

Nitrogen availability controls response of microcystin concentration to phosphorus reduction: Evidence from model application to multiple lakes

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ABSTRACT

Microcystis blooms are a global contemporary problem and the mechanisms underlying strain-level ecology (e.g. toxigenic fraction) and toxin (microcystin, MC) production are not sufficiently understood. Recent research suggests that MC synthesis depends on the availability of nitrogen and light, and that they protect toxigenic cells against damage by H₂O₂. The non-toxigenic strains employ the alternative strategy of enzymatic degradation of H₂O₂. Thus, MC-producing cells may have an advantage at high nitrogen and light availability. A model based on this mechanism was able to reproduce the observed patterns of toxigenic fraction and MC concentration in Lake Erie. However, it is unclear if this mechanism also applies to other systems. We investigated this by modeling nine different cases (i.e. lakes, stations, years). The model can reproduce observed patterns (toxigenic fraction, concentration of MC, biomass, nutrients and if available H₂O₂) for all cases, which constitutes support for the proposed mechanism. To explore lake management, we simulated single and dual nutrient (i.e. nitrogen and/or phosphorus) reduction, which predicts two types of outcomes. For lakes with nitrogen limitation at some time during the blooming season (e.g. Lake Erie) a phosphorus only reduction does not reduce MC concentration proportionally and may even increase it. Reducing phosphorus lowers biomass, which increases nitrogen and light availability and raises MC production and toxigenic fraction. For lakes with replete nitrogen (e.g. Lake Taihu) MC concentration is predicted to decrease. Here, further nitrogen availability will not increase MC production. These results advance mechanistic understanding of *Microcystis* strain ecology and toxin production and provide guidance for management.

1. Introduction

Cyanobacterial blooms in lakes and reservoirs are a global problem. Of special concern is the production of cyanotoxins: cyanobacteria can produce a variety of harmful oligopeptides, which may affect ecosystem processes and pollute drinking and recreational waters. Human impact like enhancing global warming and intensifying agricultural fertilization and the associated discharge of nitrogen and phosphorus into water bodies has promoted cyanobacterial biomass and often their dominance in phytoplankton communities and its toxicity (reviewed in Huisman et al., 2018). *Microcystis* is a commonly occurring genus of freshwater cyanobacteria, which has been observed to increasingly dominate freshwater phytoplankton communities (reviewed in Harke et al., 2016). Communities of *Microcystis* are typically composed of a number of toxigenic and non-toxigenic strains. The toxin-producing cells synthesize the hepatotoxin microcystin (MC) in varying quantities (reviewed in Dick et al., 2021). Accordingly, both variations in the toxigenic fraction

of a *Microcystis* community and MC cell quotas (i.e. fgMC/cell) affect MC concentrations (i.e. µgMC/L).

Previous research has identified numerous factors associated with patterns in MC production and toxigenic fraction. For instance, light is needed to provide energy (i.e. photosynthesis) to synthesize the MC peptide and nitrogen is an essential component of the MC molecule (Van Der Westhuizen and Eloff, 1985; Van De Waal et al., 2009; Watanabe and Oishi, 1985). Beside the effect on MC production, nutrient availability has also been shown to modulate *Microcystis* community composition, including the proportion of toxigenic to non-toxigenic strains (Davis et al., 2009). For example, nitrogen availability was found to correlate positively with the growth of toxigenic *Microcystis* strains in lake studies (Beversdorf et al., 2015; Monchamp et al., 2014; Yoshida et al., 2007).

Progress has also been made on understanding the biological role of MCs. Specifically, some studies indicate that MCs function intracellularly to protect against oxidative stress, for instance by binding

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covalently with the cysteines of RuBisCo (Zilliges et al., 2011). Consistent with that, MC producing cells are more resistant against naturally occurring concentrations of H₂O₂ than non-toxicogenic strains (Dziallas and Grossart, 2011; Zilliges et al., 2011). The non-toxicogenic strains, however, might defend themselves by a different strategy of actively degrading H₂O₂ via enzymes. There are various antioxidant enzymes, like heme catalases and peroxidases, but in *Microcystis* those enzymes are mostly restricted to peroxiredoxins (e.g. Type 2 Peroxiredoxins - T2prx) (Dick et al., 2021). There is evidence that the toxicogenic strain has less of these enzymes (Dick et al., 2021) or that they are not expressed under ambient conditions (Schuurmans et al., 2018). It should be pointed out that, at high (algicidal treatment level) H₂O₂ concentration, the enzymatic degradation strategy applied by the non-toxicogenic strain is more effective in defending against oxidative stress (Schuurmans et al., 2018). The past studies with *Microcystis* have focused on H₂O₂, but other reactive oxygen species (ROS) exist (e.g. superoxide anion and hydroxyl radical) in cyanobacteria, and their relation to MCs remains to be explored.

In general, the past research suggests that nitrogen availability and light affect the production of MCs and the resulting MC quota, which protects against H₂O₂ damage. This in turn affects the toxicogenic strains' fitness relative to the non-toxicogenic strain, which relies on enzymatic degradation of H₂O₂. Several case studies showed that phosphorus reduction resulted in a decrease in cyanobacterial biomass in lakes (Schindler et al., 2016) and this naturally results in an increased availability of other resources, as nitrogen and light, but whether toxin concentrations reduce proportionally to biomass were ultimately not quantified. In the absence of field observation and the need to make management decision today, we have to rely on models simulating those mechanisms. Several existing models simulate MC concentrations but usually do not include variable MC quota and/or multiple strains (e.g. Jähnichen et al., 2001; Long et al., 2001), which is essential for designing strategies and limits our ability to effectively manage waterbodies.

To investigate this mechanism, it was implemented in a numerical model (herein after simply referred to as "the model", Hellweger et al., 2022). The model is a dynamic, mechanistic, molecular-level model for *Microcystis* biomass and MC production. It simulates a population of individual *Microcystis* cells, being either toxicogenic or non-toxicogenic, and extracellular compounds (i.e. nutrients, MCs). Individual cells experience natural concentration of H₂O₂ which may damage proteins and affect growth. As defense strategy, the toxicogenic cells produce MCs depending on the availability of nitrogen and light, which serves as protection for their proteins whereas the non-toxicogenic cells degrade H₂O₂ enzymatically.

Hellweger et al. (2022) applied the model to Lake Erie, which serves as critical drinking water source. The lake regularly experiences dense *Microcystis* blooms and concomitant high MC concentrations, which has led to a management plan of reducing 40% of the phosphorus input (US Environmental Protection Agency, 2018). The model was able to reproduce the observed patterns of toxicogenic fraction and MC concentration in this system. Simulations of the planned phosphorus reduction predict that, although the biomass will be reduced, the MC concentration will increase. By reducing phosphorus, relatively more nitrogen and light will be available per cell, leading to a higher MC production and concomitant MC quota which in turn results in better protection against H₂O₂ damage for the toxicogenic strain and hence a higher fitness and proportion in the community. Those two factors (higher MC quota and higher toxicogenic fraction) counteract the decrease in biomass and result in an increase in MC concentration in the lake (i.e. biomass × MC quota × toxic fraction = MC concentration). That study put into question the usefulness of the probably most common lake management approach of reducing phosphorus input for reducing MC concentration and led to some criticisms (for scientific debate see comment: Huisman et al., 2022; response: Hellweger et al., 2022b; comment: Stow et al., 2022; response: Wilhelm et al., 2022).

The aim of this study was to test whether the proposed mechanism applies to other systems and if the prediction that a phosphorus only reduction results in MC concentration increase is universal. Therefore, the model was applied to eight additional cases and simulations of nutrient reduction (i.e. phosphorus only, nitrogen only or dual) were performed. The model calibrations support the mechanisms included in the model and the management simulations show that there are two types of response depending on the nitrogen availability prior to the management action.

2. Materials & methods

2.1. Model description

A detailed description of the model is provided in Hellweger et al. (2022a) and its supplementary information, and a brief overview is presented here. The agent-based model simulates individual *Microcystis* cells, including transcription and translation of selected representative genes and their metabolic interaction. For example, nitrate is transported into the cell via transporter protein NrtA. Inside the cell, it is reduced to nitrite and further to ammonium by reductases NarB and NirA. Ammonium is further assimilated via the GS-GOGAT cycle to glutamine and glutamate, which serves as substrate for MC synthesis. Photosynthesis is performed by a hypothetical gene representing a photosynthetic unit and the RuBisCo complex (i.e. PSURbCL). It produces G3P as photosynthetic end-product which is also a substrate for MC synthesis. PSURbCL and other enzymes may be damaged by H₂O₂, unless MCs are bound to the protein.

The model differentiates between toxicogenic and non-toxicogenic cells based on their strategies to deal with H₂O₂, i.e. protection via MCs versus defense via enzymatic degradation. In the toxicogenic cells, MCs are produced non-ribosomally by the *mcy* gene cluster (i.e. McyD). The expression of the *mcyD* gene is light, temperature, nitrogen stress (NtcA:2OG) and McyD protein levels dependent. The synthesis of MCs by the McyD protein requires G3P and glutamate. A subsequent binding of MCs to the PSURbCL complex serves as protection against damage from H₂O₂. Oppositely in the non-toxicogenic cells, T2prx as representative of all H₂O₂ degrading enzymes is produced, which is not included in the toxicogenic cells. In this way, the two alternative strategies of toxicogenic and non-toxicogenic cells to deal with oxidative stress are modeled. Natural *Microcystis* communities are diverse, consisting of numerous strains with varying traits like MC production and others (Dick et al., 2021). This diversity is not explicitly simulated because of competitive exclusion (i.e. paradox of plankton) (Hutchinson, 1961). To investigate general dynamics of the toxicogenic fraction, the diversity is simplified to one representative toxicogenic and non-toxicogenic strain for each model application as it was done before (Caen et al., 2024; Van De Waal et al., 2011). However, across systems, the modelled strains are differently calibrated, reflecting varying strains and traits.

In nature, MCs occur in a variety of congeners that have different toxicity, and it is known that nitrogen availability affects their composition (e.g. Agha et al., 2013; Chaffin et al., 2023; Taranu et al., 2019; Van De Waal et al., 2009). However, the mechanisms underlying their relative production is still insufficiently known. Hence, the model does not distinguish among different congeners.

The cellular metabolism of the modeled cells depends on the availability of resources like nutrients, light etc. Here, a coupled Lagrangian-Eulerian approach is used, meaning cells are simulated individually but nutrient and H₂O₂ pools are modeled as concentrations. Numerous individual toxicogenic and non-toxicogenic cells are modeled from which the population dynamics emerge. The model simulates strain-specific *Microcystis* dynamics and predicts their development under varying environmental conditions. It can be used to predict scenarios of *Microcystis* genotype succession and MC production in lake ecosystems.

The *Microcystis* model used here is a mechanistic model. It includes known biological mechanisms (e.g. photosynthesis, gene expression)

and those related to MC based on numerous laboratory studies. This mechanistic model explicitly relates mechanisms to observed patterns, i. e. causality.

2.2. Site selection

Data were compiled from published field observations of *Microcystis* dynamics. Measurements from the pelagic zone of freshwater bodies (i. e. lakes, reservoirs, rivers) were included. In some cases, multiple datasets from the same system (i. e. different stations and/or years) were available. Datasets needed to fulfill the following criteria to be included: time-resolved data (more than three data points in one growing season, for details see SI) including *Microcystis* biomass and basic limnological data (i. e. DIN, SRP) as well as two out of the three parameters: toxigenic fraction (*mcy* genes as share of total *Microcystis* 16S rDNA or cyanobacterial 16S rDNA when *Microcystis* was dominant), MC and H₂O₂ concentrations. Datasets where additional analysis would be necessary to extract the information needed for the model (i. e. qPCR for toxigenic

fraction were performed but results not provided as number) were included.

Datasets were obtained from the Google Scholar web search engine using the search terms “microcystis” AND “genotype succession” AND “microcystin”, whereas only publications written in English were included. The search resulted in 199 hits (December 2021) which were manually checked against the above criteria. From those publications, all references and citations were audited, resulting in 23 potential sites (see table 1). In addition to the original Lake Erie application (Station W12, 2014), 8 datasets were selected for modeling (boldly written in table 1) as follows. Two additional datasets for Lake Erie were chosen to validate and compare the model to the original simulation and investigate spatial and temporal heterogeneity (i. e. different station and year). Additionally, two datasets from Lake Taihu were selected due to its societal and scientific relevance. Then, Daecheong Reservoir, Sulejow Reservoir and Forez Fish Lake were selected based on the accessibility and availability of data. Data were digitized from the publications using an online free plot digitizer (WebPlotDigitizer 4.8 - Copyright

Table 1

Field data for *Microcystis* toxigenic fraction and MC concentration. Boldly written data sets were selected for model application. The circular symbol (●) indicate the presence and the line (-) the absence of this particular parameter in the dataset.

lake/reservoir	station(s)	year(s) (20XX)	ID	biomass (e)	int. MC (f)	ext. MC (f)	tox. F. (g)	H ₂ O ₂	NO ₃	NH ₄ ⁺	PO ₄ ³⁻	source	note
Agawam	-	05/06	-	Mi	●	-	D	-	●	●	●	Davis et al. (2009)	
Champlain	-	06	-	Mi	●	-	D	-	●	●	●	Davis et al. (2009)	
Copco	CR01	07	-	Mi	●	-	B	-	-	-	-	Bozarth et al. (2010)	
Daecheong	Chusori	12	DR 12	Mi	x	x	J	●	●	●	●	Joung et al. (2016)	a), d)
Durgakund	-	10	-	Ph	●	-	A	-	●	●	●	Srivastava et al. (2012)	
Erie	W4, W12	14, 15	LE W12 14 LE W04 14 LE W12 15	Ph	●	●	D	●	●	●	●	h)	a), b), c)
Forez Fish Lake	V1-V6	08	FFL 08	Mi	●	-	D	-	●	●	●	Pobel et al. (2012)	
Dongjituo Fish pond	-	17	-	Mi	●	●	A	-	●	●	●	Bi et al. (2019)	
Funil	-	11/12	-	Ch	x	x	B	-	●	●	●	Guedes et al. (2014)	
Grangent	-	06	-	Mi	●	-	B	-	-	-	-	Sabart et al. (2010)	
Hartbeespoort	-	04/05	-	Ch	x	x	B	-	●	●	●	Conradie and Barnard (2012)	
Hoedong	-	11	-	Ch	●	●	E	-	●	●	●	Park et al. (2018)	
Lakshmikund	-	10, 11	-	Ch	●	●	B	-	●	●	●	Singh et al. (2015)	
Mill Pond	-	06	-	Mi	●	-	D	-	●	●	●	Davis et al. (2009)	
Roodeplaat	-	04/05	-	Ch	x	x	E	-	●	●	●	Conradie and Barnard (2012)	
Ronkonkoma	-	05	-	Mi	●	-	D	-	●	●	●	Davis et al. (2009)	
San Juan	-	13, 14	-	Ch	●	●	E	-	●	-	●	Lezcano et al. (2018)	
Sankuldara	-	10, 11	-	Ch	●	●	D	-	●	●	●	Singh et al. (2015)	
Sulejow	Tresta,	09, 10	SR 09	Ch	●	-	A	-	●	●	●	Gagala et al. (2014)	a)
	Bronislawow		SR 10										
Taihu	N2,	13,	LT N2	Mi	●	-	B	-	●	●	●	Hu et al. (2016),	a)
			13										
	S4	10	LT S4	Ch			D					Li et al. (2014)	
			10										
Tri An	TA1-5	17	-	Ch	●	●	A	-	●	-	●	Pham et al. (2021)	
Wannsee	-	00	-	Mi	●	●	B	-	●	●	●	Kurmayer et al. (2003)	
Yaacoub Al Mansour	-	09, 10	-	Mi	●	-	B	-	●	-	●	Hammou et al. (2018)	

a) Additional datasets available from this site.

b) H₂O₂ concentrations from (Cory et al. 2016).

c) Data on toxigenic fraction not available for Lake Erie, station W12, year 2015.

d) H₂O₂ concentrations from (Yoon et al., 2021), calculated mean from the years 2017–2019.

e) Abbreviations: Mi – *Microcystis* spp cell counts; Ph – phycocyanin concentration; Ch – chlorophyll a concentration.

f) The symbol x indicates a combined analysis of extracellular and intracellular MCs.

g) Ratio of *mcy* gene cluster to *Microcystis* 16 s rDNA or rRNA. Letter indicate analyzed gene of *mcy* cluster (*mcyA-J*).

h) Input data for Lake Erie from <https://www.ncei.noaa.gov>, toxigenic fraction from (Yancey et al., 2022).

2010–2021 Ankit Rohatgi automeris.io). Additional data were gathered including temperature, daylength, PAR (i.e. photosynthetically active radiation), station depth and flow rate (Table S1).

2.3. Model application

The model was set up and calibrated to nine cases following the same general approach used in the original Lake Erie simulation. Specifically, a zero-dimensional box around the sampling station was modeled. No lake nutrient budget or an account for spatial heterogeneity within the lakes were developed. External nutrient loadings may not reflect the input into the model region due to internal sources and sinks, like sediment flux, and varying water column transport. Therefore, nutrient input into the system was estimated using a simplified approach, based on the observed total nitrogen and phosphorus concentrations and an *a priori* mass balance, as done previously (Hellweger et al., 2022; Mayerhofer et al., 2021). For example, if the observed total nitrogen concentration between two sampling times increases, nitrogen must have entered into the system, so we added sufficient loading to account for this increase (also considering outflow and settling losses, details in (Hellweger et al., 2022 and SI). This approach limits the model, e.g. for quantitatively connecting external loadings to in-lake processes, but it is sufficient for the purpose of our study. The model was calibrated against observations of biomass, nutrients, MC and (if available) H₂O₂ concentrations, and the toxigenic fraction. This includes adjustment of parameters like the temperature optimum for growth or sensitivity towards damage by H₂O₂ (see table S2 and S3). Recalibration is common for biological models, and necessary in this case as field *Microcystis* strains vary across scale and time, showing different genetic and physiological traits (reviewed in Dick et al., 2021). For these simulations, 3–26 parameters were adjusted and 95% of all parameters were not changed from the original application to Lake Erie. Model parameters were generally within literature ranges (where available) except for temperature optima for growth and MC production in the application to Forez Fish Lake, which experiences relative low temperatures (average 17.8 °C).

Biomass dynamics are affected by a number of bottom-up and top-down factors and the model accounts for several of those explicitly. For example, nitrate concentration varies dynamically based on loading, uptake by *Microcystis*, nitrification, etc. Other factors, like grazing by zooplankton or viral lysis are not explicitly simulated. Specifically, the model does not include a state variable for herbivorous zooplankton that varies dynamically due to e.g. grazing on phytoplankton. Rather, grazing is modeled using an assigned first-order loss rate. Also, limitation by other factors (e.g. iron, CO₂ etc.; Ji et al., 2020; Nagai et al., 2007) is included as an assigned factor. In the original Lake Erie simulation, the observed biomass dynamics could be reproduced with a constant grazing rate and other limitation factor. However, in reality grazing and other limitation factors are expected to vary dynamically (e.g. a phytoplankton bloom followed by a zooplankton bloom, i.e. Lotka-Volterra equations). It was not always possible to reproduce observed biomass dynamics with constant factors in the model applications presented here, and they were therefore assigned on a weekly basis, as it has been done previously (Bucci et al., 2012; Hellweger et al., 2008; 2019). To test for potential effects of those adjusted parameters on the analysis, both approaches were applied to the Lake Erie, station W12, 2014 case and compared. Also, various alternate calibration strategies were explored for a single case (see below). This simplified approach to grazing and other limitation is justified in this case as we are primarily interested in the mechanisms underlying toxigenic fraction and MC production (where all factors are explicitly included), and not in total *Microcystis* biomass dynamics. Although it is common in water quality modeling to not explicitly resolve higher trophic levels, it should be highlighted that those factors may change in some management cases. Specifically, the nutrient reduction scenarios simulated here assume that loss by zooplankton grazing does not change, although it may

actually be expected to decrease along with phytoplankton biomass.

To evaluate the fit of model and data, a pattern-oriented modeling approach was used (Grimm and Railsback, 2012; Hellweger et al., 2022). The basic idea is to identify observed patterns, defined as a trend from one quantity towards another (e.g. MC concentration increases during month X), and see if the model reproduces it (e.g. also shows an increase at that time). The focus is on the patterns (i.e. changing trends) versus the absolute quantity or magnitude. For instance, it is less relevant whether a measured peak concentration is captured by the model but rather that the model also shows a peak at that time. Pattern oriented modeling is a common method for evaluating complex agent-based model (Gallagher et al., 2021). Also, other more commonly used metrics exist for judging model performance like the root mean square error (RMSE) and normalized RMSE (NRMSE). However, they are difficult to apply to complex models with multiple patterns. One disadvantage of the RMSE is that it gives a disproportionately large weight to high errors, making it more sensitive to outliers. In the datasets used in this study, some outliers are present which are suspect to errors in the data (discussed below), so RMSE may not be the best choice for evaluating the model results.

When calibrating a complex model, several parameter combinations may produce a good fit of model and data. However, simulations using different parameter sets may result in substantially different predictions, a problem referred to as equifinality (Beven 2006; 2019). To explore the effect of this problem in the model applications here, five different parameter sets were developed for one of the cases, namely the Korean Daecheong Reservoir. Specifically, the five parameter sets differ in one parameter compared to the basecase, either controlling biomass mostly via nutrients, via grazing or via other undefined factors or MC production mostly via light. This equifinality testing serves as a test of the robustness of the model, instead of a large-scale sensitivity analysis that cannot be performed with such a complex and computationally demanding model.

3. Results and discussion

3.1. Model-data comparison

The model was calibrated to fit observed patterns of biomass, MC concentration, toxigenic fraction, nutrient and (if available) H₂O₂ concentrations. In Fig. 1, the comparison of data (i.e. symbols) and model output (i.e. line) for toxigenic fraction and MC concentrations is presented. Overall, the model could reproduce the observed patterns. For example, the model reproduced 35 of the 39 patterns (89 %, and 18 of the observed 20 peaks) in MC concentration and 20 of the 22 patterns (89 %) of the toxigenic fraction. This constitutes further support for the proposed mechanism implemented in the model. The alternate calibration of the original Lake Erie application (LE W12 14) showed a similar pattern as the original calibration and observations.

As an example, the application to Daecheong Reservoir (DR 12) is discussed (Fig. 1H, solid lines and Fig. 2, for other cases see SI, section 2 ‘Model application’). The study site Chusori is located at one arm of the long-branched reservoir in Korea. In 2012, three *Microcystis* blooms occurred in the phosphorus limited waterbody, accompanied by three peaks of MC concentrations. The model could reproduce the observed dynamics of biomass (Fig. 2A), although those were to some extent “dialed in” by the assigned grazing rates (see Methods). The highest peak in biomass was measured in the bloom in August. Here, the model predicted a lower experienced light level due to self-shading which led to a reduced *mcyD* gene expression. The resulting low MC quota (<0.1 mmol/molC) caused a lower protection level of toxigenic cells against damage from H₂O₂, leading to a reduction in the toxigenic fraction, which is also evident in the observations (Fig. 1H and 2B yellow line). For MC concentration (Fig. 1H and 2C, purple solid line), the model also matched the temporal pattern, but the very high peak concentration of 90 µg/L was not reached (depicted with asterisk in Fig. 1H and 2C, see

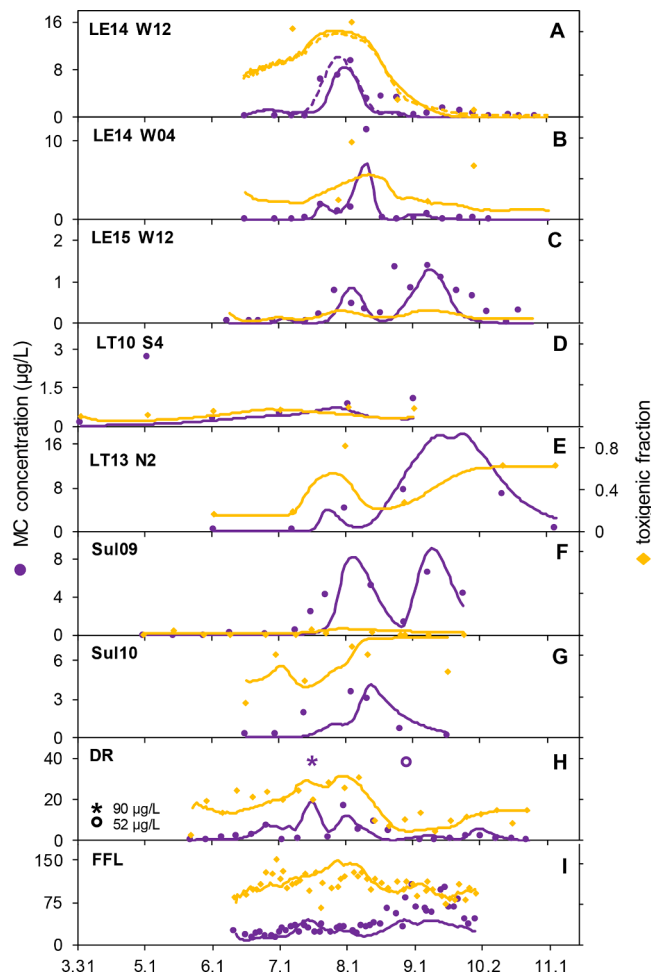


Fig. 1. Model-data comparison. Timeseries of microcystin (MC) concentration (purple, primary Y axis, note varying scale) and toxicogenic fraction (yellow, secondary Y axis) for nine model applications. Symbols depict field data, lines show model simulations. Multiple lines show different model calibrations. For LE W12 14 dashed lines show published predictions from Hellweger et al. (2022), solid lines show prediction with new parameterization. For DR 12 five parameter sets are shown, differing in one parameter compared to the basecase (solid line), either controlling MC production via light (dotted line), biomass via nutrients (dashed line), biomass via undefined factor (long dash-dotted line) or biomass via grazing (long-dashed line). Abbreviations: LE – Lake Erie, LT – Lake Taihu, SR – Sulejow Reservoir, DR – Daecheong Reservoir, FFL – Forez Fish Lake. W04, W12, N2, S4 are stations in the respective lake, number indicate year 20XX.

paragraph in Methods on pattern-oriented modelling). Another very high MC concentration of 52 $\mu\text{g/L}$ was measured in the beginning of September. The model did simulate a (very small) peak but did not reach the observed magnitude. At this time, low biomass and a reduction in toxicogenic fraction was observed. These contradicting trends suggest an MC quota 30 times higher than in the same population a few days before. Such high plasticity within a population is very unlikely (reviewed in Chorus and Welker, 2021) and suggests a possible error, for example by sampling surface scum. Over the entire time period, the modeled H_2O_2 concentration were underestimated (Fig. 2D). The model includes photochemical H_2O_2 production (estimated after Cory et al., 2016) and biological production by *Microcystis* cells. However, it excludes H_2O_2 generation by other photo- and heterotrophic organisms during photosynthesis and respiration (e.g. Marsico et al., 2015; Zepp et al., 1987). In times when *Microcystis* was not dominant in a system those sources may have been significant and responsible for the discrepancy. The resulting lower oxidative stress in the model application was

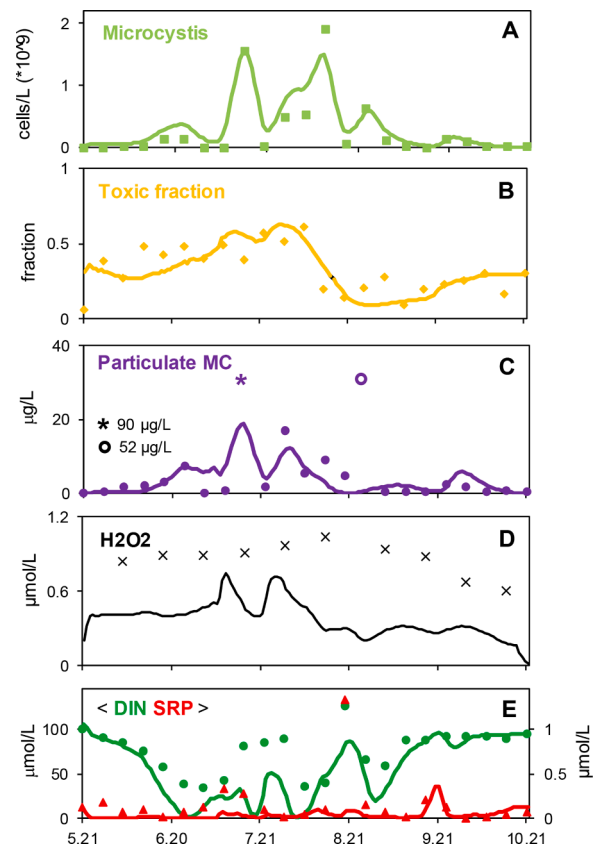


Fig. 2. Model application Daecheong reservoir, station Chusori, 2012 (DR 12). A: Data-model comparison including cyanobacterial biomass (A), toxicogenic fraction (B), intra- and extracellular microcystin concentrations (C), H_2O_2 concentrations (D) and nutrient concentrations (E) are displayed. Symbols indicate field data, lines show model simulations.

compensated for with increased H_2O_2 sensitivity of toxicogenic and non-toxicogenic cells.

A further substantial discrepancy between model and data is evident. In May 2010, a MC concentration of ca. 2.7 $\mu\text{g/L}$ was measured in Lake Taihu center at station S4 which could not be reproduced by the model (Fig. 1D). However, also here is some indication for human error during sampling or laboratory analysis. For instance, no accompanying peak in biomass or toxicogenic fraction was observed, resulting in an unrealistic high MC quota and MC:chlorophyll ratio which is ten times higher than the average measured in the previous year (Wang et al., 2012). For detailed discussion see SI.

3.2. Nutrient reduction scenarios

To explore lake management, nitrogen, phosphorus or dual reductions were simulated. The results are summarized as predicted change in biomass (i.e. phycocyanin -PCN, green symbol in Fig. 3) and toxin concentrations (i.e. MC, red symbol in Fig. 3). A reduction in nutrients has a direct effect on biomass and an indirect effect on light (i.e. self-shading), which affects photosynthesis and toxin production. Temperature also affects biomass and MC, but that is not expected to change as a result of the nutrient reduction scenarios. A 40 % nutrient loading reduction resulted in a decrease of biomass in most of the cases, up to 39 % depending on the extent of the respective nutrient being a limiting growth factor in the system. In four cases (A, C, F, I in Fig. 3) a nitrogen only reduction showed a decrease in biomass greater or to the same extend as a phosphorus only reduction. However, MC concentration showed a varied response. In the following, the cases are divided into two groups, those which were partially nitrogen limited and those which

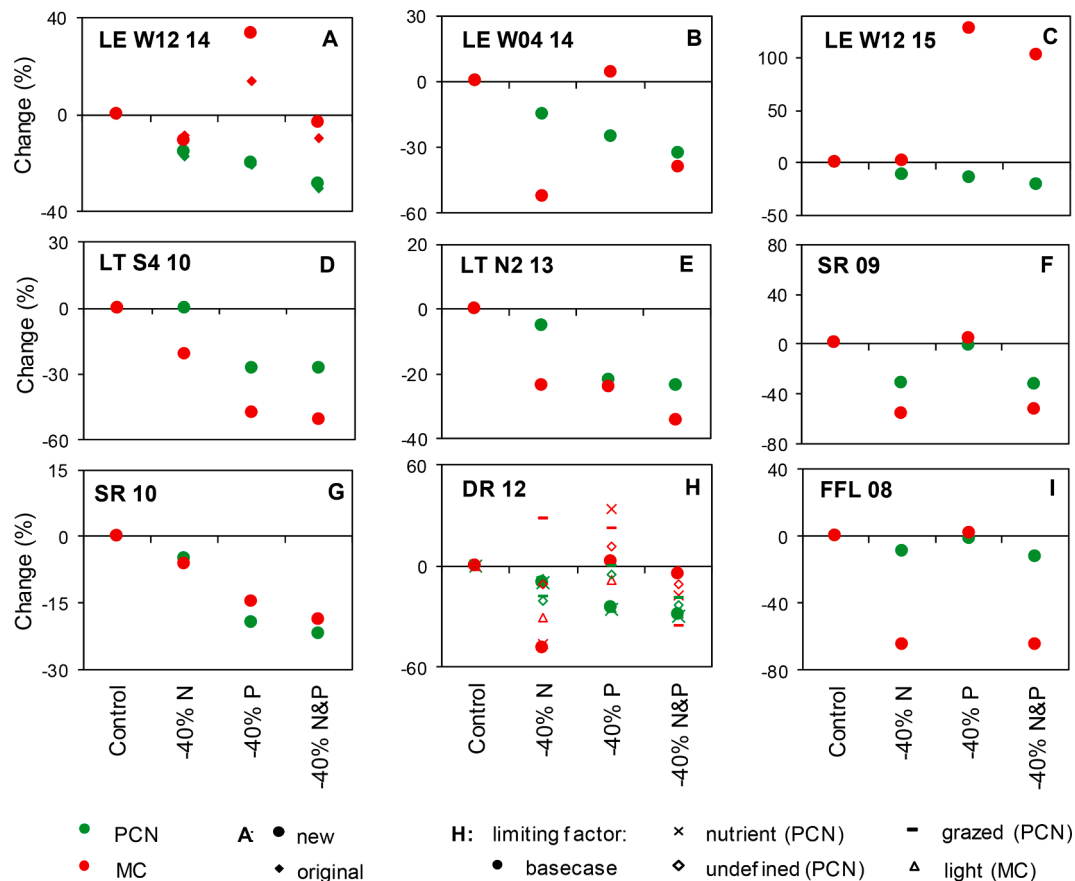


Fig. 3. 40% nutrient reduction scenarios. Model prediction of changes in biomass (phycocyanin/PCN, green) and toxin concentrations (microcystin/MC, red) when 40 % nitrogen, phosphorous or both is reduced. Change is based on average concentrations across the growing season. All simulations indicate a reduction in biomass, simulations for toxin reduction show varied response (see text). Multiple symbols show different model calibrations. For LE W12 14 (A) close diamonds show published predictions from Hellweger et al. (2022), close circles show predictions with new parameterization. For the DR 12 (H) five parameter sets are shown, differing in one parameter compared to the basecase (close circles), controlling either biomass via nutrients (crosses), via grazing (lines) or MC production via light (open triangles). Abbreviations: LE – Lake Erie, LT – Lake Taihu, SR – Sulejow Reservoir, DR – Daecheong Reservoir, FFL – Forez Fish Lake. W04, W12, N2, S4 are stations in the respective lake, number indicate year 20XX. Note the varying scale of the Y axis.

were nitrogen replete. Specifically, in cases when DIN dropped below a critical value of $\sim 2 \mu\text{molN/L}$ at any time during the growing season (i.e. seasonal minimum DIN concentration - min. DIN), those cases experienced nitrogen limitation. Oppositely, when DIN concentrations were high over the entire growing season, never dropped below $15 \mu\text{molN/L}$ prior to the reduction, the lakes and reservoirs were considered nitrogen replete. These values are in the range reported in literature of ca. $6.5 - 7.2 \mu\text{molN/L}$ for nitrogen limitation or $13 \mu\text{molN/L}$ for co-limitation (Kolzau et al., 2014; Maberly et al., 2002). The results of the nutrient reduction scenarios were also presented as predicted change in biomass and MC concentrations versus min. DIN concentration under 40% phosphorus only reduction in Fig. 4, illustrating the grouping of the cases against nitrogen limitation of the systems.

Six cases experienced partial nitrogen limitation (A, B, C, F, H, I in Fig. 3) For those, the simulation of nitrogen only or dual reduction, predicted that MC concentrations decreased along with a reduction in biomass for most cases. Only for Lake Erie, station W12, 2015 (Fig. 3C) the model predicted no change and an increase in MC concentrations under nitrogen only and dual reduction, respectively. Here, the predicted reduction in biomass led to a higher transluency which translated in a higher MC cell quota since in this case MC production is mainly light limited. When phosphorus only reduction was simulated, the model did not predict a reduction in MC concentration despite the reduction in biomass (see also Fig. 4). A higher availability of nitrogen and light due to lower biomass led to an increase in MC production (i.e. MC quota). This increased protection against H_2O_2 damage and a fitness

advantage for the toxigenic over the non-toxigenic strain resulted in a higher proportion of toxin-producing cells in the community. Higher MC cell quota along with higher toxigenic fraction explained the contrasting patterns of biomass and MC concentrations. This result was consistent with the original predictions for Lake Erie.

In three cases, nitrogen was replete over the growing season (D, E, G in Fig. 3). For these cases a reduction in nitrogen resulted in no or only slight reduction in biomass (0–5 %). The simulation of phosphorus only or dual reduction predicted a decrease of MC concentration along with biomass (which is different from the cases with partial nitrogen limitation, see also Fig. 4). Here, increased availability of nitrogen, caused by phosphorus reduction and a resulting reduced biomass, did not translate in a higher MC production and shift in toxigenic fraction.

These mechanisms were also evident in simulations of gradual reduction of phosphorus loading (figure S1B-S9B). Here, phosphorus load reduction was accompanied by a reduction in MC concentrations only if min. DIN exceeded a critical value, indicating nitrogen was not limiting in the system. If nitrogen was limiting, the MC concentration was predicted to increase or remain constant even as the biomass is decreasing, as per the above mechanism. Once nitrogen was not limiting, which may be at very low phosphorus concentration, the model also predicts MC concentration to decrease.

The nutrient reduction simulations showed that both nitrogen and light can affect the production and concentration of MC. To quantify the relative effect of nitrogen versus light on MC quota, a sensitivity analysis (i.e. $\pm 10 \%$ nitrogen or light) was performed (see table S7). In general,

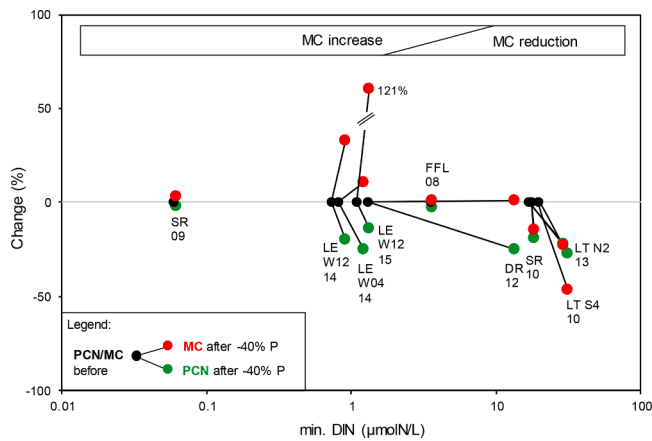


Fig. 4. 40% phosphorous only reduction scenario. Model prediction of changes in biomass (phycocyanin/PCN, green) and toxin concentrations (microcystin/MC red) when 40 % phosphorous is reduced. Change is based on average concentrations across the growing season. All simulations indicate a reduction in biomass. When the minimum dissolved inorganic nitrogen (min. DIN) concentration in the growing season prior to the phosphorous reduction is below 2 μM (i.e. nitrogen limitation) the model predicts an increase in MCs, when the min. DIN is above 15 μM the model predicts a decrease in MCs. Note the logarithmic scaling of X axis. Abbreviations: SR – Sulejow reservoir, LE – Lake Erie, FFL – Forez Fish Lake, DR – Daecheong Reservoir, LT – Lake Taihu. W04, W12, N2, S4 are stations in the respective lake. Number indicate year 20XX.

it is difficult to disentangle the effect of nitrogen and light on MC cell quota as they are closely interconnected. For example, a reduction of nitrogen lowers biomass which indirectly effects light due to self-shading. Therefore, the response to nutrient load reductions as well as the relative importance of nitrogen and light is case specific and difficult to generalize.

3.3. Equifinality simulations

For complex models, multiple parameter sets may result in a good fit between model and data but potentially result in different predictions (i. e. equifinality). Five alternate parameter sets representing different growth and MC production limitations were tested for the Daecheong Reservoir case. The model was able to reproduce the patterns in the field data with all parameter sets (Fig. 1H and figure S8, several lines). To test the effect of different model parameterizations on the prediction of management scenarios, 40% reduction of nitrogen, phosphorus or dual reduction were simulated (Fig. 3H, several symbols). The model predicts a reduction of biomass in all nutrient reduction scenarios with all five parameter sets, differing in magnitude (Fig. 3H, green symbols). MC concentrations were also predicted (Fig. 3H, red symbols). When nitrogen only or dual nutrients were reduced, the model predicted a decrease also in MC concentrations for most cases. Only when grazing was the main limitation for biomass, MC concentrations were predicted to increase (Fig. 3, dash). In this case, MC production was mainly light inhibited, being enhanced with light available due to reduced biomass. When phosphorus only was reduced, the model predicted an increase or no change in the MC concentration, resulting from higher nitrogen and light availability. This was consistent with the previous simulation of Lake Erie. For the case when MC production was mainly light limited (Fig. 3, triangle), the model predicted a slight reduction of MC concentration. However, the reduction in MC concentration was less than the reduction in biomass. As in the other cases, reduced biomass released resources but here, only the additional availability of light (not also nitrogen) drove the increased MC production. Hence, the effect on toxic fraction was lower.

Summarizing, the equifinality simulations provided further support for the mechanism implemented in the model, as the main conclusions

drawn from the predictions were generally robust to alternate calibrations.

3.4. Limitations and outlook for modeling

The present model incorporates a hypothesized biological role for MCs and reproduces observations of toxic fraction and MC concentration in Lake Erie and other systems. It thus constitutes a substantial advance over other / previous models (e.g. Jähnichen et al., 2001; Long et al., 2001). However, it is still simplified and lags behind current biological knowledge. Specifically, the model does not consider MC congeners (e.g. MC-LR, MC-RR etc.) and the effect of nutrient availability on their composition and resulting toxicity which has been investigated in several studies (e.g. Agha et al., 2013; Chaffin et al., 2023; Taranu et al., 2019; Van De Waal et al., 2009). Also, the biological and/or ecological role of MCs seems complex and diverse (Omidi et al., 2018) and may exceed the protective effect against H_2O_2 damage implemented into the model. For instance, recent studies show that MCs are involved in the subcellular localization of RuBisCo towards the cell membrane indicating MCs being involved in the process of CO_2 fixation which may be relevant in times of CO_2 limitation (Barchewitz et al., 2019; Guljamow et al., 2021). However, from an ecological or management perspective it may not be critical to explicitly account for the correct mechanism(s). The highly conserved cyanotoxin gene cluster for the non-ribosomal toxin production apparatus (Rantala et al., 2003; Tillett et al., 2000) indicate some beneficial function of MCs for the cells. It is also clear that MC production is a function of nitrogen availability (Van De Waal et al., 2009; Watanabe and Oishi, 1985). Thus, the general pattern emerging from the present model, i.e. higher MC cell quota in times of sufficient nitrogen and light availability, implying an advantage for the toxicogenic cells and resulting increase in toxicogenic fraction, may also describe other potential function(s) of MC. This also suggests a simpler model may be useful and we are presently working on developing such a code. Another advantage of a simpler model is a reduced simulation time. Simulations with the current modeling require about 60 h, which prohibits performing replicate simulations and allow statistical analyses of the predictions. Another limitation of the model is that it does not include species other than *Microcystis*. This may be especially relevant for applications to systems where *Microcystis* is not dominant, or where this may change due to management. The implementation into a whole lake model including more ecosystem components would be the next logical and interesting step.

3.5. Implications for lake management

The model by Vollenweider (1968) showed a correlation of phosphorus loading and trophic state of a water body. Accordingly, phosphorus reduction has traditionally been used by lake managers to counteract eutrophication (e.g. reviewed in Schindler et al., 2016). And indeed, in several field applications a phosphorus reduction has tremendously reduced the phytoplankton biomass, for instance in Lake Tegel, Lake Schlachtensee and Lake Washington (Fastner et al., 2016; Schindler et al., 2016). Likewise, the model used here predicts a reduction of biomass for reduced phosphorus input in all nine cases. However, the effect of phosphorus only reduction on MC quota and community composition and resulting total MC concentrations has just started to be explored. The present *Microcystis* model suggests that a phosphorus only reduction may not be beneficial or even harmful in some cases. An increase of total MC concentrations is predicted for lakes and reservoirs experiencing partial nitrogen limitation as the higher N:P ratio increases MC production and favors the toxicogenic strains.

Similarly, other studies investigated the effect of an increase in nitrogen availability in lakes and drinking water reservoirs on phytoplankton composition and found increasing toxicity in the water. For instance, studies indicate that more toxic cyanobacteria species, as for instance *Microcystis*, profit under low phosphorus and high nitrogen

condition (reviewed in Gobler et al., 2016; Harke et al., 2016). Firstly, *Microcystis* has good phosphorus storage capacity being advantageous over other potentially non-toxicogenic species under low phosphorus condition (Harke et al., 2012; Harke and Gobler, 2013). Secondly, they profit from the high availability of dissolved inorganic nitrogen in the water phase whereas diazotrophic species lose their advantage of surviving low nitrogen condition by fixing atmospheric nitrogen (Paerl et al., 2014). A concrete example illustrates the complexity of nutrient loadings and toxicity, although it is not related to *Microcystis* or the mechanism explored here. In Lake Tegel the reduction of total phosphorus loading and following decline of phytoplankton biomass has affected toxicity. Here, reduced phosphorus loadings have tremendously decreased phytoplankton biomass. Lower plankton density increased light permeability and other resources, promoting the proliferation of benthic species, for instance water moss (*Fontinalis antipyretica*). The moss can host the anatoxin-a producing cyanobacterium *Tychonema* spp. which resulted in an actual increase of those toxins in the water body (Fastner et al., 2018). Those examples illuminate the complexity of ecological systems and that anthropogenic interventions may have unforeseeable ramifications. Hence, a linear assumption of lower phosphorus equals lower biomass equals lower toxicity may disregard important biological mechanism or ecological interactions, and their consequences.

4. Disclosure

The authors declare no competing interest. All authors have approved the final article.

5. Conclusions

The *Microcystis* model was able to reproduce observed patterns of biomass, MCs and toxicogenic fraction for nine different cases. This supports the mechanism implemented into the model which considers different strategies of toxicogenic and non-toxicogenic strain to deal with H_2O_2 , namely protecting enzymes via MCs or actively degrading H_2O_2 . Additionally, we show simulations of 40% nutrient reductions and the predicted change in biomass and MC concentration for all cases.

As main finding, we present a figure showing predicted changes in biomass and MC concentration under 40 % phosphorus only reduction as a function of minimum DIN concentration during the growing season prior to the reduction. Two groups are present, those cases which experience partial nitrogen limitation during the growing season and those cases which are nitrogen replete. In cases of low minimum DIN concentrations, i.e. when there is nitrogen limitation, the model predicts an increase of MC concentration when phosphorus only is reduced. Lower phosphorus results in lower biomass but higher nitrogen availability and transparency, which translates into higher MC cell quota. This benefits the toxin producing cells, the toxicogenic fraction increases. High MC quota and increasing toxicogenic fraction counteracts the reduced biomass and total MC concentration is predicted to increase (i.e. biomass \times MC quota \times toxic fraction = MC concentration). However, if nitrogen is replete in a system over the entire growing season, the cells are always nitrogen saturated. Additional nitrogen due to reduced biomass under phosphorus reduction does not translate into higher MC cell quota and toxicogenic fraction. The MC concentration in the lake is predicted to decrease along with biomass.

Generally, our study questions the validity and usefulness of the paradigm phosphorus equals biomass equals toxicity (Wilhelm et al., 2022), and highlights the need to explicitly consider the mechanisms underlying strain succession and MC production in management.

Supplementary information

Additional information is available on 1) modeling details, including input data and parameters, and 2) model output, including figures of

model-data comparisons and simulations of nutrient reduction scenarios.

CRedit authorship contribution statement

Charlotte Schampera: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ferdi L. Hellweger:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.hal.2024.102711](https://doi.org/10.1016/j.hal.2024.102711).

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