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Combined nanoTiO$_2$ and nitrogen effects on phytoplankton: a mesocosm approach

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Abstract

The aquatic ecosystem is the ultimate sink for consumer and industrial waste discharge that contains nanometals such as titanium dioxide nanoparticle (n-TiO$_2$). In this environment, nutrient availability and nanometals influence phytoplankton community structure and function. In a mesocosm experiment, we evaluated the interactive effect of n-TiO$_2$ (48 mg L^{-1}) and two nitrogen (N) levels (limited, LN; and replete, HN) on the phytoplankton community structure (biomass, species diversity and richness, algal species divisions), biochemical composition (carbohydrates, proteins, and lipids), and antioxidant response (peroxidase activity, POD). n-TiO$_2$ decreased total phytoplankton biomass, and its combination with HN led to the highest decrease. Species diversity was not affected by N level, n-TiO$_2$, and their interaction, while species richness decreased in combined n-TiO$_2$ and HN treatment. All these recorded effects of n-TiO$_2$ on the phytoplankton community structure were enhanced by increasing temperature over time. LN initially reduced phytoplankton carbohydrate content but increased by the presence of n-TiO$_2$ and its interaction with N levels. Total protein and lipid content were not affected by n-TiO$_2$ or its interaction with N levels. POD activity was increased by the interaction between n-TiO$_2$ and the N levels tested. Our results indicate that the influence of n-TiO$_2$ on the phytoplankton community was dependent on the concentration of N. Also, phytoplankton carbohydrate content and community structure varied with increasing water temperature. A few species thrived concerning biomass during exposure to the LN + n-TiO$_2$ (*Scenedesmus quadricauda*, *Coelastrum reticulum*, and *Microcystis* sp.) and HN + n-TiO$_2$ (*Microcystis* sp.) treatments. Members of the Chlorophyta were generally susceptible to the presence of n-TiO$_2$ regardless of the N level. Thus, the presence of n-TiO$_2$ in aquatic ecosystems can alter phytoplankton community structure and dynamics.

Keywords Titanium dioxide nanoparticle · Nitrogen level · Mesocosm · Phytoplankton · Algal biomolecules · Temperature

Introduction

Metal nanoparticles have different physicochemical properties from their bulk metal forms. They have a relatively large surface-area-to-volume ratio that gives them higher reactivity (Elsaesser and Howard 2012; Hou et al. 2019). Their physical and chemical properties are different from those of their bulk form due to their small particle size, which ranges from 1 to 100 nm (Sharma 2009). These changes in properties bring with them advantages for an array of industrial applications (Horikoshi and Serpone 2013), leading to increased manufacture of synthetic metal nanoparticles (Charitidis et al. 2014; Rastogi et al. 2017). The increased use of metal nanoparticles comes with a concomitant increase in the aquatic environment, which serves as the ultimate sink for many anthropogenic and industrial waste discharges (Kaegi et al. 2008). As it is currently poorly understood, variations in chemical and physical properties of metal-based nanoparticles may influence their stability and enhance their potential toxicity to biota (Pérez et al. 2009; Kahlon et al. 2018).

Titanium dioxide nanoparticles are among the top five most used metal nanoparticles (Shukla et al. 2011). Properties such as chemical and thermal stability, high photocatalytic activity under ultraviolet light and visible light, make them excellent candidates for incorporation into several products such as antimicrobial agents, cosmetics, sunscreens, paints (Sharma, 2009), and water purification agents (Wu et al. 2014). Emerging evidence shows that n-TiO$_2$ has variable toxicities to phytoplankton species (Miller et al. 2012) and these toxicities are to a great extent influenced by changes

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in physical and chemical conditions of the aqueous environment (Dauda et al. 2017; Zhang et al. 2018). Specifically, the toxicity of n-TiO_2 to *Chlorella vulgaris* was influenced by changing nitrogen levels in the cultures (Dauda et al. 2017), and n-TiO_2 may regulate the transformation and transport of sediment phosphorus in the aquatic environment (Luo et al. 2010). Unfortunately, the effect of this nanometal on phytoplankton community has not received the desired attention. Not negating the importance of single-model systems, the predictions of n-TiO_2 ecotoxicity cannot be comprehensively made with them (Niinemets et al. 2017).

Single-model systems where the effect of just one stressor is studied provide a necessary and vital understanding of the impact of stressors on organisms. However, such studies are often limited to the outcome of the effects of an individual stressor at a time. There are several studies on the effect of metal nanoparticles on phytoplankton (Cardinale et al. 2012; Miller et al. 2012; Marchello et al. 2018). Still, the interaction of nutrients (e.g., nitrogen) with titanium dioxide nanoparticles has received little to no attention (Dauda et al. 2017). Besides, most of the available studies were small laboratory scaled, under controlled conditions, and involved a single life cycle of the phytoplankton. Although Das et al. (2014) conducted a field study of the interaction of silver nanoparticle with phosphorus on phytoplankton, and Simonin et al. (2018) conducted experiments to elucidate the interactions of nutrients with engineered nanometal containing pesticides in a wetland ecosystem, there are no studies on the interplay between n-TiO_2 and nitrogen variation on phytoplankton community structure and dynamics and biochemical composition.

The concentration of nitrogen and its interaction with different metal forms determines the absence or presence of phytoplankton in a water body (Roessink et al. 2008). Such interactions may lead to adverse effects on the growth, reproduction, and population dynamics of phytoplankton, thereby altering aquatic ecosystem balance. The responses to novel stressors may cause modifications in species composition and diversity and can lead to asynchronous peak activities of organisms at different trophic levels (Niinemets et al. 2017). Assessing the combined effects of multiple stressors on ecological systems has become an essential focus for environmental scientists in the continuing effort to ascertain the relative importance of varied physical, chemical, and biological forces that drive the interactions within biological communities (Interlandi 2002). Therefore, we hypothesize that the interactive effects of nitrogen variation and n-TiO_2 will alter primary production, phytoplankton community structure and dynamics, and biochemical composition. This study aimed to investigate changes in biomass, phytoplankton community structure and dynamics, and biochemical composition during exposure to n-TiO_2 under limited and replete nitrogen conditions. Exploring this crucial lacuna is vital to the understanding of the under-reported roles of n-TiO_2 in aquatic environments with varying nitrogen trophic status.

Methods and methods

Source of algae inoculum

The mesocosm comprised water and phytoplankton collected from the Ahmadu Bello University Reservoir and Sewage Oxidation Pond in March 2017. Inoculum samples were collected from these sources because the Ahmadu Bello University reservoir at the time of sample collection was in an oligotrophic state, and the mixture gave a good representation of the algal flora of the region.

Experimental design

Twelve (12) mesocosms were constructed and situated in the departmental pond of the Department of Botany, Ahmadu Bello University Zaria, Kaduna State, Nigeria. The mesocosms were constructed using plastic baskets fitted with polyethylene bags, and empty plastic bottles served as floats. The baskets were held together by passing a long rope in-between them at equal distances, which were subsequently fastened at poles located at the far ends. Approximately 15 L of water containing algae was transferred into each of the 12 mesocosms. Sampling was done weekly for 4 weeks for biochemical composition and phytoplankton community structure and dynamics analyses.

Treatments

Titanium (IV) oxide-anatase nano-powder (n-TiO_2) (particle size < 25 nm) was purchased from Sigma-Aldrich (CAS number 637254; Sigma-Aldrich, USA), and nitrogen (N) was provided as $NaNO_3$. For the detailed characterization of the nanoparticles used, we relied on the manufacturer's specifications. These matched some published characterization of the exact n-TiO_2 (CAS no. 637254; Sigma-Aldrich, USA) by Sadiq et al. (2011), Vicari et al. (2018) and Zhang et al. (2018). All these studies confirmed that the n-TiO_2 had anatase phase predominantly. The as-received average size of particles through TEM analysis was ~ 20 nm (Sadiq et al. 2011). The average particle size was < 25 nm using X-ray diffraction with the Debye–Scherrer equation (Sadiq et al. 2011; Zhang et al. 2018), SEM (Sundararaghavan et al. 2019), and TEM (Srivastava et al. 2011). The zeta potential of the n-TiO_2 was reported to be + 9.12 mV (Zhang et al., 2018) and + 37.4 mV (Vicari et al. 2018); hence, it may be considered to form a stable colloidal suspension.

The treatments were low nitrogen (LN) comprising lake water (control) without adding N or n-TiO_2, LN + n-TiO_2 (48.00 mg L^{-1} n-TiO_2), high nitrogen (HN; 1.90 mg L^{-1} N), and HN + n-TiO_2 (1.90 mg L^{-1} N + 48.00 mg L^{-1} n-TiO_2). The preliminary effective concentration 50 (EC_{50}) value of n-TiO_2 on *Chlorella vulgaris* was reported to be 7.98 mg L^{-1} (Dauda et al. 2017). Although the current predicted environmental concentration of n-TiO_2 is lower than that used in the present study (Gottschalk et al. 2009), its release into the environment is expected to continue with the increased use of metal nanoparticles. After sample collections on each sampling day, the HN and n-TiO_2 concentrations in the mesocosms were readjusted to the initial concentrations to maintain a consistent input of N and n-TiO_2, simulating pulsed waste discharges into water bodies. The LN and HN concentrations employed in this study represent those found in oligotrophic and hypertrophic freshwater N levels, respectively (Smith et al. 1999). All experiments were carried out in triplicate.

Concentrations of n-TiO_2 in the cultures were measured as the total concentration of the titanium (Ti) metal using an Agilent 4200 Microwave Plasma—Atomic Emission Spectroscopy system (Agilent Technologies, USA). Titanium standards were prepared with Calibration mix 1 for AA and ICP-OES (Ti 100 mg L^{-1} in 2% HNO_3 + 0.5% HF) obtained from Agilent Technologies (USA). The N concentration in the medium was quantified colorimetrically as NO_3.N per the method outlined in APHA (1999). The nominal and measured pre-adjusted N and n-TiO_2 levels in the water throughout the study are in Table S1 (see supplementary data Table S1).

Data collection

During each weekly sampling, the water in the mesocosm was carefully mixed, and then the pH and temperature of the mesocosm were taken using a multiparameter water quality portable Hanna instrument (model no. H1991300). Also, 100 mL aliquots were collected per week. Out of the 100 mL, 20 mL subsamples were preserved in Lugol's iodine solution for microscopic analysis and identification using keys provided by Bellinger and Sigee (2010). Phytoplankton cell counts were done using the drop count technique (Chia et al. 2012). The remaining 80 mL of each collected sample was divided into four centrifuge tubes containing 20 mL each. They were then centrifuged at 224×*g* for 10 min, the supernatant was discarded, and the pellets resuspended in pre-cooled phosphate buffer (pH 7.8) and frozen (− 20 °C). These samples were used for the carbohydrate (Liu et al. 1973) and total lipid (Bligh and Dyer 1959) analyses. Prior to total protein quantification (Bradford 1976) and peroxidase (POD) enzyme activity assay (Reddy et al. 1996), total protein was extracted according to Dauda et al. (2017). The POD activity assay method relied on the oxidation of pyrogallol to a colored product called purpurogallin. The colored product was measured spectrophotometrically at 430 nm, and the absorbance was equivalent to the activity of the enzyme. Carbohydrate, protein, lipid, and peroxidase activity analyses were done only on samples collected at weeks two and four.

Data analyses

Repeated measures ANOVA was used to test for the differences in the carbohydrates, protein, lipids, peroxidase activity, and cell density of the phytoplankton community exposed to the different treatments. Prior to ANOVA tests, Mauchly's sphericity and Shapiro–Wilk tests for sphericity and normality evaluation were done. In cases where sphericity was violated, the Greenhouse–Geisser correction was used to adjust the degrees of freedom and reduce type 1 error. Species richness was determined by the number of species per treatment using the Shannon diversity index (vegan package in R) (Oksanen et al. 2018). One-way ANOVA was used to test for the differences in species richness and species diversity between the treatments. Principal component analysis (PCA) was used to determine the relationship between the biochemical parameters and cell density, using data from week 2 and week 4. The association between algal species responses and the environmental factors was evaluated using canonical correspondence analysis (CCA). Only significant environmental variables (selected using the adonis function of the vegan package in R) were used for the CCA, and the significance of CCA was determined using 999 unrestricted permutations. Statistical analyses were performed at $p < 0.05$ with R for Windows (R Core Team 2018).

Results

Biomass and community structure

n-TiO_2 ($p < 0.001$) and its interaction with N ($p < 0.001$) significantly affected the total biomass of the phytoplankton community (Table 1). Nitrogen enrichment (HN) did not significantly affect the total phytoplankton biomass, while the LN condition steadily increased it from the beginning to the end of the experiment (day 28) (Fig. 1). Measured N of the LN treatment increased in the course of the experiment (see Table S1). The presence of n-TiO_2 combined with HN continuously reduced the total phytoplankton biomass from week one to week four. Under LN + n-TiO_2, algal biomass increased until day 21, after which it decreased.

Overall, 26 species of phytoplankton belonging to 5 divisions (Chlorophyta, Bacillariophyta, Cyanophyta, Euglenophyta, and Dinophyta) were observed in the mesocosms during the study. Phytoplankton species diversity

Table 1 Analysis of variance summary results for biochemical parameters, total phytoplankton biomass, and environmental parameters of phytoplankton exposed to different N and n-TiO_2 treatments over time. Values represent *F* values and those in parentheses represent *P* values

Parameter	N	n-TiO_2	N*n-TiO_2
Total phytoplankton biomass	3.28 (0.07)	16.81 (0.00)	18.00 (0.00)
Carbohydrate	59.10 (0.01)	23.73 (0.04)	24.58 (0.03)
Protein	0.34 (0.61)	0.32 (0.63)	0.01 (0.94)
Peroxidase	10.31 (0.85)	3.31 (0.21)	20.92 (0.04)
Lipid	0.34 (0.61)	0.32 (0.63)	0.01 (0.94)
Temperature	0.32 (0.85)	1.39 (0.31)	3.50 (0.06)
pH	3.51 (0.06)	0.68 (0.62)	0.42 (0.78)

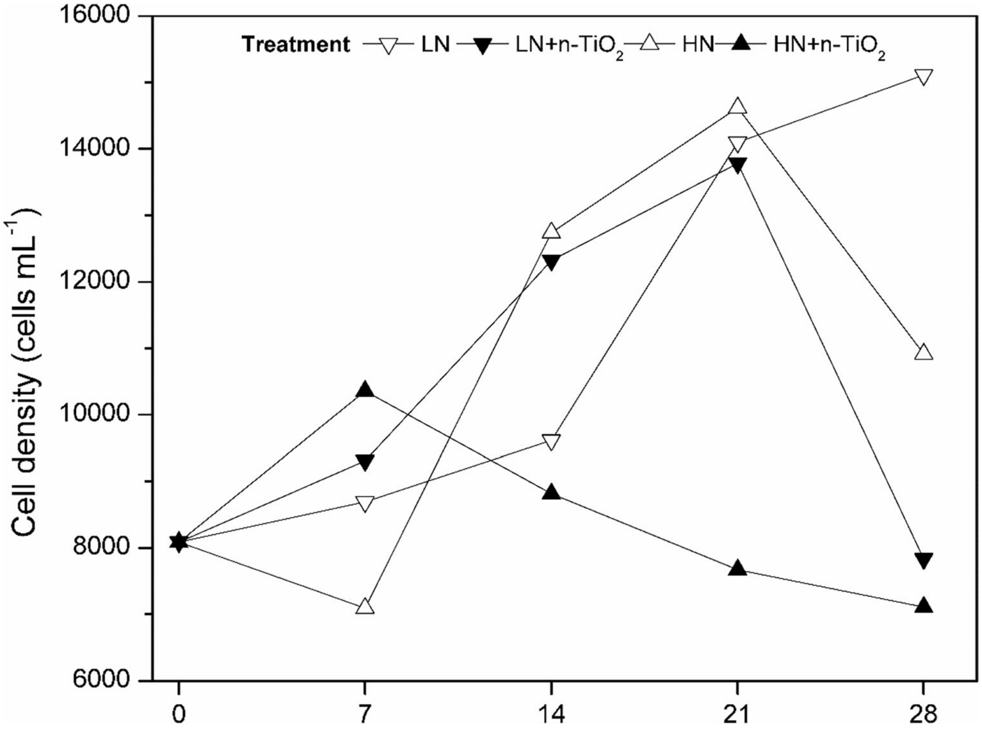
was not significantly affected by the treatments (Fig. 2), while species richness was significantly influenced (Fig. 3). The LN treatment had the highest species richness, followed by LN + n-TiO_2 and HN. Exposure to the HN + n-TiO_2 treatment resulted in the lowest species richness. Members of the Chlorophyta division (over 60%) dominated other phytoplankton divisions under all the treatment conditions, followed by Cyanophyta, Bacillariophyta, Euglenophyta, and Dinophyta (Fig. 4). At the end of the experiment (day 28), the relative abundance of phytoplankton populations revealed that *Microcystis* sp. had the highest abundance per cell density in mesocosms with the HN + n-TiO_2 (Fig. 5).

The first two CCA ordination axes accounted for 95.22% of the total variation, and three environmental variables (temperature, pH, and time) significantly defined the distribution of phytoplankton in the different treatments (Fig. 6). The canonical coefficients showed that temperature and time of exposure to the treatments were the most critical variables in the ordination axis 1. The LN, LN + n-TiO_2, HN, and HN + n-TiO_2 treatments were all significantly associated with higher temperature values over time. Increased pH was related to only HN + n-TiO_2. *Pediastrum boryanum* and *Pediastrum tetras* populations were positively associated with LN, LN + n-TiO_2, HN + n-TiO_2, and temperature. *Fragilaria* sp., *Ceratium* sp., and *Euglena* sp. populations were positively related to LN, HN, and temperature. LN and low temperature favored the abundance of *Oedogonium* sp., while *Pseudo-nitzschia* sp. was associated with high pH and HN + n-TiO_2.

Biochemical parameters

The N levels, n-TiO_2, and their combinations significantly affected total phytoplankton carbohydrate content (Table 1). Specifically, the LN condition significantly lowered

Fig. 1 Total algal biomass as a function of nitrogen and titanium dioxide nanoparticle treatments over 4 weeks



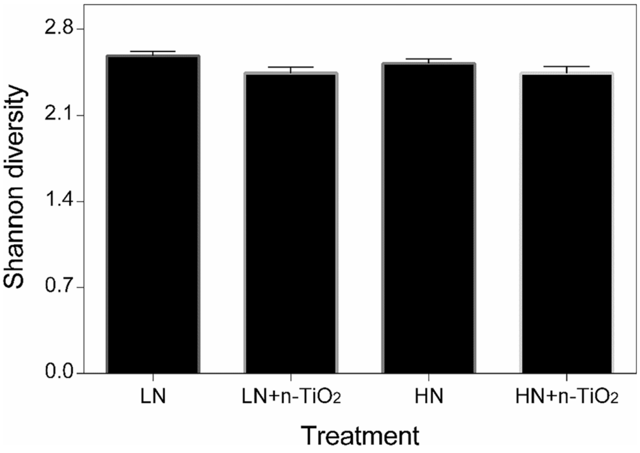


Fig. 2 Shannon diversity of phytoplankton as a function of nitrogen and titanium dioxide nanoparticle treatments over 4 weeks

carbohydrate content on day 14, while the reverse occurred on day 28. Also, HN and the combination of the N levels with n-TiO_2 led to higher carbohydrate content (Fig. 7a). All the treatments did not significantly alter the protein and lipid content of the phytoplankton throughout the study (Table 1) (Fig. 7 b and c). Neither N nor n-TiO_2 significantly influenced the peroxidase enzyme activity of the phytoplankton community, but their interactive effect was significant (Table 1) (Fig. 7d).

The first two axes of the PCA explained 55.6% of the total variation (Fig. 8). The carbohydrate and protein content of the phytoplankton had a positive relationship with HN and LN + n-TiO_2 on the second principal axis, and cell density had a significant positive association with lipid content and HN condition. Peroxidase activity significantly correlated with HN + n-TiO_2.

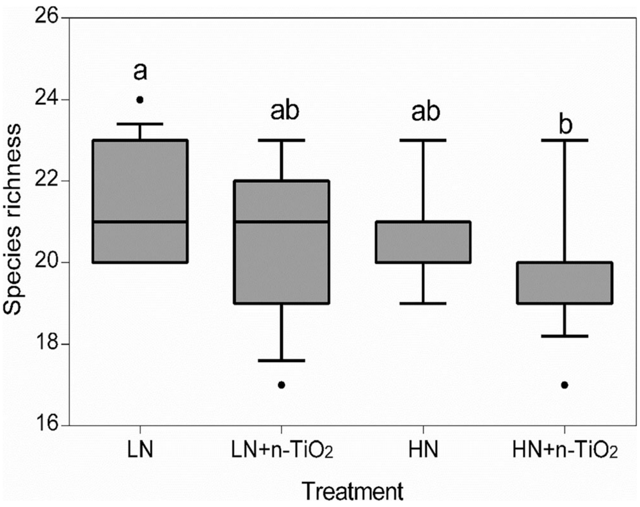


Fig. 3 Phytoplankton species richness as a function of nitrogen and titanium dioxide nanoparticle treatments over 4 weeks

Discussion

Although there was a general decrease in total phytoplankton biomass on the last day of the experiment under HN, the phytoplankton populations were able to grow and multiply, irrespective of the N level. Similar to our results, phytoplankton growth under low nitrogen conditions have been reported by other authors under field conditions (Conan et al. 2007). Phytoplankton species diversity was similar irrespective of the treatment conditions. This means that regardless of the N condition and the presence of n-TiO_2, several phytoplankton species will be able to thrive. Similar to our finding, Conan et al. (2007) recorded a diverse phytoplankton community under LN in a mesocosm experiment.

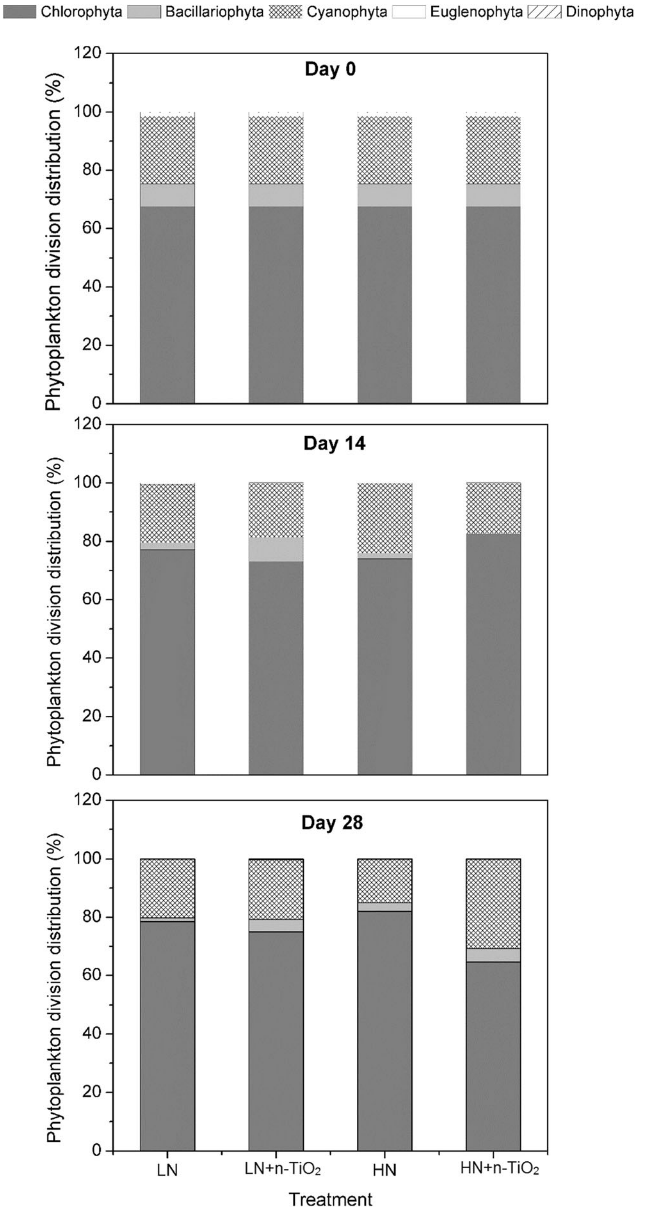


Fig. 4 Proportion (%) of phytoplankton division abundance at different time points under the different treatment conditions

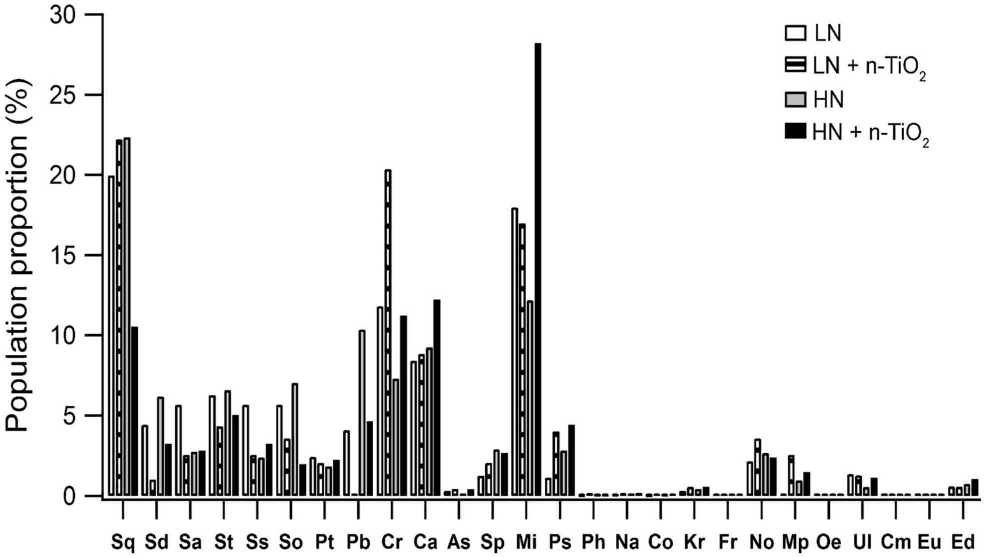


Fig. 5 Phytoplankton species population proportion under the different treatment conditions at the end of the experiment (day 28). **Sq** (*Scenedesmus quadricauda*), **Sd** (*Scenedesmus disciformis*), **Sa** (*Scenedesmus acuminatus*), **St** (*Scenedesmus tenuispina*), **So** (*Scenedesmus ornatus*), **Ss** (*Scenedesmus* sp.), **Cr** (*Coelastrum reticulum*), **Ca** (*Coelastrum astroideum*), **Pt** (*Pediastrum tetras*), **Pb** (*Pediastrum boryanum*), **As** (*Ankistrodesmus spiralis*), **Kr** (*Kirchneriella* sp.), **Mp** (*Microspora* sp.), **Oe** (*Oedogonium* sp.), **Ul** (*Ulothrix* sp.), **Ed** (*Eudorina* sp.), **Co** (*Cosmarium* sp.), **Sp** (*Spirogyra* sp.), **Ps** (*Pseudo-nitzschia* sp.), **Na** (*Navicula* sp.), **Fr** (*Fragilaria* sp.), **Mi** (*Microcystis* sp.), **No** (*Nostoc* sp.), **Ph** (*Phacus* sp.), **Eu** (*Euglena* sp.), **Cm** (*Ceratium* sp.)

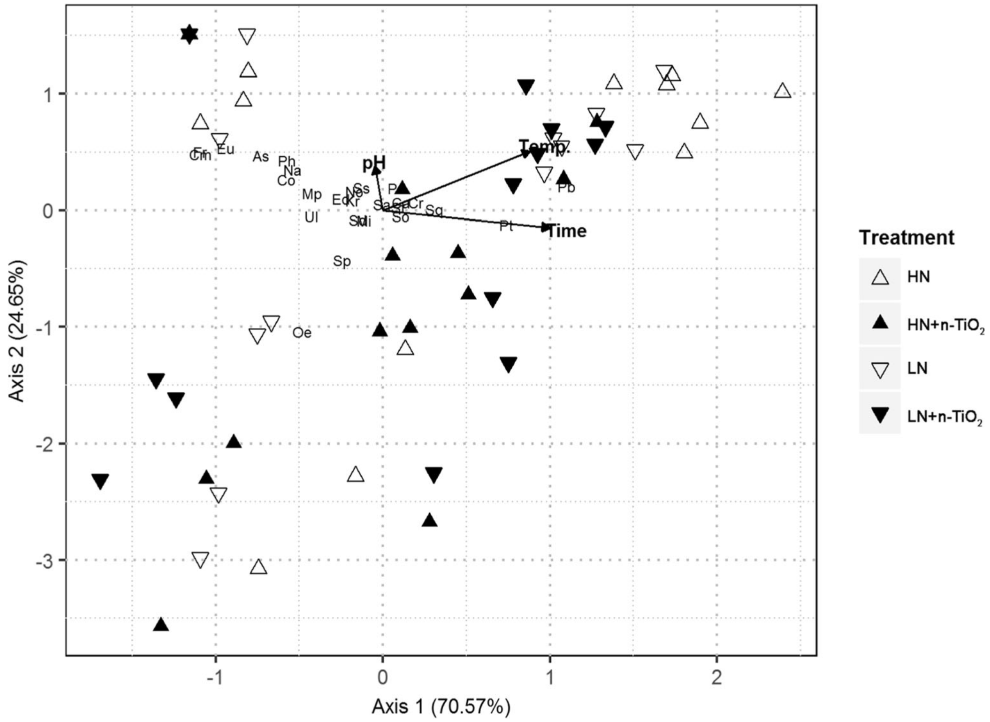


Fig. 6 Canonical correspondence analysis of environmental factors and phytoplankton species abundance as a function of nitrogen and titanium dioxide nanoparticle treatments. **Sq** (*Scenedesmus quadricauda*), **Sd** (*Scenedesmus disciformis*), **Sa** (*Scenedesmus acuminatus*), **St** (*Scenedesmus tenuispina*), **So** (*Scenedesmus ornatus*), **Ss** (*Scenedesmus* sp.), **Cr** (*Coelastrum reticulum*), **Ca** (*Coelastrum astroideum*), **Pt** (*Pediastrum tetras*), **Pb** (*Pediastrum boryanum*), **As** (*Ankistrodesmus spiralis*), **Kr** (*Kirchneriella* sp.), **Mp** (*Microspora* sp.), **Oe** (*Oedogonium* sp.), **Ul** (*Ulothrix* sp.), **Ed** (*Eudorina* sp.), **Co** (*Cosmarium* sp.), **Sp** (*Spirogyra* sp.), **Ps** (*Pseudo-nitzschia* sp.), **Na** (*Navicula* sp.), **Fr** (*Fragilaria* sp.), **Mi** (*Microcystis* sp.), **No** (*Nostoc* sp.), **Ph** (*Phacus* sp.), **Eu** (*Euglena* sp.), **Cm** (*Ceratium* sp.)

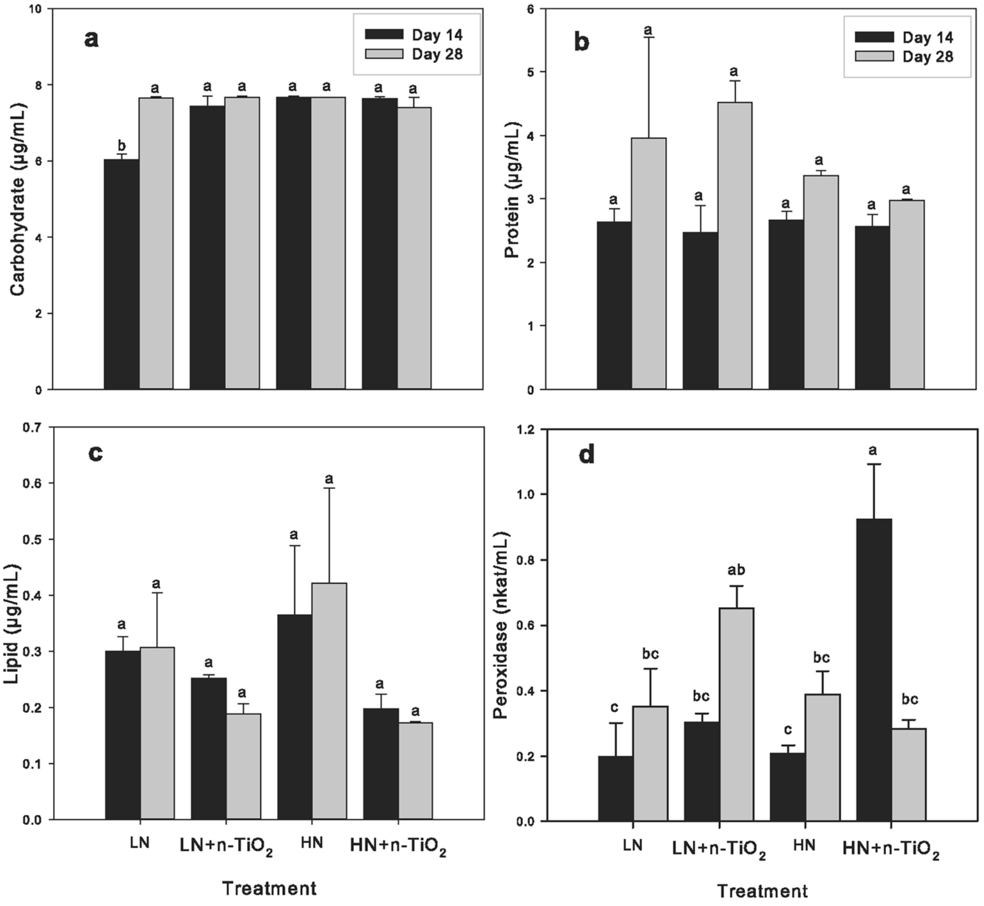


Fig. 7 Carbohydrate content (**a**), protein content (**b**), lipid content (**c**), and peroxidase enzyme activity (**d**) of phytoplankton community exposed to different nitrogen and titanium dioxide nanoparticle treatments

Nitrogen concentrations in the LN mesocosms increased over the study period, supporting an increase in phytoplankton biomass. The increase in N concentration in the LN mesocosms can be attributed to nitrogen fixation by diazotrophs. Depending on the primary form of nitrogen, availability, and levels in the environment, N fixation is

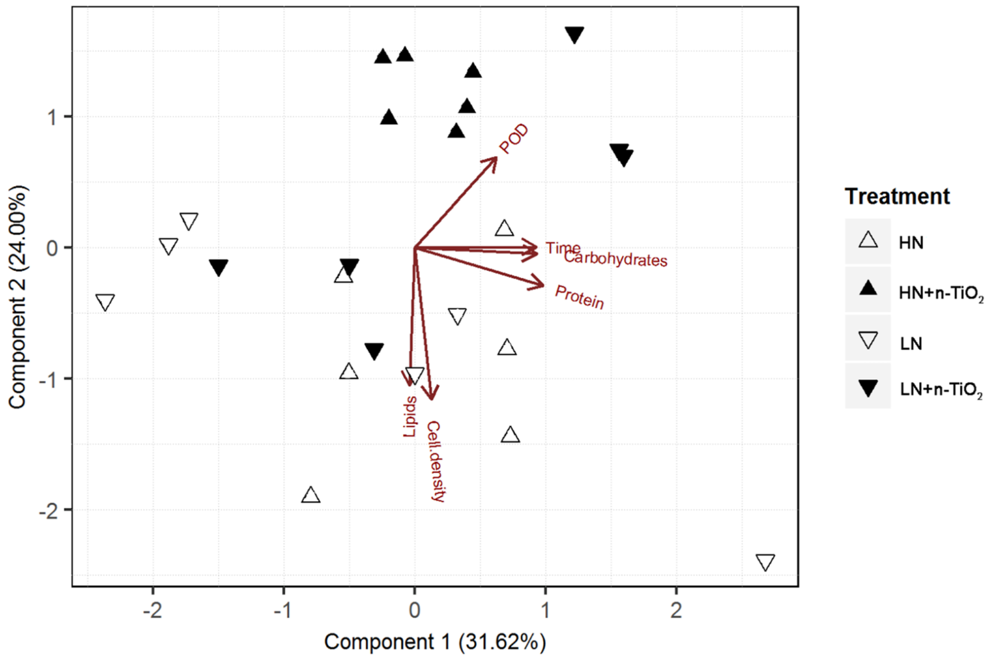


Fig. 8 Principal component analysis biplot of the biochemical parameters and cell density of phytoplankton as a function of nitrogen and titanium dioxide nanoparticle treatments

initiated to meet the specific needs of diazotrophs (Scott et al., 2008). The majority of diazotrophic cyanobacteria, such as *Nostoc* sp. that form blooms, substantially contribute to the speciation and availability of N for primary production in aquatic ecosystems (Scott and Grantz 2013). *Nostoc* sp. was the only diazotrophic cyanobacteria recorded in this study. Similar to a mesocosm where there is no water flow, Scott et al. (2008) recorded a 37% N load due to atmospheric N fixation during summertime in a watershed with low stream flow. This outcome suggests that even without anthropogenic N loading, N concentrations in mesocosms can rise after a while. Aside from diazotrophic cyanobacteria, our mesocosms experiments contained heterotrophic and anaerobic bacteria (data not shown), and the benthic fixation by these bacteria is a major source of N in many oligotrophic tropical lagoons and oligotrophic lakes (Howarth et al. 1988). The dissolved organic carbon excreted through active exudation by phytoplankton under nutrient limitation (Livanou et al. 2017) is readily available for the consumption and growth of heterotrophic bacteria (Morana et al. 2014; Thornton 2014).

The presence of n-TiO_2 and the interaction of N with n-TiO_2 reduced total phytoplankton biomass. This reduction in biomass indicates an impairment in cell division and growth. Similar to our findings, Das et al. (2014) observed that the interaction between silver nanoparticles and phosphorus variation led to 70–90% lower biovolumes of phytoplankton during a field study. Cardinale et al. (2012) also reported that n-TiO_2 hinders algal growth rates, respiration, and gross primary production, but these effects can vary depending on species-specific metabolic functions. Nitrate is also reduced through denitrification in the presence of n-TiO_2 (Lozovskii et al. 2010; Yang et al. 2013) and this reduction process is even faster under UV light (Shaban et al. 2016). These processes in our mesocosms could explain the negative outcome of n-TiO_2 and its interaction on the phytoplankton biomass production and species richness.

Contrary to the indifference recorded in species diversity index under the different treatments, species richness, which is the number of species per unit of area, was highest under LN. This suggests that the phytoplankton reproduced and multiplied best under LN, followed by HN and LN + n-TiO_2 conditions. The HN + n-TiO_2 treatment supported the lowest species richness and the highest reduction of phytoplankton biomass. On the contrary, more cyanobacteria occurred, primarily dominated by *Microcystis* sp., a harmful bloom-forming species, under HN + n-TiO_2 treatment (Wang et al. 2015; Xiao et al. 2018). This result suggests that in addition to already known contributors, changing global climate and eutrophication, to the increased frequency and magnitude of algal blooms, the combinations of high nutrients with metal nanoparticles such as titanium dioxide are playing a significant but under-reported role in the formation of Cyanobacterial harmful algal blooms (CyanoHABs). This interaction may be leading to substantial ecosystem alterations per phytoplankton community structure with severe cascading effects on higher level organisms and loss of vital biodiversity.

In addition to the effects of the treatments (LN, LN + n-TiO_2, HN, and HN + n-TiO_2) on the phytoplankton community, temperature changes substantially contributed to their collective influence. Global temperatures are expected to continue to increase over the next decades due to the degradation of the ozone layer by human activities. As a response, the increases in temperature are already known to support the excessive proliferation of bloom-forming cyanobacteria such as *Microcystis* (Rasconi et al. 2017; Scherer et al. 2017). This positive relationship between rising temperatures and *Microcystis* sp. bloom formation is further enhanced by the presence of n-TiO_2 and high nitrogen concentration, as revealed in the present study. This portrays a serious near-future environmental problem because the use of engineered nanometals is on the increase, which means levels close to that investigated in the present study may be found in aquatic ecosystems in the future.

Changes in phytoplankton biochemical composition have a cascading effect on the health of the aquatic food web (Peltomaa et al. 2017). Our results revealed that changing N levels in the presence of n-TiO_2 significantly affected the levels of carbohydrate in the phytoplankton community compared with total lipid and protein content. The LN + n-TiO_2, HN, and HN + n-TiO_2 treatments increased the total carbohydrate content of the phytoplankton community. This finding suggests that the presence of n-TiO_2 and its interaction with N increased the synthesis and accumulation of carbohydrates by phytoplankton cells irrespective of their cell densities. The accumulation of this storage biomolecule in phytoplankton has been reported to be a physiological adaptation to stress (Chia et al. 2015; Liefer et al. 2019). The accumulation of carbohydrates is caused by stunted growth of algal cells, where in place of growth, the cells invest in the accumulation of carbohydrates to store excess fixed carbon produced by unbalanced carbon and nitrogen metabolism (Chia et al. 2015). Also, carbohydrate production is linked to signaling triggers during high production of reactive oxygen species alongside the production of specific ROS scavengers, and at sufficient quantities, carbohydrates also act as direct ROS scavengers (Liang et al. 2020). In agreement with our result, Marchello et al. (2018) also reported an increase in carbohydrate content in *Chlorella sorokiniana* in the presence of n-TiO_2.

One of the major effects of metal nanoparticles on algae is the induction of oxidative stress (Bundschuh et al. 2016; Chen et al. 2019). The production of reactive oxygen species (ROS) in the presence of nanoparticles is influenced by algal species composition (Chen et al., 2019). Peroxidase is an antioxidant enzyme produced by cells to neutralize the effect of H_2O_2 (Davidson et al. 2015), which is a common ROS produced under stress conditions. Our result shows that changing N levels do not upregulate POD activity in the phytoplankton community over time, while the interaction of N with n-TiO_2 triggers higher activities of the enzyme in a time-dependent manner. We found similar responses during laboratory experiments with *C. vulgaris*, where the microalga was exposed to replete and limited nitrate conditions combined with n-TiO_2 exposure (Dauda et al. 2017). In this study, lowering nitrogen concentrations resulted in higher POD activity in the presence of n-TiO_2 compared with the treatment with replete levels of nitrogen. The decrease in POD enzyme activity recorded after 4 weeks under the HN + n-TiO_2 condition probably implies a failure of the POD enzyme defense mechanism of the phytoplankton cells after prolonged exposure to this condition. In line with this finding, Akter et al. (2018) suggested that oxidative stress resulting from metal nanoparticles can damage the antioxidant defense capacity of cells. Thus, aquatic ecosystems with LN and high levels of n-TiO_2 can induce oxidative and cellular damage to phytoplankton populations.

Conclusion

In conclusion, our results revealed that phytoplankton growth, biochemical composition, and antioxidant response are influenced by changing N conditions and the presence of n-TiO_2. However, the effect of n-TiO_2 on phytoplankton community structure and dynamics were mostly dependent on the concentration of N in the mesocosm. We observed that the effects of the treatment conditions were enhanced by rising temperatures, leading to the formation of a *Microcystis* sp. bloom at the end of the study. Therefore, the interaction of n-TiO_2 with N levels in oligotrophic and eutrophic aquatic ecosystems is capable of changing phytoplankton community structure and dynamics.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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