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Dispersal, location of bloom initiation, and nutrient conditions determine the dominance of the harmful dinoflagellate *Alexandrium catenella*: A meta-ecosystem study

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Abstract

Harmful algal blooms (HABs) are globally increasing in number and spatial extent. However, their propagation dynamics along environmental gradients and the associated interplay of abiotic factors and biotic interactions are still poorly understood. In this study, a nutrient gradient was established in a linear meta-ecosystem setup of five interconnected flasks containing an artificially assembled phytoplankton community. The harmful dinoflagellate *Alexandrium catenella* was introduced into different positions along the nutrient gradient to investigate dispersal and spatial community dynamics. Overall, total algal biovolume increased, while community evenness decreased with increasing nutrient concentrations along the gradient. *Alexandrium* was able to disperse through all flasks. On the regional scale, diatoms dominated the community, whereas on the local scale the dinoflagellate showed higher contributions at low nutrient concentrations and dominated the community at the lowest nutrient concentration, but only when initiated into this flask. A control treatment without dispersal revealed an even stronger dominance of *Alexandrium* at the lowest nutrient concentration, indicating that dispersal and the associated nutrient exchange may weaken dinoflagellate dominance under low nutrient conditions. This study presents a first approach to experimentally investigate spatial dynamics and ecological interactions of a harmful dinoflagellate along an environmental gradient in a meta-ecosystem setup, which has the potential to substantially enhance our understanding of the relevance of dispersal for HAB formation and propagation in combination with local environmental factors.

Worldwide, harmful algal blooms (HABs) are increasing in number and in spatial extent, which can have severe negative impacts on coastal ecosystems and may lead to serious economic and ecological losses (e.g., Trainer et al. 2010; Paerl et al. 2018; Stauffer et al. 2019). While harmful algae comprise a wide variety of different taxa, most harmful species belong to the dinoflagellates (Smayda and Reynolds 2003). HAB formation and persistence depend on a variety of abiotic environmental factors, such as light intensity, nutrient availability, and composition (Anderson et al. 2002; Granéli and Turner 2006; Wells et al. 2015), as well as on biotic interactions, such as competition (e.g., Granéli and Hansen 2006) and grazing (e.g., Turner 2006). HAB propagation and expansion on a regional scale is further determined by transport via ocean currents (e.g., Anderson et al. 2005; Giddings et al. 2014; Bialonski et al. 2016) and other natural hydrographic phenomena, such as eddies or upwelling events that potentially retain or introduce nutrients into the system, respectively (Kudela et al. 2005; Trainer et al. 2010). Upwelling systems are highly dynamic and subject to a strong variability of dissolved nutrient concentrations and ratios, depending on water column stratification, upwelling frequency, and intensity.

The Southern California Coast (US) represents a coastal upwelling region belonging to the eastern boundary current system. A variety of different HAB species frequently occur in this system that strongly vary in their spatial and temporal occurrence, as well as in their bloom duration and magnitude (Trainer et al. 2010). Diatoms often bloom during or shortly after upwelling events in nutrient-rich waters when mixing is high, while dinoflagellates dominate after nutrient depletion and stabilization of the water column, that is, when mixing decreases and stratification increases (Langlois and Smith 2001; Kudela et al. 2005; Anderson et al. 2008). Such temporal successions from diatoms to dinoflagellates, which are tightly coupled to hydrography and nutrient dynamics, are also commonly

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observed in other systems (e.g., the Bornholm Basin, van Beusekom et al. 2009; or Georges Bank, Gettings et al. 2014). Diatoms are known to be better competitors for dissolved inorganic nutrients compared to dinoflagellates, as they mostly have higher maximum nutrient uptake and growth rates, whereas marine dinoflagellates generally exhibit lower nutrient affinities and maximum growth rates (Litchman et al. 2007). However, dinoflagellates have evolved different strategies to increase their competitive strength under nutrient-poor conditions, including the storage of organic and inorganic nitrogen forms (Collos et al. 2004; Maguer et al. 2007; Dagenais-Bellefeuille and Morse 2013), or mixotrophic feeding, that is, the ingestion of organic particles in addition to photosynthesis (Jeong et al. 2005*a*,*b*; Yoo et al. 2009). Apart from increased nutrient uptake, mixotrophy may decrease competitive pressure through the consumption of potential competitors (Thingstad et al. 1996). This strategy and the ability to migrate in the water column, allowing them to take up nutrients from deeper water layers, likely allows dinoflagellates to persist, despite the relatively noncompetitive parameters for nutrient uptake and growth (Eppley et al. 1969; Smayda 1997). Moreover, some dinoflagellates are known to produce lytic allelopathic metabolites that can negatively influence growth, reproduction, and survival of co-existing organisms (e.g., Granéli et al. 2008; Tillmann and Hansen 2009), which further increases their competitive success under low nutrient conditions. These secondary metabolites may also immobilize or lyse potential prey organisms, which are subsequently caught and ingested (e.g., Blossom et al. 2012). More recent studies using (in situ) metatranscriptomics and metabolic profiles demonstrated the expression of such specific algal traits that may provide a competitive advantage for dinoflagellates in natural blooms, further elucidating the mechanisms that potentially govern diatom and dinoflagellate dominance and regulate bloom development in the natural environment (e.g., Zhang et al. 2019; Metegnier et al. 2020; Yu et al. 2020).

In the past decades, an increasing number of dinoflagellate bloom events has been recorded in California, including blooms of *Alexandrium catenella*, *Akashiwo sanguinea*, *Cochlodinium fulvescens*, *Ceratium* sp., *Dinophysis* sp., and *Lingulodinium polyedra*, depending on different environmental factors (Jester et al. 2009; Lewitus et al. 2012). Species of the genus *Alexandrium* produce saxitoxins, which can cause mass mortality of fish, birds, and marine mammals due to the accumulation of the toxins within the food web (Cembella et al. 2002; Jester et al. 2009; Lefebvre et al. 2016), as well as paralytic shellfish poisoning (PSP) outbreaks in humans through the consumption of contaminated seafood. Within the California Current system, *Al. catenella* is the dominant PSP-toxin producer (Trainer et al. 2010). Several species of this genus including *Al. catenella* also produce allelopathic substances, harming potential competitors, and consumers (Tillmann et al. 2008, 2009; John et al. 2015; Busch 2016). Furthermore, *Alexandrium* spp. have been shown to feed mixotrophically (Jeong et al. 2010) and to be able to store nutrients (Collos et al. 2004), potentially promoting their dominance especially under nutrient-depleted conditions (see earlier).

Dispersal via ocean currents facilitates the propagation of HABs, while local environmental factors determine their competitive success and persistence. Field studies on HABs provide important information on which factors potentially promote bloom formation, propagation, and demise, while lacking causality and capturing biotic interactions within the plankton community. Experimental laboratory studies, on the other hand, may elucidate the relevance of certain environmental factors for particular HAB taxa/species and causal relationships between them, but are often conducted with single species or strains and rarely consider interactions within a more complex community. In natural systems, HABs are transported along various physical and chemical gradients, where plankton community composition and thus competitive interactions are likely to change, which may in turn alter the competitive success of HAB species. However, a deeper understanding of the relationship among dispersal of HAB species, abiotic environmental factors, and biotic interactions along these gradients and how they determine HAB population dynamics remain elusive.

Experimental meta-community and meta-ecosystem setups have widely been used to study the role of dispersal for ecological interactions and spatial dynamics in communities. Meta-communities are defined as sets of local habitats or patches, which are linked via dispersal of potentially interacting species, while meta-ecosystems represent connected ecosystems which are linked by the spatial flow of material, energy as well as the flow of organisms (Loreau et al. 2003; Leibold et al. 2004). Many meta-community studies used patches with discrete boundaries (Logue et al. 2011). In nature, however, environmental factors and biotic interactions mainly change along continuous spatial gradients. Therefore, other studies, for example Gülzow et al. (2019), used linearly interconnected patches to study the effects of gradually distributed nutrients on marine phytoplankton communities. Meta-community or meta-ecosystem setups have become an invaluable tool in ecology to study the interplay of dispersal and species interactions on local and regional scales; however, such approaches have, to our knowledge, hitherto not been used to investigate spatial dynamics of HABs.

In this study, we investigated spatial dynamics and ecological interactions of a Californian strain of the harmful dinoflagellate species *Al. catenella* along a gradient of dissolved inorganic nutrients. We inoculated an artificial phytoplankton community representing four potentially co-occurring species of the Southern California Bight into linearly connected meta-ecosystems. These systems were either set up with a nutrient gradient or with constant nutrient conditions. After an initial establishment phase of the phytoplankton, *Alexandrium* was introduced into different positions of the meta-ecosystem to investigate its invasion

success and propagation patterns along the different nutrient regimes in a community context. Dispersal among the flasks allowed *Alexandrium* to invade all patches. In addition, controls without *Alexandrium* (with and without dispersal) and without dispersal (with and without *Alexandrium*) were established to elucidate interactive effects of dispersal and nutrient regime on *Alexandrium* dynamics.

We tested multiple hypotheses regarding both community and *Alexandrium* population dynamics at experimentally simulated local and regional scales. Our first hypothesis (H1) was that at a local scale, total algal biovolume will increase and evenness will decrease along the gradient of increasing nutrient concentrations, while equal biovolume and evenness will be found in meta-ecosystem patches without nutrient gradient. We would also predict (H2) that (a) *Alexandrium* biovolume contribution will be higher in close proximity to its inoculation position, (b) will increase with decreasing nutrient concentrations, especially when inoculated there, and (c) will decrease with dispersal of competitors and nutrients into patches with low nutrient concentrations. As for regional processes, we hypothesized (H3) that there would be no difference in total biovolume with or without a nutrient gradient, as the total nutrient amount in meta-ecosystems is the same, while evenness would be higher in meta-ecosystems containing a nutrient gradient, as increased heterogeneity of resources increases potential resource niches and thus species coexistence. We would also predict on a regional scale (H4) that (a) the inoculation position will affect *Alexandrium* biovolume only in meta-ecosystems containing a nutrient gradient, increasing its total biovolume contribution when inoculated under low nutrient concentrations, and thus (b) resulting in higher regional biovolume contributions in meta-ecosystems containing a nutrient gradient compared to homogeneous nutrient conditions. Finally, we hypothesized that (H4c) dispersal of dominant competitors and nutrients will decrease *Alexandrium* biovolume contribution also at the regional scale.

Materials

Meta-ecosystem design

A linear meta-ecosystem consisting of five interconnected flasks was established to investigate trophic interactions and propagation via dispersal of *Al. catenella* along a nutrient gradient in comparison to a system with constant nutrient conditions. For this system, 50-mL Erlenmeyer flasks (DURAN), which were customized with glassy tube attachments on two sides, were connected with 6-cm-long silicon tubes (5 mm Ø, TYGON). The connections between the flasks were kept closed with locking clips (Bevara, IKEA), while dispersal was allowed by opening those clips for 2 min daily. All meta-ecosystem sets were placed and fixed randomly on a shaking table (Laboshake, Gerhardt) under constant light (80 μE m^{-2} s^{-1}) and temperature (18°C) conditions in a climate chamber with a 12 h:12 h day : night rhythm. Different dispersal times combined with different shaking speeds were tested prior to the experiment. A dispersal time of 2 min per day at 80 rpm was chosen to ensure dispersal of all species, also of nonmotile species like diatoms, while maintaining the nutrient gradient over time. Controls without dispersal were also set up to investigate dispersal effects on community dynamics (with nutrient gradient only, as the closed intermediate position represented the constant nutrient controls). Here, the clips remained closed throughout the entire experiment. Other controls included meta-ecosystems without *Alexandrium* (with and without dispersal, see later), resulting in a total of 36 meta-ecosystems (24 in the first run, 12 in the second run) with 5 interconnected flasks each (see later).

Algae cultivation and medium preparation

All taxa used in this experiment were isolated from the coast of Southern California (Research Group of Prof. David A. Caron, University of Southern California, Los Angeles). Species from three different taxonomic groups were selected, differing in size, motility, and nutrient requirements (Table 1), including the diatoms *Thalassiosira* sp. and *Leptocylindrus* sp., the cryptophyte *Rhodomonas abbreviata*, the dinoflagellates *Prorocentrum micans* (considered nonharmful), and *Al. catenella* (considered harmful). The *Alexandrium* strain used in our study was classified as *Al. catenella* (group I, Garneau et al. 2011). The taxonomic identity of this species has been debated recently (John et al. 2014*a*,*b*; Fraga et al. 2015; Prud'homme van Reine 2017), and we use the currently accepted taxonomy of *Al. catenella* in this study.

Species biovolume (μm^3) was used as a proxy for biomass. Individual cell volumes were determined microscopically (Zeiss Axiophot) by measuring the lengths and widths of

Table 1. List of algal species included in the meta-ecosystems

Species	Taxonomic group	Individual biovolume (μm^3 per cell)	Grouped as
Alexandrium catenella	Dinophyceae	11,388	Harmful algae
Prorocentrum micans	Dinophyceae	9895	Community species
Rhodomonas abbreviata	Cryptophyceae	274	Community species
Thalassiosira sp.	Bacillariaphyeae	22,313	Community species
Leptocylindrus sp.	Bacillariaphyeae	1069	Community species

30 randomly chosen individuals for each species and calculating their biovolume according to specific geometrical shapes (Hillebrand et al. 1999). Prior to the setup of the experiment, initial cell concentrations (cells mL^{-1}) and accordingly biovolume (μm^3 mL^{-1}) of all stock cultures were determined. Stock cultures of all phytoplankton species were maintained in f/2 medium (Guillard and Ryther 1962; Guillard 1975) that was prepared from 0.2 μm filtered and autoclaved North Sea water, which was taken from the Jade Bay (Wilhelmshaven, Germany). Cultures were nonaxenic and kept in culture flasks (TC-Flasks T75, Sarstedt) in a constant environment of 18°C and a light intensity of 80 $\mu mol\ m^{-2}\ s^{-1}$ in a 12 : 12 h light : dark regime.

Prior to the experiment, five different culture media were prepared using sterilized North Sea water. Vitamins and trace metals were added according to the f/2 medium (Guillard and Ryther 1962; Guillard 1975). Nutrient compositions were adjusted to the Redfield-Brzezinski ratio (Brzezinski 1985) with concentrations increasing evenly from medium one (M1) to medium five (M5; Table 2). Seven days prior to the experiment, cultures were preincubated at intermediate nutrient concentrations (medium M3, Table 2) in order to prevent additional nutrient input to the experiment when adding the cultures, but at the same time allow algal growth within the preincubation time.

Experimental setup and sample analysis

The meta-ecosystems were divided into two sets: the nutrient gradient treatment ("NUTgrad": M1–M5, Table 2) and the constant nutrient treatment ("NUTconst": M3, Table 2). NUTgrad treatments were filled from flask 1 to flask 5 with medium M1–M5 (Table 2), respectively, with increasing nutrient concentrations. NUTconst treatments were filled with M3 only, which equals the intermediate medium of the gradient treatments. Thus, the NUTgrad and the NUTconst meta-ecosystems contained the same total amount of nutrients on a regional scale (across all five flasks) and only differed in the distribution of nutrients along the meta-ecosystem.

All flasks of both sets were inoculated with a phytoplankton community consisting of *Rhodomonas*, *Prorocentrum*, *Thalassiosira*, and *Leptocylindrus* (Table 1) with each alga initially contributing equal biovolume to the community to compensate for cell size variability (evenness = 1). A total algal biovolume of 4x106 µm3 ml^{-1} was added to each flask, inoculum and medium summing up to a total volume of 55ml per flask. Due to the complex meta-ecosystem setup entailing 36 sets of 5 interconnected flasks (see later) placed on shaking tables, we had to limit our experimental volume as a compromise between setting up as many experimental units at the same time as possible in order to avoid too many sequential experimental runs over time, and an appropriate experimental volume. Comparably small volumes are commonly used for such experiments studying dynamics in artificial phytoplankton communities (e.g., Gülzow et al. 2019) and all of the species used in our study grew well in this volume. Hammes et al. (2010) found no evidence of a volumetric bottle effect on microbial batch growth (volumes ranging from 20 to 1000 mL), and we are therefore confident that such bottle effects can be neglected in our study as well.

All flasks of all meta-ecosystems were sampled every 3rd day by removing 15% of the total volume (8.25 mL) of each flask. Using a semicontinuous culture method, these 15% of the volume were replaced with the respective medium (M1–M5, Table 2) to retain respective nutrient conditions. Locking clips were kept closed during the samplings to avoid mixing due to differences in the filling level.

After an establishment phase of 9 d to allow phytoplankton communities to develop according to different nutrient regimes, four treatments were set up, differing in the inoculation position of *Alexandrium*. The harmful dinoflagellate was either added into position "1" (low nutrient concentration, medium M1: NUTgrad1, NUTconst1), position "3" (intermediate nutrient concentration, medium M3: NUTgrad3, NUTconst3), position "5" (high nutrient concentration, medium M5: NUTgrad5, NUTconst5) or into all positions ("all," NUTgradAll, NUTconstAll), resulting in a 4×2 factorial design, with three replicates for each combination at the meta-ecosystem level (24 meta-ecosystems, 120 units total; Fig. 1). Irrespective of experimental treatment, *Alexandrium* was inoculated with a total biovolume of 1×10^6 μm^3 mL^{-1} in each meta-ecosystem, which equaled the initial biovolume the other species were set up with. This biovolume was either added to only one flask of the meta-ecosystem (treatments NUTgrad1, NUTconst1, NUTgrad3, NUTconst3, NUTgrad5, NUTconst5) or divided between the five flasks of the meta-ecosystems (treatments NUTgradAll, NUTconstAll), resulting in equal regional biovolume of *Alexandrium* (Fig. 1). The refilling volume of fresh medium was adjusted at day 9, when *Alexandrium* was added to the flasks to avoid exceeding the total volume of 55 mL (4.88 mL *Alexandrium* culture and 3.37 mL medium were added to the respective inoculation positions; only the NUTgradAll and NUTconstAll treatments received 0.976 mL of *Alexandrium* culture and 7.274 mL medium).

Table 2. Initial nutrient concentrations of all experimental media.

Medium	*N* ($\mu mol\ L^{-1}$)	Si ($\mu mol\ L^{-1}$)	P ($\mu mol\ L^{-1}$)
M1	13.44	12.60	0.84
M2	50.08	46.95	3.13
M3	86.72	81.30	5.42
M4	123.36	115.65	7.71
M5	160.0	150.0	10.0

N, total nitrogen, including ammonia, nitrate and nitrite; P, phosphate; Si, silicate.

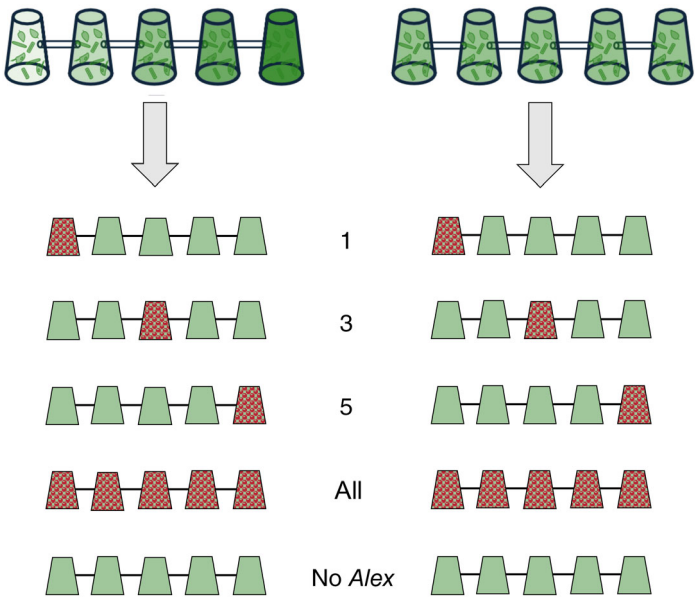

Fig. 1. Setup of the experiment: nutrient gradient on the left, constant nutrient conditions on the right; red flasks represent the different inoculation positions of *Alexandrium* ("1", "3", "5," and "all"), "no *Alex*" represents the control treatment without the addition of *Alexandrium*.

In this first experimental set, dispersal was allowed in all treatments by opening all connections between the flasks of a meta-ecosystem for 2 min d^{-1} (see earlier). An additional set of control treatments was established to investigate the effect of *Alexandrium* (*Alexandrium* presence vs. absence in the community) and dispersal (dispersal vs. no dispersal) within the system, using the same protocol as described before. Due to constraints regarding space and handling, not all experimental meta-ecosystems could be run at the same time. Therefore, this second experimental set was conducted 6 months after the initial experiment. For the NUTgrad conditions, controls were set up in triplicate without dispersal with and without the addition of *Alexandrium*, and with dispersal, but without the addition of *Alexandrium*. In the treatment without dispersal, containing *Alexandrium*, only the "All" treatment was set up, adding equal biovolume of the species to all flasks (corresponding to the biovolume that was added to the single flasks to positions 1, 3, and 5 in the first part of the experiment), as without dispersal, the other treatments where *Alexandrium* was added only to particular flasks were redundant. For the NUTconst conditions, also a control with dispersal, but without the addition of *Alexandrium* was set up. However, no additional control without dispersal was established, as the intermediate nutrient treatments (M3) that were set up without dispersal with and without *Alexandrium* for the NUTgrad conditions were the same as they would have been in all flasks of the NUTconst conditions and therefore served as control. In total, 12 meta-ecosystems with 5 flasks each were set up for the second experimental run, resulting in 60 individual flasks. All treatments and controls of both experimental sets ran for a total duration of 33 d, as after an initial increase, total algal biovolume steadily decreased, reaching almost initial inoculation concentrations by day 33 of the experiment (Supporting Information Fig. S15).

Of the removed samples (15%, 8.25 mL every 3rd day, see earlier), 3.25 mL was used to measure in vivo Chlorophyll *a* (Chl *a*) with a Fluorometer (TURNER DESIGNS, AquaFluorTM). Subsequently, the same samples were preserved with 10% Lugols iodine solution in amber glass bottles for microscopic cell counting. Samples were also taken for the analysis of dissolved inorganic nutrients. However, due to technical issues, unfortunately, all dissolved nutrient samples of this experiment had to be discarded.

Depending on the total cell concentration of each flask, subsamples ranging from 0.5 to 3 mL were used to determine algal biovolume and community composition. Subsamples were counted in Utermöhl sedimentation chambers (Utermöhl 1958) under an inverted microscope (DM IL LED, Leica) at ×100 magnification. A total of 300–400 cells were counted per sample in a minimum of 10 randomly chosen grids.

Statistical analyses

All statistical analyses and graphs were generated using R version 3.4.3 (R Development Core Team 2017) and the following packages: vegan, ggplot2, lme4, grid, plyr, reshape, lattice, pbkrtest, boot, MuMIn, and car. Data were analyzed for the local patch level as well as for the entire meta-ecosystem, that is, across all five patches (regional scale, average of each meta-ecosystem) at three different time points of the experiment. We chose to analyze particular time points rather than analyzing the whole course of the experiment (including time as a factor), as we observed transient dynamics over the course of the experiment driven by algal-nutrient interactions (see later). These time points included (1) the point just before *Alexandrium* was introduced into the system (day 9) to characterize the differently evolved phytoplankton communities to which *Alexandrium* was added; (2) an intermediate sampling date, at which *Alexandrium* had the strongest impact, that is, showed the highest relative contribution to the phytoplankton community in the course of the experiment (day 18); and (3) the last sampling day of the experiment when algal biomass had decreased to initial levels again (day 33).

On the local scale, the effects of nutrient conditions (position along the meta-ecosystem) and distance to the inoculation position on total algal biovolume, evenness, and the biovolume contribution of *Alexandrium* were tested using a linear mixed model ("lme4" package) for all three time points. Confidence intervals (95%) for model coefficients were calculated using a bootstrapping procedure ("boot" package). The model included the experimental unit (the meta-ecosystems consisting of connected flasks) as a random factor to account for dependencies between the flasks via dispersal. To analyze the effects of the inoculation position on the different response variables, we calculated the distance to the initial

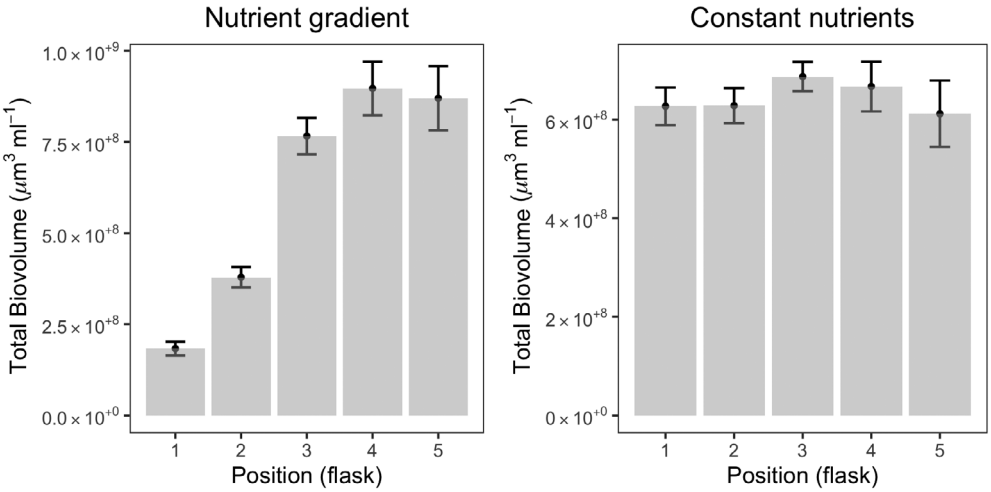

Fig. 2. Local total biovolume (mean ± standard error) at day 9 along the different flasks of the meta-ecosystems with and without a nutrient gradient directly before *Alexandrium* was introduced (note that $n = 12$, as 12 sets of meta-ecosystems were set up for each nutrient treatment [NUTgrad vs. NUTconst] to ensure three replicates for each of the subsequent *Alexandrium* inoculation treatments ["1", "3," "5," "all"]).

inoculation position for each patch and used this distance as a continuous variable in the model. Analyses were conducted separately for the controls as well as for the different sets of meta-ecosystems with and without a nutrient gradient (NUTgrad and NUTconst), since we tested the effect of the different positions in the model, which represents the nutrient gradient for NUTgrad, but constant nutrient conditions for NUTconst. In an additional analysis, we combined data from both parts of the experiment in order to test for the effect of dispersal on the local biovolume contribution of *Alexandrium*. Data were transformed if residuals were distributed heterogeneously. Marginal and conditional r^2 values were calculated according to Nakagawa and Schielzeth (2013) ("MuMIn" package) in order to assess the model fit. The bootstrapped confidence intervals indicate that model coefficients significantly differ from zero at a significance level of $\alpha = 0.05$ if their upper and lower values do not include zero. Controls without dispersal were not connected and therefore functioned as independent units. They were tested in a one-way ANOVA with the position as the explanatory variable and the same response variables as in the linear mixed model.

In order to test the effects of inoculation position and nutrient conditions (gradient NUTgrad, constant NUTconst) at the regional level on total algal biovolume, evenness, and on the percentage of *Alexandrium*, two-way ANOVAs were conducted for the three time points and for the controls separately. A one-way ANOVA was conducted for the analysis of day 9, where only the effect of nutrient conditions was tested, since the factor "inoculation position" did not yet play a role. In case of significant ANOVA results, we conducted post hoc tests in order to determine differences in effect size across treatment levels using the Tukey's "honest significant difference" test. Data were transformed if homogeneity of variances and/or Gaussian distribution were not given. For data transformations, we applied the maximum-likelihood approach of Box and Cox (1964) to select the most suitable transformation exponent using the R function powerTransform(). The regional analyses of the controls without *Alexandrium* were conducted with a nonparametric Kruskal–Wallis analysis since homogeneity of variances was not given for any of the time points.

Results

Local dynamics

Within the nutrient gradient (NUTgrad) treatments, the flask position, that is, the nutrient concentration level, influenced all response variables at all time points analyzed. Total algal biovolume significantly increased with increasing nutrient concentrations in the meta-ecosystems subject to the nutrient gradient with and without dispersal (Fig. 2; Table 3a; Supporting Information Tables S1, S2 and Figs. S3, S8, S10), supporting the first part of hypothesis H1 stating a biovolume increase along the gradient of increasing nutrients. This pattern also supported the effectiveness of our nutrient gradient until the end of the experiment, which could unfortunately not be substantiated by nutrient data (see earlier). When dispersal was allowed, not only nutrients dispersed, but also all species propagated through the system and potentially reinvaded communities. Therefore, communities without dispersal were even more distinct regarding biovolume and species composition compared to systems allowing for dispersal (Figs. 3, 4, 5; Supporting Information Figs. S5, S9, S11). This effect was particularly visible between the controls without *Alexandrium* (Supporting Information Figs. S5, S9, S11a,c).

Concordant with the second part of hypothesis H1 regarding decreasing evenness along the nutrient gradient, local evenness indeed decreased with increasing nutrient availability in the NUTgrad treatment (Table 3a). After inoculation of

Table 3. Linear mixed model results of local analyses of total biovolume, evenness and percentage of *Alexandrium* of days 9, 18, and 33. The factor "distance" is a measure for the distance to the inoculation patch of *Alexandrium*. (**a**) Model estimates, 95% confidence intervals and effect sizes for the nutrient gradient treatments (NUTgrad), where nutrient conditions differed between patches (factor "Position"). (**b**) Model estimates, 95% confidence intervals and effect sizes for the constant nutrient treatments (NUTconst), where nutrient conditions were equal in all patches (factor "Position"). Confidence intervals indicating model estimates significantly different from zero are represented in bold.

(a) Response	Day	Transformation	Coefficient	Estimate	Confidence interval		R^2 marginal	R^2 conditional
Biovolume	9	None	Position	15,393,427	**12,855,272**	**17,890,230**	0.665	0.745
	18	Log	Distance	−0.059	−0.166	0.043	0.463	0.766
			Position	0.445	**0.356**	**0.527**		
	33	Log	Distance	0.103	**0.009**	**0.198**	0.680	0.827
			Position	0.559	**0.485**	**0.635**		
Evenness	9	Log	Position	−0.301	**−0.364**	**−0.238**	0.529	0.648
	18	None	Distance	0.027	−0.004	0.057	0.494	0.676
			Position	−0.117	**−0.144**	**−0.092**		
	33	None	Distance	−0.045	**−0.075**	**−0.018**	0.280	0.556
			Position	−0.057	**−0.079**	**−0.033**		
Percentage of *Alexandrium*	18	Log	Distance	−0.379	**−0.552**	**−0.203**	0.495	0.612
			Position	−0.529	**−0.671**	**−0.382**		
	33	Log	Distance	−0.352	**−0.488**	**−0.232**	0.544	0.767
			Position	−0.514	**−0.621**	**−0.416**		

(b) Response	Day	Transformation	Coefficient	Estimate	Confidence interval		R^2 marginal	R^2 conditional
Biovolume	9	None	Position	71,394	−1,910,193	2,141,408	0.000	0.026
	18	None	Distance	1,751,472	−7174	3,653,221	0.072	0.342
			Position	1,017,880	−2,575,694	453,240		
	33	None	Distance	1,131,367	**901,784**	**1,367,013**	0.680	0.827
			Position	170,370	−12,945	358,801		
Evenness	9	None	Position	0.000	−0.009	0.010	0.000	0.086
	18	None	Distance	−0.020	**−0.037**	**−0.002**	0.077	0.365
			Position	0.005	−0.009	0.020		
	33	None	Distance	−0.073	**−0.094**	**−0.053**	0.327	0.767
			Position	−0.003	−0.018	0.012		
Percentage of *Alexandrium*	18	Log + 1	Distance	−0.307	**−0.385**	**−0.238**	0.548	0.642
			Position	0.044	−0.018	0.103		
	33	None	Distance	−1.329	**−1.745**	**−0.895**	0.335	0.638
			Position	−0.073	−0.384	0.256		

all phytoplankton species with equal biovolume (evenness = 1), local evenness decreased within the first days of the experiment in all treatments. This decrease was accompanied by an overall strong increase in total biovolume, which was largely influenced by the rapid growth of the diatom *Thalassiosira* (Figs. 3, 4, 5). It became the dominant species in almost all flasks, especially under high nutrient conditions, and therefore strongly determined local Chl *a* and total biovolume patterns (Supporting Information Figs. S1, S2).

For meta-ecosystems with constant nutrient conditions (NUTconst), neither algal biovolume nor evenness differed among different flask positions, as expected from Hypothesis H1.

After the introduction of *Alexandrium* into different positions of the meta-ecosystems, the relative biovolume of *Alexandrium* always remained significantly higher closer to the initial inoculation position across the nutrient treatments and all different inoculation positions (Table 3), as stated in hypothesis H2 (a). Furthermore, a significant positive relationship between the distance of the inoculation patch of *Alexandrium* and the total algal biovolume was found for both the NUTconst and the NUTgrad treatments, that is, total algal biovolume was lower in flasks closer to the inoculation patch of the harmful dinoflagellate. However, this relationship was only significant on day 33 (Table 3; Supporting Information Figs. S8, S10). The NUTconst treatment allowed to observe direct effects of

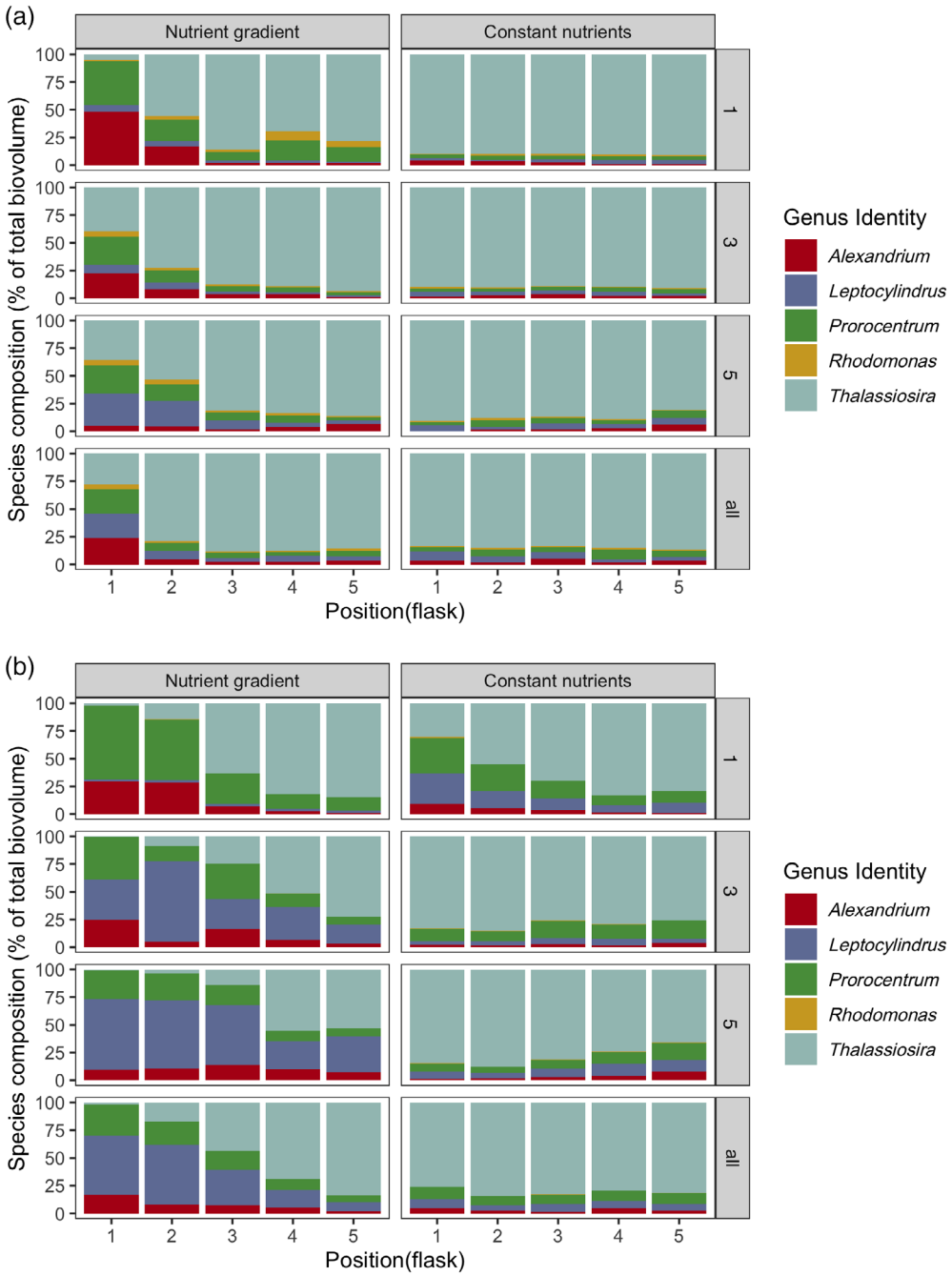

Fig. 3. Local species composition (as percentage of the total biovolume) at day 18 (**a**) and day 33 (**b**). Results of the different *Alexandrium* inoculation positions (1, 3, 5, all) are divided into rows, different nutrient treatments into columns (left—NUTgrad, right—NUTconst). Within the nutrient gradient (NUTgrad) treatments (left), position 1 represents the lowest, position 5 the highest nutrient concentration.

Alexandrium on other phytoplankton species without the interaction of altered nutrient supply in different flasks (NUTgrad). Different species responded differently to the introduction of *Alexandrium* in the NUTconst treatments. While *Prorocentrum* biovolume did not differ between local flasks, *Rhodomonas* showed the strongest reaction to the *Alexandrium* introduction and strongly decreased in high *Alexandrium* biomass patches. Both diatom species showed a small decrease in biovolume in those patches where *Alexandrium* was introduced (Fig. 3), resulting in a significant increase in evenness closer to the inoculation position of *Alexandrium* (for days 18 and 33 in the NUTconst treatment, and day 33 in the NUTgrad treatment; Table 3), whereas evenness remained much lower in controls without *Alexandrium* addition (Fig. 4a,b).

As stated in hypothesis H2 (b), the relative biovolume contribution of *Alexandrium* significantly increased with decreasing

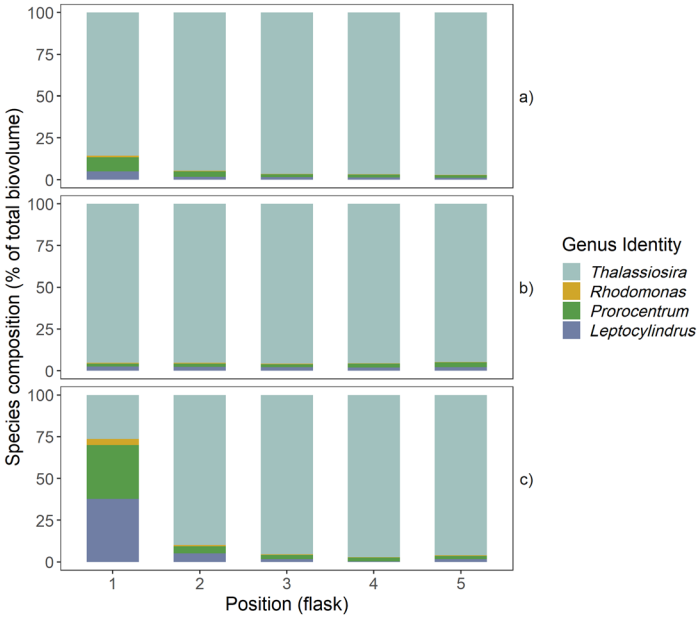

Fig. 4. Local species composition (as percentage of the total biovolume) at day 18 of the control without *Alexandrium* addition. (**a**) Control with dispersal for the NUTgrad treatment, where position 1 represents the lowest, position 5 the highest nutrient concentration. (**b**) Control with dispersal for the NUTconst treatment, where all positions represent equal nutrient concentrations. (**c**) Control without dispersal. Position 1 represents the lowest, position 5 the highest nutrient concentration. Position 3 further represents the control for the NUTconst treatments.

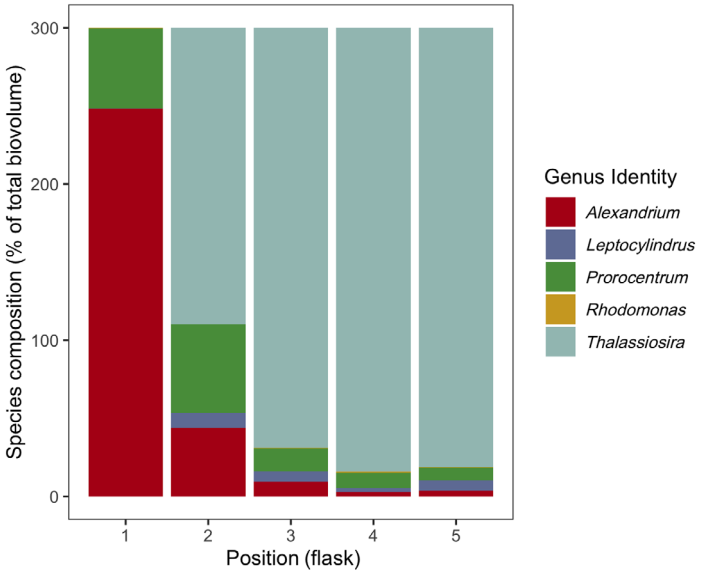

Fig. 5. Local species composition (as percentage of the total biovolume) at day 18 of the control without dispersal. Position 1 represents the lowest and position 5 represents the highest nutrient concentration. Position 3 further represents the control for the NUTconst treatments.

nutrient concentrations within the NUTgrad treatments at days 18 and 33 (Table 3a; Figs. 3, 5), supporting this hypothesis. The relative biovolume increase of *Alexandrium* was mainly driven by an absolute biovolume decrease of the dominant diatom *Thalassiosira* (Supporting Information Fig. S1a). The highest relative biovolume of *Alexandrium* in treatments with dispersal reached almost 50% of the total algal biovolume at day 18 in the lowest nutrient concentration of the NUTgrad treatment, where *Alexandrium* was initially inoculated (inoculation position 1; Fig. 3a), corresponding to cell concentrations of ~ 619 cells mL^{-1}. In the NUTgrad treatments without dispersal, *Alexandrium* became even more dominant at the lowest nutrient concentration, making up more than 75% of the total algal biovolume, which corresponded to cell concentrations of ~ 712 cell mL^{-1} (Fig. 5; Supporting Information Fig. S2). Supporting hypothesis H2 (c), stating that dispersal decreases *Alexandrium* dominance, the dinoflagellate contributed significantly lower portions to total algal biovolume when dispersal was allowed compared to the same flask position without dispersal (Figs. 3, 5; Table 4). While *Alexandrium* mostly increased evenness through the reduction of the great dominance of *Thalassiosira*, it decreased evenness in this patch under the lowest nutrient conditions by outcompeting *Thalassiosira*. In addition, also *Leptocylindrus* and *Rhodomonas* decreased to extremely low biovolume. In the control without dispersal and without *Alexandrium*, the other dinoflagellate, *Prorocentrum* dominated the community together with the diatom *Leptocylindrus* (Fig. 4c), resulting in increased evenness compared to the controls without *Alexandrium* with dispersal (Fig. 4a,b).

Regional dynamics

Treatment effects concerning single flasks (such as increasing nutrient concentrations in the NUTgrad treatment) collapse at a regional scale, where average biovolume across all five flasks of a meta-ecosystem is considered. Therefore, only the treatment effects of "nutrient gradient" (NUTgrad vs. NUTconst) and "*Alexandrium* inoculation position," as well as the control treatments (with/without dispersal, with/without *Alexandrium*) could be analyzed at the regional scale.

After experimental setup, total algal biovolume increased in all treatments up to day 6, after which both algal biovolume and accordingly Chl *a* concentration slowly decreased (Supporting Information Fig. S15). According to hypothesis H3, we expected total algal biovolume to be equal at the regional scale irrespective of constant or gradient conditions along the five flasks of the meta-ecosystem. This was confirmed in the controls without *Alexandrium*, where total algal biovolume showed no significant differences between the NUTgrad and the NUTconst treatment. For treatments with *Alexandrium*, total algal biovolume was significantly higher in the NUTconst treatments compared to the NUTgrad treatment at day 18 ($p < 0.01$, Table 5), while no significant difference could be detected by the end of the experiment anymore (Table 5; Supporting Information Fig. S15).

The overall high local impact of *Thalassiosira* was also reflected in regional dynamics, showing a strong dominance of

Table 4. Linear mixed model results of local analyses on the effect of dispersal on relative biovolume contribution of *Alexandrium catenella*. Confidence intervals indicating model estimates significantly different from zero are represented in bold.

Response	Transformation	Coefficient	Estimate	Confidence interval		R^2 marginal	R^2 conditional
Percentage of *Alexandrium*	Log 10	Dispersal yes (D)	−0.946	**−1.500**	**−0.343**	0.527	0.571
		Position (P)	−0.510	**−0.647**	**−0.357**		
		D * P	0.280	**0.114**	**0.434**		

Table 5. ANOVA results for days 9, 18, and 33 for regional total biovolume, evenness, and percentage of *Alexandrium*. Lambda represents the exponent used in the Box–Cox transformation. Significant *p*-values ($p \leq 0.05$) are marked in bold.

Response	Day	Transformation (lambda)	Coefficient	*dfN*	*F*	*p*
Biovolume	9	0.61	Gradient (G)	1	1.960	0.175
			Residuals	22		
	18	0.9	Inoculation position (IP)	3	0.584	0.634
			Gradient (G)	1	9.782	**0.006**
			IP * G	3	0.455	0.718
			Residuals	16		
	33	0.58	Inoculation position (IP)	3	0.539	0.662
			Gradient (G)	1	2.503	0.133
			IP * G	3	0.082	0.969
			Residuals	16		
Evenness	9	−0.88	Gradient (G)	1	0.971	0.335
			Residuals	22		
	18	−0.52	Inoculation position (IP)	3	1.529	0.245
			Gradient (G)	1	4.807	**0.044**
			IP * G	3	0.197	0.897
			Residuals	16		
	33	−0.34	Inoculation position (IP)	3	0.251	0.859
			Gradient (G)	1	7.172	**0.017**
			IP * G	3	2.208	0.127
			Residuals	16		
Percentage of *Alexandrium*	18	−0.88	Inoculation position (IP)	3	0.298	0.826
			Gradient (G)	1	8.798	**0.009**
			IP * G	3	0.578	0.638
			Residuals	16		
	33	−1.33	Inoculation position (IP)	3	0.362	0.781
			Gradient (G)	1	7.039	**0.017**
			IP * G	3	0.241	0.867
			Residuals	16		

this diatom across the meta-ecosystems throughout the entire experiment (Fig. 6). As a result, regional evenness was generally very low, but increased toward the end of the experiment (Supporting Information Fig. S16). We expected regional evenness to be higher in NUTgrad meta-ecosystems (Hypothesis H3), as increased heterogeneity of resources increases niche availability, thus promoting species coexistence. Supporting this part of hypothesis H3, regional evenness was indeed significantly higher in the NUTgrad meta-ecosystems compared to the NUTconst meta-ecosystems at days 18 and 33 in treatments including *Alexandrium* ($p < 0.05$, Table 5; Supporting Information Fig. S16). In the controls with dispersal, but without *Alexandrium*, evenness also tended to be higher in the NUTgrad meta-ecosystems compared to the NUTconst meta-ecosystems at day 33 (Supporting Information Fig. S17); however, this difference was not significant.

Neither the inoculation position nor the interaction between inoculation position and nutrient gradient significantly affected *Alexandrium* contribution ($p > 0.05$, Table 5), refuting hypothesis H4 (a). According to hypothesis H4 (b), *Alexandrium*

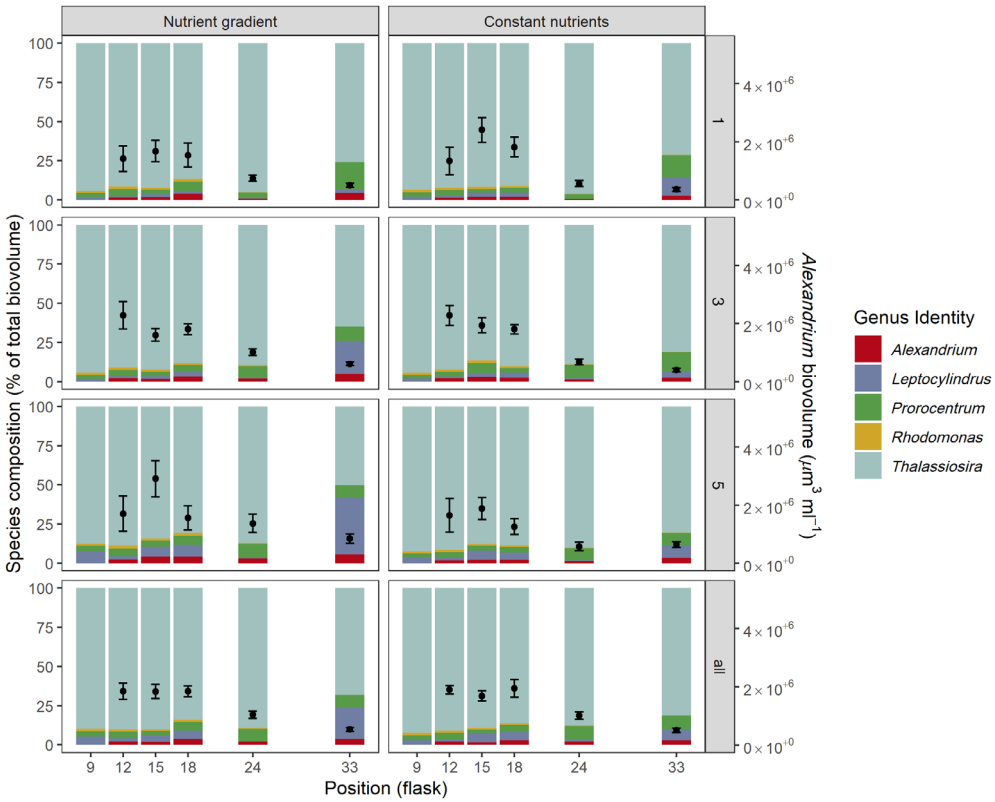

Fig. 6. Regional species composition (as percentage of the total biovolume) and average biovolume of *Alexandrium* (μm^3 mL^{-1}, mean ± standard error) over time. Results of the different *Alexandrium* inoculation positions (1, 3, 5, all) are divided into rows, different nutrient treatments into columns (left—NUTgrad, right—NUTconst).

contributions were expected to be higher in the NUTgrad treatments compared to the NUTconst treatments, as low nutrient concentrations were expected to promote this dinoflagellate, especially when initiated under these conditions. This hypothesis could be supported, as the relative regional biovolume of *Alexandrium* was higher in the NUTgrad treatments at days 18 and 33 compared to the NUTconst treatments ($p < 0.05$, Table 5). This effect was even stronger in the control treatments without dispersal, supporting hypothesis H4 (c) (data not shown).

While relative biovolume of the dinoflagellate was overall very low and did not change much over time on the regional scale, total biovolume of *Alexandrium* showed more variation. It slightly increased after inoculation at day 9, when inoculated into positions 1 and 5, reaching the highest biovolume at day 15 (Fig. 6). When *Alexandrium* was inoculated into position 3 and into all positions at the same time, neither treatment (NUTgrad, NUTconst) showed an increase in *Alexandrium* biovolume (Fig. 6).

Discussion

Overall, our meta-ecosystem experiment revealed complex regional and local distribution patterns of the harmful dinoflagellate *Al. catenella*. *Alexandrium* was able to disperse quickly between all connected patches, demonstrating the applicability of this meta-ecosystem setup to investigate spatial dynamics of this HAB species, and showing that the dinoflagellate was able to invade all of the differently shaped phytoplankton communities. Despite partly low concentrations, *Alexandrium* persisted in all flasks until the end of the experiment. Its relative biovolume contributions were strongly determined by its inoculation position, by nutrient conditions as well as by competitive interactions within the phytoplankton community. *Alexandrium* exhibited highest dominance closest to its inoculation position and at low nutrient concentrations after diatom biovolume had reached a maximum and was decreasing again. Dispersal appeared to counteract *Alexandrium* dominance, as its biovolume contribution was even higher without dispersal.

On the local scale, total algal biovolume first strongly increased with increasing nutrient availability, which was mainly driven by diatoms and especially by *Thalassiosira*. Diatoms often have higher maximum uptake rates for nutrients compared to other phytoplankton groups as well as high maximum growth rates, which generally make them good competitors for dissolved nutrients (Litchman et al. 2007). Hence, they often benefit from turbulent waters and upwelling conditions,

where nutrients and especially silicate concentrations are high (Margalef 1978; Smayda 1997; Seubert et al. 2013). Under these conditions, they are therefore likely to become dominant in the phytoplankton community and outcompete other algal groups such as dinoflagellates, which are usually weaker competitors at high nutrient conditions and additionally exhibit lower growth rates than diatoms (Margalef 1978; Smayda 1997). Likewise, in our experiment, the initial rapid growth of *Thalassiosira* was followed by a steady decrease of biovolume when nutrients became deplete. Community structure changed and other phytoplankton species increased in proportion, especially the dinoflagellates *Alexandrium* and *Prorocentrum*, in particular under low nutrient conditions. When dominating the community at day 18 of our experiment (with and without dispersal), absolute *Alexandrium* cell concentrations (600–700 cells mL^{-1}) were in the upper range of those reported for natural bloom concentrations along US coasts (Garneau et al. 2011 and references therein).

Overall, *Thalassiosira* dominated the community at higher nutrient concentrations, whereas *Alexandrium* was more dominant at low nutrient concentration, which is in accordance with previous studies (e.g., Anderson et al. 2012). In a nitrogen-depleted environment, *Alexandrium* potentially has an advantage over other phytoplankton species due to its capability to accumulate and store nitrogen (Collos et al. 2004). In addition, its ability to produce allelopathic substances and to feed mixotrophically on other phytoplankton and bacteria in our non-axenic communities might have been beneficial under low nutrient conditions, thus promoting the dinoflagellate. In a preliminary experiment, the *Alexandrium* strain used in our study ingested cells of *Rhodomonas* (strain used in our study) and proved to have allelopathic effects on several microalgae, including *Tetraselmis* sp., and different *Rhodomonas* strains, including the one used in our experiment (Busch 2016, data not shown for the latter). Therefore, the observed decrease in *Rhodomonas* biovolume when *Alexandrium* contribution was high in our experiment could have been caused by either mixotrophic feeding or by the production of harmful allelopathic substances. Preliminary experiments further revealed that *Alexandrium* was not able to feed on the diatoms used in our study (data not shown). However, both diatom species decreased in patches where *Alexandrium* was added. Comparing the different inoculation positions after *Alexandrium* introduction with the *Alexandrium* free control showed a clear decrease in diatom dominance, and this effect was stronger under low nutrient conditions. This might be an indication for an allelopathic effect of *Alexandrium* also on the diatoms. Lytic effects of *Alexandrium* spp. on other phytoplankton species are highly variable, even among strains of the same species (Tillmann et al. 2009; Blossom et al. 2012). For the same strain used in our study, Busch (2016) found an effect concentration (EC50) of 566 cells mL^{-1} for the target cryptophyte *Rhodomonas salina*, indicating 50% of cell lysis in that *Alexandrium* concentration. Tillmann et al. (2009) investigated the intraspecific variability of lytic activity in different *Alexandrium tamarense* strains, using the same target species as Busch (2016). They found a large variability of EC50 concentrations among different *Alexandrium* strains, suggesting an intermediate lytic capacity of the *Al. catenella* strain used in our study. Despite these considerations, however, in our experiment it was unfortunately not possible to disentangle the specific mechanisms that actually determined the observed patterns in the mixed algal community (competition for nutrients, allelopathic effects, mixotrophy).

While *Alexandrium* biovolume slightly increased at the beginning, it decreased toward the end of the experiment. The dinoflagellate's relative contribution over time, however, increased much stronger than its absolute biovolume, as its contributions to total phytoplankton biomass highly depended on growth and proportion of the diatom *Thalassiosira*. These results are in agreement with observed spatial and temporal patterns in the field. Anderson et al. (2008) showed that in the California Bight (Santa Barbara channel) diatoms dominated the community in seasonally stratified and nutrient-rich surface water, especially after upwelling events. After nutrient depletion and stabilization of the water column, dinoflagellates became the dominant group in the community (Anderson et al. 2008). Such temporal successions from diatoms to dinoflagellates, which are tightly coupled to hydrography and nutrient dynamics of the system, are a common phenomenon also observed in other habitats like, for example, the Bornholm Basin (van Beusekom et al. 2009) or Georges Bank (Gettings et al. 2014). The coupling between nutrient conditions and phytoplankton community structure can, however, also be found along spatial nutrient gradients. For instance, Mercado et al. (2014) found a strong decreasing gradient of nutrients and chlorophyll from the coast to offshore in the Northwestern Alboran Sea (Western Mediterranean Sea). In this area, coastal surface waters were strongly dominated by (< 50 μm) diatoms, whereas offshore communities were dominated by dinoflagellates, which were mainly caused by a decrease in diatom biomass (Mercado et al. 2014).

Dispersal was also an important factor shaping phytoplankton community structure in our study. While dispersal ensured propagation of all species and therefore maintained species diversity in all flasks, it weakened the dominance of *Alexandrium*. In controls without dispersal, the dinoflagellate contributed higher portions to total algal biovolume at low nutrient concentrations compared to treatments where dispersal was possible. This was likely due to the fact that not only algal species dispersed, but also nutrients were reintroduced from patches with high nutrient concentrations to low nutrient patches, promoting diatom growth. A lack of dispersal, though, led to lower biovolume and finally the extinction of *Thalassiosira*, and also a strong decrease of *Leptocylindrus*, especially at the lowest nutrient concentrations, enabling *Alexandrium* to become dominant. It has to be noted, though, that only one dispersal regime was tested in this experiment, and higher or lower dispersal rates may have resulted in a different outcome.

Although *Alexandrium* dispersed into all flasks within the first few days of the experiment, irrespective of inoculation position, its relative abundance was always higher closer to its own inoculation patch throughout the entire experiment. This indicates that the point of initiation or introduction of a HAB species into a local habitat plays an important role in determining its dominance in addition to local environmental constraints. In natural environments, hydrodynamics including horizontal ocean currents play a pivotal role in the propagation of phytoplankton. Bialonski et al. (2016), for instance, demonstrated in a study on the connectivity of different areas of the Southern Californian Bight that HAB propagation and success were tightly coupled to transport via ocean currents, as well as to local environmental conditions such as nutrient input via, for example, upwelling events. Transport by prevailing ocean currents is a well-known mechanism for HAB propagation (Franks and Keafer 2003), as previously shown, for instance, for *A. tamarense* (Franks and Anderson 1992) and *Alexandrium fundyense* in the Western Gulf of Maine (e.g., Anderson et al. 2005). Other hydrographic phenomena, such as upwelling events, may also contribute to HAB bloom formation; not only indirectly via introducing dissolved inorganic nutrients from deeper water layers into surface layers, but also directly by introducing phytoplankton resting stages, such as dinoflagellate cysts into surface waters, which may act as seeding populations for a potential bloom of the respective species (e.g., Smayda and Trainer 2010). Hence, different hydrographic phenomena may contribute to the introduction of HAB species into different habitats, where their competitive success is further determined by local environmental conditions.

Total algal biovolume at the regional scale was also strongly determined by the high biovolume *Thalassiosira* achieved, especially under high nutrient conditions in the meta-ecosystems containing a nutrient gradient. Across different nutrient regimes (NUTgrad vs. NUTconst), total algal biovolume hardly differed for most treatments and timepoints, except for day 18, where it was higher under constant nutrient conditions (NUTconst) than under gradient conditions (NUTgrad). Despite the strong local dominance of *Thalassiosira* at high nutrient concentrations in the NUTgrad treatment, the enhanced evenness at lower nutrient concentrations led to a higher regional community evenness in meta-ecosystems containing a nutrient gradient (NUTgrad) compared to constant nutrient conditions (NUTconst). Likewise, Matthiessen et al. (2010), who studied the effects of dispersal and resource heterogeneity on marine benthic microalgae, found a higher regional richness, diversity, and evenness under resource (light) heterogeneity compared to homogenous resource distribution. In both studies, environmental heterogeneity promoted diversity and evenness through the reduction of dominance.

Overall, our study revealed weaker effects of *Alexandrium* on a regional scale than on a local scale. The inoculation position of the dinoflagellate neither affected total algal biovolume, nor the relative regional biovolume contributions of *Alexandrium*. Due to the rapid growth of the diatom *Thalassiosira*, which dominated the entire experiment especially in the high nutrient flasks of the NUTgrad treatment and all of the flasks under constant nutrient conditions, the relative contribution of all other species including *Alexandrium* decreased. However, due to its dominance under low nutrient conditions in the NUTgrad treatment, the relative contributions of *Alexandrium* at a regional scale were overall higher in the NUTgrad treatments compared to the NUTconst treatments.

Conclusion

This study presents, to our knowledge, a first experimental approach to study harmful algae ecology in a spatial context in a meta-ecosystem framework. The setup we used proved to be suitable for investigating ecological interactions and HAB dynamics along an environmental gradient, along which inoculation position, dispersal, and the local nutrient regime determined the population dynamics of our target species *Al. catenella*. Our study revealed very different results on a local and on a regional scale, as regional contributions of *Alexandrium* were low, whereas the dinoflagellate showed very distinct patterns of distribution on a local scale. Its dominance was patchy and constrained to low nutrient conditions. Likewise, in natural environments, HABs often occur in a patchy distribution, reflecting a mixture of transport/hydrography and local environmental conditions. The inoculation position of *Alexandrium* strongly determined its biovolume contribution over the entire course of the experiment, emphasizing that the specific location of where a bloom species is introduced, matters. However, only one dispersal regime was tested in this study, and it is conceivable that the relevance of inoculation position diminishes with increasing dispersal rate. Our study provides a valuable first step in conducting experimental research on a particular HAB species/strain in a plankton community context incorporating spatial dynamics. However, we have to point out that our experimental system was rather simple, not considering higher trophic levels or other environmental factors than nutrients. Also, phytoplankton populations typically yield a high genetic diversity with a variety of different genotypes and phenotypes (Bachvaroff et al. 2009; Alpermann et al. 2010; John et al. 2015). *Alexandrium* populations are composed of a much higher diversity in genotypes and phenotypic traits, even within a single bloom (John et al. 2015), while our experiment only looked at a single strain. Further experimental studies on harmful algae in a more complex ecological and spatial context are required to advance our understanding of their bloom dynamics, which in turn can help to improve our predictive abilities regarding HAB formation and demise.

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Conflict of Interest

None declared.

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