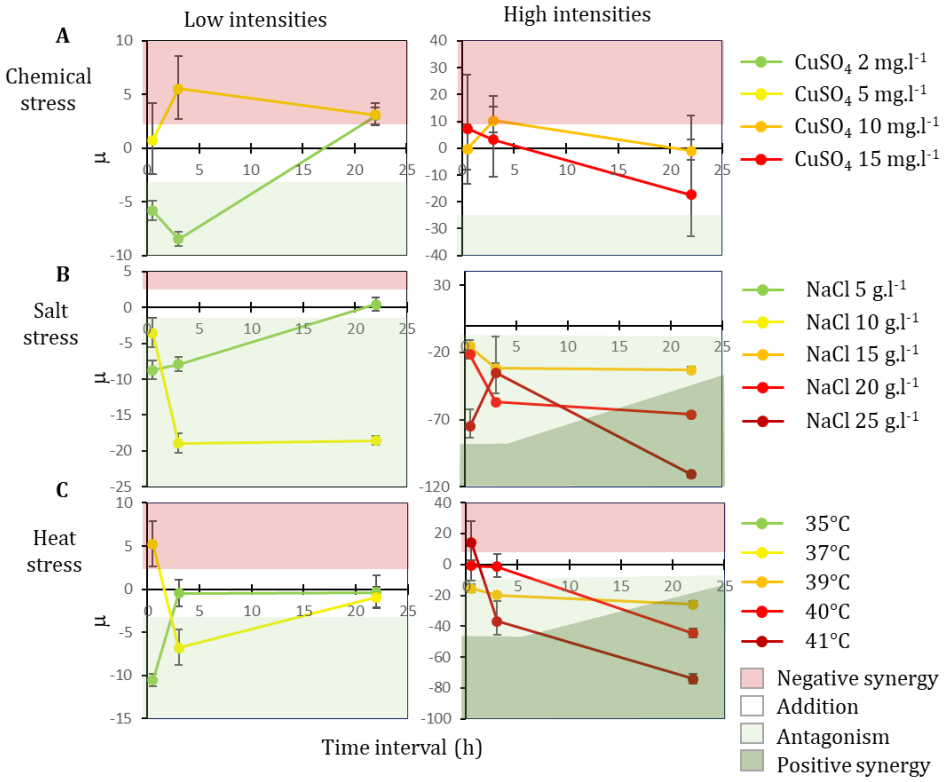


Figure 7: Parameter μ in function of the time interval between disturbances (0.5, 3 and 22h) for each nature of disturbance (A: chemical stress, B: salt stress and C: heat stress) and for each intensity. Colored areas represent different cases of interactions between disturbance (see above for μ interpretation)

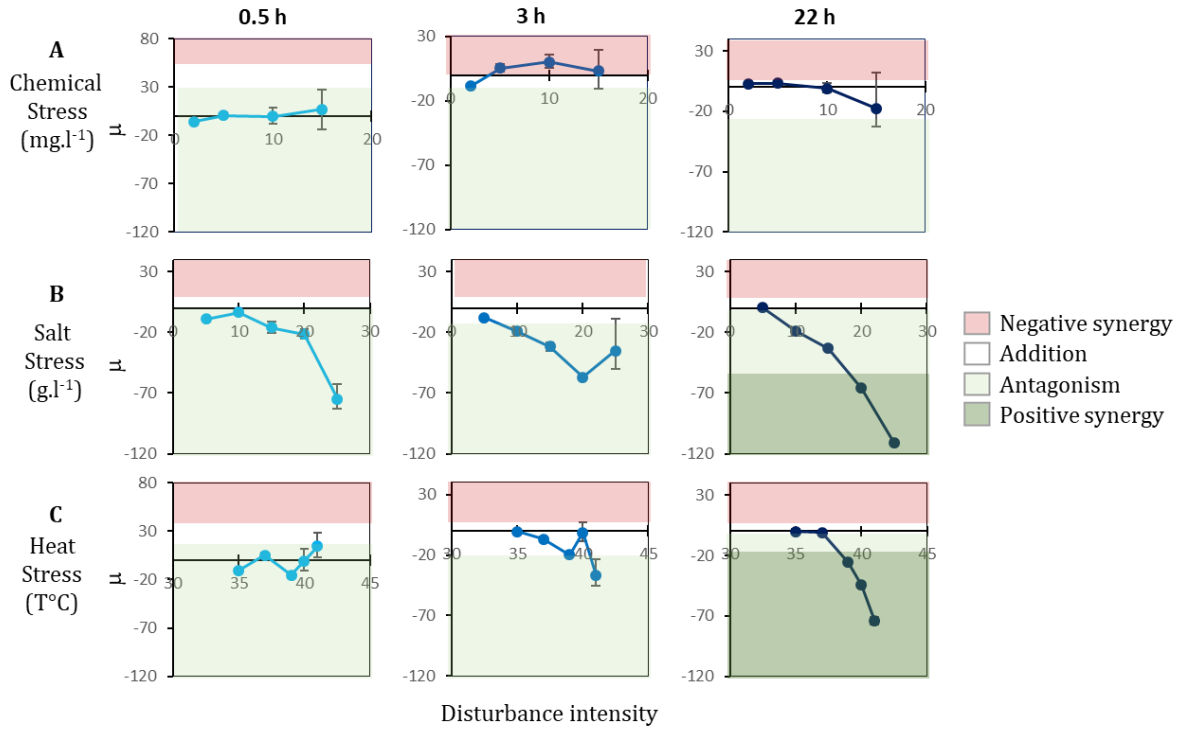


Figure 8: Parameter μ in function of the disturbance intensity for each nature of disturbance (A: chemical stress, B: salt stress and C: heat stress) and for each tested time interval between disturbance. Colored areas represent different cases of interactions between disturbance (see above for μ interpretation)

Table 1: Values of Δ_1 (loss of gross primary production compared to the control 24-h after the first disturbance) and μ, which indicates if the loss of primary production is constant during the sequence of disturbances ($\mu = 0$, in bold, no interaction between disturbances), increases (> 0, in red, negative synergistic interactions) or decreases ($-\Delta_1 < \mu < 0$, in light green, antagonistic interactions or $\mu < \Delta_1$, in dark green, positive synergistic interactions). These values are given for all the time intervals and for each disturbance intensity.

		0.5h		3h		22h	
		Δ_1	μ	Δ_1	μ	Δ_1	μ
Chemical stress	$CuSO_4$ 2 $mg.l^{-1}$	7.6	-5.8 [-6.7;-4.9]	7.8	-8.5 [-9.1;-7.8]	-3.8	3 [2.2;3.8]
	$CuSO_4$ 5 $mg.l^{-1}$	22.8	**0.7 [-2.4;4.2]**	11.2	5.6 [2.7;8.6]	9.1	3.1 [2.1;4.2]
	$CuSO_4$ 10 $mg.l^{-1}$	51.6	**-0.3 [-8.1;8.9]**	26	10.3 [5.8;15.6]	29.6	**-1 [-4.5;3.1]**
	$CuSO_4$ 15 $mg.l^{-1}$	84	**7.3 [-13.3;27.5]**	64.6	**3.2 [-10.7;19.3]**	64.6	**-17.4 [-32.7;12]**
	$CuSO_4$ 20 $mg.l^{-1}$	96.4	-47.3 [-64.5;-22.2]	86.5	-77.9 [-81.1;-74.2]	86.5	-72 [-77.1;-65.1]
Salt stress	NaCl 5 $g.l^{-1}$	10.8	-8.7 [-10.1;-7.4]	9.1	-7.9 [-8.9;-6.9]	2.4	**0.4 [-0.5;1.4]**
	NaCl 10 $g.l^{-1}$	16.6	-3.6 [-5.6;-1.5]	21.3	-19 [-20.2;-17.6]	19.3	-18.6 [-19.2;-17.9]
	NaCl 15 $g.l^{-1}$	37.1	-15.8 [-20.2;-10.8]	35.5	-31.7 [-35.1;-28.1]	36.5	-33.1 [-35.6;-30.5]
	NaCl 20 $g.l^{-1}$	40.2	-21.5 [-24.8;-17.7]	59.4	-57 [-59;-54.8]	59.9	**-66 [-67.5;-64.5]**
	NaCl 25 $g.l^{-1}$	84.2	-75 [-83.2;-62.2]	80.1	-35.1 [-50.2;-8.5]	88.3	**-110.8 [-112.8;-108.5]**
Heat stress	35°C	10.6	-10.5 [-11.3;-9.8]	3.9	**-0.5 [-2;1.1]**	-1.2	**-0.4 [-2.2;1.6]**
	37°C	0.3	5.2 [2.7;7.9]	8.2	-6.8 [-8.8;-4.7]	3.3	**-1 [-2.1;0.3]**
	39°C	18.8	-15.4 [-18.2;-12.5]	23.4	-19.9 [-20.7;-19]	21.7	**-25.8 [-27.6;-23.9]**
	40°C	34	**-0.8 [-10.4;11.7]**	31.4	**-1.5 [-8.3;6.6]**	35.8	**-44.6 [-47.1;-41.8]**
	41°C	47.2	14.2 [2.9;28.1]	70.5	-36.9 [-45.7;-23.6]	64.7	**-74.3 [-77;-71.2]**

4. Discussion

The chosen time interval of 24 h to study resilience of microbial communities was sufficient in our studies as shown by the response dynamic to each single disturbance. The response observed over time varied greatly depending of the nature of the disturbance, with a lower resilience after a chemical stress than after salt or heat stress, despite the latter induced a higher impact on gross photosynthesis. These first analyses also highlighted a transitory positive effect of heat stress on photosynthesis for the lowest intensities. Such observations are consistent with other results that highlighted a positive effect of warming on algal primary production if the temperature exposure remained in the thermal niche of species (Angilletta, 2009).

The aim of this study was to analyse the response of phototrophic biofilms to repeated disturbances of the same nature, depending on stress intensity and time interval between repetition. More specifically, we analysed the interactions between these properties using a modelling approach. It appears that the μ parameters, used to describe the interaction, was strongly dependant on the nature of the stress. Indeed, there were no clear trends for chemical stress, while for salt and heat stress, the effects were clearer. For these two natures of disturbances, it appeared that μ depended on the time interval between disturbances, as the longer the time between repetitions was, the more the effects resulted in additive effects. This is consistent with the hypothesis of Gunderson et al., (2016), which predicted an independency of disturbance events when communities had enough time to recover. However, our hypothesis that longer time interval favour additive effect is only partially verified as for highest concentrations, positive interactions (antagonisms or positive synergisms) are observed**.** Such effects induced by stress intensity at long time interval pointed out a reduced effect of stress repetitions, in accordance with (Vinebrooke et al., 2004) which suggested that under exposure to multiple stressors, communities can exhibit an increased co-tolerance to the secondary stressor due to "stress-induced community tolerance". On the contrary, the opposite was observed for the chemical stress as repeated exposure mainly resulted in an additive or negative synergy between events on gross primary production. The positive interactions observed for salt and heat stress at high intensities as time interval between successive disturbance increased, might be explained by the importance of disturbance frequency in phenotypic plasticity of organisms. Indeed, it has been shown that for an advantageous

plasticity, the time interval between stress must be long enough to allow physiological or morphological adaptations (Stomp et al., 2008), otherwise it results in energetic costs (Chevin and Hoffmann, 2017).

Our second hypothesis that for the same time interval, higher intensities favours interactions was verified only for salt and heat stress, with generally antagonisms or positive synergisms observed. A possible explanation of such effect is that under high stress condition, such as high salinity stress, the synthesis of reactive oxygen species increases (Juneau et al., 2015), which have been shown to enhance acclimation toward stress in photosynthetic organisms (Khorobrykh et al., 2020). It is likely that in our study, the low intensities disturbances remained under the tolerance range of biofilms, so no physiological response are involved. It suggest that even short time intervals between repetitions are sufficient to re-establish homeostasis in communities, resulting in independent responses to successive disturbances (Gunderson et al., 2016). However, beyond a disturbance threshold, physiological response emerged if the time interval between disturbances is sufficient. For instance, the synthesis of certain heat shock proteins involved in organism's tolerance to heat stress need hours to set up, as it has been demonstrated in some algae (Tukaj and Tukaj, 2010). However, for the chemical stress (copper), μ parameter values remained low compared to other natures of disturbances for each tested intensity, resulting in few interactions between disturbances.

Altogether, these finding highlighted that effects between successive disturbances strongly depended on the nature of the events, validating our third hypothesis. Generally, few interactions between disturbances were observed for chemical stress, while successive salt or heat disturbances mainly resulted in antagonistic or positive synergistic interactions. This is certainly due to the different modes of action on organisms. For instance, a study conducted on soil bacteria exposed to repeated salt stress showed an increased stability of communities (Berga et al., 2017), while another one pointed out a reduced stability when marine communities were exposed to successive hypoxia stresses (Villnäs et al., 2013). Particularly, copper is known for its algicide effect, so it is not surprising that repeated disturbances of this nature had stronger effect on gross primary production. Contrary to a previous study, we did not observe an enhanced tolerance to copper stress when biofilms were previously exposed to it. Indeed, the authors shown that when biofilms were previously exposed to relatively high concentrations of Cu (10

µg.L-1), phototrophic communities exhibited a better tolerance to a second exposure (Tlili et al., 2011). This might be due to the fact that in their experiment, the first exposure lasted long enough (5 weeks) to allow cells acclimation.

Our study shows that depending on the nature of the successive disturbances, and according to the disturbance regime (time interval and intensity of the disturbances), both additive and interactive effects between events can be observed. This highlights the difficulties to predict the effects of changes in disturbance regimes resulting in increased intensities and frequencies of extreme events. Further studies should focus on development of prediction methods.

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Supplementary information

Table S1: Concentrations of each algal culture in the algal mix used in the experiment. The cell concentration of the mix was 5.10^6 cell.ml^{-1}.

Algal strain	Concentration (cell.ml^{-1})
Eolimna subminuscule	1.1e^6
Fistulifera saprophila	1.4e^6
Gomphonema parvulum	0.55e^6
Navicula pelliculosa	1.4e^6
Nitzschia palea	0.55e^6

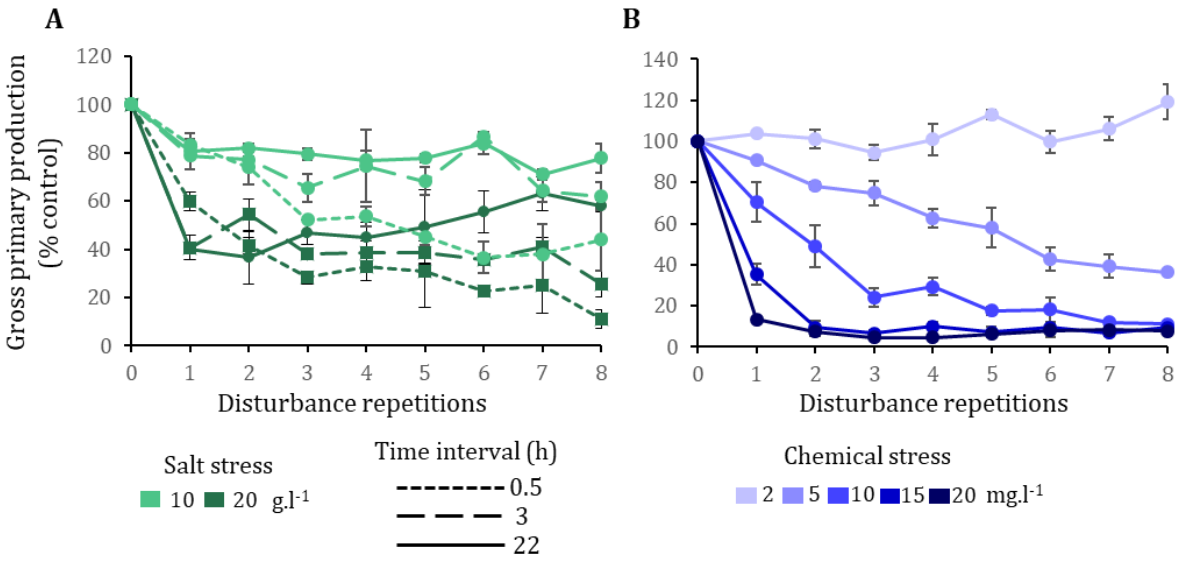


Figure S1: Example of trajectories of gross primary production (% control) for salt stress (A) and chemical (copper, CuSo4) stress (B). Graph A represents the Rl values for two salinity concentration at each time interval tested (mean ± SD, n=3), and graph B represents Rl values for the chemical stress at time interval 22h.

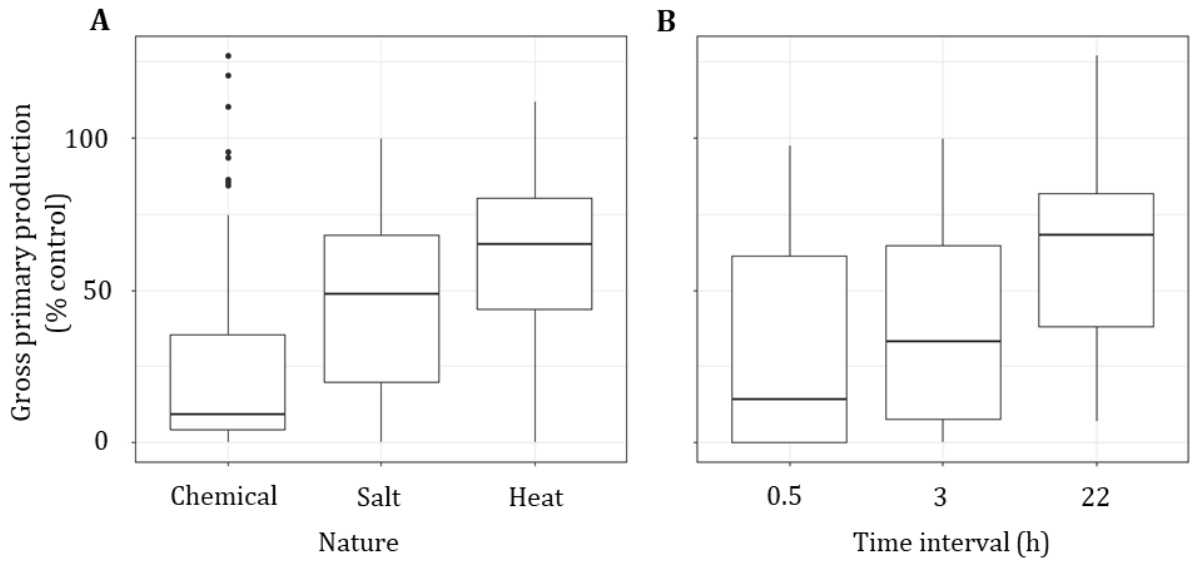


Figure S2: Resilience of gross primary production (% control) at the end of the 8 successive disturbances depending on the nature of the disturbance (A) and time interval (B).

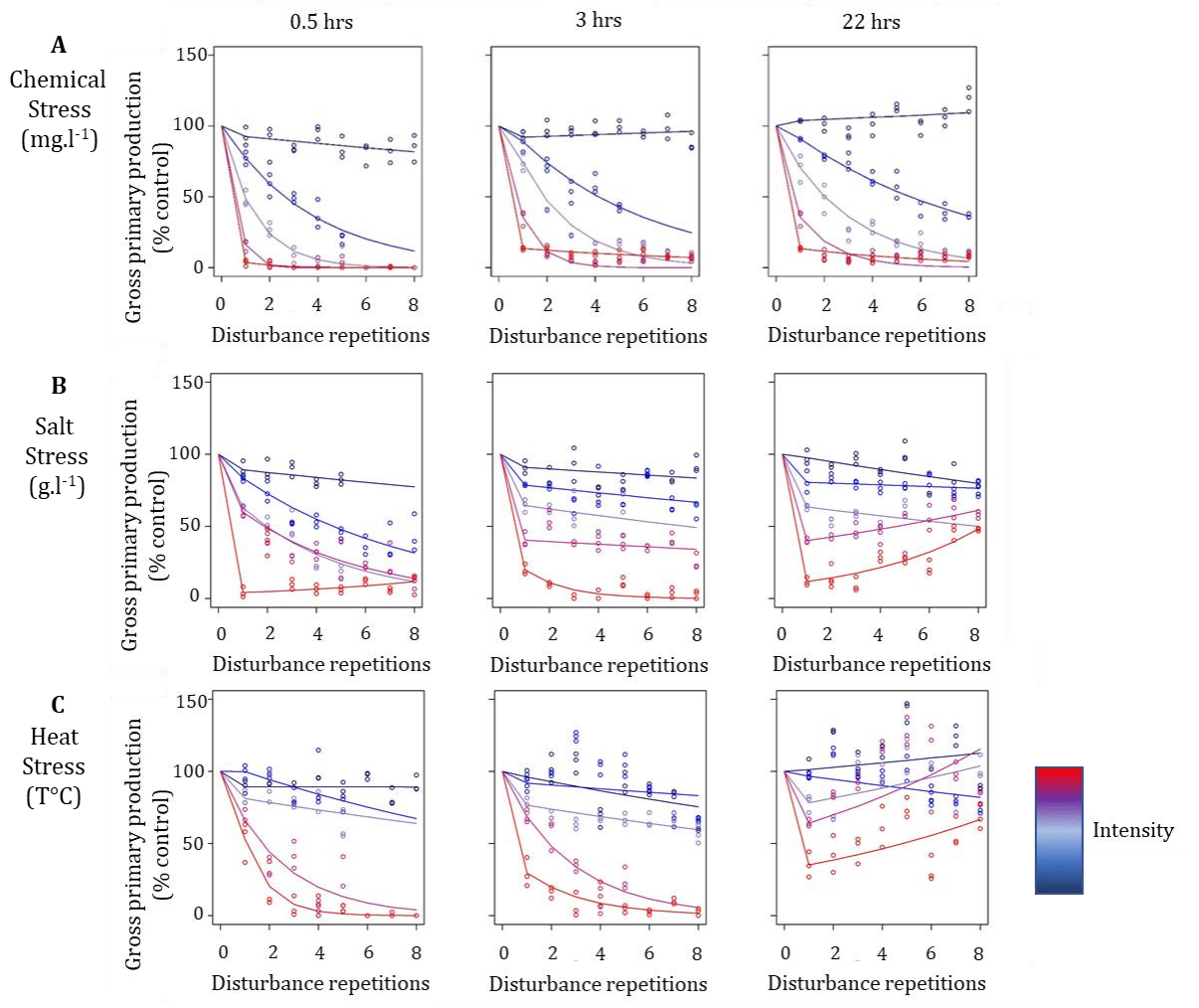


Figure S3: Representation of resilience of gross primary production (% control), from the model $Rl_{n+1} = f(n)$, as a function of number of disturbance repetitions. Curves are colored according to the disturbance intensity.

Chapitre 4

Influence de l'histoire d'intermittence hydrique sur la sensibilité des biofilms à un contaminant complexe

Chapitre 4 : Influence de l'histoire d'intermittence hydrique sur la sensibilité des biofilms à un contaminant complexe

Contexte

L'intermittence hydrique, déjà fréquente dans les régions arides et semi-arides, est exacerbée par le changement climatique et la demande accrue en eau pour les activités humaines. Ces phénomènes sont de plus en plus intenses et affectent de plus en plus de cours d'eau considérés comme pérennes (Sabater et al., 2016).
Le processus d'assèchement est une perturbation séquentielle lors de laquelle la diminution de l'écoulement de l'eau provoque une transition progressive d'un système lotique vers un système lentique dont la hauteur d'eau décroit (Datry et al., 2017). Cette baisse du niveau d'eau s'accompagne de changements dans la qualité physico-chimique de l'eau, avec par exemple une augmentation de la température et une diminution de la concentration en oxygène. Lors de la remise en eau, les contaminants contenus dans les sols, sont lessivés par les précipitations et atteignent les cours d'eau par ruissellement.



L'Aveyron asséché avec mare résiduelle (Gué de Picquecos, Tarn et Garonne, Sud Ouest France) en Août 2022.

Plusieurs études ont évalué les réponses des biofilms phototrophes de rivière à l'assèchement ou aux contaminants. L'assèchement a un fort impact sur la structure et le fonctionnement autotrophe (Acuña et al., 2015, McKew et al., 2011), mais le retour de l'eau réactive rapidement toutes les activités biologiques (Timoner et al., 2012). La réponse des biofilms à la contamination est plus difficilement prédictible puisque la réponse varie d'un contaminant à l'autre (Dalton et al., 2015, Zhong, Zhao, et Song, 2020, Halstead et al., 2014).

Si les études consacrées à la réponse des communautés microbiennes à des perturbations simultanées se sont multipliées ces dernières années, la question des perturbations séquentielles reste moins abordée. Les résultats des travaux existants sur l'effet d'une perturbation en réponse à une autre perturbation antérieure sont d'ailleurs contrastés, et montrent soit une amplification des effets négatifs (Tabor-Kaplon et al., 2006), soit un effet bénéfique de la perturbation antérieure sur la réponse à la seconde (Sjöstedt et al., 2018). Cet effet bénéfique peut être dû à la sélection d'espèces généralistes lors de la première perturbation, qui présentent une co-tolérance (Chen et al., 2021), au bien à de la tolérance acquise au stress : lors du premier stress, les espèces présentes dans la communauté ont été préparées pour résister au second (Rillig et al., 2015). Au contraire, une amplification des effets négatifs de la deuxième perturbation peut être liée à une corrélation négative entre les tolérances des espèces aux deux perturbations (Vinebrooke et al., 2004).

Objectifs et hypothèses

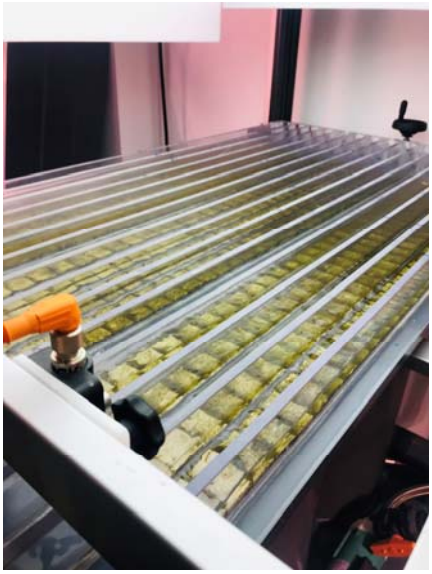
Dans ce chapitre, l'objectif est de quantifier dans quelle mesure la sensibilité des biofilms phototrophes à un contaminant agricole complexe est modulée par les conditions antérieures d'intermittence de la rivière. Pour répondre à cette question, des biofilms ont été exposés, dans des microcosmes de laboratoire, à des séquences d'assèchement qui varient en températures et durées d'assec. Puis, lors de la remise en eau, les biofilms ont été contaminés selon un gradient de concentrations.

Mon hypothèse est :
La première perturbation (la séquence d'assèchement) diminue la sensibilité des biofilms au contaminant via la sélection d'espèces co-tolérantes. C'est-à-dire que l'assec combiné à une augmentation de la température sélectionne les espèces capables de tolérer la contamination.

Matériel et méthodes

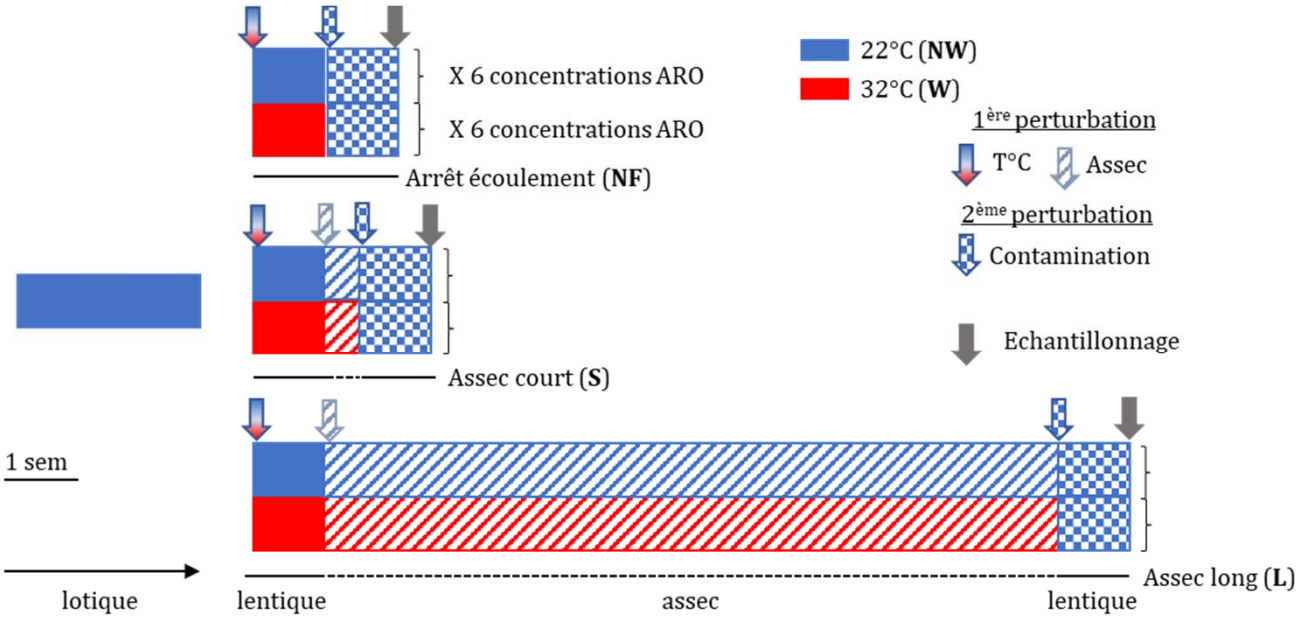
Des biofilms échantillonnés en rivière ont été cultivés en canaux en laboratoire à 22 °C. A l'issu de la période de croissance, les coupons de céramiques colonisés par le biofilm on été déposés dans des cristallisoirs, et placés à 22°C (NW) ou 32°C (W) pendant 1 semaine, pour simuler la période d'arrêt de l'écoulement. Ensuite, 1 tiers des cristallisoirs a subi un assec de 3 jours et un autre tiers a subi un assec de 12 semaines avant d'être contaminé. Les cristallisoirs restant ont directement été contaminés avec un contaminant mimant un lessivat agricole (agricultural run-off, ARO).

Le mélange de contaminants utilisé contenait des nitrates (KNO_3), du cuivre ($CuSO_4$), un élément trace métallique utilisé pour les vignes ou en agriculture biologique, ainsi que 3 pesticides organiques : le tébuconazole (fongicide), le terbuthylazine (herbicide) et le pirimicarbe (insecticide).
Lors de la contamination, les biofilms ont été exposés à 6 concentrations graduelles allant de 0, 1, 10, 50, 100 et 250 fois la plus faible concentration.



Dispositif de canaux utilisé pour la croissance des biofilms

Design expérimental



6 histoires de perturbations : **NW-NF**, **W-NF**, **NW-S**, **W-S**, **NW-L** et **W-L**

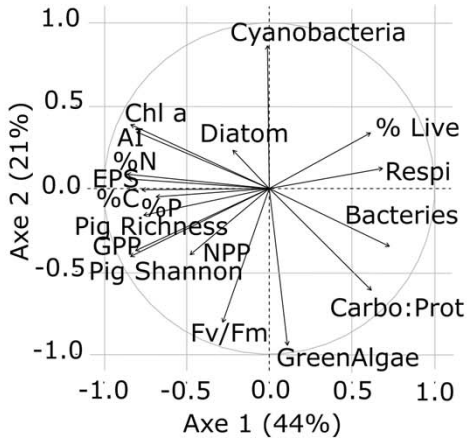
Paramètres mesurés

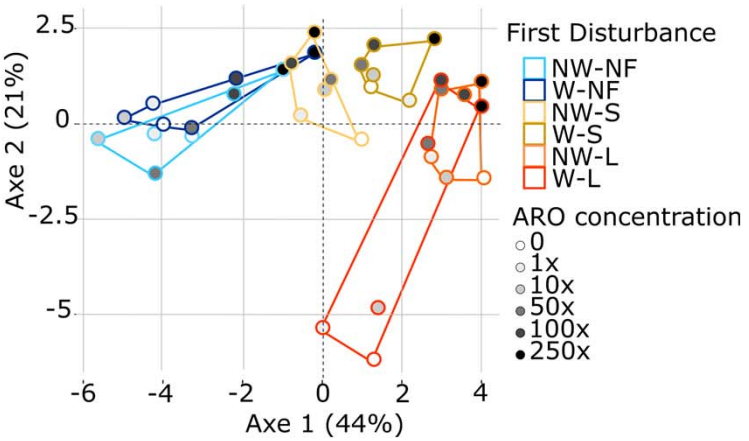
- Biomasse (sèche et algale)
- Contenu en EPS
- Composition pigmentaire
- Stœchiométrie
- Flux d'oxygène : Productivité primaire brute (GPP), nette (NPP) et respiration (R)
- Activité photosynthétique (F_V/F_M)
- % bactéries vivantes

Analyses des données

Après avoir estimé l'impact de la première perturbation (i.e. réchauffement et/ou assec), la sensibilité des biofilms au contaminant a été quantifiée en comparant les pentes des réponses à la contamination, en fonction de l'histoire de perturbation.

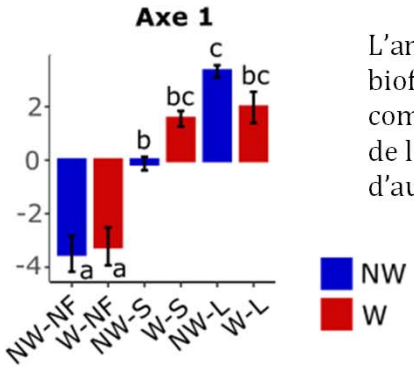
Résultats





L'analyse en composante principale permet de séparer les descripteurs du compartiment phototrophe (à gauche sur le cercle des corrélations), du compartiment hétérotrope (à droite). La représentation montre un effet de la durée d'assec sur le biofilm.

Effets combinés de l'assec et du réchauffement

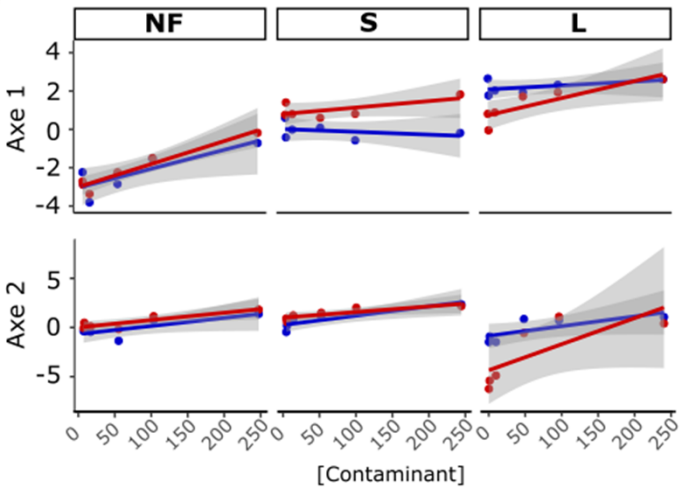


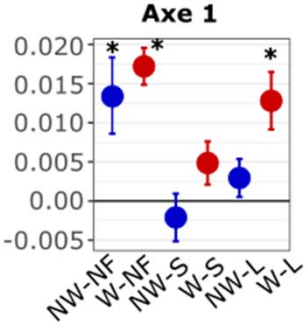
L'analyse de l'axe 1 de l'ACP met en évidence que lorsque les biofilms ont subi un assec avant la contamination, les communautés deviennent plus hétérotrophes. L'effet de la durée de l'assec n'est visible que lorsque les communautés n'ont pas subi d'augmentation de température lors de l'assec (i.e. NW)

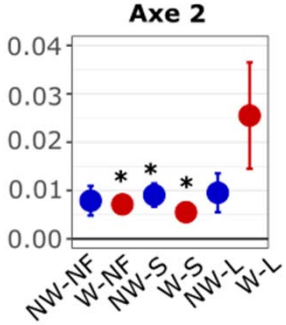
Effet du contaminant

Plus la pente en réponse à la contamination est forte, plus la sensibilité à ARO est grande.

ARO induit une augmentation de l'hétérotrophie (les valeurs de l'axe 1 augmentent) sur les biofilms non asséchés, de même qu'après un assec long avec augmentation de température. En revanche, il n'y a pas d'augmentation de l'hétérotrophie due à ARO après un assec court et après un assec long non « réchauffé ».







Les valeurs de pentes sont représentées pour chaque axe en fonction de la perturbation antérieure.

Pour les biofilms ayant subi une première perturbation d'intensité intermédiaire, le contaminant induit un changement de la composition algale, avec une augmentation de la proportion de cyanobactéries (visible sur l'axe 2).

Conclusions et Perspectives

De manière générale, les effets d'ARO, une semaine après le pulse de ARO, sont relativement faibles sur tous les biofilms. Cependant, la sensibilité des communautés à ARO dépend en partie de l'histoire de perturbation :

- **Les biofilms n'ayant pas subi de perturbation antérieure** à la contamination montrent une perte de diversité algale et de la productivité primaire brute, alors que l'hétérotrophie augmente.

- **Les biofilms ayant subi une augmentation de température** ont une réponse globalement similaire, même si le réchauffement a tendance à limiter la perte de diversité algale et à amplifier le basculement vers un système hétérotrophe.

Quand la température de l'eau augmente avant un pulse de contaminant, une sélection d'espèces co-tolérantes à la contamination peut s'opérer et ainsi réduire les effets négatifs, comme cela a été montré pour des éléments trace métalliques (Pesce et al., 2018), bien que des résultats opposés aient aussi été observés pour la même nature de perturbation (Lambert et al., 2017). Ces résultats contradictoires pourraient s'expliquer par des différences de composition spécifique des biofilms, et donc des propriétés de co-tolérances aux perturbations appliquées.

- **Les biofilms ayant subi un assec** montrent, suite à celui-ci, une forte modification de la composition de la communauté et de la structure générale du biofilm avec une perte de biomasse. De plus, la productivité primaire brute diminue fortement avec l'assec au profit d'une augmentation de la respiration. Les communautés déjà fortement impactées par l'assec sont globalement assez peu sensibles à ARO. Cette diminution varie cependant en fonction de la température et des variables structurelles ou fonctionnelles ciblées (résultats détaillés dans l'article).

→ Validation partielle de l'hypothèse

Les résultats montrent que la première perturbation est celle qui impacte le plus les biofilms. Il est possible que l'assec ait entrainé une sélection d'espèces généralistes, ou une réponse physiologique qui ait permis à la communauté de mieux résister à la contamination.

Cette étude met en évidence l'importance de considérer les conditions hydrologiques antérieures subies par une communauté microbienne lorsqu'on évalue sa sensibilité à un contaminant, et plus généralement la nécessité de prendre en compte le stress hydrique dans la gestion des risques chimiques en milieu aquatique. Elle met en avant la nécessité d'un maintien minimal de l'écoulement pour maintenir le bon fonctionnement des communautés microbiennes phototrophes, car l'assèchement est la perturbation qui impacte le plus les biofilms.

SEFS 12 Symposium for European Freshwater Sciences

Présentation orale à la SEFS12

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The sensitivity of aquatic microbial communities to a complex agricultural contaminant depends on previous drought conditions

Published in ***Water Research***

Camille Courcoul[1*], Joséphine Leflaive[1], Jessica Ferriol[1], Stéphanie Boulêtreau[1]

[1] Laboratoire d'Ecologie Fonctionnelle et Environnement de Toulouse, Université de Toulouse, CNRS UMR 5245, INPT, UPS, Toulouse, France

*Corresponding author

camille.courcoul@univ-tlse3.fr

Abstract

In intermittent rivers, which represent a prominent part of worldwide rivers, aquatic organisms are exposed to sequential disturbances including flow cessation, potentially associated with water warming, desiccation process and flow resumption. At flow resumption, pollutants stored in soil and washed by rainfalls can reach fresh waters. The interaction between contamination and river intermittency is poorly understood. In this study, we aimed at understanding in what extent the intensity of dry period combined or not to water warming drives the sensitivity of aquatic communities to a complex agricultural run-off (ARO) during rewetting. Phototrophic biofilms, at the basis of freshwater food webs, were chosen as a model of community. Biofilms grown in laboratory were first exposed to a disturbance crossing two temperature conditions (not warmed, 22°C or warmed, 32°C) and three dry periods (no drying, short (3 days), or long (3 months)). Then they were exposed to a chemical mix of nitrates, copper and 3 pesticides at 6 gradual concentrations. Various descriptors associated with biofilm structure and function were assessed one week after ARO addition. When undisturbed biofilms were exposed to ARO, they shifted toward a more heterotrophic state as they lost algal richness and diversity, and gross primary production tended to decrease. Warming alone only slightly modified the sensitivity of biofilms to ARO, with lower effects on algal richness and a trend to increase the effect on gross primary production. In contrast, the association of warming and a dry period strongly modified the sensitivity to ARO, certainly due to the selection of generalist species and/or physiological acclimation

inducted by the first disturbance. This study emphasizes the importance of considering water intermittency in the management of the ecological risk of chemicals in aquatic ecosystems.

Keywords: global change, desiccation process, agricultural runoff, sequential disturbance, phototrophic biofilms

1. Introduction

Intermittent rivers represent more than 50% of rivers worldwide (Datry, Larned, and Tockner, 2014), they can be found on every continent, within every climate (Messager et al., 2021). A river is considered intermittent or nonperennial if its flow ceases and the riverbed dries out a part of the year (Datry et al., 2017a). Even if this phenomenon is frequent and naturally occurs in arid and semiarid regions because of low precipitation events and strong direct evaporation, it is exacerbated by climate change and human uses and is expected to increase both in space and duration (Sabater et al., 2016). The prevalence of intermittency requires to adjust the classical model of river for a more accurate understanding and prediction of river functioning, biodiversity and vulnerability.

Intermittency is a complex phenomenon, which covers contrasted situations. During the desiccation process, the water flow first decreases and the system undergoes a slow transition from a lotic toward a lentic system (Datry et al., 2017b). The decrease of water level induces modifications of water quality, depending notably on the hydrological drivers and mass balance in the system (Mosley, 2015). For instance, temperature increase has been observed in many studies during drought, up to 7 degrees in a regulated river (Ha et al., 1999). After this first step, the riverbed completely dries out and there is a shift toward terrestrial habitat for some days to several months before rewetting occurs (Sabater et al., 2016). Depending on land use practices, agricultural contaminants stored in soils during drought can be leached by the precipitation events and reach fresh waters through runoff, exposing aquatic communities to a cocktail of pollutants during flow resumption. A post-drought increase in nitrate concentration was thus observed in several studies (see Mosley, 2015). In this context, flow intermittency can be considered

as a sequence of different disturbances occurring before, during and after the dry period, that can affect all the river biocenosis.

Benthic microbial communities which cannot circumvent such disturbances are particularly exposed to the consequences of river intermittency. Phototrophic biofilms are benthic microbial communities made up of both phototrophic and heterotrophic cells embedded in an extracellular polymeric matrix. These communities are of major importance in ecosystem functioning as they are key primary producers in many rivers, and are involved in organic matter and nutrient cycling (Battin et al., 2016). Their short generation time makes them "early warning systems" to many stressors (Sabater et al., 2007), especially to chemical stressors from agriculture practices like fertilizers, trace metals such as copper widely used for vineyard culture, but also organic pesticides (herbicides, insecticides, fungicides). Biofilm structural and functional responses to independent chemical stressors have been well studied. For instance, nutrients enhance biofilm growth rate and functioning but can also induce significant changes in community structure by favouring filamentous green algae (Dalton, Boutin, and Pick, 2015). Compared to planktonic algae, biofilms exhibit an important tolerance to copper contamination with usually few effects on functioning even at high concentration (2 $mg.L^{-1}$) (Zhong, Zhao, and Song, 2020). In contrast, they tend to be negatively impacted by organic herbicides, although the effect of combinations of pesticides are more difficult to predict (Halstead et al., 2014). More generally, the chemical complexity of agricultural run-off makes difficult to predict its effects on biofilm, as the interactions of stressors do not result in a simple addition of stressor effects (Pesce, Bouchez, and Montuelle, 2011).

Warming and desiccation are the main effects of river flow drying-up. With global change, intensity (i.e. duration) of dry periods is expected to increase, and increasing temperatures intensify the severity of drought (Chiang, Mazdiyasni, and AghaKouchak, 2021). When temperature increases, metabolic activities are enhanced. For instance, under warming, biofilms use more carbon substrates (Ylla, Canhoto, and Romaní, 2014) and basal fluorescence and enzymatic activity are enhanced (Romero et al., 2018). Conversely, desiccation appears to have negative impacts on biofilm structure and functioning. During desiccation, photosynthetic processes weaken regardless of the disturbance duration, while heterotrophic activities and community respiration could persist longer depending on the humidity degree remaining in biofilm (Acuña et al., 2015).

Besides those functional responses, it has been shown that desiccation tends to induce changes in both algal and bacterial communities (McKew et al., 2011; Sabater et al., 2016).

Some studies have investigated the response of microbial communities to a stressor after a prior exposure to another disturbance, but with contrasted results. In some cases, the community was then more affected (e.g. Tobor-Kapłon et al., 2006) while in others, the exposure to a first disturbance decreased the effects of the second one, the microbial community showing antagonistic responses to the successive disturbances (e.g. Sjöstedt et al., 2018). Antagonistic responses can be explained by different mechanisms. First, some species in the community can exhibit co-tolerance to both disturbances (Vinebrooke et al., 2004) with the selection of generalist species that are usually less sensitive to environmental changes (Chen et al., 2021). Acquired stress resistance can also be implied: after a first disturbance, communities have been prepared to resist a second one (Rillig et al., 2015). On the opposite, additive or synergic effects can be observed when there is a negative correlation between species tolerance to each disturbance (Vinebrooke et al., 2004). Therefore, the outcome of the disturbance sequence for a microbial community strongly depends on the order of disturbances. This was experimentally demonstrated in soil microbial communities by manipulating the sequence of three different disturbances (heat, freeze-thaw and anoxia; Calderon et al., 2018).

In intermittent rivers, rewetting appears to be a hot biogeochemical moment (McClain et al., 2003), i.e. shortly after the water returns, biogeochemical processes are reactivated and all biological activities are exacerbated, including primary production and respiration (Timoner et al., 2012). It appears important to understand in which extent phototrophic biofilms, that are critical for the whole river functioning, could be sensitive to contaminants leached by precipitations during rewetting. Some studies may provide a partial response. Proia et al. (2013) have shown that flow reduction and interruption amplified the effects of one contaminant, the triclosan, on river biofilms. Corcoll et al. (2015) highlighted the cumulative effects of the exposure to a mixture of pharmaceuticals followed by a dry period. However, these previous studies do not allow to predict the sensitivity to a complex agricultural run-off (ARO) of biofilms that have experienced a complete drying. Such a complex ARO, whose components (nutrients, herbicide, insecticide, fungicide, heavy metal) may have antagonistic effects, has been already shown to induce either positive or neutral effects on biofilm biomass (Allen et al., 2021).

This study aims at quantifying how the sensitivity of phototrophic biofilms to a complex agricultural runoff is modulated by river intermittency history. We conducted a microcosm experiment where we first manipulated both water warming and the duration of the dry period and then, we exposed produced biofilms to a gradient of ARO concentrations during rewetting. The objective was to understand in what extent the first disturbance (dry period and/or warming) drives the structural and functional biofilm response to the exposure to contaminant. Our hypothesis was that the first disturbance will decrease biofilm sensitivity to contamination through the selection of co-tolerant species.

2. Material and methods

Complex phototrophic biofilms were grown in the laboratory. They were next exposed to a typical dryness sequence that consisted in a period of no flow (NF) followed by a short dry period (S) or a long dry period (L) combined or not with warming (NW or W) (Fig. 1). Next, biofilms were exposed to ARO-enriched water at gradual concentrations to mimic rewetting with gradually contaminated water.

Experimental design

Biofilm growth

Natural biofilms were sampled in February 2020 in the river Garonne (43.396635, 1.300394, South-West France). Biofilms were scrapped with a toothbrush on field and were stored in river water at 4°C. Back to the laboratory, biofilm suspension was filtered on a 500-µm sieve to remove large mineral particles, and was used to inoculate artificial mini-channels designed for biofilm culture (Loustau et al., 2019). The temperature in the room was 22°C, and the flow velocity was 0.15 $m^3.min^{-1}$, light intensity was 70 $\mu mol.m^{-2}.s^{-1}$ (16h/8h light/dark periods). Biofilm were grown on ceramic coupons (5.76 cm^2). Volvic® water was used as culture medium. Twice a week, in addition to a supply with demineralized water to compensate evaporation, nutrients were added to maintain concentrations of the Volvic® water (final concentration in channel tanks: $N\text{-}NO_3^-$: 1.64 $mg.L^{-1}$, $P\text{-}PO_4^{3-}$: 30 $\mu g.L^{-1}$ and SiO_2: 32 $mg.L^{-1}$).

First disturbance

After 16 days of growth, biofilms experienced a no-flow period for one week either at 22°C (NW) or 32°C (W) to mimic the first step of dryness process. Colonised ceramic coupons were thus dispatched in 36 crystallizers (15 coupons per crystalliser) filled with 600 ml of Volvic® water in 2 distinct thermostatic chambers (ventilation 100%) in the same lighting conditions than during growth. After this first week, produced biofilms were exposed to contrasted hydric stress at both temperatures: one third of coupons were dried up for a short period (3 days), one third were dried up for a long (84 days) period before contamination, and the remaining third was not dried up and directly exposed to contamination. Dry period durations applied are in the range of dry durations, extending from 1 to 150 days, observed in Mediterranean streams (Colls et al., 2019), and within the range of what can be observed in French intermittent rivers (Snelder et al., 2013).

Second disturbance

The contaminant cocktail used was the same as in Allen et al. (2021). It consisted in a mix of nitrates (KNO_3), copper ($CuSO_4$) that is a metallic trace element used as algicide, and three organic pesticides (PESTANAL®analytical standards, Merck, Darmstadt, Germany): an insecticide inhibiting acetylcholinesterase (Pirimicarb, CAS number 23103-98-2), an herbicide inhibiting photosynthesis (Terbuthylazine, CAS number 5915-41-3) and a fungicide inhibiting sterol synthesis (Tebuconazole, CAS number 107534-96-3). The pulse of contaminant was applied in crystallizer water at six concentrations of 0, 1, 10, 50, 100 and 250 times the lowest concentration (Table SI1). Maximal concentrations (250x) were: $N\text{-}NO_3^-$ 750 $mg.L^{-1}$, Cu 3.5 $mg.L^{-1}$, PC 1.25 $mg.L^{-1}$, TBA 0.25 $mg.L^{-1}$ and TCZ 7.5 $mg.L^{-1}$. Stock solutions were prepared in demineralized water for KNO_3 and $CuSO_4$ and in dimethyl sulfoxide (DMSO, CAS 67-68-5, Merck) for organic pesticides. DMSO (0.01 $mg.L^{-1}$) alone was added to uncontaminated crystallizers. After one week of exposure to contaminant, coupons were removed and scrapped in Volvic® water (4 mL per coupon). The obtained biofilm suspension was used for the measure of all the descriptors.

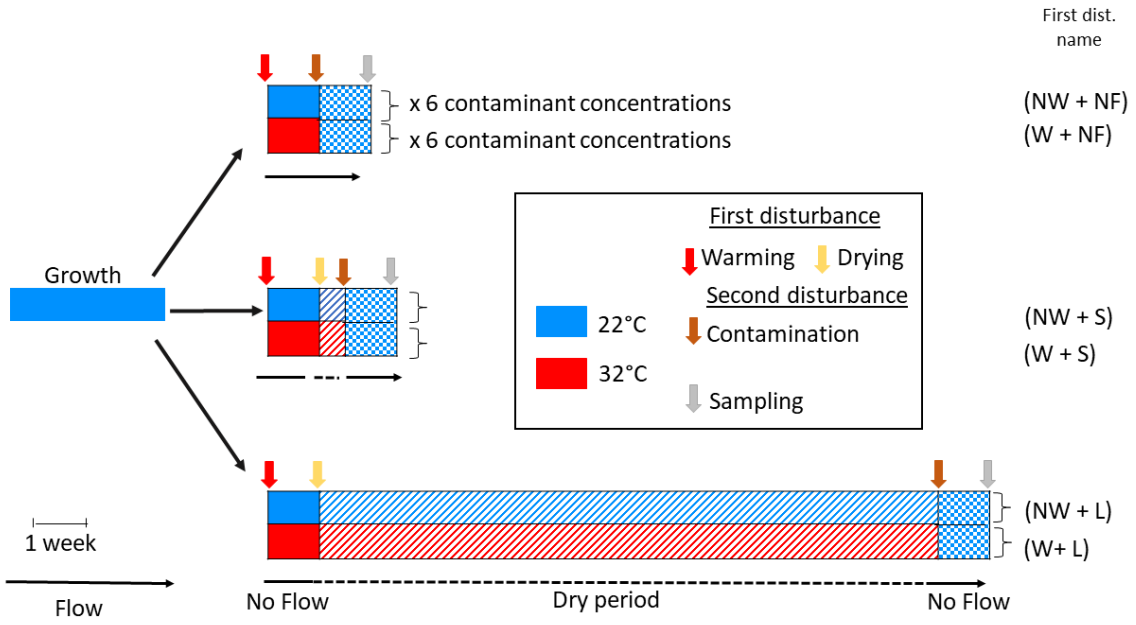

Figure 1: Experimental design (n=36). After a 2-week growth in normal flow conditions, biofilms were exposed to no-flow (NF) for a week at 22°C (NW, blue) or 32°C (W, red) (filled cases). Then, some biofilms were kept dried (hatched cases) for 3 days (short period, S) or 12 weeks (long period, L) at the same temperature than previously, before rewetting and addition of contaminant (chequered cases). Biofilm sampling and measurements were performed 1 week after contamination.

Measured descriptors

18 descriptors were measured on biofilm suspension to assess the structure of algal and bacterial communities and their functioning: the chlorophyll *a* content (Chl *a*), the autotrophic index (AI), the pigment content (main primary producer groups, pigment richness and diversity), the bacterial density, the stoichiometric composition (Carbon, Nitrogen and Phosphorus %), the quantity of extracellular polymeric substances (EPS), the carbohydrate-to-protein ratio in EPS to bring out changes in EPS composition depending on environmental conditions (Fang et al., 2014), oxygen fluxes to assess primary production and respiration, and the algal and bacterial cell physiological states (maximal quantum yield of photosystem II and % of live bacteria).

Biofilm dry mass was assessed to normalise other descriptors. One ml of each biofilm suspension was centrifuged (10 000 g, 5 min) in pre-weighed tubes. Then supernatant was removed and the pellet was oven-dried (65°C, 24h) before being weighed.

Pigment analysis

1.5 mL of biofilm suspensions were centrifuged (10 000 g, 5 min at 4°C) and pellets were stored at -80°C until pigment extraction. After freeze-drying, pellets were extracted in 2 mL of methanol (2% of ammonium acetate 1 M) (Capdeville et al., 2019). After sonication during 3 min, samples were incubated at -20°C for 15 min before another sonication step. After centrifugation (10 000 g, 5 min at 4°C), supernatants were stored at 4°C. This extraction was repeated 1 to 3 times, as long as the supernatant was still coloured. Then 1 mL of each extract were filtered with a 0.2 µm PTFE membrane syringe filter (VWR International, United States). Each pigmental composition was analysed using HPLC (LC1200 series, Agilent Technologies, United States). Specific pigment concentration was quantified by comparing each peak area of the chromatogram with those of known standards using the ChemStation software (Agilent technologies). Specific pigments were summed to obtain the proportion of each primary producer group: lutein, chlorophyll *b*, violaxanthin, neoxanthin and astaxanthin for green algae; fucoxanthin, diadinoxanthin, chlorophylls *c1* and *c2* and diatoxanthin for diatoms; myxoxanthophyll, echinenone and zeaxanthin for cyanobacteria (Majdi et al., 2011; Schlüter et al., 2006). In addition to Chl *a* quantification (reported by coupon area) used for assessing the algal biomass, a relative autotrophic index (AI) was calculated, as the ratio between Chl *a* concentration and biofilm dry weight. Finally, we calculated pigment richness (number of pigments detected) and Shannon pigment diversity index: $S=-\Sigma[Pi \ln(Pi)]$ with Pi the proportion of each pigment.

Extracellular polymeric substances

Extracellular polymeric substances (EPS) were assessed by summing both proteins and carbohydrates content. EPS were extracted on fresh samples as described by Loustau et al. (2019). After a washing step, extraction was first carried out in 1x PBS with a 37% formamide treatment. Following incubation for 60 min at 4°C at slow shaking (150 rpm),

tubes were centrifugated (8 000 g, 5 min, 4°C) and supernatant stored in 2-mL tubes. The pellets were then resuspended in 1x PBS with 50 $g.gDW^{-1}$ of cation exchange resin (CER, Dowex Marathon). Another incubation of 90 min was carried out as described in the first step. After centrifugation, supernatants were extracted, and pellets were washed another time in PBS to ensure good extraction. Supernatants tubes were stored at -20°C before quantification. The Pierce™ BCA protein assay kit (*N° 23223*, Sigma Aldrich) was used to quantify proteins content in EPS (Smith et al., 1985). Bovine serum albumin (BSA) was used as a standard, with 10 concentrations ranging from 0 to 1 $g.L^{-1}$. To increase sensitivity, microplates were incubated for 30 minutes at 60°C (Walker, 2009). After 20 min of cooling, optical density (OD) was measured at 540 nm (Synergy Mx Biotek). The anthrone method was used to quantify carbohydrates (Gaudy, 1962), using glucose as standard with concentrations ranging from 0 to 0.1 $g.L^{-1}$. After the same incubation and cooling times than for proteins, OD was measured at 620 nm. Results expressed in standard equivalents were normalised to sample biomass.

Flow cytometry

We used the Live/Dead BacLight viability kit (ThermoFisher) to discriminate between both living and dead bacterial cells and to estimate total bacterial density (number of cells by coupon area). Propidium iodide only enters bacteria with damaged membranes, while Syto9 stain labels all bacteria in green, those with intact membranes and those with damaged membranes. One mL of fresh samples was filtered on a 40-µm pore size mesh after sonication and diluted 6 times before coloration in NaCl at 0.85%. Dying was applied directly in 96-well microplates. The final volume was 200 µL per well. After a 15-min incubation in the dark, bacterial counts were performed in a microplate using a Guava® EasyCyte™ cytometer at a flow rate of 0.59 $\mu L.s^{-1}$. We recorded a maximum of 5000 events per sample. The green and red fluorescence were used to discriminate live and dead cells. Finally, the percentage of live bacterial cells was quantified using the guavaSoft software (version 3.3).

Stoichiometric composition

Total carbon, nitrogen and phosphorus contents in biofilms were measured in 1-mL biofilm suspensions oven-dried at 60°C for 24h. Carbon and nitrogen contents were determined by elemental analysis using a CHN analyser (Flash 2000, Thermo Scientific). For phosphorus quantification, samples were previously oxidized with potassium persulfate in an acid environment in autoclave to mineralize matter, and phosphorus content was determined following the molybdate-blue method (Koroleff, 1983) using a spectrophotometer at 880 nm (Uvi Light XT5, SECOCAM, Alès, France).

Primary production and respiration

Biofilm primary production and respiration were analysed by measuring changes in oxygen concentration over time using FireSting oxygen sensors (PyroScience). Measurements were conducted under agitation in 10-mL transparent vials containing 1 mL of biofilm suspension and 9 mL of Volvic water, and closed with a gas tight lid. Dissolved oxygen concentration within each vial was monitored every 5 seconds over 10 minutes using oxygen logger software. Net primary production (NPP) ($mgO2.h^{-1}.mgDW^{-1}$) was determined by measuring dissolved oxygen changes over a 5-min period of daylight (neon lamps, 90 $\mu E.m^{-2}.s^{-1}$). The rate of community (autotrophic and heterotrophic) respiration (R) was then determined as the change in oxygen concentration over a 5-min incubation period in the dark. The GPP (gross primary production) was calculated by summing NPP and R.

Fluorescence measurement

PhytoPAM measurements (Phytoplankton Analyzer, Heinz Walz GMBH, Effeltrich, Germany) have been carried out on 1/10 diluted 200-µL biofilm suspensions. The maximal quantum yield of photosystem II (PSII) or ΦPSII was used to assess algal physiological state. This metric depends on the algal species present in the biofilm, but also on the physiological state of cells as it decreases when algae are stressed or damaged (Roháček and Barták, 1999). Measurements of ΦPSII were done under dark-adapted state (Baker, 2008). ΦPSII ($0<\Phi PSII<1$) corresponds to the ratio F_V / F_M, where $F_V = F_M - F_0$ with

F_M the maximal fluorescence and F_0 the minimal fluorescence of PSII (Roháček and Barták, 1999).

Data analysis

All analyses were performed using R software version 3.6.1 (R Development Core Team). For all response variables, normality was tested, and log-transformed if necessary. A PCA was performed in order to visualize the effects of the first disturbance and contamination on biofilm global structure and functioning (Fig. 2). Effects of dry period duration (D) and warming (W) were tested with PERMANOVAs. The two first component (PC1 and PC2) of the PCA were extracted and coordinates were used as summary variables to describe the response of biofilms. Two-ways ANOVAs (D x W) and Tukey Post-hoc tests were performed on each variable as well as on PC1 and PC2.

In a second time, we assessed biofilm sensitivity to ARO by comparing the slopes of biofilm response to contaminant concentrations depending on the first disturbance biofilms were exposed to. Therefore, linear regressions were conducted on every variable as a function of contaminant concentrations. Variables were standardised using z-score to enable comparison between effects. A significant regression (t-test) indicates a significant effect of the increasing contaminant concentration on the variable. Regression slopes, as well as standard deviations, were extracted for each first disturbance treatment, and plotted together to compare the effects.

3. Results

Biofilm response to dry period and warming

PERMANOVA analysis demonstrated a significant effect of dry period duration ($p < 0.001$) on the global biofilm response. Such an effect was clearly described by the first PCA component (Fig. 2). The longer the dry period was, the more biofilm shifted toward heterotrophy (Fig. 3, Table SI2). These results were consistent with those observed on univariate analyses performed on each variable (Fig. 3). There was a significant negative effect of dry period duration on phototrophic descriptors (Table SI2): Chl *a* and AI gradually decreased with increasing dry period duration (ANOVA, $p < 0.001$, Fig. 3). Both pigment richness and diversity significantly decreased in dried biofilms ($p < 0.001$, Fig.

3). Green algae proportion increased in biofilms having experienced the long dry period (NW+L and W+L) (p = 0.004, Fig. 3, Table SI2), to the detriment of cyanobacteria (p = 0.01, Fig. 3, Table SI2). This corresponds to the effects observed on PC2 (Fig. 2, Fig. 3). Biofilm EPS quantity was significantly higher in previously undisturbed biofilm than in dried biofilms (p < 0.001, Fig. 3) with a lower carbohydrate-to-protein ratio in EPS compared to biofilms exposed to the long dry period (p < 0.001, Fig. 3, Table SI2). Bacterial concentration was highly enhanced in long dry period duration treatments (p < 0.001, Fig. 3). Heterotrophic activity represented by respiration (R) and the % of live bacteria increased while phototrophic activity represented by GPP and NPP sharply decreased with increasing dry period duration (ANOVA, p < 0.001, Fig. 2, Fig. 3, Table SI2). Consistently, the proportion of live bacteria significantly increased in dried treatments, and this effect was significantly reduced in warmed treatments (Table SI2, Fig. 3). In terms of biofilm stoichiometry, increasing dry period duration induced significant decreases in C, N, and P proportions (p <0.001, Table SI2), while significant differences between undisturbed and dried biofilms could only be observed on nitrogen content in W+L and NW+L treatments (post-hoc analysis, Fig. 3).

In addition, a significant effect of the interaction term between dry period duration and warming was observed on PC1 and PC2 (ANOVA, p < 0.04, Table SI2). Diatoms exhibited a negative response to warming with significantly less diatom-specific pigments after exposure to warming (p < 0.001, Fig. 3, Table SI2). A negative effect of warming was observed on bacterial density after the longest dry period (p < 0.001, Fig. 3, Table SI2).

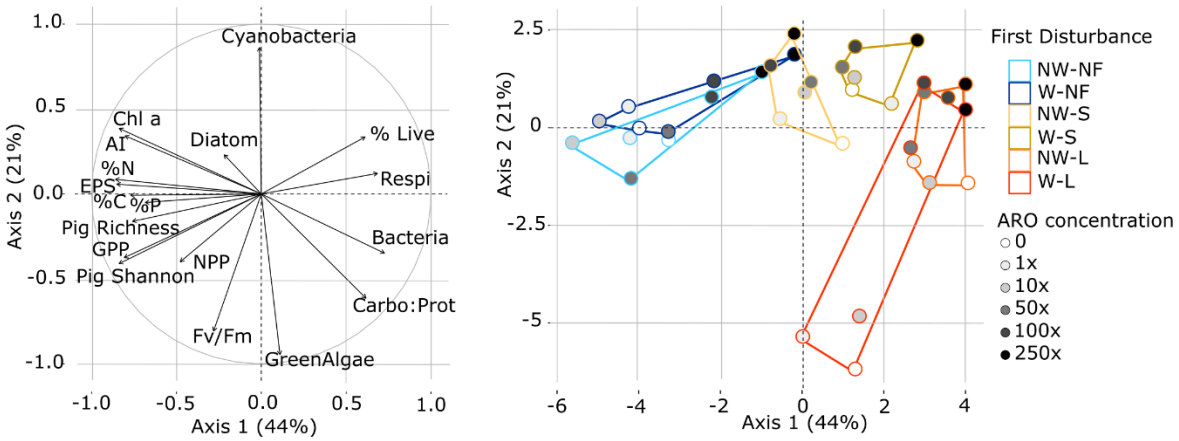

Figure 2: PCA representation of biofilm descriptors. Sample plots are grouped depending on dryness treatments and are coloured depending on ARO concentration samples were

exposed to. 18 descriptors have been used to conduct the PCA analysis. Chl *a* = chlorophyll *a*, AI = autotrophic index, EPS = extrapolymeric substances, Pig Shannon = Shannon index of pigments, Pig Richness = pigments richness, Fv/Fm = maximal quantum yield of photosystem II, GPP = gross primary production, NPP= net primary production, Respi = respiration.

Biofilm sensitivity to ARO contamination

The contaminant used to mimic ARO consisted in a mix of nitrates (KNO_3), copper ($CuSO_4$) and three organic pesticides (an herbicide, a fungicide and an insecticide). The response of the different descriptors to contamination was assessed by analysing the estimated regression slopes between each variable and the contaminant concentrations when the regression was significant (Table SI3, Fig. SI1).

ARO contamination induced a significant increase in PC1 value in the treatments NW-FN, W-NF and W-L (Fig. 4), which indicates an increase in biofilm heterotrophy (Fig. 2). Contamination had no impact on PC1 values of the other treatments. The effect of contaminant on PC2 was significant for biofilms that were previously dried for a short time and those subjected to warming alone (NF-NW, $p < 0.05$). Similar slope values were observed for all the other treatments except for W-L. Contamination had no impact on the algal compartment, except sporadic positive ones on biofilm Chl *a* in biofilms previously exposed to a short dry period without warming (NW-S treatment, Fig. 4, $t = 2.797$, $p < 0.05$) and on AI in undisturbed biofilms (NF-NW, Fig. 4, $t = 5.016$, $p < 0.01$). Contamination increased EPS production in biofilms that had been subjected to a short dry period (NW-S and W-S, Fig. 4, $t < 3.77$, $p < 0.02$). In previously undisturbed biofilms, pigment richness (Fig. 4, $t = -4.937$, $p < 0.01$) and the Shannon diversity index based on pigments (Fig. 4, $t < -3.265$, $p < 0.05$) significantly decreased with contaminant concentration, while there was no significant response in previously disturbed biofilms. Biofilms previously subjected to a long dry period with warming exhibited a shift from green algae to cyanobacteria (Fig. 4). This response to ARO contamination was confirmed on the second PCA axis (Fig. 2): low-ARO contaminated (0, 1x, 10x) samples dominated by green algae are clearly distinct from high ARO contaminated (50x, 100x, 250x) samples dominated by cyanobacteria.

When biofilms were previously exposed to warming, the total carbon content decreased with contamination (Fig. 4, $t < -2.771$, $p < 0.05$), but this sensitivity to contamination

diminished with increasing dry period duration. The response of bacterial viability to contamination was also modulated by previous warming (Fig. 4) with bacterial viability increasing with contamination in W-NF ($t = 10.10$, $p < 0.001$) and W-L ($t = 3.618$, $p = 0.05$) treatments. Respiration also tended to be stimulated by the contaminant when biofilms had been previously exposed to warming (Fig. 4). F_V/F_M decreased with contamination in biofilms previously dried (Fig. 4, $t < -2.734$, $p < 0.05$). On the contrary, gross primary production (Fig. 4) and net primary production (Fig. 4) tended to decrease with contamination only when biofilms were not previously dried, though it was significant only for the W-NF treatment ($t < -3.582$, $p < 0.023$).

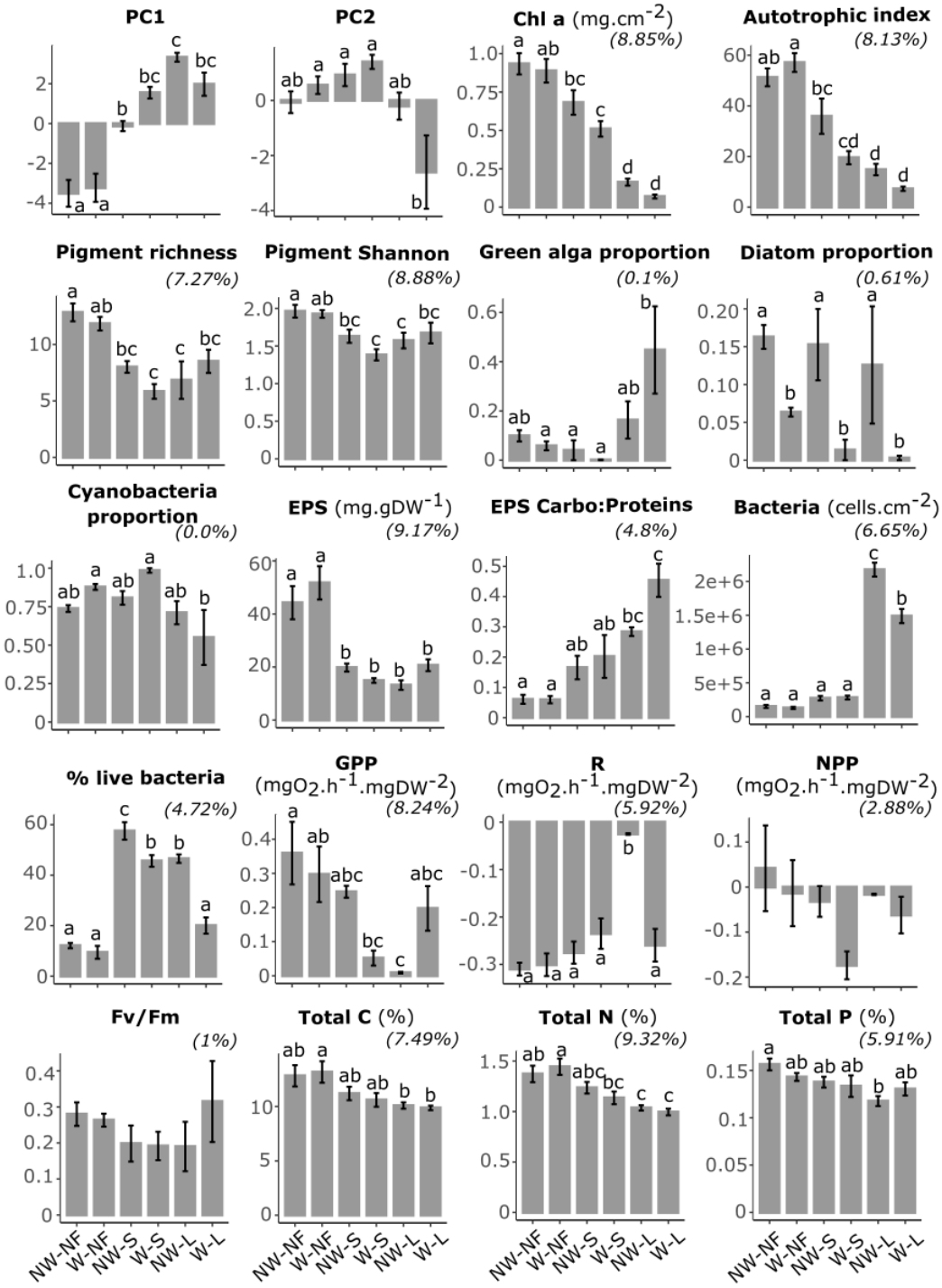

Figure 3: Barplot representations of the effects of the first disturbance on biofilm descriptors. Values represent the mean ± standard error (n = 6) PC1 and PC2 coordinates of the PCA respectively, and of each variable according to the 6 first conditions of dry period duration (NF, S or L) and warming (NW or W). Different letters mean significant statistical differences (level = 0.05). Percentage in parentheses represents the absolute contribution of the variable to the PC1.

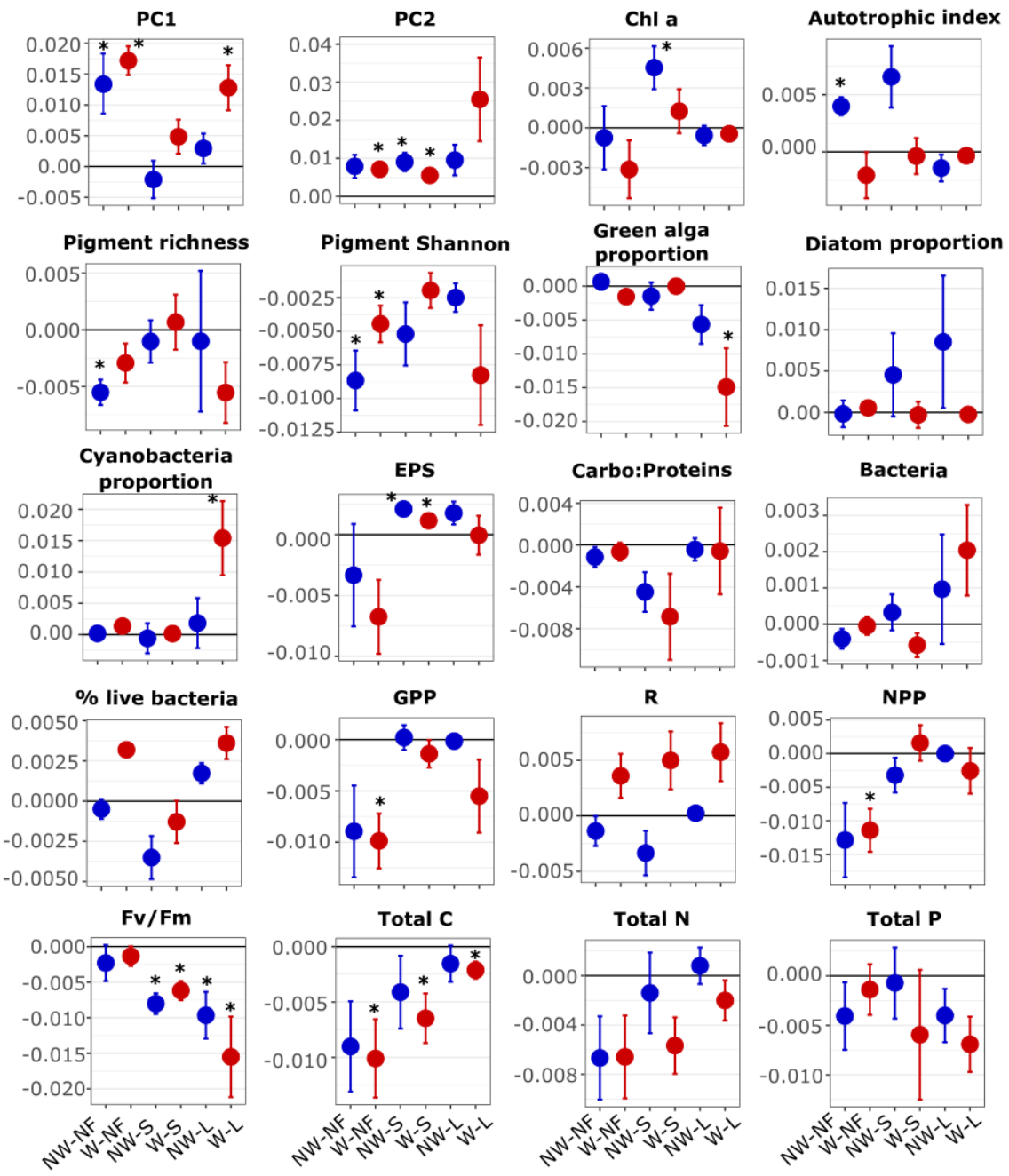

Figure 4: Biofilm sensitivity to ARO contamination depending on the first disturbance. Sensitivity to contamination is represented by the slope of regressions between measured parameter and contaminant concentrations for the 6 first conditions of dry period duration (NF, S or L) and warming (NW or W). It represents the mean (± standard error, n = 6) slopes of regressions between each parameter and contaminant concentration. No warmed and warmed treatments are represented by blue and red circles, respectively. Asterisks are for significant statistical differences (level = 0.05)

4. Discussion

The prevalence of intermittency in world rivers (Messager et al., 2021) makes necessary to take into account their specificities in the assessment of aquatic ecosystem vulnerability to anthropogenic stressors. The aim of this study was to assess if intermittency would alter the sensitivity of a river microbial community to a complex contamination. In addition to dryness, we considered the effect of water warming associated to water flow cessation. Though the response of biofilm to a synthetic agricultural-run off (ARO) was moderate, our results show that it is strongly driven by biofilm history of disturbance.

Response to ARO in previously undisturbed biofilms

The biofilms produced for the experiment were mainly dominated by cyanobacteria and in a lesser extent by diatoms, and were characterized by a positive net primary production. When exposed to increasing ARO contamination, such phototrophic communities lost algal richness and diversity, and GPP tended to decrease, while heterotrophy was enhanced (Fig. 4, PC1). Loss in pigment richness and diversity was likely linked to the removal of species sensitive to ARO, notably to copper and herbicide that were expected to negatively affect microalgae (Sousa, Chow, and Pompêo, 2019). Despite this effect, previously undisturbed biofilms globally exhibited rather low sensitivity to ARO, given the very high concentrations applied, with values reaching 750 $mg.L^{-1}$ of $N\text{-}NO_3$, 3.5 $mg.L^{-1}$ of Cu and 250 $\mu g.L^{-1}$ of terbuthylazine.

Different reasons, such as ARO complexity, biofilm abilities to resist, and experimental timing may explain such rather weak effects. First, the pesticides toxic effects could be counterbalanced by positive effects of N inputs at lowest ARO concentrations, as shown by Aristi et al., (2016), and therefore could mitigate pesticides impact. Additionally, N inputs may have increased heterotrophy by providing an alternative electron acceptor that may promote heterotrophic activities (denitrification) in the anoxic deeper layers of biofilms. Such a compensation between negative and positive effects of ARO components depending on ARO concentration is also consistent with the negative impact we observed on GPP at the highest ARO concentrations. Resistance to organic pesticides and heavy metals is notably linked to the EPS matrix which adsorbs those pollutants (Flemming et al., 2016), and to biofilm thickness that limits contact between microbial cells and pesticides for the deeper layers (Davies, 2003). The biofilm biomasses reached in this

study correspond to those of mature natural biofilms (Boulêtreau et al., 2006) and EPS production is similar to the one of classic biofilms (Kroll et al., 2014), so that such resistance mechanisms may occur. Finally, biofilms were likely to exhibit a partial recovery since they were sampled one week after ARO addition, and algae can recover very fast from herbicide exposure, as already shown for atrazine, a photosynthesis inhibitor like terbuthylazine (Laviale et al., 2011).

Response to ARO in biofilms pre-exposed to warming

Water temperature can dramatically increase over flow cessation, desiccation and fragmentation into isolated pools (Gómez et al., 2017). In accordance with field personal observations, we experimented how a +10°C heatwave could modulate ARO sensitivity. Despite the high intensity of the heatwave, biofilm response to ARO contamination was not strongly impacted by warming. The response of warmed but undried biofilms to ARO was globally similar to the one of previously undisturbed biofilms. However, pigments richness decrease was weaker, respiration tended to be enhanced and GPP was inhibited by ARO in biofilms pre-exposed to warming. This indicates that a warming event before contamination would tend to mitigate algal simplification but would amplify biofilm functioning shift toward heterotrophy. Switches to heterotrophy due to warming have been observed in natural biofilms in intermittent rivers (Acuña et al., 2015). This is partly explained by the higher metabolic response of respiration than photosynthesis to temperature (Boulêtreau et al,. 2012). Weak effects of warming observed on biofilm pigment composition do not rule out possible replacements of algal or bacterial species according to their thermal preferences, since the taxonomic composition of the communities was not assessed here.

Many studies have considered the effect of contaminants depending on temperature, but in most cases the stressors were applied simultaneously rather than sequentially. Nutrient supply, combined with warming, induced changes in structure with enhanced bacterial growth (Degerman et al., 2013). In this study, authors have shown that increasing nutrient concentration enhanced growth, with a stronger positive effect when the temperature exposure was higher (+4°C). Increasing temperature (+3, +6 and +10°C) also reduced the negative effects of toxic organic pollutants (Larras et al., 2013). When applied before contamination, warming could select species that are tolerant to heavy metal exposure and reduce the effects of the latter (Pesce et al., 2018). On the opposite,

Lambert et al., (2017) noticed that the sensitivity of phototrophic biofilms to copper increased after warming. These contradictory observations all fit with the theory of co-tolerance developed by Vinebrooke et al. (2004). When two disturbances are applied successively, the first disturbance can either increase the tolerance to the second disturbance through antagonist effects (Rillig et al., 2015; Li et al., 2014) or increase the sensitivity through synergic effects. The lower decrease in pigment richness compared to undisturbed biofilms and the weak global warming effect we observed on contaminated biofilms pre-exposed to warming could be explained by a positive correlation between warming and contamination effects on species tolerance. Species more tolerant to ARO could have been already selected by warming pre-exposure. All these experimental results suggest that the outcome of successive disturbances is far from easy to predict depending on the initial community composition, species properties, and the nature and chronology of the disturbances.

Response to ARO in previously dried biofilms

Non-perennial rivers and streams cover a wide spectrum of flow conditions from rarely intermittent rivers periodically experiencing flow cessation during a few days to ephemeral streams that only temporally flow after intense rainfall. Consistently, we exposed biofilms to two contrasted durations, a short dry period of 3 days and a long dry period of 3 months. Dry period is a very strong disturbance for the aquatic organisms forming phototrophic biofilms, inducing a high mortality rate in algae (Barthès et al., 2015a; Souffreau et al., 2010). However, even after quite long dry periods, biofilms are able to quickly recover and resume their functioning (e.g. Timoner et al., 2012). Fast recovery is partly due to the ability of some species to form resting spores to resist dry period (Hargraves and French, 1983) and to become active again within hours and days of flow resumption (Robson et al., 2008). High functional resilience is often coupled with a low structural resilience, with marked modification of the composition of both algal and bacterial components (Barthès et al., 2015b; Gottlieb, Richards, and Gaiser, 2005). In our experiment, biofilms exposed to a dry period showed deep modifications of their composition, well visible on the PCA first component, with less chlorophyll *a* biomass, less EPS, lower pigment diversity, and more bacteria. Dry period also resulted in changes in biofilm productivity, with a consistent decrease in GPP. This trend toward heterotrophy is likely also promoted by the release of nutrients by dead algal cells.

In previously dried biofilms, there was almost no effect of ARO contamination visible on the PCA first component. The contamination tended to mitigate the effect of the first disturbance on EPS production: EPS were reduced by half with dryness, whatever its duration, but slightly increased with ARO contamination. This makes sense since EPS are known to play a role in algal protection against heavy metals (Flemming et al., 2016). Dryness also led to lower ARO sensitivity of pigment richness and diversity, similarly to what was observed for biofilms pre-exposed to warming. This can be explained by the selection of co-tolerant species, as hypothesized for warming. It may also be linked to the fact that dry period had already a very strong effect on pigment diversity. The maximum quantum yield of PSII (F_V/F_M) was negatively impacted by ARO in dried biofilms in contrast with undisturbed or biofilms pre-exposed to warming. F_V/F_M value can reflect the nature of the main algal groups present in biofilm but ARO did not induce clear changes in green algae, cyanobacteria and diatom proportions. Therefore, the observed decrease in F_V/F_M suggests dry period would amplify the deterioration of the physiological state of algae (Laviale, 2008) probably due to pesticide exposition (Tiam et al., 2015). In terms of functioning, previously dried biofilms exhibited a global lower sensitivity to contaminant than undisturbed biofilms, with notably NPP and GPP less affected by ARO. This apparent reduced sensitivity is consistent with some previous studies. For instance, Calapez et al., (2020) found that after a combined exposure to no-flow and to sewage contamination, both the negative effect of contaminant on algae and the positive effect on bacteria were less pronounced than after contaminant exposure alone. Similarly, it has been shown that after intermittency, biofilms were able to maintain biodegradation of a certain pharmaceutical when exposed to emerging contaminants, while biofilms exposed to permanent flow were not (Rožman et al., 2018). It should be noted that in these two studies, biofilms were exposed to complex contaminants. On the contrary, Proia et al. (2013) observed that flow intermittency increased biofilm sensitivity to triclosan, suggesting that the toxicity of pollutants may be exacerbated following a drought. In the present study, the regressions point out that the physical disturbance induced by the dry period had the highest impact on biofilms and that the chemical stressor did not significantly intensify drought-induced changes. A similar observation emerged from the meta-analysis performed by Arenas-Sánchez et al. (2016) on studies focusing on the co-occurring effects of water scarcity and chemical pollution on micro-

and macro-organisms in aquatic ecosystems. This highlights the necessity to prioritize at least minimal water support in river management.

Response to ARO in biofilms previously exposed to both disturbances

As seen on the PCA, the combination of warming and dry period induced a strong modification of biofilm algal composition: green algae replaced cyanobacteria and diatoms which dominated undisturbed biofilm. This modification of algal composition may explain why those biofilms exhibited highest F_V/F_M than undisturbed biofilms, since green algae have highest F_V/F_M than cyanobacteria (Suggett et al., 2009). When these previously disturbed biofilms were exposed to ARO, the proportion of green algae (and F_V/F_M) significantly decreased and cyanobacteria proportion increased. This stronger response to ARO, quite visible on the PCA plot may be explained by a higher sensitivity of green algae to ARO (Ma, 2005).

Conclusion

As a whole, these results show very clearly that previous warming and/or dryness modulate biofilm response to a complex contaminant added during rewetting. We hypothesized that the first disturbance would decrease biofilm sensitivity to contamination. This was only partially observed since warming decreased the ARO effects on biofilm composition but amplified them on biofilm functioning. Dry periods significantly reduced the contaminant effects. Our findings show that the first disturbance was the one impacting the most biofilms. The low sensitivity of dried biofilm to ARO is certainly due to the selection of generalist species or the induction of physiological responses by the first disturbance that allow biofilm to resist contamination during rewetting. Our study emphasizes the importance of considering the hydrological history of a microbial community when assessing its sensitivity to a contaminant, and more generally, the requirement to consider water scarcity in the risk assessment of chemicals in aquatic ecosystems.

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Supplementary information

Table SI1: final concentrations of each contaminant added per crystallizer, expressed in $mg.L^{-1}$. TBA = terbuthylazine, PC = pirimicarb and TCZ = tebuconazole

		N-NO3	Cu	TBA	PC	TCZ
ARO concentration	C	0	0	0	0	0
	1x	3	0,014	0,001	0,005	0,03
	10x	30	0,14	0,01	0,05	0,3
	50x	150	0,7	0,05	0,25	1,5
	100x	300	1,4	0,1	0,5	3
	250x	750	3,5	0,25	1,25	7,5

Table SI2: two-ways ANOVA outputs (Var ~ Dry period duration (D) * Warming (W)). Significant results are represented in bold.

	R^2 adjusted	**P-value model**		**SS**	**Df**	**F-value**	**P-value**
			Structure				
PC 1	0.82	**<0.001**	D	226.17	2	75.961	**<0.001**
			W	0.36	1	0.240	0.628
			D x W	13.77	2	4.625	**0.018**
			Residuals	44.66	30		
PC 2	0.35	**0.003**	D	40.59	2	8.117	**0.002**
			W	1.69	1	0.677	0.417
			D x W	17.36	2	3.471	**0.044**
			Residuals	75.01	30		
Chl *a*	0.84	**<0.001**	D	3.85	2	94.817	**<0.001**
			W	0.10	1	4.759	**0.037**
			D x W	0.02	2	0.600	0.556
			Residuals	0.61	30		
AI	0.80	**<0.001**	D	1.14E+04	2	64.990	**<0.001**
			W	3.29E+02	1	3.758	0.062
			D x W	7.51E+02	2	4.289	**0.023**
			Residuals	2.62E+03	30		
EPS	0.71	**<0.001**	D	7.61E+03	2	42.332	**<0.001**
			W	1.02E+02	1	1.134	0.295

			D x W	3.06E+02	2	1.701	0.200
			Residuals	2.70E+03	30		
Bacteria	0.96	**<0.001**	D	2.12E+13	2	439.268	**<0.001**
			W	4.87E+11	1	20.205	**<0.001**
			D x W	9.29E+11	2	19.264	**<0.001**
			Residuals	7.23E+11	30		
Green Algae	0.30	0.01	D	0.54	2	6.851	**0.004**
			W	0.04	1	1.054	0.313
			D x W	0.21	2	2.649	0.087
			Residuals	1.19	30		
Diatoms	0.25	0.02	D	0.01	2	0.836	0.443
			W	0.13	1	14.999	**0.001**
			D x W	0.00	2	0.138	0.872
			Residuals	0.26	30		
Cyanobacteria	0.25	0.02	D	0.44	2	5.428	**0.010**
			W	0.02	1	0.607	0.442
			D x W	0.21	2	2.559	0.094
			Residuals	1.22	30		
Pigment diversity	0.63	**<0.001**	D	3.45E+03	2	30.433	**<0.001**
			W	1.40E+02	1	2.471	0.127
			D x W	1.37E+02	2	1.208	0.313
			Residuals	1.70E+03	30		
Pigment richness	0.52	**<0.001**	D	206.72	2	18.812	**<0.001**
			W	2.25	1	0.410	0.527
			D x W	23.17	2	2.108	0.139
			Residuals	164.83	30		
Total C	0.31	0.01	D	57.54	2	10.089	**<0.001**
			W	0.21	1	0.074	0.787
			D x W	1.34	2	0.234	0.792
			Residuals	5.54	30		
Total N	0.53	**<0.001**	D	0.93	2	21.010	**<0.001**
			W	0.00	1	0.218	0.644
			D x W	0.05	2	1.034	0.368
			Residuals	0.67	30		
Total P	0.26	0.01	D	0.00	2	6.733	**0.004**
			W	0.00	1	0.076	0.785
			D x W	0.00	2	1.784	0.185
			Residuals	0.01	30		

Fv/Fm	_	_	D	0.04	2	0.838	0.443
			W	0.01	1	0.453	0.506
			D x W	0.04	2	0.822	0.449
			Residuals	0.68	30		
GPP	0.40	**<0.001**	D	0.34	2	8.463	**0.001**
			W	0.00	1	0.230	0.635
			D x W	0.23	2	5.640	**0.008**
			Residuals	0.60	30		
R	0.72	**<0.001**	D	0.17	2	23.804	**<0.001**
			W	0.03	1	9.775	**0.004**
			D x W	0.14	2	19.291	**<0.001**
			Residuals	0.11	30		
NPP	_	_	D	0.08	2	2.258	0.122
			W	0.06	1	3.243	0.082
			D x W	0.02	2	0.461	0.635
			Residuals	0.55	30		
%Live	0.90	**<0.001**	D	1.00E+04	2	131.692	**<0.001**
			W	1.69E+03	1	44.297	**<0.001**
			D x W	8.65E+02	2	11.370	**<0.001**
			Residuals	1.14E+03	30		
Carbo:proteins	0.64	**<0.001**	D	0.58	2	28.803	**<0.001**
			W	0.04	1	4.195	**0.049**
			D x W	0.05	2	2.405	0.107
			Residuals	0.30	30		

Table SI3: two-ways ANOVA outputs (Var ~ Dist1 *ARO). Significant results are bold represented.

	R2 adjusted	**p-value model**		**SS**	**Df**	**F-value**	**P-value**
			Structure				
PC1	0.94	**<0.001**	Dist1	240.3	5	97.113	**<0.001**
			ARO	19.48	1	39.365	**<0.001**
			Dist1*ARO	13.3	5	5.387	**0.002**
			Residuals	11.88	24		
PC2	0.68	**<0.001**	Dist1	59.64	5	9.651	**<0.001**

			ARO	32.65	1	26.412	**<0.004**
			Dist1*ARO	12.70	5	2.056	0.106
			Residuals	29.67	24		
Chl *a*	0.87	**<0.001**	Dist1	30.35	5	46.891	**<0.001**
			ARO	0.01	1	0.044	0.835
			Dist1*ARO	1.54	5	2.383	0.069
			Residuals	3.11	24		
AI	0.87	**<0.001**	Dist1	28.91	5	45.858	**<0.001**
			ARO	0.32	1	2.546	0.124
			Dist1*ARO	2.75	5	4.358	**0.006**
			Residuals	3.03	24		
EPS	0.76	**<0.001**	Dist1	26.19	5	21.924	**<0.001**
			ARO	0.21	1	0.865	0.362
			Dist1*ARO	2.87	5	2.401	0.067
			Residuals	5.73	24		
Bacteria	0.97	**<0.001**	Dist1	33.92	5	200.514	**<0.001**
			ARO	0.02	1	0.630	0.435
			Dist1*ARO	0.25	5	1.449	0.243
			Residuals	0.81	24		
Green Algae	0.63	**<0.001**	Dist1	14.02	5	7.660	**<0.001**
			ARO	4.14	1	11.305	**0.003**
			Dist1*ARO	8.05	5	4.398	**0.006**
			Residuals	8.79	24		
Diatoms	0.26	**<0.001**	Dist1	21.08	5	9.631	**<0.001**
			ARO	0.81	1	1.855	0.186
			Dist1*ARO	3.40	5	1.553	0.211
			Residuals	10.50	24		
Cyanobacteria	0.54	**<0.001**	Dist1	12.46	5	5.362	**0.002**
			ARO	2.63	1	5.649	**0.026**
			Dist1*ARO	8.77	5	3.772	**0.012**
			Residuals	11.15	24		
Pigment richness	0.54	**<0.001**	Dist1	20.47	5	8.806	**<0.001**
			ARO	1.83	1	3.939	0.059
			Dist1*ARO	1.55	5	0.665	0.653
			Residuals	11.16	24		
Pigment diversity	0.76	**<0.001**	Dist1	24.03	5	20.149	**<0.001**
			ARO	3.57	1	14.947	**<0.001**
			Dist1*ARO	1.68	5	1.404	0.258

			Residuals	5.73	24		
Total C	0.62	**<0.001**	Dist1	14.30	5	7.605	**<0.001**
			ARO	8.71	1	23.158	**<0.001**
			Dist1*ARO	2.97	5	1.580	0.204
			Residuals	9.02	24		
Total N	0.66	**<0.001**	Dist1	20.87	5	12.252	**<0.001**
			ARO	3.63	1	10.657	**0.003**
			Dist1*ARO	2.33	5	1.365	0.272
			Residuals	8.18	24		
Total P	0.30	**0.037**	Dist1	12.71	5	3.641	**0.014**
			ARO	4.14	1	5.925	**0.023**
			Dist1*ARO	1.39	5	0.400	0.844
			Residuals	16.76	24		
Fv/Fm	0.57	**<0.001**	Dist1	3.91	5	1.816	0.148
			ARO	14.45	1	33.560	**<0.001**
			Dist1*ARO	6.30	5	2.926	**0.034**
			Residuals	10.34	24		
GPP	0.66	**<0.001**	Dist1	17.03	5	10.089	**<0.001**
			ARO	5.16	1	15.275	**<0.001**
			Dist1*ARO	4.71	5	2.789	**0.040**
			Residuals	8.10	24		
R	0.82	**<0.001**	Dist1	26.66	5	29.110	**<0.001**
			ARO	0.76	1	4.140	0.053
			Dist1*ARO	3.18	5	3.472	**0.017**
			Residuals	4.40	24		
NPP	0.49	**0.002**	Dist1	7.86	5	3.048	**0.029**
			ARO	6.33	1	12.286	**0.002**
			Dist1*ARO	8.44	5	3.277	**0.021**
			Residuals	12.37	24		
%Live	0.96	**<0.001**	Dist1	32.09	5	151.445	**<0.001**
			ARO	0.08	1	1.922	0.178
			Dist1*ARO	1.82	5	8.566	**<0.001**
			Residuals	1.02	24		
Carbo:proteins	0.68	**<0.001**	Dist1	24.13	5	15.222	**<0.001**
			ARO	1.56	1	4.932	**0.036**
			Dist1*ARO	1.69	5	1.069	0.402
			Residuals	7.61	24		

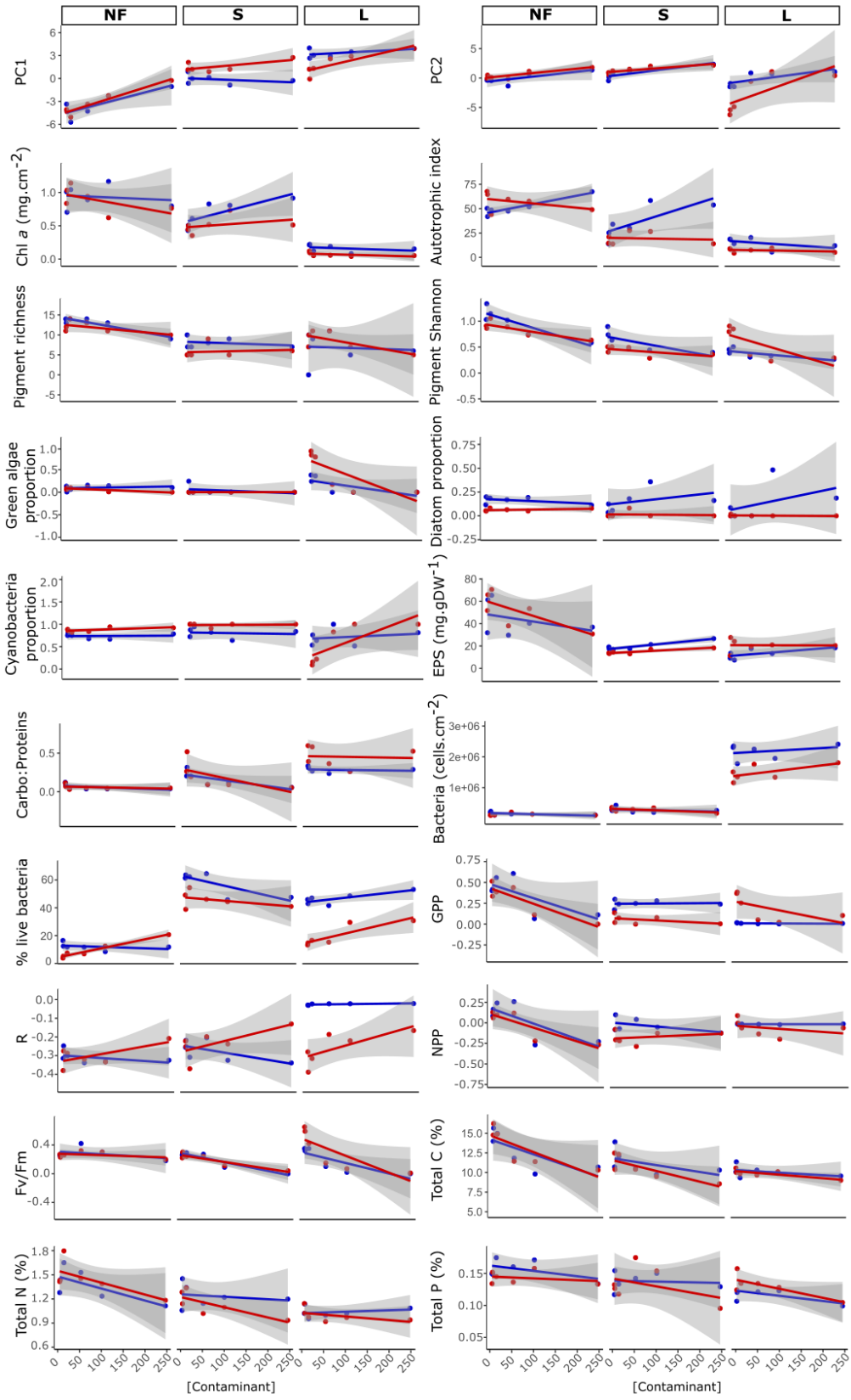


Figure SI 1: regression plots of each measured parameter as a function of contaminant concentration. for each drying duration (NF = no dry period. S = short dry period. L = long dry period) and for warmed (red) and no warmed (blue) treatments.

Chapitre 5

Influence de l'histoire thermale sur la stabilité du biofilm phototrophe dans une rivière intermittente

Chapitre 5 : Influence de l'histoire thermale sur la stabilité du biofilm phototrophe dans une rivière intermittente

Contexte

Lors d'une séquence d'intermittence hydrique, les biofilms phototrophes sont soumis à l'augmentation des températures, d'abord de l'eau puis de l'air, en plus de la dessication. L'influence de cette augmentation de température sur la dynamique de la réponse des biofilms lors de la remise en eau est méconnue. Or, cette histoire thermale est également susceptible de modifier la stabilité des biofilms lors de la remise en eau, notamment lorsque l'eau est contaminée.

Les effets des contaminants et de la température ont été assez largement étudiés, mais de manière simultanée plutôt que séquentielle. Les différentes études montrent que l'interaction contaminant * température dépend de la nature du contaminant qui est appliqué. Par exemple, l'augmentation de température diminue la tolérance des biofilms à une exposition au cuivre (Morin et al., 2012) mais augmente la tolérance algale aux pesticides organiques (Larras et al., 2013). Un enrichissement en nutriment combiné à une augmentation de température augmente la biomasse des phototrophes (Lürling et al., 2018).



L'Aveyron asséché avec mare résiduelle (Gué de Picquecos, Tarn et Garonne, Sud Ouest France) en Août 2022.

De plus, l'effet de l'assec sur les biofilms est plutôt bien documenté dans la littérature (Romaní et al., 2013, Acuña et al., 2015) mais ces travaux ne permettent pas de prédire comment l'histoire thermale de ces communautés microbiennes influence leur stabilité, d'autant plus lorsque le contaminant est complexe.

L'exposition à une température élevée avant la contamination pourrait induire une co-tolérance ou une co-sensibilité (au sens de la *negative co-tolerance* décrite par Vinebrooke et al., 2004) des communautés et donc, des interactions entre les deux perturbations qui soient synergiques ou antagonistes.

Dans le chapitre précédent, j'ai mis en évidence que la résistance des biofilms à un contaminant complexe dépend de la combinaison antérieure température * assec. La résistance à ARO semble globalement augmenter suite à l'assec mais ce résultat varie selon les conditions d'intermittence et les variables considérées (Courcoul et al., 2022). Si ces résultats semblent suggérer un effet d'héritage de la température sur la stabilité du biofilm au contaminant, ils doivent être testés.

Objectifs et hypothèses

L'objectif est d'évaluer comment l'histoire thermale d'une situation d'intermittence hydrique influence la stabilité de la structure et du fonctionnement de biofilms phototrophes exposés à un contaminant lors de la remise en eau. Pour répondre à cet objectif, des biofilms cultivés en microcosmes ont été soumis à une augmentation de température (+10°C) ou pas lors d'un assec long, puis contaminés lors de la remise en eau avec un contaminant complexe mimant un lessivat agricole selon un gradient de concentrations.

Mes hypothèses sont :

1. L'histoire thermale modifie la stabilité des biofilms après l'assec.
2. L'histoire thermale modifie la stabilité des biofilms exposés au contaminant, et cet effet varie en fonction de la concentration en contaminant.
3. L'effet de l'histoire thermale diminue au cours du temps.

Matériel et méthodes

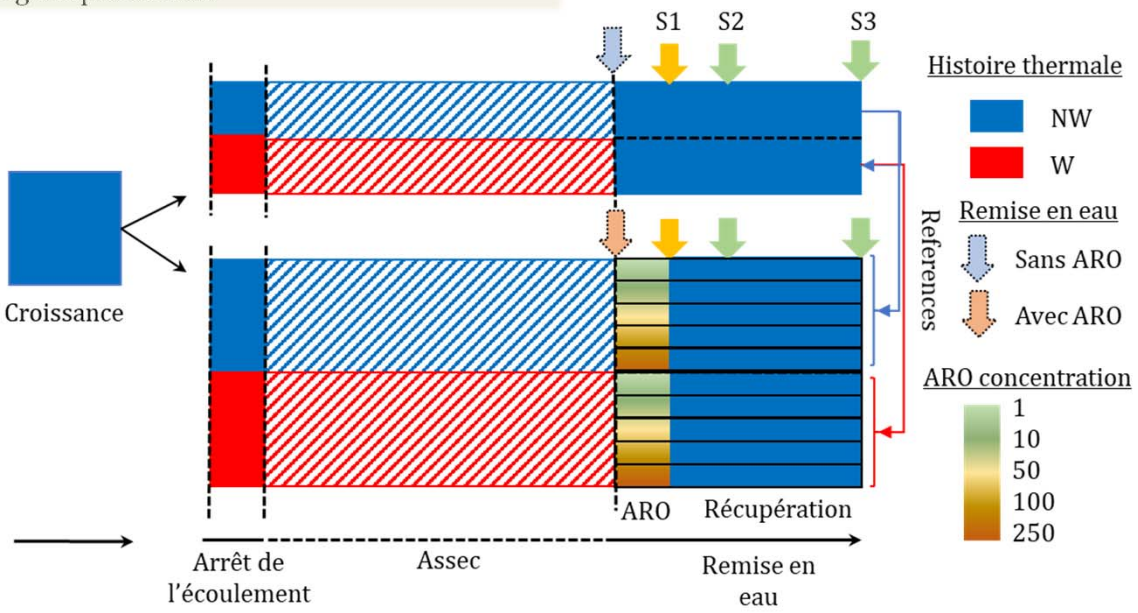
Des biofilms échantillonnés en rivière ont été cultivés en canaux au laboratoire sur des coupons de céramique. A l'issue de la période de croissance, les biofilms ont été transférés dans des cristallisoirs, puis placés à 22°C (NW) ou 32°C (W) pendant 1 semaine, pour simuler la période d'arrêt de l'écoulement. Les biofilms ont ensuite subi un assec de 12 semaines aux mêmes températures d'exposition que lors de la période d'arrêt de l'écoulement. Enfin, tous les biofilms ont été remis en eau à 22°C avec de l'eau non contaminée ou de l'eau contaminée à 5 différentes concentrations de ARO pendant 1 semaine. Plusieurs prélèvements (S1, S2, S3) ont été réalisés pour évaluer la dynamique temporelle de la réponse.

Le mélange de contaminants utilisé, et les concentrations étaient les mêmes que dans le chapitre 4.



Biofilms sur coupons de céramiques, asséchés en cristallisoirs

Design expérimental



N=36 échantillons=2T°C * 6 concentrations * 3 réplicats pour chaque prélèvement
S1 : prélèvement de fin de perturbation; S2 : 1 semaine après la fin de la perturbation; S3 : 3 semaines après la fin de la perturbation

Paramètres mesurés

- Biomasse (sèche et algale)
- Analyses de pigments
- Composition des EPS
- Composition élémentaire
- Composition et diversité des eucaryotes et des procaryotes
- Flux d'oxygène (GPP, NPP, R)
- Activité photosynthétique (F_V/F_M)
- Biolog

Analyses des données

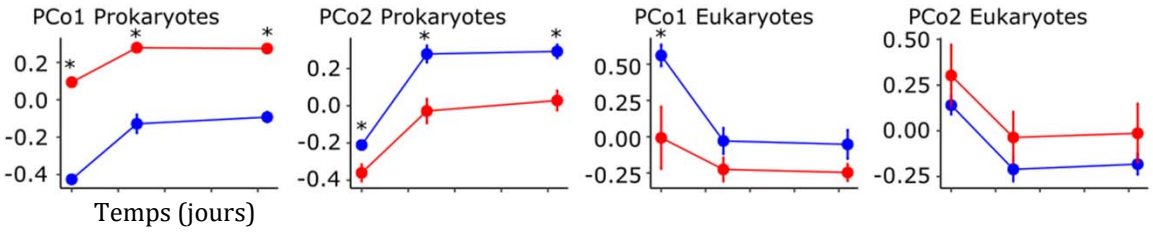
Des analyses ont d'abord été réalisées sur les biofilms non contaminés. La réponse de chaque variable (univariée et multivariée) a été comparée pour chaque prélèvement entre les biofilms précédemment exposés aux 2 températures afin de mettre en évidence l'effet de l'histoire thermale sur la dynamique de réponse à l'assec.

Ensuite, pour les biofilms contaminés, la réponse de chaque variable a été comparée prélèvement par prélèvement à celle des biofilms non contaminés exposés à la même histoire thermale (référence). Cela a permis de mettre en avant les différences éventuelles d'impact du contaminant selon l'histoire thermale, au cours du temps.

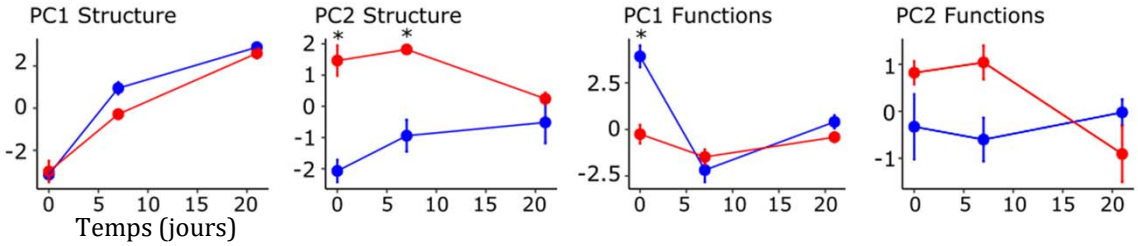
Résultats

Influence de l'histoire thermale sur la dynamique après l'assec

L'histoire thermale a un fort effet sur la composition des communautés après un assec. Cet effet persiste dans le temps pour les procaryotes, ce qui montre que l'histoire thermale pendant l'assec conditionne la composition de la communauté procaryote à plus long terme.

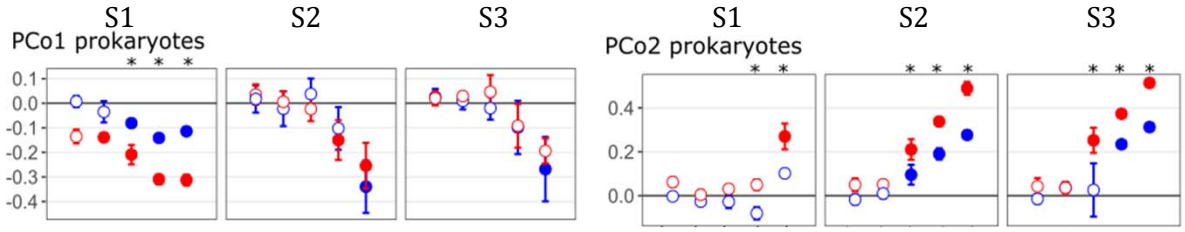


Après l'assec, il y a des différences dans la structure générale et le fonctionnement liés à l'histoire thermale, mais ces effets disparaissent au cours du temps → la période de récupération fait disparaitre les différences liées à l'histoire thermale.

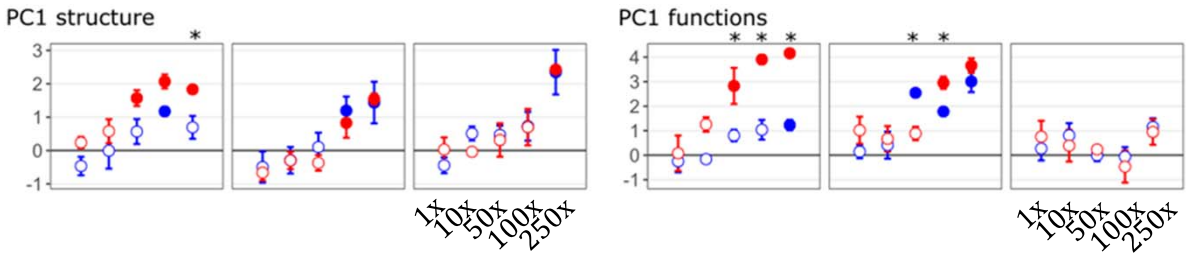


Influence de l'histoire thermale sur la dynamique de la réponse à un contaminant après l'assec

En fin de perturbation (S1) l'exposition aux fortes température augmente les changements liés à ARO par rapport à la référence sur l'axe 1.
Sur l'axe 2, les effets liés au contaminants sont plutôt visibles pendant la période de récupération. Pour les plus fortes concentrations, quand les biofilms ont été exposés à la forte température pendant l'assec, les changements engendrés par le contaminant sont plus marqués.



L'histoire thermale a relativement peu d'effet sur la réponse de la structure générale à ARO, malgré une nette tendance à observer des changements plus forts pour les fortes concentrations en fin de perturbation (S1). Ces effets disparaissent au cours du temps de récupération.
Les effets de ARO sur le fonctionnement des biofilms sont assez similaires, mais avec un effet important de l'histoire thermale à S1. Une forte résilience fonctionnelle est observée puisqu'à S3, il n'y a pas de différence significative.



Conclusions et Perspectives

Influence de l'histoire thermale sur la dynamique après l'assec

De manière générale, l'histoire thermale influence la trajectoire de récupération des biofilms suite à l'assec

- Des différences importantes entre les biofilms ayant des histoires thermale contrastées sont mises en évidence sur la composition des communautés bactériennes et ces différences subsistent au cours de la période de récupération.
- L'histoire thermale influence la composition des communautés eucaryotes après l'assec, mais elles convergent au cours de la période de récupération
- Les différences induites par l'histoire thermale sur le fonctionnement en fin de perturbation disparaissent au cours du temps de récupération

Comme attendu, l'histoire thermale module la stabilité des biofilms suite à l'assec, cependant, contrairement à ce qui était attendu, l'exposition à des températures élevées lors de l'assec n'a pas intensifié les processus hétérotrophes (Freixa et al., 2017, Romero et al., 2019).

→ Validation partielle de l'hypothèse 1

Influence de l'histoire thermale sur la stabilité face à l'exposition au contaminant après un assec

- **L'exposition à une augmentation de température lors de l'assec amplifie les effets du contaminant** par rapport aux références, avec notamment une augmentation des processus hétérotrophes.

→ Validation de l'hypothèse 2

Ces différences entre histoires thermales diminuent lors de la période de récupération, mettant en évidence une forte résilience fonctionnelle des biofilms. Cependant, la composition des communautés bactériennes continuent à diverger entre histoires thermales.

→ Validation de l'hypothèse 3

Cette étude met en évidence l'influence importante de l'histoire thermale des communautés sur la réponse des biofilms, qui est d'autant plus importante que la concentration en contaminant à laquelle les communautés sont exposées est importante. Cela suggère que l'exposition à de fortes températures lors de l'assec sélectionne des espèces sensibles à la contamination. Dans le contexte actuel de changement global, l'augmentation des évènements d'intermittence hydrique et l'exposition à des contaminants complexes augmentent, menaçant l'intégrité de l'écosystème.

Thermal history influences the stability of phototrophic biofilms exposed to agricultural run-off in intermittent rivers

Camille Courcoul[1], Joséphine Leflaive[1], Anne-Sophie Benoiston[2], Jessica Ferriol[1], Stéphanie Boulêtreau[1]

[1] Laboratoire d'Ecologie Fonctionnelle et Environnement de Toulouse, Université de Toulouse, CNRS UMR 5245, INPT, UPS, Toulouse, France

[2] Laboratoire Evolution et Diversité Biologique (EDB UMR 5174), Université de Toulouse, CNRS, IRD

1. Introduction

The notion of community stability refers to how communities withstand (resistance) and/or recover (resilience) from a disturbance. Past conditions are considered as an important driver of community stability. For instance, thermal history was shown to influence the stability of various communities in response to heat stress. Fishes (Olsen et al., 2021), invertebrates (Galbraith et al., 2012; Giomi et al., 2016), microalgae (Courcoul et al., 2022a) or bacterial communities associated to coral (Shiu et al., 2017) acclimated to warm temperatures are more tolerant to heat stress. However, to our knowledge, no study investigated the influence of thermal history on the response to a disturbance other than heat stress.

The terms of "legacy" or "memory" have been widely used in literature to describe the effects of past environmental conditions or the remaining effects of a past disturbance (Franklin et al., 2000; Zweifel and Sterck, 2018). It can occur either in the form physiological adaptations to prior conditions / disturbance or by the presence of surviving material (individuals or matter) right after a disturbance (Johnstone et al., 2016). When successive disturbances occur, the legacy of a previous disturbance can affect the stability of a community to the second one (Buma, 2015). Such phenomenon has been quite extensively studied in forest ecology (Seidl et al., 2014; Trammell et al., 2011),

but remains poorly understood in microbial ecology (Azarbad et al., 2016; Jurburg et al., 2017) with the notable exception of soil ecosystems.

Successive disturbances can act additively, the first disturbance having no influence on the response to the second one. On the opposite, the disturbances can interact so that the effects of the second disturbance are amplified or reduced, especially when there is a negative or positive co-tolerance of species to the disturbances (Vinebrooke et al., 2004). When the absolute effect of the two disturbances is 'more-than' the expected additive effect, the interaction is synergistic and when it is 'less-than', the interaction is antagonistic (Piggott et al., 2015). The latter case can be explained by acquired stress resistance following the first disturbance (Rillig et al., 2015). This is well illustrated by the observation that the recovery of soil microbial communities after a heat stress or a freeze stress was higher or lower, respectively, when this stress was preceded by a heat stress (Jurburg et al., 2017). Another study highlighted a better functional resistance of lake bacterioplankton to salt stress when communities were previously exposed to a peak of low pH (Sjöstedt et al., 2018). The nature, the chronology of events, and the time interval between two events appear to drive the response of communities to sequential compounded disturbances (Calderón et al., 2018; Dal Bello et al., 2019). This makes it difficult to predict the outcome of successive interactions from the effects of individual disturbances (Rillig et al., 2019).

The majority of existing works focused on soil microbial communities, and in a lesser extent on planktonic communities. Much less studies have been carried out on benthic microbial communities. The latter are yet one of the main primary producers in many freshwater ecosystems, and are involved in biogeochemical cycles making them important drivers of ecosystem functioning (Battin et al., 2016). These benthic communities are more and more exposed to flow intermittency (Messager et al., 2021), which is accompanied by the succession of various disturbances. During the intermittency process, as the water flow decreases, the benthic communities experience transitions from a lotic toward a lentic system, then toward a terrestrial habitat due to complete drying of riverbed. When the flow ceases, very high temperatures can be reached in the remaining water because of the reduced thermal inertia (Colls et al., 2019; Gómez et al., 2017; Morrill et al., 2005) and physicochemical conditions are strongly modified (Gómez et al., 2017). Once flow resumes after the dry period, it is very likely that contaminants

stored in soils leach out and reach flowing waters by runoff, exposing communities to several pollutants, depending on land uses (Dahm et al., 2003; Passeport et al., 2013).

The structure and functioning of phototrophic biofilms are strongly impacted by desiccation (e.g. Amalfitano et al., 2008; Romaní et al., 2013), but they are able to rapidly recover (e.g. Acuña et al., 2015; Timoner et al., 2014a). While the direct effect of dry period on biofilms is relatively well known, it is not clear how the temperature they experience during the flow interruption and dry period influences their trajectory of recovery. It was shown that the severity of flow intermittency (i.e. solar radiation and temperatures) experienced during the dry period in the field negatively influences algal biomass and biofilm metabolism (Colls et al., 2019). In contrast, biofilms exposed in microcosms to dry period in combination with increased temperature were similarly impacted as biofilms exposed to dry period alone (Courcoul et al., 2022b). However, the exposure to high temperature before and during the dry period significantly increased the resistance of biofilms to a complex contaminant for some structural and functional parameters (Courcoul et al., 2022b). These results suggest a legacy of warming on biofilm stability to a chemical disturbance.

Since during intermittency biofilms are particularly exposed to heatwaves, whose duration, intensity and frequency are expected to increase with climate change (Hoegh-Guldberg et al., 2018), it is critical to understand in what extent high temperature would affect their recovery from dry period and their stability when exposed to another disturbance. To achieve this objective, we performed a microcosm experiment where we first exposed biofilms to a flow intermittency sequence (no flow then a dry period) at two contrasting temperatures, and then monitored their recovery and assessed their stability. For the latter, we exposed biofilms to a chemical disturbance, i.e. a complex agricultural run-off, for 1 week. We compared warmed and non-warmed biofilm structural and functional responses at three times following rewetting and contamination (Fig. 1). We first hypothesized that thermal history modifies the biofilm response trajectory to flow intermittency by enhancing heterotrophy. Our second hypothesis is that thermal history modulates the stability of biofilms exposed to contamination and this modulation depends on the contaminant concentration (i.e. disturbance intensity). Last, we hypothesized the influence of thermal history on biofilm response decreases with recovery time.

2. Material and methods

Phototrophic biofilms were grown in the laboratory from natural communities. Following this growth step, they were exposed to a flow intermittency sequence under two different temperature conditions (control or warmed) (Fig. 1). Finally, biofilms were rewetted with or without agricultural run-off at 5 different concentrations.

Experimental design

Natural biofilms were sampled in the river Garonne (43.396635, 1.300394, South-West France) to inoculate mini-channels in which biofilms were grown on ceramic coupons in standard conditions at 22°C, at 70 µmol.m^{-2}.s^{-1} for 16h per day, with a flow velocity 0.15 m^3.min^{-1}, as described in Courcoul et al. (2022). After 16 days of growth, biofilms experienced 1 week of no-flow followed by 12 weeks of dryness, either at 22°C (non-warmed, NW) or 32°C (warmed, W) to mimic two contrasted thermal histories (Fig. 1). For that, the colonised ceramic coupons from channels were randomly distributed into 36 crystallizers (15 coupons per crystallizer) filled with 600 ml of Volvic® medium and placed in two thermostatic chambers (18 crystallizers per chamber, light/night cycle of 16h/8h, 70 µmol.m^{-2}.s^{-1}). After one week, all crystallisers were carefully emptied and kept dry for 12 weeks. During rewetting, biofilms were all placed at 22°C. Thirty crystallizers were exposed to an agricultural run-off (ARO) at 5 concentrations (6 crystallisers per concentration) while the 6 others were not contaminated. The used agricultural contaminant consisted in a complex mixture of nitrates (KNO_3), copper ($CuSO_4$) and three organic pesticides (PESTANAL® analytical standards, Merck, Darmstadt, Germany): the inhibiting photosynthesis herbicide Terbuthylazine, the fungicide Tebuconazole and the insecticide Pirimicarb. The contaminant was applied at concentrations of 1, 10, 50, 100 and 250 times the lowest concentration (see Table S1). One week after the pulse of contaminant, the medium was changed in each crystallizer with Volvic® water to allow recovery in uncontaminated conditions. During this recovery period, the medium was refilled every 2 days with demineralized water to compensate evaporation, and enriched in nutrients at the same concentrations than during biofilm growth phase (See Supplementary information section). Biofilm resistance to contamination was assessed by sampling biofilms at the end of the contamination exposure (S1), and biofilm recovery was assessed by sampling biofilms 1 week and 3 weeks later (S2, S3, Fig. 1). At each sampling date, 5 coupons were scrapped in 20 mL Volvic® water.

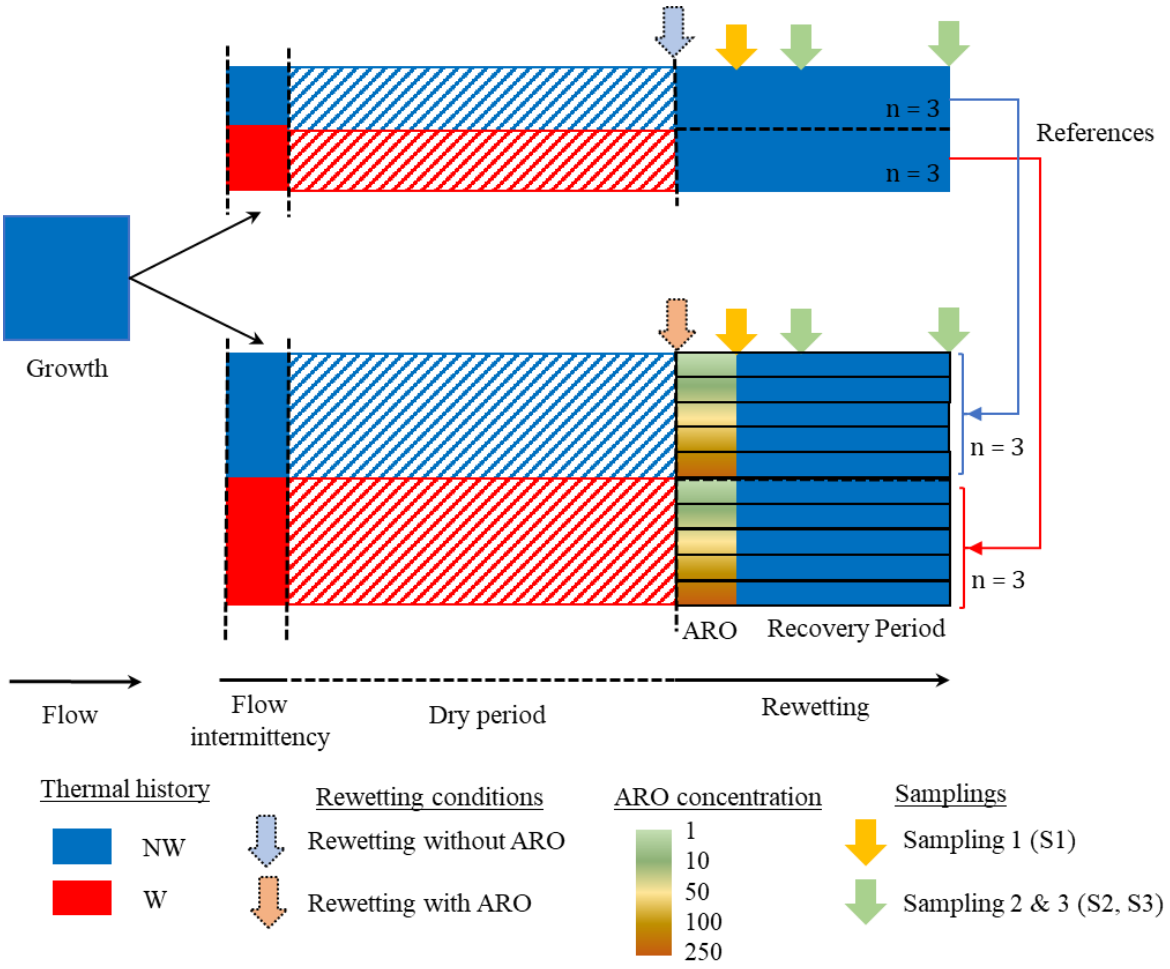


Figure 1: Experimental design. After 16 days of growth under normal flow conditions (22°C), biofilms were kept at 22°C (NW) or warmed at 32°C (W) for 1 week under no-flow conditions and, then kept dried for 12 weeks without changing the temperature. At the end of the dry period, all the biofilms were rewetted at 22°C for 1 week, without (top timeline, blue arrow) or with addition of ARO (bottom timeline, orange arrow) at 5 concentrations (from 1 to 250 times the lowest concentration). Biofilms sampling and measurements were conducted at the end of the contamination period (S1, yellow arrows), and after 2 and 4 weeks of rewetting (S2, S3, green arrows). Samples from the top timeline were used to understand how thermal history influences biofilm recovery to flow intermittency. Samples from the bottom timeline were used to understand how thermal history influences biofilm stability to ARO after a flow intermittency sequence, by comparison with the appropriate reference without ARO.

Measured parameters

Several descriptors were measured on biofilm suspensions to assess the structure of biofilm communities and their functioning: the chlorophyll *a* content (Chl *a*), the proportion of main algal groups, the prokaryotic and eukaryotic community compositions, the stoichiometric composition (C:N and C:P), the quantity of extracellular polymeric substances (EPS), the fungal biomass, biofilm primary production and respiration, algal physiological state and the heterotrophic functional diversity based on carbon source degradation.

Pigment analysis

1.5 mL of biofilm suspensions were centrifuged (10 000 g, 5 min at 4°C) and pellets were stored at -80°C until pigment extraction. After freeze-drying, pellets were extracted in 2 mL of methanol (2% of ammonium acetate 1 M) (Capdeville et al., 2019). The samples were sonicated during 3 min and incubated at -20°C for 15 min before another sonication step. After centrifugation (10 000 g, 5 min at 4°C), supernatants were stored at 4°C. This extraction was repeated 1 to 3 times, as long as the supernatant was still coloured. Then 1 mL of each extract was filtered with a 0.2 µm PTFE membrane syringe filter (VWR International, United States). Pigment composition was analysed using HPLC (LC1200 series, Agilent Technologies, United States). Specific pigment identity and concentration were quantified by comparing each peak area of the chromatogram with those of known standards using the ChemStation software (Agilent technologies). Specific pigments were summed to obtain the proportion of each primary producer group: lutein, chlorophyll *b*, violaxanthin, neoxanthin and astaxanthin for green algae; fucoxanthin, diadinoxanthin, chlorophylls *c1* and *c2* and diatoxanthin for diatoms; myxoxanthophyll, echinenone and zeaxanthin for cyanobacteria (Majdi et al., 2011; Schlüter et al., 2006).

Prokaryotic and eukaryotic composition and diversity

DNA extraction

DNA was extracted using the Qiagen DNeasy® Blood & Tissue extraction kit (Qiagen, Venlo, Netherlands) following the modified manufacturer's protocol designed for facilitating the lysis of Gram-positive bacteria. Extractions were carried on 0.5 ml of biofilm suspension (8 mg on average). Total DNA was eluted in 70 µL of elution buffer,

and quantified by spectrophotometry (Nanodrop® 1000, Willmington, USA). DNA was visualized on gel (Agarose 1%).

DNA amplification and sequencing

Prokaryotic composition was assessed through the amplification of the V4 region of the 16S rRNA gene using the primers used in the Earth Microbiome Project: c515F 5'-GTGYCAGCMGCCGCGGTAA -3' (Caporoso et al. 2011) and 806R 5'-GGACTACNVGGGTWTCTAAT-3' (Apprill et al. 2015). PCR was performed from 2 µL of extracted DNA in a 20 µL mixture including 10 µL AmpliTaq Gold Mastermix® (Thermo Fisher Scientific, Waltham, USA), 0.16µg/µL^{-1} BSA, and 0.5 µM of each primer and following this program: initial denaturation at 95°C for 10 min, followed by 35 cycles at 95°C for 30 sec, at 50°C for 30 sec, and at 72°C for 1min 45, ending with a final extension at 72°C for 10 min.

The V9 region of the 18S rRNA gene was amplified to assess eukaryotic community composition using the primers used in the Earth Microbiome Project: 1391F 5'-GTACACACCGCCCGTC -3' and EukBr 5'-TGATCCTTCTGCAGGTTCACCTAC -3' (Amaral-Zettler et al. 2009). PCR was performed from 0.8µL of extracted DNA in a 20 µL mixture including 8 µL AmpliTaq Gold Mastermix® (Thermo Fisher Scientific, Waltham, USA) and 0.2 µM of each primer and following this program: at 94°C for 3 min, followed by 35 cycles at 94°C for 45 sec, 57°C for 60 sec, and 72°C during 90 sec, ending with 72°C for 10 min.

Some combinations of 8 bp tags were also added at the 5' end of forward and reverse primers to identify samples after sequencing step. PCR effectiveness was evaluated by including positive and negative samples. PCR was performed in triplicate on each sample. We collected 8 µL of amplicons per sample and negative controls (2µl for positive control) and pooled them.

The sequencing was performed on the external Illumina platform Fasteris Genesupport SA (Plan-les-Ouates, Illumina). The pool dedicated to quantify prokaryotic diversity was sequenced in one run using the 2x250 bp MiSeq and the pool dedicated to quantify eukaryotic diversity in another run using the 2x150 bp MiSeq Micro chemistry.

We used the OBITools package developed by Boyer et al., (2016) to process and filter the Illumina sequences. First, paired-end reads were assembled, and low-quality sequence were removed (fastq average quality scores < 40). After demultiplexing, identical

sequences were dereplicated. Then, sequences with non-suitable length were filtrated (<100 bp). Finally, the sequences were clustered using a similarity threshold of 97%.

Data curation and taxonomic assignation were performed with the *metabaR* R package (Zinger et al., 2021) and data analyses with the *phyloseq* R package (McMurdie and Holmes, 2013). Before curation, the number of reads for 109 samples was 15 782 376 for prokaryotes and 2 958 361 for eukaryotes. Taxonomic assignation of both prokaryotic and eukaryotic sequences was made from the Silva 138 SSU database (Quast et al., 2013) with *silva_annotator* function. Potential extraction contaminants, spurious and non-target motus, PCR outliers and tag-jumps were flagged, and removed from the dataset. Note that for prokaryotic sequences, 2 PCR outliers were identified and removed. Finally, after data curation 15 139 926 prokaryotic sequences, clustered in 16 115 OTUs and 2 776 520 eukaryotic sequences clustered in 1 070 OTUs were analysed.

Phyla with less than 10 occurrences were filtered and a prevalence threshold of 5% of total samples was applied. Specific richness and Shannon diversity index were estimated on each clean dataset. Relative abundances of remaining OTU were calculated at the phylum, class and genus level. The final number of OTUs analysed was 5677 for Prokaryotes and 323 for Eukaryotes.

Stoichiometric composition

Total carbon, nitrogen and phosphorus contents in biofilms were measured in 1-mL biofilm suspensions oven-dried at 60°C for 24 h. Carbon and nitrogen contents were determined by elemental analysis using a CHN analyser (Flash 2000, Thermo Scientific). For phosphorus quantification, samples were previously oxidized with potassium persulfate in an acid environment in autoclave to mineralize matter, and phosphorus content was determined following the molybdate-blue method (Koroleff, 1983) using a spectrophotometer at 880 nm (Uvi Light XT5, SECOCAM, Alès, France).

Extracellular polymeric substances

EPS were assessed by summing both biofilm protein and carbohydrate contents. EPS were extracted on fresh samples as described by Loustau et al. (2019). Briefly, after a washing step, extraction was first carried out in 1X PBS with a 37% formamide treatment. Following incubation for 60 min at 4°C at slow shaking (150 rpm), tubes were centrifugated (8 000 g, 5 min, 4°C) and supernatants stored in 2-mL tubes. The pellets

were resuspended in 1x PBS with 50 g.gDW^{-1} of cation exchange resin (CER, Dowex Marathon). Another incubation of 90 min was carried out as described in the first step. After centrifugation, supernatants were extracted, and pellets were washed another time in PBS to ensure good extraction. Supernatants tubes were stored at -20°C before quantification. The Pierce™ BCA protein assay kit (*N° 23223*, Sigma Aldrich) was used to quantify protein content in EPS (Smith et al., 1985). Bovine serum albumin (BSA) was used as a standard, with 10 concentrations ranging from 0 to 1 g.L^{-1}. To increase sensitivity, microplates were incubated for 30 minutes at 60°C (Walker, 2009). After 20 min of cooling, optical density (OD) was measured at 540 nm (Synergy Mx Biotek). The anthrone method was used to quantify carbohydrates (Gaudy, 1962), using glucose as standard with concentrations ranging from 0 to 0.1 g.L^{-1}. After the same incubation and cooling times than for proteins, OD was measured at 620 nm. Results expressed in standard equivalents were normalised to sample dry mass.

Ergosterol

The fungal biomass was estimated through quantification of ergosterol (Gessner et al., 1991). 5-mL of biofilm suspension was freeze-dried before extraction. Then, the dry biomass was weighed and conditioned in sealable glass pipes. 5-mL of MeOH-KOH (8 g.L^{-1}) was added in the pipes, and incubated at 4°C overnight. In addition, controls were prepared with 100 µL of ergosterol solution (200 µg.L^{-1}) and incubated in the same conditions. The next day, tubes were incubated at 80°C for 30 minutes in water bath. After cooling, 1-mL of HCl (0.65 M) was added in samples. After conditioning of vac cartridges (OASIS HLB Cartidges, Waters™), 3 mL of samples were dried for 1 h under gentle pressure (0 to -5 bar). Extracts were eluted with 1.4-mL of isopropanol in pre-weighted vials. Filled vials were weighted again to calculate the mass of extract, and kept at 4°C before analyses through HPLC (HPLC 306 KONTRON and DETECTOR 340 KONTRON). The concentration of ergosterol was estimated by dividing the peak area by the coefficient of the calibration line. The quantity of ergosterol per unit area was calculated from the total amount of ergosterol in the extract divided by the biofilm quantity per unit area.

Primary production and respiration

Biofilm primary production and respiration were analysed by measuring changes in oxygen concentration over time using FireSting oxygen sensors (PyroScience, Aachen, Germany). Oxygen measurements were conducted under agitation in 10-mL transparent

vials containing 1 mL of biofilm suspension and 9 mL of Volvic® water, and closed with a gas tight lid. Dissolved oxygen concentration within each vial was monitored every 5 seconds over 10 minutes using oxygen logger software. Net primary productivity (NPP) ($mgO2.h^{-1}.cm^{-2}$) was determined by measuring dissolved oxygen changes over a 5-min period of daylight (neon lamps, 90 $\mu E.m^{-2}.s^{-1}$). The rate of community (autotrophic and heterotrophic) respiration (R) was then determined as the change in oxygen concentration over a 5-min incubation period in the dark. The GPP (gross primary productivity) was calculated by summing NPP and R.

Algal physiological state

PhytoPAM measurements (Phytoplankton Analyzer, Heinz Walz GMBH, Effeltrich, Germany) have been carried out on 1/10 diluted 200-µL biofilm suspensions. The maximal quantum yield of photosystem II (PSII) or F_V /F_M was used to assess algal physiological state, where $F_V = F_M - F_0$ with F_M the maximal fluorescence and F_0 the minimal fluorescence of PSII (Genty et al., 1989). This metric depends on the algal species present in the biofilm, but also on the physiological state of cells as it decreases when algae are stressed or damaged (Roháček and Barták, 1999). Measurements of F_V /F_M were done under dark-adapted state (Baker, 2008).

Heterotrophic functional diversity

The ability of heterotrophic microorganisms to metabolize carbon substrates was measured with Biolog Ecoplates™ microplates (Biolog Inc., Hayward, CA, USA). The metabolization of a substrate results in tetrazolium reduction and purple coloration (Garland and Mills, 1991). Each microplate consists of three replicates of 31 carbon sources and three wells with no substrate used as a blank. 1 mL of biofilm suspension was diluted in 5 mL of Volvic® water, and filtrated on Whatman GF/F filter. 150 µL of this suspension were inoculated in each well. Microplates were incubated for 5 days in the dark at 22°C. The optical density (OD) at 590 nm was measured at the end of inoculation period using a microplate reader (Biotek Synergy HT). For each plate, the OD was corrected by the mean OD measured for blank wells. Each substrate was assigned to a guild according to its chemical nature: amine, amino-acid, carbohydrate, carboxylic acid or polymer (Choi and Dobbs, 1999). An activity percentage was calculated for each guild after substrate quantities were equally weighted to the largest one (Leflaive et al., 2005). The average colour well development (AWCD) was calculated for each sample by dividing

the sum of the net ODs by the number of substrates (n = 31). The Shannon heterotrophic diversity index (-Σ pi ln(pi)) was calculated for each sample with pi as the OD/ Σ OD.

Statistical analyses

Statistical analyses were performed with R software (version 3.6.1, R development Core Team). Data set was analysed in two steps as explained in the Figure 1. First, only uncontaminated samples were considered to assess the influence of thermal history on the biofilm recovery to the flow intermittency. Then, we analysed the effect of thermal history on the response of biofilms to contamination after flow intermittency.

The most abundant prokaryotic and eukaryotic phyla and classes were compared between samples using ANOVA, in order to identify changes in community composition over time and between thermal histories. In addition, changes in community compositions were assessed through principal coordinate analyses (PCoA) conducted on Bray-Curtis dissimilarities for both prokaryotes and eukaryotes OTUs. Differences across thermal conditions and sampling dates were tested using PERMANOVAs using *adonis* function provided in {vegan} package. For each PCoA, the two first axis were extracted and used as synthetic variables.

Similarly, a principal component analysis (PCA) was performed on structure variables: Chl *a* ($mg.cm^{-2}$), EPS ($mg.cm^{-2}$), fungal biomass ($mg.cm^{-2}$), the proportions of phaeopigments, green algal and cyanobacterial specific pigments, C:N and C:P ratios, the proportions of *Proteobacteria* and *Firmicutes* which were the most represented bacterial groups, and Shannon diversity indexes of both prokaryotes and eukaryotes. Another PCA was conducted on functional variables: F_V/F_M, GPP ($mgO_2.h^{-1}.cm^{-2}$), Respiration ($mgO_2.h^{-1}.cm^{-2}$), AWCD, the number of carbon substrates metabolized and the Shannon heterotrophic functional diversity index. Differences across thermal conditions and sampling dates were also tested through PERMANOVAs. The two first axis were also extracted and used as synthetic variables.

For the first step, two-factor ANOVAs (Thermal history x Sampling date) were carried out to test for interactions. For the second step, differences in biofilm responses induced by ARO contamination was calculated compared to its reference (same thermal history and sampling date). The influence of thermal history on the effect of ARO was tested for each

concentration and each sampling date with ANOVA. ANOVA condition applications were systematically verified using Shapiro test for residual normality, Breusch-Pagan test for homoscedasticity and Durbin-Watson test for autocorrelation. The significative threshold was 0.05.

3. Results

Influence of the thermal history on biofilm recovery to flow intermittency

Prokaryotic and eucaryotic composition and diversity

After one week of rewetting (S1), prokaryotic communities of uncontaminated samples were mainly made up of *Proteobacteria,* especially *Gammaproteobacteria* and *Firmicutes* especially *Bacilli* in both NW and W conditions (Fig 2a, 2c, Table S2, S3). Over time (at S2 and S3), the abundance of *Bacteroidota*, especially *Bacteroidia*, increased in both thermal conditions ($p = 0.004$), while it decreased for both *Proteobacteria* and *Firmicutes* ($p = 0.01$ and $p = 0.002$, respectively). When considering the classes, *Gammaproteobacteria* decreased overtime to the benefit of *Alphaproteobacteria* ($p < 0.001$), for both NW and W conditions. The relative abundances of *Proteobacteria* and *Firmicutes* depended on thermal history. Indeed, the percentage of *Proteobacteria* was lower for W treatments compared to NW ones ($p = 0.01$), while the opposite occurred for *Firmicutes* ($p = 0.005$). At S2 and S3, there were significantly more *Bacilli* in W than in NW treatments ($p = 0.002$). Prokaryotic diversity increased over time ($p = 0.003$), with biofilms sampled at S1 significantly less diversified ($H' = 2.96 \pm 0.27$) than those sampled at S2 and S3 ($H' = 3.61 \pm 0.49$ and 3.68 ± 0.35, respectively). There was no effect of the thermal history on the prokaryotic diversity.

After one week of rewetting (S1), eukaryotic communities were mainly dominated by *Ciliophora* (class of *Intramacronucleata*) for biofilms from both NW and W conditions (Fig. 2b, 2d, Table S2, S3). At S2 and S3, the abundance of *Ciliophora* decreased in all samples ($p < 0.001$), while the relative abundances of *Chloroplastida* (mainly *Trebouxiophyceae*) and *Amoebozoa* increased ($p = 0.004$ and $p = 0.001$, respectively). Eukaryotic diversity remained constant over time in W samples ($H' = 1.92 \pm 0.54$), while it significantly increased between S1 ($H' = 0.50 \pm 0.5$) and S2 ($H' = 2.42 \pm 0.28$) in NW samples ($p = 0.04$), and remained different at S3 ($H' = 2.54 \pm 0.34$). We found no OTU related to Diatoms in our samples consistently with pigment analyses.

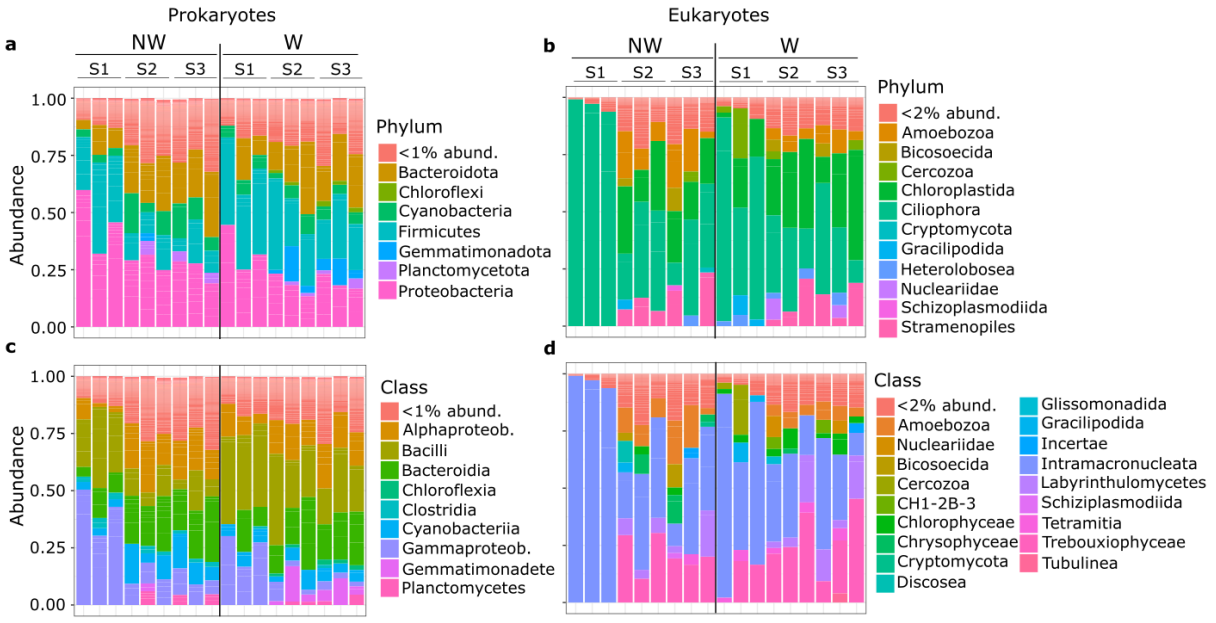


Figure 2: Prokaryotic (a and c) and eukaryotic (b and d) community composition (in relative abundances) of the uncontaminated biofilms. Biofilms were organised according to their thermal history (NW or W) and their sampling date (S1, S2 or S3). The most abundant (>1% reads for prokaryotes and > 2% for eukaryotes) are shown for phylum (top) and class (bottom).

The prokaryotic community structure was affected by both the thermal history (PERMANOVA performed on Bray-Curtis distances, $p = 0.001$, $F = 10.78$, $r^2 = 0.31$) and the sampling date (PERMANOVA, $p = 0.001$, $F = 4.98$, $r^2 = 0.29$) (Fig. 3a). Prokaryotic community structures in NW and W biofilms remained significantly different along the sampling dates ($p < 0.001$ for both PCo1 and PCo2, Fig. 3c). The prokaryotic community structures significantly changed between S1 and S2 in both thermal histories ($p < 0.001$). The structure of eukaryotic community depended on both thermal history (PERMANOVA, $p = 0.005$, $F = 4.63$, $r^2=0.18$) and sampling date (PERMANOVA, $p = 0.004$, $F = 3.93$, $r^2 = 0.30$) (Fig. 3b). Nevertheless, the effect of thermal history was only visible at S1, on the PCo1 ($p = 0.005$, Fig. 3d). There was a significant effect of sampling date on PCo2, with S1 communities being significantly different than S2 and S3 communities ($p = 0.02$).

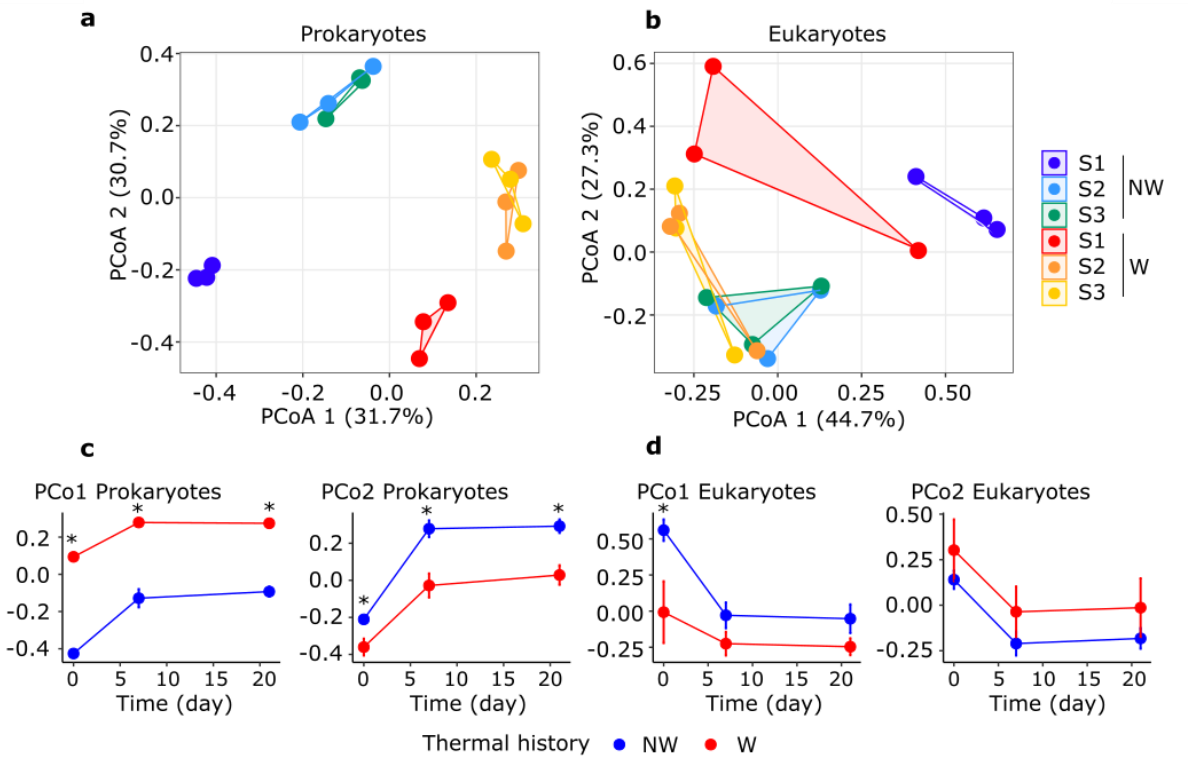


Figure 3: Bray-Curtis dissimilarities between prokaryotic communities (a) and eukaryotic communities (b) of uncontaminated biofilms, represented along two first axes of PCoAs. Samples are grouped by thermal history (NW or W) and sampling date (S1, S2 or S3) (n=3). The dynamics of PC1 and PC2 coordinates (mean ± SE, n = 3) over time are represented below for prokaryotes (c) and eukaryotes (d). Asterisks represent significant differences between NW and W treatments at the same sampling date.

Biofilm general structure

All the data describing biofilm structure were also analysed with PCA. The first PCA axis well discriminated samples according to their chl *a*, phaeopigments, ergosterol and their proportion in *Firmicutes* on the left side of the plan, opposed to cyanobacteria proportion, EPS content, C:N, C:P ratios, and community diversities on the right side (50.3% variance explained, Fig. 4a). The second axis was positively correlated with the proportion of green algae and negatively correlated with the Proteobacteria content (18.4% variance explained). The biofilm general structure varied between sampling dates (PERMANOVA, $p < 0.001$, $F = 11.20$, $r^2 = 0.59$), but there was no effect of thermal history (Fig. 4a). Biofilm coordinates on the first component significantly increased over time, with no effect of thermal history (ANOVA, $p < 0.001$, Fig. 4c). One week after rewetting (S1), biofilms were

characterized by a high quantity of chl *a*, ergosterol, a high proportion of phaeopigments and *Firmicutes*. From S1 to S3, biofilms exhibited a gradual increase in EPS quantity, C:N and C:P ratios, and a decrease in chl *a* (Fig S1). The second axis discriminated biofilms according to their thermal history for S1 and S2 (Fig. 4c) (ANOVA, $p < 0.001$), but this effect was no more visible at S3. Warming promoted green algae after the flow intermittency, and the proportion of green algal specific pigments remained higher in W biofilms at S1 and S2, but significantly decreased ($p = 0.002$) to reach the same level than in NW biofilms at S3 (Fig S1). On the contrary, cyanobacteria specific pigments were significantly higher in NW biofilms at S2, but their proportion increased at S3 in W biofilms ($p = 0.023$) to reach similar values than in NW biofilms (Fig S1).

Biofilm functioning

A second PCA was performed with the data related to biofilm structure. The first PCA axis discriminated between autotrophic and heterotrophic variables, with GPP and F_V/F_M represented on the left side of the plan while variables related to carbon metabolization and oxygen consumption were on the right side. The second axis was positively correlated with the functional heterotrophic diversity. The permutational multivariate analyses showed that thermal history and sampling date interacted to significantly discriminate clusters (PERMANOVA, $p = 0.023$, $F = 4.91$, $r^2 = 0.27$) (Fig. 4b). This translated into significant changes in the first principal component over time only for NW biofilms. At S1, the biofilms in NW were significantly more heterotrophic compared to biofilms in W treatments, with lower GPP and F_V/F_M (Fig. S1). These differences disappear from S2. There were no significant differences on the second axis.

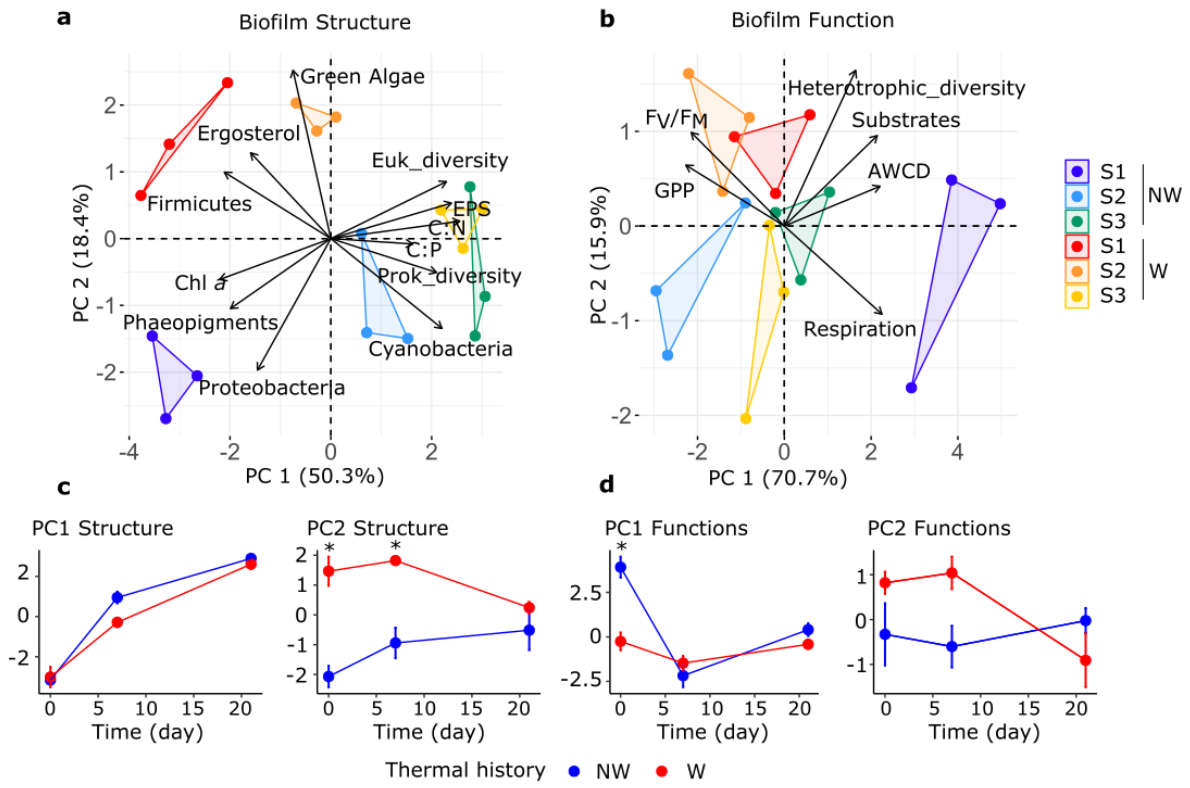


Figure 4: Principal component analysis (PCA) on performed on variables related to the global structure (a) and functions (b) of uncontaminated biofilms, represented along two first axes of PCAs. Arrows represent the measured variables. Samples are grouped by thermal history (NW or W) and sampling date (S1, S2 or S3) (n=3). The dynamics of PC1 and PC2 coordinates (mean ± SE, n = 3) over time are represented below for biofilm structure (c) and functions (d). Asterisks represent significant differences between thermal histories.

<u>Influence of the thermal history on biofilm stability to contamination after a dry period</u>

The stability of warmed and non-warmed biofilms was assessed at the end of the dry period by exposing the biofilms to an agricultural run-off. The prokaryotic community structure was affected by the contaminant (PERMANOVA, $p = 0.001$, $F = 8.91$, $r^2 = 0.17$), the thermal history (PERMANOVA performed on Bray-Curtis distances, $p = 0.001$, $F = 28.0$, $r^2 = 0.11$) and the sampling date (PERMANOVA, $p = 0.001$, $F = 34.47$, $r^2 = 0.26$). One week after the contamination (S1), ARO induced significant changes in prokaryotic communities, including a decrease in *Firmicutes* ($p < 0.001$), while it increased the proportion of *Proteobacteria* in contaminated samples ($p < 0.001$). One week following

the end of the contamination (S2), the effect of ARO on *Firmicutes* and *Proteobacteria* remained at S2 and S3 ($p < 0.001$). The analyse of the first axis of the PCoA (Fig. 5a) indicates that at S1 ARO induced significant changes on prokaryotic community structure from 10X ($p < 0.001$, Fig. 5c), with a significant amplification of these effects in the W biofilms from 50X ($p = 0.004$). At S2 and S3 ARO effects were still significant only for the highest concentrations, with no more effect of the thermal history. On the second axis of the PCoA, the effect of ARO on biofilms increased over time, since it was only significant at 250X at S1 but was significant from 50X at S2 and S3. Warming treatment increased ARO effects from 100X at S1 and from 50X at S2 and S3.

The eukaryotic community structure was affected by the contaminant (PERMANOVA, $p = 0.001$, $F = 6.93$, $r^2 = 0.18$), the thermal history (PERMANOVA performed on Bray-Curtis distances, $p = 0.001$, $F = 16.1$, $r^2 = 0.08$), and the sampling date (PERMANOVA, $p = 0.001$, $F = 15.57$, $r^2 = 0.16$) (Fig. 5b). There were no significant effects of ARO on dominant groups at S1, but significant changes occurred at S2 for biofilms exposed to the highest concentration of ARO (250X): an increase in the proportion of *Ciliophora* ($p = 0.003$), and a decrease in *Chloroplastida* and *Amoebozoa* proportions, compared to uncontaminated references ($p = 0.005$ and $p = 0.04$ respectively). At the end of the recovery period, there were no more any significant differences between treatments. Looking at PCoA axes, there were only few significant effects of ARO, mainly at S2 and S3 on the second axis for the 2 highest concentrations (Fig. 5d). The thermal history only had marginal effects.

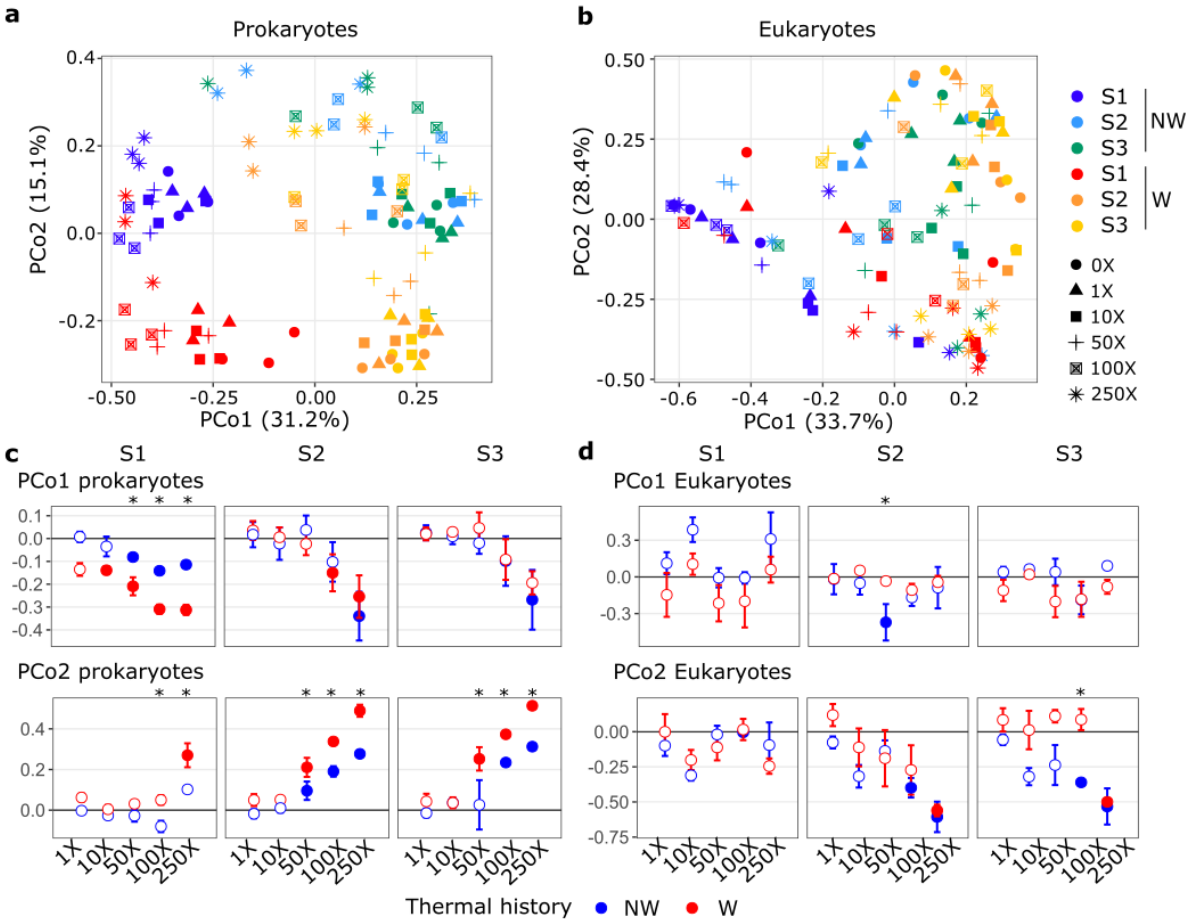


Figure 5: Bray-Curtis dissimilarities between prokaryotic communities (a) and eukaryotic communities (b) of all samples, represented along two first axes of PCoAs. Samples are grouped by thermal history (NW or W) and sampling date (S1, S2 or S3) (n=18). Differences between the PCoA coordinates of each biofilm and the corresponding uncontaminated reference (NW or W) are represented for each sampling date and each concentration for prokaryotes (c) and eukaryotes (d), (mean ± SE, n = 3). Solid round represents significant difference between references and contaminated samples. Asterisks on the top of each plot represent significant differences between thermal history.

The first axis of the PCA conducted on global biofilm structure (Fig. 6a) was negatively correlated to community diversity, stoichiometric composition, and EPS content, and positively correlated to the proportion of phaeopigments, of *Proteobacteria*. The second axis discriminated biofilms with higher cyanobacterial proportion on the top of the plan from biofilms with higher green algal proportion and the fungal biomass on the bottom.

High ARO concentrations (i.e. 50X, 100X and 250X) induced significant increases in PC1 coordinates compared to uncontaminated reference biofilms at S1 ($p < 0.001$, Fig. 6c). This response was amplified by W treatment ($p < 0.001$). Both effects of ARO and thermal history progressively decreased over time as biofilms recovered. However, no resilience was observed at S3 in biofilms exposed to 250X. ARO induced a significant increase in PC2 coordinates from 50X at S1, that was stronger in W biofilms ($p < 0.001$). This response diminished at S2 and disappeared or was reversed for the highest concentrations at S3.

The PCA conducted on functional variables well discriminated autotrophic and heterotrophic processes on the first axis, with GPP and F_V/F_M negatively correlated and respiration and heterotrophic functional diversity positively correlated (Fig. 6b). ARO induced significant increase in PC1 coordinates at high concentrations, mainly for W biofilms at S1 (Fig. 6d). Such changes suggested that ARO enhanced heterotrophic processes in W biofilms. At S2, changes due to ARO were more complex with opposite influence of thermal history according to ARO concentrations ($p = 0.01$), even if biofilms remained more heterotroph than uncontaminated biofilms at high ARO concentrations. The effect of thermal history did not persist over time as there was no longer significant difference between W and NW samples at S3.

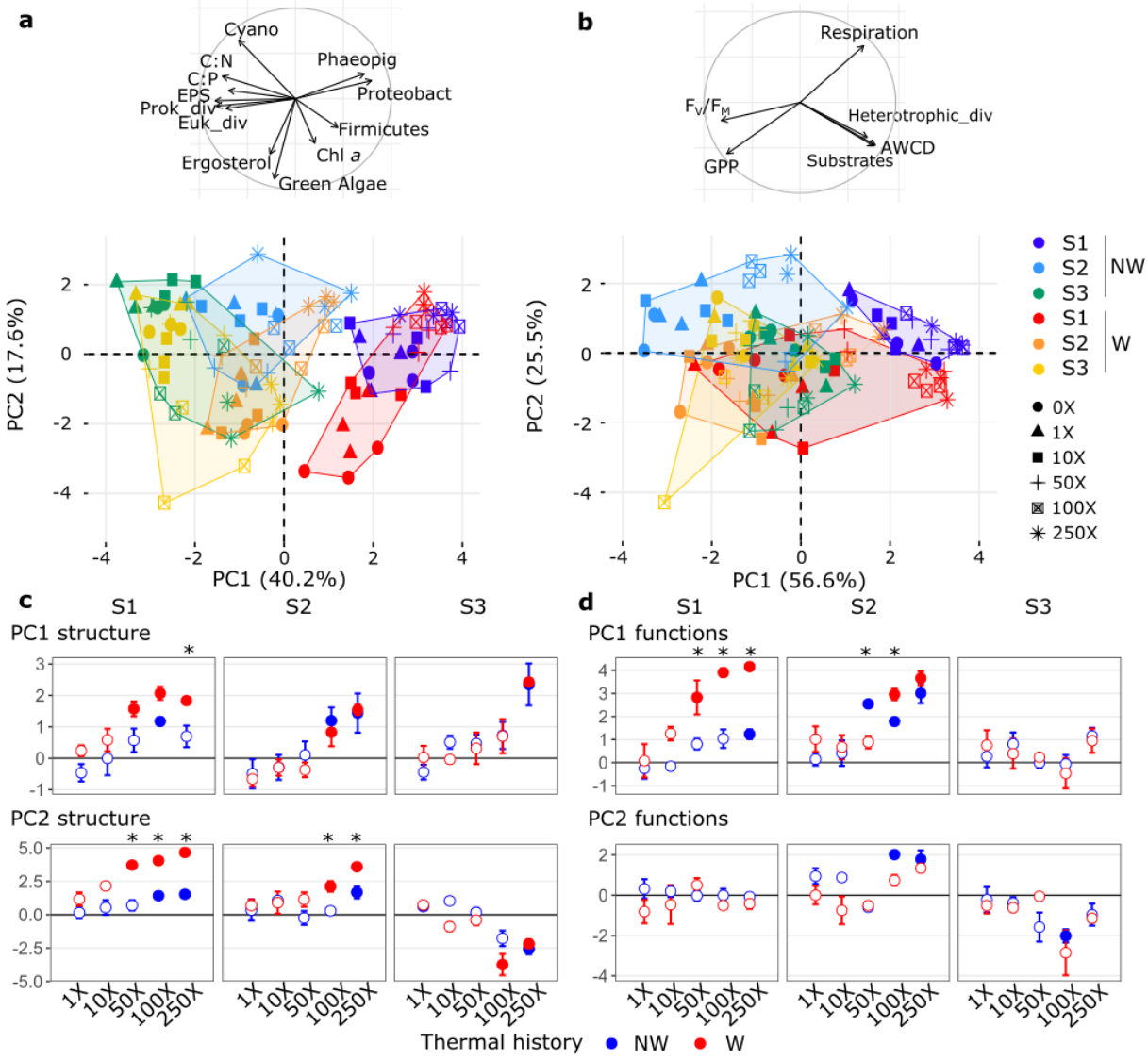


Figure 6: Principal component analysis (PCA) on global biofilm structure (a) and functions (b) of all samples, represented along two first axes of PCAs. Arrows representing the measured variables. Samples are grouped by thermal history (NW or W) and sampling date (S1, S2 or S3) (n=18). Differences between the PCA coordinates of each biofilm and the corresponding uncontaminated reference (NW or W) are represented for each sampling date and each concentration (mean ± SE, n = 3) for structure (c) and functions (d) PCAs. Solid rounds represent cases where the difference between references and contaminated samples is significant. Asterisks on the top of each plot represent significant differences between thermal histories.

4. Discussion

In this study, we aimed at understanding how thermal history influences the response of phototrophic biofilms to flow intermittency and their stability, i.e. their ability to withstand and recover from another disturbance. For the latter, we used a complex contaminant as a disturbance likely to occur at rewetting.

Influence of the thermal history on biofilm recovery to flow intermittency

As a first hypothesis, we expected warming during no-flow and dry periods to modify the trajectory of biofilms during rewetting. More precisely, warming was supposed to amplify biofilm heterotrophy, which is a well-known transitory response of biofilms to dry periods (Freixa et al., 2017; Romero et al., 2019). Our assumptions were only partially met. The thermal history strongly impacted the biofilm composition, structure and function assessed 1 week after rewetting. However, heterotrophy was not enhanced and the effect of thermal history was only transitory as almost no difference could be observed 4 weeks after rewetting. The noticeable exception is the prokaryotic composition of biofilms which kept diverging according to the thermal history. Indeed, biofilms were globally dominated by *Proteobacteria*, *Bacteroidetes* and *Firmicutes*, which are phyla commonly dominating stream biofilm communities (Battin et al., 2016), but their composition significantly changed after drying and during rewetting, especially after warming. *Exiguobacterales (Bacilli)* which are known to generally benefit from low-flow periods (Timoner et al., 2014b), have replaced *Burkholderiales (Gamma-Proteobacteria)* in warm samples after drying.

The recovery of biofilms was monitored during 4 weeks, we cannot exclude the possibility of a convergence of community composition after a longer time. This seems however unlikely. The 4-week monitoring represents a significant part of the biofilm growth cycle, which should be a few weeks, in the nutrient conditions of our experiment (Biggs, 1996). It seems rather that the community was placed on a new trajectory of succession, which may be considered as a new ecological state. This is consistent with the notion of ecological resilience proposed by Holling (1973), but it only concerns the composition of biofilm and not its functioning. Thanks to the very high functional redundancy of prokaryotes (Louca et al., 2018), similar functions are similarly performed by a different set of species. Such a decoupling between the responses of biofilm structure and biofilm

functioning was observed in several other studies (Barthès et al., 2015; Bundschuh et al., 2017).

Cyanobacteria and green algae which are known to be more resistant to desiccation than diatoms (Sabater et al., 2017) benefitted from flow intermittency. Exposure to a high temperature during flow intermittency did not enhanced heterotrophy compared to non-warmed thermal history. On the contrary, rewetting promoted green algae in W biofilms along with GPP and higher Fv/Fm. These effects were transitory and disappeared during recovery period. Biofilms were similar both in terms of general structure and functioning after 3 weeks of rewetting. The higher Fv/Fm values in W biofilms may be explained by their higher ratio green algae/cyanobacteria at S1 (see Fig. S1). Indeed, Fv/Fm depends on the physiological state of algae (Roháček and Barták, 1999) but also on the algal group, and optimum values are lower for cyanobacteria than for diatoms (Suggett et al., 2009). However, this may be only a partial explanation because the Fv/Fm values are the same in W and NW biofilms at S2 while the proportions of cyanobacteria and green algae are very different.

Influence of the thermal history on biofilm stability after a dry period

The monitoring of biofilms following their contamination indicated that the biofilms that experienced warm condition during the dry period were less resistant and less resilient than the biofilms kept in cooler conditions. This effect was especially visible for the highest concentrations of contaminant, which led to more heterotrophy. This confirms our second hypothesis. This might be explained by the increased sensitivity of biofilms to stressors when far from their optimal tolerance (Heugens et al., 2008). This may be particularly true for phototrophic organisms, photosynthesis being one of the most heat-sensitive cellular processes (Glatz et al., 1999). This may be the reason of the modification of the balance between phototrophy and heterotrophy. The enhanced sensitivity to the contaminant after exposure to warming during flow intermittency might also be linked to the selection of species less resistant to the contamination.

These results are not fully consistent with the observation that generally warming buffers the effects of other stressors, including contaminants in freshwater ecosystems (Jackson et al., 2016). For instance, it has been shown that nutrient supply combined with warming (+5°C or +4°C) increased phototrophic biomass (Lürling et al., 2018) and bacteria growth rate (Degerman et al., 2013). However, this tendency depends on the nature of the

contaminant applied. For instance, rising temperatures improved biofilm tolerance to herbicides (Larras et al., 2013), while it decreased it when biofilms were exposed to copper (Morin et al., 2017). It should be noted that in most of those studies contaminant and warming were applied simultaneously rather than sequentially.

Here, we exposed the biofilms to a very complex contaminant, in order to mimic an agricultural run-off. Compared to uncontaminated references, ARO increased algal mortality (high proportion of phaeopigments), and biofilm heterotrophic processes. In addition, the proportion of green algae and fungal biomass significantly decreased with increasing ARO concentration. It was expected since the contaminant cocktail contained both an herbicide and a fungicide. A similar effect on benthic green algae was observed in microcosms mimicking shallow lake for low concentrations of the same ARO contaminant (Allen et al., 2021). However, combined effect of nutrients, trace metal element and organic pesticides on phototrophic biofilms are difficult to predict, and depend on the observed parameters. For instance, the response of microbial communities involved in litter decomposition to combined exposure to fungicide and nutrient depended on the fungicide concentration (Gardeström et al., 2016). In another study, the combined effect copper and nutrients or herbicide and nutrients depended on the biofilm compartment that was considered (autotrophs vs. heterotrophs) (Tlili et al., 2010).

As expected in our third hypothesis, the effect of thermal history progressively decreased during the recovery period, which may be related to the very high resilience ability of biofilms (Acuña et al., 2015). However, the transitory (excepted for prokaryotic composition) effect of thermal history observed during the recovery to the dry period lasted longer when biofilms were exposed to ARO, notably for the highest concentrations. The partial loss of stability for the biofilms represents a 'silent effect' of warming during river intermittency: it is no visible per se but is revealed after exposure to a novel disturbance. This highlights the existence of unpredictable and underestimated consequences of global warming on freshwater ecosystems, already considered as one of the most threatened ecosystems by multiple stressors (Carpenter et al., 2011).

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Supplementary information

Supplement A

Biofilms were grown in artificial mini-channels. Three modules designed for biofilm cultivation were used (Loustau et al., 2019). Each one was composed by three independent blocks corresponding to three separated closed circulating loops of water for the supply of four mini-channels. The temperature in the room was 22°C, and the flow velocity of 0.15 $m^3.min^{-1}$. A circadian cycle of light/dark periods corresponding to 16h/8h was applied (70 $\mu mol.m^{-2}s^{-1}$). Biofilm were grown on ceramic coupons (5.76 cm^2). Volvic® water was used as culture medium. Twice a week, in addition to a supply with demineralized water to compensate evaporation, nutrients were added inside loops to maintain nutrient concentrations of the Volvic® water (final concentration in tanks: N-NO_3^-: 1.64 $mg.L^{-1}$, P-PO_4^{3-}: 30 $\mu g.L^{-1}$ and SiO_2: 32 $mg.L^{-1}$). As blocks were independent, some coupons were regularly dispatched between channels during growth to limit heterogeneity between separated loops. After 16 days of growth, ceramic coupons were randomly dispatched between in 36 crystallizers filled with Volvic® water and placed in thermal chambers. Half of the samples (n=18) were maintained at 22°C, while the other half was exposed to +10°C warming (32°C), to induce differences in biofilms thermal histories. After one week, water was cautiously removed with an air pump to mimicry desiccation, and biofilms were exposed to a dry period of 12 weeks, during which they remained at whether 22°C or 32°C to maintain the differences in thermal histories. After the period of flow intermittency, crystallizers were re-watered with Volvic® water at 22°C and part of the samples were exposed to gradual concentrations of a complex contaminant mimicking an agricultural run-off made of nitrates, copper and three organic pesticides (see Table S1). Contamination were carried out in triplicates, resulting in a total of 12 treatments: 2 thermal histories x 6 rewetting conditions (1 control + 5 ARO concentrations). One week after the rewetting with or without ARO exposure, the medium was renewed in every crystallizer with clean Volvic® water, and biofilms were maintained under the same conditions than during growth phase for the entire recovery period: 16h/8h day/night period at 70 $\mu mol.m^{-2}s^{-1}$, with nutrient amendment twice a week at initial medium concentrations.

Table S1: final concentrations of each contaminant added per crystallizer, expressed in $mg.L^{-1}$. TBA = terbuthylazine, PC = pirimicarb and TCZ = tebuconazole. TBA, PC and TCZ solutions were prepared in DMSO. To ensure the solvent had no effect, the same quantity of DMSO was added in uncontaminated samples.

		N-NO3	Cu	TBA	PC	TCZ
ARO concentration	C	0	0	0	0	0
	1x	3	0,014	0,001	0,005	0,03
	10x	30	0,14	0,01	0,05	0,3
	50x	150	0,7	0,05	0,25	1,5
	100x	300	1,4	0,1	0,5	3
	250x	750	3,5	0,25	1,25	7,5

Tables S2: Communities composition by Phyla for uncontaminated samples (mean % ± SD) for prokaryotes and eukaryotes. Only dominant Phyla are presented, the less represented have been summed in the columns "Other".

	Sampling 1		Sampling 2		Sampling 3	
	NW	W	NW	W	NW	W
Phyla	Prokaryotic Phyla					
Bacteroidota	9.13 (±4.45)	9.86 (±9.86)	24.75 (±3.26)	21.61 (±10.19)	27.98 (±7.20)	22.54 (±3.01)
Cyanobacteria	4.18 (±0.34)	8.40 (±1.80)	13.66 (±5.90)	8.52 (±3.23)	12.59 (±3.27)	8.37 (±0.90)
Firmicutes	33.65 (±8.43)	39.38 (±2.95)	14.10 (±3.74)	30.35 (±11.29)	14.11 (±6.12)	24.80 (±4.28)
Proteobacteria	51.53 (±13.54)	40.38 (±8.78)	38.92 (±3.53)	26.83 (±4.89)	36.41 (±6.54)	27.91 (±4.50)
Others	1.51	2.02	8.58	12.69	8.91	16.39
	Eukaryotic Phyla					
Amoebozoa	0.09 (±0.07)	1.11 (±0.67)	15.66 (±7.52)	8.95 (±2.26)	18.10 (±9.74)	10.25 (±2.24)
Chloroplastida	1.07 (±0.87)	14.66 (±10.88)	25.87 (±9.41)	34.69 (±7.48)	21.86 (±3.08)	32.55 (±18.23)
Ciliophora	96.94 (±2.52)	67.35 (±26.03)	37.24 (±12.75)	31.49 (±10.53)	30.99 (±17.45)	30.32 (±19.95)
Others	1.89	16.87	21.23	25.14	29.04	26.87

Tables S2. Communities composition by Classes for uncontaminated samples (mean % ± SD) for prokaryotes and eukaryotes. Only dominant Classes are presented, the less represented have been summed in "Other" columns.

	Sampling 1		Sampling 2		Sampling 3	
	NW	W	NW	W	NW	W
Class			Prokaryotic Phyla			
Alphaproteobacteria	7.98 (±3.94)	13.0 (±4.23)	26.33 (±4.68)	19.65 (±1.62)	23.86 (±3.95)	22.68 (±3.67)
Bacilli	29.94 (±7.28)	38.21 (±2.55)	11.75 (±4.62)	29.30 (±11.13)	11.35 (±6.05)	23.36 (±5.01)
Bacteroidia	9.13 (±4.45)	9.86 (±9.85)	24.74 (±3.26)	21.61 (±10.19)	27.98 (±7.20)	22.54 (±3.01)
Cyanobacteriia	4.18 (±0.34)	8.40 (±1.81)	13.66 (±5.90)	8.52 (±3.23)	12.59 (±3.27)	8.37 (±0.90)
Gammaproteobacteria	43.56 (±9.76)	27.38 (±7.77)	12.59 (±1.31)	7.18 (±4.63)	12.55 (±2.85)	5.23 (±1.16)
Others	5.22	3.16	10.94	13.74	11.67	17.83
			Eukaryotic Phyla			
Amoebozoa	0	0	11.19 (±2.27)	7.04 (±1.97)	15.32 (±7.79)	8.09 (±1.05)
Chlorophycea	0.63 (±0.14)	3.09 (±1.29)	2.29 (±1.94)	6.34 (±5.00)	3.16 (±1.35)	6.55 (±4.43)
Intramacronucleata	96.94 (±2.52)	67.36 (±26.03)	37.24 (±12.75)	31.49 (±10.53)	30.99 (±17.45)	30.32 (±19.95)
Labyrinthylomycetes	0	0	7.05 (±2.26)	9.68 (±9.63)	10.33 (±8.48)	13.11 (±7.72)
Trebouxiophyceae	0	11.56 (±10.04)	23.55 (±11.33)	28.33 (±9.61)	18.69 (±1.90)	26.00 (±18.21)
Others	1.98	17.53	18.68	17.11	23.36	15.93

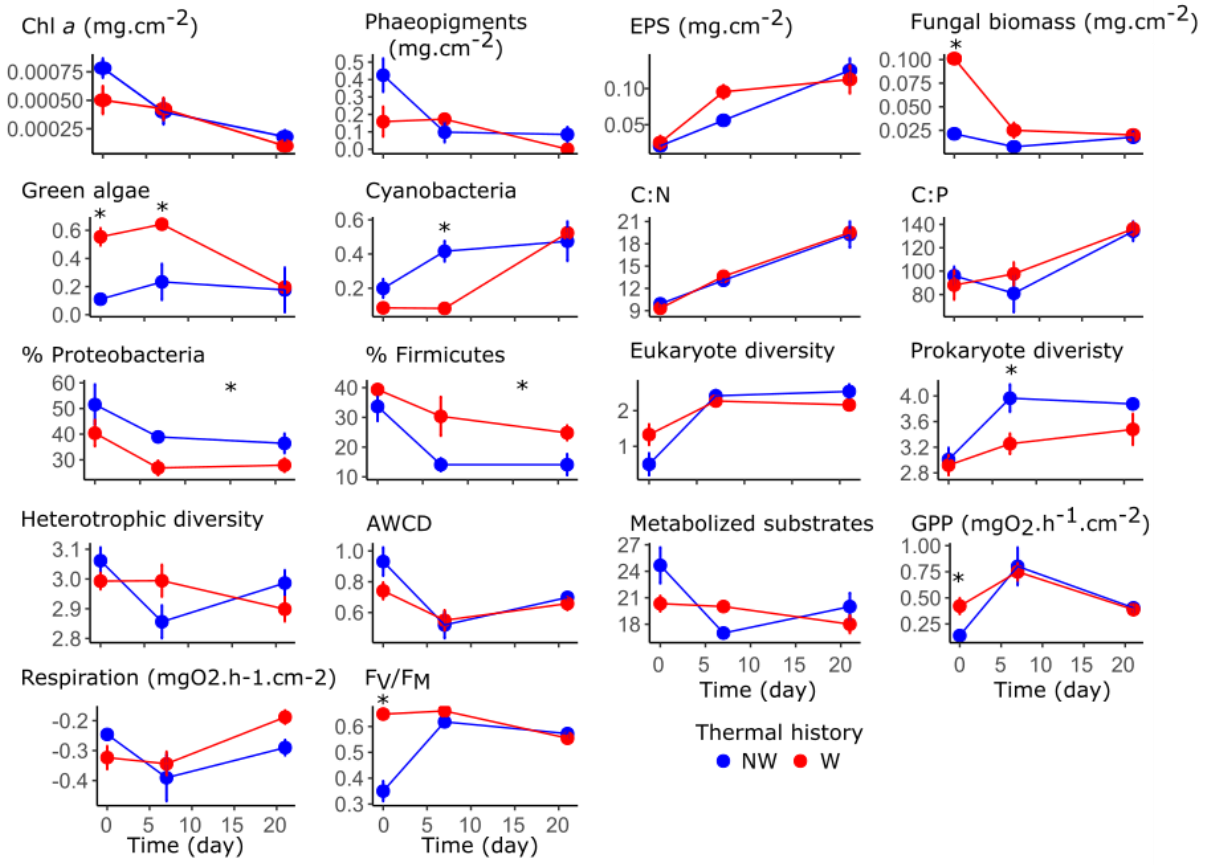

Figure S1: Evolutions of response variables (mean ± SE, n = 3) over time (S1, S2 and S3) for uncontaminated biofilms. Asterisks represent significant differences between thermal histories.

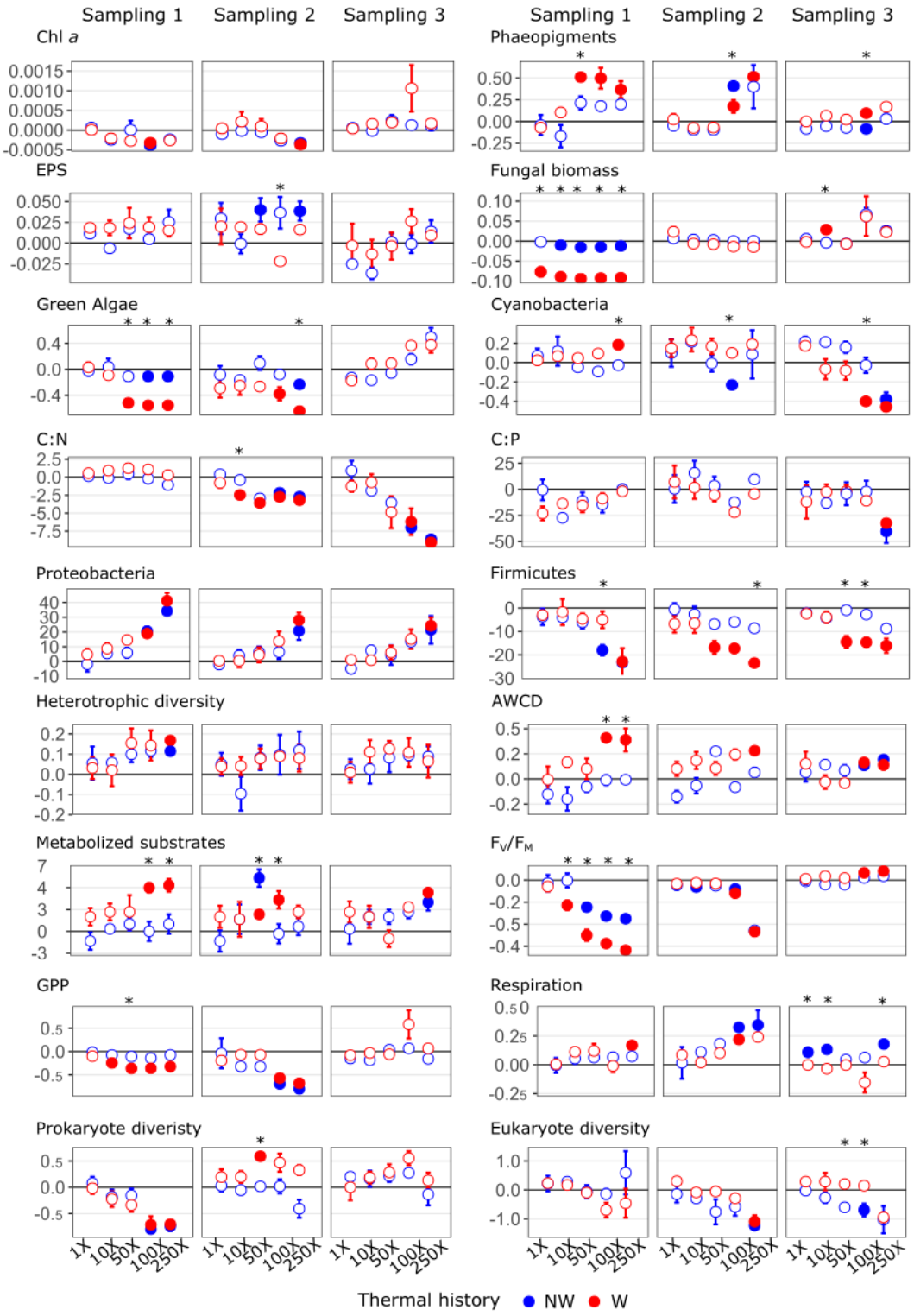

Figure S2: Changes in response variables compared to uncontaminated references (mean ± SE, n = 3) are represented below for each contaminant concentration (1X, 10X, 50X, 100X and 250X) at each sampling date (S1, S2 and S3). Solid rounds represent cases where the difference between references and contaminated samples is significant. Asterisks on the top of each plot represent significant differences between thermal histories.

Conclusion générale

Conclusion générale

Dans le contexte du changement global, les écosystèmes subissent à la fois des modifications du climat, entrainant par exemple l'augmentation du nombre des vagues de chaleurs ou des changements dans les patrons de précipitations, ainsi qu'une augmentation des pressions anthropiques telles que l'exposition à des contaminants. En agissant conjointement, ces facteurs de stress modifient les régimes de perturbations auxquels les communautés et les écosystèmes sont soumis (Turner, 2010). Ma thèse avait pour but d'évaluer et de comprendre les effets de ces changements de régimes de perturbation sur la stabilité des communautés microbiennes aquatiques benthiques. J'ai pour cela manipulé plusieurs niveaux de complexité de perturbation et différents niveaux d'organisation biologiques (i.e. populations et communautés). Les expérimentations que j'ai menées en laboratoire sur des biofilms de complexités variables (monospécifique, communautés synthétiques simplifiées, communautés complexes cultivées en canal ou communautés issues du milieu naturel) ont permis d'identifier les facteurs importants qui contribuent à la stabilité des biofilms soumis à des perturbations associées au changement global. J'ai principalement abordé cette question sous 3 angles (Figure 15) : l'importance des caractéristiques propres aux communautés (1), le rôle des conditions antérieures et l'histoire de perturbations dont notamment l'histoire thermale (2) ainsi que l'effet des propriétés des séquences de perturbations (3).

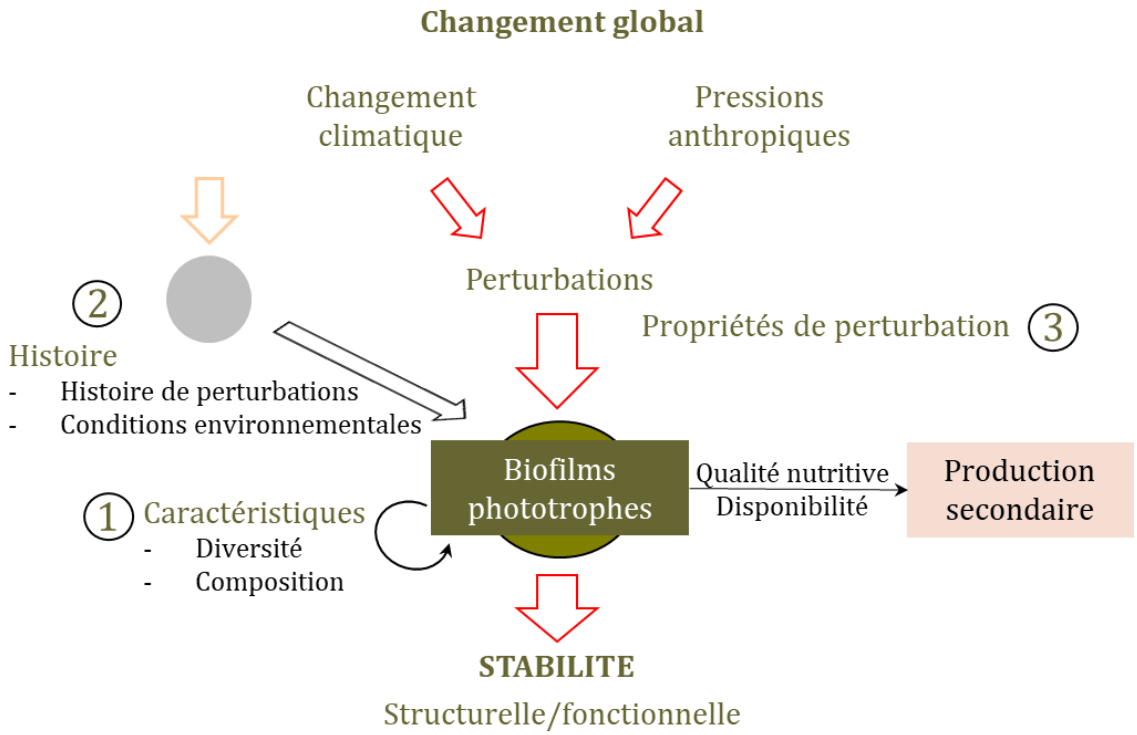

Figure 15 : Schéma récapitulatif des principales questions abordées lors de ma thèse pour comprendre l'influence des caractéristiques des communautés (diversité et composition), de leur histoire (perturbations ou conditions environnementales antérieures) et des propriétés des perturbations sur la stabilité structurelle et fonctionnelle des biofilms et leur capacité à soutenir la production secondaire.

Dans le **premier chapitre** de ma thèse, j'ai mis en évidence l'importance conjointe de la diversité / composition de la communauté algale et des conditions thermiques antérieures dans la réponse de la photosynthèse à un stress thermique unique et intense. Mon travail confirme que ces deux composantes sont certes essentielles, mais surtout qu'elles n'influent pas de la même manière la réponse au stress. En effet, **l'histoire thermale** joue un rôle déterminant dans la capacité de la communauté à tolérer le stress, les communautés acclimatées aux températures les plus élevées tolérant des stress plus intenses et/ou plus longs. La **diversité** quant à elle joue un rôle majeur dans la forme de la réponse : plus la diversité est importante, plus la réponse au stress est graduelle et tamponnée, grâce au phénomène d'assurance (Yachi and Loreau, 1999).

Le **deuxième chapitre**, dans lequel des biofilms de 4 **compositions** algales initiales contrastées ont été exposés à deux durées d'assec, de 3 jours et 2 semaines, avait

pour objectif de comprendre dans quelle mesure la composition taxonomique et biochimique de chaque communauté et **l'intensité** de la perturbation influencent la qualité des biofilms phototrophes en tant que ressource pour des consommateurs et plus généralement leur capacité à soutenir la production secondaire. Mon travail démontre que l'assec diminue fortement la qualité nutritionnelle potentielle d'un biofilm, mais que la durée d'assec a peu d'effet. Un assec de durée très courte suffit à réduire significativement la qualité potentielle d'une ressource essentielle pour les niveaux trophiques supérieurs. Le second point clé soulevé dans ce chapitre est que la trajectoire de récupération quelques semaines après l'assec dépend de la composition initiale des communautés. Ce sont notamment les changements dans les proportions relatives entre diatomées et cyanobactéries qui conditionnent l'augmentation de la qualité potentielle pour certains biofilms, la diminution pour d'autres ou encore le retour vers une qualité similaire à celle de l'état initial. En revanche, les conditions d'assec et de résilience testées n'impactent pas aussi nettement la production secondaire qu'elles affectent la qualité nutritionnelle potentielle des biofilms.

Ces deux premiers chapitres ont permis de préciser sur quelles composantes de la stabilité les caractéristiques propres aux communautés agissent dans le cadre d'une perturbation unique (Figure 16). Il est intéressant de noter que les variations naturelles saisonnières de températures, i.e. l'histoire thermale, contribuent à augmenter la tolérance des algues, via l'acclimatation des souches à des conditions particulières mais aussi en agissant sur la composition des assemblages et la sélection d'espèces thermo-tolérantes. Comme montré dans le deuxième chapitre, la résilience des biofilms à l'assèchement dépend très largement de la composition initiale des communautés. Ensemble, ces résultats montrent à quels points l'histoire de perturbations en modulant la tolérance des biofilms à une perturbation, mais aussi leur composition, peut influencer les composantes de la stabilité des communautés microbiennes.

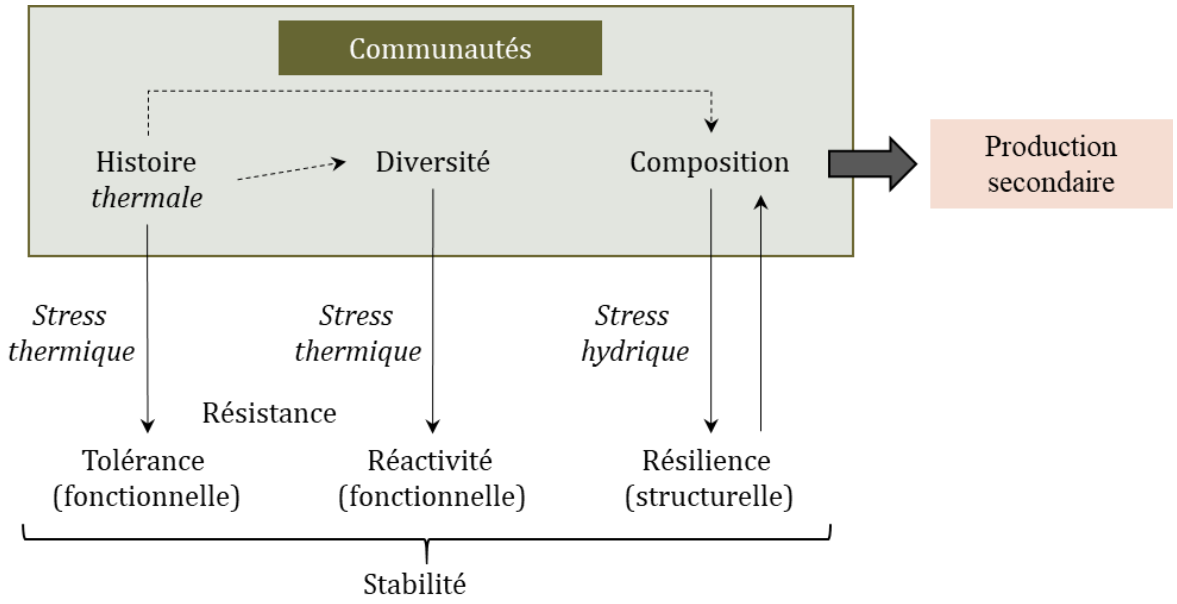

Figure 16 : principaux résultats des chapitres 1 et 2. Résumé des caractéristiques qui influencent la stabilité des biofilms en réponse à une perturbation unique (stress thermique ou assèchement). L'effet de l'assec sur la stabilité des communautés en tant que ressource a été évalué sur la production secondaire.

Dans la suite de ma thèse, je me suis focalisée sur les propriétés des régimes de perturbations qui influencent la résistance et la résilience des biofilms, en travaillant cette fois-ci non plus sur des perturbations isolées mais en analysant des successions ou des séquences de perturbations de natures identiques ou différentes.

Le **chapitre 3** était consacré à l'étude de la réponse de biofilms phototrophes simplifiés à des répétitions de **perturbations identiques**. Je me suis attachée à identifier les interactions éventuelles entre les perturbations successives au cours de ces **séquences**, en manipulant la durée de **l'intervalle** de temps entre les répétitions, **l'intensité** des perturbations, ainsi que la nature des perturbations appliquées. J'ai ainsi mis en évidence que ces interactions dépendent très fortement de la nature de la perturbation testée, selon que le stress est thermique, chimique ou salin. J'ai également démontré qu'une même nature de perturbation peut conduire à des interactions totalement différentes (pas d'interaction, antagonisme, synergie entre perturbations) selon l'intensité et la fréquence des perturbations. Enfin, l'effet de l'intervalle de temps dépend très fortement de l'intensité des perturbations. L'ensemble de ces résultats

démontre que les interactions entre perturbations sont peu prédictibles, même dans le cas extrêmement simplifié de plusieurs perturbations de même nature et de même intensité.

Enfin, l'objectif des deux derniers chapitres était de comprendre comment l'histoire de perturbations modifie la stabilité des biofilms qui se trouvent exposés à une seconde perturbation de nature chimique (Figure 17). Pour atteindre cet objectif, j'ai utilisé une séquence de perturbation réaliste associée au processus d'assèchement des cours d'eau (lentification, assèchement, réchauffement). Ces deux chapitres s'appuient sur une même expérimentation dont le déroulement initial a été fortement modifié par la crise sanitaire du printemps 2020, m'obligeant à revoir le design expérimental en cours d'expérience. De ce fait, les résultats ont dû être scindés en 2 chapitres distincts.

Dans le chapitre 4, j'ai étudié **l'effet des conditions hydrologique et thermale sur la sensibilité** (ou résistance) des biofilms exposés à un contaminant complexe. Cette expérience a permis de démontrer que le régime de perturbations antérieures conditionne la sensibilité des biofilms phototrophes à un lessivat agricole. Ce travail démontre l'importance de prendre en compte l'histoire d'intermittence hydrique dans l'évaluation du risque chimique dans les écosystèmes aquatiques.

Dans le **chapitre 5**, je me suis plus spécialement intéressée à l'effet de **l'histoire thermale** lors de l'intermittence (lentification et assec) sur la stabilité de biofilms exposés à un contaminant complexe. L'histoire thermale oriente fortement la réponse des communautés au contaminant, une température plus élevée lors de l'assec augmente les effets du contaminant. En revanche, ce chapitre montre que l'effet de l'histoire thermale est très transitoire puisque les trajectoires fonctionnelles des biofilms ayant eu des histoires thermales différentes convergent après seulement 4 semaines de remise en eau. A l'inverse, les effets sur la résilience structurelle persistent dans le temps, notamment pour la composition des microorganismes procaryotes. Le découplage structure-fonction observé est très certainement lié à de la redondance fonctionnelle.

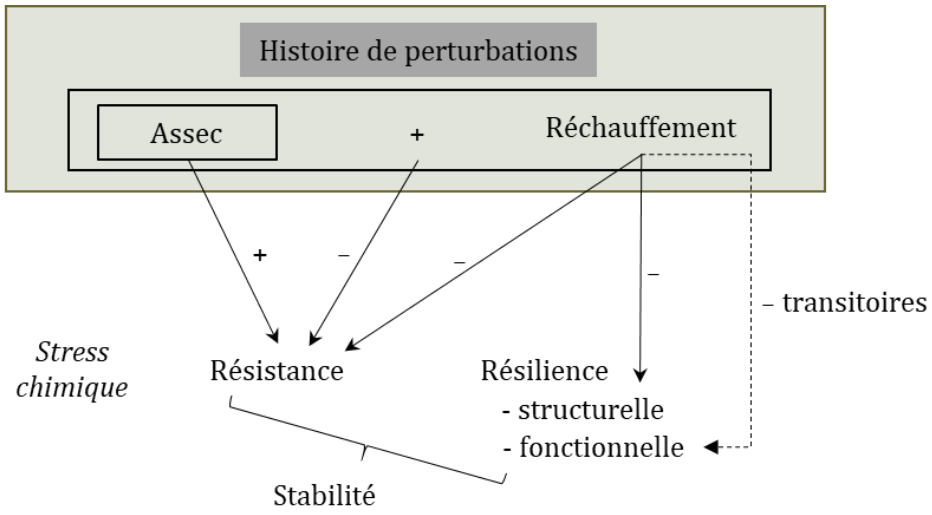

Figure 17 : principaux résultats des chapitres 4 et 5. Lorsque les biofilms sont exposés à un contaminant complexe après un assec, la durée d'assec et le contexte thermal antérieur modulent la résistance des biofilms au contaminant. Plus la première perturbation a un fort impact, moins l'effet du contaminant est fort. L'histoire thermale des biofilms lors de l'assèchement influence la stabilité des biofilms lors de la remise en eau.

Dans le milieu naturel, une perturbation complètement isolée relève de l'exception. Le plus souvent, une perturbation précède ou est suivie d'une autre perturbation, formant ou non une séquence selon l'échelle de temps considérée. Il est alors nécessaire, pour comprendre et anticiper la réponse d'une communauté à une perturbation donnée, de prendre en compte **l'histoire de perturbations**. Contrairement à la plupart des travaux relatifs à la résilience microbienne, qui se focalisent sur les effets de perturbations uniques ou simultanées, dans ce travail **je me suis attachée à travailler sur des séquences de perturbations complexes dont les effets sur la stabilité microbienne sont encore largement méconnus.** J'ai montré que les perturbations successives peuvent agir indépendamment, mais que des interactions positives ou négatives entre perturbations sont très fréquentes lorsque les perturbations sont rapprochées. De même, l'histoire de perturbations module significativement la stabilité des biofilms, mais le sens de l'effet dépend de la perturbation considérée et de son intensité.

Si mes travaux apportent des premiers éléments de compréhension, ils pointent la complexité des réponses et la difficulté de prévoir la stabilité des communautés

microbiennes. Ils soulèvent également des questionnements sur la stratégie à adopter pour concevoir des expérimentations permettant de mesurer la stabilité des communautés microbiennes en laboratoire et des perspectives de transposition des résultats obtenus en laboratoire vers le milieu naturel dont je souhaite discuter ci-après.

J'ai utilisé deux types d'approches lors de mes expériences : des expositions à des stress et des expériences de courtes durées, ou bien des expériences à plus long terme lors desquelles les biofilms ont été perturbés et la stabilité a été suivie pendant plusieurs semaines. Les deux approches présentent des avantages et des inconvénients. Les expériences à moyen terme ont permis de manipuler des assemblages microbiens assez complexes, représentatifs de communautés naturelles, dans des conditions environnementales assez réalistes alors que les expériences de courtes durées ont garanti la répétabilité et la réplication. Chaque expérience de mon travail est le résultat d'un compromis entre ces 3 dimensions : la représentativité du modèle biologique utilisé, le réalisme du design, et la possibilité de multiplier les conditions et de répéter les designs (Figure 18).

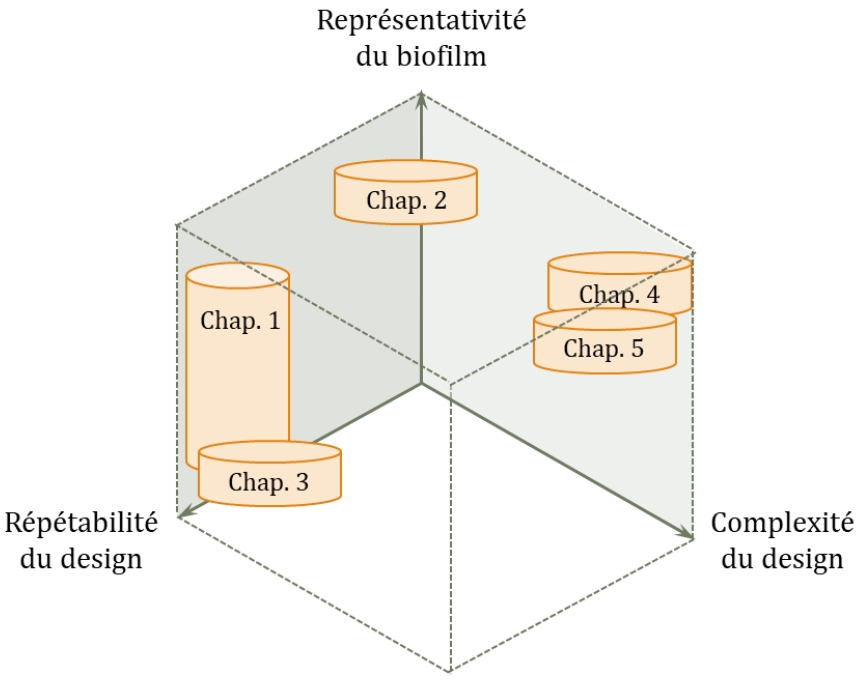

Figure 18 : Compromis entre les 3 aspects importants dans l'étude de la stabilité des communautés microbiennes. Un espace à 3 dimensions a été construit en se basant sur la complexité du design, sa répétabilité en testant de multiples conditions, mais aussi la représentativité naturelle du biofilm.

Dans les expériences à court terme (1 à 10 jours), utilisées dans les chapitres 1 et 3, des approches plus systémiques, c'est-à-dire globales, ont été employées. Dans ces expériences, la réponse du biofilm a été analysée à partir d'une seule variable intégrative (production primaire ou activité photosynthétique) avec une simplification de la structure du biofilm ou de sa complexité dans certaines conditions. La répétabilité des protocoles et leur simplicité permet de tester un très grand nombre de conditions de perturbations, aussi bien pour des biofilms collectés dans le milieu naturel que pour des souches de laboratoires, en répliquant facilement les mesures. Les résultats obtenus pour les différents modèles biologiques possédant différents niveaux de complexité peuvent être aisément comparés. Ces approches où un grand nombre de conditions sont manipulées permettent de tester l'existence de patterns généraux de la stabilité des systèmes en réponse à des perturbations.

Ces stratégies expérimentales aboutissent à la construction de modèles transposables à d'autres types de communautés ou organismes et utilisables dans divers domaines. C'est le cas par exemple de la dose thermale dans le chapitre 1, qui s'est avérée être une méthode pertinente pour évaluer et prédire l'effet de stress thermiques sur des communautés issues du milieu naturel. Cette approche semble être un outil prometteur pour prédire les impacts des vagues de chaleur estivales sur l'intégrité des communautés microbiennes. Cette méthode, venant du domaine médical et initialement développée pour évaluer l'effet de la température sur la viabilité et l'efficacité photosynthétique de cultures d'algues en réacteurs pour les bio-carburants (Béchet et al., 2017), pourrait être appliquée à d'autres types de communautés microbiennes, mais aussi à d'autres natures de perturbations. De la même manière, le modèle généré dans le chapitre 3 est un moyen original de décrire les interactions entre les propriétés d'une séquence de perturbations (son intensité, l'intervalle de temps entre les répétitions, le nombre de répétitions et la nature du stress appliqué). Jusqu'à présent, les études mettaient principalement en évidence le sens des interactions entre les facteurs de stress, mais aucun outil n'était disponible pour décrire l'influence et les interactions entre les propriétés de perturbations sur la stabilité des communautés (Philippot et al., 2021).

Dans les autres chapitres, des biofilms complexes ont été cultivés dans des canaux artificiels en laboratoire, permettant la mise en place de l'organisation tri-dimensionnelle caractéristique de ces communautés microbiennes. Ce changement d'échelle spatiale, en passant de microcosmes de quelques millilitres à des canaux alimentés par des réservoirs de 50 litres, favorise un changement d'échelle temporelle. En effet, il est alors envisageable de maintenir pendant plusieurs semaines les communautés et un suivi à plus long terme de la stabilité en réponse aux perturbations est réalisable. Dans ces expériences, j'ai utilisé des approches multi-paramètres qui, par opposition aux chapitres 1 et 3, permettent une compréhension plus détaillée des changements observés dans les communautés. De nombreuses variables ont été mesurées, générant une quantité de données importante. L'intérêt de cette approche est que cela permet de comprendre plus finement les mécanismes mis en jeu que lorsqu'une seule variable intégrative est mesurée mais cela pose la question de la gestion et du tri des informations générées pour en extraire les tendances significatives et pertinentes. Cette difficulté est contournée par l'utilisation des méthodes d'analyses multivariées qui permettent de synthétiser la réponse en gardant le maximum d'informations possibles, tout en omettant volontairement la part d'information non expliquée. Les variables synthétiques générées ainsi se sont avérées très intégratrices, puisqu'elles expliquaient une grande part de l'information. A mon sens, les deux approches sont complémentaires, et doivent être choisies en fonction des objectifs recherchés. Pour mieux identifier et comprendre les mécanismes impliqués dans la stabilité des communautés microbiennes, l'approche multi-paramètres est la plus pertinente. Pour généraliser la réponse des systèmes et dégager des grands patterns, il est plus judicieux de se limiter à la mesure de paramètres intégrateurs clés, tels que la productivité primaire brute.

L'expérimentation en laboratoire permet de s'affranchir des incertitudes liées au milieu naturel mais implique aussi que les expériences se déroulent pendant un temps limité. Le choix d'une échelle de temps appropriée est une question critique dans l'étude de la résilience. Cette question de la temporalité de la résilience a été avancée par Shade et al. (2012) pour expliquer, en autres, le fait que sur 148 études analysées, une résilience par rapport à l'état avant perturbation n'était que très rarement observée. Dans mes travaux, les durées de suivi de la résilience ont été choisies en fonction du

dimensionnement du dispositif expérimental et de la durée de la perturbation. Ainsi, dans le chapitre 3, la résilience de biofilms cultivés dans des microcosmes de 2 ml a été mesurée 24 h après une perturbation de 2 h. Dans le dernier chapitre, j'ai étudié la résilience des biofilms à l'assèchement pendant 4 semaines, après un assec s'étendant sur 12 semaines. Ce choix de durée peut paraitre questionnable, la perturbation étant 3 fois plus longue que la période de récupération. Cependant, dans mon chapitre 5, une résilience au moins partielle a été mise en évidence au cours des 4 semaines considérées, et cette durée a été suffisante pour mettre en évidence des divergences ou convergences selon les traitements dans les trajectoires des communautés lors de la récupération. Cela est certainement lié au fait que dans mes conditions expérimentales non limitées en nutriments, 4 semaines est une échelle de temps permettant au biofilm d'approcher sa biomasse maximale. En effet, dans le cycle de croissance des biofilms en milieu naturel, la phase de croissance compte entre 2 et 12 semaines, en fonction des conditions nutritives, sa durée étant d'autant plus longue que les concentrations en nutriments sont faibles (Biggs, 1996).

Un autre point à soulever lorsque que l'on étudie la stabilité des communautés microbiennes est la définition du point de référence, la résilience étant toujours mesurée par rapport à un état de référence (Grimm and Wissel, 1997). Typiquement, deux types de références peuvent être utilisés : soit l'état du système avant perturbation, soit un système contrôle non perturbé étudié en parallèle tout au long de l'expérience (Todman et al., 2016). Au cours de ma thèse, j'ai été confrontée au fait que le choix de la référence pouvait être problématique et influencer fortement les résultats pour des perturbations de longue durée. En effet, les biofilms étant des systèmes extrêmement dynamiques, les contrôles non perturbés divergent par rapport à l'instant de la perturbation, et le choix de la référence est alors très critique. Dans mes travaux, j'ai pris le parti d'utiliser une référence non perturbée simultanée, choix guidé par le fait que la remise en eau après assec ne pouvait être réalisée exactement dans les mêmes conditions que la phase de croissance. La référence m'a permis de prendre en compte ces changements. Il est également possible de considérer qu'il n'existe pas d'état de référence, mais plutôt des dynamiques de référence (Grimm and Wissel, 1997). Dans le cas des biofilms, ou autres systèmes avec plusieurs états stables, il n'y a que très rarement un retour à un état de référence prédéfini, ainsi les métriques servent plutôt à quantifier le changement

engendré par la perturbation plutôt que le retour à un état de référence (Jentsch et al., 2002).

Les analyses de composition basées sur le séquençage de l'ADN du dernier chapitre de ma thèse ont mis l'accent sur la forte redondance fonctionnelle au sein des biofilms : en dépit des changements dans la composition des communautés, les fonctions réalisées suite à la perturbation étaient les mêmes que par rapport à la référence (Allison and Martiny, 2008). Cette observation remet aussi en perspective la pertinence de l'application des concepts distincts de résilience ingénieure et de résilience écologique aux communautés microbiennes ayant une très forte résilience fonctionnelle. En effet, l'orientation vers l'un ou l'autre de ces deux types de résilience dépend de la variable observée. Ainsi, dans mon cas, lorsque l'on considère la composition des communautés bactériennes, le système change d'état et traduit une résilience de type écologique. Cependant, aucun changement significatif dans la composition des eucaryotes n'est mis en évidence, de même que sur les fonctions remplies par la communauté, ce qui correspond à une résilience de type ingénieure pour les fonctions mesurées dans l'expérience. Ces résultats contrastés montrent les limites de l'application de certains concepts d'écologie générale aux modèles microbiens.

La perspective générale de mes travaux est de transposer les résultats observés en laboratoire au milieu naturel. Cette transposition implique un changement d'échelle, à la fois spatiale et temporelle, en replaçant les biofilms au sein de l'écosystème. Dans le cadre de l'effet des conditions thermiques, il s'agira, par exemple, de prendre en compte le fait que lors d'épisodes de vagues de chaleur, l'augmentation des températures de l'eau se déroule de manière progressive, générant un gradient d'expositions à diverses températures. Si j'ai bien caractérisé la sensibilité des biofilms à un stress thermique brutal (chapitre 1), il reste nécessaire de comprendre comment les biofilms répondent à de tels événements plus progressifs. Mes travaux suggèrent pour le moment que la sensibilité des biofilms pourrait être réduite lors de ces successions, les stress thermiques agissant de façon antagoniste, mais cela est fortement dépendant des caractéristiques de la séquence de perturbations (chapitre 3) et n'exclut pas l'existence de seuils au-delà desquels il n'y a plus de résilience.

D'autre part, à l'échelle du tronçon de rivière, les perturbations ne s'appliquent pas nécessairement de façon homogène. La séquence d'assèchement peut être incomplète localement, avec des zones d'écoulement interrompu sans assèchement complet, conduisant à une forte hétérogénéité spatiale. Lors de la remise en eau, l'hétérogénéité de la composition spécifique au sein du biofilm augmente aussi (Ledger et al., 2008), ce qui s'accompagne d'une hétérogénéité dans la qualité nutritionnelle (chapitre 2). Cependant, la qualité potentielle d'une ressource varie d'un consommateur à l'autre, en fonction des besoins spécifiques. La transposition des résultats au milieu naturel impliquera l'intégration de la diversité des consommateurs et donc de considérer d'autres taxa, pour estimer plus globalement les effets de l'intermittence hydrique sur la production secondaire. Il est probable que les effets diffèrent d'un consommateur à l'autre. Le gammare utilisé dans l'expérience du chapitre 2 est un modèle facile à maintenir en laboratoire et avec lequel mes collaborateurs avaient déjà fait des mesures de qualité alimentaire. Néanmoins, il s'agit plutôt d'un déchiqueteur qui, bien qu'omnivore, consomme préférentiellement la matière organique allochtone, et plus marginalement le biofilm, qui lui apporte des éléments nutritifs essentiels (Crenier et al., 2017). La transposition au milieu naturel nécessitera également de se placer sur une échelle de temps plus longue, et d'évaluer les effets de changements dans la qualité potentielle d'une ressource à plus long terme sur d'autres paramètres, tels que la reproduction (DeMott, 1998; Gulati and Demott, 1997; Schälicke et al., 2019). Il faudra également intégrer des aspects plus complexes de la séquence de perturbation. Par exemple, lorsque le niveau de l'eau diminue au début de la séquence d'assèchement, une augmentation de la concentration en contaminants dans l'eau est possible à cause de la baisse des capacités de dilution (Guasch et al., 2010; Mosley, 2015). Il s'agira d'intégrer davantage les mécanismes liés au fonctionnement des rivières, notamment les processus d'immigration issus des communautés situées plus en amont et qui peuvent jouer un rôle important dans la stabilité des communautés suite à un assec (Sabater et al., 2017). Enfin, la transposition au milieu naturel devra aussi prendre en compte l'importance des effets indirects des perturbations via la modification des interactions biotiques avec les compétiteurs ou les niveaux trophiques supérieurs, que j'ai pu mettre en évidence lors de mon stage de master 2 (Allen et al., 2021, voir annexe).

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Disentangling the direct and indirect effects of agricultural runoff on freshwater ecosystems subject to global warming: A microcosm study

Joey Allen[a,b,*], Elisabeth M. Gross[b], Camille Courcoul[a], Stéphanie Bouletreau[a], Arthur Compin[a], Arnaud Elger[a], Jessica Ferriol[a], Sabine Hilt[c], Vincent E.J. Jassey[a], Martin Laviale[b], Bastian H. Polst[d], Mechthild Schmitt-Jansen[d], Herwig Stibor[e], Vinita Vijayaraj[b], Joséphine Leflaive[a]

[a] Laboratoire écologie fonctionnelle et environnement, Université de Toulouse, CNRS, INPT, UPS, Toulouse, France
[b] Université de Lorraine, CNRS, LIEC, F-57000 Metz, France
[c] Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany
[d] Helmholtz-Centre for Environmental Research – UFZ, Dept of Bioanalytical Ecotoxicology, Leipzig, Germany
[e] Department of Biology II, Ludwig-Maximilians-University Munich, Planegg-Martinsried, Germany

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ABSTRACT

Aquatic ecosystems are exposed to multiple stressors such as agricultural run-off (ARO) and climate-change related increase of temperature. We aimed to determine how ARO and the frequency of its input can affect shallow lake ecosystems through direct and indirect effects on primary producers and primary consumers, and whether warming can mitigate or reinforce the impact of ARO. We performed a set of microcosm experiments simulating ARO using a cocktail of three organic pesticides (terbuthylazine, tebuconazole, pirimicarb), copper and nitrate. Two experiments were performed to determine the direct effect of ARO on primary producers (submerged macrophytes, periphyton and phytoplankton) and on the grazing snail *Lymnaea stagnalis*, respectively. Three different ARO concentrations added as single doses or as multiple pulses at two different temperatures (22°C and 26°C) were applied. In a third experiment, primary producers and consumers were exposed together to allow trophic interactions. When functional groups were exposed alone, ARO had a direct positive effect on phytoplankton and a strong negative effect on *L. stagnalis*. When exposed together, primary producer responses were contrasting, as the negative effect of ARO on grazers led to an indirect positive effect on periphyton. Periphyton in turn exerted a strong control on phytoplankton, leading to an indirect negative effect of ARO on phytoplankton. Macrophytes showed little response to the stressors. Multiple pulse exposure increased the effect of ARO on *L. stagnalis* and periphyton when compared with the same quantity of ARO added as a single dose. The increase in temperature had only limited effects. Our results highlight the importance of indirect effects of stressors, here mediated by grazers and periphyton, and the frequency of the ARO input in aquatic ecosystems.

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1. Introduction

Ecosystems are severely affected by anthropogenic stressors (Barnosky et al., 2011; Butchart et al., 2010) and aquatic ecosystems are particularly threatened (Reid et al., 2019). In this context, it is crucial to determine the mechanisms by which the impact of stressors on specific organisms can alter ecosystem state and functioning (Bruder et al., 2019). Aquatic ecosystems are exposed to multiple anthropogenic stressors (Jackson et al., 2016; Ormerod et al., 2010) partially originating from local sources such as agricultural activities (Ippolito et al., 2015; Malaj et al., 2014; Stehle and Schulz, 2015). Agricultural run-off (ARO) is not only responsible for high nutrient input, but it also contains multiple xenobiotics such as herbicides, insecticides, fungicides and trace metals that might affect ecosystems and interact with other stressors in an unpredictable way (Schriever et al., 2007). Nutrient and pesticide input to adjacent waterbodies through ARO is highly dependent on the strength and frequency of rain events and agricultural practices. Therefore, aquatic ecosystems are frequently exposed to multiple pulses of nitrates and pesticides with variable intensity (Daouk et al., 2013; Liess et al., 1999; Reinert et al., 2002),

* Corresponding author.
E-mail address: joeyallen@orange.fr (J. Allen).

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further complicating the assessment and prediction of ARO effects. Existing studies on ARO revealed severe effects on aquatic ecosystem processes such as primary production and ecosystem respiration (Peters et al., 2013). Multiple pulse exposure to pollutants and nutrients can modulate the effects compared to a single or continuous exposure (Davis et al., 2019; Reinert et al., 2002; Varga et al., 2019). In addition to local stressors, global warming affects aquatic ecosystems by influencing the metabolic rates of organisms (Brown et al., 2004; Gillooly et al., 2001) as well as their sensitivity towards stressors (Cuco et al., 2016; Debecker et al., 2017; Delnat et al., 2019). All the different stressors may have interactive effects, but determining how multiple stressors interact is challenging (Jackson et al., 2016), especially in complex systems where biotic interactions lead to indirect effects (Bruder et al., 2019).

Shallow lakes are ideal ecosystems to address the aforementioned challenges, as they are the most abundant waterbodies worldwide (Verpoorter et al., 2014), often exposed to ARO (Hijosa-Valsero et al., 2016) and prone to warming (Adrian et al., 2009). The state of a shallow lake ecosystem is shaped by biotic interactions among and between primary producers and higher trophic levels, and thus they respond non-linearly to nutrient loading with rapid shifts between two alternative stable states: a macrophyte-dominated clear-water state and a phytoplankton-dominated turbid state (Scheffer and van Nes, 2007; Scheffer et al., 1993). Such shifts have severe consequences for several ecosystem functions (Hilt et al., 2017) and services (Janssen et al., 2020). Both warming and exposure to ARO have been shown to affect macrophytes (Mckee et al., 2002), phytoplankton (Donald et al., 2011; Huisman et al., 2018; Paerl and Huisman, 2008; Zhao et al., 2019a), periphyton (Piggott et al., 2015) and primary consumers (Schaum et al., 2018; Zhang et al., 2018). Increasing temperature and nutrient loading were predicted to increase the probability of a shift toward the turbid state (Moss et al., 2011; Scheffer et al., 2015), while the effects of other pollutants including pesticides, trace metal elements, trace organics, pharmaceuticals and vanity products remained largely unexplored (Phillips et al., 2016). Combining expertise from ecology and ecotoxicology can help elucidating the complexity of stressor and biotic interactions in shallow lakes, e.g. by using microcosm experiments providing a higher ecological realism than single species exposed to stressors such as pollutants. Such experiments will reveal direct and indirect effects on interacting functional groups in aquatic communities, an essential prerequisite to predict and eventually mitigate the impact of global warming by controlling local stressors (Scheffer et al., 2015).

Here, we investigated in microcosms the combined effect of global warming and ARO on two trophic levels (primary producers and their consumers) representative of shallow lake ecosystems. We hypothesised that 1) ARO and warming will affect primary producer and consumer development. Particularly, we expected that ARO will cause a shift towards a phytoplankton dominance and that warming will increase this ARO effect. We also expected that 2) direct effects on some organisms will affect others indirectly through biotic interactions. We further hypothesise that 3) multiple pulses of ARO will favour the overall resistance of the ecosystem in comparison to a single pulse treatment with the same overall amount of pollutant added to the microcosms, because a more progressive input of toxicants would allow organisms to acclimate to this stressor. The three microcosm experiments were performed mimicking ARO containing nitrate, copper and typical organic pesticides with different exposure modes (addition of ARO by a single or multiple pulses) and warming following the IPCC RCP 8.5 scenario (+4°C, IPCC, 2014). The effects of these stressors were evaluated on microcosms containing only the primary producers, only the grazers or both with the overall aim to gain a mechanistic understanding of direct and indirect effects of two major environmental stressors affecting shallow aquatic ecosystems. We used structural equation modelling to determine further causal linkages between these stressors and observed effects.

Table 1
Final concentration of the contaminants in the microcosms. For the experiment 2, only 1X and 3X treatments were used.

	Single addition			Multiple pulses	
	1X	2X	3X	XX	XXX
Terbuthylazine (µg L^{-1})	1	2	3	2	3
Tebuconazole (µg L^{-1})	30	60	90	60	90
Pirimicarb (µg L^{-1})	5	10	15	10	15
Cu (µg L^{-1})	14	28	42	28	42
N-NO_3 (mg L^{-1})	3	6	9	6	9

2. Material and methods

Three microcosm experiments were performed. In Experiments 1 and 2, respectively, primary producers and grazers were exposed independent of each other to ARO and warming, while in experiment 3, they were exposed together to allow for trophic interactions. The microcosms were incubated at two different temperatures and exposed to a cocktail of pesticides, copper and nitrate designed to mimic the effect of ARO (see Table 1 for ARO composition). Six different ARO treatments were used: a control without ARO; three treatments corresponding to the ARO at the lowest concentration (X), twice the lowest concentration (2X), and thrice the lowest concentration (3X) as a single addition at the beginning of the experiment; two treatments with two or three pulses of ARO at the lowest concentration (X) with four-day intervals between each addition (multiple pulse treatments XX and XXX, with the same final amount of ARO as in 2X and 3X, respectively. Target temperatures were set at 22°C (controls representing typical temperate lake summer temperatures e.g. Mckee et al., 2002; Sand-Jensen et al. 2019) and 26°C (representing the future climate warming scenario). We therefore used 12 different combinations of ARO and temperature: cold-control, heated-control, cold-1X, heated-1X, cold-2X, heated-2X, cold-3X, heated-3X, cold-XX, heated-XX, cold-XXX, heated-XXX with 5 replicates for each condition. In experiment 2, with grazers alone, only the 1X and 3X treatments at both temperatures were used. In addition, the concentration of nitrates was reduced since nitrates would not be consumed in the absence of primary producers (Table 1).

The ARO cocktail contained terbuthylazine (CAS number 5915-41-3), a triazine photosynthesis-inhibiting herbicide (Lorente et al., 2015), tebuconazole (CAS number 107534-96-3), a triazole fungicide known to affect biofilm and phytoplankton (Artigas et al., 2014), pirimicarb (CAS number 23103-98-2), a carbamate insecticide inhibiting acetylcholinesterase (Krieger, 2010), and copper ($CuSO_4$, CAS number 7758-98-7), a trace metal used in fungicidal, bactericidal and algicidal preparations (Lamichhane et al., 2018). All pesticides were PESTANAL® analytical standards (Merck, Darmstadt, Germany). Concentrations were based on published EC values (EC10, 20, 50) for each organism group, available SSD data and some unpublished data on the toxicity of all ARO components. From these data, we identified an effective concentration for each ARO pollutant (excluding nitrate) equivalent to the EC20 for chronic exposure for the most sensitive group, which was used as the highest concentration in the subsequent experiments (Table 1). Stock solutions of organic pesticides were prepared in Dimethyl sulfoxide (DMSO, CAS 67-68-5, Merck). The same amount of DMSO was added in all microcosms at a final concentration of 0.125%. Copper and nitrate (KNO_3) solutions were prepared in MilliQ water.

2.1. **Experiment 1 -** *primary producers alone*

In experiment 1, 8-L microcosms contained submerged macrophytes (*Myriophyllum spicatum* L., *Elodea nuttallii* Michx., *and Potamogeton perfoliatus* L), planted in artificial sediments (OECD TG 239 (2014) modified to replace nutrient salts by 5 g kg^{-1} nettle powder as fertilizer), and ten strains of microalgae (SI table S1). Macrophytes were introduced first to allow rooting before being submitted to competition with phytoplankton and periphyton. Algae were introduced in the microcosms 9 days after macrophytes and 8 days before ARO addition and temperature increase, just after a complete water renewal to remove initial differences in algae and nutrient levels that had developed in the microcosms. Achieved temperatures were 22.5 ± 0.4°C (unheated) and 25.7 ± 0.4°C (heated microcosms). Microcosms were maintained during 28 days after ARO addition with regular water and nutrient addition until final sampling. Air temperature was maintained at 22°C, light at 70 µmol m^{-2} s^{-1} (16:8 h light:dark cycle). Translucent polypropylene strips were placed in each microcosm for periphyton development monitoring. More information on microcosm settings are provided in the supplementary material.

2.2. Experiment 2 – grazers alone

The pond snail *Lymnaea stagnalis* was exposed to ARO at 22 ± 0.2°C and 26 ± 0.2°C in the absence of any primary producer. This species was selected because it is commonly found in shallow freshwater ecosystems, feeding primarily on benthic algae and aquatic plants (Elger et al., 2009). It is also a good model in ecotoxicology (Amorim et al., 2019) recently normalized as OECD test guideline (OECD 2016). *Lymnaea stagnalis* was obtained from a nearby pond and bred in the laboratory for several generations to obtain a parasite-free population. Individuals were selected to minimise size differences, measured and weighed (mean shell length ± SD = 13.7 ± 1.3 mm, mean total weight = 202 ± 58 mg). Each snail was placed in a 12-cm diameter, 30-cm high cylindrical glass aquarium filled with 1 L of Volvic® water with temperature control as in experiment 1. Snails were starved for 12 h before simultaneous addition of ARO and ten pre-weighed 12-mm diameter lettuce discs (organically grown). After 24 h, the remaining leaf discs were collected and freeze-dried. Additional lettuce discs were used to determine initial dry mass, and the dry mass of lettuce consumed by each snail was determined. The snails were then fed *ad libitum* with lettuce for 21 days. Water evaporation in the aquaria was compensated by addition of deionised water twice a week. The light cycle was 16:8 light:dark with only 5 µmol m^{-2} s^{-1} irradiance to control possible algal development. At the end of the experiment, snails were starved for 12 h before a new feeding test was performed with 10 pre-weighed 14-mm diameter lettuce discs and stopped after 16 h to avoid complete consumption of the food provided. The snails were then measured and weighed.

2.3. **Experiment 3 -** *primary producers and consumers*

We used the same experimental set up as in experiment 1 but added one individual of *L. stagnalis*, one individual of zebra mussel (*Dreissena polymorpha*) and twenty *Daphnia magna* neonates in each microcosm. Snail individuals collected as in experiment 2 had an initial mean (± SD) shell length of 18.7 (± 2.2) mm and a mean total fresh mass of 0.4 (± 0.1) g including shell. Zebra mussels collected in a lake were placed in aquaria for 1 week before being transferred to the microcosms and measured using an electronic calliper (16.0 ± 1.2 mm). Individuals were selected to be as homogenous in size as possible. *D. magna* were bred at the laboratory. Macrophytes, algae and primary consumers were introduced in the mesocosms 10, 7 and 3 days before addition of ARO, respectively, to allow for settlement without stressors.

In addition, several adjustments were made due to observations in the first experiment and changes in material availability. The organic pesticides were prepared as a 10-fold more concentrated solution, and therefore, the amount of DMSO added to the microcosms was ten times lower (0.01%). $CaCO_3$ at 7.5 g kg^{-1} was added in the sediment of each microcosm as a buffer to limit potential effects of acidity on the primary consumers. An additional plastic strip was added. Mean water temperatures were 21.4 ± 0.3°C and 23.7 ± 0.3°C in the non-heated and heated microcosms, respectively. Final sampling was done 21 days after the addition of ARO as it corresponded to the maximal development of phytoplankton in experiment 1.

2.4. Measured parameters

At the end of each experiment, water was collected and filtered at 0.7 μm (GF/F). In the filtered water, concentrations of nitrate and dissolved phosphorus were determined by ion chromatography (Dionex Ics-5000$^+$, Sunnyvale, CA, USA) and by ammonium molybdate spectrophotometric method, respectively (AFNOR 1990). The plastic strips were cut at 20 cm from the bottom, and all *L. stagnalis* eggs and juveniles present on the strips were collected and counted. Periphyton on these strips was scraped and collected with 20 mL of Volvic® water. From the resulting suspension, subsamples of 1.5 mL were centrifuged (10000 g, 10 min) in 2 mL tubes. The filters and pellets were stored at -80°C until photosynthetic pigment analysis.

Phytoplankton and periphyton pigments were extracted to estimate total planktonic and periphytic algae biomass and the proportion of diatoms, green algae and cyanobacteria. Pigment extraction was carried out on periphyton and phytoplankton samples after freeze-drying by the addition of 2 mL methanol buffered with 2% ammonium acetate 1 M, followed by an initial sonication step (3 min at 4°C) and 15 min of incubation at -20°C, and then a second sonication step. Pigment extracts were recovered after centrifugation (10000 g, 5 min, at 2°C). The extraction steps were repeated until obtained extracts were no longer visibly coloured. All the extracts of the same sample were pooled. Extracts were filtered through a 0.2-μm PTFE membrane syringe filter (VWR International, United States), and analysed by HPLC. HPLC analysis of pigments was performed as described by Capdeville et al. (2019). Pigments specific to a particular group of primary producer (diatoms: fucoxanthin, diatoxathin and diadinoxanthin; green algae: chlorophyll *b*, lutein, violaxanthin and neoxanthin; cyanobacteria: zeaxanthin, echinenone and myxoxanthophyll) were used to assess variation of the different groups of microalgae (Mackey et al., 1996; Majdi et al., 2011). The total P concentration in periphyton biomass was determined following the ammonium molybdate spectrophotometric method after persulphate digestion in acidic conditions of freeze-dried periphyton subsamples following AFNOR guidelines (AFNOR 1990).

Macrophytes were handled as described in Gross et al. (2018). Macrophytes were removed from the sediments and rinsed in water to remove attached particles and epiphytes. All the plants of one species were spread in a large tray equipped with a ruler, and a picture was taken. Roots and aboveground parts were separated and rapidly dried. Aboveground parts were then weighed to estimate macrophyte biomass. Visible signs of macrophyte consumption by grazers were recorded based on the pictures taken.

In experiment 3, adult *L. stagnalis* were collected, weighed and measured. Individuals of *D. polymorpha* were collected and measured. The presence of dead *L. stagnalis* or *D. polymorpha* was recorded. Due to a rapid disappearance of *D. magna* in all microcosms, no measurement was possible.

2.5. Statistics

ARO concentration, exposure mode (single or multiple pulses of ARO addition) and temperature effects on the different measured parameters were analysed using an ANCOVA with ARO concentration as a continuous variable and temperature and exposure mode as categorical independent variables. The effect of exposure mode was analysed using only 2X/XX and 3X/XXX data with contrast analysis. The magnitude of the effects was measured using Hedges' g (Hedges, 1981) effect size calculated from *t*-statistics derived from the ANCOVA according to Berben et al. (2012) using the R package {esc}.

2.6. Structural equation modelling

To explore the simultaneous effect of ARO and warming on the interactions between macrophytes, phytoplankton, periphyton and grazers, we built a path analysis subjected to structural equation modelling (SEM; Grace et al., 2010). SEM has been developed as a powerful tool for inferring cause-effect relationships among variables via proposed hypotheses tested against data (Grace et al., 2012; Grace and Irvine, 2020). Furthermore, SEM permits the evaluation of direct and indirect effects within a network of variables (Bruder et al., 2019). This approach has been successfully applied to identify the mechanistic pathway underlying the effect of different stressors on aquatic ecosystems (Alexander et al., 2016, 2013; Majdi et al., 2014; Rodrigues et al., 2018). Indeed, as a framework for quantitative analyses, SEM is an extension of univariate mediation analyses taking into account the effect of variable X on variable Z through its effect on Y (Grace and Irvine, 2020; Reisner et al., 2013). Using *a priori* knowledge, we built a full model of cause-effect relationships based on hypothetical pathways supported by literature (Table 1). Notably, the full model included a direct pathway between ARO exposure and phytoplankton biomass, and several indirect pathways via competition with periphyton or macrophytes (See Supplementary Figure S6). The hypothetical relationships between variables (Table 1) was then tested on the correlation matrix of experimental data (Grace and Irvine, 2020; Reisner et al., 2013). We considered all available variables to investigate the interactions among trophic levels. Using *a priori* knowledge, a full path analysis model was created including simultaneously several hypothetical pathways (Table 2) through which ARO and warming could affect macrophytes, phytoplankton, periphyton and grazers. We only used the biomasses of macrophytes, phytoplankton, periphyton and grazers from experiment 3 (with primary producers and consumers) to perform this analysis. For the primary consumers, only *L. stagnalis* size increase was used as *D. magna* did not develop and no growth was observed on *D. polymorpha*. The different directions and kind of interactions among trophic levels were tested. Then, successive full models were simplified by step-wise exclusion of variables and/or linkages with non-significant weights and non-significant co-variance, as estimated by Akaike Index Criterion (AIC), until a minimal adequate model showing specific linkages remained. The best model was selected based on its coherence with the direct effect observed in experiments 1 and 2. The adequacy of the final model was determined using quality indicators such as Chi^2, root mean square error of approximation (RMSEA), standardised root mean square residual (SRMR), adjusted goodness of fit index (aGFI) and comparative fit index (CFI) (Fan et al., 2016). SEM was performed using the R package {sem}.

3. Results

3.1. Experiment 1: Effects of stressors on primary producers without consumers

ARO exposure and elevated temperature had no effect on total macrophyte aboveground biomass (Fig. 1). The exposure mode did not change the effect on macrophytes (Fig. 1). The mean final macrophyte dry mass per microcosm was 0.61 ± 0.24 g with values for individual microcosms ranging from 0.11 to 1.23 g (Supplementary material Fig. S1).

Phytoplankton biomass varied between 2 and 238 µg L^{-1} Chl *a*, and increased with increasing concentrations of ARO ($p = 0.041$; Fig. 1). Clear-water states with low phytoplankton (< 50 µg L^{-1} of Chl *a*) were observed in all treatments, but highly turbid water with high phytoplankton (> 100 µg L^{-1} of Chl *a*) was observed only at the two higher ARO concentrations. The exposure mode did not change the effect of ARO on phytoplankton. Warming had no significant effect on phytoplankton (Fig. 1) but favoured green algae (Fig. 2).

ARO exposure and warming had no significant effect on periphyton biomass, with a mean value of 1.08 ± 0.71 µg cm^{-2} of Chl *a*. However, we observed a trend for higher periphyton biomass ($p = 0.060$) with ARO and a higher periphyton biomass with multiple pulses as compared to single additions of the ARO ($p = 0.046$, Fig. 1). ARO induced an increase of the cyanobacterial pigment/Chl *a* ratio in both periphyton and phytoplankton (Fig. 2). ARO had no significant effect on the total amount of P contained in periphyton (Supplementary material Fig. S5).

Table 2
Links tested in the SEM. Experiment 1: primary producers alone, experiment 2: grazers alone.

Interaction	Effect	Hypothesis	Observed direct effect
ARO → macrophytes	+/-	Fertilisation by nitrates/toxicity of pesticides and Cu	
ARO → periphyton	+/-	Fertilisation by nitrates/toxicity of pesticides and Cu	Non-significant effect but slight positive trend observed in experiment 1
ARO → phytoplankton	+	Fertilisation by nitrates/toxicity of pesticides and Cu	Positive effect observed in experiment 1
ARO → grazers	-	Toxicity of pesticides and Cu	Negative effect observed in experiment 2
Temperature → macrophytes	+/-	Enhanced metabolisms/heat stress	No effect in experiment 1
Temperature → periphyton	+/-	Enhanced metabolisms/heat stress	No effect in experiment 1
Temperature → phytoplankton	+/-	Enhanced metabolisms/heat stress	No effect in experiment 1
Temperature → grazers	+/-	Enhanced metabolisms/heat stress	Very limited effect in experiment 2
Grazer → biofilm	-	grazing	
Grazer → macrophytes	-	grazing	Few damaged macrophytes
Biofilm → phytoplankton	-	Light & nutrient competition	
Phytoplankton → periphyton	-	Light & nutrient competition	
Phytoplankton → macrophytes	-	Light & nutrient competition	
Macrophytes → phytoplankton	-	Light & nutrient competition	
Macrophytes → periphyton	-	Light & nutrient competition	
Biofilm → macrophytes	-	Light & nutrient competition	

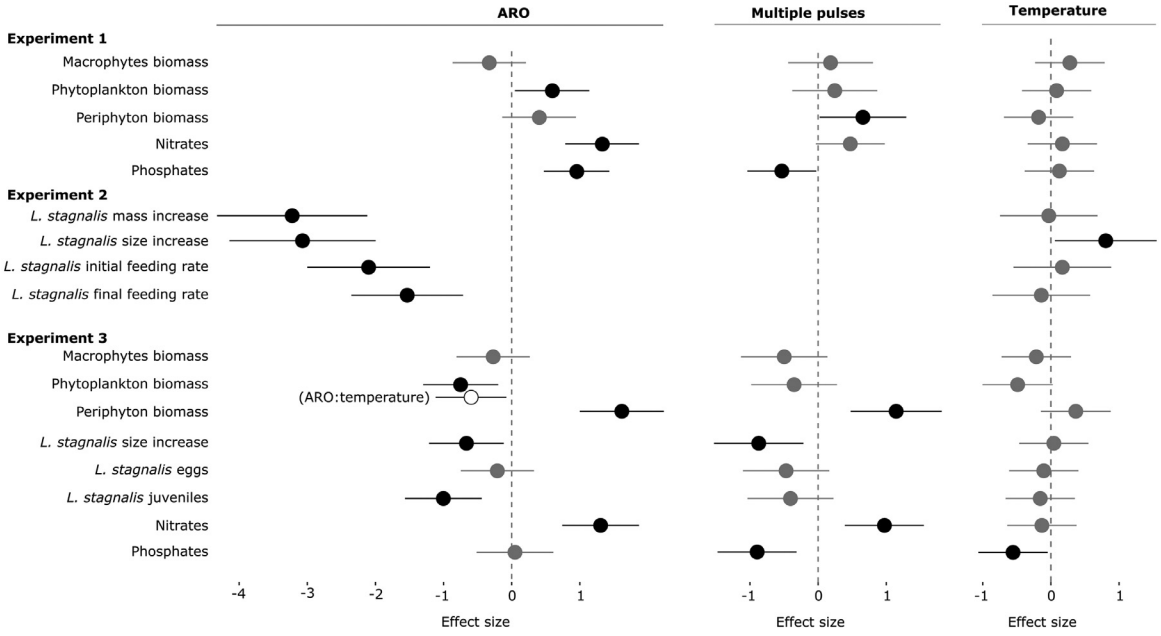

Fig. 1. Effect size (Hedges' g ± 95% confidence interval) on biomass or growth parameters in each experiment for the three factors tested: ARO total concentration, exposure mode (*i.e.* single vs. multiple pulses for a similar ARO concentration) and temperature. Open symbols show interaction effect when significant. Grey symbols: non-significant effects, black symbols: significant effects.

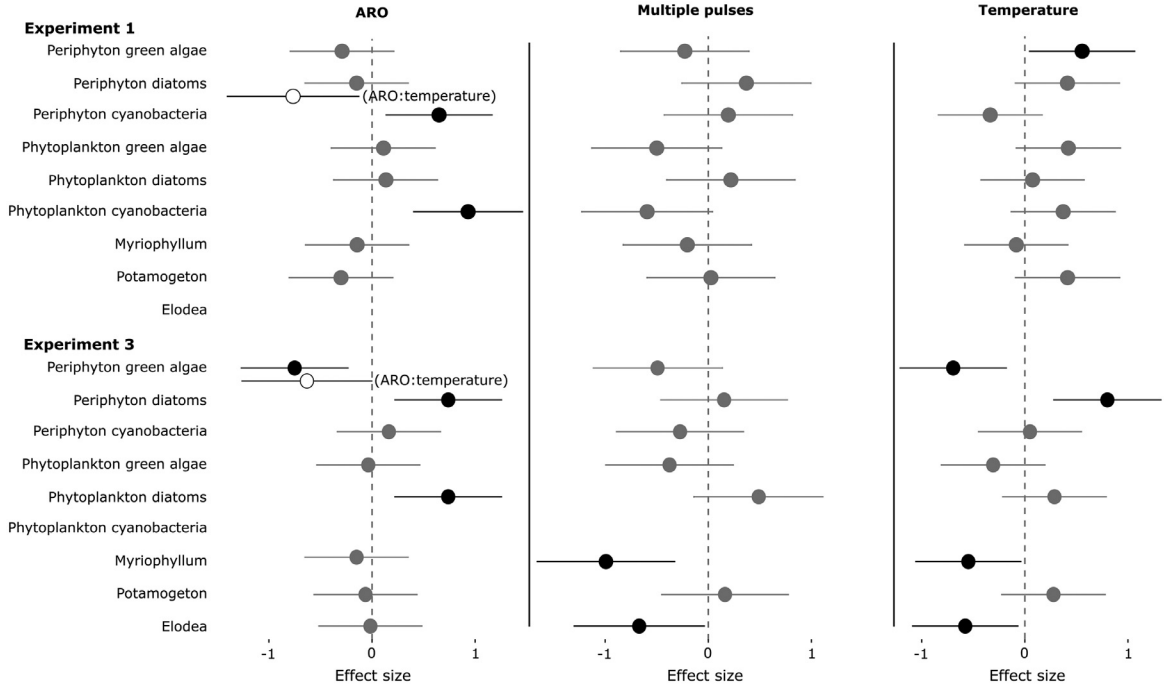

Fig. 2. Effect size (Hedges' g ± 95% confidence interval) on the composition of periphyton, phytoplankton and macrophyte communities for the three factors tested: ARO total concentration, exposure mode (*i.e.* single vs. multiple pulses for a similar ARO concentration) and temperature. Variations of green algae, diatom and cyanobacteria biomass was assessed by taxa specific pigment/Chl a ratio. Cyanobacteria were absent in phytoplankton. Macrophyte biomass was separated into *M. spicatum, P. perfoliatus* and *E. nuttallii* biomass. Open symbols show interaction effect when significant. Grey symbols: non-significant effects, black symbols: significant effects.

At the end of the experiment, concentrations of soluble reactive phosphate ($p < 0.001$) and nitrates ($p < 0.001$) were significantly higher in microcosm water with higher ARO additions. Repeated addition of ARO resulted in significantly lower dissolved phosphate concentrations compared to treatments with single ARO additions ($p = 0.04$), while only a marginal difference was recorded for nitrate concentrations ($p = 0.07$, Fig. 1).

3.2. Experiment 2: Effects of stressors on grazers

ARO exposure strongly reduced *L. stagnalis* growth with a significant effect on its increase in mass and length (Fig. 1). The snail mass increased by 603 ± 32 mg without ARO and by only 184 ± 44 mg at the highest ARO concentration. Warming positively influenced length increase but had no effect on snail mass (Fig. 1).

ARO induced a significant reduction ($p < 0.001$) of the consumption rates of *L. stagnalis* at the beginning of the exposure (from 8.14 ± 0.79 mg day^{-1} in the controls to 2.68 ± 0.62 mg day^{-1} in the 3X treatment). Similarly, after 3 weeks of exposure, consumption rates of *L. stagnalis* were 35% lower when exposed to the highest ARO dose (from 17.9 ± 1.9 mg day^{-1} in the control to 11.6 ± 2.4 mg day^{-1} at the 3X ARO concentration). No effect of warming on consumption rate was observed (Fig. 1).

3.3. Experiment 3: Effects of stressors on interacting primary producers and consumers

Macrophyte biomass did not respond to ARO addition (independent of the exposure mode) nor warming (Fig. 1). In this experiment, ARO concentration negatively affected phytoplankton biomass (Fig. 1), and these effects were stronger at the lower temperature indicating an interaction between ARO exposure and warming (Fig. 1). The highest phytoplankton biomasses were recorded in the cold control microcosms (cold: 84.8 ± 36.0 µg L^{-1} Chl *a*, warm: 45.0 ± 14.4 µg L^{-1} Chl *a*).

Conversely, ARO concentration caused a strong increase of periphyton biomass (Fig. 1). Periphyton biomass was very low in control conditions (0.22 ± 0.07 µg cm^{-2} Chl *a* and 0.38 ±0.19 µg cm^{-2} Chl *a* for cold and warm temperatures, respectively) but was significantly higher with ARO, especially when added as multiple pulses (Fig. 1). At the highest ARO concentration, periphyton biomass reached 2.90 ± 0.61 µg cm^{-2} Chl *a* (cold) and 3.51 ± 0.67 µg cm^{-2} Chl *a* () when ARO was added as multiple pulses (XXX), while it reached only 2.05 ± 0.60 µg Chl *a* cm^{-2} (in cold) and 2.11 ± 0.78 µg Chl *a* cm^{-2} (in warm) when ARO was added as a single dose (3X). ARO, and particularly exposure to multiple pulses, increased the P content of periphyton ($p < 0.001$ and $p = 0.019$). Warming did not significantly affect final periphyton biomass ($p = 0.48$).

D. magna neonates disappeared in all microcosms except six microcosms where *D. magna* population develop but also disappeared before the end of the experiment. ARO strongly affected the mussels, all of which died at the higher ARO concentration (independent of frequency of addition), while they survived in all control microcosms. No clear increase in mussel size was observed in any of the microcosms where they remained alive.

The snails survived in all the microcosms except one. A noticeable size increase (65.8 ± 35.4%) and the production of a high number of eggs (up to 489 eggs in one microcosm, but several microcosms with no visible eggs) were observed. Juvenile snails were also observed, principally in the controls (supplementary material). ARO strongly reduced the growth of *L. stagnalis* (Fig. 1). Multiple pulses led to a higher reduction of growth than a single addition of ARO (Fig. 1). Warming had no significant effect on snail growth or reproduction. The number of snail eggs was not affected by ARO treatments, but the number of juveniles was strongly reduced by ARO concentration independent of the exposure mode (Fig. 1). Damage due to snail feeding on *P. perfoliatus* was observed in 80% of the controls; in 44%, 20% and 30% of the microcosms in the 1X, 2X and 3X treatments; and in 0% of the microcosms in the multiple pulses (XX and XXX) treatments.

ARO and warming induced a decrease of the proportion of green algae and an increase of diatoms in the periphyton (Fig. 2). ARO also caused an increase of the diatom pigment in the phytoplankton. Warming negatively affected the growth of *M. spicatum* and *E. nuttallii* but not of *P. perfoliatus*. Repeated addition of ARO had a negative effect on macrophytes when compared with a single addition (Fig. 2).

Nitrate concentrations at the end of the experiment increased significantly with increasing ARO concentrations ($p < 0.001$). This effect was stronger in treatments with repeated ARO addition as compared to single additions ($p < 0.001$, Fig. 1). Concentrations of soluble reactive phosphate were lower in treatments with multiple pulses as compared to single additions. Warming led to significantly lower dissolved phosphate concentrations ($p = 0.02$, Fig. 1)

3.4. Structural equation modelling

A first SEM model was constructed including all possible relationships among measured variables and between the measured variables and experimental parameters (Table 2, supplementary material Fig. S6). We did not find a significant relationship between warming and any measured parameter in the SEM performed, and therefore removed warming effects in the model. In the resulting model (supplementary material Fig. S6), only periphyton biomass and snail growth were directly affected by ARO. To explore further the direct and indirect effects of ARO on macrophyte or phytoplankton dominance, we used a third model considering only the effect of periphyton on phytoplankton and of periphyton on macrophytes without the respective reciprocal effects (Fig. 3).

Our model showed a strong positive effect of ARO concentration on periphyton development not only directly (path = 0.59, $p < 0.001$; Fig. 3) but also indirectly through the negative effect of ARO on periphyton grazers (Fig. 3). Indeed, ARO decreased grazer growth, which in turn reduced grazer consumption of periphyton leading finally to an increased periphyton development (Fig. 3). We

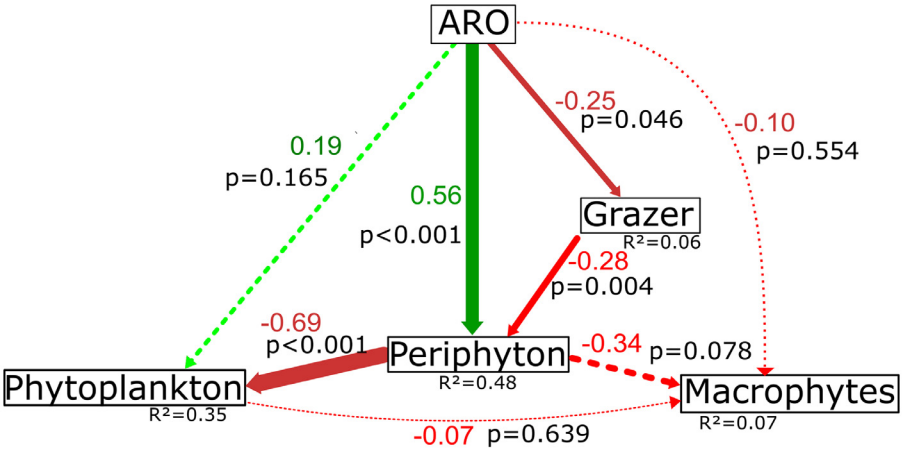

Fig. 3. Path diagram of the selected SEM model. Red arrows indicate negative relations between variables. Green arrows indicate positive relations between variables. Dashed arrows represent non-significant relations between variables. Significant threshold was set at $p = 0.05$. R^2 are the proportion of variation explained by the model for each response variable.

also found a strong negative effect of periphyton on phytoplankton development (path = -0.69, $p < 0.001$; Fig. 3). Therefore, phytoplankton development was indirectly affected by ARO through its effect on periphyton. Our model further indicated no significant relationship between macrophyte biomass and other parameters.

4. Discussion

The combination of experiments with one and two trophic levels allowed us to assess direct and indirect effects of two major environmental stressors, climate warming and agricultural run-off (ARO) on the functioning of shallow aquatic systems. Confirming our first hypothesis, we showed direct effects of ARO on primary producers and primary consumers. We further revealed the structuring role of periphyton and their grazers *(L. stagnalis)* in these systems often described only by the alternative dominance of submerged macrophytes or phytoplankton (Scheffer and van Nes, 2007; Scheffer et al., 1993). Repeated exposure to ARO, as can be expected for shallow aquatic systems in an agricultural landscape, enhanced these effects. In contrast to our expectations, warming by approx. 3.5°C did not enhance the negative effects, probably because our tested temperature increase did not affect primary producers directly. Our results underline the importance to increase ecological complexity when evaluating the effect of agricultural run-off including pesticides on aquatic systems, as single species tests would not be able to reveal all direct and indirect effects. In the following, we will detail how the results matched with our initial hypotheses.

4.1. Direct effects of ARO and warming on single trophic levels

To test our first hypothesis, experiments 1 and 2 were designed to determine the effect of the stressors without trophic interactions. We observed a positive effect of ARO on phytoplankton growth. This positive effect may be linked to the presence of nitrate in the ARO because it is consistent with what can be expected with nitrate addition in P-rich environments (Donald et al., 2011; Leavitt et al., 2006; Paerl and Scott, 2010). The low amount of remaining nitrate at the end of the experiment further supports a possible effect of nitrate. This increase in phytoplankton biomass did not lead to a strong negative competitive effect on macrophytes, as could be expected, and there was no shift to a typical phytoplankton-dominated state. However, such shifts can be expected under *in situ* conditions, when higher water depth and longer duration result in significant shading of macrophytes by phytoplankton.

The most remarkable direct effect of ARO was a strong inhibition of growth and feeding of the snail *L. stagnalis*. This is consistent with the known effect of pesticides and copper on this species (Amorim et al., 2019; Tufi et al., 2016). Indeed, Tufi et al. (2016) observed acetylcholinesterase inhibition on *L. stagnalis* exposed to 3.62 µg L^{-1} of pirimicarb. In our experiment, pirimicarb concentration ranged from 5 to 15 µg L^{-1}. Tebuconazole and copper are linked to embryotoxicity in terrestrial snails (*Helix aspersa*; Druart et al., 2012). The highest concentration of Cu used in our study (42 µg L^{-1}) is in the range of the estimated EC50 on juvenile *L. stagnalis* growth rate in a 28-day experiment (Vlaeminck et al., 2019). Nitrate cannot be responsible for the negative effect observed because the concentration in our experiment was more than 50-fold below the toxic concentration for any freshwater mollusc (Soucek and Dickinson, 2012).

The hypothesis that warming will promote phytoplankton dominance could not be confirmed. Warming did not affect primary producers nor snail feeding activity. This result is surprising because warming in the range of our experiments brings both chlorophytes and cyanobacteria closer to their optimum temperature (Lürling et al., 2013), and snail activity was expected to increase with warming in the tested range (Schaum et al., 2018; Zhang et al., 2018). However, a lack of warming effects on macrophytes was also reported in other studies, for instance, McKee et al., (2002) observed no temperature-induced change in the development of macrophytes in a 2-year mesocosm experiment with a 3°C warming above ambient temperature. Similarly, Feuchtmayr et al. (2019) found no effect on primary producers using a 4°C temperature elevation, in a 1-year experiment where temperature followed natural variations. Primary producers may have been driven more by nutrient limitation (although we regularly added nutrients) than by temperature. Indeed, an antagonistic interaction between warming and nutrient limitation have been observed in several studies (Birk et al., 2020; Richardson et al., 2019). Also, studies with an impact of temperature on grazers (Schaum et al., 2018; Zhang et al., 2018) used a higher temperature difference than our study.

4.2. Importance of indirect effects mediated by biotic interactions in a more complex system

In experiment 3, including both primary producers and grazers, the net effect of ARO on phytoplankton development was negative and thus opposite to the single trophic level experiment, probably due to the toxic effects of ARO on periphyton grazers that released periphyton from grazing pressure. By this mechanism, ARO had an indirect positive effect on periphyton, in addition to the direct positive effect of ARO on periphyton indicated by the SEM model. A strong negative relationship was also observed between periphyton and phytoplankton probably due to competition between the two functional groups for nutrients (more specifically for phosphorus because a decrease in phytoplankton biomass was observed while nitrate was added with the ARO).

The negative effect of ARO on *L. stagnalis* was reduced in experiment 3 (with primary producers and grazers), where the microcosms also included primary producers, compared to experiment 2 (grazers alone). Different factors may explain this reduced effect. Notably, the other organisms present may also have led to a reduced exposure of snails due to increased adsorption and degradation of toxicants. Periphyton also represents a higher quality food than lettuce, leading to a decrease in the sensitivity to toxicants (Fidder et al., 2018; Vlaeminck et al., 2019). Beside effects of food quality, the available food in terms of periphyton biomass in the control microcosms was very low. Snail growth may have been food limited, thus reducing the magnitude of the observed effect of ARO. This is supported by the fact that *L. stagnalis* grazing on *P. perfoliatus* was more frequently observed in the control microcosms than in treatments with higher periphyton biomass, indicating a limitation in the availability of periphyton that they preferentially graze. This effect of resource limitation on snail growth may have led to an underestimation of ARO effects on *L. stagnalis.*

This may have also resulted in an underestimation of the indirect effect of ARO on periphyton mediated by snail grazing as well as a lower direct effect than estimated with the SEM. Moreover, this is supported by the fact that ARO did not strongly affect periphyton in experiment 1 (primary producers only), so a strong direct effect is unlikely.

The inhibition of phytoplankton development by periphyton that we observed is consistent with other mesocosm studies that attributed this inhibition to allelopathic effects of filamentous algae (Trochine et al., 2011) or to nutrient competition (Kazanjian et al., 2018). Indeed, a higher periphyton biomass in ARO treatments retained more P, which was not available for phytoplankton development.

The role of periphyton and their grazers is not considered in classical models of alternative stable states in shallow eutrophic lakes (Scheffer and van Nes, 2007; Scheffer et al., 1993). Yet, shading effects of periphyton on macrophytes (Jones and Sayer, 2003; Phillips et al., 2016; Roberts et al., 2003) and positive effects of snail grazing on macrophytes are well known (Mormul et al., 2018). Our results contrasted with theory predicting the increase of phytoplankton biomass and the regression of macrophytes in shallow lakes as a result of eutrophication and warming (Kosten et al., 2012; Mooij et al., 2007; Moss et al., 2011; Scheffer and van Nes, 2007). However, they support modelling studies (Genkai-Kato et al., 2012; Jäger and Diehl, 2014) indicating that also periphyton can control the development of phytoplankton. This suggests a key role of periphyton and their grazers in macrophyte-phytoplankton interactions, as already outlined by Phillips et al. (1978), reinforced in Phillips et al. (2016). We therefore consider that periphyton should receive more attention among the steering factors controlling alternative stable states in shallow lakes.

We show here that while some stressors are expected leading to higher phytoplankton abundance due to direct effects, complex interactions can lead to completely different results. Generally, the monitoring of lake ecological quality is based on an indicator of eutrophication, including for example measurement of phytoplankton Chl *a* (Laplace-Treyture and Feret, 2016). The complex effect of ARO observed in this study can remain undetected by lake monitoring programs although the functioning of the system has been truly altered. Similarly, while different environmental parameters are used to predict the occurrence of cyanobacterial blooms (Zhao et al., 2019a), biotic interactions are not included. Such indirect effects must be take into account, and therefore multiple components of the ecosystems should be used for ecosystem health assessment (Zhao et al., 2019b), together with a good understanding of mechanistic pathways linking stressors to observed effects.

4.3. ARO arriving in multiple pulses increases effects

In our third hypothesis, we expected that exposure to multiple pulses, compared to a single addition of the same quantity of pollutants, would allow the system to remain below a critical threshold, thus allowing the organisms to acclimate and reducing the possibility of a shift towards the dominance by phytoplankton. The stronger negative effect of multiple pulses on snail growth might be due to a delayed recovery after multiple exposures. The positive effect on periphyton development is most probably an indirect effect caused via the direct negative effects on grazers. However, a similar nearly significant trend was observed in the first experiment. Therefore, the positive effect on biofilm might be, partially, a direct effect of ARO, particularly the recurrent nitrate. A few studies have considered single or repeated additions of toxicants. Pulses of herbicide have been suggested to modulate the impact on macrophytes and algae of a subsequent exposure, while it depends strongly on the ability of the organisms to recover from previous exposure (Vallotton et al., 2008, 2009; Varga et al., 2019). The stronger effect of the multiple-pulse exposure on the grazers and periphyton can be due to a rapid degradation of the pesticides or an interaction between pesticides or copper and compounds present in the microcosms (e.g. extracellular polymeric substances produced by biofilm) or changes in copper redox states (Coutaud et al., 2018) that limit the ARO effect. In an experiment with similar microcosms, using ARO concentrations equivalent to our 3X treatment but with only the primary producers present, 54% of terbuthylazine, 67% of pirimicarb and 88% of tebuconazole were recovered in the water after 20 days (data not shown). It is therefore important to consider that ARO input to lakes as multiple pulses can possibly increase the effect of agricultural run-off. Indeed, multiple pulses can better reflect the real contamination patterns of aquatic ecosystems (Daouk et al., 2013; Reinert et al., 2002).

5. Conclusion

Shallow lakes are complex ecosystems influenced by a strong benthic-pelagic coupling. They are considered in a good ecological status when dominated by submerged macrophytes, while dominance of phytoplankton or a massive development of periphytic algae are signs of degradation. A few studies have already addressed of the potential of eutrophication and/or climate warming for shifts between alternative stable states typical for these systems. Yet, our study is the first to investigate the impact of single or multiple pulses of agricultural run-off composed of organic and inorganic pesticides and nitrates in the context of climate warming. We tested the first two trophic levels, primary producers and primary consumers, and show that negative effects can foster either phytoplankton or periphyton development. Our findings underline the importance to go beyond simple laboratory systems using only single species or functional groups. Single species tests cannot account for the complexity in these systems due to multiple direct and indirect effects acting in parallel. Our series of microcosm experiments based on individual and combined trophic levels coupled with SEM allowed us to disentangle direct and indirect effects of two major global stressors, climate warming and agricultural run-off (ARO) on the development of macrophytes, phytoplankton and periphytic algae. We showed that direct positive effects of ARO on phytoplankton were completely reversed by indirect effects mediated by biotic interactions such as the competition between periphyton and phytoplankton or herbivory by grazers on periphyton. Effect of ARO on grazers such as *L. stagnalis* strongly affected the balance between functional groups of primary producers, and this effect was enhanced when ARO arrived in multiple pulses. Experiments with more complex systems allowing to take into account biotic interactions and different scenarios of stressor exposure are a more reliable basis for ecosystem assessment and management.

Declaration of Competing Interest

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

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Supplementary materials

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Influence du régime de perturbations sur la stabilité de communautés microbiennes aquatiques dans un contexte d'intermittence hydrique

Résumé : Le changement global et les modifications des régimes de perturbations qui en découlent impactent la stabilité des communautés. Les communautés microbiennes sont des acteurs majeurs du fonctionnement des écosystèmes. En rivière, les biofilms phototrophes participent activement au recyclage de la matière organique et assurent une part très significative voire principale de la production primaire. Identifier les facteurs qui influencent leur stabilité et analyser leur réponse aux multiples perturbations qui affectent les écosystèmes aquatiques est donc crucial. Ce travail de thèse vise ainsi à comprendre comment les propriétés des perturbations (nature, intensité, intervalle de temps entre perturbations successives…) ainsi que les caractéristiques des communautés et leur histoire influencent la stabilité des communautés microbiennes. Pour répondre à cette question, j'ai étudié la résistance et la résilience, structurelles et fonctionnelles, de biofilms phototrophes exposés à des perturbations associées à l'intermittence hydrique (lentification, assèchement, stress thermique, contamination) en manipulant la complexité des assemblages microbiens (souches algales, communautés synthétiques et naturelles) et la complexité des séquences de perturbations (évènements uniques ou séquentiels). Grâce à mes différentes expériences réalisées en laboratoire, j'ai démontré comment la diversité des communautés et leur histoire thermale augmentent la capacité des biofilms à résister à des stress brefs et intenses de température. J'ai aussi montré que la composition initiale des communautés algales conditionne la stabilité de la qualité nutritionnelle des biofilms après un assec. J'ai analysé les effets de l'histoire de l'intermittence hydrique d'une rivière sur la sensibilité des biofilms phototrophes à un contaminant agricole complexe. Enfin, mon travail a permis de démontrer que des perturbations identiques successives de même nature ont des effets combinés très différents (additifs, antagonistes ou synergiques) sur la résilience fonctionnelle des biofilms qui dépendent de l'intensité et de l'intervalle de temps entre les évènements.

Mots clés : Biofilms phototrophes, séquences de perturbations, stabilité, résistance, résilience, changement global

Influence of the disturbance regime on the stability of aquatic microbial communities in a context of hydric intermittency

Summary: Global changes and resulting changes in disturbance regimes threatened community stability. Microbial communities are major actors of ecosystem functioning. In rivers, phototrophic biofilms contribute significantly to the recycling of organic matter and are one of the most important primary producers. It is therefore crucial to identify the drivers of their stability and to analyse their response to the multiple disturbances aquatic ecosystems are exposed to. My thesis work aims at understanding how disturbance properties (nature, intensity, time interval…) and communities' features and their history influence the stability of microbial communities. To answer this question, I studied both structural and functional resistance and resilience of phototrophic biofilms exposed to disturbances associated with hydric intermittency (lentification, drying, thermal stress, contamination), by manipulating the complexity of microbial communities (isolated strains, synthetic or natural communities) and the complexity of disturbance sequences. Thanks to several laboratory experiments, I showed how the diversity and history of communities increase biofilm resistance to short heat stress. I also demonstrated that the initial algal composition of biofilms determines the stability of the biofilm nutritional quality after a dry period. I also analysed the effects of the history of hydric intermittency on the stability of phototrophic biofilms exposed to a complex contaminant mimicking an agricultural run-off. Finally, I was able to evidence that successions of identical disturbances had very different combined effects (additive, antagonistic or synergistic) on biofilm functional resilience according to the intensity and time interval between each event.

Keywords: Phototrophic biofilms, sequential disturbances, stability, resistance, resilience, global change