Determination of critical processes underlying the resilience of aquatic microbial communities under distinct resource availabilities

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Julio Alberto ALEGRE STELZER

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- Department F.-A Forel for Environmental and Aquatic Sciences & Institute for Environmental Sciences, University of Geneva, 66 Blvd. Carl Vogt, 1211 Geneva, Switzerland.
- Department of Ecosystem Research, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 301, 12587 Berlin, Germany.
- Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands

1st Reviewer:	Professor Dr. Rita Adrian
2nd Reviewer:	Professor Dr. Justyna Wolinska

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Live long and prosper!

Declaration of Independence

Herewith I certify that I have prepared and written my thesis independently and that I have not used any sources and aids other than those indicated by me.

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Affiliations:

Julio A. A. Stelzer [a, b, c, d]

Ian D. Jones [e]

Petra M. Visser [h]

Alena S. Gsell [d]

Jérôme Kasparian [f]

Rita Adrian [b], [c]

Bas W. Ibelings [a]

Jorrit P. Mesman [a,f,g]

Stéphane Goyette [f]

Daniel F. McGinnis [a]

Lisette N. de Senerpont Domis [d]

Vasilis Dakos [i]

[a] *Department F.A. Forel for Environmental and Aquatic Sciences and Institute for Environmental Sciences, University of Geneva, Geneva, Switzerland*

[b] *Department of Biology, Chemistry, and Pharmacy, Freie Universität Berlin, Berlin, Germany*

[c] *Department of Ecosystem Research, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*

[d] *Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands*

[e] *Faculty of Natural Sciences, Biological & Environmental Sciences, University of Stirling, Stirling, UK*

[f] *Group of Applied Physics and Institute for Environmental Sciences, University of Geneva, Geneva, Switzerland*

[g] *Department of Ecology and Genetics, Uppsala University, Uppsala, Sweden*

[h] *Department of Freshwater and Marine Ecology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94248, 1090 GE Amsterdam, The Netherlands*

[i] *Institut des Sciences de l'Evolution, University of Montpellier, Montpellier Cedex, France*

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Summary

Freshwater systems have always been under the most variate disturbance regimes. However, the number of multi-stressors and the frequency/intensity of stochastic *pulse perturbations* they will face in the future is predicted to increase. Stochastic *pulse perturbations* such as storms and floods are well known to temporarily disrupt ecosystem services that are fundamental for global existence within short time scales - as drinking water supply, irrigation, and food provision. Eutrophication - one of the main freshwater *press perturbations* deteriorating water quality - may enhance harmful cyanobacterial blooms, modify the structure of food webs, and alter long-term ecological stability, resulting in substantial ecological, social, and economic losses. Despite all our knowledge on the effects of stochastic events and eutrophication operating in isolation, we know little about how their interactions can shape the *resilience* of aquatic ecosystems.

The aim of this thesis is to identify general processes of recovery and response pathways taking place after mortality pulse perturbations and assess how *resource availability* can modify them. For that, the existing literature was reviewed, and experiments were executed using aquatic systems of different ecological complexity. Chapter 2 starts with a global review of how climate change may modify in-lake processes that are prone to escalate into *feedback loops* of relevance to freshwater biogeochemistry (i.e., mixing regimes modifying nutrient upwelling). Chapter 3 explored if *Early-Warning Signals of Regime Shift* (EWS) could produce sufficient mechanistic empirical evidence to be used as a reliable methodological framework for quantifying *Ecological Resilience*. For that, we reviewed the literature for aquatic experiments that have designed interactions between *pulse* and *press perturbations*. Using the lessons learned from both reviews, the experiment of Chapter 4 used an eco-evolutionary microcosm with complex intraspecific interactions to focus on methodological constraints of using *recovery rate* and *time* for addressing *engineering* and *ecological resilience*. Also, this experiment investigated the role of resource availability on the processes ruling the recovery from *pulse perturbations* at a community-alike level. Last, in chapter 5, ecological complexity was scaled up using mesocosms filled with natural lake water to identify key processes involving the effect of nutrient availability on the microbial community response to *pulse perturbations*. Here the focus was on how eutrophication changes the phytoplankton *resistance* and *resilience* to mortality events that cause internal nutrient turnover. Together, these four chapters provided the core of the thesis.

Overall, we identified that climate change can indeed create *feedback loops* capable of altering the nutrient dynamics in lakes, as well as the direction of change depending on the lake characteristics. We scrutinized processes that can lead to an increase of total nutrients in the epilimnion in the long

run while creating longer periods of dissolved nutrient limitation within a season. Those processes are related to In-lake mixing regimes, which are susceptible to atmospheric conditions and responsible for the internal mixing of nutrients in lakes. Therefore, the presence of *feedback loops* in lake dynamics might become increasingly relevant under ongoing climate change.

When reviewing the reliability of EWS to address the loss of *ecological resilience* in aquatic systems, we observed that the field is still at the stage of proof-of-concept. Complex experiments designed to assess the interaction between *pulse* and *press perturbations* were virtually absent. Most experiments considered either a *pulse* or a *press perturbation* affecting the system, and often without a mechanistic understanding of why an EWS was observed or not. Amongst all EWS, *recovery rate* or *time* were the most capable of bringing together how long-term changes in the system affect the response to stochastic pulse perturbations. *Recovery rates* showed reliable results when predicting population collapses; however, the translation of individual populational-level *recovery rates* to more complex community-based ecosystem dynamics is far from consolidated.

Moving on into process-based approaches, our experiments highlighted that the effects of *resource availability* can be decomposed into two distinguished processes of *recovery*. One is the capability of the system to fully recover from disturbance. Another is the *recovery* pattern that the system will embody after the pulse perturbation. In both experiments we observed the importance of biotic interaction for determining how the system responds to perturbations and the effect of resource availability on shaping the direction of those interactions. *Competition*, *facilitation*, *intrinsic growth rates*, population *turnover*, the complexity of ecological interactions, and rates of *energy transfer*, all played a role in the processes underlying *'resilience'* (*latu sensu*).

The eco-evolutionary microcosm taught us that *resource availability* modulates the speed of the recovery process (*rate* and *time*) but not necessarily the level of *recovery*. Systems with low *resource availability* showed weaker *recovery rates* and longer *recovery times* for both compositional and functional dimensions compared to when resources were high. However, all tested systems showed substantial plasticity in absorbing and recovering from a *pulse perturbation* (complete *recovery*). Therefore, the slower recovery process caused by low *resource availability* did not change “the measure of the persistence of the system” or “their ability to absorb change and disturbance and still maintain the same relationships between populations or state variables" – the formal definition of *ecological resilience* (Holling, 1973). When dealing with systems of different *resource availability*, *ecological* (*sensu* Holling) and *engineering resilience* (*sensu* Pimm) may potentially decouple in time. Therefore, we must be especially careful when using *recovery* from perturbation as a proxy of *ecological resilience* if *resource availability* is the main driver of ecosystem change.

When scaling up the *trophic complexity* to a mesocosms level, we observed that *pulse perturbations* increased the amount of autochthonous nutrients in the system, selecting for '*small and fast-growers*' at first. As more resources were allocated in the system before the *pulse perturbation*, more nutrients were released during the *mortality event*; and with more dissolved nutrients available for uptake, higher peaks of phytoplankton concentration were observed as a response to the *pulse perturbation*. However, the level of *resource availability* in the mesocosms did not change the *resistance* or the *recovery* from a mortality *pulse perturbation*. Together, the chlorophyll-a displacement declined after each sequential *pulse perturbation* despite increases in *resource availability*. The more perturbed the system was, the less responsive it became, and this can be interpreted as a possible community pathway towards *stability*. The mesocosms experiment indicated the capability of the system to adapt to sequential *pulse perturbations* in a way to reduce its impacts and that *resource availability* and sequential *pulse perturbations* are antagonistic forces shaping the response of the system to mortality events.

Both experiments executed in this thesis presented a convergent story. *Resource availability* did not change the capability of the systems to *recover* from *pulse perturbations*, despite changing their compositional structure and process rates. However, the evidence that *resource availability* is unlikely to influence the capability of systems to *recover* should not lead to underestimations of the risks related to changing process rates in ecological systems. Systems under low *resource availability* might become more vulnerable to changes in the frequency of pulse perturbations, while systems with high resource availability may present stronger responses immediately after the *pulse perturbation* (i.e., peaks of Chl-a concentrations). This is an important consideration when managing waterbodies with strict regulations (i.e., drinking water provision) since systems with a surplus of *resource availability* may more easily trespass regulatory guideline values (e.g., maximum Chl-a threshold levels for safe water supply) following a *pulse perturbation*, while oligotrophic systems may become especially vulnerable to changes in the frequency of sequential weather-related *pulse perturbations*.

Zusammenfassung

Süßwassersysteme waren schon immer den unterschiedlichsten Störungsregimen ausgesetzt. Es wird jedoch vorhergesagt, dass die Anzahl der Multi-Stressoren und die Häufigkeit/Intensität der stochastischen Impulsstörungen, denen sie in Zukunft ausgesetzt sein werden, zunehmen werden. Es ist bekannt, dass stochastische Impulsstörungen wie Stürme und Überschwemmungen Ökosystemdienstleistungen, die für die globale Existenz von grundlegender Bedeutung sind, innerhalb kurzer Zeitspannen vorübergehend stören können - z. B. Trinkwasserversorgung, Bewässerung und Nahrungsmittelversorgung. Eutrophierung - eine der wichtigsten Störungen der Süßwasserpresse, die die Wasserqualität verschlechtert - kann schädliche Cyanobakterienblüten fördern, die Struktur von Nahrungsnetzen verändern und die langfristige ökologische Stabilität beeinträchtigen, was zu erheblichen ökologischen, sozialen und wirtschaftlichen Verlusten führt. Trotz all unserer Kenntnisse über die Auswirkungen von stochastischen Ereignissen und Eutrophierung, die isoliert voneinander auftreten, wissen wir nur wenig darüber, wie ihre Wechselwirkungen die Widerstandsfähigkeit aquatischer Ökosysteme beeinflussen können.

Ziel dieser Arbeit ist es, allgemeine Erholungsprozesse und Reaktionswege zu identifizieren, die nach Störungen durch Mortalitätsimpulse ablaufen, und zu bewerten, wie die Ressourcenverfügbarkeit diese verändern kann. Zu diesem Zweck wurde die vorhandene Literatur gesichtet, und es wurden Experimente mit aquatischen Systemen unterschiedlicher ökologischer Komplexität durchgeführt. Kapitel 2 beginnt mit einem globalen Überblick darüber, wie der Klimawandel Prozesse im See verändern kann, die sich zu Rückkopplungsschleifen entwickeln können, die für die Biogeochemie des Süßwassers von Bedeutung sind (z. B. Mischungsregime, die den Nährstoffauftrieb verändern). In Kapitel 3 wurde untersucht, ob Frühwarnsignale für Regimeverschiebungen (Early-Warning Signals of Regime Shift, EWS) genügend mechanistische empirische Beweise liefern können, um als zuverlässiger methodischer Rahmen für die Quantifizierung der ökologischen Widerstandsfähigkeit verwendet werden zu können.

Zu diesem Zweck haben wir die Literatur zu aquatischen Experimenten durchgesehen, bei denen Wechselwirkungen zwischen Impuls- und Druckstörungen untersucht wurden. Auf der Grundlage der Erkenntnisse aus beiden Übersichten wurde im Experiment in Kapitel 4 ein ökoevolutionärer Mikrokosmos mit komplexen intraspezifischen Interaktionen verwendet, um die methodischen Einschränkungen bei der Verwendung von Erholungsrate und -zeit für die Untersuchung der technischen und ökologischen Belastbarkeit zu untersuchen. Außerdem untersuchte dieses Experiment die Rolle der Ressourcenverfügbarkeit für die Prozesse, die die Erholung von

Impulsstörungen auf einer gemeinschaftsähnlichen Ebene bestimmen. In Kapitel 5 schließlich wurde die ökologische Komplexität mit Hilfe von Mesokosmen, die mit natürlichem Seewasser gefüllt waren, erhöht, um die Schlüsselprozesse zu identifizieren, die die Auswirkungen der Nährstoffverfügbarkeit auf die Reaktion der mikrobiellen Gemeinschaft auf Impuls-Störungen betreffen. Hier lag der Schwerpunkt darauf, wie die Eutrophierung die Resistenz und Widerstandsfähigkeit des Phytoplanktons gegenüber Mortalitätsereignissen verändert, die einen internen Nährstoffumsatz verursachen. Zusammen bilden diese vier Kapitel den Kern der Arbeit.

Insgesamt haben wir festgestellt, dass der Klimawandel in der Tat Rückkopplungsschleifen schaffen kann, die die Nährstoffdynamik in Seen verändern können, und dass die Richtung der Veränderung von den Eigenschaften des Sees abhängt. Wir haben Prozesse untersucht, die langfristig zu einem Anstieg der Gesamtnährstoffe im Epilimnion führen können und gleichzeitig längere Perioden der Begrenzung gelöster Nährstoffe innerhalb einer Saison verursachen. Diese Prozesse hängen mit dem Durchmischungsregime im See zusammen, das von den atmosphärischen Bedingungen abhängt und für die interne Durchmischung von Nährstoffen in Seen verantwortlich ist. Daher könnte das Vorhandensein von Rückkopplungsschleifen in der Seedynamik bei fortschreitendem Klimawandel zunehmend an Bedeutung gewinnen.

Bei der Überprüfung der Verlässlichkeit von EWS im Hinblick auf den Verlust der ökologischen Widerstandsfähigkeit aquatischer Systeme haben wir festgestellt, dass sich das Feld noch im Stadium des Proof-of-Concept befindet. Komplexe Experimente zur Bewertung der Wechselwirkung zwischen Impuls- und Pressestörungen waren praktisch nicht vorhanden. In den meisten Experimenten wurde entweder eine Impuls- oder eine Druckstörung untersucht, die das System beeinflusste, oft ohne ein mechanistisches Verständnis dafür, warum ein EWS beobachtet wurde oder nicht. Von allen EWS waren die Erholungsrate oder die Zeit am ehesten in der Lage zu erfassen, wie sich langfristige Veränderungen im System auf die Reaktion auf stochastische *Impulsstörungen* auswirken. Erholungsraten zeigten verlässliche Ergebnisse bei der Vorhersage von Populationszusammenbrüchen; die Übertragung von individuellen Erholungsraten auf Populationsebene auf komplexere gemeinschaftsbasierte Ökosystemdynamiken ist jedoch noch lange nicht gefestigt.

Unsere Experimente haben gezeigt, dass die Auswirkungen der Ressourcenverfügbarkeit in zwei unterschiedliche Erholungsprozesse zerlegt werden können. Der eine ist die Fähigkeit des Systems, sich vollständig von einer Störung zu erholen. Der andere ist das Erholungsmuster, das das System nach der Impulsstörung verkörpern wird. In beiden Experimenten konnten wir beobachten, wie wichtig biotische Interaktionen sind, um zu bestimmen, wie das System auf Störungen reagiert, und wie sich die Verfügbarkeit von Ressourcen auf die Richtung dieser Interaktionen auswirkt. Konkurrenz, Erleichterung, intrinsische Wachstumsraten, Populationsumsatz, die Komplexität ökologischer Interaktionen und die Energieübertragungsraten spielten alle eine Rolle bei den Prozessen, die der "Resilienz" (latu sensu) zugrunde liegen.

Der ökoevolutionäre Mikrokosmos lehrte uns, dass die Ressourcenverfügbarkeit die Geschwindigkeit des Erholungsprozesses (Geschwindigkeit und Zeit), aber nicht unbedingt das Ausmaß der Erholung beeinflusst. Systeme mit geringer Ressourcenverfügbarkeit wiesen sowohl bei der Zusammensetzung als auch bei den funktionalen Dimensionen geringere Erholungsraten und längere Erholungszeiten auf als Systeme mit hoher Ressourcenverfügbarkeit. Alle getesteten Systeme zeigten jedoch eine beträchtliche Plastizität bei der Absorption und Erholung von einer Impulsstörung (vollständige Erholung). Der langsamere Erholungsprozess, der durch die geringe Ressourcenverfügbarkeit verursacht wurde, änderte daher nicht "das Maß für die Persistenz des Systems" oder "seine Fähigkeit, Veränderungen und Störungen zu absorbieren und dennoch die gleichen Beziehungen zwischen Populationen oder Zustandsvariablen aufrechtzuerhalten" - die formale Definition der ökologischen Resilienz (Holling, 1973). Bei Systemen mit unterschiedlicher Ressourcenverfügbarkeit können sich ökologische (im Sinne von Holling) und technische Belastbarkeit (im Sinne von Pimm) möglicherweise zeitlich entkoppeln. Daher müssen wir besonders vorsichtig sein, wenn wir die Erholung von Störungen als Indikator für die ökologische Resilienz verwenden, wenn die Ressourcenverfügbarkeit die Haupttriebkraft für Veränderungen im Ökosystem ist.

Beim Hochskalieren der trophischen Komplexität auf die Ebene der Mesokosmen konnten wir beobachten, dass die *Impulsstörungen* die Menge an autochthonen Nährstoffen im System erhöhten, wobei zunächst kleine und schnell wachsende Arten bevorzugt wurden. Da vor der Impulsstörung mehr Ressourcen im System vorhanden waren, wurden während des Mortalitätsereignisses mehr Nährstoffe freigesetzt; und da mehr gelöste Nährstoffe zur Aufnahme zur Verfügung standen, wurden als Reaktion auf die Impulsstörung höhere Spitzenwerte der Phytoplanktonkonzentration beobachtet. Das Niveau der Ressourcenverfügbarkeit in den Mesokosmen änderte jedoch nichts an der Widerstandsfähigkeit oder der Erholung von einem Mortalitätsimpuls. Insgesamt nahm die Chlorophyll-a-Verschiebung nach jeder aufeinanderfolgenden Impulsstörung ab, obwohl die Verfügbarkeit der Ressourcen zunahm. Je stärker das System gestört wurde, desto weniger reagierte es, was als möglicher Weg der Gemeinschaft zur Stabilität gedeutet werden kann. Das Mesokosmen-Experiment zeigte, dass das System in der Lage ist, sich an sequenzielle *Impulsstörungen* so anzupassen, dass die Auswirkungen reduziert werden, und dass Ressourcenverfügbarkeit und sequenzielle *Impulsstörungen* antagonistische Kräfte sind, die die Reaktion des Systems auf Mortalitätsereignisse bestimmen.

Beide Experimente, die im Rahmen dieser Arbeit durchgeführt wurden, zeigten eine konvergente Geschichte. Die Ressourcenverfügbarkeit änderte nichts an der Fähigkeit der Systeme, sich von *Impulsstörungen* zu erholen, obwohl sich ihre Zusammensetzungsstruktur und Prozessraten änderten. Der Nachweis, dass die Ressourcenverfügbarkeit keinen Einfluss auf die Erholungsfähigkeit von Systemen hat, sollte jedoch nicht dazu führen, dass die Risiken im Zusammenhang mit veränderten Prozessraten in ökologischen Systemen unterschätzt werden. Systeme mit geringer Ressourcenverfügbarkeit könnten anfälliger für Änderungen der Häufigkeit von *Impulsstörungen* werden, während Systeme mit hoher Ressourcenverfügbarkeit unmittelbar nach der Impulsstörung stärkere Reaktionen zeigen können (z. B. Spitzenwerte der Chl-a-Konzentration). Dies ist eine wichtige Überlegung bei der Bewirtschaftung von Gewässern mit strengen Vorschriften (z. B. bei der Trinkwasserversorgung), da Systeme mit einem Überschuss an Ressourcenverfügbarkeit nach einer Impulsstörung leichter die vorgeschriebenen Richtwerte (z. B. maximale Chl-a-Grenzwerte für die sichere Wasserversorgung) überschreiten können, während oligotrophe Systeme besonders anfällig für Änderungen der Häufigkeit aufeinander folgender wetterbedingter *Impulsstörungen* sein können.

Résumé

Les systèmes d'eau douce ont toujours été soumis à des régimes de *perturbations* les plus variés. Cependant, le nombre de facteurs de stress multiples et la fréquence et l'intensité des *perturbations* sous forme *d'impulsion* stochastique (*stochastic pulse perturbations*) auxquelles ils seront confrontés à l'avenir devraient augmenter. Les *perturbations d'impulsion* stochastiques telles que les tempêtes et les inondations sont bien connues pour perturber temporairement les services écosystémiques qui sont fondamentaux pour la vie de l'Humanité mondiale dans des délais courts - comme l'approvisionnement en eau potable, l'irrigation et la production alimentaire. L'eutrophisation - l'une des principales *perturbations continue* (*press perturbation*) d'une masse d'eau douce qui détériore la qualité de l'eau - peut favoriser les proliférations de cyanobactéries nocives, modifiant la structure des réseaux trophiques, et altérer la stabilité écologique à long terme, entraînant des pertes écologiques, sociales et économiques substantielles. Malgré toutes nos connaissances sur les effets des événements stochastiques et de l'eutrophisation opérant de manière isolée, nous avons peu de connaissances sur la façon dont leurs interactions peuvent façonner la *résilience* des écosystèmes aquatiques.

L'objectif de cette thèse est d'identifier les processus généraux de rétablissement et les réponses adéquates à mettre en place après des *perturbations d'impulsion* pour mortalité et d'évaluer comment la *disponibilité des ressources* (*resource availability*) peut les modifier. Pour cela, la littérature existante a été revue et des expériences ont été réalisées en utilisant des systèmes aquatiques de complexité écologique différente. Le chapitre 2 commence par un examen complet de la façon dont le changement climatique peut impacter les processus lacustres susceptibles de modifier la composition biogéochimique de l'eau (c'est-à-dire les régimes de mélange modifiant la remontée des nutriments). Le chapitre 3 explore si les *signaux d'alerte précoce du changement de régime* (*early-warning signals of regime shift EWS*) pourraient produire des preuves empiriques mécanistiques suffisantes pour être utilisées comme cadre méthodologique fiable pour quantifier la *résilience écologique* (*Ecological Resilience*). Pour cela, nous avons passé en revue la littérature sur les expériences aquatiques qui ont conçu des interactions entre les *perturbations d'impulsion* et *continue*. En utilisant les leçons tirées des deux revues, l'expérience du chapitre 4 a utilisé un microcosme éco-évolutif avec des interactions intra spécimens complexes pour se focaliser sur les contraintes méthodologiques d'utilisation du *taux de récupération* (*recovery rate*) et du *temps de récupération* (*recovery time*) pour aborder *l'ingénierie* et la *résilience écologiques* (*engineering and ecological resilience*). En outre, cette expérience a étudié le rôle de la *disponibilité des ressources* sur les

processus gouvernant le rétablissement des communautés après des *perturbations d'impulsion*. Enfin, dans le chapitre 5, la complexité écologique a été mise à l'épreuve en utilisant des *mésocosmes* remplis d'eau de lac naturel pour identifier les processus clés impliquant l'effet de la *disponibilité des nutriments* sur la réponse de la communauté microbienne aux *perturbations d'impulsion*. Ici, l'accent a été mis sur la manière dont l'eutrophisation modifie la résistance et la *résilience* du phytoplancton aux événements de mortalité qui provoquent le renouvellement interne des nutriments. L'ensemble de ces quatre chapitres représente le cœur de ma thèse.

Dans l'ensemble, nous avons identifié que le changement climatique peut en effet créer des *boucles de rétroaction* (*feedback loops*) capables de modifier la dynamique des éléments nutritifs dans les lacs, ainsi que la direction du changement en fonction des caractéristiques de ces derniers. Nous avons examiné les processus qui peuvent conduire à une augmentation des nutriments totaux dans l'épilimnion à long terme, tout en créant des périodes plus longues de limitation des nutriments dissous au cours d'une saison. Ces processus sont liés aux régimes de mélange dans le lac, qui sont sensibles aux conditions atmosphériques et responsables du mélange interne des nutriments dans les lacs. Par conséquent, la présence de ces *boucles de rétroaction* dans la dynamique des lacs pourrait devenir de plus en plus pertinente dans le cadre du changement climatique en cours.

Lors de la revue de la fiabilité des EWS pour faire face à la perte de *résilience écologique* dans les systèmes aquatiques, nous avons observé que le domaine est toujours au stade de "preuve de concept" ("*proof of concept*"). Des expériences complexes conçues pour évaluer l'interaction entre les *impulsions* et les *perturbations continue* étaient pratiquement absentes. La plupart des expériences ont considéré soit une *impulsion*, soit une *perturbation* continue affectant le système, et souvent sans une compréhension mécanistique de la raison pour laquelle un signal précoce a été observé ou non. Parmi tous les EWS, le taux de récupération ou le *temps de récupération* étaient les plus à même de montrer comment les changements à long terme du système affectent la réponse aux *perturbations* l'*impulsion* stochastiques. Les *taux de récupération* ont montré des résultats fiables lors de la prévision des effondrements de population ; cependant, la traduction des *taux de récupération* individuels au niveau de la population en dynamiques écosystémiques communautaires plus complexes est loin d'être établie.

Passant aux approches basées sur les processus, nos expériences ont mis en évidence que les effets de la *disponibilité des ressources* peuvent être décomposés en deux processus distincts. Le premier processus est responsable de la capacité du système à se remettre complètement d'une *perturbation*. Et le second processus est le modèle de récupération que le système incarnera après la *perturbation* de l'*impulsion*. Dans les deux expériences, nous avons observé l'importance de l'interaction biotique pour déterminer comment le système répond aux *perturbations* mais également sur l'effet de la *disponibilité des ressources* sur la direction de ces interactions. La concurrence (*competition*), la facilitation (*facilitation*), les taux de croissance intrinsèques (*intrinsic growth rates*), le renouvellement de la population (*population turnover*), la complexité des interactions écologiques et les taux de transfert d'énergie, ont tous joué un rôle dans les processus sous-jacents à la « *résilience* » (*sensu lato*).

Le microcosme éco-évolutif nous a appris que la *disponibilité des ressources* module la vitesse du processus de récupération (le taux et la temporalité) mais pas nécessairement le niveau de récupération. Comparé aux systèmes avec une disponibilité des ressources élevée, les systèmes avec une faible *disponibilité des ressources* ont montré des *taux de récupération* plus faibles et des *temps de récupération* plus longs pour les dimensions compositionnelles et fonctionnelles. Cependant, tous les systèmes testés ont montré une plasticité substantielle dans l'absorption et la récupération d'une *perturbation d'impulsion* (récupération complète). Par conséquent, le processus de récupération plus lent causé par la faible *disponibilité des ressources* n'a pas changé « *la mesure de la persistance du système* » ou « *leur capacité à absorber les changements et les perturbations et à maintenir les mêmes relations entre les populations ou les variables d'état* » - la définition formelle de la *résilience écologique* (Holling, 1973).

Lorsqu'il s'agit de systèmes de *disponibilité de ressources* différentes, la *résilience écologique* (*sensu* Holling) et la *résilience de l'ingénierie* (sensu Pimm) peuvent potentiellement se découpler dans le temps. Par conséquent, nous devons être particulièrement prudents lorsque nous utilisons le rétablissement après une *perturbation* comme indicateur de la *résilience écologique* si la *disponibilité des ressources* est le principal moteur du changement de l'écosystème.

Lorsque l'on augmente la complexité trophique au niveau des *mésocosmes*, nous avons observé que les *perturbations d'impulsion* augmentaient la quantité de nutriments autochtones dans le système, en sélectionnant d'abord les « *small and fast-growers* ». Comme plus de ressources étaient allouées dans le système avant la *perturbation d'impulsion*, plus de nutriments étaient libérés pendant l'événement de mortalité ; et avec plus de nutriments dissous disponibles pour l'absorption, des pics plus élevés de concentration de phytoplancton ont été observés en réponse à la *perturbation d'impulsion*. Cependant, le niveau de *disponibilité des ressources* dans les *mésocosmes* n'a pas modifié la résistance ou la récupération après une *perturbation d'impulsion* de mortalité. Globalement, le déplacement de la chlorophylle-a a diminué après chaque *perturbation d'impulsion* séquentielle malgré l'augmentation de la *disponibilité des ressources*. Plus le système était perturbé, moins il devenait réactif, ce qui peut être interprété comme une voie communautaire possible vers la stabilité.

L'expérience de *mésocosmes* a indiqué que la capacité du système à s'adapter aux *perturbations d'impulsion* séquentielles de manière à réduire ses impacts, et que la *disponibilité des ressources* et les *perturbations d'impulsion* séquentielles sont des forces antagonistes qui façonnent la réponse du système aux événements de mortalité.

Les deux expériences exécutées dans cette thèse ont présenté un résultat convergent. La *disponibilité des ressources* n'a pas modifié la capacité des systèmes à récupérer des *perturbations d'impulsion*, malgré la modification de leur structure de composition et de leurs taux de traitement. Cependant, la preuve que la *disponibilité des ressources* est peu susceptible d'influencer la capacité des systèmes à se rétablir ne devrait pas conduire à une sous-estimation des risques liés à l'évolution des taux de processus dans les systèmes écologiques. Les systèmes à faible disponibilité de ressources pourraient devenir plus vulnérables aux changements de fréquence des *perturbations d'impulsion*, tandis que les systèmes à haute disponibilité de ressources peuvent présenter des réponses plus fortes immédiatement après la *perturbation d'impulsion* (c'est-à-dire des pics de concentrations de Chl-a). Il s'agit d'une considération importante lors de la gestion des plans d'eau soumis à des réglementations strictes (c'est à dire l'approvisionnement en eau potable), car les systèmes disposant d'un surplus de ressources disponibles peuvent plus facilement dépasser les valeurs indicatives réglementaires (par exemple, les niveaux de seuil maximal de Chl-a pour l'approvisionnement en eau potable) suite à une *perturbation par impulsion*, tandis que les systèmes oligotrophes peuvent devenir particulièrement vulnérables aux changements de fréquence des perturbation par impulsion séquentielles liées aux conditions météorologiques.

Chapter 1

General Introduction

Significance Statement

A centenary question among ecologists is how species can co-exist under the same resource availability and what implications it has on *ecological stability*. Resource availability has been broadly studied from the perspective of maintenance of biodiversity (Tilman, 1982), productivity of ecosystems (Cardinale et al., 2006), and ecosystem functioning (Hooper et al., 2005), but not so often studied from the perspective of perturbation ecology; in special for aquatic systems. In an era of raising pervasive ecological disturbances, there is a pressing need to understand the factors constituting community response to perturbations and *resilience* dynamics. Aquatic systems are currently being pressed by a multitude of environmental stressors as mean temperature rising (Korell et al., 2020), acidification (Dudgeon et al., 2006), brownification (Williamson et al., 2015, Kritzberg and Ekström, 2012), eutrophication (Downing, 2014, World Water Assessment Programme, 2009, Carr and Neary, 2008), species invasion (Dudgeon et al., 2006), and habitat suppression (Kraemer et al., 2021), making them one of the most vulnerable ecosystems in the current era. Aquatic multi-stressors *per see* can create temporary disfunction of ecosystem services by triggering harmful cyanobacterial blooms (Huisman et al., 2018, Paerl and Huisman, 2009), reducing *ecological stability* (Rosset et al., 2014, Chapin et al., 2000), changing the structure (Scheffer et al., 1993) and physicochemical characteristics (Kraemer et al., 2021) of the waterbody resulting in substantial socio-economic losses (Dodds et al., 2009, Hoagland et al., 2002) and ecological debt (Figueiredo et al., 2019, Moyle and Leidy, 1992).

Concomitantly to those pressures, the frequency and intensity of natural stochastic disturbances are predicted to increase in the contemporary world (Woolway et al., 2021, Bell et al., 2018). Stochastic *pulse perturbation*s as storms and floods are well known to temporarily disrupt many ecosystem services that are fundamental for global existence in short time scales - as drinking water supply, food provision, and use for irrigation (Ummenhofer and Meehl, 2017, Khan et al., 2015, Cann et al., 2013, WHO, 2011). Unfortunately, being hit by a stochastic perturbation is unavoidable in most cases. Hence, there is an increasing need to understand if pressured systems would have sufficient potential to resist and recover from stronger and/or more frequent *pulse perturbations*. Unrevealing the processes ruling short-term responses to *pulse perturbations* is a straightforward way to assess the consequences of long-term environmental pressures and an unavoidable task if we intend to minimize disruption of services throughout adaptive management plans in a changing world.

A detailed understanding of disturbance responses needs to go beyond associations (e.g., correlations and deterministic statistical tools) and incorporate features of the disturbances and community traits capable of mechanistically explaining the processes involved in *resilience*, *resistance*, and *stability*

(Cairns et al., 2020). Recent works have demonstrated the prevalence of biotic drivers over abiotic ones on the dynamics of ecosystem *stability* (Valencia et al., 2020, Isbell et al., 2013) and also the capability of ecosystems to internally absorb perturbations among their biological network, reducing the net effect of those disturbances (Brown et al., 2016, Connell and Ghedini, 2015). Understanding how the effects of perturbations percolate through the ecosystem is vital to understanding the risks and benefits of human-driven control efforts in restoring and conserving populations and communities (Cairns et al., 2020). Although freshwater systems have always been under the most variate disturbance regimes, the escalation in the number of multi-stressors and frequency-intensity of stochastic *pulse perturbations* that they will face in the future highlights water security as a fundamental contemporary problem to be tackled.

Perturbation Ecology and The Yellow Brick Road for Risk Assessment in a Changing World

Perturbation ecology is the branch of ecology focused on how an exogenous event can modify the current course of an ecosystem, assessing how the system was before, during, and after the event happened. Perturbation ecology may focus on the immediate effect of a *pulse perturbation* (i.e., changes in bioturbidity after a storm event) or the long-term consequences of it (i.e., macrophyte uprooting with consequent phytoplankton dominance – see Scheffer and van Nes (2007)). Perturbation ecology may also focus on how the combined effect of sequential *pulse perturbations* can potentially drift a system towards an alternative ecological pathway (succession) - the legacy effect (Jacquet and Altermatt, 2020, Cuddington, 2011); or how long-lasting changes in environmental pressures can modify the functioning of the system.

Perturbations are essentially categorized into two groups according to their dynamics in the system (Bender et al., 1984). A *pulse perturbation* is a relatively instantaneous alteration of the physicochemical or biotic elements of the system that dissipates in the short term (Figure 1, left), allowing the system to go back to its pre-perturbation state if the main features of the system are preserved (e.g., species composition, habitat structure). Common examples are storms, wildfires, flash floods, and epidemics. A *press perturbation*, on the other hand, is a sustained alteration that does not dissipate or leave the system, forcing the system to adapt in a way to accommodate it (Figure 1, right). As a consequence of *press perturbations*, the system assumes a slightly different equilibrium (which can, in fact, be a dynamic equilibrium). This is the case of most environmental stressors that are continuous over time as the increase in global surface temperature, habitat suppression, and changes in trophic state. Due to its inherent capability of directing the system over time, ecologists often label *press perturbations latu sensu* as `driving forces`.

Moreover, pulse and *press perturbations* substantially differ in the biotic responses they induce in the system (Table 1). *Pulse perturbations*, except in rare cases, are only identifiable if they can force the system to leave its current equilibrium. The exception is when the observer is aware of the existence of a *pulse perturbation*, but its effect is unidentifiable in the system (e.g., a light wind hitting a stratified lake without inducing mixing in the water column) – a situation that opens room for philosophical debates over the actual existence of a 'perturbation'. *Pulse perturbations* will necessarily change the behavior of the system for a brief and often transient period, however, without necessarily changing the long-term trends of the system. A didactic example is a population in exponential growth exposed to a stochastic mortality event. The mortality event might delay the population reaching its carrying capacity, but only in exceptional cases this mortality event *per see* will stop or modify the exponential growth itself. Therefore, due to its inherent behavior, all *pulse perturbations* can be pragmatically identified by a displacement followed by a recovery period; which can happen to its full or not depending on the nature and magnitude of the perturbation (Hillebrand and Kunze, 2020) and the current '*resilience*' level of the community (Scheffer et al., 2012).

The *press perturbations*, on the other hand, may not necessarily bring the system out of equilibrium but will fundamentally change the equilibrium of the system over time (Graham et al., 2021). A simple analogy to explain it compares the effect of aging to intense physical activity on the metabolic stress of a given organism. After intense physical activity, the hypothetical organism may feel exhausted and with low reserves of carbohydrates in its body - an unpaired situation compared to its normal condition. Briefly after the event, the organism experiments the highest levels of metabolic stress, which rapidly reduces with rest until leveling up with the pre-activity condition. An organism can have multiple moments of intense physical activity within a lifetime, which itself fades away due to aging. Aging, on the other hand, continuously increases the levels of

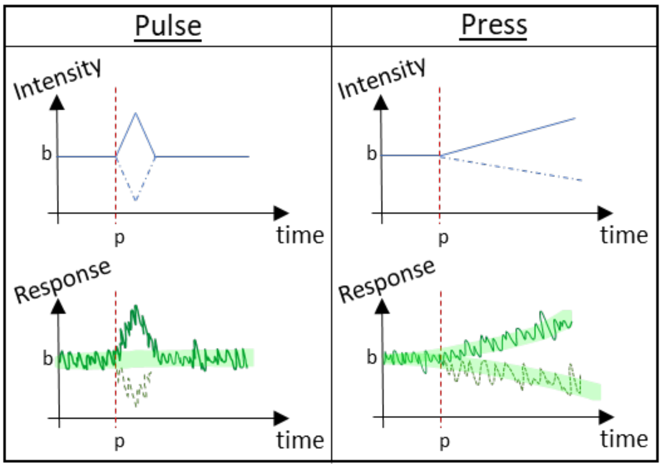

Figure 1. Inherent differences between pulse and press perturbations regarding the nature of the perturbation and the responses it causes in the system. The systems are initially at their baseline level 'b' for a given perturbation intensity (e.g., wind speed) and measurable response variable (e.g., Chl-a concentration). At the moment 'p', the intensity of the perturbation changes, inducing a response of the system. In a pulse type perturbation, the system tends to temporarily leaves its equilibrium (shaded green) before returning to it. In a press perturbation, the system tends to modify the equilibrium over time.

basal metabolic stress (under rest conditions) in the body. For instance, the basal metabolic stress of a child will be lower than an old individual, even without any intense physical activity taking place. Aging permanently changes the baseline of the system, while intense physical activity temporarily moves the system out of its baseline. In this example, physical stress is the *pulse perturbation*, and aging is the press. The same rationale applies to *press perturbations* at the environmental level. With a *press perturbation*, the system may never leave its baseline state – unless hit by a *pulse perturbation* – but the baseline is prone to change over time. Therefore, it is fundamentally important to understand that the processes involved in response to pulse and *press perturbation*s are distinct, and therefore, demand different strategies to be observed, analyzed, and quantified (Stelzer et al., 2021, Arnoldi et al., 2018, Hastings, 2010, Hastings, 2004). These two types of perturbations are also intimately connected with the most used '*Resilience' frameworks* discussed in this thesis and should be used as theoretical guidance for reasoning the ecological outcomes further described here.

Table 1. Comparison between different types of perturbation. O=may or may not.

	Pulse	Press
Temporal aspect (perturbation)	Short-term	Long-term
Perturbation leaves the system	Yes	No
System leaves equilibrium	Yes	O
System changes equilibrium	O	Yes
Response of the system	Displacement and recovery from perturbation (partial or full)	Changes in the baseline over time (modification of the functional aspect of the system)
Timeframe of the response	Short- to long-term	Long-term
Examples	Storms, wildfires, mortality events	Mean global temperature, habitat suppression, eutrophication

Obviously, as in many other ecological fields, the categorization of perturbations between press and pulse is a mere essentialism to convey a comprehensive message and insight process-based argumentations. The fact is that in nature, it might be hard to distinguish what is a press and a *pulse perturbation* for different reasons. For instance, the temporal aspect of press and *pulse perturbations* is species-dependent. A pulse for one species (e.g., fish) might be a press for another (e.g., phytoplankton). Therefore, the definition of press and *pulse perturbations* should be applied according to the characteristics of the studied system ('*resilience*' of what? to what? and compared to when?). Moreover, press and *pulse perturbations* can hardly be defined as unifactorial design in nature. For instance, a storm *pulse perturbation* might be decomposed into multiple components, including water mixing, dilution, sediment resuspension, nutrient upwelling, and superficial runoff. Each component could be classified as an individual perturbation and have its own singular intensity and duration. Therefore, perturbations are themselves a combination of multiple stressors often labeled under the umbrella of the same causing agent. Also, press and *pulse perturbations* will occur simultaneously in nature (at the same time and space), sometimes making it impossible to disentangle their effects without experiments (Paine et al., 1998). The research focus might assume two distinct perspectives while combining these two types of perturbations into an experimental approach. First, the experiment may focus on how the *press perturbation* may affect the system's response to the *pulse perturbation* or even the characteristics of the *pulse perturbation* itself. Second, the experiment may focus on how the *pulse perturbations* may accelerate or delay the long-term changes induced by the *press perturbation*. For example, the study may rely on how eutrophication modifies the recovery of the phytoplankton community after a storm event in a drinking water reservoir; or on how storms can accelerate or slow down the eutrophication process with consequent phytoplankton dominance in a drinking water reservoir. Since both perspectives are complimentary from the ecological perspective – and may very often be possible using the same dataset - the combination of press and *pulse perturbations* become paramount to gather information on how systems respond to both types of perturbations in a fast-changing world

Unfortunately, perturbation experiments are often costly and laborious (Gross and Bluthgen, 2020), what make challenging their execution using ecological relevant scales in time and space. Nevertheless, the fact that ecosystems are constantly being hit by *pulse perturbations* –anthropogenic or not - creates a solid opportunity for studying the effect of environmental pressures (*press perturbation*s) based on their ecosystem responses. Natural stochastic perturbations as floods, fires, storms, droughts, or man-made stochastic perturbations as changes in flow regime, water quality, sanitation measures, biomanipulation, among others, produce a rich scenario for studying the combined role of pulse and *press perturbation* on the *stability* of ecosystems. Identifying opportunity costs for assessing long-term responses to perturbations in places where water management actions are needed or already in place creates a profitable opportunity to learn from ongoing processes. The expertise required for making profit out of such opportunity-costs is halfway through and include good practices between managers and scientists (Visser et al., 2015, Matthijs et al., 2012), a solid experimental framework capable of joining theoretical expectations with empirical information (Stelzer et al., 2021, Carpenter et al., 2014), methodological tools capable of gathering the quality data (Pimm et al., 2019, Donohue et al., 2016), and process-based or probabilistic statistical frameworks capable of reasoning the data (Spears et al., 2017). In a nutshell, Hodgson et al. (2015) proposed that in any study of *resilience* it is crucial to (i) define and model the system; (ii) define and measure the system state that is at risk; (iii) define the stable states to which the system might recover; and (iv) define the magnitude, frequency, and structure of disturbance. These four points are also well established on most environmental impact and risk assessments, creating the possibility of merging the scientific gathering of information with the biomanipulation produced during the environmental licensing processes and execution phase itself. By walking together, scientists and managers would take advantage of ongoing ecosystem-level manipulations, reducing the overall costs of large whole-system experiments while possibly increasing the number of studied systems in time and space.

Resource Availability in the Context of Freshwater Perturbations

A resource can be broadly described as any biotic or abiotic component of a system that sustains the life cycle of an organism or population, including nutrients, light, water, food supply, space, among many others (Hutchinson, 1957). The combination of different resources is at the core definition of ‘niche’ - the role an organism plays in a system - and therefore, resource availability is one of the main factors determining the ecological dynamics of populations, communities, and ecosystems. While natural resources are generally limited and likely to become scarce in a global scenario, many inland waters are regionally experiencing a surplus of resources, especially nutrients coming from adjacent areas. An iconic example is the phosphate, which is predicted to be limiting enough to have severe implications for global food security in the next centuries (Alewell et al., 2020, Koppelaar and Weikard, 2013) at the same time that accumulates in lakes and reservoirs in levels capable of disrupting ecosystem functioning (Huisman et al., 2018, Monchamp et al., 2018).

The diversity–*stability* hypothesis states that a greater diversity of traits occurs in species-rich communities, providing a greater likelihood that species and ecosystem functions can be maintained during environmental disturbances (Loreau and de Mazancourt, 2013, McCann, 2000, Peterson et al., 1998, MacArthur, 1955). Since resource availability directly affects community composition, it may also indirectly impact how ecosystems absorb environmental perturbations. Some studies have associated the high allochthonous resource availability to higher levels of biodiversity and trophic complexity (Siqueira et al., 2021, Liu et al., 2021, Cardinale et al., 2009), while others have identified the opposite trend (Chesson, 2000, Walker, 1995, Tilman, 1982, Ehrlich and Ehrlich, 1981). Further explanations for this antagonism are extensively discussed by Duffy (2009) and Hillebrand and Matthiessen (2009) and are mainly associated with deterministic observations of the studied systems rather than with process-based inferences. Yet, neither whether resource availability increases or decreases biodiversity, nor the role of biodiversity on ‘*ecological stability*’ are the central themes of this thesis, but to which extent resource availability may directly change the *stability* of (eco)systems at a given biodiversity level. High resource availability is expected to foster recovery by producing favorable conditions for growth after *pulse perturbations*, which may reduce the time a population remains affected by a *pulse perturbation*. On the other hand, high resource availability may also skew the recovery from perturbation towards the dominance of *'fast-growers',* resulting in delayed recovery from perturbations at a community level. Scrutinizing how resource availability (i) modifies the ecological processes involved in the recovery from *pulse perturbations* and (ii) affects the *stability* of the system facing *pulse perturbations* are key to foreseeing the complex interactions between climate and nature expected on the Anthropocene.

The Polyvalence of Resilience Concept and its Application to Management

The field of *Resilience* in ecology is dominated by two clearly interconnected schools that unfortunately diverged in thinking in the last 50 years, named '*ecological resilience'* and '*engineering resilience'* (Table 2). To understand these divergences, it is important to trace back to the origin of both different views. Both concepts of '*resilience'* started to be nurtured around the '50s with the pioneering contributions of Odum (1953), MacArthur (1955), and Elton (1958) towards the concept of '*ecological stability*'. '*Ecological stability',* however, is still today a highly mutable term with numerous subjective definitions. Back in 1997, Grimm and Wissel (1997) have already identified more than 160 definitions and 40 metrics of *stability* in one of the very first reviews on the topic. Not surprisingly, both classified '*ecological stability'* as *'one of the most nebulous terms in the whole of ecology'*. When understanding the confusing arena of '*ecological stability'* during the nurturing of '*resilience'* concepts, it is almost natural that emerging schools would drift towards far different pathways (Van Meerbeek et al., 2021). '*Resilience'* is a concept originally derived from the broader - and unbounded - framework of '*ecological stability'* (Figure 2), and this may have fostered the core of the contemporary contradiction involving both *resilience* theories in ecology. The understanding of '*ecological stability'* with all its components was far incomplete, naive, and therefore unfeasible to be coined under immutable definitions at such an early stage of development.

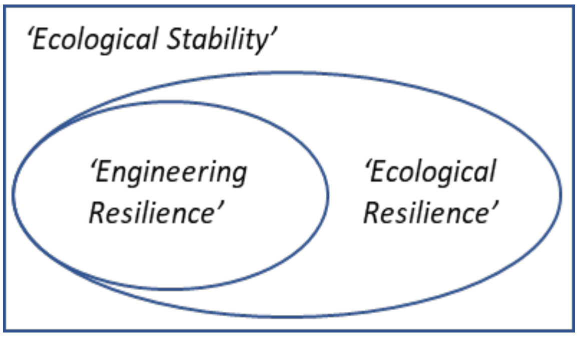

Figure 2. Conceptual visualization of the overlap between stability and the two mains schools of resilience.

Table 2. The definitions of 'resilience' and 'resistance' according to each resilience school.

	Resilience	Resistance
Ecological *(focused on press perturbations = driving force)* *sensu* Holling	"a measure of the persistence of systems and of their ability to absorb change and disturbance and still maintain the same relationships between populations or state variables."	*Capability of a system to sustain an ecological state despite the effect of a *pulse perturbation*.
Engineering *(focused on pulse perturbations)* *sensu* Pimm	Capability to return to the pre-perturbation condition.	Capability of a system to remain within equilibrium despite the presence of a perturbation.

**term not described in the literature. The description is a logical inference to the factorial design. Resistance is neglected from the perspective of ecological resilience, while it is fundamental in engineering resilience.*

'Ecological resilience' was formally presented to the scientific community by Holling (Holling, 1973) as a criticism towards the concept of equilibrium developed in the late '20s by Lotka-Volterra. However, it became widely popular in the last decades, when *resilience* and *catastrophic shifts* started to be a subject of broad interest for management and science. Being resilient under the '*Ecological resilience*' framework, however, is a rather qualitative aspect of the system, being mostly used as a conceptual framework focused on the non-equilibrium of ecological systems (Table 3). *Tipping points*, *critical thresholds*, *regime shifts*, and *alternative stable states* are terms commonly embraced by '*ecological resilience'*. Nevertheless, the existence of *tipping points* (Hillebrand et al., 2020), *alternative states* (Capon et al., 2015), and persistent shifts in ecosystem proprieties (Hillebrand and Kunze, 2020) are defended today to be an exception rather than the rule in natural systems. '*Ecological resilience'* has also raised some fundamental criticism towards its temporal scale of assessment – measurements often happen in different timescales than originally proposed by the theory (Hastings, 2004) - and within system's characteristics that are hard to quantify (Van Meerbeek et al., 2021, Donohue et al., 2016, Newton, 2016). Some contemporary scientists even state '*ecological resilience*' as a valuable framework to explore the theoretical background of ecological processes, but being of little empirical use (Beisner, 2012).

A couple of years after the consolidation of '*ecological resilience'*, Pimm (Pimm, 1984) steered a more pragmatic *resilience* school focused on quantifiable aspects of an ecosystem (Pimm et al., 2019). Unlike '*ecological resilience'*, '*engineering resilience'* assumes that after the dissipation of the perturbation (therefore, a *pulse perturbation*), the system shall recover to its pre-disturbed condition.

With this assumption in mind, a set of tools were developed in a way to quantify *resilience* as the recovery to equilibrium after a perturbation (e.g., Donohue et al. (2016), Pimm (1984)).

Despite shared grounds and interest, these two theories have developed in parallel. In fact, the assumption of equilibrium or non-equilibrium is the only fundamental difference between them and is mostly related to temporal scales of assessments in the system (Hastings, 2004). While *engineering resilience* focuses on the immediate effect of a *pulse perturbation*, ecological *resilience* focuses on the long-term trends of the system while mainly induced by a *press perturbation* (Table 3, although ecological *resilience* allows room for interpreting the system with or without *pulse perturbations*, as discussed in Figure 3).

Table 3. Comparative table between the main aspects of both schools of resilience.

	Ecological *Resilience*	*Engineering Resilience*
Type of perturbation focused on	Press	Pulse
Temporal aspect	Long-term trends	Short-term responses
Stability aspect focused on	Non-equilibrium	Equilibrium
Nature of the framework	Qualitative	Quantitative
Ecological level of measurement	Populations to ecosystems	Organisms to communities
Keywords	Regime shifts and tipping-points	Recovery (*resilience*) and resistance
Sensu	Holling	Pimm

Later, a strong field surged with well-structured ideas to finally calculate '*ecological resilience'* based on deterministic and univariate statistical indicators of system's *stability*. This novel approach was labeled '*Early-Warning Signals of Regime Shift'* (EWS), in allusion to the catastrophic transitions proposed by Holling (Holling, 1973) through the illustration of the basins of attraction (see Scheffer et al. (2001)). Among many EWS, van Nes and Scheffer (2007) proposed that rates of recovery from sequential *pulse perturbations* – as defined by Pimm (1984) – could be used as a strong indicator of proximity to critical transitions – a core aspect of *resilience* as proposed by Holling (1973) and exemplified by May (1977) and (Scheffer, 1990). This was a fundamental step towards the overlap of both theories since it compartmentalized '*resilience'* concepts into '*ecological stability'* (Figure 2). Many works started to apply recovery from *pulse perturbations* to infer '*ecological resilience'* (as discussed in chapter 3), which could potentially equip environmental managers with methods for preventing impacts on the provision of ecosystem services instead of solely mitigating it.

Understanding the polyvalence of *resilience* concepts is paramount for properly reasoning any study inferring *stability* metrics. Thus, this thesis presents a heuristic interpretation of '*resilience'* that respects well-established terms and couple them into a framework where its different facets are combined into a process-based definition of '*resilience'* (Figure 3). In a very simplistic and pragmatic way, '*engineering resilience*' measures the dynamics of the ball while '*ecological resilience*' measures the dynamics of the basin of attraction. Moreover, *pulse perturbations* are responsible for moving the ball, while *press perturbation*s are responsible for modifying the landscape of the basin of attraction. Together, '*ecological resilience'*, '*engineering resilience', and 'press and pulse framework'* form a powerful theoretical platform with almost null contradictions and capable of tackling dynamics of the system in distinct temporal and spatial scales and over the most variate type of stressors.

However, this thesis does not intend to host a discussion of how *resilience* should be interpreted but rather to construct a practical framework where different concepts are considered. While concepts are idiosyncratic, ecological processes are deeply bounded to evolutionary processes. Therefore, the sensible objective of unifying processes and not concepts may give a more comprehensive understanding of how ecosystems change in time and space.

Glossary of Commonly Used Terms in the Thesis

The glossary Figure 3 shows a two-dimensional representation of how *resilience* processes interact with each other. The Figure 3 is suggested to be interpreted within a continuum of time for a single system while interpreting it as a snapshot of different systems is also possible. It is fundamental to notice that different *resilience* concepts are associated with different types of perturbation. While *engineering resilience* explicitly demands a *pulse perturbation* that brings the system out of equilibrium, *ecological resilience* depends on a *press perturbation* that pushes the system towards the area of bi-*stability*. Thus, loss of *resilience* for ecological and *engineering resilience* have different meanings. The first means the proximity to a tipping point; the further the incapability to return to pre-perturbation condition. The terms described in the Figure 3 are described below and will repeatedly appear in this thesis.

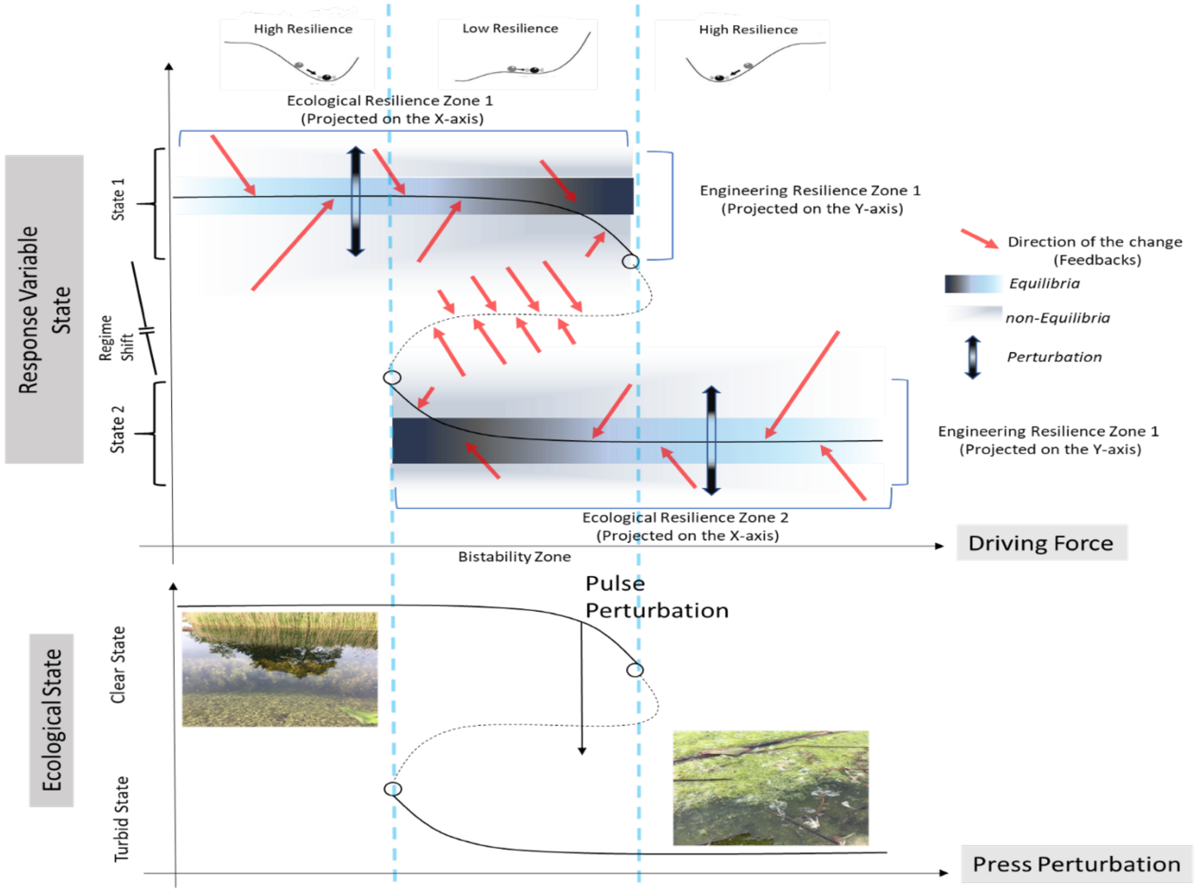

Figure 3. Upper: An integrative view of the different facets of resilience. The solid black line represents the system itself, represented by a response variable or proxy (y-axis) responding to changes in a driving force (x-axis) over time. As further the system is pushed - going from the blue to the black part of the colored bar - the closer it gets to a tipping point (open circle). When reaching the tipping point, any further increase in the driving force results in a rapid change to another state (regime shift). Therefore, changes in color from blue to black indicate loss of ecological resilience as a function of changes in the driving force – moving to a shallower basin of attraction (top figures). The basins of attraction are projected on the x-axis, represented by the Ecological Resilience Zones 1 and 2. Bistability zone is the area where both states can co-exist under the same level of the driving force, either as alternative stable states or whilst flickering between states. Mind that bistability may occur in space (similar lakes in different locations) but rarely in time, the reason why is suggested to interprete it as a temporal continuum. Furthermore, the system may also be hit by perturbations that bring it out of its zone of equilibrium (represented by the blue-black colored bar). Once out of equilibrium, internal feedbacks may enforce the system to return to its pre-perturbed condition or push it to a different state. The range of displacement from equilibria and recovery back to it defines the zone of interest for engineering resilience, with the intensity of displacement of the responsible variable projected on the y-axis. The intersection between engineering resilience and ecological resilience is where EWS "recovery" takes place. The graph shows the representation of a single shift in state. However, systems with multiple states should be seen as a continuum with multiple stepwise representations of the plot. The main terms of the figure are explained in the text. Lower: Empirical example of a regime shift in shallow lakes. The nutrient enrichment (press perturbation) erodes the basin of attraction, enforcing a shift from a clear state dominated by submerged macrophytes to a turbid state dominated by phytoplankton. This process is independent of any pulse perturbation; however, the nutrient enrichment erodes the basin of attraction of the lake, making the lake more vulnerable to a regime shift caused by a stochastic pulse perturbation (i.e., storm).

Forces

(Pulse) Perturbation – a temporary stress on the system that alters the arrangement of biotic and abiotic elements, potentially pushing the system out of equilibrium. Some examples of perturbations are turbidity and flood pulses caused by a storm, heatwaves, or sudden mortality events (e.g., due to contamination). If the perturbation is too strong, a regime shift may unfold as a consequence of it.

The effect of perturbations is represented by the projection of vertical arrows on the Y-axis in Fig. 1 (as a quantitative value of the response variable).

(Press perturbation) Driving force – an inherent component of the system on which its change pushes the system away from its actual ecological state. Common examples of driving forces are changes in nutrients ((oligo)eutrophication), climate change-driven changes in the physicochemical characteristics of the waterbody (temperature, pH), and changes in bottom-up and top-down control (abundance of top-predators). If the changes in the driving force are too sudden and happen with steep rates, it may bring the system out of equilibrium, triggering a perturbation (e.g., nutrient resuspension from the sediment). The driving force is represented in the diagram by the X-axis (quantitative value).

State variable

Regime shift – an abrupt and often persistent change in the functioning of the ecosystem (ecological state). It can be caused either by changes in the driving force, due to a strong or additive perturbations or the combination of all. Examples of a well-known regime shift are the transition from turbid to clear water in shallow lakes and lentic to lotic states of rivers; however, it can also represent the extinction of a species (going from present to absent).

Processes

Resistance (to perturbation) – Capability of a system to remain within the zone of equilibrium despite the presence of a potential perturbation. Resistance is a fundamental component of *engineering resilience*. As more resistant a system is, the stronger the perturbation has to be for bringing it out of equilibrium.

Engineering resilience – Capability of a system to return to the zone of equilibrium after being destabilized by a perturbation. Often measured as metrics of recovery and commonly used as a synonym of recovery rate. It is associated with quantitative characteristics of the system (e.g., time or length of the recovery after a storm).

Ecological resilience – Capability of a system to sustain an ecological state when pushed either by a driving force or a strong perturbation. Sometimes the term is exchangeable with ecological *stability* or “resistance to changes in the state”. Contrary to *engineering resilience*, it is more associated with a qualitative characteristic of the system (e.g., the shape of the basin of attraction as the capability to sustain a clear state in lakes).

For a more in-depth view of the glossary presented here, see (Van Meerbeek et al., 2021, Pimm et al., 2019, Hodgson et al., 2015, Carpenter et al., 2014).

This Thesis

The objective of this thesis is to better understand how resource availability, as a *press perturbation*, may change how aquatic communities respond to *pulse perturbations* (Table 4). Our primary goal was to identify general processes and pathways taking place after a mortality *pulse perturbation* and assess the effect of resource availability on them. For that, the thesis reviewed the existing literature and executed experiments using systems of different ecological complexity (single species eco-evolutionary microcosms and mesocosms manipulations using natural communities). The chapter 2 contains a global revision of how climate change may modify in-lake processes that are prone to escalate into feedback loops of relevance for freshwater biogeochemistry (i.e., mixing regimes and nutrient upwelling). Simultaneously, in chapter 3, the thesis explores if Early-Warning Signals of Regime Shift (EWS) could produce sufficient empirical evidence to be used as a reliable methodological framework for inferring levels of *Ecological Resilience*. This review was used as the foundation for defining which methodologies would be used to parametrize recovery from perturbations on the experiments of the following chapters. Chapter 4 used an eco-evolutionary bacterial system with complex intraspecific interactions to focus on methodological constraints of using recovery rate and time for addressing *engineering* and *ecological resilience*. Also, this experiment investigated the role of resource availability on the recovery from *pulse perturbations* at the population level (how the press modifies the response to the *pulse perturbation*). Later in chapter 5, ecological complexity was scaled up by using natural lake water to study the effect of nutrient availability on the microbial community response to *pulse perturbations*. Here, the perspective of using the *pulse perturbations* to better understand the effect of the *press perturbation* on the system *stability* (recovery and resistance) was applied. An overview of the structure and approaches used in this thesis is presented in Figure 4.

Table 4. Thesis delineation of the research questions and hypotheses for each thesis chapter.

Chapter	Questions	Hypotheses
2	To what extent can long-term atmospheric changes induce permanent shifts in lake biogeochemistry and functioning?	**(1)** Climate change can induce changes in lake mixing regimes and produce self-sustaining conditions to modify aquatic ecosystems permanently. **(2)** Changes in mixing regimes can induce long-term changes in resource availability through nutrient dynamics, potentially modifying aquatic ecosystems.
3	Can experiments bridge the gap between theory and application of Early-Warning Signals?	(**1**) EWS have been extensively studied in experiments, and the mechanistic basis of its functioning is well understood. (**2**) EWS are applicable with the same reliability to different ecological levels, ranging from organisms to communities.
4	How does resource availability modify the intraspecific ecological interactions to produce distinct recovery patterns after a single mortality *pulse perturbation*?	(**1**) low resource availability reduces the recovery rate and therefore increases the time needed for the system to recover. **(2)** Recovery rate and time are associated with the asymptotic recovery of the system (*ecological resilience*).
5	How does eutrophication modify the ecological *stability* of the microbial community during serial mortality *pulse perturbations*?	(**1**) Eutrophication increases maximum displacement after a *pulse perturbation*. (**2**) Eutrophication reduces the level of recovery a system can reach (baseline change). (**3**) Eutrophication increases resistance against *pulse perturbations*.

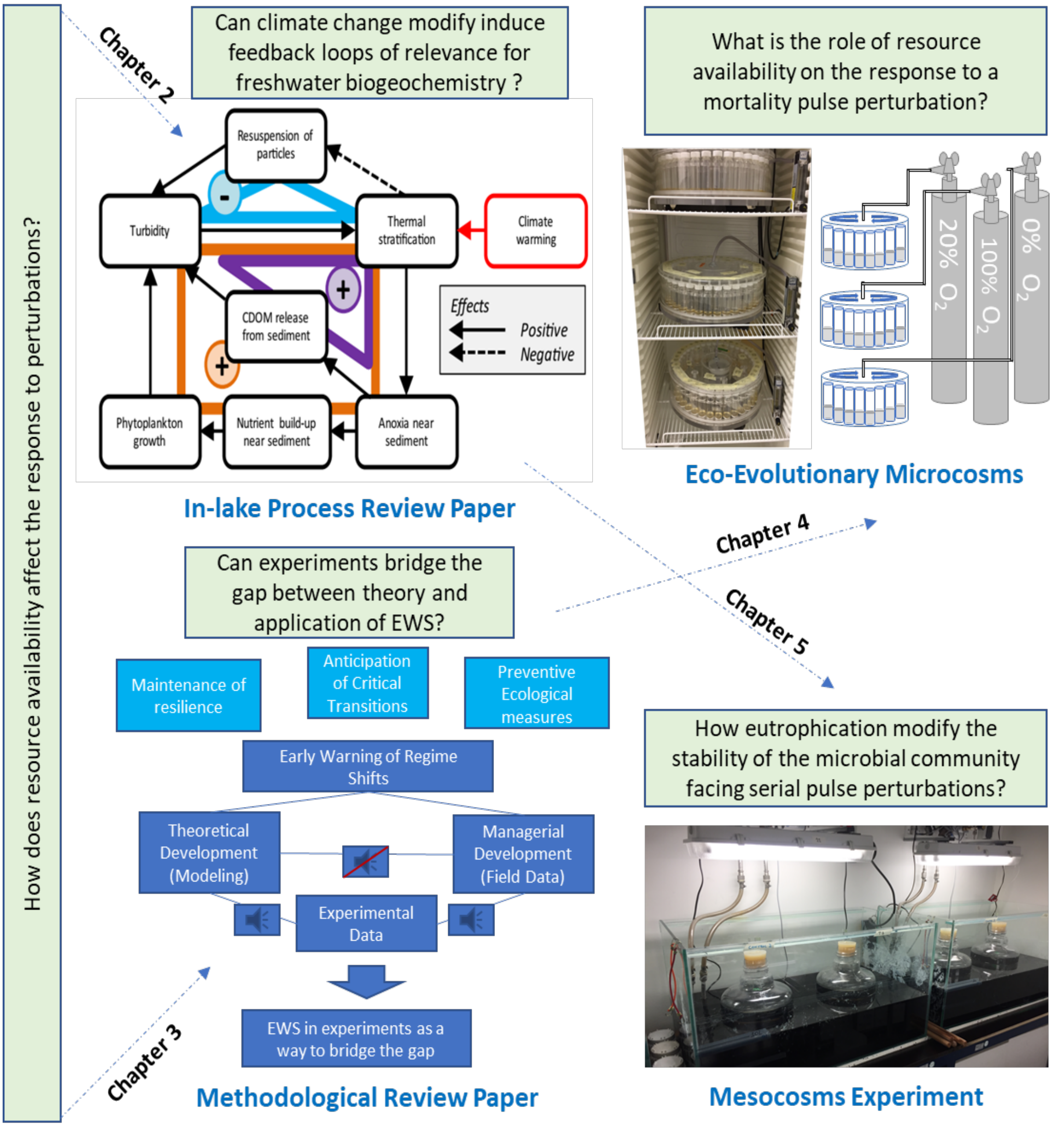

Figure 4. Infographic of the thesis structure and the approaches used to investigate the role of resource availability on resilience from perturbations.

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Chapter 2

The Role of Internal Feedbacks in Shifting Deep Lake Mixing Regimes Under a Warming Climate

Jorrit P. Mesman; **Julio A. A. Stelzer**; Vasilis Dakos; Stéphane Goyette; Ian D. Jones; Jérôme Kasparian; Daniel F. McGinnis; Bas W. Ibelings (2021) The role of internal feedbacks in shifting deep lake mixing regimes under a warming climate. **Freshwater Biology**, 66, 1021-1035, https://doi.org/10.1111/fwb.13704

Abstract

1. Climate warming is causing changes in the physics of deep lakes, such as longer summer stratification, increased water column stability, reduced ice cover, and a shallower depth of winter overturns. An ultimate consequence of warming would be a transition to a different mixing regime. Here we investigate the role of physical, chemical, and biological feedback mechanisms that unfold during a shift in mixing regime, and whether these feedbacks could prompt and stabilise the new regime. Although climate, interannual temperature variation, and lake morphometry are the main determinants of a mixing regime, when climate change causes shifts in mixing regime, internal feedback mechanisms may gain in importance and modify lake ecosystem functioning.
2. We review the role of these feedbacks in three mixing regime shifts: from polymictic to seasonally stratified, from dimictic to monomictic, and from holomictic to oligomictic or meromictic.
3. Polymictic lakes of intermediate depth (*c*. 3-10 m mean depth) could experience seasonal stratification if a stratification event triggers phytoplankton blooms or dissolved organic matter release, reducing transparency and therefore further heating the surface layer. However, this feedback is only likely to have influence in small and clear lakes, it would be easily disturbed by weather conditions, and the resulting stratified state does not remain stable in the long term, as stratification is lost in winter.
4. The ice-albedo feedback might cause an accelerated shift from ice-covered (dimictic) to ice-free (monomictic) winters in sufficiently deep (mean depth 50 m or more) lakes, where temperature memory is carried over from one winter to the next. Nevertheless, there is an ongoing debate into whether this process can persist during natural weather variations and overcome self-stabilising mechanisms such as thermal insulation by snow. The majority of studies suggest that a gradual transition from dimictic to monomictic is more likely than an abrupt transition.
5. A shift from a holomictic to a meromictic regime can occur if anoxia is triggered by incomplete mixing and an increase in deep-water density - through the accumulation of solutes - exceeds a density decrease by hypolimnetic warming. A shift to meromixis would strongly alter the biology of a lake and might be difficult to reverse. If solutes accumulate only minimally in the hypolimnion, an oligomictic regime is formed, in which years with complete and incomplete mixing alternate.

6. Understanding the importance of feedback mechanisms and the role of biogeochemistry when lakes shift in mixing regime could lead to a better understanding of how climate change affects lake ecosystems.

Keywords: Climate change, meromixis, mixing regime, stratification, water transparency

Introduction

Temperatures in lakes all over the world have been rising over the past century as a consequence of global warming (O'Reilly et al., 2015). This warming has resulted in an overall increase in thermal stability, with longer periods of summer stratification and steeper thermoclines, restricting exchange of substances between the epi- and hypolimnion (Shimoda et al., 2011; Kraemer et al., 2015). Climatic trends driving the thermal stability in deep lakes have also had profound impacts on lake chemistry and biology. For instance, reduction of deep mixing can result in the depletion of oxygen (anoxia) in the hypolimnion (Schwefel et al., 2016). A longer duration of stratification and reduction of deep mixing can increase the heterogeneity of vertical nutrient profiles, with nutrient-rich deep waters and nutrient-poor surface waters (Winder and Sommer, 2012; Schwefel et al., 2019). In turn, this altered vertical nutrient distribution affects lake biota such as phytoplankton and fish (O'Reilly et al., 2003; Winder and Sommer, 2012). Moreover, observational studies of lake thermal structure and numerical climate simulations have pointed towards climate-induced shifts in mixing regime (Box 1), implying structural changes in lake ecosystems (Peeters et al., 2002; Ficker et al., 2017; Shatwell et al., 2019; Woolway and Merchant, 2019).

In the present review paper, we look at the physical, chemical, and biological consequences of climate warming and increased density stratification in deep lakes, defined here as lakes that stratify during at least one season. We then identify internal feedbacks that can reinforce (positive feedbacks) or slow down (negative feedbacks) shifts between mixing regimes. The scope of this paper only includes regime shifts where such feedback loops were identified in the existing literature, or where they could be constructed using individual processes, and considers mixing regime shifts in the context of increasing atmospheric temperatures. The potential importance of feedbacks is well illustrated by the alternative macrophyte- (clear-water) and algae-dominated (turbid) states in shallow lakes (Ibelings et al., 2007). Regime shifts between these two states involve feedback loops between turbidity, nutrients, and trophic interactions that retain either state, also in the face of changing external processes such as eu-/oligotrophication or perturbations such as storms (Scheffer, 1998; Scheffer et al., 2001).

In deep, stratified lakes the vertical distribution of oxygen, nutrients, and phytoplankton are strongly influenced by density stratification, which hints at the potential of mixing regimes to act as important drivers of ecosystem functioning. Mixing regimes are primarily driven by physical processes, and therefore under direct influence of climate change (Livingstone, 2008; for definitions of mixing regimes, see Box 1). Mixing regimes in deep lakes differ from one another in several physical, chemical, and biological aspects (e.g. Boehrer and Schultze, 2008; Adrian et al., 2009; North et al., 2014).

According to the classical view on mixing regimes (Hutchinson and Löffler, 1956; Lewis Jr, 1983), local climate and morphometry are the main factors determining the mixing regime of a lake. However, factors other than depth and climate, such as transparency (Brothers et al., 2014) and solute content (Boehrer and Schultze, 2008), can also influence lake mixing. Conversely, mixing regimes might influence these factors. Thus, lake-internal feedbacks could stabilise and even determine the mixing regime, especially in situations where morphometry and climate can support multiple mixing regimes. It is in these situations that mixing regime shifts are to be expected, and already unfolding, in response to ongoing climate change. If self-sustaining feedback mechanisms hold the new regime in place, shifts in mixing regime may prove to be resilient.

Quantitative observations and numerical simulations specifically focusing on shifts in mixing regime by factors other than temperature are scarce, as long-term observations and detailed studies are needed to observe such shifts and identify the drivers. However, individual processes that could lead to feedback loops stabilising mixing regimes, are well described. In what follows, we review the literature on the physical trends related to increased duration and strength of density stratification, and the chemical and biological consequences thereof (Figure 1). Following this literature review, we derive processes at play during a transition in mixing regime and discuss their interaction in typical lake regime shifts. The observed feedbacks are brought together, visualised, and placed into the perspective of shifts in mixing regime under increasing temperatures. We also discuss the limitations of the relevance of each feedback and specify for what types of lakes these feedbacks may be considered. In this way, we believe our review provides new and pertinent information on how climate warming may affect lake ecosystems, extending beyond direct effects of temperature alone.

Box - Types of lake mixing regimes

Categorising lakes on the basis of their mixing regime is a well-established practice (Forel, 1880; Hutchinson and Löffler, 1956). Lakes are classified according to the number of mixing events per year and the degree of mixing. Depending on local climate, depth, salinity, and lake morphology, a lake mixes a certain number of times per year (never - amictic, once -monomictic, twice - dimictic, three or more times - polymictic), either completely – always from top to bottom (holomixis) –, only sometimes from top to bottom (oligomixis) or always partially (meromixis). Shallow lakes tend to be *polymictic*, i.e. they mix multiple times per year, although below what depth a lake is to be considered shallow has been the topic of discussion (see Padisák and Reynolds, 2003). In most cases, the occurrence of polymixis is used to define a lake as shallow. Depending on lake fetch, transparency, and wind speeds, polymixis tends to occur below mean depths of 3 to 20 m (Padisák and Reynolds, 2003; Kirillin and Shatwell, 2016). This shallowness makes the lake prone to mixing events, either wind-induced or caused by convective cooling, although stratification events lasting multiple days or weeks are also possible (Mischke, 2003; Wilhelm and Adrian, 2008).

The presence of long-term, i.e. over at least a season, density stratification is used here to define what constitutes a deep lake. In deep lakes, seasonal temperature variation largely controls the mixing regime. Near the poles, lakes, for now, have permanent ice cover (*amictic lakes*) or only experience inverse stratification (i.e. cold above warmer water, as the maximum density of freshwater is achieved at 4°C) and these *cold monomictic* lakes only mix in summer. Moving to lower latitudes, winter temperatures are still low enough for inverse stratification and ice formation, but air temperatures in summer are high enough to allow formation of a warm epilimnion; these are *dimictic* lakes, that mix before and after a winter period with inverse stratification. Where winters are not cold enough for ice formation, stratification only occurs in summer and deep lakes only mix in winter: these are *warm monomictic* lakes (Lewis Jr, 1983). The absence of strong seasonal temperature variation in tropical regions causes a different yearly pattern near the equator, with a more dynamic development of the epilimnion. However, mixing seasons often still exist as a result of seasonal patterns in radiation, rainfall, or wind, and tropical deep lakes are classified as warm monomictic, following Lewis Jr (1996). In the main text the term monomictic refers to warm monomictic lakes.

Winter mixing does not necessarily reach the deepest location of the lake. Complete mixing is called *holomixis* and incomplete mixing is termed *meromixis*. In permanent or “true” meromictic lakes, stratification is caused by an increased concentration of solutes that raises the density of the deep water, for example by sea water or saline groundwater influx (Hutchinson, 1957; Gulati et al., 2017). The two chemically different layers do not mix for multiple years. However, in many temperate and tropical deep lakes, mixing depths vary year-to-year and complete winter mixing occurs at varying frequencies, ranging from on average once every year to once every 5 decades. These lakes are not holomictic, but no permanent chemical stratification is formed either. In this paper, we define these lakes as *oligomictic* (following Lewis Jr, 1973). We reserve the term *meromictic* for lakes with chemically different layers and stable density stratification due to the effect of solutes (following Gulati et al., 2017).

We therefore define the mixing regime of a lake both in terms of the frequency of mixing (poly-, di-, monomictic) and the extent of mixing (holo-, oligo-, meromictic). For a more complete description of mixing regimes and potential further subdivisions, we refer the reader to Boehrer and Schultze (2008).

Physical, Chemical, and Biological Consequences of Enhanced Stratification

Water temperatures and stratification

(Figure 1: P1, P2, P3) The increase in global surface air temperature (IPCC, 2014) has an impact on lake temperature and water column stratification. Global surveys of surface water temperatures report an increase in epilimnetic temperatures with rates roughly between 0.2 and 1°C per decade (Shimoda et al., 2011; Kraemer et al., 2015; O'Reilly et al., 2015). The temperature difference between epi- and hypolimnion often increases, causing longer and stronger thermal stratification in summer (Fang and Stefan, 1999; Foley et al., 2012; Kraemer et al., 2015). Even when temperature differences remain the same, the density difference becomes greater with warming, as the water density-temperature relation is steeper at higher temperatures (Wetzel, 2001). As the density difference between epi- and hypolimnion increases, mixing of the two layers is reduced, which further heats the surface layer and increases density differences, as less heat is transported downwards. The stability of the water column is often expressed as the Schmidt stability (i.e. the potential energy stored in stratification per unit area) (Schmidt, 1928; Idso, 1973). Between 1970 and 2010, average Schmidt stability in lakes worldwide has increased by up to 25% (Kraemer et al., 2015).

(Figure 1: P2) A larger density difference between the epilimnion and hypolimnion increases the local stability of the water column. An increase in stability reduces the vertical turbulent diffusivity K_z, which indicates the rate of vertical mixing (Ravens et al., 2000; Wüest et al., 2000). Hence, a stronger stratification implies that dissolved substances less easily traverse the thermocline, promoting separation between surface and bottom waters.

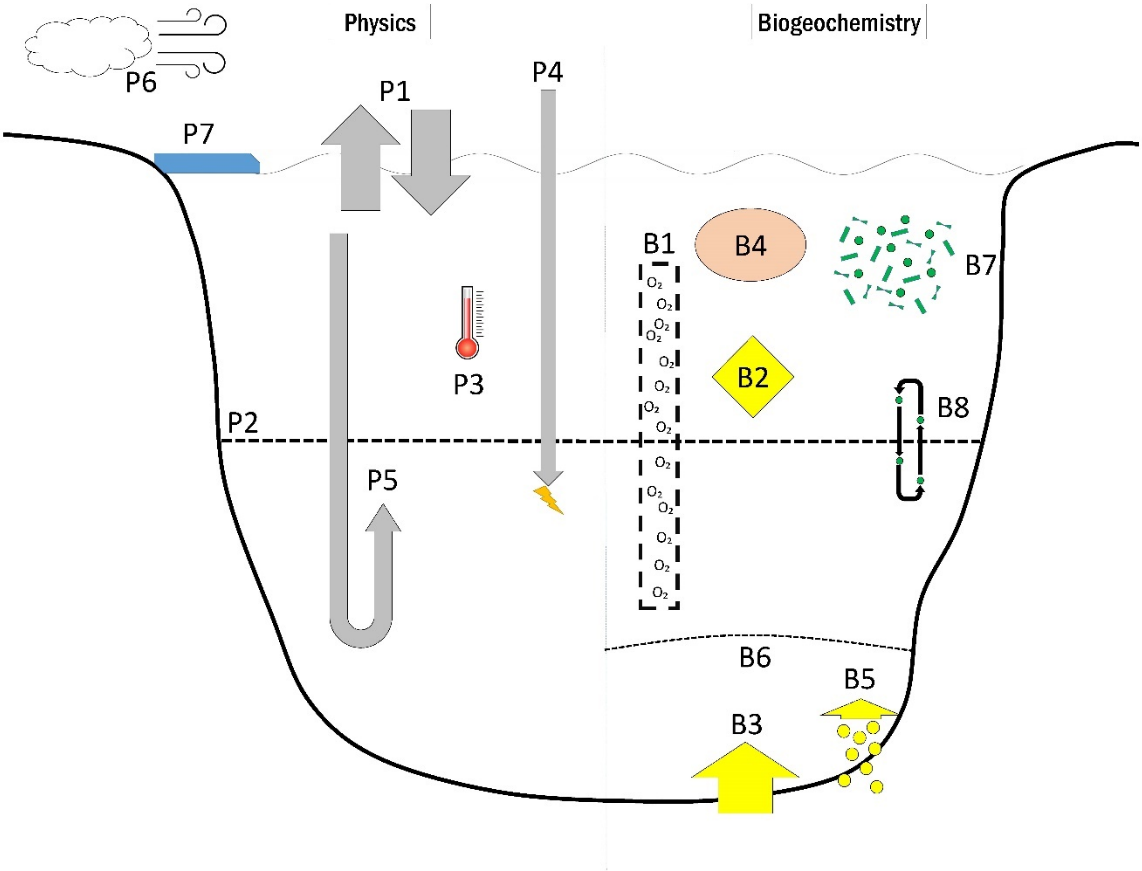

Figure 1: Overview of the physical and biogeochemical components and processes in deep lakes considered in the text. P denotes a physical and B a biogeochemical process. Energy fluxes at the air-water interface (P1) represent the interaction between climate and the lake. Thermal

stratification (P2) is important for transport between water layers and is formed primarily by higher water temperatures (P3) in the surface layers compared to bottom layers. Light penetration (P4) causes heating of surface layers and is essential for phytoplankton growth. Deep-water mixing (P5) can occur as a result of strong convective cooling and marks the end of the stratified season. Wind stress (P6) also promotes mixing and deepening of the mixed layer. Ice cover (P7) affects surface heat fluxes and reduces effects of wind on the lake interior. Oxygen concentration (B1) is linked to many chemical and biological processes in the water column. Nutrient concentrations (B2) in the epilimnion are essential for the growth of phytoplankton. Nutrients and other type of solutes can be released from the sediment (B3). Coloured dissolved organic matter (CDOM, B4) reduces light penetration in the water column. Greenhouse gases can be emitted from the sediment (B5). If the deep-water layers of a lake are heavier than the overlying water due to solute content, meromixis is formed (B6). Phytoplankton biomass (B7) grows through consumption of resources such as nutrients and light in the photic zone of the lake. Some cyanobacteria have variable buoyancy (B8) that enables uptake of nutrients from below the thermocline, or they may use their buoyancy to form deep chlorophyll maxima in the metalimnion of the lake.

Oxygen dynamics in deep lakes

(Figure 1: P2, P4, P5, B1) Oxygen sources (reaeration and photosynthesis) are mainly restricted to the epi- and metalimnion (Wetzel, 2001; Obrador et al., 2014; Giling et al., 2017b), and in most lakes oxygen is constantly being depleted in the hypolimnion, especially near the sediment. Generally, deep convective mixing is often the major source of oxygen replenishment in the deep-water layers (Straile et al., 2003), although river intrusion can also notably affect hypolimnetic oxygen conditions (Fink et al., 2016). The extent of the oxygen-depleting processes in the water column and the sediment, the volume of the hypolimnion, and the sediment area to hypolimnion volume ratio define the rate at which oxygen concentrations fall after installation of the thermocline (Schwefel et al., 2018). Hypolimnia of highly productive systems have a higher oxygen depletion rate (Rippey and McSorley, 2009; Müller et al., 2012). Deeper lakes contain more oxygen due to a thicker hypolimnion, and oxygen depletion rates tend to decrease with depth, so deeper lakes are less prone to become anoxic in one summer (Müller et al., 2012; Schwefel et al., 2018). However, they are less likely to experience complete vertical mixing, and climate warming further decreases this likelihood. In the case of incomplete mixing, the oxygen is only partially replenished and the hypolimnion will experience lower oxygen levels the following year. Therefore, a shift from holomictic to oligomictic behaviour implies a greater risk of anoxic conditions, for productive lakes in particular.

(Figure 1: P3, P5, B1) Numerous observations of hypolimnetic anoxia are attributed to shifts in the extent of mixing exist in both temperate (Foley et al., 2012; Ito and Momii, 2015) and tropical regions (O'Reilly et al., 2003; Fukushima et al., 2017), and climate change is expected to amplify this trend (Peeters et al., 2002; Fang and Stefan, 2009; Sahoo et al., 2013). While eutrophication is often seen as the main cause of anoxia, changes in deep-water mixing can be at least as important in deep lakes (Schwefel et al., 2016). Aside from the increase in stratification, climate change can affect hypolimnetic oxygen through increased temperatures as well, as mineralisation and metabolic rates are higher at higher temperatures, in the order of a 3%-6% increase per °C (Fang and Stefan, 2009;

Gudasz et al., 2010). In this way, hypolimnetic warming could increase the intensity of oxygen depletion in the hypolimnion and sediments (Straile et al., 2003).

Influence of anoxia on nutrient distribution and other substances

(Figure 1: B1, B2, B3) Anoxia near the sediment can induce enhanced internal phosphorus loading through reduction of the benthic redox potential, so that iron-bound phosphate is released from the sediment (Søndergaard et al., 2003). While this enhanced release can be relevant on short time scales, on seasonal (or longer) time scales internal P budgets are mostly dependent on settling and mineralisation rates, as well as sediment characteristics (Hupfer and Lewandowski, 2008). During stratification, P tends to accumulate in the hypolimnion as nutrients are not mixed into the photic zone while mineralisation in the sediment and pelagic continues. When deep mixing occurs, large amounts of P can enter the photic zone, potentially boosting productivity (Lehmann et al., 2015; Lepori et al., 2018). Nitrogen can also be released from the sediment under anoxic conditions in the form of ammonium (Wetzel, 2001). Denitrification occurs in anoxic hypolimnia and, especially, sediments (Wetzel, 2001). It is a major loss term of nitrogen in lakes. When anoxia of the hypolimnion is ended by a mixing event, nitrogen, like phosphorus, can enter the photic zone and boost productivity. However, large losses of nitrogen to the atmosphere can occur through denitrification, as nitrate-rich water is brought in contact with the anoxic sediment (De Brabandere et al., 2015; Lehmann et al., 2015).

(Figure 1: P5, P6, B1, B2, B3) The stratification that is at the root of this build-up of nutrients in the hypolimnion, however, also prevents nutrients from entering the epilimnion, as increased stratification implies that dissolved substances are retained more in their respective layers. Reduced entrainment of deeper water layers and less intense winter mixing could cause decreased nutrient concentrations in the epilimnion, despite the higher nutrient concentrations in the hypolimnion (Yankova et al., 2017; Schwefel et al., 2019). As nutrients accumulate in the hypolimnion because of increased stratification with climate change, the amount of nutrients released to the epilimnion when complete mixing does occur goes up.

(Figure 1: B1, B3, B4, B5, B6) Apart from nitrogen and phosphorus, other substances are affected by hypolimnetic shortages of oxygen as well. Iron-oxide-bound carbon can be released as coloured dissolved organic matter (CDOM) under anoxia when iron is reduced (Hamilton-Taylor et al., 1996; Brothers et al., 2014). In the deep layers of meromictic lakes, the anoxic conditions enable the occurrence of reduction processes involving iron, manganese, and sulfide, which are often essential for creating and maintaining meromixis (Friedrich et al., 2014; Lehmann et al., 2015; Schultze et al., 2017). These elements can occur in dissolved form under anoxic conditions and accumulate in deep-water layers of meromictic lakes, where they increase deep-water density (Imboden and Wüest, 1995; Gulati et al., 2017). Strong stratification and anoxia can also induce more methane emissions from lakes (Grasset et al., 2018; Vachon et al., 2019). Like dissolved solutes, dissolved methane and dissolved carbon dioxide can occur in high concentrations in deep, anoxic water layers, and affect density, with methane reducing and carbon dioxide increasing water density (Imboden and Wüest, 1995; Schmid et al., 2002). The effect of dissolved gases on density can be especially important in lakes where there is a high influx of gases from the sediment, for example as a result of volcanic activity (Schmid et al., 2002).

Influence of mixing dynamics on lake phytoplankton

(Figure 1: B2, B7) A change in nutrients in the epilimnion will strongly control phytoplankton development. As mentioned above, increased stratification might actually reduce nutrient levels in the surface water of lakes. Longer stratification in such a case means a longer period of nutrient limitation for phytoplankton (Yang et al., 2016) and therefore a bigger advantage for species that efficiently use or store nutrients (Winder and Sommer, 2012), and potentially for mixotrophic species, which have access to additional organic nutrient sources (Jansson et al., 1996). However, the effects of increased stratification and warming of surface waters differ between oligotrophic and eutrophic systems. In oligotrophic systems, a higher metabolic rate driven by higher temperatures in combination with nutrient shortage can lead to lower levels of biomass compared to colder temperatures, while in eutrophic systems, the higher temperature may boost growth and biomass (Jöhnk et al., 2008; Kraemer et al., 2017).

(Figure 1: P2, P4, B7, B8) Stratification also affects the phytoplankton's ability to remain near the surface and in the euphotic zone. Formation of a thermocline reduces the depth over which phytoplankton is mixed, effectively increasing their chance to remain in the photic zone (Huisman et al., 1999). At the same time, however, stratification reduces turbulence and vertical mixing deeper down in the water column, and sinking becomes a major loss term for many dense phytoplankton species (Diehl et al., 2002). A lower water viscosity at higher temperatures (Hutter and Jöhnk, 2004) increases sinking rates and facilitates migration through buoyancy regulation (Paerl and Huisman, 2009). Stronger density stratification and suppression of turbulence thus may give an advantage to motile phytoplankton species (Huisman et al., 2004; Winder and Hunter, 2008).

(Figure 1: P5, B2, B7, B8) Higher nutrient concentrations in the hypolimnion do not directly promote phytoplankton growth because of the lack of light at depth, with the exception of phytoplankton species that produce a deep chlorophyll maximum in the metalimnion (e.g. *Planktothrix rubescens*), or buoyancy regulators that are perceived to make excursions into the hypolimnion (e.g. *Microcystis*)

(Fee, 1976; Paerl and Huisman, 2009). The strong vertical heterogeneity in nutrient levels induces the possibility that mixing events causing entrainment of hypolimnetic water into the epilimnion can lead to spikes of epilimnetic nutrient concentrations (Lehmann et al., 2015), stimulating phytoplankton blooms (Giling et al., 2017a). These events can be caused by extreme weather events such as storms, cold spells, or river floods (Jennings et al., 2012). With increased stratification in summer, the amount of energy needed for these deep mixing events increases, but the nutrient pulse after such an event tends to be stronger (Coats et al., 2006).

Shifts in Mixing Regime

In this section, we identify which processes could form positive or negative feedbacks that could lead to a shift in mixing regime. As these shifts are already unfolding and likely to continue into the future (Woolway and Merchant, 2019), it is important to assess which changes to expect and if they are able to self-amplify under a given condition. The shifts in mixing regime that are treated here are: (1) from a polymictic to a seasonally stratified regime; (2) from a dimictic to a monomictic regime, where ice cover and inverse stratification in winter are disappearing; and (3) from a holomictic to an oligomictic or a meromictic regime. Here we investigate if and under what conditions feedback mechanisms can reinforce shifts in mixing regimes.

Two other shifts in mixing regime can also be expected with climate change, mediated through changes in hydrology. In lakes where water level is projected to decrease with climate change, a shift from stratified to polymictic can be expected if the water level falls below a critical value to sustain seasonal stratification (Zohary and Ostrovsky, 2011; Kirillin and Shatwell, 2016). Increase in water level could cause a shift in the opposite direction. Both temperature and water level can be a driver of a shift between polymictic and stratified regimes, and the feedbacks we discuss in the following section apply to both. In saline lakes, a reduction of freshwater inflow can cause a shift from meromictic to holomictic, as the freshwater layer on top of the heavier saline layer diminishes (Gertman and Hecht, 2002; Kaden et al., 2010) and vice versa with an increase in precipitation (Melack and Jellison, 1998). We are not aware of literature that describes feedbacks from the new mixing regime to the hydrological input, or changed biogeochemical conditions under the new regime that affect the vertical salt distribution in a way that affects the new regime's stability. Our view is therefore that such a response of the saline lake mixing regime is a direct function of the discharge and seasonality of the external inflow (although a threshold response is possible), and because of the lack of known internal feedbacks, we will not treat this regime shift further.

Shift from a polymictic to a seasonally stratified regime

Lakes of intermediate depth (c. 3–10 m mean depth, Kirillin and Shatwell, 2016) can support both polymictic and seasonally stratified (dimictic or monomictic) regimes, based on morphometry, transparency, wind speed, and annual mean solar radiation flux (Kirillin and Shatwell, 2016). A shift from polymixis to seasonal stratification might occur as a result of climate warming in these lakes (Kirillin, 2010; Woolway and Merchant, 2019). This trend can be amplified by reduced water transparency and lower wind speeds in summer (Shatwell et al., 2016). If transparency is reduced, less energy penetrates to deeper layers, as more solar radiation is absorbed near the surface. This can result in warming of the surface layer, cooling of the hypolimnion, and overall stronger stratification (Jones et al., 2005; Tanentzap et al., 2008), but the influence of transparency on stratification is significantly stronger in smaller lakes due to a lower contribution of wind mixing to turbulence formation (Fee et al., 1996). A decrease in transparency can be caused by phytoplankton growth or increased CDOM content, for example as a consequence of catchment-based inflow of nutrients or organic matter. CDOM loading from peatlands or forests may increase as a function of climate change, for example through increased decomposition rates at higher temperatures (Jennings et al., 2010). Wind is a crucial factor in exchanging heat between atmosphere and lakes by inducing mixing (Imboden and Wüest, 1995), and can be a decisive factor for hypolimnetic temperature trends. Indeed, decreasing wind speeds cause a cooling of the hypolimnion by reducing heat transfer to deep-water layers (Magee and Wu, 2017). Regional trends in wind speed might have the potential to cause a shift in mixing regimes (Woolway et al., 2019). However, wind forcing is external to the lake system, and we are not aware of literature describing feedbacks between wind forcing and lake conditions that reinforce either the polymictic or stratified mixing regime.

Periods with warm and calm weather are promoted by climate change, and can induce stratification events in polymictic lakes, lasting multiple days or even weeks (Wilhelm and Adrian, 2008). However, strong inter-annual variation in the duration of stratification within the same lake has been documented that cannot be explained by temperature changes alone (Riis and Sand-Jensen, 1998; Brothers et al., 2014). Water transparency was determined to be a major factor of shifts in mixing regime in the studies of Riis and Sand-Jensen (1998), Brothers et al. (2014), and Shatwell et al. (2016), and is, as stated, influenced by phytoplankton growth and CDOM content.

In this paper, we are interested in feedbacks that would stabilise a newly established stratified regime, such as mechanisms that would perpetuate lower transparency. Brothers et al. (2014) described such a feedback in a eutrophic German lake with an average depth of 1.7 m (maximum 2.9 m) and a surface area of 3.3 ha. Strong rainfall flooded surrounding peatlands, leading to increased CDOM and nutrient concentrations and higher water levels (about 1 m) in the lake. Transparency was reduced due to a combination of increased CDOM and phytoplankton, which caused stratification and anoxia near the sediment, and promoted internal loading of CDOM and nutrients from the sediment, stabilising the stratified state. For a different lake, Riis and Sand-Jensen (1998) describe almost a doubling of the duration of stratification over a period of 40 years due to increased CDOM concentrations in an oligotrophic Danish lake of 8.1 m mean depth (maximum 12 m) and a surface area of 12 ha, but no stabilising feedbacks were identified. Model simulations of two eutrophic German lakes (maximum depths 8 and 9.5 m) by Shatwell et al. (2016) suggested that phytoplankton can have a decisive influence on mixing regimes in lakes of intermediate depth. The presence or absence of a clear-water phase in spring could change the mixing regime for that year. Again, no feedbacks are described. Still, in shallow lakes a heatwave or period of calm can trigger a period of stratification and potentially cause anoxia when oxygen depletion is sufficiently high, followed by nutrient release to the photic zone when stratification ends (Wilhelm and Adrian, 2008). When the accumulated nutrients become available to phytoplankton during stratification, for example due to buoyancy regulation, the ensuing bloom could reduce transparency, leading to stronger heating of the upper water layers (Jones et al., 2005), and thus stabilise the stratified regime.

However, despite the crucial role of transparency in regulating thermal stratification and the study of Brothers et al. (2014) showing the potential of the transparency-reduction feedback in environmental data, this feedback might only apply to a select set of lakes. Below, we give three arguments why the likelihood that this feedback will cause bi-stability of polymixis and seasonal stratification might be limited: (1) the feedback can regularly be overridden by external perturbations unless a specific set of lake conditions, regarding morphometry and transparency, is present; (2) stratification hinders exchange between sediment and surface water, effectively weakening the feedback; and (3) there is a reset of lake conditions in winter and no carry-on of the feedback to the next year.

The feedback therefore requires a specific set of conditions to effectively influence lake mixing regime. Lakes that are too shallow cannot sustain seasonal stratification as commonly recurring convective or wind mixing events break down stratification completely. Conversely, deeper lakes often already have a stratified regime, thus restricting bi-stability to lakes with an intermediate depth range (c. 3-10 m, Kirillin and Shatwell, 2016). In lakes larger than approximately 5 km^2, wind and convective mixing are the decisive factors to determine the depth of stratification and transparency only has a minor effect (Fee et al., 1996), whereas Kirillin and Shatwell (2016) report a decreasing effect of transparency on mixing regime above a lake length of about 1-10 km based on an analysis of 379 lakes. Therefore, the feedback described above would only be relevant in small lakes of intermediate depth. Additionally, Persson and Jones (2008) show that in already turbid water, a change in transparency has little effect on thermal stability, which would suggest that the transparency-reduction feedback requires an initially low turbidity. If all light was already absorbed in the mixed layer before the reduction of transparency unfolds, a further drop in transparency would not make a large difference in heat distribution (Persson and Jones, 2008). Along the same lines, a sensitivity analysis in the modelling study by Shatwell et al. (2019) indicated that the effect of varying transparency on stratification is strongest in small lakes with low to medium (up to *c*. 1.0 m^{-1}) extinction coefficients.

Stratification restricts exchange of dissolved materials between deep and shallow water layers. Therefore, sediment release of CDOM and nutrients would only marginally reach the epilimnion and affect light penetration. Nutrients would reach the surface layer after a mixing event, as is supported by findings of blooms after the end of stratification events (Wilhelm and Adrian, 2008), but this would break the transparency-reduction feedback. Buoyant cyanobacteria could – potentially - use the nutrients in the hypolimnion and move across the thermocline into the light to grow (Paerl and Huisman, 2009), keeping the feedback loop intact, although the reality of this remains under discussion (Bormans et al., 1999). In the study of Brothers et al. (2014), sediment release of substances did influence the pelagic despite consistent stratification, but the role of the thermocline was not discussed. The presence of a thermocline limits turbulence reaching the sediment, so stratification reduces resuspension of particles that sink to the bottom and promotes sedimentation losses. Formation of stratification can thus reduce particle-based turbidity. The reduced turbidity in turn decreases stratification, completing a negative feedback loop (Figure 2a). If the turbidity is caused by sinking particles (e.g. non-buoyant phytoplankton cells), this negative feedback inhibits sustenance of the stratified state.

Lastly, in winter, phytoplankton biomass is low and complete mixing occurs, so the feedback loop is broken. Therefore, there is no carry-over of mixing regime from year to year. Years with enhanced stratification could easily be followed by a year with polymictic behaviour since the occurrence of seasonal stratification in one year does not influence the likelihood of stratification in the next year. Stratification can be triggered by a period of warm and calm weather, which makes timing a relevant issue. A heatwave in spring/early summer can affect the mixing regime for the rest of the year, but a similar event at the end of summer has only a brief effect.

Summarising, in a polymictic lake of intermediate depth, a seasonally stratified regime can establish under lower wind speeds, decreased transparency, or a higher water level (Kirillin and Shatwell, 2016). Changes in transparency can strongly influence thermal stability (Persson and Jones, 2008; Tanentzap et al., 2008), and even shift mixing regimes (Brothers et al., 2014; Shatwell et al., 2016). Feedback loops could sustain the stratified state (Figure 2a), which might cause a sudden shift from a polymictic to a seasonally stratified regime for a particular year (Figure 2b). However, as discussed above, there is a suite of reasons why these feedbacks may not be dominant in most lakes.

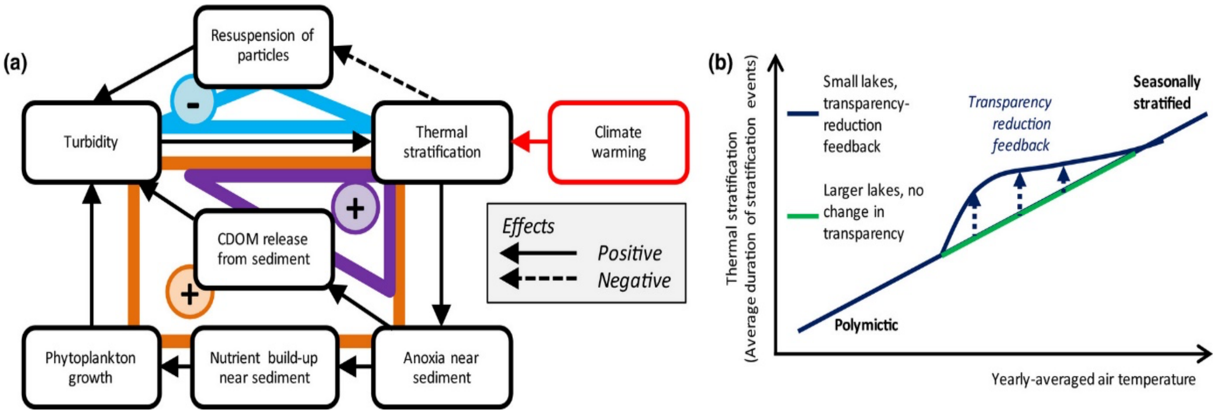

Figure 2: (a) The associated feedbacks for a shift from a polymictic to a seasonally stratified regime. Solid arrows denote a stimulating (positive) effect and dashed arrows a reducing (negative) effect. The effect of climate warming is shown in red. Three feedback loops are potentially formed. Both the purple and the orange feedback loops are positive (i.e. self-reinforcing). The blue, negative feedback loop is activated when the turbidity that supports the stratification is reduced because of sinking particles. (b) State diagram of a shift from a polymictic lake of intermediate depth to a seasonally stratified regime. Climate warming increases the duration of stratification events. When these periods become long enough to trigger a reduction in transparency, in some years there might be a sudden jump to a longer stratified period. This is most likely to occur in small lakes, where transparency has the strongest control on stratification patterns (blue line). If the turbidity is caused by sinking particles, sedimentation of these particles will result in clearer water and the breakdown of stratification. In larger lakes, or lakes where the positive feedbacks are only weak, the increase in duration of stratification will be more linear (green line). In a seasonally stratified regime, a reduction in transparency will not, or only marginally, increase the length of the stratified period.

Shift from a dimictic to a monomictic regime

Near the poles, loss of ice cover in deep lakes is likely to turn amictic lakes into cold monomictic lakes, and cold monomictic into dimictic lakes (Nõges et al., 2009). In temperate regions, it forces the two separate mixing events at the end of autumn and the end of spring into one period with a more-or-less uniform temperature profile; a shift from dimixis to monomixis (Ficker et al., 2017; Sharma et al., 2019).

For a freshwater lake of a given morphometry, weather conditions and water temperature determine whether ice forms or not (Leppäranta, 2015). Climate change drives the atmosphere towards warmer conditions, but due to natural variation in weather, perpetuation of ice-free conditions after one ice-free winter is unlikely unless water temperatures express a memory of previous winters. Such a memory might be established due to the large thermal heat capacity of deep lakes and a dominant effect of ice-albedo. Ice has a higher albedo than water (i.e. ice reflects more shortwave radiation), reducing heating of an ice-covered lake. When ice disappears, the surface water warms faster through absorption of solar radiation (Austin and Colman, 2007). A modelling study on the Laurentian Great Lakes under a prescribed weather cycle, atmospheric noise, and slow climatic forcing, showed that ice in deep lakes can prevent lake warming by its high albedo and promotes ice cover in following years (Sugiyama et al., 2017), which we define as a memory effect. Once ice disappeared, deep water layers warmed up to a larger degree, making it harder for water temperature to reach freezing levels in following years.

Only sufficiently deep lakes have the necessary thermal heat capacity to transfer the effect of ice cover to the next winter; Sugiyama et al. (2017) investigated lakes with an average depth of at least 50 m, but do not give a minimum depth required to generate a memory effect. Bi-stability occurred in ranges of annual mean air temperatures of *c*. 0.5°C (for lakes of 50 m depth) and *c*. 1.5°C (for lakes of 150 m depth). Outside of these ranges, the lakes were always ice-covered, or always ice-free, regardless of the ice cover in previous winter.

Apart from its higher albedo, ice insulates the lake from the atmosphere, limiting heat loss to the atmosphere in winter (Leppäranta, 2015; Zhong et al., 2016). This insulation works in an opposite direction as the ice-albedo feedback (Figure 3a), and it weakens the memory effect. The relative importance of both processes is still disputed, but Sugiyama et al. (2017) find a dominant ice-albedo feedback with a one- to three-column model. In contrast, at a higher spatial resolution, Zhong et al. (2016) and Ye et al. (2019) did not find a dominant memory effect and state that the role of ice albedo is small, suggesting a smoother transition from dimictic to monomictic with a warming climate. All three modelling studies were performed in a similar environment (the Great Lakes region) and actively investigated the role of ice-albedo in determining thermal structure. Similar to Zhong et al. (2016) and Ye et al. (2019), the modelling study of Shatwell et al. (2019) reported a gradual shift from ice-covered to ice-free winters in two lakes of 20-30 metres mean depth in Europe, rather than an abrupt shift. Other studies on the ice-albedo feedback and thermal memory in lakes are scarce. However, this topic has been addressed for sea ice; modelling studies suggest that the response of sea ice to atmospheric temperature changes might show lags, but is not truly bi-stable (Ridley et al., 2012; Li et al., 2013), and therefore a gradual loss of ice cover is to be expected.

In brief, although the role of ice albedo in lakes is still debated, it is generally considered that memory effects of ice cover in lakes have only a minor influence. In case of a dominant memory effect caused by a large thermal heat capacity and a dominant ice-albedo feedback, shifts in mixing regime between monomictic and dimictic lakes would have a sudden nature (Figure 3b, blue lines). If destabilising positive feedbacks are weak, a smoother transition from monomictic to dimictic is expected, with alternating dimictic and monomictic years (Figure 3b, green line). Both the ice-albedo feedback and the insulation effect could be relevant for the shifts from amictic to cold monomictic and from cold monomictic to dimictic as well. However, literature on these mixing regime shifts is limited.

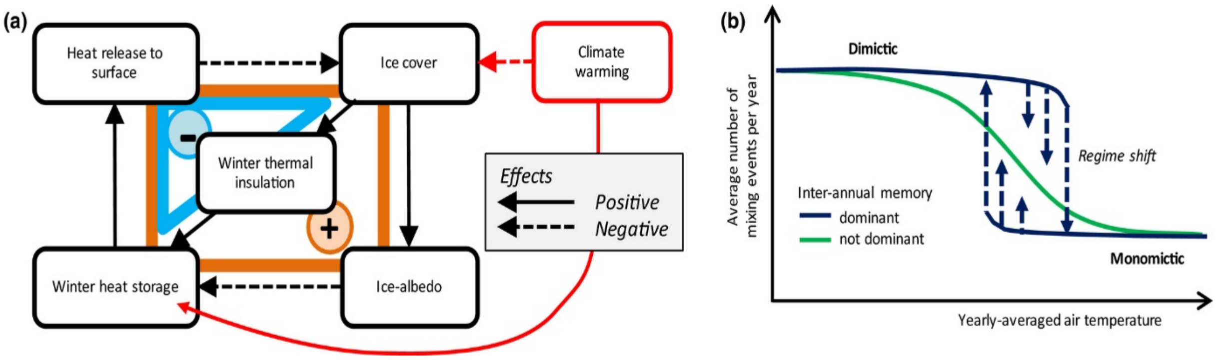

Figure 3: (a) The associated feedbacks for a shift from a dimictic to a monomictic regime. Solid arrows denote a stimulating (positive) effect and dashed arrows a reducing (negative) effect. The effect of climate warming is shown in red. Two feedback loops are formed. The blue feedback loop is negative (i.e. self-stabilising), while the orange loop is positive (self-reinforcing). (b) State diagram of a shift from a dimictic to a monomictic regime. Inter-annual thermal memory is supported by a large heat storage capacity and the positive feedback in (a). In case of a dominant memory effect, shifts in mixing regime would have a sudden nature (blue line and arrows), while if the memory effect is not dominant (green line), there is a smoother transition, with alternating dimictic and monomictic years. The length of the dotted arrows denotes the probability of a regime shift from one mixing regime to another.

Shift from a holomictic to an oligo- or meromictic regime

Some lakes that are (becoming) monomictic are experiencing less complete mixing events, and decreases in the maximum mixing depth as indicated by oxygen profiles (North et al., 2014; Saulnier-Talbot et al., 2014). Hydrodynamic models driven by climate scenarios predict this trend to continue, resulting in a progressively decreasing maximum mixing depth (Matzinger et al., 2007; Sahoo and Schladow, 2008; Schwefel et al., 2016). Complete mixing – i.e. top to bottom - can even disappear entirely and maximum mixing depth could decrease by up to 80% (Matzinger et al., 2007; Sahoo and Schladow, 2008). Perroud and Goyette (2010) predict a decrease in duration of fully mixed conditions for the peri-alpine Lake Geneva (Switzerland/France). These findings imply that complete mixing in monomictic lakes will decrease in the future and parts of the hypolimnion can stay isolated from the atmosphere for multiple years.

However, in oligomictic lakes there is an interplay between the increase in hypolimnetic temperature and the frequency of complete mixing events. The hypolimnion slowly heats over the year as a result of the geothermal heat flux and as warmer water from the epilimnion is gradually mixed into the deeper layers by turbulence. Incomplete winter mixing fails to cool the hypolimnion, resulting in a warming trend. This increase in hypolimnetic temperature facilitates complete mixing in subsequent years, as less cooling is required for an overturn. Additionally, the higher thermal expansivity of water at higher temperatures can increase the likelihood of complete mixing in lakes with elevated salt concentrations in the hypolimnion (Matzinger et al., 2006). When deep mixing finally occurs during a colder winter, hypolimnetic temperatures show a sudden drop and the resistance to full mixing in subsequent years would increase again. This is why this process is referred to as a sawtooth pattern

(Livingstone, 1993; 1997). This pattern has been observed in several deep lakes (e.g. Straile et al., 2003; Coats et al., 2006; Lepori and Roberts, 2015), and might facilitate sporadic overturn events even under milder temperatures. Climate warming is predicted to lengthen the warming periods and reduce the frequency of turnovers and subsequent cooling (Livingstone, 1997). In accordance with the sawtooth-pattern feedback, a decrease in the extent of winter mixing is expected during ongoing climate warming. However, if a new plateau in air temperatures is reached, the frequency of complete mixing is likely to return to its previous level as hypolimnetic temperatures catch up with winter temperatures, unless meromixis develops.

In both oligomictic and meromictic lakes, oxygen replenishment in the hypolimnion is strongly reliant on sporadically occurring winter mixing, and a reduced frequency of overturns means an increased likelihood of anoxia (Foley et al., 2012; Schwefel et al., 2016). As a secondary effect of the increased anoxia, internal nutrient loading and harmful effects for lake productivity and fish can be expected as a lake shifts from a holomictic to an oligomictic regime (O'Reilly et al., 2003). Internal loading in stratifying lakes changes the distribution of nutrients in the system, by increasing the concentration in the hypolimnion, but affecting the epilimnion only to a lesser extent. As eutrophication also increases oxygen depletion in the hypolimnion, eutrophication and climate warming both increase the risk of anoxia.

Model studies of deep-water mixing predict a reduced frequency of complete turnovers or even a complete disappearance, but as a gradual trend (Danis et al., 2004; Sahoo et al., 2013; Schwefel et al., 2016). The sawtooth pattern of hypolimnetic temperatures in oligomictic lakes facilitates deep mixing events after years with incomplete mixing (Livingstone, 1993). However, most of the studies above did not include an effect of solutes (i.e. salinity) on water density - a decisive factor in the formation of meromixis (Boehrer and Schultze, 2008; Camacho et al., 2017). Meromictic lakes have a denser, chemically different water layer below the hypolimnion, which is rarely mixed into the upper layers. Meromictic lakes can behave strikingly differently from thermally stratified lakes in terms of temperature profile, water renewal, chemistry, and ecology (Gulati et al., 2017; Lepori et al., 2018). The causes of meromixis are diverse (see Gulati et al., 2017), but a common characteristic is that the density difference is sustained. For example, in case of high iron concentrations below an oxic water layer, iron that is mixed into oxic water tends to precipitate and sink back into the anoxic water, where it dissolves again and maintains the density stratification (Boehrer and Schultze, 2008). Internal processes like this make meromixis generally a very stable mixing regime.

Endogenic meromixis is a form of meromixis that is sustained by decomposition and increased concentrations of dissolved substances by biogeochemical cycles in the deep water layer (for full explanation, see Boehrer and Schultze, 2008). This leads to the hypothesis of anoxia as a potential trigger for endogenic meromixis (Hutchinson, 1957; Julià et al., 1998). In this situation, the onset of anoxia sets off the formation of a heavier water layer by an increased build-up of solutes in the hypolimnion and complete mixing becomes too infrequent to distribute these solutes through the water column. This build-up of solutes suppresses further mixing and could cause a more abrupt and permanent formation of meromixis. In Lake Lugano (Italy/Switzerland), a large (49 km^2) and deep (maximum depth 288 m) per-alpine lake, endogenic meromixis might have formed as a result of anthropogenic eutrophication (Lepori et al., 2018). The possibility of meromixis caused by climate change has not been addressed often in scientific literature. Julià et al. (1998) mentioned climate-induced anoxia as a potential cause of meromixis in the Spanish Lake La Cruz. In a modelling study of the oligotrophic, monomictic Lake Ohrid (North Macedonia/Albania, maximum depth 289 m), Matzinger et al. (2007) found that above an atmospheric warming rate of 0.02°C/yr, hypolimnetic temperature increase would fall behind surface water warming rates. Additionally, solute accumulation in the hypolimnion would further increase the density of deep waters, preventing complete overturns in future scenarios (Matzinger et al., 2007). The increase in solute concentration must be strong enough to offset a reduction of density by hypolimnetic warming (Figure 4a). Several physical lake processes can mix surface water into the hypolimnion, even when a chemical gradient is present, therefore reducing density differences. Wind-induced internal waves increase turbulence around the thermocline, and therefore mixing between both layers (Imboden and Wüest, 1995). Differential cooling is the process where shallow areas experience fast convective cooling and this water mass moves downwards along the lake slopes in cases where morphology is suitable. This can lead to transport of water from the surface to deep water layers (Peeters et al., 2003; Ambrosetti et al., 2010). Intrusion of river inflow is another potentially important factor for deep-water renewal (Ambrosetti et al., 2010). Depth of river intrusion depends on inflow water temperature and lake thermal structure, amongst others (Fink et al., 2016). High sediment densities in the inflow can increase water density and allow penetration into the hypolimnion (Loizeau and Dominik, 2000; Fink et al., 2016). In lakes where these processes cause significant deep-water renewal, a shift to meromixis is less probable. Formation of meromixis due to climate warming is also not likely in dimictic or polymictic lakes where complete mixing is frequent, but could occur in monomictic lakes in multiple climate zones.

It is difficult to determine in which lakes a shift towards meromixis is most likely to occur. Prime candidates are lakes that did not experience prolonged periods of anoxia and incomplete mixing until now, but are susceptible to experience these with the effects of climate change. Deeper lakes in particular seem to be vulnerable due to their tendency to mix incompletely (Danis et al., 2004).

However, these are not the only factors, as also the chemical composition of the sediment and underlying bedrock determines the nature and quantity of compounds that can be released under anoxic conditions (Del Don et al., 2001; Boehrer and Schultze, 2008). Additionally, Matzinger et al. (2007) suggest that meromixis is stimulated under a high rate of warming, which puts lakes at risk that lie in parts of the world that are experiencing rapid warming. Altogether, the likelihood of formation of meromixis with climate change is not obvious, and lake-specific approaches are necessary to evaluate this risk. Modelling efforts such as the one by Matzinger et al. (2007) are a promising approach, which should be supplemented by lake-specific information on sediment release rates of major density-affecting solutes.

In summary, as a holomictic lake warms, the likelihood of incomplete mixing increases, especially under rapid warming rates (Matzinger et al., 2007). This creates an oligomictic regime, where the sawtooth pattern (Livingstone, 1997) may result in complete mixing in some years. This oligomictic regime increases the chance of anoxia and solute accumulation in the hypolimnion, potentially culminating in a sudden shift to meromixis (Figure 4b). Once a lake becomes meromictic, it might be difficult to reverse this change (Lepori et al., 2018). Lakes with the potential of strong solute release from the sediment and weak deep-water renewal are most susceptible to such a shift.

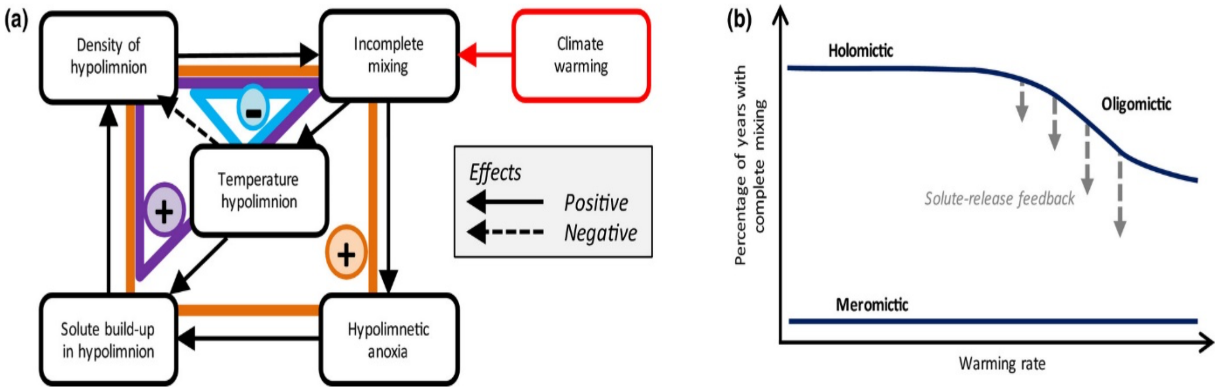

Figure 4: (a) The associated feedbacks for a shift from a holomictic to an oligo- or meromictic regime. Solid arrows denote a stimulating (positive) effect and dashed arrows a reducing (negative) effect. The effect of climate warming is shown in red. The negative (self-stabilising) feedback loop, in blue, causes a decrease in the density of the hypolimnion after incomplete mixing, while the positive (self-reinforcing) feedback loops, in purple and orange, stimulate a density increase. (b) State diagram of a shift from a holomictic to either an oligomictic or a meromictic regime. When incomplete mixing occurs as a result of warming, an oligomictic regime is formed. This increases the chances of forming anoxia and solute accumulation, potentially resulting in a meromictic regime if the density increase by solute build-up (positive feedbacks) exceeds the density decrease by hypolimnetic warming (negative feedback). The length of the dotted arrows denotes the probability of a regime shift from an oligomictic to a meromictic regime, but the possibility for this shift depends on lake-specific characteristics. A shift is most likely to occur in lakes with little deep-water renewal, where large quantities of solutes can be released from the sediment.

Conclusion

Climate change can cause shifts in the mixing regime of deep lakes, which will change their behaviour in terms of physics, chemistry, and biology. In this study, we investigated whether certain feedbacks could affect these regime shifts. The investigated potential shifts are from polymictic to seasonally stratified, from dimictic to monomictic, and from holomictic to oligo- or meromictic. All these shifts in mixing regime display reinforcing feedback mechanisms, but these feedbacks are likely to be relevant under certain conditions only. In lakes of intermediate depth, polymixis and seasonal stratification can be alternative states, based on transparency and wind sheltering. If a period of stratification can trigger a strong reduction in transparency, for example due to phytoplankton bloom formation or release of CDOM, a stratified regime can form. However, this feedback is valid only under a narrow range of lake conditions, and the stratified state is not carried over from year to year, which is a full reset occurs each year. Dimictic lakes can become monomictic due to loss of ice cover. One study found that a shift from dimixis to monomixis could show bi-stability because of the ice-albedo feedback coupled to thermal heat capacity in sufficiently deep (at least 50 m average depth) lakes, but other studies in lakes and the sea suggest the opposite. The majority of studies would predict only a minor influence of memory effect of ice cover on lake dynamics, and therefore a gradual shift from mostly ice-covered to mostly ice-free winters. A climate-induced shift from holomixis to meromixis can occur if a density increase of the deep water layer by solute build-up outweighs a density decrease due to hypolimnetic warming. Such a shift would have profound influences on aquatic ecosystems, but more research is needed on this topic to assess where and when this can happen. If incomplete mixing does not result in a net density increase of the deep waters, an oligomictic regime is formed.

Although climate, interannual temperature variation, and morphometry are the main determinants of a mixing regime, transparency, water level, and internal feedbacks can facilitate and stabilise shifts between mixing regimes. Without denying the value of studies investigating mixing regime shifts driven by warming alone, we hope this paper places those findings in the perspective that several other components of lake ecosystems can influence mixing regimes as well. A change in mixing regime can mean a step-change in a physico-chemical parameter (e.g. anoxia) that can feed back to the vertical density distribution or heat budget. If we consider these processes as well, we may get a better understanding of how climate change affects lake mixing regimes. Given the great importance of mixing regime for functioning of lakes, more knowledge on the likelihood of transitions and the stability of such changes would be important for lake management. Observations of shifts in mixing regime due to internal lake processes are important, but may be hard to realise due to the long timescales and required data involved. For each of the three mixing regime shifts studied in this paper, modelling approaches have made important contributions. Inclusion of biogeochemistry in modelling could lead to further advances when investigating the shifts polymictic-stratified and holomictic-meromictic. The study of feedback loops in lake processes has so far been focussed mainly on shallow lakes (e.g. Scheffer, 1998). Our review is one of the first studies to systematically explore the potential feedbacks occurring in deep lakes, as well as the climate dependency of these mechanisms.

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Author’s Contributions

All authors conceived the ideas and designed methodology; JPM collected the data; JPM analyzed the data; JPM led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Chapter 3

Early Warning Signals of Regime Shifts for Aquatic Systems: Can Experiments Help to Bridge the Gap Between Theory and Real-World Application?

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Chapter 4

Resource Availability Decouples the Linkage Between Engineering and Ecological Resilience

Julio A. A. Stelzer; Jorrit P. Mesman; Bastiaan W. Ibelings (2022) Resource availability decouples the linkage between engineering and ecological resilience. *In preparation*

Abstract

It has been proposed that reduced recovery rates from pulse perturbations may serve as a proxy for the loss of ecological resilience. Yet, this proposition may not hold when changes in resource availability affect community composition, directly impacting how ecosystems respond to environmental pulse perturbations. In terrestrial and aquatic systems, an increase in resource availability has often been associated with a consequent loss of ecological resilience. On the other hand, high resource availability is expected to foster recovery by producing favorable conditions for growth after pulse perturbations. This creates a paradoxical situation because short-term expectations may diverge from long-term trends in the system when associating recovery from pulse perturbations to a loss of ecological resilience. Here we used *Pseudomonas fluorescens* grown under different oxygen concentrations (key limiting resource) to address how resource availability modulates recovery patterns from a mortality pulse perturbation, looking at functional (cell density) and compositional (ecotype distribution) dimensions of the system. Systems with low resource availability displayed slower *recovery rates* and longer *recovery times* for both dimensions. Also, low resource availability created a condition of evolutionary stasis (limitation of adaptive radiation), making the composition of the systems mostly determined by priority effects. At high resource availability, we observed the opposite. *Recovery* was fast and temporal differences in fitness translated into a dynamic ecotype dominance during the recovery period. However, irrespective of resource availability, all the systems recovered to levels superior to 90% of the baseline condition in both compositional and functional dimensions (*asymptotic recovery)*, indicating substantial plasticity of the systems in absorbing and recovering from a pulse perturbation (sustaining its ecological state). Therefore, recovery from perturbation may become a poor proxy of ecological resilience if resource availability drives system change, because resources modulate the recovery *rate* and *time* but not necessarily the level of the *asymptotic recovery*.

Keywords: Resilience, recovery, resource availability, pulse perturbations, Pseudomonas fluorescens, eco-evolutionary dynamics, early-warning signals.

Introduction

Different metrics of recovery from pulse perturbations have been proposed as indicators that a biological system is losing resilience (Dai et al., 2013, van Nes and Scheffer, 2007). The longer the *recovery time* or weaker the *recovery rate* after perturbation (*engineering* resilience - *sensu* Pimm (1984)), the lower is the resilience of the system (*ecological* resilience – *sensu* Holling (1973)). However, resource availability directly affects community composition (Tilman, 1994), which may impact how ecosystems respond to environmental perturbations. In shallow lake ecosystems, high resource availability for a prolonged period of time (eutrophication) may push them towards a turbid cyanobacteria-dominated state with loss of aquatic biodiversity and impaired ecosystem functioning (Scheffer, 2004). In terrestrial systems, high resource availability also has been shown to reduce plant biodiversity with consequent loss of ecosystem functioning in long-term studies (Harpole et al., 2016, Isbell et al., 2013). In both situations, the long-term increase in resource availability (e.g., nutrients) is associated with the reduction in biodiversity and consequent loss of *ecological* resilience (*sensu* Holling, see Gunderson (2000)). On the other hand, resource availability is also expected to play a direct role in the recovery from short-term pulse perturbations, the typical scenario in which *engineering* resilience (*sensu* Pimm) is quantified. One primary functional aspect of recovery is to reconstitute density levels of the system close to the pre-perturbation condition through individual populational (re)growth (Arnoldi et al., 2018) – a function in which resource availability is paramount. High levels of resource availability tend to increase the rates of recovery (see Jentsch and White (2019)) by creating favorable conditions for fast population regrowth immediately after the pulse event (Shade et al., 2012, Haddad et al., 2008). Moreover, high resource availability may reduce the burden of overall metabolic costs employed on specialized traits used for resource competition, which often occur at expenses of energy allocated for growth. With these aforementioned processes combined, systems with high resource availability would be expected to recover faster. This creates a paradoxical situation when linking recovery from pulse perturbations to losses of ecological resilience; because short-term expectations (i.e., faster recovery rates and high engineering resilience) may diverge from long-term trends (i.e., loss of *ecological* resilience) in the system (Arnoldi et al., 2018, Hastings, 2010).

A number of studies investigated the inference that the rates of recovery from pulse perturbations can be translated into an early-warning signal for loss of *ecological* resilience, either by comparing systems in time (e.g., Veraart et al. (2011), Drake and Griffen (2010)) or space (e.g., Dai et al. (2015), Sirota et al. (2013)). The validity of this inference showed conflicting results that escalated with the ecological complexity of the studied system (see Stelzer et al. (2021)). As more trophic levels and organismal interactions were included in the experimental designs, lower the reliability of the above-mentioned inference. A valid argument is that assessing and comparing *recovery rates* in complex ecological networks is entangled with a plethora of ecological processes from organismal to ecosystem level that might be hard to translate using a single indicator (i.e., *recovery rates*) (Scheffer et al., 2015). Hence, studies of recovery from pulse perturbation using complex biological assemblages (e.g., mesocosms) are often approached from two distinct perspectives, a functional one that prioritizes the recovery in functions operating at the community level (e.g., recovery in community biomass) and a compositional one that prioritizes the recovery of the individual populations within the community. These two perspectives have been shown to result in far distinct recovery processes and assumptions of state of recovery (Hillebrand and Kunze, 2020), making the selection of a single attribute of the system as a proxy for whole system behavior a challenging task (see Spears et al. (2017)). Nevertheless, for inferences at the population level, recovery from pulse perturbation has shown so far reliable relations between *engineering* and *ecological* resilience under similar resource availability conditions (e.g., Dai et al. (2015), Veraart et al. (2011)).

The study of recovery from experimental pulse perturbations has been described as an appropriate model for simulating ecological responses to many natural disturbances, such as floods, forest fires, and disease outbreaks; and has been widely applied in experimental ecology (Arnoldi et al., 2018, Haddad et al., 2008). Unlike other statistical indicators that requires long-term monitoring data to show trends in *ecological resilience* (e.g., moving averages, autocorrelation, and variance), recovery from pulse perturbations may only require a few intervals of high-frequency data for it. Thus, this approach may become a valuable tool to investigate potential ecological disruptions caused by changes in environmental conditions where long-term data is nonexistent or unfeasible to implement. However, before being able to bridge theory and application - and given the empirical evidence that the correspondence between recovery metrics and *ecological resilience* may fail in complex ecological networks, together with the theoretical prediction of a paradoxical interaction between *engineering* and *ecological* resilience due to changes in resource availability - we would require a mechanistic understanding of how different levels of resource availability affect recovery from pulse perturbations in isolation from other environmental pressures.

Pseudomonas fluorescens grown in static (unperturbed) microcosms has been successfully and extensively used as an experimental eco-evolutionary model system (McDonald et al., 2009, Bantinaki et al., 2007, Spiers et al., 2003, Rainey and Travisano, 1998). It has contributed to research on biodiversity (Loudon et al., 2015, Rainey and Travisano, 1998), the intermediate disturbance hypothesis (Buckling et al., 2000), and interspecific niche competition (Chu et al., 2021), among others. What controls the *P. fluorescens* experimental model is the need for oxygen, which becomes only available at the Air/Liquid interface (AL-interface) if kept static (Koza et al., 2011). The lack of mixing results in oxygen depletion in the broth, enforcing selection towards evolution strategies that enable oxygen uptake at the AL-interface. Therefore, any ecotype in the system that evolved from the single ancestor would have to represent an adaptation towards functional traits that allows them to either compete or cooperate towards oxygen uptake at the AL-interface (Rainey and Rainey, 2003). Comparing systems that have evolved from a single ancestor and are fully adapted to their environmental conditions guarantees that no other major perturbations are pressuring the system when the pulse perturbations take place. This makes *P. fluorescens* an ideal empirical model to unbiasedly assess how community-alike responses to pulse perturbations are shaped by different levels of resource availability – a scenario virtually impossible to isolate in other types of experiments.

Here we grew *P. fluorescens* in microcosms experiencing different oxygen concentrations to address how resource availability can modulate recovery patterns from a pulse perturbation. We tested the hypothesis that low resource availability would reduce the *recovery rate* and increase the *time* needed for the system to recover compared to when resource availability was high. We associated these indicators with “the measure of *the persistence of systems and of their ability to absorb change and disturbance and still maintain the same relationships between populations or state variables*" – the formal definition of ecological resilience (Holling, 1973) – by assessing changes in the capacity of the systems in achieving recovery levels compared to the pre-perturbation condition of the systems (*asymptotic recovery*) in its functional and compositional dimensions. Further, we discuss whether the use of recovery metrics to compare systems with different resource conditions is a coherent ecological approach from the perspective of *ecological resilience*.

Materials and Methods

Ecology within the *P. fluorescens* experimental model

The experimental model starts from a single ancestor (ecotype) that builds up diversity by adaptive radiation over different mutation pathways (Lind et al., 2015, McDonald et al., 2009, Spiers et al., 2003) in response to the environmental conditions and ecological interactions. The novel habitat is promoted by the formation of an acetate cellulose biofilm (mat) in the AL-interface, which supports multiple ecotypes with different functional traits. The *P. fluorescens* mat is composed of three main ecotypes: the Smooth morphotype (SM), ancestor type charceterised by fast growth rates; Wrinkly Spreaders (WS), responsible for the structural productiontion of the mat used to colonize the AL-interface; and Fuzzy Spreaders (FS), who have a low oxygen demand and form rafts below the mat.

This simplifies much more complex interactions at the genotypic level, which are unnecessary at the '*recovery from perturbation'* scope of this study (an advanced descriptive of the ecotypes is given in Ferguson et al. (2013)). The formation of the biofilm involves the evolution of cooperation – *de novo* and in real-time – from an ancestral state that is asocial and unicellular (SM, Rainey and Kerr (2010), Rainey and Rainey (2003)). The eco-evolutionary dynamics of the mat follow the tragedy of the commons (Hardin, 1968), where SM increase their fitness based on the exploitation of adaptive traits from its WS competitor, reducing the overall adaptability of the system (Rainey and Kerr, 2010). The system also includes facilitation with SM fostering the fixation of FS through its competition against WS (Ferguson et al., 2013). Ultimately, the diversity in the system is well-known to be maintained through negative frequency-dependent selection (Spiers et al., 2002) and built on kin selection (Rainey and Rainey, 2003). Such a simple system evolving from a single ancestor with many processes of ecological relevance and complex evolutionary feedbacks makes *P. fluorescens* unique for debugging ecological insights.

Microcosms foundation and sampling

Pseudomonas fluorescens SBW25 wild type (SM ecotype) was grown in standard microcosms as described by Rainey and Travisano (1998). Briefly, 5 mL of KB liquid broth (initial pH ≈ 7.2 ± 0.2) was dispensed into glass vials and inoculated with ca. 10^7 *P. fluorescens* CFU (Colony Forming Units) to rapidly achieve carrying capacity. The experiment was conducted under static conditions at 28^o C to allow adaptive radiation under three different oxygen atmospheres (levels of resource availability). For this, vials were placed inside gas-tight Perspex incubators and continuously flushed with nitrogen, air, or pure oxygen at 0.5 L min^{-1} (respectively "Low", "Control", and "High" resource availability treatments). Note that even when the chamber was flushed with nitrogen, residual oxygen was present in the system (i.e., dissolved oxygen in the broth, vial headspaces, and when opening the chamber for sampling); therefore, no treatment was anoxic. Destructive sampling of replicate vials occurred daily on the 7 days preceding the pulse perturbation (described below) and at 0, 8, 15, 30, and 48h after the pulse perturbation to assess recovery of CFU concentration and ecotype diversity. Serial dilutions were made in 96-well plates and the plating volume varied from 20 – 100 µL, depending on the treatment and age of the cosm. Plating was done by the "Copacabana Method" using 0.3 mm sterilized glass beads to evenly spread the bacterial community across 1.5% Agar KB plates. Plates were incubated for 48h at 28^oC under atmospheric air conditions before counting their ecotypes using a stereoscopic microscope. The experiment ran for 216 hours and sampling was done in triplicate.

Mortality pulse perturbation and the effect of resource availability on the recovery

To quantify the different aspects of recovery showed by the ecotypes and total CFU concentration in the microcosms under different atmospheric conditions, we induced a 98% non-selective mortality event. Mortality was mimicked by destroying the bacterial cellulose mat through vortexing and transferring 2%v/v of the system to fresh media. As quantitative metrics of recovery we estimated: (i) the time needed for the system to reach 50% of its maximum recovery potential *(recovery time)*, (ii) the maximum level of recovery the system reached after perturbation *(asymptotic recovery),* and (iii) the parameter controlling the maximum rate of the recovery following the pulse perturbation *(recovery rate)*. For this, a baseline was defined as the period between the fixation of the three main ecotypes (SM, WS, and FS) and the moment just before the pulse perturbation. Within this window, the $\log_{10}$ concentration of SM, WS, and total CFU were averaged and used as the '*100%-recovery*' reference point. For defining the '*0%-recovery*' reference point, samples were taken in triplicate immediately after the pulse mortality event and averaged for each ecotype and the total CFU concentration as above. The concentrations of SM, WS, and total CFU were then normalized using this *0-100% scaling*, and the metrics of recovery were assessed as a '*% of recovery compared to the baseline*'. This approach allows the comparison of recovery metrics from systems with different carrying capacities caused by distinct resource availability. Metrics of recovery were not calculated for FS since their negligible concentration compared to SM and WS caused a stochastic appearance in the timeseries.

Experimental modeling of recovery metrics

Recovery time, *asymptotic recovery*, and *recovery rate* were calculated by fitting logistic regression models to the timeseries describing their recovery trajectories. For that, we used the package Aomisc (Onofri, 2020) in the software R (R Core Team, 2020). The logistic regression used is shown in Figure 1, and each parameter can be treated as a metric of recovery.

"Y" is the *% of recovery compared to the baseline* reference value, while "X" is the *time after perturbation* (hours). The inflection point "e" represents the end of the exponential recovery and beginning of logarithmic recovery, and as later it happens in time, slower is the process of recovery (*recovery time)*; the higher asymptote "d" represents the plateau where the system stabilizes after recovery, therefore, determining to which extent (%) the system recover compared to the pre-event condition (*asymptotic recovery)*; the parameter controlling the slope at the inflection point "b" modifies the maximum rate of recovery in the system, and for mathematical reasons, the lower the value, the faster the recovery is (*recovery rate)*. The lower asymptote "c" represents the starting point of the recovery process, which is equal for all the cosms (0%), and therefore, is not informative as a recovery metric in this study. The estimates of each recovery metric were plotted as density distributions using a gaussian curve.

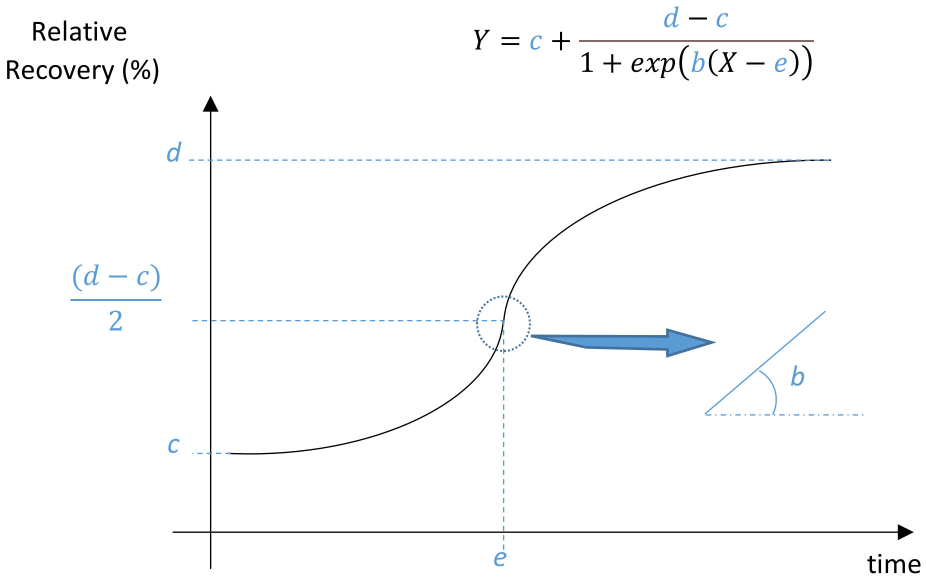

Figure 1. Application of logistic regression models for assessing recovery from pulse perturbations. d=higher asymptote; c=lower asymptote; e=recovery time; b=recovery rate.

Results

As expected, the three main *P. fluorescens* ecotypes arose through evolution within three days of microcosms foundation and showed a clear and consistent baseline between 72h and 168h (Figure 2, *left*). The bacterial concentration immediately after the pulse perturbation was also compatible with the 98% mortality event as expected and was successfully used to calculate the *0%-recovery reference*. All treatments and ecotypes showed recovery superior to 90% of the values observed in the baseline period by the end of the experiment, but the three treatments showed different recovery patterns depending on their oxygen resource availability (Figure 2, *right*).

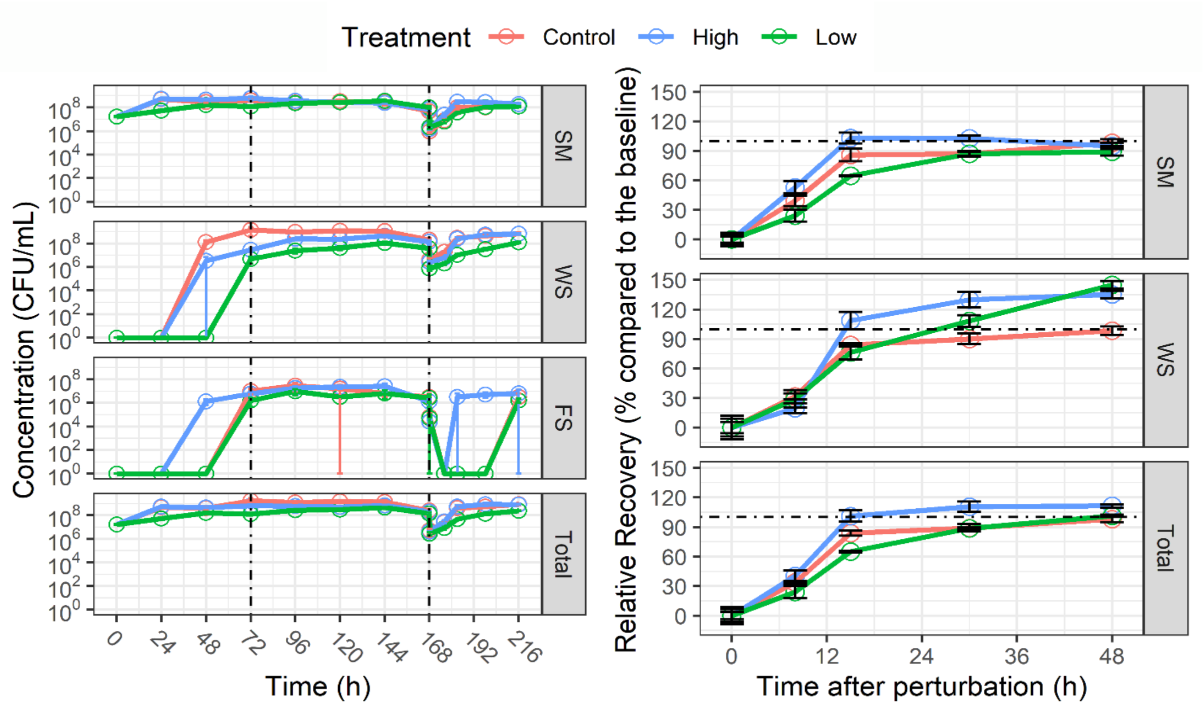

Figure 2. Timeseries of the different ecotypes exposed to different resource availabilities during the pulse perturbation experiment. Left: Concentrations (CFU/mL) of the three ecotypes (SM, WS, and FS) and the total CFU concentration in the microcosms. The period between the vertical black lines determines the baseline period. Right: The relative recovery compared to the baseline, where time=0 represents the period immediately after the mortality pulse perturbation, and the horizontal dash-dotted line denotes 100% recovery. Error bars represent the mean ± 1 S.E.M.

The metrics of recovery were quantified from the functional (total CFU concentration) and compositional dimension (individual ecotypes) since a recovery in function does not necessarily imply a recovery in composition. For both dimensions, low resource availability produced remarkably different patterns of response to the pulse perturbation compared to the other treatments. Low resource availability increased the *recovery time* after perturbation and weakened the *recovery rate* (Figure 3). Also, the microcosm community under low resource availability showed fewer changes in community composition since the microcosm foundation (evolutionary stasis, Figure 4). Under high resource availability, the system recovered with a stronger response to the pulse perturbation, in less time, and to a greater level than when resources were low (Figure 3). The *asymptotic recovery* of microcosms under high resource provision reached levels that surpassed the baseline condition for both ecotypes and total CFU concentration (complete recovery). However, the high resource availability under pure oxygen did not change the *recovery time* or *recovery rate* of the system as a whole (functional dimension) compared to the control treatment while changing its compositional recovery (recovery from the individual ecotypes) (Figure 3). High resource availability increased the turnover rate after the pulse perturbation, with different ecotypes dominating different periods of the recovery (Figure 4). The recovery was initially led by SM, who dominated the system at the early stages of recovery, afterward slowly being overtaken by WS, finally returning to the pre-disturbed community composition. This process was distinguished from all other treatments. The *asymptotic recovery* of the ecotypes (compositional) for the control and low nutrient condition showed contrasting behaviors. SM recovered to levels close to the baseline in the control treatment (Figure 3,*c*), while WS ecotype recovered to a level higher than the baseline condition when resource availability was low (Figure 3,*f*). Despite the reduced rate and longer time needed to recover the systems under low resource availability, these showed similar *asymptotic recovery* compared to the control from a functional perspective (Figure 3,*i*).

The steep recovery rate of the SM ecotype under high resource availability resulted in an imprecise "b" parameter (Figure 3,*a*). The recovery happened too fast (less than 15h), causing most sampled points to be either within the lower or at the higher asymptote. Due to the low number of sampling points during the log-lag trajectory of recovery (binomial behavior of the curve), the standard deviation was high, resulting in a flat probability distribution of the “b” parameter. Nonetheless, the visual inspection of the logistic regression and statistical output of the model converged towards a strong *recovery rate* compared to the other treatments (supplementary material). All the logistic regression models showed good representativity of the empirical data, with pseudo-R^2>0.9 (Ecotypes vs. treatments).

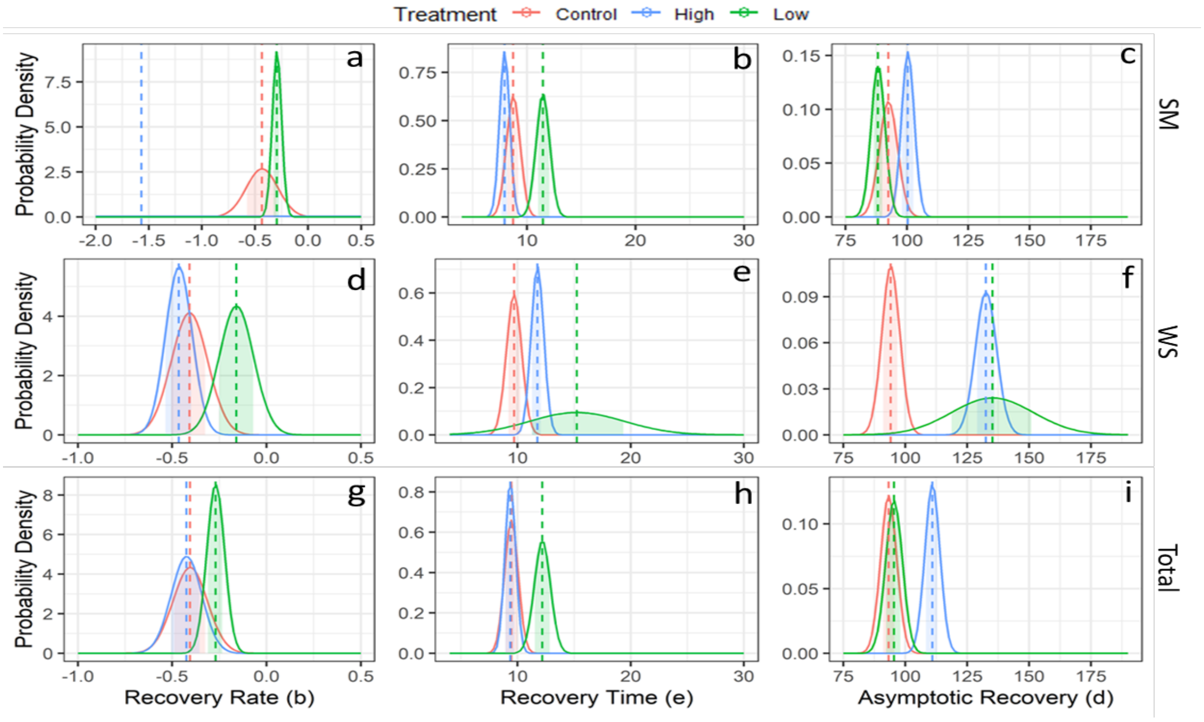

Figure 3. Probability density distribution of the logistic regression parameters used as metrics of recovery from a single mortality pulse perturbation. Treatments refer to the 3 different levels of resource availability; SM and WS are the dominant ecotypes, and Total refers to the system as a whole (WS, SM, and FS). Vertical dashed lines represent the mean parameter distribution and the shaded area ± 1 s.d. X-axis units: Recovery rate = 1/hour; Recovery time = hours; Asymptotic Recovery = % recovery compared to the baseline.

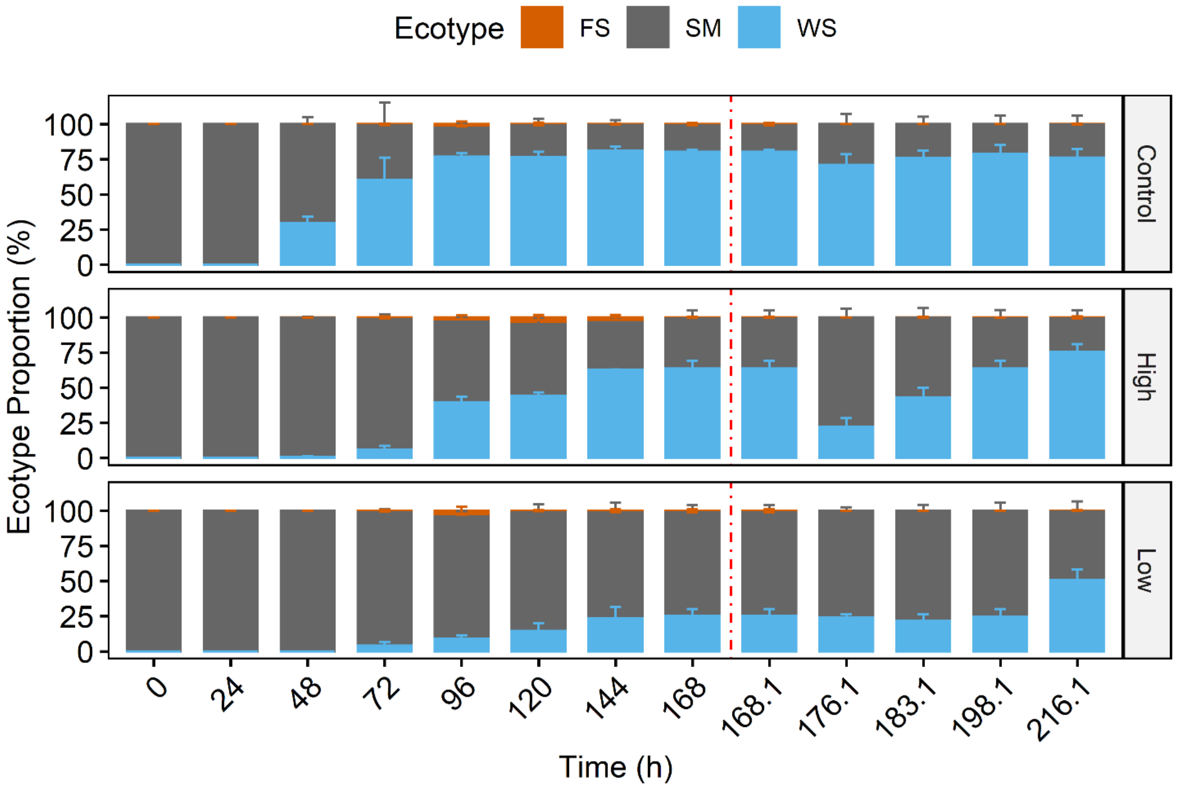

Figure 4. Ecotype distribution during the experiment. Control, high and low are the levels of resource availability, and FS, SM, WS are the three ecotypes. The vertical dashed line in red represents the moment of the mortality pulse perturbation. Error bars are mean ± 1 S.E.M.

Discussion

Oxygen is vital for the development of *P. fluorescens* (which is strictly aerobic) but only available at the AL-interface through atmospheric diffusion. The adaptive radiation of *P. fluorescens* in static microcosms starts when dissolved oxygen is depleted in the broth (Buckling et al., 2000, Kassen et al., 2000, Rainey and Travisano, 1998) and mutants evolve new traits allowing oxygen uptake at the AL-interface. This induces a strong selective pressure towards traits that allow the microcosm community to interact for the resource uptake at this layer. By manipulating the oxygen availability at the AL-interface, we indirectly modeled the nature and intensity of the ecological interactions during the microcosm development, resulting in distinct ecotype distributions. Comparing systems that have evolved from a single ancestor under different resource availability creates an unique situation where all ecotypes are fully adapted to the environmental conditions, ensuring that no other major perturbations were pressuring the systems when the mortality pulse perturbation took place (i.e., sustaining biodiversity levels and composition that would not occur naturally at a given resource availability scenario). This approach allows for an unbiased understanding of how community-alike responses to pulse perturbations are shaped by different levels of resource availability *per se*, instead of assessing the response of communities that are already struggling to accommodate changes in resource availability (e.g., multi-stressors) – This situation, despite fundamental, is virtually impossible to isolate in other types of experiments.

The different patterns of recovery in ecological systems are often attributed to three factors that are frequently entangled: the level of resources available for recovery after the pulse perturbation, the strength and complexity of ecological interactions, and the community composition at the time of perturbation. The perturbation in the systems with low resource availability resulted in weaker *recovery rates* and longer *recovery times* for both compositional and functional dimensions. We attribute this behavior to the simple fact of lacking sufficient resources for basic metabolic needs (Brown et al., 2004, Enquist et al., 2003). For instance, the bacterial cellulose produced by WS is metabolically expensive (Knight et al., 2006, MacLean et al., 2004, Rainey and Rainey, 2003), requiring energy that could potentially be allocated to growth (Spiers, 2007). When resource limitation was severe, the recovery of WS was delayed, also delaying the recovery of SM who depends on the WS mat formation to colonize the AL-interface and uptake oxygen for its own metabolic needs. The combination of poor recovery from both ecotypes under low resource availability (compositional recovery) resulted in a late and weaker functional recovery . Such processes exemplify the importance of ecological interactions as facilitation during the recovery period, even when organisms are predicted to compete for resources after reaching the carrying capacity of the system. Low resource availability also created a condition of evolutionary stasis during the adaptive radiation, which remained throughout the perturbation period. Despite the clearly adaptive traits of WS for mat formation at the AL-interface allowing enhanced oxygen uptake, the SM ecotype remained dominant before the pulse perturbation when resources were low . We believe this pattern was created due to the reduced fitness advantage provided by the mat production when oxygen is also scarce at the AL-interface (insufficient returns on the expensive investment in bacterial cellulose production). This may have created an environment where the differences in fitness between SM and WS were very low (see Hubbell (2001)), potentially resulting in a microcosm composition where priority effects play a larger role (Zhou et al., 2014).

Systems where priority effects mainly rule the process of recovery from pulse perturbations are expected *per se* to be vulnerable to ecological drift (random responses) due to the lack of a clear successional pathway for compositional recovery (Cairns et al., 2020). In addition, systems with low resource availability took longer to recover, making them susceptible to any increase in the frequency of pulse perturbations. If sequential pulse perturbations hit the system in a frequency higher than the period needed for recovery, the system may produce cumulative additive effects capable of permanently changing the ecological state of the system (see Dai et al. (2013)). This process is of increased relevance since stochastic pulse perturbations like weather-related events are predicted to increase within the near future (Woolway et al., 2021, Bell et al., 2018, Harris et al., 2018). In a broader scenario, the combination of these processes may culminate into fragile systems vulnerable to ecological drifts caused by sequential stochastic pulse events.

At high resource availability the patterns of recovery were far distinct. The systems showed a stronger response to the pulse perturbation, recovering in less *time*, and with more complex compositional dynamics. The adaptive traits that conferred WS dominance during the baseline period were not enough to suppress the fast growth of SM towards the carrying capacity of the system after the pulse perturbation. This caused an inversion in community composition from a WS-dominated to a SM-dominated system during the early recovery period, which also resulted in the functional dimension of the system recovering before the compositional one . Close to reaching the carrying capacity, dissolved oxygen depletes fast in the broth and the fast growth trait of SM stopped being an ecological advantage, resulting in loss of dominance to the highly adapted WS. This is the opposite scenario from the one observed at the low resource availability, where priority effects may have partially determined the composition of the system. At high resource availability the composition of the system was determined by resource competition , where adaptive traits resulted in temporal differences in fitness that were translated into the transient dominance of the ecotypes in different periods of the recovery phase. Under air, where limited amount of resources were diffusing from the atmosphere (Loudon et al., 2015), competition between ecotypes may have been more severe than under pure oxygen. With fewer resources, WS was capable to maintain its baseline dominance during the whole recovery period, confirming its increased fitness for uptaking oxygen at the AL-interface.

Despite the different processes involved in the compositional recovery at high resource availability and the control, both showed similar functional *recovery rates* and *times*. This provides empirical evidence that assessing process rates uniquely from a functional perspective may hide important information needed to mechanistically understand how a system recovers from pulse perturbations (Arnoldi et al., 2018). Also, the estimates of *asymptotic recovery* indicated that a complete functional recovery does not necessarily mean a complete compositional recovery of all parts of the system. In this situation, a biased functional perspective may consider the system fully recovered while, in fact, it is still far from returning to its pre-disturbance community composition (Hillebrand and Kunze, 2020). Such differences in perspective may become an inherent issue *per se* when using recovery rates as a metric of ecological resilience, since subjective choices may jeopardize objective assessments. The recovery trajectories of the systems were modulated by resource availability but determined by the type and strength of ecological interactions. Therefore, the role of biotic interactions proved to be fundamentally important to generate a more comprehensive, mechanistic-level understanding of how systems respond to pulse perturbations under different levels of resource availability.

Overall, higher levels of resource availability resulted in faster *recovery rates* and shorter *recovery times.* However, all the systems tested here showed *asymptotic recovery* superior to 90%, indicating substantial plasticity of the system in absorbing and recovering from a pulse perturbation (complete recovery). The slower recovery process caused by low resource availability did not change "the measure of *the persistence of the system"* or *"their ability to absorb change and disturbance and still maintain the same relationships between populations or state variables*" – namely *ecological* resilience (Holling, 1973). Therefore, we have to be especially careful using recovery from perturbation as a proxy of ecological resilience if resource availability is the main driver of ecosystem change. When dealing with systems of different resource availability, *ecological* (*sensu* Holling) and *engineering* resilience (*sensu* Pimm) may potentially decouple in time, misguiding interpretation of the results (Hastings, 2010). This is because the levels of resource availability modulated the speed of the recovery process (*rate* and *time*) but not necessarily the level of recovery. The use of recovery metrics to address different levels of *ecological* resilience between systems with different resource availabilities - in order to compare them - may be biased by the simple fact that systems with low resource availability will inherently respond through slower processes. Still, those slower processes might be compatible with the energy budget stored in the system and the rates of energy flow involved in basic metabolic needs and ecological interactions; therefore, not compromising the capability of the system to recover and sustain its state.

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Author's Contributions

JAAS, BWI conceived the ideas and designed methodology; JAAS collected the data; JAAS, JPM analyzed the data; JAAS led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Chapter 5

Phytoplankton Responses to Repeated Pulse Perturbations Imposed on a Trend of Increasing Eutrophication

Julio A. A. Stelzer; Jorrit P. Mesman; Alena S. Gsell; Lisette N. de Senerpont Domis; Petra M. Visser; Rita Adrian; Bastiaan W. Ibelings (2022) Phytoplankton responses to repeated pulse perturbations imposed on a trend of increasing eutrophication. **Ecology & Evolution**, n.a, https://doi.org/10.1002/ece3.8675

Abstract

While eutrophication remains one of the main pressures acting on freshwater ecosystems, the prevalence of anthropogenic and nature-induced stochastic pulse perturbations is predicted to increase due to climate change. Despite all our knowledge on the effects of eutrophication and stochastic events operating in isolation, we know little about how eutrophication may affect the response and recovery of aquatic ecosystems to pulse perturbations. There are multiple ways in which eutrophication and pulse perturbations may interact to induce potentially synergic changes in the system, for instance, by increasing the amount of nutrients released after a pulse perturbation. Here, we performed a controlled press and pulse perturbation experiment using mesocosms filled with natural lake water to address how eutrophication modulates the phytoplankton response to sequential mortality pulse perturbations; and what is the combined effect of press and pulse perturbations on the resistance and resilience of the phytoplankton community. Our experiment showed that eutrophication increased the *absolute* scale of the chlorophyll-a response to pulse perturbations but did not change the *proportion* of the response relative to its pre-event condition (resistance). Moreover, the capacity of the community to recover from pulse perturbations was significantly affected by the cumulative effect of sequential pulse perturbations but not by eutrophication itself. By the end of the experiment, some mesocosms could not recover from pulse perturbations, irrespective of the trophic state induced by the press perturbation. While not resisting or recovering any less from pulse perturbations, phytoplankton communities from eutrophying systems showed Chlorophyll-a levels much higher than non-eutrophying ones. This implies that the higher absolute response to stochastic pulse perturbations in a eutrophying system may increase the already significant risks for water quality (e.g., algal blooms in drinking water supplies), even if the relative scale of the response to pulse perturbations between eutrophying and non-eutrophying systems remains the same.

Keywords: Eutrophication, Microbial ecology, Preventive management, Pulse perturbation, Recovery, Resilience.

Introduction

Perturbations are an inherent phenomenon of any socio-ecological system and can have far-reaching consequences for the state of such systems. Perturbations can be pragmatically divided into press and pulse perturbations (Bender, 1984). Pulse perturbations are relatively instantaneous alteration of the physicochemical or biotic parts of the system that dissipates shortly after (e.g., storms), allowing the system to go back to its pre-perturbation state if the main features of the system are preserved (e.g., species composition, habitat structure). This bouncing dynamic caused by a focal and transient perturbation is often refereed as "*engineering resilience*" (*sensu* Pimm, 1984). Press perturbations, on the other hand, are sustained alteration that does not dissipate or leave the system (e.g., eutrophication), forcing the system to adapt in a way to accommodate this novel pressure, often by assuming a slightly different equilibrium. When the system fails in doing it so, the increase in environmental pressures forces the ecosystem towards an abrupt and persistent change in state over prolonged timescales. This dynamic of sustaining or shifting states is refereed as "*ecological resilience*" (*sensu* Holling (1973)). Both antagonist frameworks of "*resilience*" may co-exist into a press and pulse framework, potentially aiding our understanding of how complex systems respond to the interaction of multiple perturbations.

Aquatic systems sustain a multitude of fundamental ecosystem services that are vulnerable to perturbations. Drinking water supply, irrigation, and recreation are known to be temporarily disrupted by pulse perturbations of different natures as caused by extreme weather events (WHO 2011; Khan *et al.* 2015; Ummenhofer & Meehl 2017), waterborne diseases (Cann *et al.* 2013), and chemical spills (Sengul *et al.* 2012; Anenberg & Kalman 2019). Although the most variate stochastic events have always impacted ecosystems, the frequency, intensity, and duration of weather-related pulse perturbations are predicted to increase in a changing world (Bell *et al.* 2018; Harris *et al.* 2018; Woolway *et al.* 2021). Alongside, despite efforts to control eutrophication - one of the main freshwater press perturbations deteriorating water quality -, its impact on ecosystems is also expected to rise due to climate change (Carr & Neary 2008; World Water Assessment Programme 2009; Fink *et al.* 2018; Ho, Michalak & Pahlevan 2019). Eutrophication may enhance harmful cyanobacterial blooms (Paerl & Huisman 2009; Huisman *et al.* 2018), alter long-term ecological stability (Chapin *et al.* 2000; Rosset *et al.* 2014), and modify the structure of food webs (e.g., Alexander, Vonlanthen and Seehausen (2017); van der Lee *et al.* (2021)) resulting in substantial ecological, social and economic losses (Hoagland *et al.* 2002; Dodds *et al.* 2009). With the aforementioned escalation in eutrophication combined with more prevalent stochastic pulse perturbations, water security is becoming an increasing concern as recognized by the United Nations' sustainable development goals (UN 2020).

There are multiple ways in which pulse perturbations and eutrophication as a press perturbation may interact. For instance, pulse perturbations may enforce stochastic mortality events, which initially reduces populational densities but also enhances dissolved nutrient concentrations due to cell lysis and increased turnover, promoting strong subsequent growth of primary producers (Haddad *et al.* 2008; Jacquet & Altermatt 2020). When coupled with ongoing eutrophication, the amount of dissolved nutrients in the ecosystem that can be incorporated by primary producers to fix biomass increases, possibly increasing the peak response of the phytoplankton community in response to the combined effect of press and pulse perturbations.

While pulse perturbations trigger transient events, their consequences may become permanent at the ecosystem level (Scheffer *et al.* 2001; Harris *et al.* 2018). The distinction between transient and permanent responses to pulse perturbation is dependent on the capability of the system to recover from disturbance, which is often associated with (i) the compositional and functional structure of the ecosystem (Hillebrand & Kunze 2020; Thayne *et al.* 2021), (ii) the legacy effect from repeated perturbations (Johnstone *et al.* 2016; Ryo *et al.* 2019), as well as with (iii) the rates of energy flow to higher trophic levels (Shade *et al.* 2012b; McCauley *et al.* 2018). Eutrophication may modify these aspects by inducing changes in community composition (Rigosi *et al.* 2014; Rosset *et al.* 2014) and functional structure (Jochimsen, Kummerlin & Straile 2013; Moody & Wilkinson 2019; van der Lee *et al.* 2021), which in the long run may have important implications for how an ecosystem responds to pulse perturbations. Many studies have posed that the long-term changes in community-level responses such as changes in species richness, composition, and/or dominance, directly or indirectly caused by press perturbations like eutrophication, can potentially be amplified by the effect of extreme weather events (Smale & Wernberg 2013; Boucek & Rehage 2014; Bertani, Primicerio & Rossetti 2015; Arens & West 2016), sometimes with catastrophic consequences for the conservation of ecosystems (Coumou & Rahmstorf 2012; Harris *et al.* 2018).

Despite all our knowledge on the effects of freshwater eutrophication and stochastic events operating in isolation, we know little about how eutrophication as a press perturbation affects the response and recovery of aquatic ecosystems to pulse perturbations. Stochastic pulse perturbations are often short-lived and challenging to observe in high resolution, unlike press perturbations that are persistent over time and practical to assess as part of long-term monitoring campaigns (Stelzer *et al.* 2021). This hinders the simultaneous data collection of both types of perturbation, which is, however, needed to gauge how pulse and press perturbations may interact. Experimental approaches capable of mimicking long-term press effects upon which stochastic perturbations are superimposed are a fundamental tool to increase our understanding of the topic (Yang *et al.* 2017). Moreover, comparatively little attention has been given to two other important aspects of perturbations. First, eutrophication is a dynamic process where pressure levels change over time, which is often neglected in laboratory and field experiments (Shade *et al.* 2012a; Stelzer *et al.* 2021). Second, repeated pulse perturbations produce a legacy effect carried over time in ecological communities (Johnstone *et al.* 2016; Jacquet & Altermatt 2020), creating fundamentally different responses when compared to a single perturbation response (Ryo *et al.* 2019).

To help filling the knowledge gap on how continuous eutrophication interacts with sequential pulse perturbations, we performed a controlled press and pulse perturbation experiment using mesocosms filled with natural lake water of a meso-oligotrophic lake. We started the experiment with three treatments under the same trophic state and thereafter applied two gradients of phosphate enrichment to simulate press perturbations of different rates, and a control. Together, we applied three sequential H_2O_2 shock treatments of variable intensities to induce mortality events with consequent nutrient release, mimicking the effect of pulse perturbations. With this experimental setup, we aimed to answer the following questions: (i) how does eutrophication modulate the phytoplankton response to sequential pulse perturbations? and (ii) how does eutrophication affect resistance and recovery of chlorophyll-a (Chl-a) levels after recurrent pulse perturbations?

Material and Methods

Field collection, acclimation, and build-up of the mesocosms

Lake water was collected at the southernmost basin of Maarsseveense Plassen, Maarssen, The Netherlands (52°08'28.0"N, 5°04'59.9"E). Maarsseveense Plassen is a meso-oligotrophic lake system with a surface area of 100 ha and a maximum depth of 34 m (see Swain, Lingeman and Heinis (1987) for an extensive description). Sampling consisted of taking 450 L of lake water, approximately 10 meters from the shoreline and 30-70 cm below the surface (local depth 1.3-1.6 m). A single water sample was taken on 29th March 2019 and immediately filtered over a 2 mm mesh to remove most of the mesozooplankton.

In the laboratory, the 450 L tank was left standing for 10 minutes and had the bottom layer siphoned out to remove sand and other large particles. Next, the tank was homogenized and split into two batches. One batch was used for the experiment itself, while the other batch was retained and used to compensate sampling losses during the experiment (hereafter, refill water). The refill water was treated with a single pulse of 10 mg/L H_2O_2 (EMSURE® Supelco®, Merck, Darmstadt, Germany) and kept in the dark at 4°C, both to reduce biological activity until use in the experiment. Compensating sampling losses was needed due to the frequency and volume sampled (see Sampling and data processing). The refill water was chosen over other solutions to conserve the nutrient stoichiometry of the controls as similar as possible to their initial conditions.

The experimental batch was slowly acclimated to the experimental conditions for 24 days to avoid a temperature shock in the community. The long acclimation period was set due to the differences in water temperature between Maarsseveense Plassen at the sampling day (12°C) and the proposed 20°C "summer temperature" used in the experiment. During this acclimation phase, the temperature was increased at a rate of 2.5 °C per week until reaching 20 °C, and the incident light was set to 20 µmol photons.m^{-2}.s^{-1} (measured immediately above the water surface) with a photoperiod of 16:8 h (Light: Dark) simulating the sunset: sunrise period at the time of the experiment. No significant changes in phytoplankton pigment composition and concentration were observed during the acclimation process (data not shown). Water was circulated from the bottom of the experimental batch three times a day for 15 minutes using an aquarium pump (≈600L/h) to reduce sedimentation during the acclimation.

Three days before the beginning of the experiment, the experimental batch was homogenized, divided into eighteen 10-L polycarbonate carboys (Nalgene, Rochester, New York, USA), and randomly placed inside large aquaria under identical light and temperature conditions as described above (Figure 1-a). Each carboy received a non-airtight rubber stopper to minimize aerial cross-contamination and an electromagnetic stirring system. Stirring was set to turn on three times a day – 1 hour in the morning during the sampling and 15 minutes in afternoon and night – with strength enough to resuspend sedimented particles without creating a vortex in the surface. To reduce light variability, the aquaria in which the carboys were submerged was dyed with "black pond dye" until light penetration became negligible (Secchi-disk alike tool invisible at 5 cm below the surface, see Figure 1-a).

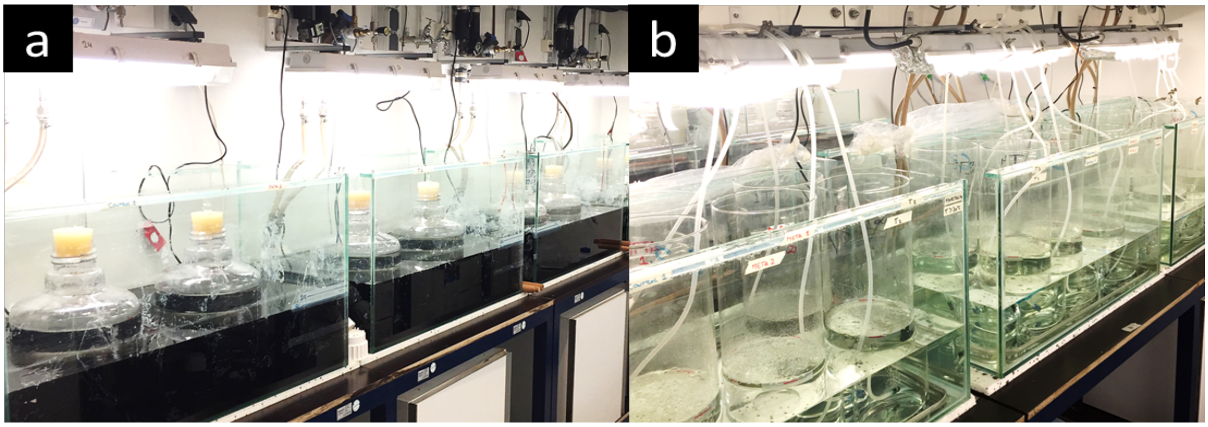

Figure 1. Experimental setup. (A) 10-L carboys were filled with lake water and placed into large aquaria for light and temperature control. Light was accessible to the mesocosms through the top of the vessels, and the water around it was dyed in black to reduce the variability of response caused by diffusive scatter of light into the mesocosms. (B) Water vessels were used for H_2O_2 pulse perturbations. Water vessels stayed at the same temperature as the experiment and were aerated overnight (12-16 h) to keep particles resuspended.

Experimental design and application of the perturbations

The experimental design consisted of a control and two nutrient enrichment treatments (press perturbations) with six replicates each (N=18). During the eutrophication process, mesocosms went through three cycles of pulse perturbations induced by a H_2O_2 shock treatment causing mortality events with consequent internal nutrient turnover. Pulse perturbations are fundamental for the quantifications of responses used here and therefore applied to all mesocosms (controls inclusive). The application of the press and pulse perturbations, sampling, and quantification of responses are described below. The experiment lasted 105 days, during which we quantified the effects of eutrophication on the community response to pulse perturbations.

Press perturbations (eutrophication)

At the beginning of the experiment, all the treatments started with the same total phosphorous concentration (TP ≈ 11.9 ± 5.3 µg/L). The control treatment remained at TP levels similar to the initial concentrations in the lake throughout the experiment, while the eutrophication treatments were exposed to three stepwise increases in TP (Figure 2-a) by adding dissolved K_2HPO_4 (CAS-16788-57-1, Merck, Darmstadt, Germany) just after inducing the pulse perturbations. One of the treatments suffered a mid-strong eutrophication process with predicted final TP = 0.41 mg/L, while the other treatment suffered a strong eutrophication process with predicted final TP=0.82 mg/L (Figure 2-a). The eutrophication treatments were set in a way that the mid-strong eutrophication treatment would go through a full period of Nitrogen:Phosphorous (N:P) co-limitation at the second perturbation cycle, while the strong treatment would abruptly shift from a P-limited to an N-limited system (see Figure 3-c for reference). The amount of K_2HPO_4 added in each perturbation cycle to reach the next anticipated nutrient level was calculated using the dilution Equation 1, based on TP values measured on the days preceding the beginning of the first perturbation cycle.

Equation 1. Dilution equation for multiple solutions, where the final concentration in the mesocosms is a function of the partial dilutions divided by the total volume of the mesocosms. Cc is the Predicted TP concentration in the mesocosms at the perturbation p; Vc is the volume left in the mesocosms after sampling; Cr is the TP concentration of the refill water; Vr is the volume of refill water used; Cs is the concentration of eutrophication solution; Vs is the volume of eutrophication solution added and Vc + Vr + Vs = 10 liters.

$$c_{c_p} = (c_{c_{p-1}} v_{c_p} + c_{r_p} v_{r_p} + c_s v_{s_p}) \div (v_{c_p} + v_{r_p} + v_{s_p}) \qquad (1)$$

Pulse perturbations

Pulse perturbations were simultaneously applied to all the mesocosms (controls inclusive) at days 4, 40, and 68, constituting three perturbation cycles (P1, P2, and P3, respectively. Figure 2-b). For this, mesocosms were homogenized, and a fraction of their volume was transferred to individual water vessels (Figure 1-b & Figure 2-c_i, r=10 cm, h=60 cm) where the H_2O_2 pulse took place. Additional to the mesocosms fraction, refill water was used to compensate for the volume lost due to sampling during the perturbation cycle (approximately 70mL every other day, Figure 2-c_{ii}). Refill water has the same physicochemical composition as the control and therefore is used to conserve nutrient stoichiometry in the mesocosms, reducing nutrient losses caused by sampling. The sum of these two volumes constituted the proportion of the mesocosms subjected to the perturbation. Each perturbation cycle had a different volume fraction split for perturbation - P_1=50%, P_2=15%, and P_3=30% - mimicking mortality events of different intensities (e.g., mortality induced to 50% of the community). The different perturbation intensities were induced as random effects in the system and not as a factorial design together with the eutrophication treatments. Hence, all the mesocosms received the same perturbation intensity at the same cycle.

The pulse perturbations themselves consisted of a single pulse of H_2O_2, acting as a shock treatment capable of bringing the system out of equilibrium. H_2O_2 leads to severe oxidative stress, inducing mortality with a consequent nutrient release when in high concentrations. Concentrations between 0.5-5 mg/L are reported to be somewhat selective, affecting cyanobacteria preferentially (Drábková *et al.* 2007). However, concentrations over this threshold are expected to induce mortality to most biological groups evenly (see Matthijs *et al.* (2012); Piel *et al.* (2021)). Here, we spiked the content of the water vessels with 10 mg/L H_2O_2 (Hydrogen peroxide 30%, EMSURE®, Supelco®, Merck, Darmstadt, Germany, Figure 2-c_{iii}) overnight under similar conditions as in the carboys (light and temperature), except for the constant aeration used to keep particles in suspension (Figure 1-b). The next day, residual H_2O_2 was measured in the water vessels using peroxide test strips Dosatest® (VWR International, Cat no. 85433.601) before returning the perturbed fraction to the original mesocosms (Figure 2-c_{iv}). Residual H_2O_2 in the water vessel was never higher than 2 mg/L and virtually absent in the mesocosms within 24 hours after completing the pulse perturbation. Since H_2O_2 is a biogenic Reactive Oxygen Species (ROS) that decays to water and oxygen in the order of a few hours to a few days (Cooper & Zepp 1990; Häkkinen, Anesio & Granéli 2004), it leaves no persistent chemical footprint in the system. After completing the pulse perturbation, any deviation from the 10-L mark in the mesocosms after returning the perturbed fraction was corrected using demineralized water to compensate for evaporation losses (mostly negligible during the experiment).

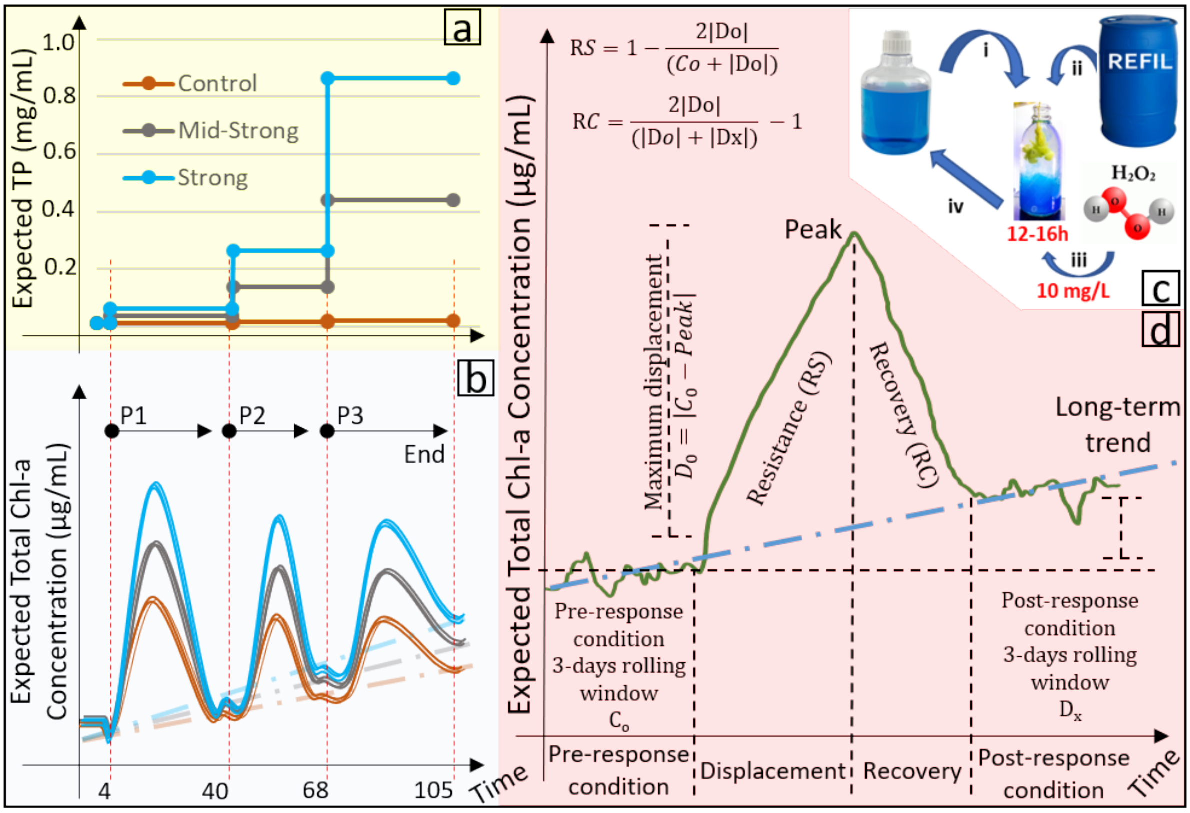

Figure 2. Infographic of the experimental design with its press and pulse perturbations, expected responses, and a conceptual example of how perturbation metrics were quantified. a) The press perturbations consisted of a gradual increase in the phosphate concentration of the mesocosms, simulating the effect of eutrophication in three different treatments. (brown) control where no eutrophication took place; (gray) mid-strong eutrophication with approximate final TP=0.41 mg/L; and (blue) strong eutrophication with approximate final TP=0.82 mg/L. The increase of the press perturbation happened in three stepwise increases during the experiment. b) The three perturbation cycles with the expected responses of the mesocosms to the combination of the press (eutrophication) and pulse perturbations (H_2O_2 mortality events). The colored solid lines represent the expected development of Chl-a during the experiment, each one with 6 replicates (n=6, N=18). The vertical red dotted line marks the exact moment of the pulse perturbation. P1, P2, and P3 were the three different perturbation cycles, each one with a different intensity and different eutrophication levels. The diagonal dashed lines indicate the expected long-term changes in Chl-a levels due to the combination of press and pulse perturbation (baseline changes). Pulse perturbations were simultaneous to the increase in the press perturbation. c) Induction of the mortality pulse perturbations with consequent nutrient turnover. First, a fraction of the mesocosms was transferred to a separate container (i). To compensate for sampling losses, refill water was added to the same container where the pulse perturbation took place (ii). The sum of these two fractions was spiked with 10 mg/L H_2O_2 overnight (iii) before returning to the original mesocosms (iv). The fraction of the mesocosms split for the perturbation determined the intensity of the perturbations, P1=50%, P2=15%, and P3=30%. d) Conceptual example of how perturbation metrics were quantified. Within an individual perturbation, the timeseries of the Chl-a response to the pulse perturbation were divided into 4 moments (x-axis). Resistance (RS), recovery (RC), and maximum displacement (Do) were individually calculated accordingly for each of the six replicates. The quantification of these metrics was used to model the effect of eutrophication (press perturbation) on the phytoplankton response to pulse perturbations. The green line represents the expected response of the system to the pulse perturbation (Time=0). The blue dashed line indicates the long-term changes in Chl-a levels due to the combination of press and pulse perturbation (baseline changes).

Sampling and data processing

Sampling was conducted every other day in the morning, one hour after the lights reached their full emission strength. Mesocosms were stirred for at least 15 minutes to allow for homogenization and particle resuspension (see Field collection, acclimation, and setup), followed by three counter-vortex movements to reduce the effect of particle size separation due to fluid drag force before sampling. A sample of 250 mL was taken from the mesocosms and transferred to multiple conical polypropylene centrifuge tubes (VWR International) for further analyses (70 mL in total). The unused sampled volumes were immediately returned to the mesocosms, reducing sampling losses as much as possible.

Pigment-based community dynamics

Subsamples for pigment-based community dynamics were kept in amber tubes, and their fluorescence was measured immediately after sampling using Phyto-PAM (Walz, Germany). Phyto-PAM estimates the relative abundances of different phytoplankton groups, i.e., cyanobacteria, green algae, and diatoms, based on the relative proportion of group-specific pigments present in the sample (Walz 2003). The conversion of relative abundances to absolute chlorophyll-a concentrations (µg/L) was calculated based on pre-determined calibration curves. We used *Microcystis aeruginosa, Chlorella* sp., and *Synedra* sp. cultures to generate the calibration curves for cyanobacteria, green algae, and diatoms, respectively. The total chlorophyll-a concentration was calculated as the sum of the three chlorophyll-a fractions. Data collection for pigment-based community dynamics was done every other day over the full length of the experiment.

Nutrients

Subsamples for dissolved nutrient analysis were immediately placed in the fridge (4°C) until concluding the fluorescence measurements on the same day. Next, 42mL of sample were filtered through a 0.7 µm GF/F filter (Whatman, UK) mounted on a vacuum filtration manifold system. The filtrate was frozen at -20°C for later quantification of dissolved nutrients (P-PO4, N-NH4, N-NO2, and N-NO3) using an autoanalyzer (QuAAtro39 Autoanalyzer, Seal, USA).

Size-based community dynamics

Community size-based biovolume distribution was assessed in every other sample (four days) using a Coulter Counter (Beckmann, Indianapolis – USA). Samples were pre-filtered with a 100 µm-mesh to avoid clogging, and particles with diameters between 2.93-60 µm were counted. The lower cut-off 2.93 µm was set to avoid counting bacterioplankton and cell debris, while the upper cut-off 60 um was determined based on the abundance of the microbial community under microscopy. Particle surface and volume were quantified and allocated to 300 size bins. Three counts of 100 µL were averaged for each sample, and the total biovolume was calculated by summing the biovolume of all individual bins within a sample. Only a few missing cyclopods, filamentous algae, and one taxon of ciliates, all with negligible biovolumes compared to the range selected, were found after a taxonomic investigation under the microscope (Supplementary material – Functional structure). The total biovolume does not distinguish autotrophs from heterotrophs, thus, representing the dynamics of the whole microbial community.

Quantification of responses

Long-term effect of the press perturbation (eutrophication) on total biovolume and total chlorophyll-a concentrations

To assess the effect of eutrophication on the biovolume build-up of the <60 µm microbial community fraction, total biovolume was averaged during the whole perturbation cycle (from one H_2O_2 to the next H_2O_2 manipulation for the Baseline, P1, P2, and P3, Figure 2-b). This was done for each replicate individually, totaling 72 averaged values (3 treatments x 6 replicates x 4 periods – including the pristine period). Therefore, each averaged value comprised the full biovolume of the microbial response to the combined effect of press and pulse perturbation, allowing a direct comparison between treatments. The relationship between biovolume build-up and total chlorophyll-a concentration was assessed using all data points available in both timeseries for each eutrophication treatment (N=466).

Short-term effects of the press perturbation (eutrophication) on the response and recovery from mortality pulse perturbations (chlorophyll-a as a proxy)

The dynamics of the response metrics preceding and following pulse perturbations were calculated based on three periods, (i) the pre-response condition, (ii) the peak of the response, and (iii) the post-event condition (Figure 2-d). Using these three periods, we calculated the maximum displacement, resistance, and recovery of total chlorophyll-a concentration for each one of the perturbations. We quantified 54 individual perturbations divided into three eutrophication treatments with six replicates, each one undergoing three perturbation cycles.

Pre-response condition (C_o) and post-event condition (D_x)

The determination of the pre-response condition was automated using a minimum of 3 days moving average (2 or more sampling points), identifying the period of least variance within the perturbation cycle. This approach could identify the period just before the response to perturbation became apparent. Thus, the pre-response condition does not necessarily represent the exact period preceding the H_2O_2 perturbation but the moment just before the system starts reacting to it. This approach is consistent with what often happens in real-world systems since the response to pulse perturbations often shows a lag-effect (Dodson, Arnott & Cottingham 2000) – a difference in timing between the perturbation and the response. Furthermore, this approach allows uniformity when assessing sequential perturbations (as in this experiment), where the pre-response condition of a given perturbation cycle is also the post-event condition of the perturbation cycle just before it (e.g., $P2_{\text{Post-event condition}} = P3_{\text{Pre-response condition}}$).

Maximum displacement (D_o)

The maximum displacement was calculated as the absolute difference in units of chlorophyll-a between the peak of the response (maximum Chl-a value at the perturbation cycle) and the pre-response condition (C_o) (Equation 2). The maximum displacement shows the *absolute* effect of the pulse perturbation given their respective eutrophication treatments rather than the *relative* effect (see resistance and recovery index). Thus, it represents the magnitude of the response to the pulse perturbation, irrespective of the Chl-a level before the response starts.

Equation 2. Calculation of maximum displacement.

$$D_0 = |C_0 - Peak| \qquad (2)$$

Resistance (RS) and Recovery index (RC)

Resistance and recovery index were calculated following the method proposed by (Orwin & Wardle 2004) with slight modifications. Both indexes are based on percent changes to the pre-response condition, holding proportionality between treatments of different trophic states. Therefore, the RS and RC allow the observation of how eutrophication modifies the intensity of response to the pulse perturbation, being a relative index that allows direct comparison between systems with different chlorophyll-a levels.

The Resistance index (RS) represents the degree of change the pulse perturbation caused in the system compared to the undisturbed situation (Equation 3) – originally proposed as an undisturbed treatment and here proposed as the pre-response condition within the perturbation cycle:

Equation 3. Calculation of the resistance index.

$$RS_{(p)} = 1 - \frac{2|D_0|}{(C_0 + |D_0|)} \qquad (3)$$

where $RS_{(p)}$ is the resistance at the perturbation cycle "p"; D_o is the difference between the pre-response condition (C_o); and the peak of disturbance (see Figure 2-d). The behavior of the index can be visualized in the supplementary material (Figure 1). The original index is restricted to values between -1 and +1, with the value of +1 showing that the perturbation had no effect on the system (maximal resistance). Lower values indicate stronger effects of the perturbation, with RS=0 representing 100% change compared to the pre-response condition (e.g., C_o=20, D_o=20, RS=0; 100% change compared to the pre-response condition level). The index scales change exponentially, and negative values indicate that the system was displaced by more than 100 % compared to the pre-response condition. To expand the index within the values of low resistance (values between 0 and -1), we rescaled it to values between 0 and 10, where 10 represents no change (original index = 1), 9 represents 100% change (original index=0) compared to the pre-response condition, and smaller values represent less resistance. The formulas used for rescaling are provided as supplementary material (Table 1), as well as the comparison between original and rescaled indexes (Figure 2 and 4).

The recovery index (RC) is based on the concept of engineering resilience as proposed by Pimm (1984), in which the system has a "single" equilibrium to which it may return after dissipating the pulse perturbation. Yet, once the perturbations are followed by changes in trophic state, we expect the post-event conditions to stabilize at a higher chlorophyll-a concentration than the pre-response condition - mainly because of the increased carrying capacity caused by the nutrient addition. The recovery index incorporates this situation into its calculation and determines to what extent the system recovers after perturbation (fully or partially), considering the pre-response condition, the post-event condition, and the maximum displacement (Equation 4).

Equation 4. Calculation of the recovery index.

$$RC_{(p)} = \frac{2|D_0|}{(|D_0|+|D_x|)} - 1 \qquad (4)$$

where $RC_{(p)}$ is the recovery at the end of perturbation cycle "p" and D_x is the post-event condition of the perturbation cycle (Figure 2-d). Similar to resistance, recovery is also originally restricted to values between -1 and +1, with the value of +1 showing that the system fully recovered after the perturbation (maximal recovery). Lower values indicate loss of recovery, with RC=0 representing 0% recovery compared to the peak of the perturbation (e.g., C_o=10, Peak=20, D_x=20, RS=0; 0% recovery compared to the peak of perturbation). As RS, the RC scale changes exponentially as further it goes from +1 (see supplementary material, Figure 3). Also, RC uses the maximum displacement as a scaling factor to calculate recovery to the pre-response condition. To expand the index within the values of high recovery (values between 1 and 0), we also rescaled it to values between 0 and 10, where 10 represents *full recovery* (original index=1), 1 represents *no recovery* (original index=0) compared to the pre-response condition, and values smaller than 1 mean that the system has drifted away after the perturbation (see supplementary material Table 1 for rescaling).

Statistics

All statistics were calculated using the R software (R Core Team 2020). Pre-response condition, maximum displacement, and recovery index were compared between treatments and perturbation cycles by linear mixed effect models (LMEM) using the package “lme4” (Bates *et al.* 2015). Resistance index and total biovolume were assessed in the same way by generalized additive mixed model (GAMM) using the package “mgcv” (Wood 2017). LMEM and GAMM were set to calculate effect size estimates of the treatments compared to the control. Thus, significance and estimates were calculated using the control as a reference. Because the control treatment does not suffer eutrophication, temporal changes in its response to pulse perturbations are assumed to be a consequence of the sequential pulse perturbations; while its differences to the eutrophying treatments, the effect of eutrophication. All the models had the number of pulse perturbations and eutrophication treatment as fixed effect terms, tested with and without interactions. The intensity of pulse perturbations (fraction of the mesocosms spared for the pulse perturbation) and temporal pseudo-replication were set as random effect terms, with the second allowing for random slopes based on the perturbation effect when model complexity allowed (Supplementary material - Statistics). Results are expressed as a function of the number of sequential pulse perturbations since neither resistance, recovery, or maximum displacement are time-dependent metrics on the way they were calculated. The effect of total biovolume and eutrophication on total chlorophyll concentration was assessed by a generalized linear model (GLM), including an interaction between the terms to include potential changes in chlorophyll-a concentrations per biovolume unit. GLM was fitted using the package “lattice” (Sarkar 2008). To comply with the assumptions for model validation, data were log10-transformed when needed. Significance values were obtained from F-distributions, and P-values <0.05 are referred to as *significant*.

Results

The overall response to pulse perturbations

The eutrophication treatments (press perturbations) successfully shifted the systems from a P-limited system to an N-limited system by the end of the experiment (Figure 3-a/b). Also, we successfully created a period of likely N:P co-limitation at the second perturbation cycle for the mid-strong treatment (Figure 3-c). The co-limitation period represents a fundamental stoichiometric difference between the two eutrophication treatments. Yet, the co-limitation period did not result in substantial differences in Chl-a responses to pulse perturbations between them. Controls remained P-limited during the whole experiment.

The H_2O_2 pulse perturbations induced mortality events with a consequent nutrient release, as observed by the abrupt drop in total biovolume (Figure 4-a) and peaks of dissolved inorganic nitrogen

(Figure 3-b) that followed the perturbations. Shortly after the induced mortality and nutrient turnover, the phytoplankton community showed a positive growth response observed by the peaks in Chl-a levels (Figure 4-b). Also, the pulse perturbation increased the total biovolume of the microbial community (2.93µm < fraction < 60µm). The increase in total biovolume also happened in the controls, where no nutrients were added during the experiment (no press perturbation). The Chl-a and total biovolume response were transient and followed a recovery period, which varied between treatments and perturbation cycles. The first pulse perturbation induced marked changes in the total biovolume of the microbial community, which contrary to Chl-a, did not recover until the end of the experiment. The combination of eutrophication and pulse perturbations resulted in systems with different functional structures (Supplementary material – Functional structure), chlorophyll-a levels, and biovolumes of the microbial community (Figure 4), which affected the pattern of response to pulse perturbation of the phytoplankton communities (Table 1). The periphyton formation was quantified in the last quarter of the experiment, with no major development observed in the data (Supplementary material – Periphyton formation).

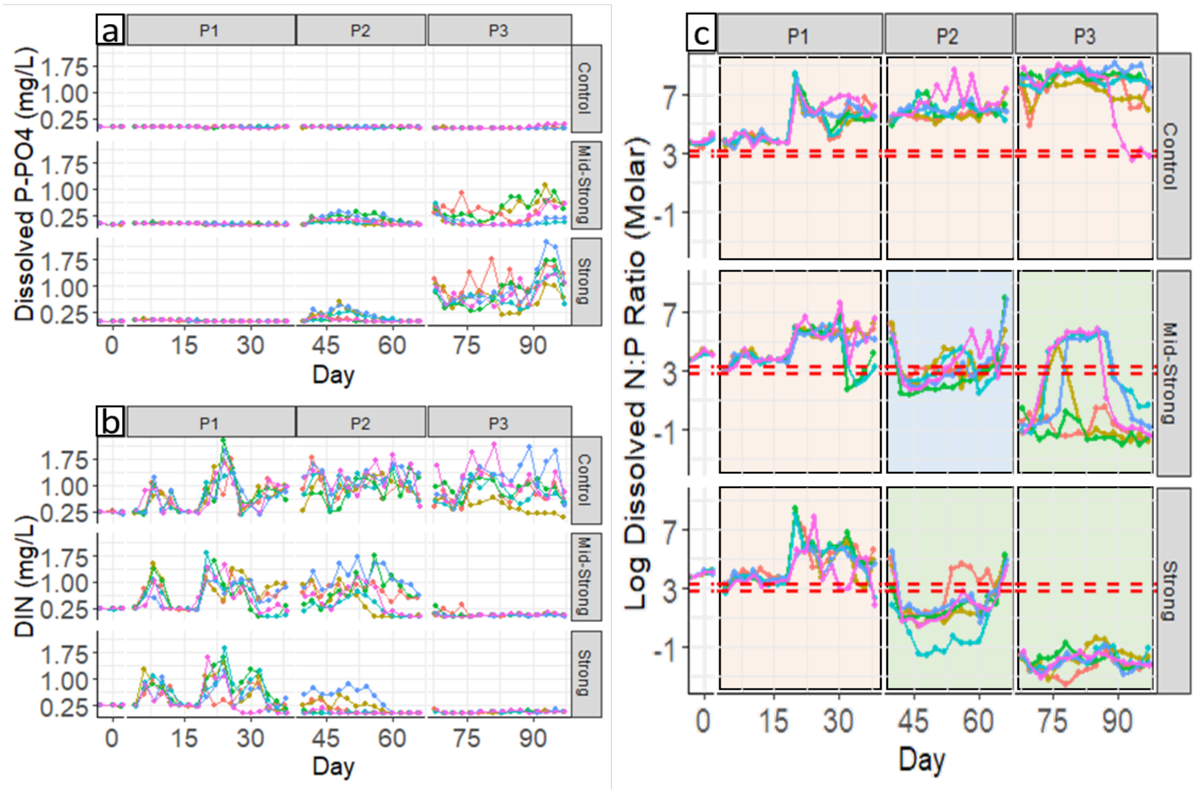

Figure 3. Timeseries of a) dissolved orthophosphate; b) dissolved inorganic nitrogen (sum of NH_4, NO_2, and NO_3); c) N:P ratio of dissolved nutrients during the three perturbation cycles of the experiment (P1, P2, and P3). Each colored line represents the temporal development of an individual replicate (n=6, N=18). Control, mid-strong and strong are the three eutrophication treatments (press perturbations). On panel c, the horizontal dashed red lines represent the N:P ratio of 16:1 (Redfield 1934) and 25:1 (Sterner 2009), and the shaded panels are an empirical indication of expected P-limited systems (in orange, when the system is set above the horizontal lines), co-limitation (in blue, when the system is set between the lines), and N-limitation (in green, when the system is set below the horizontal lines).

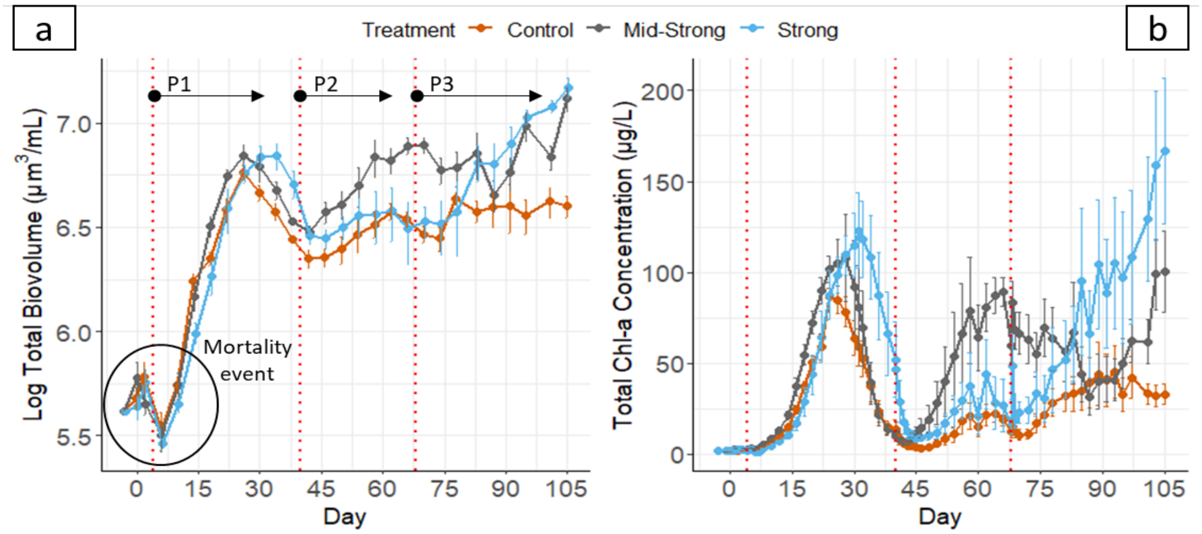

Figure 4. The microbial community responses to the combination of press and pulse perturbations along the 105 days of the experiment. a) Timeseries of total biovolume of the whole microbial community (2.93µm < fraction < 60 µm) and b) Timeseries of total chlorophyll-a concentration, a proxy for the response of the phytoplankton community. Control, Mid-Strong, and Strong are the three different levels of continuous eutrophication (press perturbation). Vertical dashed lines represent the exact moment when the mortality pulse perturbations were induced to the system; P1, P2, and P3 are the different pulse perturbation cycles. Solid lines represent the mean value of the response variable (n=6), and error bars are the mean ± 1 standard error of the mean (S.E.M).

Long-term effect of the press perturbation (eutrophication) on total biovolume and total chlorophyll-a concentrations

Perturbations induced a significant increase in biovolume of the particle fractions smaller than 60 µm ($F=5.52$, $p<0.05$, larger fractions were not quantified). Also, eutrophication interacted with the perturbations ($F=3.30$, $p<0.05$), accelerating the rate of biovolume build-up in the two nutrient-rich treatments (Figure 5-a). Biovolume showed to be positively correlated to the total chlorophyll-a concentration in the mesocosms ($p<0.001$, $R^2=0.723$), suggesting a coupled increase of biovolume and primary producers (Figure 5-b). The different eutrophication rates also changed the relation between biovolume and chlorophyll-a concentration, meaning that chlorophyll-a concentrations were higher in the eutrophied treatments than in the control treatment for the same given biovolume ($p<0.05$). Yet, these differences were small (an increase of ≈ 1.2 µg/L of total chlorophyll-a compared to the control).

Short-term effects of the press perturbation (eutrophication) on the response and recovery from mortality pulse perturbations (chlorophyll-a as a proxy)

Pre-response conditions

The total chlorophyll-a concentration in the pre-response conditions increased significantly during the experiment. From P1, P2 to P3, the Chl-a concentration increased by a factor of 6 in the controls, by a factor 26 in the Mid-Strong treatment, and by a factor of 10.5 in the Strong eutrophication treatment (an absolute increase of 9.0, 52.2, and 18.1 µg Chl-a/L, respectively). The sequential pulse perturbations showed a higher effect size for modifying total Chl-a concentrations at the pre-response condition than the eutrophication pressure itself (Supplementary material - Statistics). The increase in Chl-a levels at the pre-response conditions happened in all the treatments and was caused by the sequential pulse perturbations (F=50.01, p<0.001). Moreover, our assessment indicated that eutrophication interacted with the effect of perturbations (F=4.58, p<0.01), meaning that the rate of change in Chl-a concentration due to the repeated perturbations was amplified by eutrophication (Figure 6-a). Yet, we observed no isolated eutrophication effect in Chl-a levels changes at the pre-response conditions (F=5.19, p<n.s). The random effect size estimates of the different intensities of pulse perturbations applied to the mesocosms in the previous perturbation cycle showed a minor effect on the chlorophyll-a levels at the pre-response condition (Supplementary material - Statistics). Eutrophication and the number of perturbations explained 67% of the variance in the model, while the full model explained 75% of the observed patterns in Chl-a concentrations in the pre-response conditions (Marginal R^2= 0.676 / Conditional R^2=0.751).

Maximum displacement

The absolute increase in total Chl-a concentration between the pre-response condition and the peak of the response was higher in both eutrophication treatments compared to the controls (F=6.15, p<0.01). This means that eutrophication increased the magnitude on which the phytoplankton community responded to the pulse perturbations. Still, both eutrophication treatments responded similarly, irrespective of the nutrient co-limitation that developed in the mid-strong treatment. For both treatments, the absolute number of total chlorophyll-a units (µg/L) displaced between the pre-response condition and the peak was nearly twice as large as the control. Model estimates showed that the eutrophication treatment produced an effect size in the opposite direction of the number of perturbations (antagonistic response). While eutrophication showed a positive effect size on maximum displacement, serial perturbations resulted in a negative one. Yet, the sequential pulse perturbations hitting the system did not significantly affect the maximum displacement, despite the observed negative trend (F=1.89, p<n.s) (Figure 6-b). Part of this result is addressed by the strong random effect sizes of perturbation intensity, which corrects the fixed effect sizes of the model (Supplementary material - Statistics). A separate analysis of the effect of perturbation intensity on maximum displacement showed a significant increasing trend, indicating that indeed maximum displacement and perturbation intensity were positively correlated - and both negatively correlated to sequential perturbations (Supplementary material - Statistics). For this reason, the interaction between eutrophication levels and the number of perturbations was not tested. The model showed a

full variance explained of 55% with eutrophication and the sequential perturbations explaining 28% of the observed variance.

Resistance

Only six times out of 54 (11%), the mesocosms displaced less than double of the pre-response condition (points above the red line, Figure 6-c), indicating that the system was very susceptible to the pulse perturbations applied. The resistance significantly increased during the experiment mainly due to the impact of serial perturbations (F=22.47, $p<0.01$, $R^2=0.594$). The nutrient enrichment treatments did not significantly affect the resistance level compared to the controls (F=1.46, p<n.s), nor did eutrophication interact with the perturbations (F=2.02, p<n.s). The resistance index is calculated as a relative change between the pre-response condition and the conditions at the peak of the perturbation, therefore, normalizing the different scales of response caused by the press perturbation as observed in the maximum displacement (see Resistance (RS) and Recovery index (RC)).

Recovery

Recovery of Chl-a levels in the systems was significantly affected by the sequential perturbations (F=17.16, $p<0.01$), with some mesocosms losing the capability to recover within the timeframe of the experiment after the third perturbation cycle (Figure 6-d). We found no statistically significant indication that eutrophication reduced or interacted with sequential perturbations to reduce recovery after the pulse perturbation (F=0.90, P<n.s and F=0.182, p<n.s; respectively). The full model explained 82% of the observed variance, with eutrophication and the number of perturbations accounting for 71% (Marginal R^2= 0.708 / Conditional R^2=0.822).

Table 1. Statistical summary from the results of the perturbation metrics assessing the effect of eutrophication (press perturbation) on the Chl-a response to a mortality pulse perturbations with internal nutrient turnover. n.s=non-significant; n.a=not assessed; F and p are the F-value and p-value from the model output. GAMM=Generalized additive mixed model; LMEM=Linearized mixed effect model. The fields in bold are statistically significant.

Metric	Model	Press Perturbation (eutrophication)	Pulse Perturbation (mortality+turnover)	Interaction
Pre-response	LMEM	n.s	**F=50.01, p<0.001**	**F=4.58, p<0.01**$^{\text{mid-strong}}$
Max Displacement	LMEM	**F=6.15, p<0.01**	n.s	n.a
Resistance	GAMM	n.s	**F=22.47, p<0.01,**	n.s
Recovery	LMEM	n.s	**F=17.16, p<0.01**	n.s

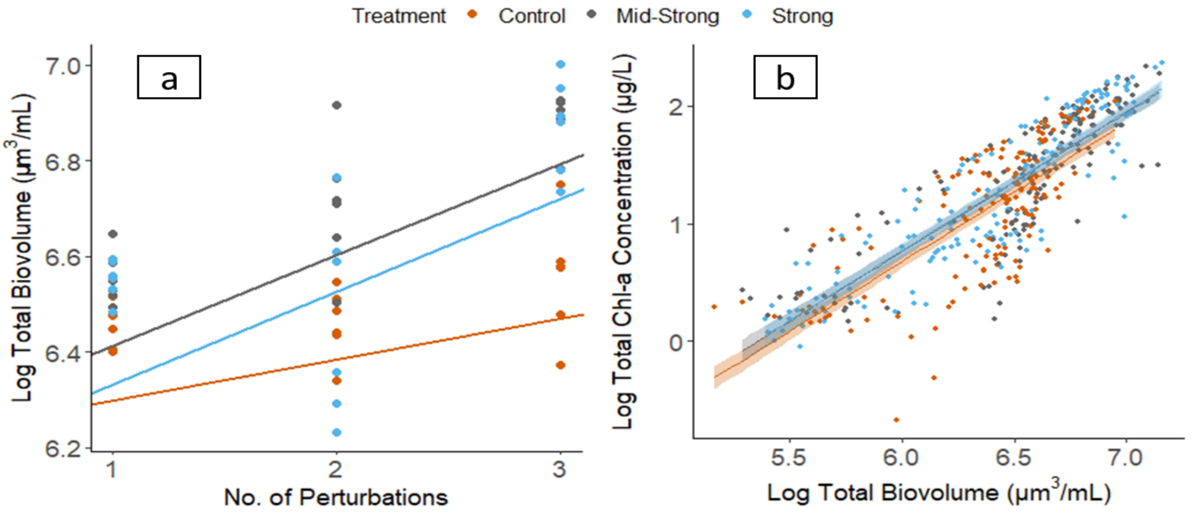

Figure 5. The effect of press and pulse perturbations on the total biovolume of the microbial community (2.93 < fraction < 60 µm) and its correlation to Chl-a levels. a) the effect of eutrophication and pulse perturbations on the biovolume accumulation during the experiment. The solid regression lines represent the full linear mixed-effect model estimates, and the dots are the empirical data (n=6). b) Generalized linear model showing the regression between total biovolume and total chlorophyll-a for each one of the eutrophication treatments (press perturbation). The solid lines represent the model estimates and the shaded area the 95% confidence interval of the model. Dots are empirical data (N=466, R^2=0.723).

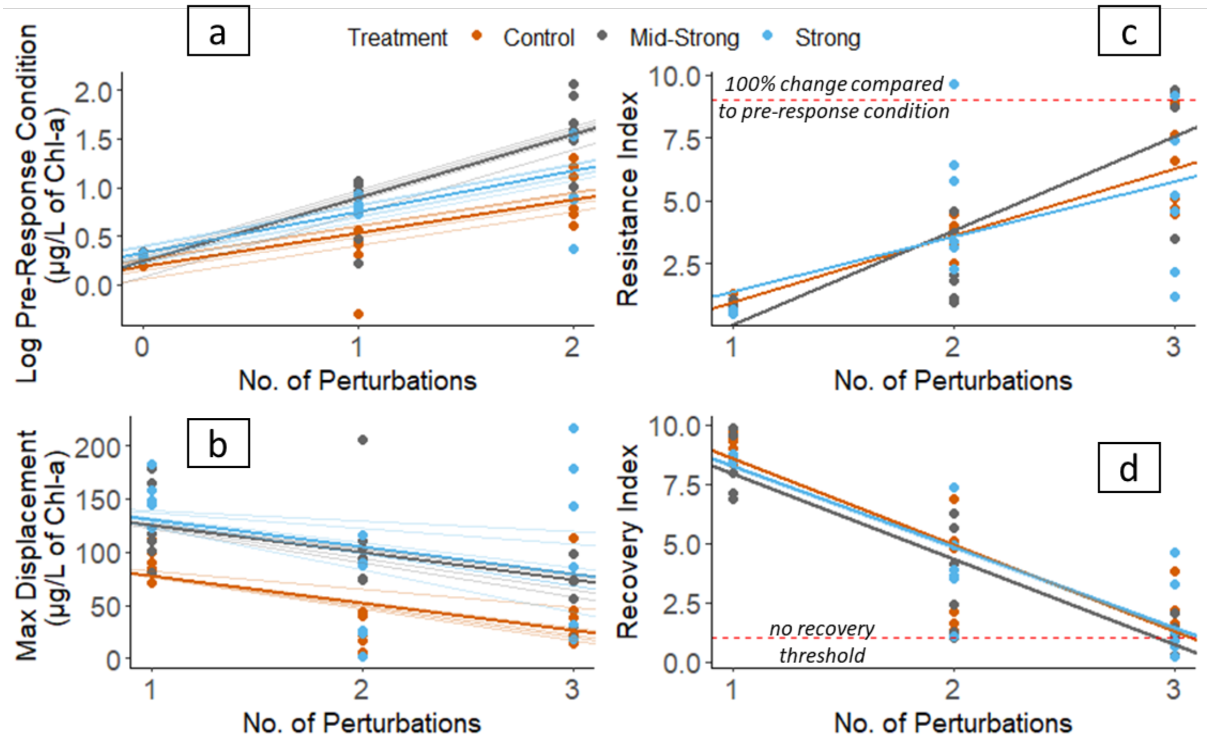

Figure 6. The effect of eutrophication on the Chl-a response to the mortality pulse perturbations. Solid regression line represents the full model estimates, and shaded lines represent the individual mesocosms estimate with or without random intercepts depending on the model complexity. Dots represent the metrics of pulse perturbation calculated from the empirical data (n=6). a) Changes in the total chlorophyll-a concentration at the pre-response condition; b) Maximum displacement of total chlorophyll-a concentration; c) Resistance Index, and d) Recovery Index.

Discussion

Here we explored the interplay between eutrophication and sequential pulse perturbations on the response of the phytoplankton community. Our results showed that resistance increased and recovery decreased after each pulse perturbation and that eutrophication *per se* can increase the magnitude of response of the phytoplankton community compared to non-eutrophying systems. Yet, we did not find evidence that eutrophication would make the phytoplankton community proportionately less resistant or less resilient to pulse perturbations than when eutrophication is absent. Although experiments and field observation explicitly considering a press and pulse framework assessing the interactive effect of eutrophication on the phytoplankton response to pulse perturbations are virtually absent, similar results were observed on the effect of droughts in eutrophying grassland (Xu *et al.* 2014; Bharath *et al.* 2020).

The H_2O_2 pulse perturbations induced community-level mortality events, creating a transient period of high autochthonous dissolved nutrient levels in the mesocosms. With dissolved nutrients available for uptake, transient peaks in chlorophyll-a concentration were observed. This response pattern was already described in other microbial communities (Haddad *et al.* 2008; Jacquet & Altermatt 2020) and advocated to describe phytoplankton responses to extreme weather events that induce mortality with consequent increases in nutrient availability and turnover. Moreover, it is similar to what is described in lakes after a storm, where phytoplankton is mixed in the water column reducing cell density; at the same time that prompts nutrient upwelling with subsequent opportunities to increase Chl-a concentrations (Stockwell *et al.* 2020).

The first perturbation cycle showed the lowest resistance of the phytoplankton community, with systems reaching chlorophyll-a levels about 40 to 60-times higher than the pre-response condition. Such an intense response was unexpected and most likely associated with the novelty of the perturbation to the microbial community (Johnstone *et al.* 2016), which has never been exposed to H_2O_2. Another possible explanation is that the chemical effect of hydrogen peroxide - as a strong oxidizer capable of breaking down stable dissolved organic matter that was not promptly bioavailable before the first perturbation – turned over more nutrients at the first perturbation cycle, thereby increasing the phytoplankton response. Also, the first pulse perturbation had the highest intensity. Yet, our results did not point to a major contribution of perturbation intensity to the resistance of the phytoplankton community on the different perturbation cycles (Supplementary material - Statistics).

As presented before, pulse perturbations *per se* permanently increased the biovolume of the microbial community within 2.93-60 µm even when the press perturbation was absent. This suggests that the community structure was permanently changed despite the observed recovery in chlorophyll-a (Supplementary material – Functional structure). Differences between compositional and functional recovery after pulse perturbations have been exhaustively reviewed and are well known to diverge in mesocosms experiments (Shade *et al.* 2012a; Hillebrand & Kunze 2020), mainly due to the lack of dispersal and differences in seeding (Hillebrand & Kunze 2020). In our mesocosms, the changes in community composition sustained the functional redundancy necessary to rebuild the Chl-a levels. Similar results are broadly described in the literature with different systems and scales (Allison & Martiny 2008; Allan *et al.* 2011; Hoover, Knapp & Smith 2014; Pennekamp *et al.* 2018). However, from the second perturbation onwards, the mesocosms partially lost their capability to fully recover within a perturbation cycle, irrespectively of the eutrophication level. These incomplete recovery patterns led to post-response conditions stabilizing at a higher chlorophyll-a concentration compared to the pre-response condition, enforcing the formation of novel baselines along the experiment. At the third perturbation cycle, some mesocosms reached a point of no recovery (transformed RC_{index}=1).

While pulse perturbations increased the amount of autochthonous nutrients in the mesocosms, eutrophication increased the amount of allochthonous nutrients. Still, despite similar patterns in nutrient availability, press and sequential pulse perturbations had different effects on the mesocosms. Mortality pulse perturbations are reported to reduce population sizes of species with lower-intrinsic growth rates, selecting for species of high-intrinsic growth rates that can rapidly recover from the pulse perturbation (Haddad *et al.* 2008). Thus, smaller primary producers known for their high intrinsic growth rates when nutrients are available (Ward *et al.* 2017) would dominate a perturbed system if top-down regulation cannot be sustained at high levels - which would act more strongly on small than large-sized phytoplankton. In the same response direction, eutrophication increases nutrient availability creating favorable conditions for fast-growing taxa (Klappenbach, Dunbar & Schmidt 2000). These long-term interactions on nutrient availability between press and pulse perturbations potentially explain the biovolume build-up within the 2.93-60 µm fraction and why the eutrophied treatments showed higher biovolumes with higher Chl-a peaks in response to pulse perturbations. However, at the same time that pulse perturbations select for small primary producers, the sequential pulse perturbations may artificially select for organisms that cope better with the pulse perturbation regime, reducing the responsiveness of the system (but note that only a part of the community was subjected to pulse perturbations). Thus, the more pulse perturbations, the higher the resistance of the community, and the lower the observed response to the internal nutrient turnover. Hence, the effect of pulse perturbations may act in either way depending on the number of perturbations suffered by the systems. Moreover, the effect of sequential pulse perturbations and eutrophication can be interpreted as forces that act in the opposite direction when modulating the community response to a stochastic event that induces internal nutrient turnover. In our experiment, the build-up of resistance due to sequential pulse perturbations was more significant than the combined effect of eutrophication and internal nutrient turnover caused by the pulse perturbations.

Our experimental mesocosms suggested that community structure was shaped to absorb the sequential pulse perturbations at the cost of increasing resistance and reducing recovery. The more perturbed the system was, the less responsive it became, and this can be interpreted as a possible community pathway towards stability (Paine, Tegner & Johnson 1998). The loss of recovery and increase in resistance driven by the serial perturbations rather than eutrophication was evidenced in multiple aspects of the system. First, the controls showed a decrease in recovery and increase in resistance, despite not having any ongoing eutrophication process. Second, the eutrophying treatments did not show any difference compared to the control at any perturbation cycle, indicating that eutrophication had no observable effect in the loss of recovery or increase in resistance. Third, the effect sizes obtained from the LMEM and GAMM showed a prevalence of repeated perturbations over eutrophication (Supplementary material, Figures 10 and 11) for determining recovery and resistance. This combination of evidence suggests that the sequential pulse perturbations can be more important than the trophic state for phytoplankton community stability. The recovery and resistance indices observed here were within the range of distribution observed in the shallow eutrophic Lake Müggelsee (Thayne *et al.* 2021) while studying the lake response to a multitude of storms across different seasons. There, the pre-response conditions partially controlled the stability of the lake when hit by extreme storm events. However, press perturbations were not explicitly considered (i.e., changes in the lake's trophic state).

Moreover, we observed that microbial communities undergoing strong eutrophication are likely to show an increase in chlorophyll-a levels after sequential pulse perturbations compared to non-eutrophying systems (nearly twice as much). Since eutrophic systems often already show higher Chl-a concentrations (Søndergaard *et al.* 2011), the combination with larger chlorophyll-a displacements may dramatically increase the immediate consequences of the pulse perturbations. This is an important consideration when managing waterbodies with strict regulatory directives (e.g., strict Chl-a concentration thresholds for water supply) because eutrophication will not only affect the functioning of the system under stable conditions (Jeppesen *et al.* 2005; Alexander, Vonlanthen & Seehausen 2017) but may also compromise water security when affected by stochastic events.

We were able to assess three pulse perturbations, while the number of perturbations hitting an aquatic system within a legacy effect window is likely higher in natural systems (Johnstone *et al.* 2016). It is possible that the effect of eutrophication on resistance and recovery of sequential perturbations would become explicit when assessing more perturbation cycles. Moreover, dispersal and multitrophic levels of natural complexity could also alter the legacy effect carried over the perturbations through species reseeding (Hillebrand & Kunze 2020) and/or a stronger top-down control of the phytoplankton community (McCauley *et al.* 2018), potentially modifying the resistance and recovery index. These processes may create significant differences between the experimental results we observed and their direct applications to natural lakes. Nevertheless, the nature of the processes described here may remain valid despite the changes in their relative importance at a whole-lake level and work as a guiding framework for future studies.

Conclusion

The press and pulse experimental framework proposed here indicated that eutrophication and sequential pulse perturbations are forces that potentially act in opposite directions, with a prevalent effect of sequential pulse perturbations in the recovery and resistance of the phytoplankton community. Eutrophication increased the *absolute* response (magnitude) of the phytoplankton community to pulse perturbations but did not change the *relative* response (intensity) compared to the pre-response condition. Nonetheless, eutrophying systems tend to operate much closer to regulatory thresholds for water quality (e.g., limit concentration of Chl-a), implying that higher absolute responses may already pose significant risks for water security even if the proportion of response in the different eutrophication levels remain the same.

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Author’s Contributions

JAAS, LNdSD, BWI, and JPM conceived the ideas and designed methodology; JAAS collected the data; JAAS, LNdSD, and JPM analyzed the data; JAAS led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Accessibility

Data and scripts are available on FigShare digital repository (https://doi.org/10.6084/m9.figshare.16646872).

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Chapter 6

General Discussion

Critical Processes Underlying 'Resilience'

A fundamental goal in experimental biology is to identify and quantify processes that may play an important role in the complexity of a natural system. Whereas experiments using simplified systems may not translate directly to the community or whole ecosystem level, they allow valuable ecological insights based on an in-depth mechanistic understanding of the processes ruling the systems. The primary objective in the thesis was to identify general processes and pathways taking place after a mortality pulse perturbation and assess the effect of resource availability on them. Studies of *resilience* often rely on quantifying physicochemical parameters to address the responses of the ecosystem. However, these parameters often produced vague information about the main players underlying the *resilience* process. The role of biotic interactions, which cannot be gained purely by assessing environmental drivers, proved to be fundamentally important for understanding the response of the perturbation experiments assessed here under different resource availabilities. Competition, facilitation, intrinsic growth rates, population turnover, complexity of ecological interactions, trophic complexity, rates of energy transfer, all played a role in the processes underlying '*resilience'* (*latu sensu*).

Ecosystems are continuously pressed by various multi stressors (driving forces) while suffering stochastic *pulse perturbations* that momentarily force them out of equilibrium (e.g., storms, heatwaves, point-source pollution events). In the *Pseudomonas fluorescens* experiment, we observed the importance of biotic interaction for determining how the system responds to perturbations and the effect of resource availability on shaping the direction of those interactions. The importance of biotic-biotic interactions on the response to perturbations has already been pointed out by other studies (Valencia et al., 2020, Brown et al., 2016, Ghedini et al., 2015, Jochimsen et al., 2013, Isbell et al., 2013). In the mesocosms experiment, we observed the capability of the system to adapt to sequential *pulse perturbations* in a way to reduce its impacts. The more perturbed the system was, the less responsive it became, and this can be interpreted as a possible community pathway towards stability (Paine et al., 1998). Also, we observed that the loss of recovery and the increase in resistance to *pulse perturbations* was driven by the serial perturbations rather than resource availability, suggesting that the sequential *pulse perturbations* can be more important than resource availability (*press perturbation*) for phytoplankton community stability.

We also observed that *pulse perturbations* increased the amount of autochthonous nutrients in the system, selecting for '*small and fast-growers*' at first. However, the direction of this process can change with the increase in resistance caused by sequential *pulse perturbations*. With fewer nutrients being released due to the mortality event, top-down regulation is likely to increase in importance during the response to *pulse perturbations*. The causes of eutrophication are often approached from a bottom-up perspective (Ansari et al., 2010, Prepas and Charette, 2003), indicating an underestimation of trophic complexity and top-down regulation on the stability of eutrophying systems. The importance of top-down regulation on the stability of ecosystems is connected with the energy transfer and allocation of biomass in higher trophic levels, as presented by McCauley et al. (2018). As an example, it is common sense to acknowledge the importance of sharks for the regulation of marine food webs, but when it comes to freshwater systems, the focus often relies on the bottom-up control of phytoplankton communities.

Lastly, our methodological revision taught us that the ecological level of the proxy used for measuring *resilience* is expected to affect how long before the actual regime shift an EWS is observed; and its potential for presenting false positives and negatives. The closer a proxy is to a population level, the further it is from representing ecosystem-level responses (Cottingham and Carpenter, 1998). On the other hand, since populations respond faster to environmental changes than communities or ecosystems, they are expected to respond to them promptly. Process-based experiments can help translate how signals from populations can be reliably upscaled to community and ecosystem levels, as well as define the best proxies for measuring a relevant *resilience* process. For instance, the assessment of a storm whose direct effects last for a couple of days will demand proxies with a high population turnover (e.g., phyto- and zooplankton), while assessment of a hurricane whose direct effects may last months will allow proxies with a lower population turnover (e.g., macrophytes or fish).

Effects of Resource Availabilities in the 'Stability' of Perturbed Systems

The effects of resource availability can be decomposed into two distinguished processes of recovery. One is the capability of the system to fully recover from disturbance, which is often associated with (i) the compositional and functional structure of the ecosystem (Thayne et al., 2021, Hillebrand and Kunze, 2020), (ii) the legacy effect from repeated perturbations (Ryo et al., 2019, Johnstone et al., 2016), and (iii) the rates of energy flow to higher trophic levels (McCauley et al., 2018, Shade et al., 2012). Second is the pattern of recovery after perturbation, which was associated with (i) the level of resources available for recovery after the pulse perturbation, (ii) the community composition at the time of perturbation, and (iii) the strength and complexity of ecological interactions. Both experiments in this thesis presented a convergent story. Resource availability did not change the capability of the systems to recover from *pulse perturbations*, despite changing the compositional structure of the systems. Yet, in both experiments, resource availability changed the pattern of response of the system to the *pulse perturbations*.

Community composition and resource availability are often entangled in nature. Assessing the effect of resource availability on the response to *pulse perturbations* in a system evolving from a single ancestor (chapter IV) provided an unbiased perspective of the isolated role of resource availability in shaping community response to *pulse perturbations*. No population can be more adapted to a given environment than in the situation where all its ecotypes evolved from adaptive radiation and fixed population by 'natural' selection at a given resource level since the original ancestor. When we tuned the oxygen availability at the Air Liquid interface in the *Pseudomonas* microcosms, we indirectly modeled the nature and intensity of their ecological interactions during the microcosm formation, resulting in distinct ecotype distributions. This provided empirical evidence that the resource availability on which the population/community developed shapes its distribution, and therefore, its response to perturbations.

The *Pseudomonas* experiment showed us that perturbation in the systems with low resource availability resulted in weaker recovery rates and longer recovery times for both compositional and functional aspects of the system. This is attributed to the simple fact of lacking sufficient resources for basic metabolic needs (Brown et al., 2004, Enquist et al., 2003). Also, low resource availability created a condition of evolutionary stasis during the adaptive radiation, which remained during the perturbation period. This created an environment where the differences in fitness between different ecotypes were very low (see the neutral theory, Hubbell (2001)), potentially resulting in a microcosm composition where priority effects play a larger role (Zhou et al., 2014). At high resource availability, recovery was stronger and faster. Also, the composition of the system was mainly determined by resource competition and facilitation, where adaptive traits resulted in an increase of fitness that was translated into the dominance of a specific ecotype. Thus, we observed that the nature and strength of ecological interactions during the perturbation showed to be fundamental for defining the patterns of recovery. Studies of the role of competition and facilitation on the recovery from *pulse perturbations* are virtually absent and certainly a profitable area of investigation.

Resource availability, however, did not change to which level the system can recover after a pulse perturbation. The slower processes induced by low resource availability were compatible with the energy budget stored in the system and the rates of energy flow involved in basic metabolic needs and ecological interactions; therefore, not compromising its '*ecological resilience*'. A potentially dangerous situation in systems with low resource availability is when sequential perturbations strike them. Globally, the frequency, intensity, and duration of weather-related *pulse perturbations* are predicted to increase (Bell et al. 2018; Harris et al. 2018; Woolway et al. 2021). If the frequency of these *pulse perturbations* increases to an extent to overlap the time needed for recovery to take place, it may produce a cumulative additive effect of *pulse perturbations* (Woolway et al., 2021). Since systems with low resource availability tend to respond slower, they might become more vulnerable to sequential *pulse perturbations*, culminating in more fragile systems. Yet, this effect would be a consequence of the simple additive effect of *pulse perturbations* and not to the loss of '*ecological*' or '*engineering resilience*'.

In the mesocosms, our approach was to start all the systems with the same resource availability and diverge their resources over time, since complex natural communities cannot be evolved from a single ancestor (in a feasible timeframe). Systems with more resources were capable of producing higher levels of biovolume. This biovolume was transformed into chemical energy and released into the system as dissolved nutrients when hit by a pulse perturbation. As more resources were allocated in the system before the perturbation, more nutrients were released during the perturbation; and with more dissolved nutrients available for uptake, higher peaks of phytoplankton concentration were observed as a response to the pulse perturbation. We observed that microbial communities undergoing strong eutrophication are likely to dramatically increase chlorophyll-a levels after sequential *pulse perturbations* compared to non-eutrophying systems (nearly twice as much). Since eutrophic systems tend to operate much closer to regulatory thresholds for water quality (e.g., Chl-a levels), higher absolute Chl-a responses triggered by stochastic pulse events may pose significant risks for water security. This is an important consideration when managing waterbodies with strict regulatory directives (i.e., drinking water provision).

Moreover, our mesocosms experiment showed that resistance increased and recovery decreased after each pulse perturbation and that eutrophication *per se* can increase the magnitude of response of the phytoplankton community compared to non-eutrophying systems. Yet, we did not find evidence that eutrophication would make the phytoplankton community proportionately less resistant or less resilient to *pulse perturbations* than when eutrophication is absent. The results from both experiments are identical in direction in this aspect. Resource availability did not make the systems more vulnerable to *pulse perturbations* or less recoverable. In view of '*ecological resilience*', both systems were capable of sustaining their pre-perturbation state, and in view of '*engineering resilience*', both systems were capable of recovering from the perturbation to a similar level for both resource availability scenarios.

Assessing Resilience when Resource Availability is Changing

One main asset of assessing recovery from perturbations is that it requires only a fraction of the data needed for making long-term inferences of how the *press perturbation* affects the system. This can be helpful when data acquisition is an issue (e.g., monitoring campaigns cannot operate continuously). Also, recovery as a metric for ecosystem stability could allow more cost-effective monitoring schemes since recovery requires only short periods of data collection, opposite to the multi-annual continuous monitoring needed for assessing long-term trends of ecosystem dynamics. A consistent challenge is that assessing recovery from perturbations may require a high-frequency sampling before, during, and after the perturbation, implying that the pulse perturbation has to be either predicted in advance or induced in the system.

Studies of recovery from *pulse perturbations* can be approached from two distinct perspectives: a functional one that prioritizes the recovery in functions operating at the community level (e.g., recovery in community biomass) and a compositional one that prioritizes the recovery of the individual populations composing the community. These two perspectives have been shown to result in far distinct recovery processes and assumptions of state of recovery (Hillebrand and Kunze, 2020), making the selection of a single attribute of the system as a proxy for whole system behavior a challenging task (see Spears et al. (2017)).

Despite its simplicity, the *Pseudomonas* experiment showed us that systems with different resource availabilities could show similar recovery in the functional aspects while presenting far different recoveries in the compositional aspects. This might become fundamentally important when the organisms at stake share some common functional traits, but may also have distinct traits giving them fairly different functions at the system level. In this situation, a biased functional perspective may consider the system fully recovered while, in fact, it is still far from recovered considering its compositional aspects (Hillebrand and Kunze, 2020). Even a simple system as the *Pseudomonas* microcosms provided empirical evidence that assessing recovery solely from a functional perspective may hide important processes needed for mechanistically understanding how a system recovers from perturbations. Moreover, the estimates of asymptotic recovery – the maximum level a system recover after a pulse perturbation - indicated that a complete functional recovery does not necessarily mean a complete compositional recovery of all parts of the system.

The interplay of recovery and resource availability most often will not allow comparison of raw data since resource availability changes the carrying capacity of the systems being compared (changes in scale). The comparison of systems will require a normalization of the metrics measured in a way to scale systems of different trophic states. Also, full recovery may not be expected when the carrying capacity of the system is changing during the serial assessment of recovery rates. This is critical because incomplete recovery directly impacts the methodological calculation of recovery rates and time (see the assumptions of *'engineering resilience'* in the introduction). The assessment of recovery rates under these circumstances requires a simulation of the post-perturbation baseline to which the system is expected to recover in a way to compensate for the incomplete recovery (i.e., stabilization in a different equilibrium within the same 'state'). Yet, Thayne et al. (2021) demonstrated that the situation of full recovery is highly unlikely to happen in complex natural scenarios, as when assessing the effect of storms in a shallow lake. When systems are already expected not to follow the theoretical pattern of full recovery, using simulated approaches to fulfill methodological needs become superfluous. This is why we decided to index recovery independently of the time in the mesocosm experiment. Assessing recovery rates in systems that are constantly changing their resource availability, and therefore in their carrying capacity, is methodologically unfeasible to a given extent due to the incomplete recovery. In alternative to indexing recovery as we have done, individual recovery rates can be calculated at a population level (as observed in the first experiment). Yet, it is well known that beacon community and ecosystem responses based on the assessment of a single populational proxy is challenging and prone to misinformation (Chapter III).

Experiments showed that univariate metrics would not be capable of addressing the full complexity embedded in the study of *resilience*. Therefore, individual assessments of recovery could be most useful as a partial indication that changes are about to happen in the studied system (Clements and Ozgul, 2018, Eason et al., 2016, Lindegren et al., 2012). A fundamental step is to merge univariate responses into comprehensive composites (e.g., indexes presented by (Clements and Ozgul, 2016, Drake and Griffen, 2010). Also, recent studies have reported promising results by using multivariate analysis – an analysis capable of collapsing the behavior of multiple variables of a complex system into an index that captures the system dynamics over time, space, or both (Fath et al., 2003) - to help us translating individual responses into a significant ecosystem-level response (see Sundstrom et al. (2017), Eason et al. (2016), Spanbauer et al. (2014), Eason et al. (2013), Karunanithi et al. (2008)). Near-natural experiments focused on process-based assessments of *resilience* would help shaping future multivariate frameworks that can be applicable to the complexity of the real world, benefiting monitoring and management.

From our understanding, the approach used in the mesocosm experiment (Chapter V) - instead of using rates, using % changes compared to the baseline - showed the best fit for the circumstances while using a community-based proxy that is directly meaningful for the research question. Moreover, we have to be especially careful using recovery from perturbation as a proxy of *ecological resilience* when resource availability is the main driver of ecosystem change. This is because resource availability in both systems studied here played an important role in determining the patterns of recovery but not to which level the system can recover after a pulse perturbation. When dealing with systems of different resource availability, ecological (*sensu* Holling) and *engineering resilience* (*sensu* Pimm) may potentially decouple over time, misguiding interpretation of the results (Hastings, 2010). Hence, the use of recovery processes to address different levels of *ecological resilience* between systems with different resource availabilities - in a way to rank them - may be flawed by the simple fact that systems with low resource availability will inherently respond throughout slower processes. The use of patterns of recovery for inferring stability of systems cannot be indiscriminate and has strict boundaries, which require previous knowledge of the system to be better understood. The use of recovery as a metric of stability only makes sense if assessing multiple *pulse perturbations* within the same system for making temporal comparisons that agree with temporal changes in the *press perturbation*. It is possible that other constraints beyond resource availability also exist when using recovery to address stability; however, they were not investigated in this thesis. We observed in both experiments that using recovery to compare systems with different resource availability is risky if an advanced process-based understanding of the mechanisms ruling the systems is not in place.

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Chapter 7

Perspective and Remarks

The High Nutrient Paradigm in Freshwater Systems

Eutrophication remains one of the main pressures acting on freshwater ecosystems, and its impact is expected to rise due to climate change (Fink et al., 2018, World Water Assessment Programme, 2009, Carr and Neary, 2008). In freshwater systems, high levels of resource availability are often translated as high nutrient levels of total nitrogen (TN) and total phosphorous (TP), which both together with Chl-a are used for determining the trophic state of the system (Chen et al., 2015). However, the coupling of trophic state with nutrient levels have multiple caveats that may misguide observational data acquisition and the development of process-based knowledge over the effect of resource availability on ecological processes:

1. TP is mostly used to determine the trophic state of freshwater systems but is rarely measured with reliable methodologies to account for TP in the whole system (e.g., system analysis). When limnology refers to TP, it frequently refers TP_{seston}, or in other words, the TP allocated in the planktonic community (sometimes only phytoplankton); instead of the TP allocated within the different trophic levels of the system. Therefore, a so-called 'oligotrophic' system may ultimately have more TP than a eutrophic system by simply having it fixed in perennial biomass. At a whole ecosystem level, a direct implication of this process is that the total energy budget in the system will be significantly different when hit by a *pulse perturbation* that induces in-system mortality. While sounding very specific, mass mortality events are less rare than expected by common sense (Fey et al., 2015).
2. The qualification of trophic state follows almost exclusively a bottom-up approach, which drives the limnological field to the pitfall of understanding eutrophication from a single perspective. A classic example is the experimental theory that fish is detrimental to the trophic state of a lake by reducing the grazing pressure on the phytoplankton community (see, Jeppesen et al. (2012) and Hansson et al. (2012)). On the other hand, the higher the number of trophic levels (McCauley et al., 2018) and community complexity (Hodapp et al., 2019), the higher the amount of total nutrients that can be fixed in biomass, reducing its bioavailability for the phytoplankton community. Therefore, top-down regulation of aquatic food webs can potentially compensate for the increase in internal nutrient load caused by eutrophication. Hence, reduced trophic complexity (e.g., caused by the removal of top predators) may imply similar or even stronger consequences in the long run towards phytoplankton dominance than nutrient addition *per see* (see the series of experimental manipulations of Paul and Peter lakes in Wisconsin – the USA, starting from Carpenter et al. (2008)).

3. Freshwater systems are often P-limited systems (Schindler et al., 2008, Elser et al., 2007), what partially explains the focus on TP when studying eutrophication. However, the release of P-limitation often results in secondary limitations of Carbon, Nitrogen, or Iron if they do not increase stoichiometrically with TP. Therefore, a continuous eutrophication process will only be observed if co-limitation between those macronutrients is maintained along the eutrophication curve. The plankton community often shifts towards a Carbon or Nitrogen limitation when P is not limiting, producing trophic bottlenecks. While phytoplankton can fix Carbon and Nitrogen, they have faster growth rates than their primary consumers in general, what can result in a transient phytoplankton bloom (Schindler, 1977). Although, this process may happen independently of the trophic state, as blooms are also described in oligotrophic lakes (Reinl et al., 2021).

Obviously, this thesis does not stage a defense of eutrophication processes or the disruption of ecological processes. Yet, the larger picture created on the scientific imaginary that resource availability has always to be minimized appears to be overstated in aquatic systems. Having access to higher levels of resource availability has increased biodiversity in experimental systems evolving from a single ancestor and reduced the likelihood of extinction the mesocosms experiment. In both trophic complexities and scales tested here, having a high level of resource availability showed to be in favor of a more even system response to the perturbations, reducing the dominance of a specific trait group and aiding the recovery process. Moreover, high resource availability was not detrimental to *ecological resilience* within the systems tested. Still, extensive experimental replication will be required in further studies to corroborate these findings, assessing and manipulating the energy flow/mass balance within the food web along with a continuous increase in resource availability while applying multiple *pulse perturbations*.

Cultural Background and the EPR Paradox in Biological Sciences Aid in Understanding why (Eco)Systems Act "Against" Human Will

The EPR paradox (acronym of the authors) is a thought experiment proposed by physicists Albert Einstein, Boris Podolsky, and Nathan Rosen, in which they argued that the description of physical reality provided by quantum mechanics was incomplete (Einstein et al., 1935). The foundation of this paradox was the incapability of deterministically explaining deviations between reality and a predicted physical outcome embedding processes that were, by the time, accepted as well understood. Above and beyond the physical character of this digression, it highlights that when the knowledge of the processes governing a system is misunderstood or absent, deterministic results become a mere probabilistic projection. In ecology, the scenario is not different. The stochasticity of ecological systems regarding their spatiotemporal development is *per se* a major incognita in environmental sciences. Perturbation ecology may provide a thoughtful framework to incorporate the "deviations from deterministic outcomes" since it introduces new processes to the systems that cannot be ignored in reality (under the expenses of falling on the EPR paradox with an incomplete reality).

A first step is to humbly accept that we know less than we think we know; and that our operational capabilities are at the same time above our comprehension and below our expectations. In other words, we have to accept that we need to know more to do more. For decades ecology faced (and sometimes overcame) several taboos, like the spatial and temporal evolution of species, the importance of eco-evolutionary dynamics, the effect of the biogeography of islands, the fundaments of meta-community/population dynamics, the importance of species interactions (beyond the simplicity of competition), the successional patterns of landscape evolution, the energy flows and de-compartmentalization of ecosystems, the importance of ethology in ecology, among many others. While originating from different sources of knowledge and theoretical schools, on the core of the initial denial that slows down ecological development is the anthropocentric perspective towards (what should be and how should behave) an ecosystem (Figure 1).

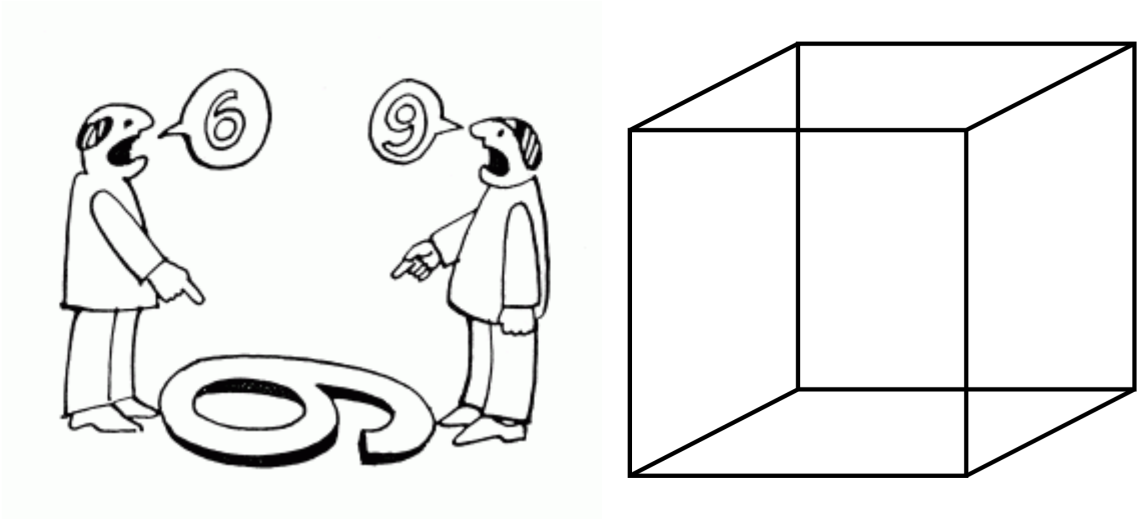

Figure 1. left: Different perspectives may have fairly distinct interpretations over the same subject. Often this situation can lead towards a progressist development of ideas and novel understandings but sometimes may become a source of unproductive argumentation and inflexibility. Right: Necker's cube as proposed by the Swiss crystallographer Albert Necker. Most people see a cube projected to the lower left at first glance but shifting to the upper right after a few seconds of observation. The flipping of the cube represents the ambiguity of assertions when based on thin assumptions, weak referential, and incomplete understanding of the system as a whole.

"No problem can be solved from the same level of consciousness that created it". Future developments in *resilience* (*latu sensu*) may embrace the mechanistic understanding of the system, which shall be interpreted from the least anthropocentric view as possible. Here, I do not argue that human needs shall be subjected to undervaluation when studying ecosystems but that the understanding of the processes ruling the ecosystem responses shall be acquired from a perspective that goes beyond human needs. The interpretation of ecophysiological traits from an anthropocentric and deterministic point of view without considering the underlying mechanisms that created and supported them is doomed to the production of artificial and incongruent knowledge, that is only applicable under a highly limited array of constraints. This is the utmost threat produced by scientific-driven data. Taxonomy based on morphological data is a historical example of a misguided interpretation of biological processes. Darwin and Wallace only acknowledged evolution when they observed it from the perspective of the organism.

In ecology, we shall walk towards the light where "Nothing in biology makes sense except in the light of … evolution" (Dobzhansky, 1973). Organisms and the consequent ecological patterns created by them have evolved uniquely and exclusively from their perception and interaction with the environment; and not from our perspective on how they perceive and interact with the environment. Interpreting ecological evidence from the organism's perspective would reduce the likelihood of incurring the prosecutor's fallacy (Boettiger and Hastings, 2012) and produce mechanistic interpretations of how a given part of the system interacts with the environment. For that, we have to be more attentive to the importance of biotic-biotic interactions in ecology, which fundamentally requires a better understanding of our biodiversity. For instance, behavioral ecology (human ecology in the further examples) has been a game-changer in preventive medicine, macroeconomics, social cohesion, marketing, and psychology. Shifting the perspective towards understanding how the organism perceives the environment (instead of how the environment interacts with the organism) creates infinite possibilities of effective management already proved in many areas of science, from which ecology may learn from. As a matter of fact, many inferences in ecology have been imported from other fields as physics, mathematics, sociology, and economics (*resilience* inclusive). Only by understanding the mechanisms ruling an ecosystem at a given time and space, we will be able to produce comprehensive rationales that are not dependent on a probabilistic framework (see, Spears et al. (2017)), and therefore, reasonably safe for decision-making.

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Chapter 8

Supplementary Material

Chapter 3 - Early Warning Signals of Regime Shifts for Aquatic Systems: Can Experiments Help to Bridge the Gap between Theory and Real-World Application?

Supplement – Dataset

The table 1 contains all the papers used in the revision. The file “Metadata” (Table 2) contains the categorization of the 24 papers used to compile this review on experimental early warning signals. Data were categorized based on the characteristics described in the original document.

Important aspects:

- When many papers were published with data coming from a single experiment, these experiments were considered not unique. The column “Unique Experiment ID” refers to the cataloging of original experiments. Non-unique experiments received the same ID according to the first published paper.
- “Time/n.a” on the driving force means that the experiment had no explicit driving force pushing the system away from its state.
- The proxy abundance refers to counts and concentrations of organisms (e.g., Cells/ml). It does not include pigment concentrations. Those were separated as Chlorophyll-a and Phycocyanin.
- Different metrics of variance (e.g., sd, CV, variance itself) were all clustered as “variance”. When fields appear in gold at the metadata excel sheet, it means that two or more metrics of the same cluster were assessed with the same proxy in the same experiment.
- Different metrics of “shape of the distribution” (e.g., kurtosis, skewness) were clustered as “shape of distribution”. When fields appear in gold at the metadata excel sheet, it means that two or more metrics of the same cluster were assessed with the same proxy in the same experiment.
- Sampling effort did not follow fixed timesteps for some experiments. Therefore, what you find in the data is an averaged sampling effort based on what was originally described in the paper.
- n.a* means no external perturbation, but a perturbation was caused by the abrupt manipulation of the driving force.

Revision of EWS from Wilkinson et al. (2018):

The paper from Wilkinson et al. (2018) described results from EWS indicators in table 3 of the paper. The paper itself assesses the capability of EWS to produce a composite signal before an algae bloom for management perspective and not for the sake of EWS development. Thus, the picking of the individual results for Proxies x Metrics combination was not explicit on the text and was obtained as below:

Proxy Chlorophyll-a:

1) Was a bloom observed (“DOY” Bloom) for Chlorophyll-a?
    a) Yes (Go to number 2)
    b) No (Disregard EWS of Regime shift)
2) Did the bloom trigger “pre-bloom alarm” (numeric values ≠ 0 and not “n.a”) using Standard Deviation or Autocorrelation as a metric for Chl-a?
    a) Yes (Result accounted as “Positive” for the given Proxy Vs. Metric combination)
    b) No (Result accounted as “Negative” for the given Proxy Vs. Metric combination)

Proxy Phycocyanin:

1) Was a bloom observed (“DOY” Bloom) for Phycocyanin?
    a) Yes (Go to number 2)
    b) No (Disregard EWS of Regime shift)
2) Did the bloom trigger “pre-bloom alarm” (numeric values ≠ 0 and not “n.a”) using Standard Deviation or Autocorrelation as a metric for Phycocyanin?
    a) Yes (Result accounted as “Positive” for the given Proxy Vs. Metric combination)
    b) No (Result accounted as “Negative” for the given Proxy Vs. Metric combination)

Note that due to the way we assessed the individual results of EWS, false positives are neglected instead of being considered “negative results”. On this way, we avoid quantifying results for EWS in situations that have not surpassed the tipping-point (expected regime shift but without confirmation)

Revision of EWS from Clements et al. (2015):

The paper from Clements et al. (2015) analyzed composites of EWS. This means that the response of individual EWS were merged into a single comprehensive signal. We could not extract individual results for each of the five metrics used as we did for the other papers. Therefore, we considered them all as “inconclusive” even though the composites succeeded in observing EWS. In a matter of effect, if those results were considered all as the success rate of abundance-based proxies would increase from 71% to 81%. Although this difference sounds significant, it has only increased the number of positive results from 22 to 25 out of 31 and would not alter the core message of the paper. Independently of how we interpreted these specific results, abundance-based proxies would still be considered a recommended line of investigation. Due to the criticism surrounding the real-world applicability of EWS we chose to be more conservative when

For further queries and data, please contact the main author (jaastelzer@gmail.com)

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Table 1. List of aquatic experimental papers compiled for assessing the reliability of Early-Warning Signals of regime shift. Different papers having the same experimental ID means that the data came from the same experiment.

Paper	Unique Experimental ID	Author	Title	Journal	Year
1	1	Robinson & Uehlinger, 2008	Experimental Floods Cause Ecosystem Regime Shift in a Regulated River	Ecol. Applications	2008
2	2	Drake & Griffen, 2010	Early warning signals of extinction in deteriorating environments	Nature	2010
3	3	Carpenter et al., 2011	Early warnings of regime shifts: a whole-ecosystem experiment	Science	2011
4	4	Veraart et al., 2012	Recovery rates reflect distance to a tipping point in a living system	Nature	2012
5	5	Dai et al., 2012	Generic indicators for loss of resilience before a tipping point leading to population collapse	Science	2012
6	3	Seekell et al., 2012	Conditional Heteroskedasticity Forecasts Regime Shift in a Whole-Ecosystem Experiment	Ecosystems	2012
7	6	Dai et al., 2013	Slower recovery in space before collapse of connected populations	Nature	2013
8	7	Sirota et al., 2013	Organic-matter loading determines regime shifts and alternative states in an aquatic ecosystem	Pro. Nat. Acad. USA	2013
9	3	Batt et al., 2013	Changes in ecosystem resilience detected in automated measures of ecosystem metabolism during a whole-lake manipulation	Pro. Nat. Acad. USA	2013
10	3	Pace et al., 2013	Zooplankton provide early warnings of a regime shift in a whole lake manipulation	Limn. & Ocean	2013
11	3	Seekell et al., 2013	Evidence of alternate attractors from a whole-ecosystem regime shift experiment	Theoretical Ecology	2013
12	3	Cline et al., 2014	Early warnings of regime shifts: evaluation of spatial indicators from a whole-ecosystem experiment	Ecosphere	2014
13	8	Soissons et al.,2014	Cover versus recovery: Contrasting responses of two indicators in seagrass beds	Marine Pollution Bulletin	2014
14	9	Benedetti-Cecchi et al., 2015	Experimental Perturbations Modify the Performance of Early Warning Indicators of Regime Shift	Current Biology	2015
15	10	Dai et al., 2015	Relation between stability and resilience determines the performance of early warning signals under different environmental drivers	Pro. Nat. Acad. USA	2015
16	12	Jarvis et al., 2016	Early warning signals detect critical impacts of experimental warming	Eco & Evolution	2016
17	13	Clements and Ozgul, 2016	Including trait-based early warning signals helps predict population collapse	Nat. Communications	2016
18	14	Sommer et al., 2017	Are generic early-warning signals reliable indicators of population collapse in rotifers?	Hydrobiologia	2017
19	15	Butitta et al., 2017	Spatial early warning signals in a lake manipulation	Ecosphere	2017

20	16	Rindi et al., 2017	Direct observation of increasing recovery length before collapse of a marine benthic ecosystem	Nature Ecology & Evolution	2017
21	17	Wilkinson et al. 2018	Early warning signals precede cyanobacterial blooms in multiple whole-lake experiments	Ecological Monographs	2018
22	18	El-Hacen et al., 2018	Evidence for 'critical slowing down' in seagrass: a stress gradient experiment at the southern limit of its range	Sci Rep	2018
23	19	Ghadami et al., 2018	Rate of recovery from perturbations as a means to forecast future stability of living systems	Sci Rep	2018
24	16	Rindi et al., 2018	Experimental evidence of spatial signatures of approaching regime shifts in macroalgal canopies	Ecology	2018

Table 2. Metadata. Compilation of all the Early-Warning Signals quantified in aquatic experiments since 2000. Experimental designs were decomposed using a press and pulse framework, where the pulse perturbations are simply called "perturbations "and the press perturbations are labeled "driving forces".

Paper	Unique Experiment ID	Main Perturbation	Secondary Perturbation	Regime of Main Perturbation	Regime of Secondary Perturbation	Driving Force	Is the Driving Force Increasing over Time?	Proxy Type	Proxy	Metric	Approximate Sampling Frequency	Approximate Length of the Experiment	Described Result	Regime Shift	Biological Complexity	Environment	Focci	Species	Reference
1	1	Floods	n.a	Multiple Pulses	n.a	Time/n.a	No	Biotic	Chlorophyll-a	Recovery	40 days	8 years	Positive	Flood Controlled / Flood Resilient Fauna	Complex	Whole-River Manipulation	Periphyton	Multiple	Robinson & Uehlinger (2008)
1	1	Floods	n.a	Multiple Pulses	n.a	Time/n.a	No	Biotic	Dry-Weight	Recovery	40 days	8 years	Positive	Flood Controlled / Flood Resilient Fauna	Complex	Whole-River Manipulation	Periphyton	Multiple	Robinson & Uehlinger (2008)
1	1	Floods	n.a	Multiple Pulses	n.a	Time/n.a	No	Biotic	Benthic Organic Matter	Recovery	40 days	8 years	Inconclusive	Flood Controlled / Flood Resilient Fauna	Complex	Whole-River Manipulation	Periphyton	Multiple	Robinson & Uehlinger (2008)
1	1	Floods	n.a	Multiple Pulses	n.a	Time/n.a	No	Biotic	Dry-Weight	Recovery	40 days	8 years	Inconclusive	Flood Controlled / Flood Resilient Fauna	Complex	Whole-River Manipulation	Seston	Multiple	Robinson & Uehlinger (2008)
1	1	Floods	n.a	Multiple Pulses	n.a	Time/n.a	No	Biotic	Chlorophyll-a	Recovery	40 days	8 years	Positive	Flood Controlled / Flood Resilient Fauna	Complex	Whole-River Manipulation	Seston	Multiple	Robinson & Uehlinger (2008)
1	1	Floods	n.a	Multiple Pulses	n.a	Time/n.a	No	Biotic	Spatial Biomass	Recovery	40 days	8 years	Positive	Flood Controlled / Flood Resilient Fauna	Complex	Whole-River Manipulation	Macroinvertebrates	Multiple	Robinson & Uehlinger (2008)
1	1	Floods	n.a	Multiple Pulses	n.a	Time/n.a	No	Biotic	Individual Biomass	Recovery	40 days	8 years	Inconclusive	Flood Controlled / Flood Resilient Fauna	Complex	Whole-River Manipulation	Macroinvertebrates	Multiple	Robinson & Uehlinger (2008)
1	1	Floods	n.a	Multiple Pulses	n.a	Time/n.a	No	Biotic	Richness	Recovery	40 days	8 years	Positive	Flood Controlled / Flood Resilient Fauna	Complex	Whole-River Manipulation	Macroinvertebrates	Multiple	Robinson & Uehlinger (2008)
1	1	Floods	n.a	Multiple Pulses	n.a	Time/n.a	No	Biotic	Abundance	Recovery	40 days	8 years	Positive	Flood Controlled / Flood Resilient Fauna	Complex	Whole-River Manipulation	Macroinvertebrates	Multiple	Robinson & Uehlinger (2008)
2	2	n.a	n.a	n.a	n.a	Food provision	Yes	Biotic	Abundance	Variance	10 days	416 days	Positive	Extinction (Population Collapse)	Simple	Microcosms	Daphnia magna	Single + feed	Drake & Griffen (2010)
2	2	n.a	n.a	n.a	n.a	Food provision	Yes	Biotic	Abundance	Autocorrelation	10 days	416 days	Positive	Extinction (Population Collapse)	Simple	Microcosms	Daphnia magna	Single + feed	Drake & Griffen (2010)
2	2	n.a	n.a	n.a	n.a	Food provision	Yes	Biotic	Abundance	Shape of the Distribution	10 days	416 days	Positive	Extinction (Population Collapse)	Simple	Microcosms	Daphnia magna	Single + feed	Drake & Griffen (2010)
2	2	n.a	n.a	n.a	n.a	Food provision	Yes	Biotic	Abundance	Spatial Correlation	10 days	416 days	Positive	Extinction (Population Collapse)	Simple	Microcosms	Daphnia magna	Single + feed	Drake & Griffen (2010)
3	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Biotic	Chlorophyll-a	Recovery	5 mins	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Phytoplankton	Multiple	Carpente et al. (2011)
3	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Biotic	Chlorophyll-a	Variance	5 mins	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Phytoplankton	Multiple	Carpente et al. (2011)
3	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Biotic	Chlorophyll-a	Variance	5 mins	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Phytoplankton	Multiple	Carpente et al. (2011)
3	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Biotic	Chlorophyll-a	Autocorrelation	5 mins	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Phytoplankton	Multiple	Carpente et al. (2011)

Paper	Unique Experiment ID	Main Perturbation	Secondary Perturbation	Regime of Main Perturbation	Regime of Secondary Perturbation	Driving Force	Is the Driving Force Increasing over Time?	Proxy Type	Proxy	Metric	Approximate Sampling Frequency	Approximate Length of the Experiment	Described Result	Regime Shift	Biological Complexity	Environment	Focci	Species	Reference
3	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Biotic	Chlorophyll-a	Shape of the Distribution	5 mins	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Phytoplankton	Multiple	Carpente et al. (2011)
3	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Biotic	Chlorophyll-a	Spectral Ratio	5 mins	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Phytoplankton	Multiple	Carpente et al. (2011)
4	4	Mortality	n.a	Multiple Pulses	n.a	Light Intensity	Yes	Biotic	Abundance	Recovery	5 mins	29 days	Positive	Extinction (Population Collapse)	Simple	Single species setup	Aphanizomenon flos-aquae	Single	Veraart et al. (2012)
4	4	Mortality	n.a	Multiple Pulses	n.a	Light Intensity	Yes	Biotic	Abundance	Autocorrelation	5 mins	29 days	Positive	Extinction (Population Collapse)	Simple	Single species setup	Aphanizomenon flos-aquae	Single	Veraart et al. (2012)
4	4	Mortality	n.a	Multiple Pulses	n.a	Light Intensity	Yes	Biotic	Abundance	Variance	5 mins	29 days	Negative	Extinction (Population Collapse)	Simple	Single species setup	Aphanizomenon flos-aquae	Single	Veraart et al. (2012)
5	5	Mortality	Osmotic Shock	Multiple Pulses	Single Pulse	Mortality Rate	No	Biotic	Abundance	Recovery	1 day	9 days	Positive	Extinction (population collapse)	Simple	Single species setup	Saccharomyces cerevisiae	Single	Dai et al. (2012)
5	5	Mortality	Osmotic Shock	Multiple Pulses	Single Pulse	Mortality Rate	No	Biotic	Abundance	Autocorrelation	1 day	9 days	Positive	Extinction (population collapse)	Simple	Single species setup	Saccharomyces cerevisiae	Single	Dai et al. (2012)
5	5	Mortality	Osmotic Shock	Multiple Pulses	Single Pulse	Mortality Rate	No	Biotic	Abundance	Variance	1 day	9 days	Positive	Extinction (population collapse)	Simple	Single species setup	Saccharomyces cerevisiae	Single	Dai et al. (2012)
5	5	Mortality	Osmotic Shock	Multiple Pulses	Single Pulse	Mortality Rate	No	Biotic	Abundance	Shape of the Distribution	1 day	9 days	Negative	Extinction (population collapse)	Simple	Single species setup	Saccharomyces cerevisiae	Single	Dai et al. (2012)
5	5	Mortality	Osmotic Shock	Multiple Pulses	Single Pulse	Mortality Rate	No	Biotic	Abundance	Variance	1 day	9 days	Positive	Extinction (population collapse)	Simple	Single species setup	Saccharomyces cerevisiae	Single	Dai et al. (2012)
6	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Biotic	Chlorophyll-a	Conditional Heteroskedasticity	1 day	4 years	Inconclusive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Phytoplankton	Multiple	Seekell et al. (2012)
6	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Biotic	Abundance	Conditional Heteroskedasticity	1 day	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Minnows	Multiple	Seekell et al. (2012)
7	6	Mortality	n.a	Multiple Pulses	n.a	Mortality Rate	No	Biotic	Abundance	Variance	1 day	5 days	Positive	Extinction (population collapse)	Simple	Single species setup	Saccharomyces cerevisiae	Single	Dai et al. (2013)
7	6	Mortality	n.a	Multiple Pulses	n.a	Mortality Rate	No	Biotic	Abundance	Autocorrelation	1 day	5 days	Positive	Extinction (population collapse)	Simple	Single species setup	Saccharomyces cerevisiae	Single	Dai et al. (2013)
7	6	Mortality	n.a	Multiple Pulses	n.a	Mortality Rate	No	Biotic	Abundance	Spatial Correlation	1 day	5 days	Negative	Extinction (population collapse)	Simple	Single species setup	Saccharomyces cerevisiae	Single	Dai et al. (2013)
7	6	Mortality	n.a	Multiple Pulses	n.a	Mortality Rate	No	Biotic	Abundance	Recovery	1 day	5 days	Positive	Extinction (population collapse)	Simple	Single species setup	Saccharomyces cerevisiae	Single	Dai et al. (2013)
8	7	n.a*	n.a	Multiple Pulses	n.a	Organic matter	Yes	Abiotic	Oxygen	Variance	1 min	4 days	Positive	Aerobic / Anaerobic	Complex	Microcosms	Physico-Chemical	Multiple	Sirota et al. (2013)
8	7	n.a*	n.a	Multiple Pulses	n.a	Organic matter	Yes	Abiotic	Oxygen	Shape of the Distribution	1 min	4 days	Negative	Aerobic / Anaerobic	Complex	Microcosms	Physico-Chemical	Multiple	Sirota et al. (2013)

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8	7	n.a*	n.a	Multiple Pulses	n.a	Organic matter	Yes	Abiotic	Oxygen	Mean	1 min	4 days	Inconclusive	Aerobic / Anaerobic	Complex	Microcosms	Physico-Chemical	Multiple	Sirota et al. (2013)
8	7	n.a*	n.a	Multiple Pulses	n.a	Organic matter	Yes	Abiotic	Oxygen	Recovery	1 min	4 days	Inconclusive	Aerobic / Anaerobic	Complex	Microcosms	Physico-Chemical	Multiple	Sirota et al. (2013)
8	7	n.a*	n.a	Multiple Pulses	n.a	Organic matter	Yes	Abiotic	Oxygen	Shape of the Distribution	1 min	4 days	Inconclusive	Aerobic / Anaerobic	Complex	Microcosms	Physico-Chemical	Multiple	Sirota et al. (2013)
9	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Biotic	Chlorophyll-a	Autocorrelation	5 mins	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Phytoplankton	Multiple	Batt et al. (2013)
9	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Biotic	Chlorophyll-a	Variance	5 mins	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Phytoplankton	Multiple	Batt et al. (2013)
9	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Abiotic	pH	Autocorrelation	5 mins	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Physico-Chemical	Multiple	Batt et al. (2013)
9	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Abiotic	pH	Variance	5 mins	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Physico-Chemical	Multiple	Batt et al. (2013)
9	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Abiotic	Oxygen	Autocorrelation	5 mins	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Physico-Chemical	Multiple	Batt et al. (2013)
9	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Abiotic	Oxygen	Variance	5 mins	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Physico-Chemical	Multiple	Batt et al. (2013)
9	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Metabolism	GPP	Autocorrelation	5 mins	4 years	Negative	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Ecosystem Metabolism	Multiple	Batt et al. (2013)
9	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Metabolism	GPP	Variance	5 mins	4 years	Negative	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Ecosystem Metabolism	Multiple	Batt et al. (2013)
9	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Metabolism	GPP	Spectral Ratio	5 mins	4 years	Negative	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Ecosystem Metabolism	Multiple	Batt et al. (2013)
9	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Metabolism	R	Autocorrelation	5 mins	4 years	Negative	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Ecosystem Metabolism	Multiple	Batt et al. (2013)
9	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Metabolism	R	Variance	5 mins	4 years	Negative	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Ecosystem Metabolism	Multiple	Batt et al. (2013)
9	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Metabolism	R	Spectral Ratio	5 mins	4 years	Negative	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Ecosystem Metabolism	Multiple	Batt et al. (2013)
9	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Metabolism	NEP	Autocorrelation	5 mins	4 years	Negative	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Ecosystem Metabolism	Multiple	Batt et al. (2013)
9	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Metabolism	NEP	Variance	5 mins	4 years	Negative	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Ecosystem Metabolism	Multiple	Batt et al. (2013)
9	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Metabolism	NEP	Spectral Ratio	5 mins	4 years	Negative	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Ecosystem Metabolism	Multiple	Batt et al. (2013)

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10	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Biotic	Abundance	Variance	1 day	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Daphnia spp.	Multiple	Pace et al. (2013)
10	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Biotic	Abundance	Autocorrelation	1 day	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Daphnia spp.	Multiple	Pace et al. (2013)
11	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Biotic	Abundance	Fitness to GARCH model	1 day	4 years	Inconclusive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Minnows	Multiple	Seekell et al. (2013)
11	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Biotic	Abundance	Fitness to BDS model	1 day	4 years	Inconclusive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Minnows	Multiple	Seekell et al. (2013)
12	3	n.a*	n.a	Multiple Pulses	n.a	Top Predator Addition	Yes	Biotic	Abundance	Spatial Variance	1 day	4 years	Positive	Planktivorous / Piscivorous	Complex	Whole-Lake Manipulation	Minnows	Multiple	Cline et al. (2014)
13	8	Mortality	n.a	Single Pulse	n.a	Nutrient Addition	No	Biotic	Abundance	Recovery	46 days	46 days	Inconclusive	Extinction (Population Collapse)	Complex	Coastal Manipulation	Seagrass	Multiple	Soissons et al., (2014)
14	9	Mortality	n.a	Single Pulse	n.a	Clipping	No	Biotic	Abundance	Variance	6 months	7 years	Positive	Canopy/Turf dominated state	Complex	Coastal Manipulation	Seagrass	Multiple	Benedetti-Cecchi et al., (2015)
14	9	Mortality	n.a	Single Pulse	n.a	Clipping	No	Biotic	Abundance	Autocorrelation	6 months	7 years	Positive	Canopy/Turf dominated state	Complex	Coastal Manipulation	Seagrass	Multiple	Benedetti-Cecchi et al., (2015)
14	9	Mortality	n.a	Single Pulse	n.a	Clipping	No	Biotic	Abundance	Shape of the Distribution	6 months	7 years	Positive	Canopy/Turf dominated state	Complex	Coastal Manipulation	Seagrass	Multiple	Benedetti-Cecchi et al., (2015)
14	9	Mortality	n.a	Single Pulse	n.a	Clipping	No	Biotic	Abundance	Recovery	6 months	7 years	Positive	Canopy/Turf dominated state	Complex	Coastal Manipulation	Seagrass	Multiple	Benedetti-Cecchi et al., (2015)
15	10	Mortality	n.a	Multiple Pulses	n.a	Food provision	Yes	Biotic	Abundance	Autocorrelation	1 day	22 days	Positive	Extinction (Population Collapse)	Simple	Single species setup	Saccharomyces cerevisiae	Single	Dai et al. (2015)
15	10	Mortality	n.a	Multiple Pulses	n.a	Food provision	Yes	Biotic	Abundance	Variance	1 day	22 days	Positive	Extinction (Population Collapse)	Simple	Single species setup	Saccharomyces cerevisiae	Single	Dai et al. (2015)
15	11	Mortality	n.a	Multiple Pulses	n.a	Mortality Rate	Yes	Biotic	Abundance	Autocorrelation	1 day	20 days	Positive	Extinction (Population Collapse)	Simple	Single species setup	Saccharomyces cerevisiae	Single	Dai et al. (2015)
15	11	Mortality	n.a	Multiple Pulses	n.a	Mortality Rate	Yes	Biotic	Abundance	Variance	1 day	20 days	Positive	Extinction (Population Collapse)	Simple	Single species setup	Saccharomyces cerevisiae	Single	Dai et al. (2015)
16	12	Mortality	n.a	Multiple Pulses	n.a	Temperature	No	Biotic	Abundance	Recovery	2.5 days	64 days	Positive	Extinction (Population Collapse)	Simple	Single species setup	Chlorella vulgaris	Single	Jarvis et al. (2016)
16	12	Mortality	n.a	Multiple Pulses	n.a	Temperature	No	Biotic	Abundance	Autocorrelation	2.5 days	64 days	Positive	Extinction (Population Collapse)	Simple	Single species setup	Chlorella vulgaris	Single	Jarvis et al. (2016)
16	12	Mortality	n.a	Multiple Pulses	n.a	Temperature	No	Biotic	Abundance	Variance	2.5 days	64 days	Positive	Extinction (Population Collapse)	Simple	Single species setup	Chlorella vulgaris	Single	Jarvis et al. (2016)
17	13	Mortality	n.a	Multiple Pulses	n.a	Food Provision	Yes	Biotic	Abundance	Autocorrelation	1 day	45 days	Inconclusive	Extinction (Population Collapse)	Simple	Microcosms	Didinium nasutum	Two	Clementz & Ozgul (2016)

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17	13	Mortality	n.a	Multiple Pulses	n.a	Food Provision	Yes	Biotic	Abundance	Density ratio	1 day	45 days	Inconclusive	Extinction (Population Collapse)	Simple	Microcosms	Didinium nasutum	Two	Clementz & Ozgul (2016)
17	13	Mortality	n.a	Multiple Pulses	n.a	Food Provision	Yes	Biotic	Abundance	Autoregressive Coefficient	1 day	45 days	Inconclusive	Extinction (Population Collapse)	Simple	Microcosms	Didinium nasutum	Two	Clementz & Ozgul (2016)
17	13	Mortality	n.a	Multiple Pulses	n.a	Food Provision	Yes	Biotic	Abundance	Recovery	1 day	45 days	Inconclusive	Extinction (Population Collapse)	Simple	Microcosms	Didinium nasutum	Two	Clementz & Ozgul (2016)
17	13	Mortality	n.a	Multiple Pulses	n.a	Food Provision	Yes	Biotic	Abundance	Variance	1 day	45 days	Inconclusive	Extinction (Population Collapse)	Simple	Microcosms	Didinium nasutum	Two	Clementz & Ozgul (2016)
18	14	Mortality	n.a	Multiple Pulses	n.a	Toxicity	Yes	Biotic	Abundance	Autocorrelation	2 days	64 days	Negative	Extinction (population collapse)	Simple	Microcosms	Brachionus calyciflorus	Single + feed	Sommer et al. (2016)
18	14	Mortality	n.a	Multiple Pulses	n.a	Toxicity	Yes	Biotic	Abundance	Recovery	2 days	64 days	Negative	Extinction (population collapse)	Simple	Microcosms	Brachionus calyciflorus	Single + feed	Sommer et al. (2016)
18	14	Mortality	n.a	Multiple Pulses	n.a	Toxicity	Yes	Biotic	Abundance	Variance	2 days	64 days	Positive	Extinction (population collapse)	Simple	Microcosms	Brachionus calyciflorus	Single + feed	Sommer et al. (2016)
18	14	Mortality	n.a	Multiple Pulses	n.a	Toxicity	Yes	Biotic	Abundance	Shape of the Distribution	2 days	64 days	Negative	Extinction (population collapse)	Simple	Microcosms	Brachionus calyciflorus	Single + feed	Sommer et al. (2016)
18	14	Mortality	n.a	Multiple Pulses	n.a	Toxicity	Yes	Biotic	Abundance	Shape of the Distribution	2 days	64 days	Negative	Extinction (population collapse)	Simple	Microcosms	Brachionus calyciflorus	Single + feed	Sommer et al. (2016)
18	14	Mortality	n.a	Multiple Pulses	n.a	Toxicity	Yes	Biotic	Abundance	Variance	2 days	64 days	Positive	Extinction (population collapse)	Simple	Microcosms	Brachionus calyciflorus	Single + feed	Sommer et al. (2016)
19	15	n.a*	n.a	Single Pulse	n.a	Nutrient Addition	No	Biotic	Phycocyanin	Spatial Variance	7 days	2 years	Negative	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Butita et al. (2017)
19	15	n.a*	n.a	Single Pulse	n.a	Nutrient Addition	No	Biotic	Phycocyanin	Spatial Shape of the Distribution	7 days	2 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Butita et al. (2017)
19	15	n.a*	n.a	Single Pulse	n.a	Nutrient Addition	No	Biotic	Phycocyanin	Spatial Autocorrelation	7 days	2 years	Negative	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Butita et al. (2017)
19	15	n.a*	n.a	Single Pulse	n.a	Nutrient Addition	No	Biotic	Phycocyanin	Frequence of Extreme Events (EVD)	7 days	2 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Butita et al. (2017)
20	16	Mortality	n.a	Multiple Pulses	n.a	Canopy Removal	No	Biotic	Abundance	Recovery	12 months	2 years	Inconclusive	Canopy/Turf dominated state	Complex	Coastal Manipulation	Seagrass	Multiple	Rindi et al., (2017)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Phycocyanin	Variance	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Phycocyanin	Autocorrelation	5 mins	3 years	Negative	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Abiotic	Oxygen	Variance	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)

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21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Abiotic	Oxygen	Autocorrelation	5 mins	3 years	Negative	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Chlorophyll-a	Variance	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Chlorophyll-a	Autocorrelation	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Phycocyanin	Variance	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Phycocyanin	Autocorrelation	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Abiotic	Oxygen	Variance	5 mins	3 years	Negative	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Abiotic	Oxygen	Autocorrelation	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Chlorophyll-a	Variance	5 mins	3 years	Negative	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Chlorophyll-a	Autocorrelation	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Abiotic	Oxygen	Variance	5 mins	3 years	Negative	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Abiotic	Oxygen	Autocorrelation	5 mins	3 years	Negative	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Chlorophyll-a	Variance	5 mins	3 years	Negative	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Chlorophyll-a	Autocorrelation	5 mins	3 years	Negative	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Phycocyanin	Variance	5 mins	3 years	Negative	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Phycocyanin	Autocorrelation	5 mins	3 years	Negative	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Abiotic	Oxygen	Variance	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Abiotic	Oxygen	Autocorrelation	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Chlorophyll-a	Variance	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)

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21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Chlorophyll-a	Autocorrelation	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Phycocyanin	Variance	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Biotic	Phycocyanin	Autocorrelation	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Abiotic	Oxygen	Variance	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
21	17	n.a	n.a	n.a	n.a	Nutrient Addition	Yes	Abiotic	Oxygen	Autocorrelation	5 mins	3 years	Positive	Algal bloom	Complex	Whole-Lake Manipulation	Cyanobacteria	Multiple	Wilkinson et al. (2018)
22	18	Mortality	n.a	Single Pulse	n.a	Tide Level	No	Biotic	Abundance	Recovery	1 month	2 years	Positive	Extinction (Population Collapse)	Complex	Coastal Manipulation	Seagrass	Multiple	El-Hacen et al., (2018)
23	19	Mortality	n.a	Multiple Pulses	n.a	Mortality Rate	No	Biotic	Abundance	Recovery	1 day	16 days	Positive	Extinction (Population Collapse)	Simple	Single species setup	Saccharomyces cerevisiae	Single	Ghadami et al., (2018)
24	16	Mortality	n.a	Multiple Pulses	n.a	Canopy Removal	No	Biotic	Abundance	Variance	12 months	2 years	Positive	Canopy/Turf dominated state	Complex	Coastal Manipulation	Seagrass	Multiple	Rindi et al., (2018)
24	16	Mortality	n.a	Multiple Pulses	n.a	Canopy Removal	No	Biotic	Abundance	Variance	12 months	2 years	Positive	Canopy/Turf dominated state	Complex	Coastal Manipulation	Seagrass	Multiple	Rindi et al., (2018)
24	16	Mortality	n.a	Multiple Pulses	n.a	Canopy Removal	No	Biotic	Abundance	Autocorrelation	12 months	2 years	Inconclusive	Canopy/Turf dominated state	Complex	Coastal Manipulation	Seagrass	Multiple	Rindi et al., (2018)
24	16	Mortality	n.a	Multiple Pulses	n.a	Canopy Removal	No	Biotic	Abundance	Discrete Fourrier Transformation	12 months	2 years	Positive	Canopy/Turf dominated state	Complex	Coastal Manipulation	Seagrass	Multiple	Rindi et al., (2018)
24	16	Mortality	n.a	Multiple Pulses	n.a	Canopy Removal	No	Biotic	Abundance	Shape of the Distribution	12 months	2 years	Positive	Canopy/Turf dominated state	Complex	Coastal Manipulation	Seagrass	Multiple	Rindi et al., (2018)

Supplement – Results of all “Proxy Vs. Metric Combinations”

The tables below show all the combinations of proxies and metrics found in the review of the 24 papers assessing EWS of regime shift in aquatic experiments. The table scrutinizes all the positive, negative, and inconclusive results per each type of biological setup, as described in the original papers. Color codes are mere facilitation to observe the frequencies of each result. Results are made available as an excel sheet as well.

Table 3. Number of each individual “Proxy Vs. Metric” combinations reviewed from the 24 papers describing EWS of regime shifts in aquatic experiments using simple biological setups. (+) Positive, (-) Negative and (o) Inconclusive results as described in the original paper. Partial sums refer to the sum of the respective columns or lines.

Complexity	Simple																																										
Metric/Proxy	Abundance			Benthic Organic Matter			Chlorophyll - a			Dry-Weight			GPP			Individual Biomass			NEP			Oxygen			pH			Phycocyanin			R			Spatial Biomass			Richness			Partial sum per metric
Result	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	
Autocorrelation	7	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
Autoregressive Coefficient	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Conditional Heteroskedasticity	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Density ratio	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Discrete Fourrier Transformation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fitness to BDS model	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fitness to GARCH model	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Frequence of Extreme Events (EVD)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shape of the Distribution	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Mean	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Recovery	5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
Spatial Autocorrelation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spatial Correlation	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Spatial Shape of the Distribution	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spatial Variance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spectral Ratio	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Variance	9	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
Partial Sum per Result	23	7	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35
Total per Proxy	35			0			0			0			0			0			0			0			0			0			0			0			0						

Table 4. Number of each individual “Proxy Vs. Metric” combinations reviewed from the 24 papers describing EWS of regime shifts in aquatic experiments using complex biological setups. (+) Positive, (-) Negative and (o) Inconclusive results as described in the original paper. Partial sums refer to the sum of the respective columns or lines.

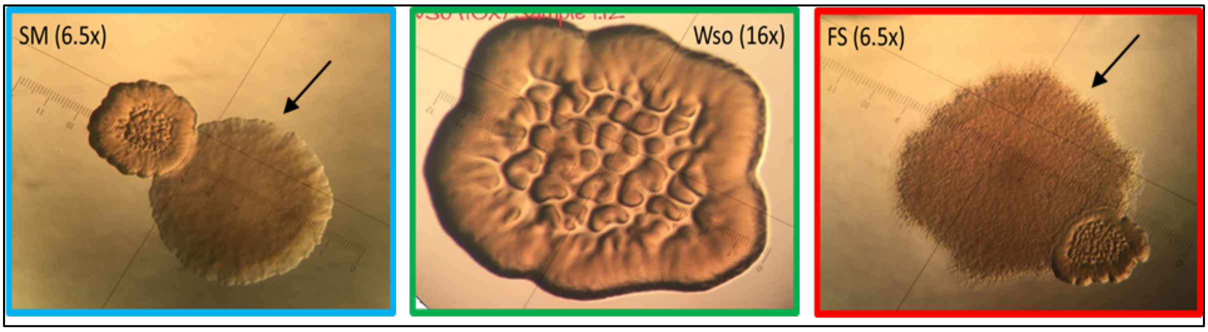
Complexity	Complex																																										
Metric/Proxy	Abundance			Benthic Organic Matter			Chlorophyll - a			Dry-Weight			GPP			Individual Biomass			NEP			Oxygen			pH			Phycocyanin			R			Spatial Biomass			Richness			Partial sum per metric
Result	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	
Autocorrelation	2	0	1	0	0	0	5	1	0	0	0	0	0	1	0	0	0	0	0	1	0	4	2	0	1	0	0	2	2	0	0	1	0	0	0	0	0	0	0	23
Autoregressive Coefficient	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Conditional Heteroskedasticity	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Density ratio	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Discrete Fourrier Transformation	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Fitness to BDS model	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Fitness to GARCH model	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Frequence of Extreme Events (EVD)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Shape of the Distribution	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
Mean	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Recovery	3	0	2	0	0	1	3	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	15
Spatial Autocorrelation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Spatial Correlation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spatial Shape of the Distribution	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Spatial Variance	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2
Spectral Ratio	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	4
Variance	4	0	0	0	0	0	5	2	0	0	0	0	0	1	0	0	0	0	0	1	0	5	2	0	1	0	0	3	1	0	0	1	0	0	0	0	0	0	0	26
Partial Sum per Result	14	0	5	0	0	1	15	3	1	1	0	1	0	3	0	0	0	1	0	3	0	9	5	3	2	0	0	7	5	0	0	3	0	1	0	0	1	0	0	84
Total per Proxy	19			1			19			2			3			1			3			17			2			12			3			1			1			

Table 5. Number of each individual "Proxy Vs. Metric" combinations reviewed from the 24 papers describing EWS of regime shifts in aquatic experiments for all types of experiments. (+) Positive, (-) Negative and (o) Inconclusive results as described in the original paper. Partial sums refer to the sum of the respective columns or lines.

Complexity	All Types of Experiments																																										
Metric/Proxy	Abundance			Benthic Organic Matter			Chlorophyll - a			Dry-Weight			GPP			Individual Biomass			NEP			Oxygen			pH			Phycocyanin			R			Spatial Biomass			Richness			Partial sum per metric
Result	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	+	-	o	
Autocorrelation	9	1	2	0	0	0	5	1	0	0	0	0	0	1	0	0	0	0	0	1	0	4	2	0	1	0	0	2	2	0	0	1	0	0	0	0	0	0	0	32
Autoregressive Coefficient	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Conditional Heteroskedasticity	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Density ratio	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Discrete Fourrier Transformation	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Fitness to BDS model	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Fitness to GARCH model	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Frequence of Extreme Events (EVD)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Shape of the Distribution	3	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
Mean	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Recovery	8	1	3	0	0	1	3	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	22
Spatial Autocorrelation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Spatial Correlation	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Spatial Shape of the Distribution	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Spatial Variance	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2
Spectral Ratio	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	4
Variance	13	1	1	0	0	0	5	2	0	0	0	0	0	1	0	0	0	0	0	1	0	5	2	0	1	0	0	3	1	0	0	1	0	0	0	0	0	0	0	37
Partial Sum per Result	37	7	10	0	0	1	15	3	1	1	0	1	0	3	0	0	0	1	0	3	0	9	5	3	2	0	0	7	5	0	0	3	0	1	0	0	1	0	0	119
Total per Proxy	54			1			19			2			3			1			3			17			2			12			3			1			1			

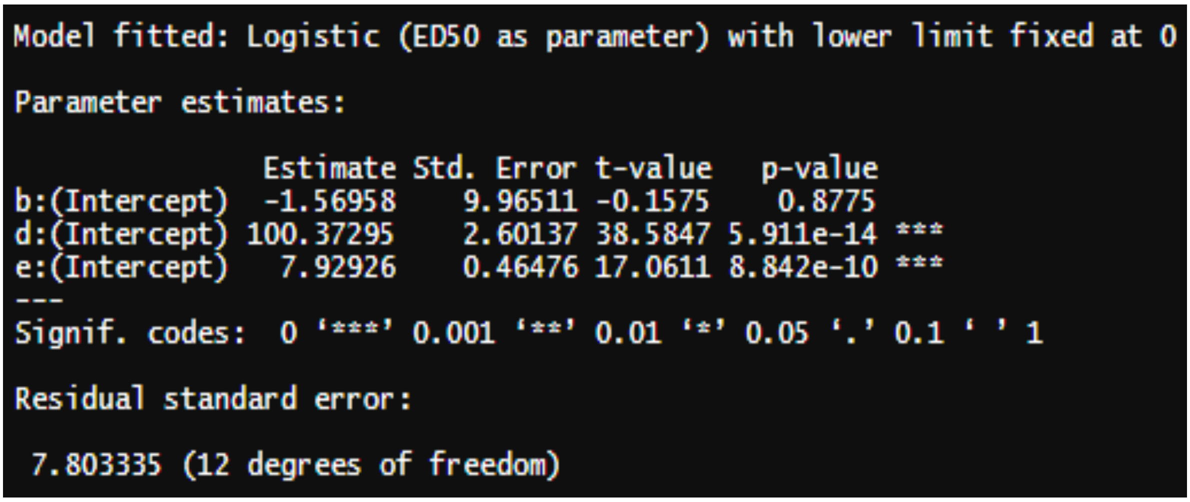
Chapter 4 - Resource Availability Decouples the Linkage between Engineering and Ecological Resilience

Supplementary Material



Supplement 1. Main ecotypes at the present study. The black arrow indicates the correct ecotype.

Supplement 2. P. fluorescens microcosms with its mat formed 96h after foundation. Microcosms started from approximately 10^7 CFU/mL from the SM ecotype.



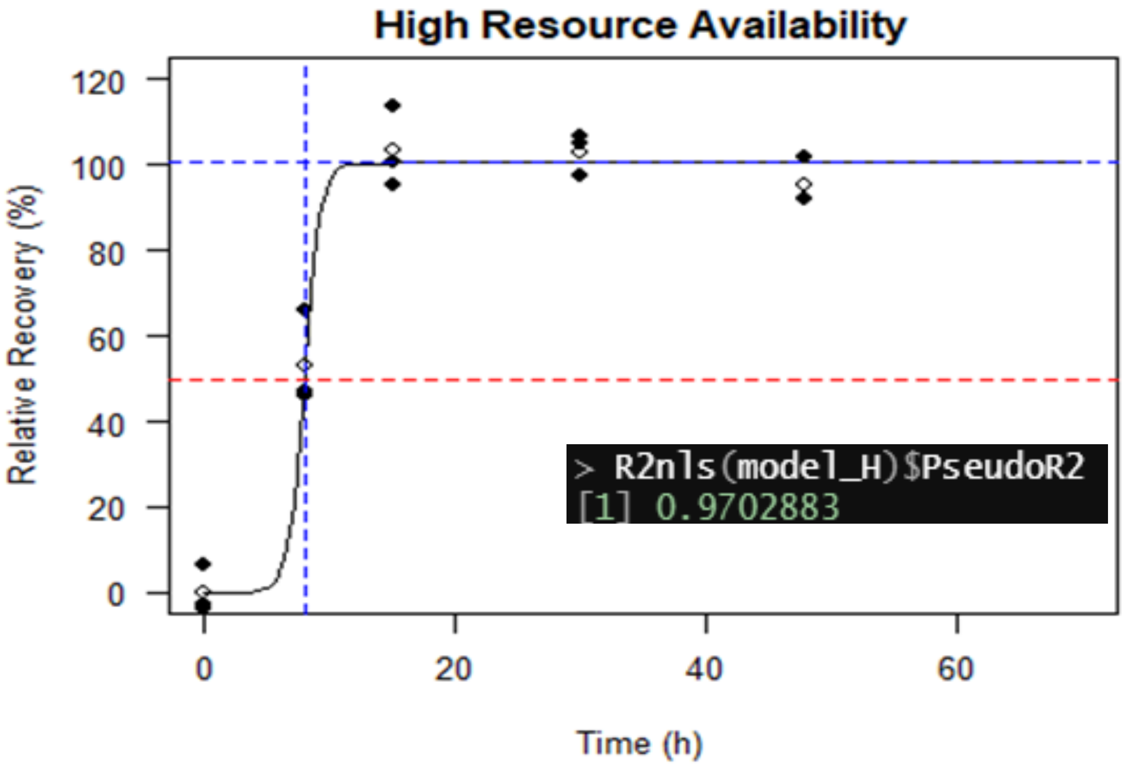
```
Model fitted: Logistic (ED50 as parameter) with lower limit fixed at 0

Parameter estimates:

                Estimate Std. Error t-value   p-value    
b:(Intercept)  -1.56958    9.96511 -0.1575    0.8775    
d:(Intercept) 100.37295    2.60137 38.5847 5.911e-14 ***
e:(Intercept)   7.92926    0.46476 17.0611 8.842e-10 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error:

 7.803335 (12 degrees of freedom)
```



```
> R2nls(model_H)$PseudoR2
[1] 0.9702883
```

Supplement 3. Model output of the logistic regression model for the SM ecotype at high resource availability. Closed dots are empirical data, and open dots are the model estimate. The horizontal red dashed line represents 50% relative recovery compared to the baseline. The horizontal blue line is the asymptotic recovery (d), and the vertical dashed line represents the time when the system reached halfway of its recovery potential. The high variability of the "b" estimate (slope at the inflection point) is caused by the few points at the log-lag phase (time=8h) in comparison to the asymptotic measurements (times=0, 15, 24, 48h).

Chapter 5 - Phytoplankton Responses to Repeated Pulse Perturbations Imposed on a Trend of Increasing Eutrophication

Methodology

Metrics for quantifying response to pulse perturbations

Table 1. Metrics for quantifying response to pulse perturbations and their formula.

Metric	Symbol	Formula	Reference
Pre-event condition	C_o	Rolling window of three days with least variance	-
Maximum Displacement	D_o	$D_0 = \|C_0 - Peak\|$	Orwin and Wardle (2004)
Resistance Index	RS	$\left(\left(\left[10^{\left(\left(1-\frac{2\|\mathrm{Do}\|}{(Co+\|\mathrm{Do}\|)}\right)*-1\right)}\right]-10\right)\right)*\text{-}1$	Modified from Orwin and Wardle (2004)
Recovery Index	RC	$= 10^{\left(\frac{2\|\mathrm{Do}\|}{(\|\mathrm{Do}\|+\|\mathrm{Dx}\|)}-1\right)}$ Where D_x=Post-event condition	Modified from Orwin and Wardle (2004)

Resistance (simulation)

Figure 1. Resistance of simulated data with displacements of different intensities using the index proposed by Orwin and Wardle (2004). RS index = 0 means 100% displacement compared to the baseline

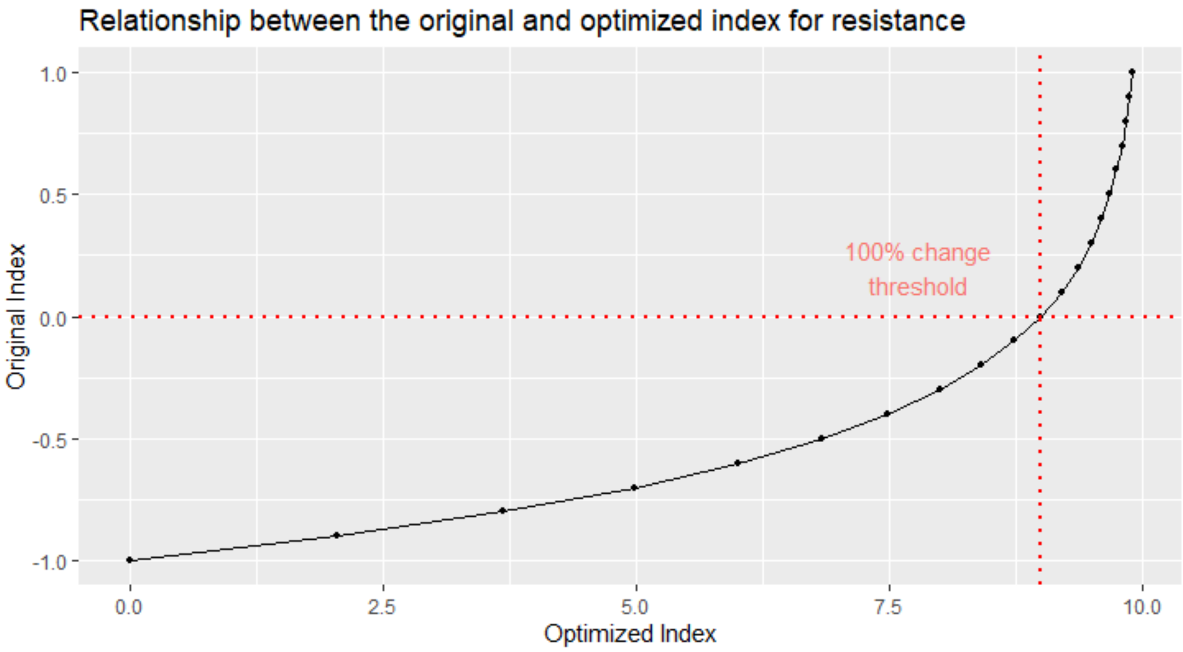


Figure 2. Relationship between the original scaling and optimized scaling used for the resistance index.

Recovery (simulation)

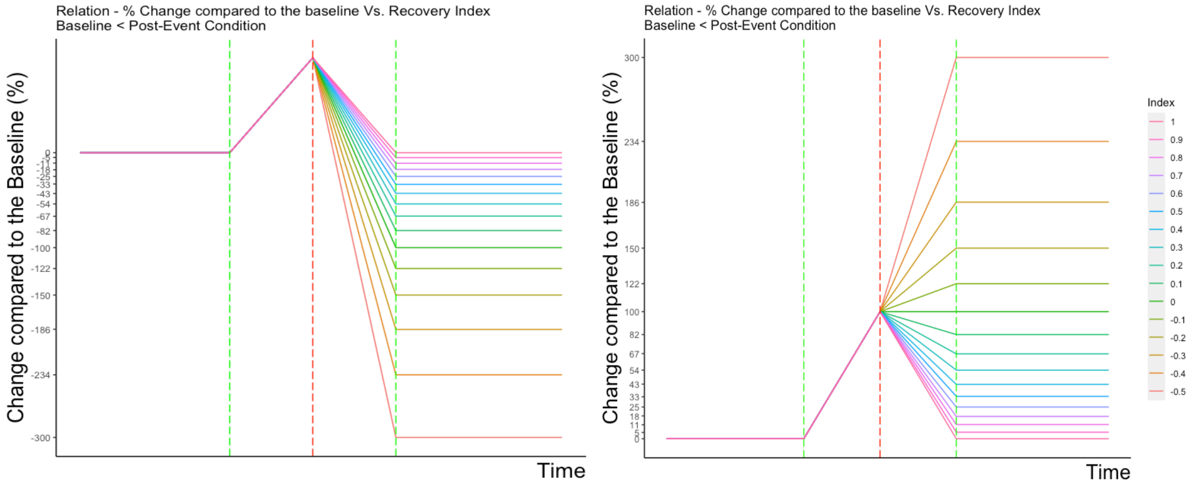


Figure 3. (left) Recovery of simulated data with post-event condition lower than the antecedent baseline. RC index = 0 means 0% recovery. In other words, the system is displaced and stabilizes at the maximum displacement level. Negative RC index values address values of post-event conditions higher than the module of max displacement. (right) Recovery of simulated data with post-event condition higher than the antecedent baseline. RC index = 0 means 0% recovery. In other words, the system is displaced and stabilizes at the maximum displacement level. Negative RC index values address values of post-event conditions higher than the max displacement.

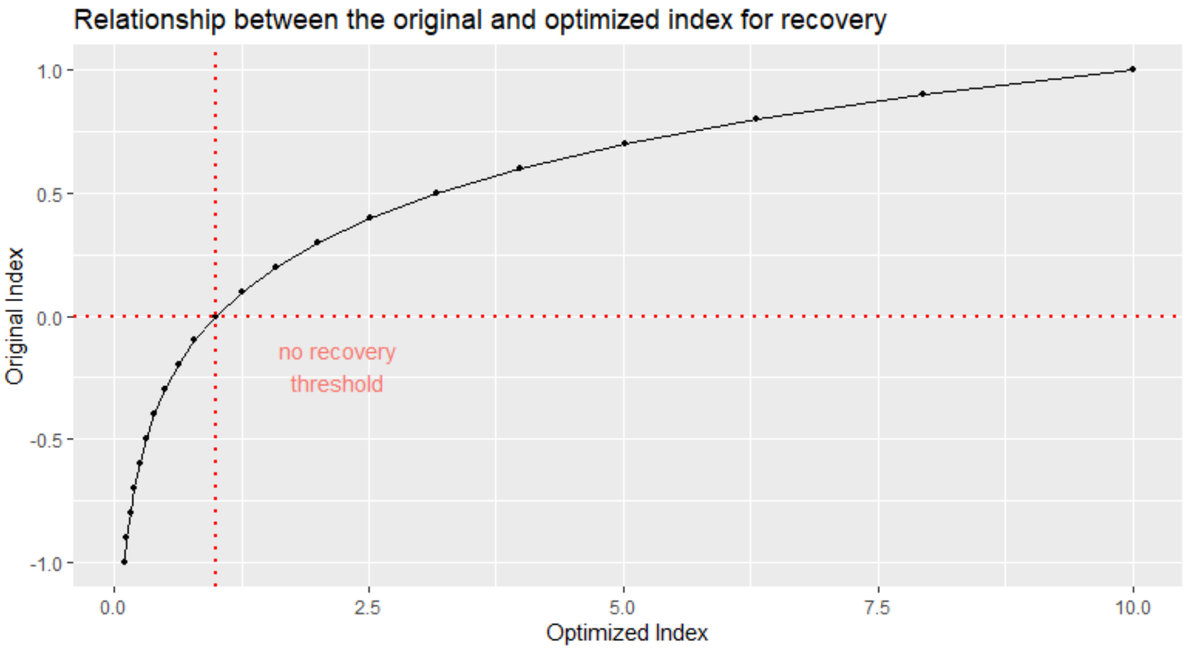


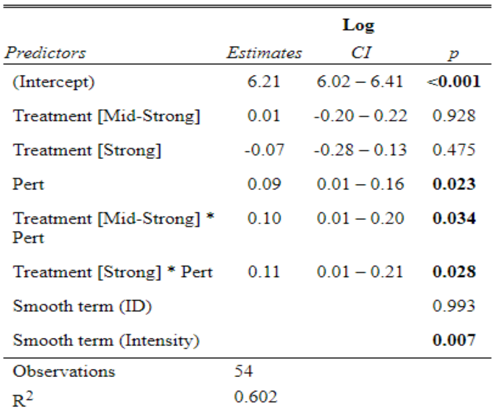
Figure 4 Relationship between the original scaling and optimized scaling used for the recovery index.

Statistics

Long-term effect of the press perturbation (eutrophication) on total biovolume and total chlorophyll-a concentrations

Biovolume

*Table 2. Model output from the generalized additive mixed model (GAMM) for total biovolume during the perturbations. Fixed effects: Eutrophication treatment and No. of perturbations; Random effects: Cosm ID (pseudoreplication) and Perturbation Intensity. [m = gam(data=df_biovolume, Log ~ Treatment * Pert + s(ID, bs = 're') + s(Intensity, bs = 're'), method = 'REML').*



		Log	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	6.21	6.02 – 6.41	**<0.001**
Treatment [Mid-Strong]	0.01	-0.20 – 0.22	0.928
Treatment [Strong]	-0.07	-0.28 – 0.13	0.475
Pert	0.09	0.01 – 0.16	**0.023**
Treatment [Mid-Strong] * Pert	0.10	0.01 – 0.20	**0.034**
Treatment [Strong] * Pert	0.11	0.01 – 0.21	**0.028**
Smooth term (ID)			0.993
Smooth term (Intensity)			**0.007**
Observations	54		
R^2	0.602		

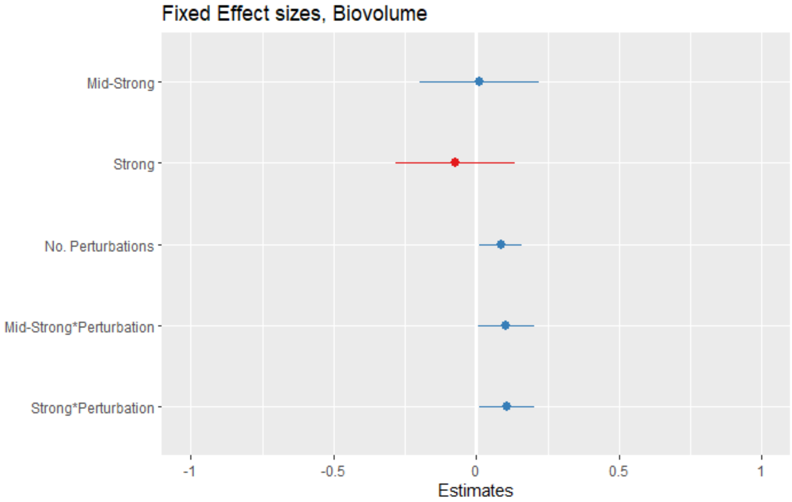


Figure 5. Model estimates for the fixed effects sizes on total biovolume. Random effects are not showed here but can be reproduced using the R.markdown and data available with the manuscript.

Total pigments in function of biovolume and treatment

Table 3. Model output from the generalized linear mixed model (GLMM) showing the relation of total biovolume and eutrophying treatments to the total concentration of phytoplankton pigments (N=466, R^2=0.723). [glm(data = df_timeseries, Log_Pig ~ Log_Bio + factor(Treatment))].

	Log Pig		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	-6.43	-6.86 – -5.99	**<0.001**
Log_Bio	1.18	1.12 – 1.25	**<0.001**
Treatment [Mid-Strong]	0.08	0.01 – 0.16	**0.027**
Treatment [Strong]	0.09	0.02 – 0.17	**0.013**
Observations	466		

Short-term effects of the press perturbation (eutrophication) on the response and recovery from mortality pulse perturbations (chlorophyll-a as a proxy)

Pre-event condition

*Table 4. Model output from the linear mixed effect model (LMEM) for pre-event conditions. Fixed effects: Eutrophication treatment and No. of perturbations; Random effects: Cosm ID (pseudoreplication) and Perturbation Intensity. [m = lmer(data=df_baseline, Log ~ Treatment * Pert + (1 | ID) + (1 |Intensity))].*

	Log		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	0.19	-0.06 – 0.44	0.137
Treatment [Mid-Strong]	0.05	-0.25 – 0.36	0.741
Treatment [Strong]	0.14	-0.16 – 0.45	0.366
Pert	0.34	0.17 – 0.52	**<0.001**
Treatment [Mid-Strong] * Pert	0.31	0.10 – 0.52	**0.004**
Treatment [Strong] * Pert	0.08	-0.13 – 0.29	0.470
Random Effects			
σ^2	0.07		
τ_{00} ID	0.02		
τ_{00} Intensity	0.01		
ICC	0.23		
N_{ID}	18		
$N_{Intensity}$	3		
Observations	54		
Marginal R^2 / Conditional R^2	0.676 / 0.751		

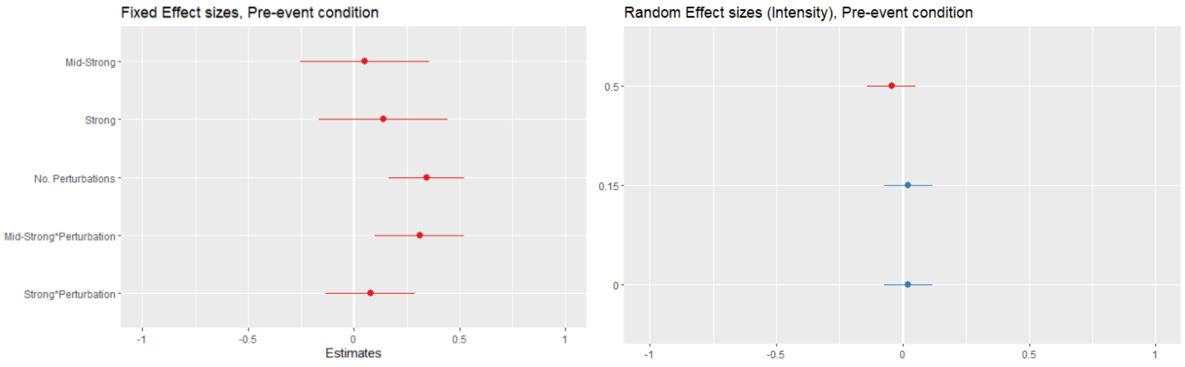


Figure 6. Model estimates for the fixed effects (left) and random effect sizes (right) for pre-event conditions. Random effects for Cosm ID are not showed here but can be reproduced using the R.markdown and data available with the manuscript.

Maximum displacement

Table 5. Model output from the linear mixed effect model (LMEM) for maximum displacement. Fixed effects: Eutrophication treatment and No. of perturbations; Random effects: Cosm ID (pseudoreplication) and Perturbation Intensity. [m = lmer(data=df_displacement, Response ~ Treatment + Pert + (Pert | ID) + (1 |Intensity))].

	Response		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	103.57	23.59 – 183.54	**0.011**
Treatment [Mid-Strong]	47.53	15.89 – 79.16	**0.003**
Treatment [Strong]	52.94	21.31 – 84.58	**0.001**
Pert	-25.58	-61.97 – 10.81	0.168
Random Effects			
σ^2	1611.42		
$\tau_{00\ \mathrm{ID}}$	31.18		
$\tau_{00\ \mathrm{Intensity}}$	586.07		
$\tau_{11\ \mathrm{ID.Pert}}$	125.33		
$\rho_{01\ \mathrm{ID}}$	-1.00		
ICC	0.37		
N_{ID}	18		
$N_{\mathrm{Intensity}}$	3		
Observations	54		
Marginal R^2 / Conditional R^2	0.285 / 0.550		

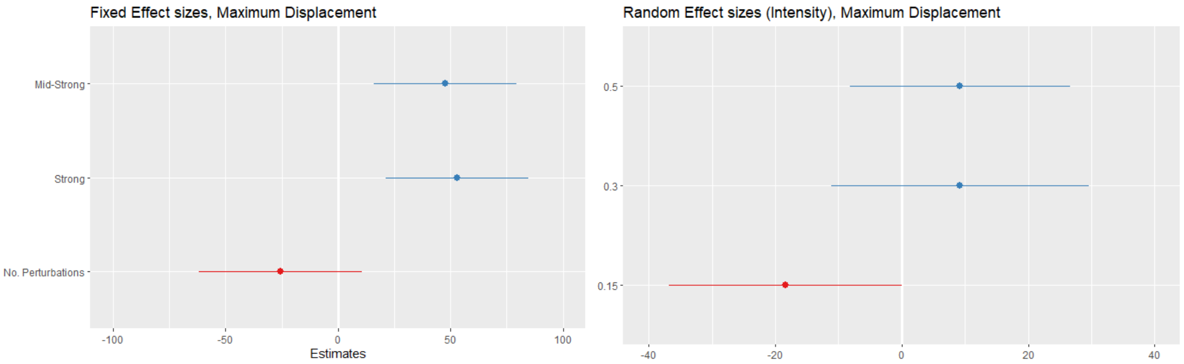


Figure 7. Model estimates for the fixed effects (left) and random effect sizes (right) for maximum displacement. Random effects for Cosm ID are not showed here but can be reproduced using the R.markdown and data available with the manuscript.

Importance of intensity

Table 6. Model output from the linear mixed effect model (LMEM) for maximum displacement. Fixed effects: Eutrophication treatment, perturbation intensity and number of perturbations. Random effect: Cosm ID (pseudoreplication). [m = lmer(data=df_displacement, Response ~ Treatment + Intensity + Pert + (1 | ID))].

	Response		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	38.00	-23.79 – 99.79	0.228
Treatment [Mid-Strong]	45.70	15.29 – 76.10	**0.003**
Treatment [Strong]	54.08	23.68 – 84.49	**<0.001**
Intensity	127.35	29.47 – 225.22	**0.011**
Pert	-12.85	-30.03 – 4.34	0.143
Random Effects			
σ^2	1870.27		
$\tau_{00\ ID}$	98.57		
ICC	0.05		
N_{ID}	18		
Observations	54		
Marginal R^2 / Conditional R^2	0.388 / 0.419		

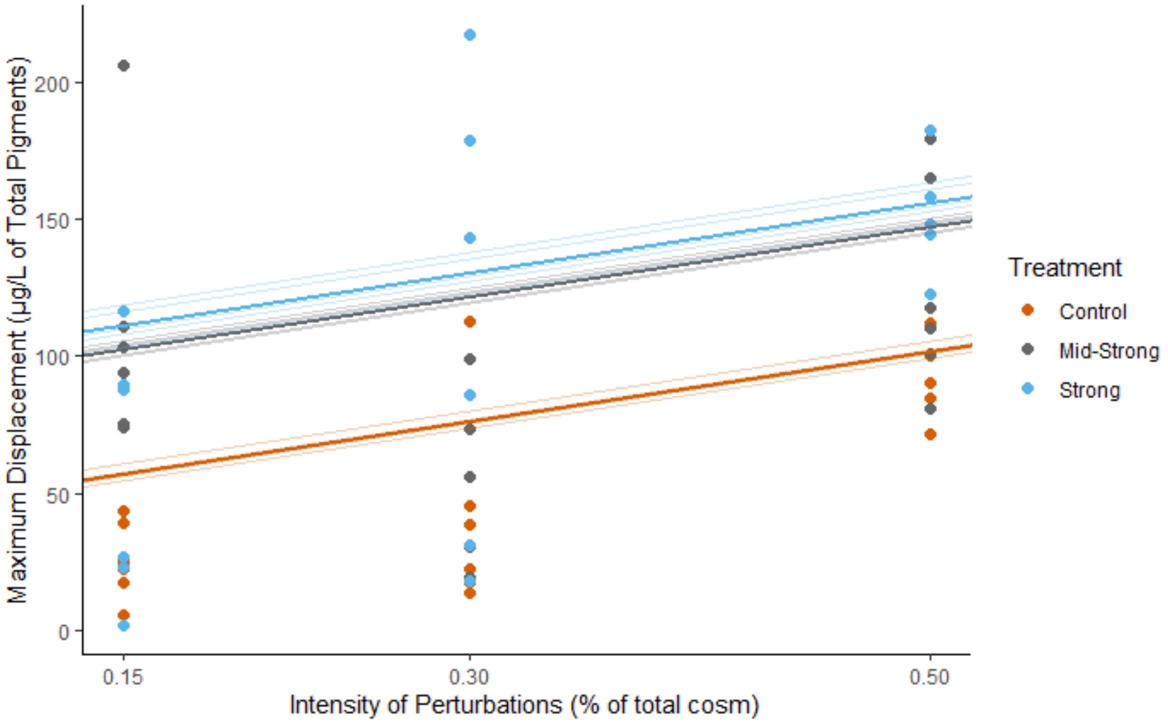


Figure 8. The effect of eutrophication on the maximum displacement after perturbation. Differently from the other assessments in the body of the manuscript, the intensity was also included as a fixed effect here. This is because we were interested in observing the additive effect of intensity and repeated pulse perturbations on the maximum displacement of the system. While the number of sequential perturbations is not apparent in the plot (z-axis), it modifies the intensity and maximum displacement relation. This can be observed in the effect sizes plot in Figure 9. Solid regression line represents the full model estimates, and shaded lines represent the individual cosm estimate without random intercepts. Shaded dots are the empirical data (n=6). Fixed effects: Eutrophication treatment, perturbation intensity, and the number of perturbations. Random effect: Cosm ID (pseudoreplication).

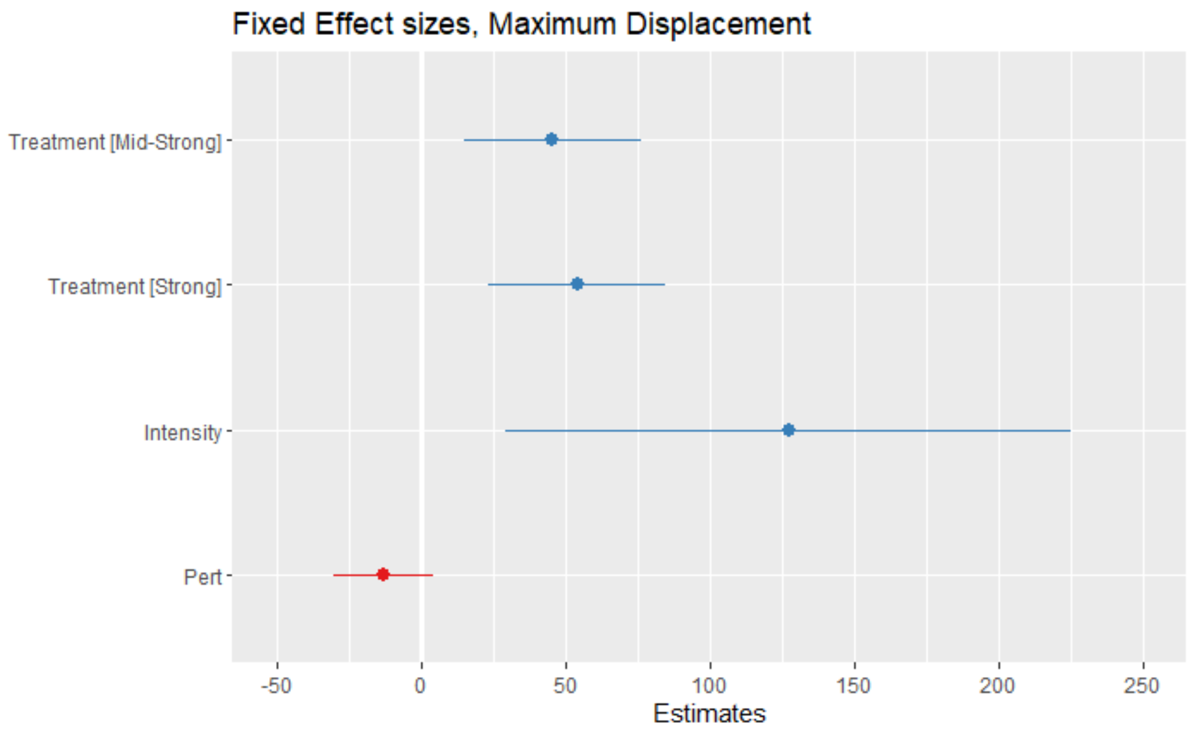


Figure 9. Model estimates for the fixed effects for maximum displacement. Note that the direction of the effect size of the number of perturbations ("Pert") is in the opposite direction of eutrophication and perturbation intensity. Random effects for Cosm ID are not showed here but can be reproduced using the R.markdown and data available with the manuscript.

Recovery index

*Table 7. Model output from the linear mixed effect model (LMEM) for Recovery Index. Fixed effects: Eutrophication treatment and No. of perturbations; Random effects: Cosm ID (pseudoreplication) and Perturbation Intensity. [m = lmer(data=df_recover, Log ~ Treatment * Pert + (1 | ID) + (1 |Intensity))].*

		Log	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	12.23	8.32 – 16.15	**<0.001**
Treatment [Mid-Strong]	-0.73	-3.28 – 1.83	0.579
Treatment [Strong]	-0.58	-3.14 – 1.98	0.659
Pert	-3.64	-5.45 – -1.83	**<0.001**
Treatment [Mid-Strong] * Pert	0.05	-1.14 – 1.23	0.940
Treatment [Strong] * Pert	0.23	-0.95 – 1.41	0.699
Random Effects			
σ^2	2.18		
$\tau_{00\ \mathrm{ID}}$	0.04		
$\tau_{00\ \mathrm{Intensity}}$	1.35		
ICC	0.39		
N_{ID}	18		
$N_{\mathrm{Intensity}}$	3		
Observations	54		
Marginal R^2 / Conditional R^2	0.708 / 0.822		

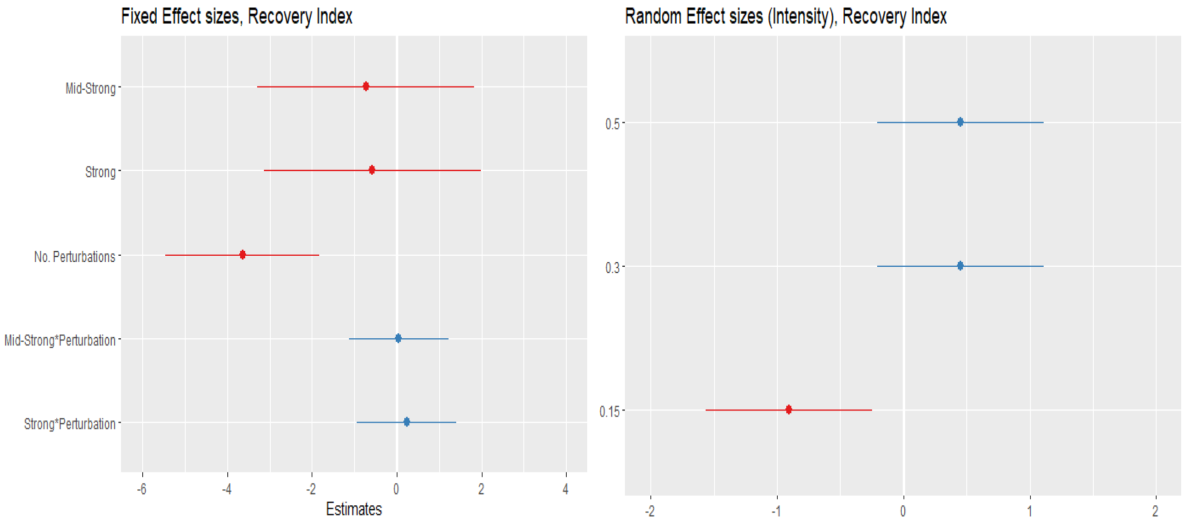


Figure 10. Model estimates for the fixed effects (left) and random effect sizes (right) for recovery. Random effects for Cosm ID are not showed here but can be reproduced using the R.markdown and data available with the manuscript.

Resistance index

Table 8. Model output from the generalized additive model (GAM) for Resistance Index. Fixed effects: Eutrophication treatment and No. of perturbations; Random effects: Cosm ID (pseudoreplication) and Perturbation Intensity. [m = gam(Log ~ Treatment + Pert + Treatment:Pert + s(Pert, ID, bs = 're') + s(Intensity, bs = 're'), data = df_change, method = 'REML')].

		Log	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	-1.69	-4.12 – 0.73	0.167
Treatment [Mid-Strong]	-1.98	-5.42 – 1.45	0.252
Treatment [Strong]	0.86	-2.57 – 4.30	0.616
Pert	2.65	1.53 – 3.77	**<0.001**
Treatment [Mid-Strong] * Pert	1.08	-0.50 – 2.67	0.176
Treatment [Strong] * Pert	-0.46	-2.05 – 1.13	0.560
Smooth term (Pert,ID)			0.829
Smooth term (Intensity)			0.859
Observations	54		
R^2	0.594		

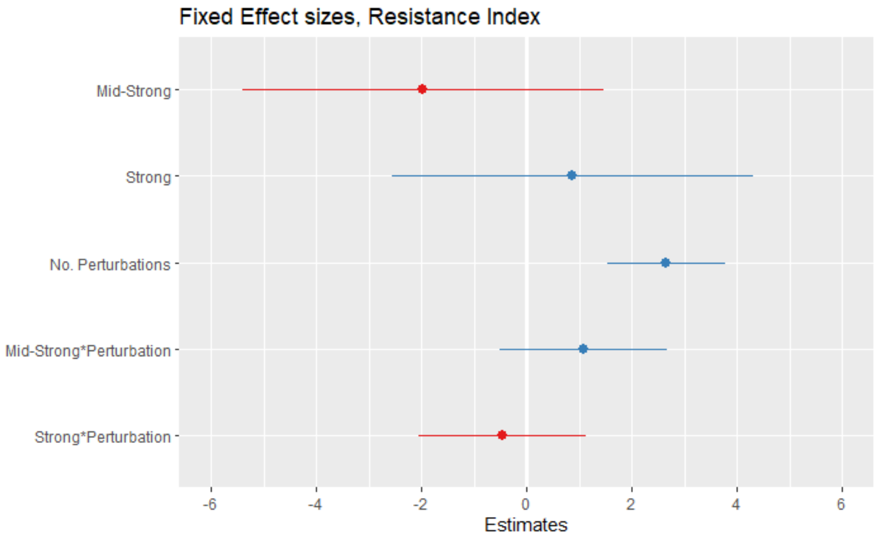


Figure 11. Model estimates for the fixed effects sizes on resistance. Random effects are not showed here but can be reproduced using the R.markdown and data available with the manuscript.

Functional Structure

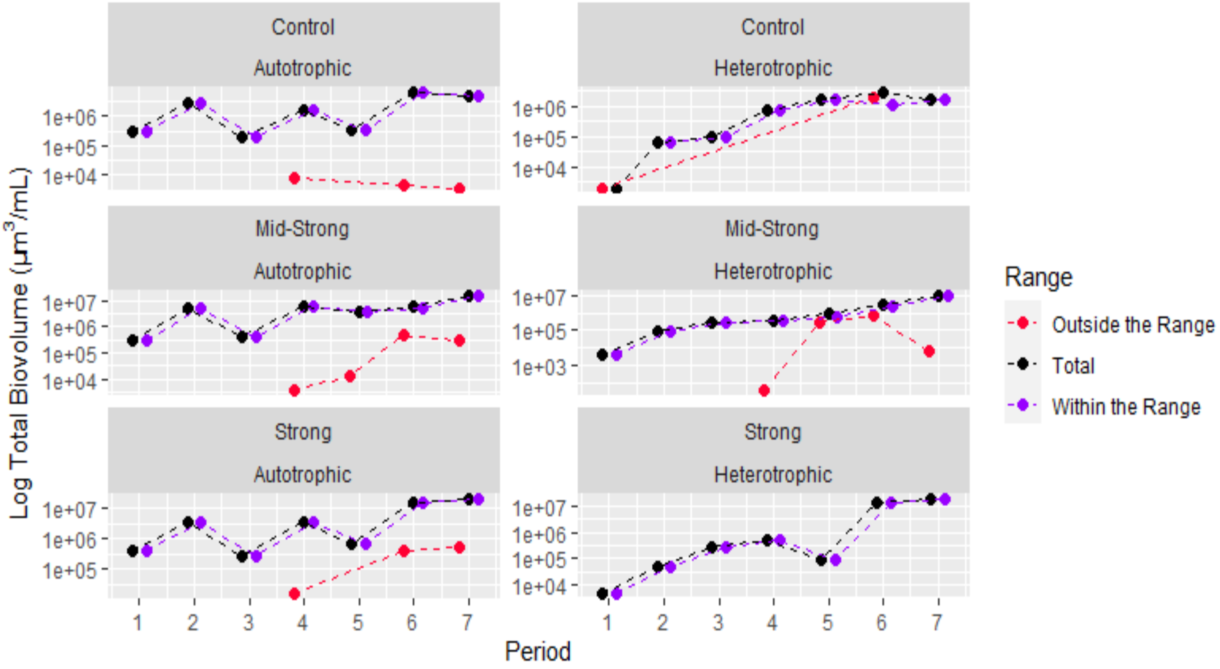


Figure 12. Comparison between the size fractions quantified using the Coulter Counter (purple, fraction 2.93 µm – 60 µm) and when analyzed using microscopy (red, fraction larger than 60 µm). The group outside the range includes filamentous algae, cyclopoids, and one taxon of ciliate larger than 60µm in diameter. The odd numbers in the x-axis represent pre-response conditions (moments of stability before perturbation), and the even numbers represent the peak of phytoplankton response measure with Phyto-PAM™. Data counted using an inverted microscope using pooled samples of the 6 replicates per treatment

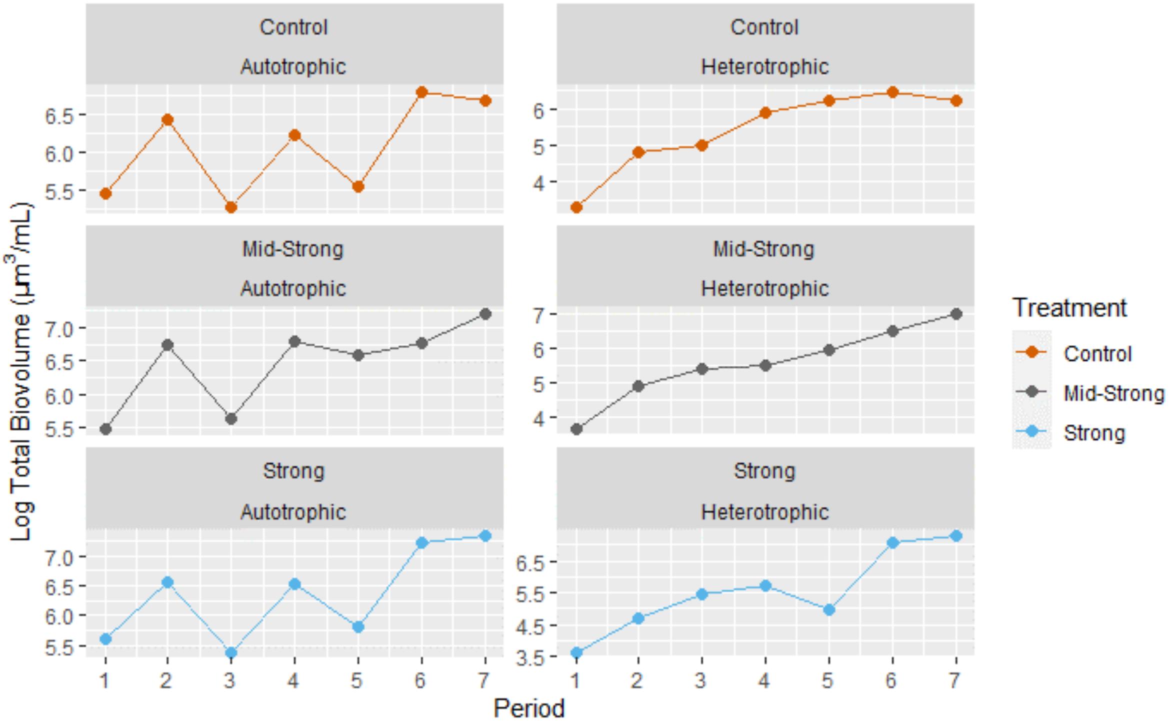


Figure 13. Timeseries of the biovolume concentration of autotrophs Vs. heterotrophs along the three perturbation cycles of the perturbation experiment. The odd numbers in the x-axis represent pre-response conditions (moments of stability before perturbation), and the even numbers represent the peak of phytoplankton response measure with Phyto-PAM™. Data were counted using an inverted microscope using pooled samples of the 6 replicates per treatment. Y-axis log10.

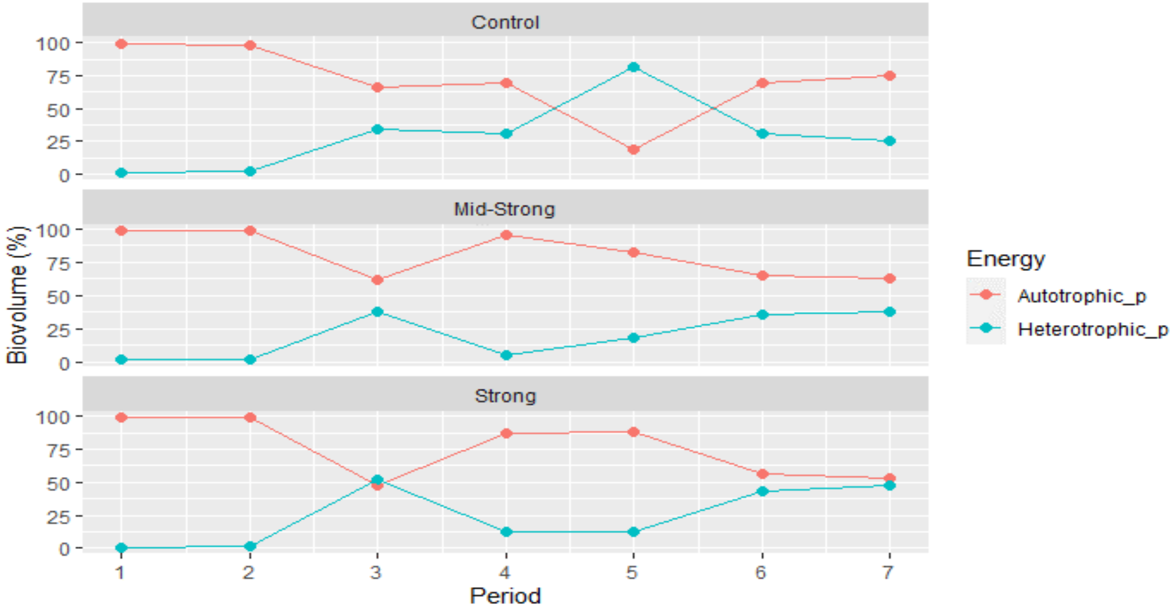


Figure 14. Timeseries of the relative biovolume concentration of autotrophs Vs. heterotrophs along the three perturbation cycles of the perturbation experiment. The odd numbers in the x-axis represent pre-response conditions (moments of stability before perturbation), and the even numbers represent the peak of phytoplankton response measure with Phyto-PAM™. Data were counted using an inverted microscope using pooled samples of the 6 replicates per treatment.

Periphyton Formation

Preparation of periphyton samples:

Squares of 15.76 cm^2 polycarbonate sheets were cut and placed at the bottom of each mesocosm coupled with the temperature loggers. Sheets were deployed just after the pulse perturbation on the 3rd cycle and removed at the end of the experiment, 17 days later (from day 78 to day 95).

For quantification, each periphyton stripe was removed and placed in a 50mL falcon with 42 ml of NaCl 0.9%. The falcons were vigorously agitated and vortexed at maximum speed at least 30 seconds to lose the periphyton from the polycarbonate sheet.

After, the solution was measured using the Phyto-PAM in the same way as phytoplankton in the experiment, using NaCl 0.9% as blank. For the dry weight, acetate cellulose filters were pre-weighted, 28mL of solution filtered, dried for 24h at 60 degrees, and re-weighted.

Dry weight and Chlorophyll-a concentrations were calculated based on the surface area of the mesocosms (diameter= 26.2 cm, height = 22 cm). The dry weight is presented as absolute mass of periphyton per container (in mg) and Chlorophyll-a as the concentration of Chl-a at the mesocosms surface if diluted in 10L volume (the content of the mesocosm).

Values were compared to the averaged Chl-a concentrations and the cumulative Chl-a concentration in the water column during the same period.

Table 9. Averaged values of periphyton and phytoplankton at the last quarter of the experiment (mean ± sd). Cum_Sum = cumulative sum of Chl-a concentration of the phytoplankton community during the 17 days of periphyton measurement.

	Measurement	Control	Mid-Strong	Strong
Periphyton	Dry Weight (mg)	53.67 ± 53.67	120.35 ± 41.04	111.82 ± 24.01
	Chl-a (µg/L) diluted in 10L mesocosm (Periphyon)	15.39 ± 8.42	34.48 ± 21.32	23.74 ± 24.81
Phytoplankton	Chl-a (µg/L)*	36.62 ± 27.96	48.15 ± 38.34	79.38 ± 72.44
	Cum_Sum (µg/L)	1977.78	2622.55	4286.47

* Note that the high sd values are due to the integration of the timeseries during the perturbation period

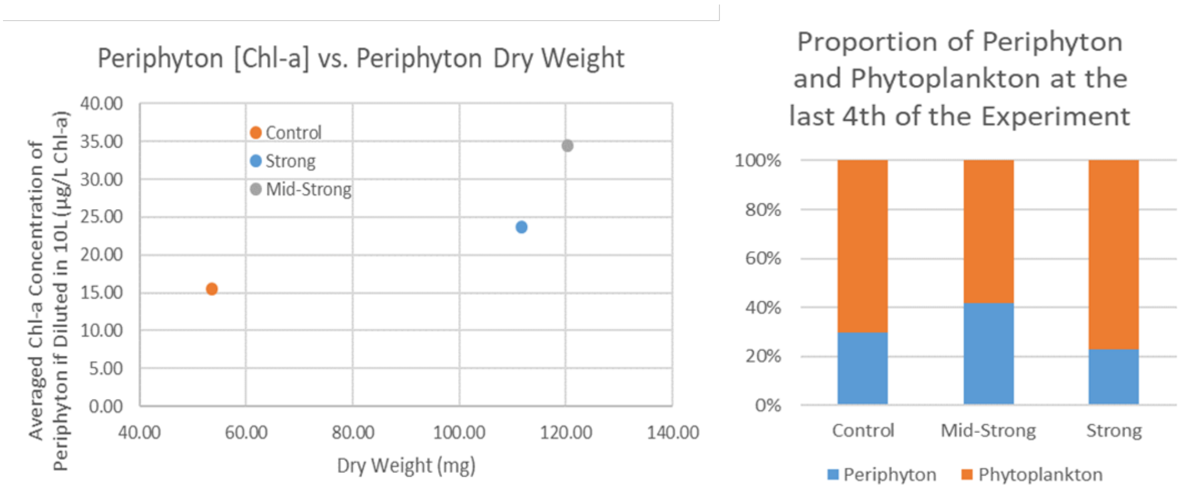


Figure 15. left: Relation between dry weight and chlorophyll-a concentrations in the periphyton community after 17 days of colonization. Note that the increase in dry weight of periphyton is poorly related to the increase in Chl-a concentrations, likely due to heterotrophic microorganisms. Right: Proportion between periphyton and phytoplankton in the last perturbation cycle. Note that the periphyton Chl-a concentration results from 17 days of accumulation, while the phytoplankton Chl-a concentration is the averaged value during the same period.

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Short CV & Publications

With post graduations in Ecosystem-Based Water Management and Resilience of Aquatic Ecosystems, my realm is to understand the processes underlying water quality and enforce self-sustaining measures that guarantee a good health of aquatic systems. Both, water and food security are paramount goals for regional sustainability and for the avoidance of water-based conflicts, therefore, being an ultimate objective of my work. Focused on Environmental Risk Assessment of pollutants and Resilience of Ecosystems in face of multiple stressors, most of my career was dedicated towards the sustainable usage of natural resources. I'm also engaged in the 'science & policy' interface regarding water legislation, focusing on "Water & Society", system analysis, and its importance for socio-economical cohesion.

Education

Ph.D. in Environmental Sciences (2017-2022)
University of Geneva, Geneva (GVA), Switzerland.
Free University of Berlin, Berlin (BER), Germany.
(EU Marie Curie International Network Training & European Joint Doctorate)
Dissertation: Determination of critical processes underlying the resilience of aquatic microbial communities under distinct resource availabilities.
Advisors: Prof. Bas Ibelings & Prof. Rita Adrian.

M.S. in Transnational Ecosystem-Based Water Management (2014-2016)
Radboud University, Nijmegen (GLD), The Netherlands.
Duisburg-Essen University, Essen (NRW), Germany.
(Radboud scholarship for talented non-EEA students and Erasmus + scholarship)
Dissertation: Effects of long- and short-term experimental salinization on methane emissions from temperate coastal wetlands.
Advisors: Dr. Sarian Kosten, Prof. Wilhelm Kuttler & Dr. Gijs van Dijk

Technical Course in Hydrology (2013)
Federal University of Rio Grande do Sul, Porto Alegre (RS), Brazil.

B.S. in Biological Sciences (2008-2012)
Federal University of Rio Grande do Sul, Porto Alegre (RS), Brazil.
(Four different national scholarships in Research & Development)
Dissertation: Usage of microbial respirometry in environmental risk assessment: A microbiological approach to ecotoxicological studies.
Advisors: Dr. Alexandre Arenzon, & Dr. Sueli Teresinha Van der Sand

Publications

11. **Stelzer, J.A.A.**, Mesman, J.P., Gsel, A.S., Senerpont-Domis, L.N, Visser, P.M., Adrian, R. & Ibelings, B.W. (2022) *Phytoplankton responses to repeated pulse perturbations imposed*

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9. **Stelzer, J.A.A.**, Mesman, J.P., Adrian, R. & Ibelings, B.W. (2021) *Early warning signals of regime shifts for aquatic systems: Can experiments help to bridge the gap between theory and real-world application?* Ecological Complexity, 47, doi:10.1016/j.ecocom.2021.100944

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7. Mesman, J.P., **Stelzer, J.A.A.**, Dakos, V., Goyette, S., Jones, I.D., Kasparian, J.M., McGinnis, D.F. & Ibelings, B.W. (2021) *The role of internal feedbacks in shifting deep lake mixing regimes under a warming climate*. Freshwater Biology, 66, 1021-1035, doi:10.1111/fwb.13704

6. Kraemer, B.M., Pilla, R.M., Woolway, R.I., Anneville, O., Ban, S.H., Colom-Montero, W., Devlin, S.P., Dokulil, M.T., Gaiser, E.E., Hambright, K.D., Hessen, D.O., Higgins, S.N., Johnk, K.D., Keller, W., Knoll, L.B., Leavitt, P.R., Lepori, F., Luger, M.S., Maberly, S.C., Muller-Navarra, D.C., Paterson, A.M., Pierson, D.C., Richardson, D.C., Rogora, M., Rusak, J.A., Sadro, S., Salmaso, N., Schmid, M., Silow, E.A., Sommaruga, R., **Stelzer, J.A.A.**, Straile, D., Thiery, W., Timofeyev, M.A., Verburg, P., Weyhenmeyer, G.A. & Adrian, R. (2021) *Climate change drives widespread shifts in lake thermal habitat. Nature Climate Change*, 11, 521-+, doi:10.1038/s41558-021-01060-3

5. Ahkola, H., Lindholm-Lehto, P., Perkola, N., Valitalo, P., Merilainen, P., Maenpaa, K., **Stelzer, J.A.A.**, Heiskanen, I., Jarvisto, J., Nuutinen, J. & Leppanen, M.T. (2021) *A preliminary study on the ecotoxic potency of wastewater treatment plant sludge combining passive sampling and bioassays*. Sci Total Environ, 758, 143700, doi:10.1016/j.scitotenv.2020.143700

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3. **Stelzer, J.A.A.**, Rosin, C.K., Bauer, L.H., Hartmann, M., Pulgati, F.H. & Arenzon, A. (2018) *Is fish embryo test (FET) according to OECD 236 sensible enough for delivering quality data*

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1. **Stelzer, J.A.A.,** Arenzon, A., van der Sand, S.T. (2012) – *Usage of microbial respirometry techniques for environmental risk assessment: a microbiological approach for studies in ecotoxicology*. Digital Repository LUME-UFRGS. Open access: http://hdl.handle.net/10183/117126. (only Portuguese).