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Exposure to nanoplastics affects the outcome of infectious disease in phytoplankton☆

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ABSTRACT

Infectious diseases of humans and wildlife are increasing globally but the contribution of novel artificial anthropogenic entities such as nano-sized plastics to disease dynamics remains unknown. Despite mounting evidence for the adverse effects of nanoplastics (NPs) on single organisms, it is unclear whether and how they affect the interaction between species and thereby lead to ecological harm. In order to incorporate the impact of NP pollution into host-parasite-environment interactions captured in the "disease triangle", we evaluated disease outcomes in the presence of polystyrene NP using an ecologically-relevant host-parasite system consisting of a common planktonic cyanobacterium and its fungal parasite. NP at high concentrations formed hetero-aggregates with phytoplankton and inhibited their growth. This coincided with a significant reduction in infection prevalence, highlighting the close interdependency of host and parasite fitness. Lower intensity of infection in the presence of NP indicates that reduced disease transmission results from the parasite's diminished ability to establish new infections as NP formed aggregates around phytoplankton cells. We propose that NP aggregation on the host's surface acts as a physical barrier to infection and, by reducing host light harvesting, may also hamper parasite chemotaxis. These results demonstrate that the consequences of NP pollution go well beyond toxic effects at the individual level and modulate the intensity of species interactions, thereby potentially eliciting diverse cascading effects on ecosystem functioning.

1. Introduction

The widespread presence of plastic in the environment has become a characteristic imprint of human population growth and industrialization (Galloway et al., 2017). Over 350 million tons of plastics are produced annually (PlasticsEurope 2019), a significant fraction of which ultimately becomes debris in inland water bodies and oceans, largely via riverine transport (Lebreton et al., 2018; van Emmerik and Schwarz 2020). The presence of plastic and its effects on aquatic ecosystems are hence of increasing interest. Direct release of plastic pellets and fibers, as well as fragmentation of plastic macro-debris, have led to the presence of smaller size (<5 mm) plastics (i.e. microplastics) in virtually all ecosystems on Earth (Gasperi et al., 2015; He et al., 2018; Lebreton et al., 2018; Peeken et al., 2018; Besseling et al., 2019; González-Pleiter et al., 2021). In addition, photochemical fractionation, biological degradation, and mechanical abrasion of microplastics can result in nano-sized plastic particles smaller than 100 nm, i.e. nanoplastics (NPs) (Lambert and Wagner 2016; Gigault et al., 2018). NPs raise special concerns due to their unique ability to cross cell membranes, penetrate organs, and bioaccumulate in organisms (Chang et al., 2020; Lehner et al., 2019; Strungaru et al., 2019). This hazard is compounded by the potential adsorption of other environmental contaminants which may amplify their toxicity (Rist et al., 2017). Unlike other forms of plastic, NPs display colloidal properties and physicochemical factors like water pH or salinity can influence their charge or aggregation, affecting their biological fate and bioavailability (Gigault et al., 2018; Nasser and Lynch, 2016; Wu

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et al., 2019; Zhang et al., 2019).

Because of these concerns, research on NP pollution has attracted increasing attention in recent years (reviewed in Koelmans et al., 2015). Numerous ecotoxicological studies have now demonstrated diverse negative effects of NPs on single organisms, especially aquatic ones. For instance, exposure to NPs has been shown to cause reductions in body size, reproduction, and survival in zooplankton (e.g. Besseling et al., 2014; Lin et al., 2019; Ward and Kach, 2009), bioaccumulation in mollusks (Casado et al., 2013), and reduced photosynthetic activity, oxidative stress, and growth inhibition in phytoplankton (Bergami et al., 2017; Bhattacharya et al., 2010; Feng et al., 2019; Wan et al., 2018). As well as their physiological effects, NPs are prone to interacting with cell walls, aggregating around phytoplankton and other suspended particles to form sestonic hetero-aggregates (Koelmans et al., 2015; Wegner et al., 2012). Hetero-aggregation can hamper phytoplankton light harvesting (via shading), interfere in exchange processes at the cell-medium interface, and affect sedimentation (Bhattacharya et al., 2010; Besseling et al., 2014; Lagarde et al., 2016).

Despite growing knowledge on the negative impacts of NPs on individual organisms or species, little has been done to elucidate how NPs affect higher levels of biological organization and lead to ecological harm. Informed predictions about the impact of nano-sized plastic at the community and ecosystem levels demand approaches beyond toxicology at the sub-organismal and individual levels (Chae et al., 2018; Koelmans et al., 2015; Segner, 2011). Most key functional aspects of ecosystems (e.g. energy flow and trophic transfer, biogeochemical cycling, or ecological succession) are largely mediated by interactions between organisms, including competition, predation, or parasitism, rather than by the fitness of single species. Biotic interactions are major components of biodiversity and indicators of ecosystem health that can be used to diagnose the extent of environmental disruption (Lafferty et al., 2008; Valiente-Banuet et al., 2015). Understanding how biotic interactions are affected by NP pollution remains paramount to predicting their underlying ecological consequences. While studies have indicated that NPs can affect predator-prey interactions and be transferred up the food web (Chae et al., 2018; Chae and An 2020), the effects of NP on other biotic interactions, such as parasitism, remain virtually unexplored.

Infectious disease, i.e. the symbiotic relationship between organisms and their parasites, is a ubiquitous and pervasive ecological interaction. With virtually every species serving as host to at least one parasite, parasitism represents the most widespread consumer strategy in nature (Price 1980; de Meeûs and Renaud 2002) and has far-reaching effects on populations, communities and ecosystems. Parasites affect host abundance by increasing mortality and decreasing fecundity (Lafferty et al., 2008), which in turns alters competition between species, and can lead to successional changes and shifts in community composition (Hudson et al., 2006). Furthermore, parasites act as engines of evolution: hosts and parasites exert strong selection on each other thus driving co-evolution, an intense and pervasive force of evolutionary change that promotes and maintains genetic diversity (Hamilton et al., 1990). Finally, parasites are also key elements of food webs (Lafferty et al., 2006; Marcogliese and Cone, 1997), being involved in most existing trophic links (e.g. Amundsen et al., 2009) and with a total biomass that can exceed that of predators (e.g. Kuris et al., 2008).

The outcomes of infectious disease processes and their underlying effects on ecosystem functioning are determined not only by the reciprocal interaction between host and parasite, but also by the external environment. This concept is captured by the disease triangle (Stevens 1960), a classic model in epidemiology used to predict disease outcomes for wildlife and in public health (e.g. Scholthof, 2007). Here, we aim to integrate for the first time the emerging environmental challenge of nano-sized plastic pollution into the disease triangle. We focus on the infection of phytoplankton by fungal parasites as a model system. Phytoplankton represents the foundation of most aquatic food webs and, being responsible for up to 50% of global carbon fixation, plays a pivotal role in global biogeochemical cycles (Falkowski 2012). Phytoplankton is commonly attacked by fungal parasites of the order Chytridiomycota (i.e. chytrids) (reviewed in Frenken et al., 2017a). Infection by chytrids is always lethal and can develop into epidemics capable of severely reducing phytoplankton populations, often causing successional changes, and mitigating and/or suppressing harmful algal blooms (Gerphagnon et al., 2015). We aim to characterize the effects of NPs on this phytoplankton-chytrid host-parasite system. More specifically, we test whether the effects of NP exposure extend beyond toxicity at the single organism level and consequently interfere in species interactions.

2. Materials and methods

2.1. Study organisms

The filamentous planktonic cyanobacterium *Planktothrix agardhii* (strain NIVA-CYA 630, isolated from Lake Lyseren, Norway in 2008), a dominant taxon commonly forming harmful algal blooms in mesotrophic to hypertrophic temperate freshwater ecosystems (Kurmayer et al., 2016), and its obligate fungal chytrid parasite *Rhizophydium megarrhizum* (strain NIVA-Chy Kol2008, isolated from Lake Kolbotnvatnet, Norway in 2008; Sønstebø and Rohrlack, 2011) were used in this study. Chytrids are characterized by a free-living life stage in the form of flagellated zoospores, which actively seek their host by chemotaxis. Once located, zoospores encyst on the host's surface and penetrate it, developing and expanding rhizoids to extract nutrients. Encysted zoospores gradually develop into sporangia (chitinaceous reproductive structures) that release new zoospores upon maturation (Ibelings et al., 2004). Cyanobacterial host cultures were maintained as non-axenic batch cultures in borosilicate glass Erlenmeyer flasks at 16 °C in Z8 medium (Kotai 1972). For experimental purposes, the host was also cultivated in FW04 medium (Nicklisch et al., 2008), a synthetic medium that provides more environmentally relevant nutrient conditions, mimicking ionic compositions typical of temperate meso-eutrophic lakes (Table S1). The chytrid was routinely cultivated at 16 °C under a continuous light intensity of 45 μmol photons m^{-2} s^{-1}, transferring zoospores to uninfected host cultures every three weeks.

2.2. Characterization of NPs

Spherical polystyrene nanoparticles with a nominal size of 100 nm (Micromod Partikeltechnologie, Rostock, Germany, product nr. 30-00-102) were used. Particles are tagged with a fluorescent marker with excitation and emission peaks at 552 nm and 580 nm, respectively. NP particles were morphologically characterized by Scanning Electron Microscopy (SEM): 1 μL of the 10 mg mL^{-1} commercial solution was dried for 30 min in a laminar flow hood at room temperature. The sample was coated with a 3 nm layer of gold using a Polaron sputter coater and observed with a digital scanning electron microscope DSM 950 operating at an electric potential difference of 25 kV.

The hydrodynamic size of the nanoparticles in both culture media (Z8 and FW04, see above) and distilled water was determined by dynamic light scattering analysis using a Malvern Zetasizer Nano ZS. The electro-kinetic Zeta (ζ-) potential (i.e. the electric

potential at the boundary between the Stern layer of firmly attached counter-ions and the diffuse layer in the liquid) was quantified by electrophoretic light scattering for each medium in the presence and absence of NPs using the same apparatus. All measurements were conducted in triplicates.

Changes in the pH of media upon NP addition were ruled out by conducting a pilot experiment, in which different NP concentrations (0, 10, and 100 mg L^{-1}) were incubated in both nutrient media, in the presence or absence of infected cyanobacteria (host initial Optical Density at 750 nm (OD_{750}) = 0.05) in five replicate flasks, and pH was measured one day before adding NPs, and on days 1, 4, 7, and 10 after NP addition.

2.3. Experimental design

Two experiments were conducted to investigate the influence of increasing NP concentrations (0, 10, 100 mg L^{-1}) on host growth and hetero-aggregation (hereafter referred to as *host-focused experiment*), and on parasite fitness traits (hereafter referred to as *infection experiment*), respectively. Both experiments were conducted under realistic (i.e. using FW04 medium) and replete (i.e. using Z8 medium) nutrient concentrations to assess the potential effect of artificially high nutrient loads (as typically used in laboratory assays) on the properties of NPs and their subsequent toxicity. For each experiment, 10 replicates containing either cyanobacteria and their chytrid parasites (*infection experiment*) or cyanobacteria only (*host-focused experiment*) were set up for each NP concentration−nutrient load combination. In addition, for the *host-focused experiment*, four replicated "abiotic" controls (i.e. containing neither host nor parasite) were included for each condition to evaluate hetero-aggregation of NPs in the absence of cyanobacteria (see below). The experiments had the following designs: *Host-focused experiment*: (3 NP concentrations * 2 nutrient loads * 10 replicates) + (3 NP concentrations * 2 nutrient loads * 4 control replicates), resulting in 84 experimental units; *Infection experiment:* (3 NP concentrations * 2 nutrient loads * 10 replicates), resulting in 60 experimental units.

The experiments were conducted in Corning Costar 24-well plates in a media volume of 2 mL each. Cyanobacteria were acclimated in the respective media and maintained at 20 °C under a continuous light intensity of 20 μmol photons m^{-2} s^{-1} for two weeks before the experiments commenced. For both experiments, the NP stock solution was sonicated at 35 kHz and 20 °C for 60 s and added as appropriate to each experimental unit containing exponentially growing cyanobacteria (OD_{750} = 0.01). Both experiments lasted seven days. During that time, plates were gently shaken by hand daily and their position randomized. All experimental units were sampled at the end of the experiment.

2.4. Host-focused experiment

Cyanobacterial growth over the course of the experiment was quantified using chlorophyll *a* concentration as a biomass proxy. Glass fiber filters containing cyanobacteria were collected at the beginning (from cultures acclimated to experimental conditions) and end of the experiment (from each individual experimental unit) and stored at −80 °C until analysis. Filters were freeze-dried in darkness, dried at 20 °C in a vacuum desiccator for 1 h, and then incubated with 1 mL dimethylformamide and 100 μL ammonium acetate (1 M) for pigment extraction. Chlorophyll *a* concentration in the resulting extracts was determined by High Performance Liquid Chromatography (HPLC) using a Waters Alliance HPLC 2695 equipped with a C18-column and a Waters Photodiode Array Detector 2996 (detection at 410 nm and 440 nm channels).

Cyanobacterial filaments and NPs showed a tendency to form macroscopic aggregates upon contact. To quantify the degree of such hetero-aggregation, repeated turbidity measurements (i.e. OD_{750}) at multiple positions within single wells were performed at day 7 post-inoculation using a multimode micro-plate reader Tecan SPARK supported by Tecan SparkControl (version 2.3) software. The plates were shaken orbitally at 510 rpm for 5 s before measurement. Fifty-two OD_{750} reads per well were taken following a circular pattern and maintaining a minimum distance of 2.1 mm from the border of the well to avoid reflection from the rim (10 flashes per reading position; 50 ms settle time between reads; well diameter 15.6 mm). The variance of OD_{750} reads within single wells was used as a proxy for the degree of hetero-aggregation in the suspension and was expressed as the magnitude of the standard deviation of the readings per well. A biomass-normalized hetero-aggregation was also calculated to account for the possibility that cultures reaching higher filament densities (due to faster growth) inherently present higher intra-well variability (due to the increased likelihood of filament entanglement). This was done by dividing the magnitude of the standard deviation by the mean absolute OD_{750} recorded for each well. Abiotic controls were used to determine the degree of hetero-aggregation of NPs in the absence of the host.

2.5. Infection experiment

Host and NP were incubated for 24 h before inoculation with parasite zoospores. This incubation time aimed to approximate natural situations, in which NPs are persistently present in the water and interact with phytoplankton, and disease outbreaks usually occur as discrete events. Parasite zoospores for experimental infections were obtained by inoculating an exponentially growing cyanobacterial culture with parasite zoospores 10 days before the start of the experiment and maintaining it under a continuous light intensity of 45 μmol photons m^{-2} s^{-1}. After a 10-day incubation, the infected culture was filtered sequentially through a sterile 5 μm nylon-mesh and a 3 μm polycarbonate filter (Whatman Nucleopore Track-Etch membrane) after Agha et al. (2018) to obtain a host-free suspension of chytrid zoospores. Zoospore density in the suspension was determined using a Sedgewick Rafter counting chamber under a Nikon Ti Eclypse inverted microscope. The filtrate was added as necessary to achieve a final zoospore concentration of 2000 mL^{-1} in the respective experimental units.

Seven days after inoculation with parasite zoospores, experimental units were fixed in 2% formaldehyde, their identities blinded, and stored at 4 °C. Parasite fitness was then evaluated using three traits: a) prevalence of infection, determined by the proportion of infected hosts identified by microscopic inspection of 200 randomly selected cyanobacterial filaments for the presence of chytrid infection; b) intensity of infection, determined by quantifying the mean number of encysted chytrids per infected cyanobacterial filament after inspecting 80 randomly selected infected individuals; c) size of sporangia (i.e. parasite reproductive structures), determined by measuring the two semi-axes of 10 fully developed sporangia per sample (only mature or empty sporangia on hosts presenting only one infection) using NIS-Elements BR 4.5 software. The volume of each sporangium was calculated as:

$$V = \pi/6 * d_1^2 * d_2$$

where d_1 and d_2 stand for the short and long semi-axes, respectively.

2.6. Brightfield and confocal microscopy

Samples of infected cyanobacteria cultivated in 100 mg L^{-1} NPs were imaged 7 days after chytrid inoculation under brightfield illumination using a Nikon Ti Eclypse inverted microscope. The same samples were stained with Calcofluor White (CFW excitation 380 nm and emission 475 nm, final concentration 2.5% (v/v)) to detect chitinaceous fungal structures, and imaged with a Zeiss LSM 710 confocal microscope using 405 nm and 550 nm excitation lasers.

2.7. Statistical analyses

Linear models were used to assess the effects of NP concentration and nutrient load on host growth, hetero-aggregation, biomass-normalized hetero-aggregation (*host-focused experiment*), and parasite fitness traits (i.e. prevalence of infection, intensity of infection, and sporangial volume; *infection experiment*). For the response variable hetero-aggregation, the presence or absence of cyanobacteria was included as an additional fixed factor. Model assumptions were confirmed by visual inspection of the residuals. Models' fixed and interactive terms, their significance, and proportion of variance explained (calculated as sum of squares quotients) are reported. Contrast tests were performed subsequently to identify significant differences between levels of the factors. P-values were corrected for multiple comparisons using the Benjamini-Hochberg method (Benjamini and Hochberg 1995). All analyses were performed using RStudio (v.1.2.1335).

3. Results

3.1. NP characterization

Scanning Electron Microscopy confirmed the spherical shape of the NP particles (Fig. S1). The hydrodynamic size of the polystyrene NPs ranged from 113 to 128 nm across culture media and tested NP concentrations (Table S2). Deviations from the 100 nm nominal size result from the association of medium molecules on the surface of the particles under the respective conditions. Electrophoretic light scattering evidenced similar ζ-potential of NP particles in both culture media and distilled water, ranging from −31 mV in Z8 medium to −23 mV in FW04 medium (Table S2). A pilot experiment did not reveal any significant variation in pH or conductivity following NP addition in either medium or in the presence or absence of cyanobacteria (data not shown).

3.2. Host-focused experiment

Host growth. The growth of cyanobacteria was significantly inhibited at high NP concentrations (Fig. 1; NPs explained 48.9% of the variance; Table 1). In the absence of NPs, cyanobacterial growth was consistently higher under nutrient replete conditions. However, at the high NP concentration (100 mg L^{-1}), no difference in growth between nutrient loads was evident, indicating that the impact of high NP concentrations on cyanobacteria overrode the effects of dissimilar nutrient availability (significant NP x Nutrient load interaction; Table 1).

Hetero-aggregation. NPs in the absence of cyanobacteria (i.e. abiotic controls) did not spontaneously initiate aggregation, whereas cyanobacteria in the absence of NPs increased overall intra-well variance in OD measurements, indicating their intrinsic tendency to entangle and form aggregates (Fig. 2A). Notably, when

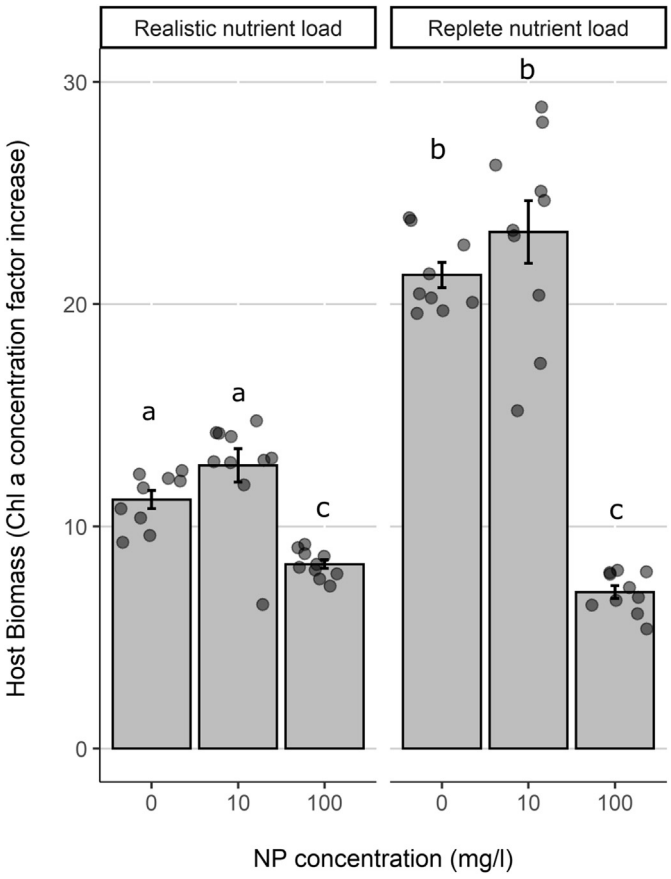

Fig. 1. Increase in chlorophyll *a* concentration over the 7-day inoculation in cyanobacterial cultures under different NP concentrations and nutrient loads. Letters indicate significantly different groups ($p < 0.05$). Error bars indicate s.e.

cyanobacteria were in contact with NPs at high concentration (100 mg L^{-1}), there was a dramatic increase in hetero-aggregation (Fig. 2A). The different degree of hetero-aggregation in different media was attributed to variation in host filament densities reached during incubation under dissimilar nutrient loads (see Fig. 1). In fact, nutrient load had no significant effect on normalized hetero-aggregation and NP concentration alone explained about 80% of the variance (Fig. 2B; Table 1).

3.3. Infection experiment

Prevalence of infection after seven days was significantly lower in the presence of high NP concentrations (100 mg L^{-1}) relative to controls (Fig. 3A). Irrespective of NP concentration, prevalence of infection was consistently lower under realistic nutrient loads relative to replete conditions (Nutrient load explained 27% of the variance; Table 2). Intensity of infection (i.e. the mean number of infections present on single infected individual hosts) followed a similar trend to prevalence of infection (Fig. 3B), although nutrient load had a smaller effect size on this trait and NP concentration was the most explanatory variable (84.5%; Table 2). Sporangial size was not affected by NP concentration or nutrient load, although a significant interaction between these factors was observed (Table 2; Fig. 3C). When plotting prevalence of infection and host growth in the absence of parasites, a positive correlation was evident (Fig. 4A). In turn, parasite transmission was inversely correlated with the degree of hetero-aggregation (Fig. 4B).

Microscopy. Hetero-aggregation of NPs on the surface of

Table 1
Linear models for fixed effects of NP concentration, nutrient load, and their interaction on host growth, hetero-aggregation, and biomass-normalized hetero-aggregation. For normalized hetero-aggregation, presence/absence of algae (P/A) was included as an additional fixed factor. Significant p-values are depicted in bold. Degree of hetero-aggregation is reported as a reduced model excluding non-significant terms, i.e. the triple interaction term.

Response variable	Factor	df	F-value	P-value	% Variance explained
Host growth	NP concentration	2	114.80	< **0.001**	48.9
	Nutrient load	1	109.86	< **0.001**	23.2
	NP x Nutrient load	2	41.57	< **0.001**	17.52
	Residuals	54			10.96
Degree of hetero-aggregation (reduced model)	NP concentration	2	114.17	< **0.001**	37.72
	Nutrient load	1	8.89	**0.0039**	1.47
	P/A	1	201.69	< **0.001**	33.32
	NP x Nutrient load	2	4.52	**0.0141**	1.49
	NP x P/A	2	39.39	< **0.001**	13.01
	Nutrient load x P/A	2	4.60	**0.0352**	0.76
	Residuals	74			12.22
Degree of hetero-aggregation normalized by biomass	NP concentration	2	99.38	< **0.001**	78.34
	Nutrient load	1	0.00	0.9900	0.00
	NP x Nutrient load	2	0.47	0.6263	0.38
	Residuals	54			21.28

cyanobacteria was evident under brightfield and confocal microscopy (Fig. 5). NPs aggregates appeared as large hetero-aggregates around living filaments, as well as empty, filament-shaped NP structures, likely produced upon filament detachment or death.

4. Discussion

The ever-increasing production and accumulation of plastic in the environment has fueled increasing efforts in recent years to understand its ecological consequences. Yet, whereas most studies have focused on the effects of plastics at the sub-organismal or individual levels, there remains a critical need to understand how these effects play out in an ecosystem context (Rochman et al., 2016). The various interactions between organisms (e.g. competition, predation, parasitism) can serve as important links in reconciling the responses of single organisms to environmental change with alterations in ecological processes occurring at higher levels of biological complexity. This study helps to bridge the gap by evaluating the effects of nano-sized plastic exposure on host-parasite interactions.

Several anthropogenic impacts on the environment have been associated with the emergence and reappearance of infectious diseases (Jones et al., 2008; Fisher et al., 2012). For instance, temperature elevation, eutrophication, and agrochemical pollution have been shown to affect dynamics of wildlife and human vector-borne diseases (Becker et al., 2015; Mordecai et al., 2017; Rohr et al., 2008). Yet, anthropogenic alterations of the environment do not necessarily promote infectious disease. Our findings show that exposure to NP can hinder chytrid disease spread in phytoplankton, as demonstrated by lower infection prevalence and intensity in the presence of high NP concentrations. A reduction was detected consistently under both replete and environmentally relevant nutrient concentrations, suggesting that this response may be independent of nutrient load in the range investigated here.

According to the disease triangle concept, disease outcome is the result of the reciprocal interaction among host, parasite, and environment (Stevens 1960). Alterations in the external environment can trigger directional effects that affect host and parasite, jointly influencing disease outcome. Infectious disease modulation by the environment can occur *via* direct or indirect effects on parasites. We define indirect effects on the parasite as those resulting from alterations in the fitness and/or physiology of the host (representing the parasite's immediate environment) that ultimately affect parasite performance and can be understood as environment → host → parasite cascades (Fig. 6). Such indirect effects could be inferred from our experiment: even in the absence of NP, low nutrient loads led to reduced host growth, which in turn resulted in decreased parasite infection prevalence relative to nutrient replete conditions (Fig. 3). This is attributable to reductions in host intracellular resources available for parasite growth and reproduction under nutritionally suboptimal conditions (Frenken et al., 2017b). Similarly, and consistent with the toxic effects of NPs on phytoplankton reported elsewhere (Besseling et al., 2014; Bergami et al., 2017), high NP concentrations inhibited cyanobacterial growth, which coincided with significant reductions in disease transmission. Toxic and/or growth inhibitory effects of NPs on the host led thereby to reduced parasite prevalence overall (Fig. 4A).

NPs tend to aggregate with other suspended particles, natural colloids, and other sestons (Bhattacharya et al., 2010). The aggregation of NPs with phytoplankton elicits another form of environment → host → parasite cascade. In our experiments, NPs formed hetero-aggregates with cyanobacterial filaments (Figs. 2 and 5). NP aggregation can affect the host in several ways, e.g. by reducing light availability, increasing sedimentation, or disrupting cell-medium exchanges (Bhattacharya et al., 2010; Koelmans et al., 2015). Conditions leading to high hetero-aggregation consistently caused reduced parasite transmission in our experiments (Fig. 4B). We envision two non-exclusive possibilities to explain this phenomenon. Firstly, aggregated NPs can constitute a physical barrier for the parasite. NP aggregates on host and chytrid surfaces were visible microscopically (Fig. 5). Since direct contact between zoospores and the host surface is necessary to initiate infection, NPs on the host's surface can act as a physical layer hindering contact between host and parasite, thereby hampering parasite encystment and, ultimately, its transmission. The reduced intensity of infection recorded under conditions of higher hetero-aggregation supports this possibility (Fig. 3B). Secondly, aggregation of NPs around phytoplankton results in shading, which might reduce host photosynthesis (Bhattacharya et al., 2010; Besseling et al., 2014). Light is a critical factor controlling chytrid transmission, with low irradiances diminishing or completely suppressing infection (Canter and Lund 1951; Bruning 1991; Tao et al., 2020). This is because free-living chytrid zoospores use photosynthetic exudates as chemical cues to find and infect new hosts (Scholz et al., 2017) Therefore, reduced light availability and/or hampered membranotropic diffusion due to NP aggregation might quantitatively or

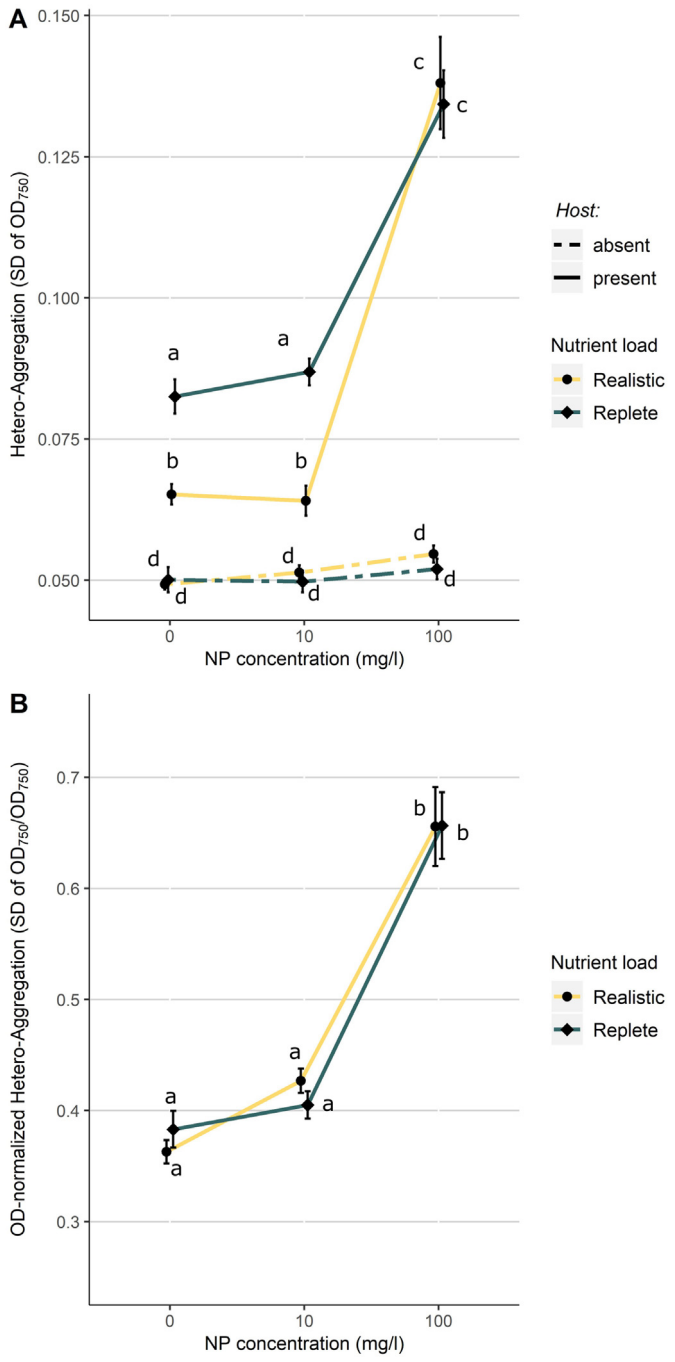

Fig. 2. Degree of hetero-aggregation (A) and biomass-normalized hetero-aggregation (B) under different NP concentrations and nutrient loads. Hetero-aggregation is expressed as the magnitude of the standard deviation recorded after 52 optical density readings at different positions within single wells. Normalization was performed by dividing this value by the mean absolute optical density recorded in each well. Letters indicate significant differences ($p < 0.05$) in contrast tests after correction for multiple comparisons.

qualitatively affect host photosynthetic exudates, thus decreasing parasite chemotactic attractions and further impacting disease transmission. Overall, infection success is strongly dependent on host fitness and susceptibility to infection, both of which can be modulated by changes in the external environment. This is reflected by the strong correlation between parasite transmission and host growth or hetero-aggregation (Fig. 4), both of which were significantly affected by NPs. These results indicate indirect effects

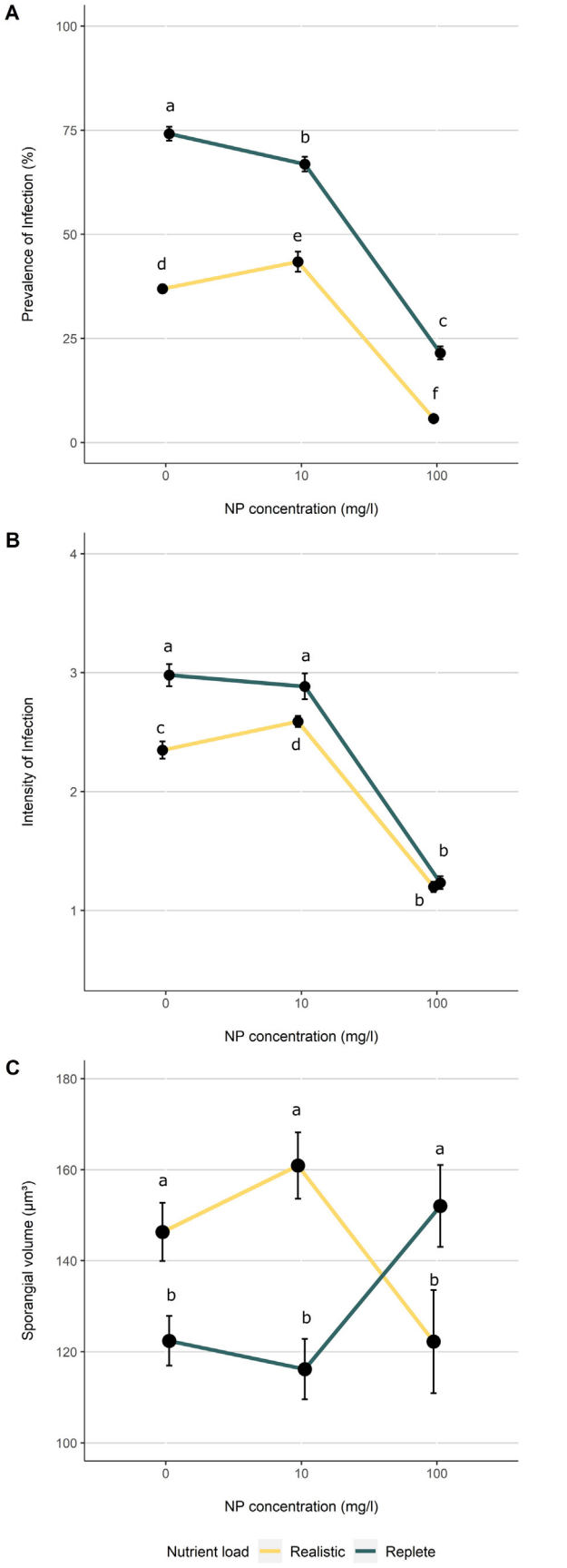

Fig. 3. Parasite traits under different NP concentrations and nutrient loads. Prevalence of infection (A), intensity of infection (B), and sporangial volume (C) 7 days after inoculation. Letters indicate significantly different groups ($p < 0.05$) in contrast tests after correction for multiple comparisons. Error bars represent s.e.

Table 2
Linear models for fixed effects of NP concentration, nutrient load, and their interaction on the studied parasite fitness traits. Significant p-values ($p < 0.05$) are depicted in bold.

Response variable	Factor	df	F-value	P-value	% Variance explained
Prevalence of infection	NP concentration	2	438.87	< **0.001**	65.3
	Nutrient load	1	368.75	< **0.001**	27.4
	NP x Nutrient load	2	22.46	< **0.001**	3.3
	Residuals	54			4.0
Intensity of infection	NP concentration	2	268.56	< **0.001**	84.5
	Nutrient load	1	28.15	< **0.001**	4.3
	NP x Nutrient load	2	8.16	< **0.001**	2.6
	Residuals	54			8.5
Sporangial volume	NP concentration	2	0.14	0.8661	0.3
	Nutrient load	1	4.00	0.0504	4.9
	NP x Nutrient load	2	11.77	< **0.001**	28.8
	Residuals	54			66.0

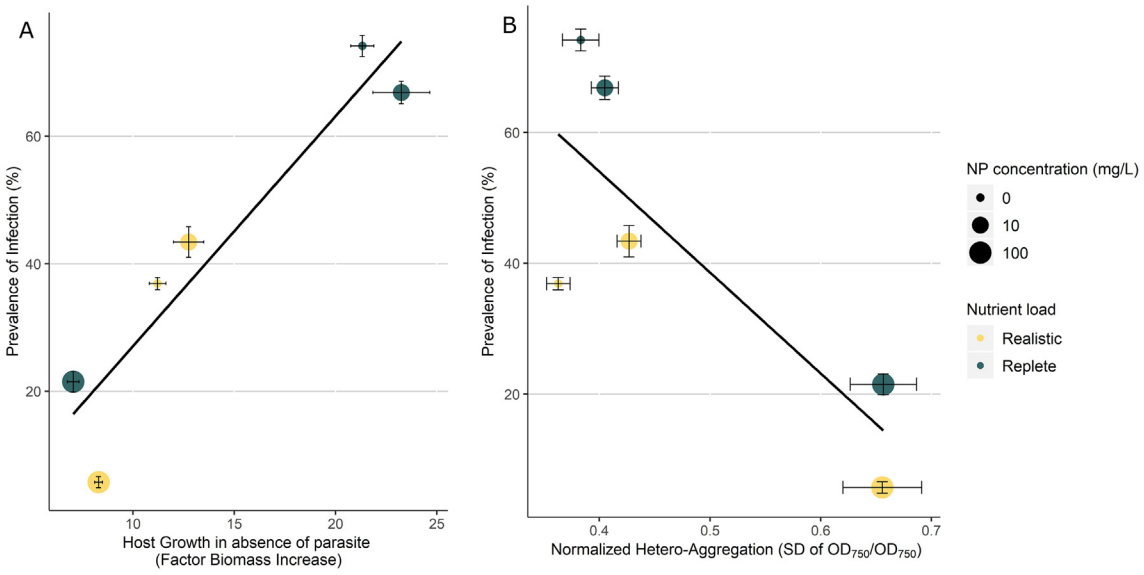

Fig. 4. Correlation between prevalence of infection and host growth ($r^2 = 0.93$) (A) and biomass-normalized hetero-aggregation ($r^2 = -0.80$) (B) under different NP concentrations and nutrient loads. Error bars indicate s.e.

on the parasite, in which NPs affect disease outcome by exerting effects on the host that ultimately impact parasite fitness (Fig. 6). Direct effects of NPs on parasites (i.e. environment → parasite → host cascades) are also likely to exist, e.g. toxicity to chytrid zoospores. Unfortunately, characterizing them in this particular host-parasite system remains challenging, since the chytrid is an obligate parasite that cannot be cultivated in the absence of its cyanobacterial host.

It is likely that plastic pollution may also affect disease outcome in other host-parasite systems (e.g. de Souza Machado et al., 2018). However, the direction of this effect might be dictated by differences in infection strategies across host-parasite systems and/or dissimilar susceptibility of antagonists to environmental stressors. For instance, parasites relying of free-living transmission stages or ectoparasites (i.e. parasites that live on the external surface of hosts) are in constant contact with the external environment and are likely to be more susceptible to environmental perturbations than endoparasites, which spend most of their life inside their hosts. Also, it has been shown that microplastic exposure suppresses host immune response in Crustacea (Sadler et al., 2019), bivalve (Détrée and Gallardo-Escárate, 2018), fish (Limonta et al., 2019), and coral (Tang et al., 2018) host systems, which could imply promotion of disease in contrast to the phytoplankton-chytrid system. In addition, an integration of our findings into realistic NP exposure scenarios is needed, in order to assess the ecological risk of NPs. Whereas predictions of the environmentally relevant NP concentrations suggest exposure concentrations in the range of the μg/L (Lenz et al., 2016) and might imply that the concentrations used in our experiment are unrealistic, these are based solely on the extrapolation of measured field concentrations of larger-sized microplastics (i.e., not nanoplastics) and are hence not free of uncertainty. The truth is that, at present, the lack of analytical methods to quantify actual NP concentrations in the environment makes it difficult to delineate the range of environmentally relevant concentrations (discussed in Besseling et al., 2019). Therefore, exploring the consequences of plastic pollution under environmentally realistic concentrations remains an urgent need to assess its ecological risks.

Addressing the effects of nanoplastic exposure on biotic interactions constitutes a first step toward linking physiological effects on single organisms with ecological consequences. Disease is an omnipresent ecological process that can alter ecosystem services and drive major ecosystem processes (Hatcher et al., 2012; Paseka et al., 2020). By inhibiting (or promoting) disease, NP contamination can have impacts extending beyond the individual fitness of host and parasite. Based on our knowledge of the biology of the

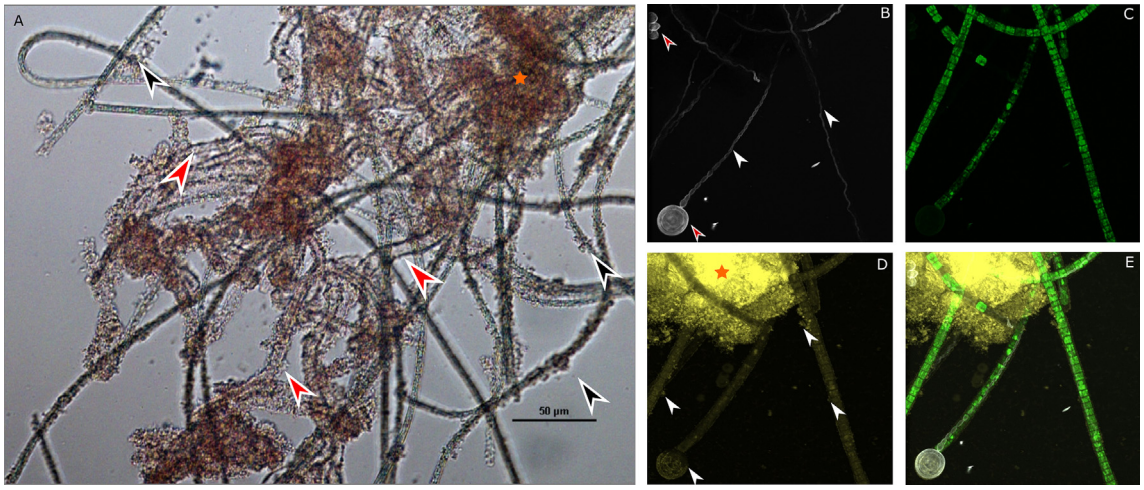

Fig. 5. Micrographs of the filamentous cyanobacterium *Planktothrix agardhii* infected by the chytrid *Rhizophydium megarrhizum* under NP exposure (100 mg L^{-1}). (A) Brightfield image showing strong NP aggregation around host filaments and deposition along their surface. NP aggregates appear as large hetero-aggregates (orange star) and around living filaments (black arrows), leaving translucent empty NP sheaths after filament death or detachment (red arrows). The scale bar in (A) applies to all panels. (B–E) Fluorescence confocal images (B) Channel collecting emission < 480 nm showing fungal rhizoids expanding along infected host filaments (white arrows) and sporangia at the filaments termini (red arrows). (C) Channel collecting emission between 480 and 550 nm, showing the autofluorescence of the cyanobacterium. (D) Channel collecting emission >570 nm, showing fluorescence signal of tagged NPs (emission 580 nm). NPs appear as large hetero-aggregates (orange star), microaggregates on the host surface (white arrows), and as smaller depositions covering cyanobacterial and chytrid surfaces. (E) All fluorescence channels stacked. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

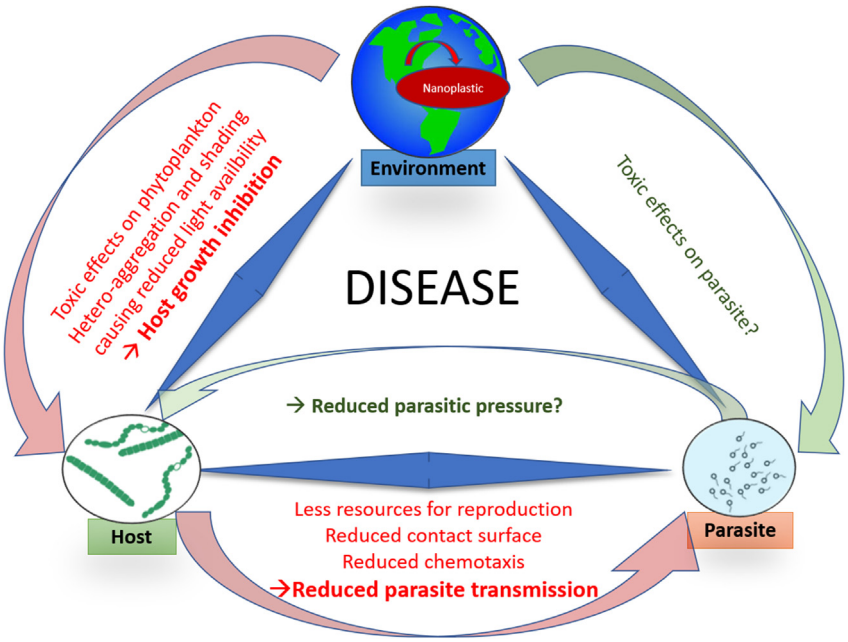

Fig. 6. Schematic representation of the effects of NP pollution on the interaction between phytoplankton hosts and their chytrid parasites in the framework of the disease triangle. Red pathways depict environment-host-parasite cascades, i.e. indirect effects of NPs on the parasite. Green pathways represent possible environment-parasite-host cascades, i.e. direct effects of NPs on the parasite. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

interaction between phytoplankton and their chytrid parasites, several mechanisms by which changes in disease severity affect ecosystem functioning can be envisioned. Firstly, chytrids act as a major biotic control factor on phytoplankton that can drive community dynamics and modulate or suppress harmful phytoplankton blooms (Frenken et al., 2017a; Gerphagnon et al., 2015). If chytrid epidemics are reduced, for example by NP exposure, this can lessen phytoplankton top-down control, with consequences for algal bloom dynamics, phytoplankton community composition, and overall primary production (Frenken et al., 2017a; Gerphagnon et al., 2015). Secondly, disease affects food web topology by modulating existing trophic interactions (Selakovic et al., 2014) and establishing new trophic links (e.g. by serving as food for other organisms; Johnson et al., 2010). Chytrids render carbon available to consumers (that is otherwise retained in poorly edible phytoplankton hosts) by serving themselves as prey for zooplankton (Agha et al., 2016; Kagami et al., 2007). In the process, phytoplankton carbon is upgraded into lipids of high nutritional value (Gerphagnon et al., 2019), and herbivory is enhanced in the system by undermining phytoplankton resistance to grazers (Frenken

et al., 2020), all of which facilitates trophic transfer in pelagic food webs. Lastly, evidence for the importance of parasitism by chytrids as a driver of phytoplankton evolution is gradually mounting. Experimental evolution assays and field surveys show that selective pressure imposed by chytrids promotes genetic diversity in phytoplankton populations (Agha et al., 2018; De Bruin et al., 2008; Gsell et al., 2013). Overall, by affecting disease processes, NPs have the potential to impact the extent and intensity of underlying ecological and evolutionary processes.

5. Conclusion

This study demonstrates that nanoplastic pollution affects the ecological interaction between organisms. Specifically, nanoplastic exposure reduced the intensity of host-parasite interactions between phytoplankton and their fungal parasites, decreasing infectious disease severity and highlighting disregarded ecological consequences of (nano)plastic pollution. Detrimental effects of nanoplastics on the host, including growth inhibition and hetero-aggregation, impact parasite fitness and reduce disease severity, exemplifying how the toxic effects of nanoplastic on individual organisms entail ecological consequences when considering interactions at higher levels of biological complexity. Given the importance of infectious disease processes for phytoplankton dynamics, trophic transfer and co-evolution, these findings point towards profound ecological consequences of plastic pollution and call for overcoming single-organism approaches and using ecological interactions between organisms as eco-toxicological endpoints in future research efforts.

Authors statement

The work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Declaration of competing interest

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

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