

Changes in photosynthetic parameters and lipid classes of N-limited freshwater microalga under cadmium exposure

Giseli Swerts Rocha (✉ swertsbio@gmail.com)

NEEA/CRHEA, University of São Paulo (USP)

Christopher C. Parrish

Memorial University of Newfoundland

Evaldo L.G. Espíndola

NEEA/CRHEA, University of São Paulo (USP)

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Abstract

In the environment, algae are exposed to non-optimal conditions and adapt their physiology to cope with adverse conditions, such as limiting nutrients or excess contaminants. The present study evaluated the changes in photosynthetic parameters and lipid classes of the freshwater green microalga *Ankistrodesmus densus* exposed to nitrogen (N) limitation and cadmium (Cd) exposure. While N is required for the algal metabolism, Cd does not present known functions in freshwater microalgae. Our results indicate that the growth rate was more affected by metal than by the nutrient. At the same time, the quenchings, i.e., fractions of light energy driven to photochemical or non-photochemical processes of photosynthesis, were not affected under the N limitation. However, in the combination of the two stressors, the changes were more pronounced, suggesting a synergism in the growth rate, chlorophyll *a* production, photochemical quenching (qP), and effective quantum yield ((Y(II))). While Cd did not affect any lipid classes, the percentage of storage lipids (triacylglycerol) was increased under N limitation and in some of the combinations of N limitation and Cd. However, in the combination of the stressors, there were some non-linear changes in structural lipids (phospholipids) and a decrease in acetone mobile polar lipids. Our data indicate that the combination of the two stressors affected algal physiology with synergism in several parameters. However, the algal photoprotective mechanisms effectively avoided photodamage, as indicated by non-photochemical quenchings (qN and NPQ) and in the quantum yield related to regulated energy loss Y (NPQ) values.

Introduction

Algae require some essential nutrients, such as nitrogen and phosphorus, for optimal growth and metabolism. Their limitation can drive some physiological changes, e.g., impairment in photosynthesis (Zhao et al. 2017), photodamage to photosystem II (PSII) (Bouchard et al. 2008), and decrease in chlorophyll (Geider et al. 1993) and in respiration (Alcoverro et al. 2000). Nitrogen is needed to form proteins (Chia et al. 2013), nucleic acids, phospholipids with nitrogenous bases, and pigment metabolism (Turpin 1991). When limitation occurs in the environment, it affects those parameters and, consequently, the metabolism of algae, while excess of N can result in algal blooms and eutrophication.

Cadmium is a non-essential metal, without known function in freshwater microalgae, which can affect photosynthesis (Bauman et al. 2009), reactive oxygen species production (Qian et al. 2009; Alho et al. 2019), cell viability, and growth (Volland et al. 2014; Echeveste et al. 2017); replace Mg in the light harvesting center (Andresen et al. 2016); displacing Ca in photosystem II (PSII)-water-oxidizing complex, altering Ca homeostasis (Andosch et al. 2012); decrease or increase chlorophyll *a* production (Marchello et al. 2018; Rocha et al. 2020, respectively); alter lipid classes and fatty acids saturation (Rocha et al. 2020) and biomass composition (Chia et al. 2013, Alho et al. 2019). In combination with other stressors, such as nutrient limitation, the effects are usually more deleterious (Chia et al. 2013), where synergism can be observed (Rocha et al. 2021).

The assessment of chlorophyll fluorescence using the pulse amplitude modulation (PAM) fluorometers (e.g., Phyto-PAM) can provide information about the effects of stressors in photosynthesis, such as nutrients (e.g., Zhao et al. 2017), metals (e.g., Baumann et al. 2009), or the combination of two stressors, such as phosphorus limitation and Cd (e.g., Rocha et al. 2021). With the values obtained with dark- or light-adapted samples, it is possible to infer about the use of photochemical energy, including heat or fluorescence dissipation mechanisms, under normal or stressful conditions. In summary, the information obtained by PAM fluorometers can provide a deeper understanding of the impacts of the stressors in the photosynthesis.

The combination of assessing different endpoints, such as photosynthetic and biochemical changes, help to elucidate the mechanisms affected under different stressful scenarios (Baracho et al. 2019). The freshwater microalga *Ankistrodesmus densus* is widely distributed worldwide (e.g., John and Tsarenko 2002; Rosset et al. 2020) and it is sensitive to several stressors, such as Cd, P, and nanoparticles (Barreto et al. 2021; Rocha et al. 2021). In a previous study (Rocha and Espíndola 2021), we could observe the interactions of N-limitation and copper excess in the photosynthetic responses of the microalga *A. densus*, where the combination of both stressors was more deleterious than the isolated effects. Thus, aiming at evaluating the impacts of the combination of acclimated N-limited *Ankistrodesmus densus* and Cd exposure, we assessed changes in growth, lipid classes and photosynthetic parameters, such as maximum and operational quantum yield, photochemical and non-photochemical quenching, and the regulated and non-regulated non-photochemical energy loss in photosystem II (PS II).

Material And Methods

The freshwater microalga *Ankistrodesmus densus* (Chlorophyceae) was obtained from the algae culture collection of the Botany Department at the Federal University of São Carlos (CCMA-UFSCar 3; São Carlos, SP, Brazil). Stock cultures were kept in WC medium (Guillard and Lorenzen 1972) without EDTA. The algae were cultured at pH 7.0 under controlled conditions of light intensity ($130 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), photoperiod (12:12 h light: dark cycle), and temperature ($22 \pm 2^\circ\text{C}$). Algal cells were acclimated to 2 different nitrogen (N) concentrations: 1mM N (control) and 0.01 mM N (1% N). Exponentially growing cells were transferred every 72 h to a fresh medium containing different amounts of N. Cells were checked under the microscope and measurements in a pulse amplitude modulated fluorometer PhytoPAM (PhytoPAM I, Walz, Germany) were done daily to confirm acclimation before starting the toxicity tests. During exposure to N-limited medium, the maximum quantum yield was decreasing slightly and after at least 5 renovations of medium where the values were similar, we considered the algae acclimated to this new condition. The acclimation period lasted for 35 days. Exponentially growing *Ankistrodesmus densus* cells N-acclimated (1 mM N – control and 0.01 mM N) were exposed for 72 h to 4 cadmium (CdCl_2 Titrisol 1000 mg L^{-1} - Merck) concentrations: 0 - control; 0.5; 1.0 and 2.0 μM Cd, which were defined after preliminary tests. Cadmium concentrations higher than 2.0 μM did not allow the growth of algae in N-limited medium. The initial concentration of algal cells in the preliminary tests and in experiments was $5 \times 10^5 \text{ cells mL}^{-1}$. Each treatment had three replicates. Nitrogen was determined as

nitrate in samples following the methodology 4500-NO₃-B described in APHA (1995). Samples were acidified with HCl 1 N and read in a spectrophotometer at 220 nm.

The metal present in the culture media was determined as described by Rocha et al. (2020). Briefly, samples with Cd were acidified with 1% HNO₃ and measured by inductively coupled plasma – optical emission spectrometry (Thermo ICP-OES, iCAP 7000; Thermo Fischer Scientific, Madison, WI, EUA), and quantified using 228.80 nm emission line. The quantification limits was 0.01 mg L⁻¹, and the detections limits were 0.00006 mg L⁻¹. Data are presented as a function of free Cd²⁺ ions, estimated through the chemical equilibrium MINEQL⁺ 4.62.3 (Environmental Research Software, Hallowell, ME, USA).

Samples were fixed with Lugol's every 24 h and counted under an optical microscope using a Neubauer hemocytometer. Growth rate was calculated according to USEPA (2012) - $\mu = [\ln(df) - \ln(di)]/t$; where: μ = specific growth rate (day⁻¹); df = cell density at the end of the experiment; di = cell density at the beginning of the experiment; t = exposure time (in days). Chlorophyll *a* was determined after filtering samples (10 mL) onto cellulose ester membranes (0.45 μ m pore size – Millipore) and extracting with DMSO (Shoaf and Lium 1976). Absorption was measured at 664 and 647 nm wavelengths in a spectrophotometer (HACH DR 5000, USA). Chlorophyll *a* concentration was calculated according to Jeffrey and Humphrey (1975).

Chlorophyll fluorescence was assessed using Phyto - PAM I equipped with an ED-101US/MP optical unit. Two mL were taken up and dark-adapted for 15 minutes to allow PS II reaction centers to oxidize completely. With the values of initial (F_0), maximum (F_M), and variable ($F_v = F_M - F_0$) fluorescence, we obtained the maximum photosynthetic efficiency of PSII ($\Phi = F_v/F_M$) at 2, 24, 48, and 72 h after metal exposure. At 72 h, the samples were exposed to continuous actinic light (128 μ mol photons m⁻² s⁻¹) to obtain the parameters of light-adapted samples (pulses every 20 s, during 15 min). With the steady-state chlorophyll fluorescence (F_s), and maximum fluorescence in light (F'_m), the calculation of the operational quantum yield ($((Y(II) = (F'_m - F_s)/F'_m))$), photochemical quenching ($((qP = (F'_m - F_s) / (F'_m - F_0))$), non-photochemical quenching [$qN = 1 - [(F'_m - F'_0) / (F'_m - F_0)]$](Juneau et al. 2002), and Stern-Volmer non-photochemical quenching ($((NPQ = (F'_m - F'_0)/F'_m))$) (Maxwell and Johnson 2000) were performed, as well as the quantum yield of regulated ($((Y(NPQ) = (F_s / F'_m) - F_s / F_m))$) and non-regulated non-photochemical ($((Y(NO) = F_s / F_m))$) energy loss in PSII (Klughammer and Schreiber 2008).

Total lipids were extracted using chloroform: methanol: chloroform-extracted water (2:1:1), as described in Rocha et al. (2018), with determination using a TLC/FID Iatroscan MK6s (Mitsubishi Kagaku Iatron Inc., Tokyo, Japan). Briefly, samples (100 mL) were filtered on baked glass fiber filters, ground with a metal rod, and lipid extractions were performed with the use of methanol: chloroform: chloroform extracted water (5:4:1) once, and the procedure was repeated three more times only with the addition chloroform. Samples were flushed with nitrogen and conserved at -20 °C, and in the following day the samples were spotted in quartz rods. After three development systems, the lipid classes aliphatic hydrocarbon (HC), wax ester/steryl ester (WE/SE), ketone (KET), triacylglycerol (TAG), free fatty acid (FFA), aliphatic alcohol

(ALC), sterol (ST), acetone mobile polar lipid (AMPL) and phospholipid (PL) were detected and compared to lipid standards from Sigma-Aldrich (USA). Analytical conditions for latroscan runs were hydrogen flow 173 mL min⁻¹, air flow 2 L min⁻¹ and scan speed 4 mm s⁻¹. Chromatograms were integrated using Peak Simple 4.88 software.

The data were tested for normality and homogeneity of variance. Statistically significant differences among treatments and controls were determined using one-way ANOVA and Tukey's post hoc at $p < 0.05$ (SigmaPlot 11, Systat). The data were obtained from three experimental replicate cultures and are presented as mean $\pm$ SD of the replicates. Two-way ANOVA was done to detect the effects of Cd and N on the parameters analyzed.

When the measured parameter in treatments with the combination of both stressors was significantly lower than that for control or the single stressor (N limitation or Cd), we assumed the independent action model to calculate the predicted and observed effects, and the synergy ratio. The predicted effects were calculated using Eq. 1; the observed effects in the mixture were calculated using Eq. 2, and the synergy ratio of N + Cd was calculated using Eq. 3, using the predicted and observed effects (Gottardi et al. 2017). The synergy ratios below 1 indicate antagonism, equal to 1 indicates additivity, and higher than 1 indicates synergism.

$$(1) \text{ Predicted} = \frac{N}{C} \times \frac{Cd}{C},$$

where N refers to the value of the endpoint obtained under N-limitation exposure; Cd, under cadmium exposure, and C refers to control.

$$(2) \text{ Observed} = \frac{Mix}{C},$$

where the Mix is the endpoint's value under the combination of N-limitation and Cd exposure.

$$(3) \text{ Synergyratio} = \frac{\text{Predicted}}{\text{Observed}},$$

where values lower and higher than 1 indicate antagonism and synergism, respectively.

Results

The growth rates were more sensitive than the chlorophyll *a* concentration in the presence of Cd in control treatments, while the N- limitation affected chlorophyll *a* concentration more (Table 1). The values obtained for maximum quantum yield at 72 h ($\Phi_M = F_v/F_m$) were affected in the presence of Cd and under N limitation, although it was one of the least affected parameters in this study. Since the values obtained for these parameters were lower in the combination of the stressors than in the control, it was possible to calculate the synergy ratio (Supplementary Table 1), which shows synergism only at the growth rate in the combination of the stressors.

Table 1

Growth rate, chlorophyll *a* concentration, and maximum quantum yield of *Ankistrodesmus densus* under nitrogen and cadmium exposure.

Nitrogen (mM N)	Cd ²⁺ (μM L ⁻¹)	Growth rate (day ⁻¹)	Chlorophyll <i>a</i> (μg mL ⁻¹)	Maximum quantum yield (Φ _M)
1.00 (C)	9.8 x 10 ⁻¹³ (C)	1.07 ± 0.12 ^a	0.60 ± 0.03 ^a	0.68 ± 0.01 ^a
1.00 (C)	0.49	0.51 ± 0.06 ^c	0.61 ± 0.03 ^a	0.65 ± 0.01 ^{ab}
1.00 (C)	0.98	0.37 ± 0.03 ^d	0.58 ± 0.04 ^a	0.64 ± 0.01 ^{bc}
1.00 (C)	1.96	0.28 ± 0.03 ^{de}	0.40 ± 0.02 ^b	0.65 ± 0.01 ^{bc}
0.01 (1%)	9.8 x 10 ⁻¹³ (C)	0.78 ± 0.06 ^b	0.31 ± 0.02 ^c	0.62 ± 0.02 ^{cd}
0.01 (1%)	0.49	0.30 ± 0.03 ^{de}	0.27 ± 0.01 ^c	0.61 ± 0.01 ^d
0.01 (1%)	0.98	0.22 ± 0.02 ^{ef}	0.40 ± 0.03 ^b	0.63 ± 0.01 ^{bcd}
0.01 (1%)	1.96	0.15 ± 0.02 ^f	0.31 ± 0.01 ^c	0.63 ± 0.01 ^{bcd}

The presence of Cd slightly decreased the values of qP in control only at the highest concentration (≈ 6% lower). In contrast, in the N limited treatment, all of the Cd concentrations decreased qP to values lower than observed in non-limited algae (Fig. 1A). The same behavior was observed in qN (Fig. 1B) and NPQ (Fig. 1C), with Cd altering these parameters only at the highest concentration in non-limited cells and in all treatments under N limitation. It is interesting to note that, contrary to what was observed for Φ_M (dark-adapted samples), the quenching parameters were not altered with less N in control (without the addition of Cd).

The complementary quenchings Y (II), Y (NO), and Y (NPQ) are shown in Fig. 2. It is possible to observe that N limitation did not change any of these parameters significantly, i.e., Y (II) decreased 6%, and Y (NO) increased 7%, and without differences in Y (NPQ) in N-limited medium compared to control. In contrast, Cd decreased the Y (II) (Fig. 2A) and increased the Y (NPQ) (Fig. 2C) in both treatments (N-limited and N-replete), with greater damage by Cd under N-limitation. The Y (NPQ) was the most affected parameter, indicating that algal photoprotective mechanisms were activated to avoid or decrease damage under Cd exposure, especially under N limitation.

The lipid class TAG increased under N limitation and in the presence of Cd (Table 2), with the highest values observed in the combination of N-limited and Cd, except at the highest metal concentration. The changes caused by Cd in the control treatment were not significantly different, except for the increase of TAG in 0.49 μM Cd²⁺ L⁻¹. The changes in PL were not linear, only being significantly affected in the combination of N limitation and 0.49 μM Cd²⁺ L⁻¹, and higher values were observed in the combination

of N-limitation and highest Cd. The HC was higher under N limitation, but without significant differences in Cd exposure or in the combination of N limitation or Cd.

Table 2

Lipid composition (% of total lipids) of *Ankistrodesmus densus* under nitrogen and cadmium exposure. Columns without a letter in common are statistically different ($p < 0.05$). HC (aliphatic hydrocarbon); SE/WE (steryl ester/wax ester); KET (ketone); TAG (triacylglycerol); FFA (free fatty acid); ALC (aliphatic alcohol); ST (sterol); AMPL (acetone mobile polar lipid); PL (phospholipid). Different superscript letters indicate statistically significant differences. *C refers to control.

Nitrogen (mM N)	Cd ²⁺ (μM L ⁻¹)	HC	TAG	FFA	ST	AMPL	PL
1.00 (C)	9.8 x 10 ⁻¹³ (C)	2.3 ± 0.2 ^a	21.6 ± 0.3 ^e	5.1 ± 1.8 ^{ab}	6.2 ± 3.0	24.5 ± 7.0 ^{ab}	40.7 ± 4.6 ^a
1.00 (C)	0.49	2.5 ± 0.3 ^a	33.2 ± 3.8 ^{cd}	6.2 ± 0.7 ^{ab}	5.3 ± 1.3	17.6 ± 1.4 ^{bc}	35.3 ± 6.5 ^{ab}
1.00 (C)	0.98	2.9 ± 0.5 ^a	25.5 ± 2.5 ^{de}	1.0 ± 1.0 ^b	8.1 ± 3.4	28.8 ± 5.0 ^a	33.7 ± 3.9 ^{ab}
1.00 (C)	1.96	3.1 ± 0.4 ^a	30.4 ± 2.2 ^{cde}	4.4 ± 3.8 ^{ab}	5.4 ± 3.6	19.1 ± 2.6 ^{abc}	37.5 ± 4.4 ^a
0.01 (1%)	9.8 x 10 ⁻¹³ (C)	5.1 ± 0.6 ^b	36.2 ± 3.3 ^{bc}	4.1 ± 1.3 ^{ab}	7.7 ± 0.9	17.9 ± 1.9 ^{abc}	29.0 ± 6.2 ^{ab}
0.01 (1%)	0.49	2.8 ± 0.3 ^a	52.4 ± 5.2 ^a	6.9 ± 1.9 ^a	2.4 ± 0.9	13.2 ± 0.6 ^c	22.2 ± 7.7 ^b
0.01 (1%)	0.98	2.8 ± 0.9 ^a	43.4 ± 5.8 ^{ab}	5.9 ± 1.6 ^{ab}	5.5 ± 0.5	10.5 ± 4.2 ^c	31.8 ± 3.5 ^{ab}
0.01 (1%)	1.96	3.0 ± 0.5 ^a	28.2 ± 0.4 ^{cde}	6.4 ± 1.9 ^{ab}	7.9 ± 2.3	12.0 ± 4.2 ^c	42.4 ± 4.0 ^a

Discussion

The growth was affected under the combination of both stressors, especially when combined, with synergistic effects. In the study of Alho et al. (2019), the authors observed a decrease in the growth of *Raphidocelis subcapitata* and an increase in chlorophyll *a* concentration under Cd exposure. Although the substitution of Mg by Cd can occur in chlorophyll molecule (Küpper et al. 1996), it is interesting that there was no synergism in all of the combinations of N and Cd stress in chlorophyll *a* concentrations (Supplementary Table 1), indicating that the amount of N available affected the amount of pigment more. These data are different from those obtained by Rocha and Espíndola (2021), where synergism occurred in the combination of N-limitation and Cu exposure. The higher interference of N limitation on the amount of pigment can be related to N's use in pigment metabolism (Turpin 1991).

The slight decrease in maximum quantum yield under Cd exposure does not corroborate previous studies, where this parameter was a sensitive endpoint for the microalga *Raphidocelis subcapitata* (Alho et al. 2019) and *Micrasterias denticulata* (Andosch et al. 2012). The combination of the two stressors was not synergistic (Supplementary Table 1), differing from our previous study where N-limited algae exposed to copper were more affected than isolated stressors (Rocha and Espíndola 2021), suggesting a metal-specific response. The maximum quantum yield, which can provide information about the physiological state of algae, being proportional to the PS II activity, and affected under environmental stress (Krause and Weis 1991), was the least sensitive parameter evaluated, corroborating Parkhill et al. (2001) who do not recommend its use for nutrient acclimated algae. This highlights the importance of the previous acclimation to nutrient-limited media since some authors observed a decrease in Φ_M of about 35% when changing the culture directly from nutrient replete to nutrient-depleted media (White et al. 2011).

With regards to the quenchings, less N was responsible for the higher impacts on the presence of Cd in the qP, qN, and NPQ parameters, indicating that less N was not enough to change these parameters, differing from the study by White et al. (2011), where the removal of nitrate from BG 11 medium resulted in a 6-fold increase in the values of NPQ of *Chlorella* sp. However, with the addition of metal, more damages were observed in N-limited cells than in N-replete cells, suggesting a synergistic effect (Supplementary Table 1). The slight decrease (about 10%) in Φ_M and lower values of qP and higher qN and NPQ in nitrogen-limited cells under Cd exposure can indicate photodamage protection and no photoinhibition in the tested conditions (Cosgrove and Borowitzka 2011; Malapáscua et al. 2014).

The slight decrease in qP in control cells exposed to Cd corroborates the data of Echeveste et al. (2017) at the concentrations close to those analyzed here, while the combination of N-limitation and Cd decreased qP about 23%. This could indicate decreased PS II electron transport rate once less energy is used for electron transport (Juneau et al. 2002) and a higher proportion of closed reaction centers (Mallick and Mohn 2003). The parameters qN and NPQ were more sensitive than qP with the addition of Cd in control (an increase of 15% and 60%, respectively). In comparison, the combination of N-limitation and Cd increased 115% and 200%, respectively, indicating a higher sensitivity of NPQ. The increase in NPQ can indicate protection from photodamage (Baracho et al. 2019) and is related to the increase in heat dissipation (Lombardi and Maldonado 2011), which is compensated by the decrease in Y (II), as observed in this study.

The effective quantum yield – Y (II) – was more sensitive than the maximum quantum yield, corroborating data by Echeveste et al. (2017) in *Chlorolobion braunii* and by Alho et al. (2019) in *Raphidocelis subcapitata*, and differing from those by Wang and Dei (2006), where the response of the two parameters in *Chlamydomonas reinhardtii* was similar. Its decrease under N limitation and highest Cd exposure can be related to the decrease in qP in this scenario, with a similar decrease in percentage ($\approx 23\%$). On the other hand, Y (NO) (Fig. 2B) was the least affected quenching, indicating that there was no change in the passive dissipation of heat and fluorescence under any source of stress (Cd or N), indicating that mechanisms used to dissipate energy passively were not activated, and, consequently, the ability of algae to cope with the excess of illumination was not affected (Klughammer and Schreiber

2008). Comparing the results of the present study with a previous one with the species *Selenastrum gracile* (Rocha et al. 2020), we suggest that Y (NO) and Y (NPQ) can be species-specific, i.e., the mechanisms of active heat dissipation ((Y(NPQ))) were activated in *A. densus* under Cd or N stress, while in *S. gracile*, the passive mechanisms of dissipation ((Y(NO))) were activated under Cd exposure, which indicates a higher photoprotective capacity of *A. densus* compared to *S. gracile*, and there is no indication of damages in photoprotection mechanisms of *A. densus* under the tested Cd or N concentrations, isolated or combined.

With regard to the lipid composition, the AMPL decreased about 50% under Cd exposure combined with N limitation, corroborating data by Chia et al. (2013) in the microalga *Chlorella vulgaris*. The availability of nutrients can affect biomass quality (Rocha et al. 2018), and the increase of TAG under stress is well documented in the literature. It can be related to increased energy reserves to cope with adverse scenarios (Cooksey 2015), including N limitation (Huang et al. 2019), probably due to N's ability to change biochemical pathways (Touzet et al. 2007). On the other hand, it is interesting to note that there were some shifts between which one was the most representative lipid class; under control or extreme stress, PL was the main class, while under moderated stress - less N and lower amount of metal - TAG was the most representative, i.e., PL decreased in the N limited and Cd combination, however it increased at the highest combination, with a decrease in TAG, indicating investment in structural lipids to reduce damage to the structure of the algae under more elevated stress (Rocha et al. 2020).

In summary, Cd and N limitation affected the growth rate, chlorophyll *a*, and the maximum quantum yield. N limitation did not affect the quenchings related to the use of photochemical energy ((qP, qN, NPQ, Y(II), and Y (NPQ))), while Cd did. N limitation increased the amount of lipid classes HC and TAG, with Cd not exerting any significant influence in any of the lipid classes. Still, N limitation and Cd stress combined resulted in greater changes than only Cd or N limitation, with a synergism under N limitation and Cd exposure in the growth rate, qP, and Y (II) parameters. In addition, the higher increase observed in qN, NPQ, and Y (NPQ) suggest a synergism in these parameters and that the photoprotective mechanisms were efficient in avoiding photodamage, minimizing photoinhibition. More N allows better driving of light energy, as observed in light-adapted samples; however, P limitation was more deleterious than N limitation in photosynthetic parameters of *A. densus* in our previous study (Rocha et al. 2021). The AMPL was the most affected lipid class in the combination of N limitation and Cd; and HC was the most affected lipid class under N limitation. However, for the other lipid classes, it was not possible to observe a clear trend of changes, i.e., while higher values of TAG were observed under N limitation and two moderated concentrations of Cd, at the combination of N limitation and the highest Cd concentration, values were not different from the control; and PL was significantly lower only at the combination of N limitation and the lowest Cd concentration. Based on our results, we suggest more studies dealing with nutrient limitation and excess of other contaminants to obtain more information on the effects of these mixtures in the photosynthesis of microalgae.

Declarations

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Data will be made available under request

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Figures

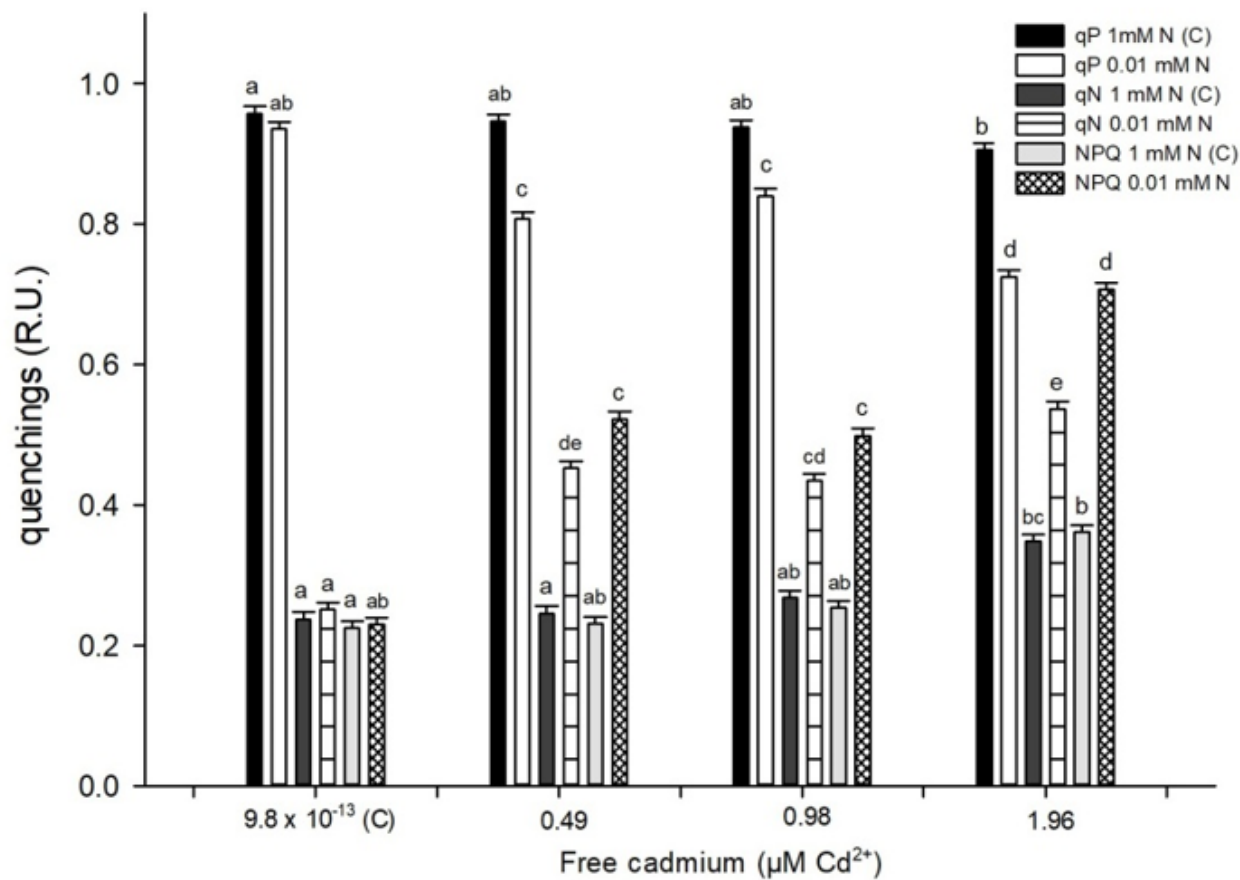


Figure 1

Quenchings of *Ankistrodesmus densus* at 72 h exposed to different Cd and N concentrations. Photochemical quenching (qP) and non-photochemical quenchings (qN and NPQ). Separate statistical analysis were performed for each quenching evaluated.

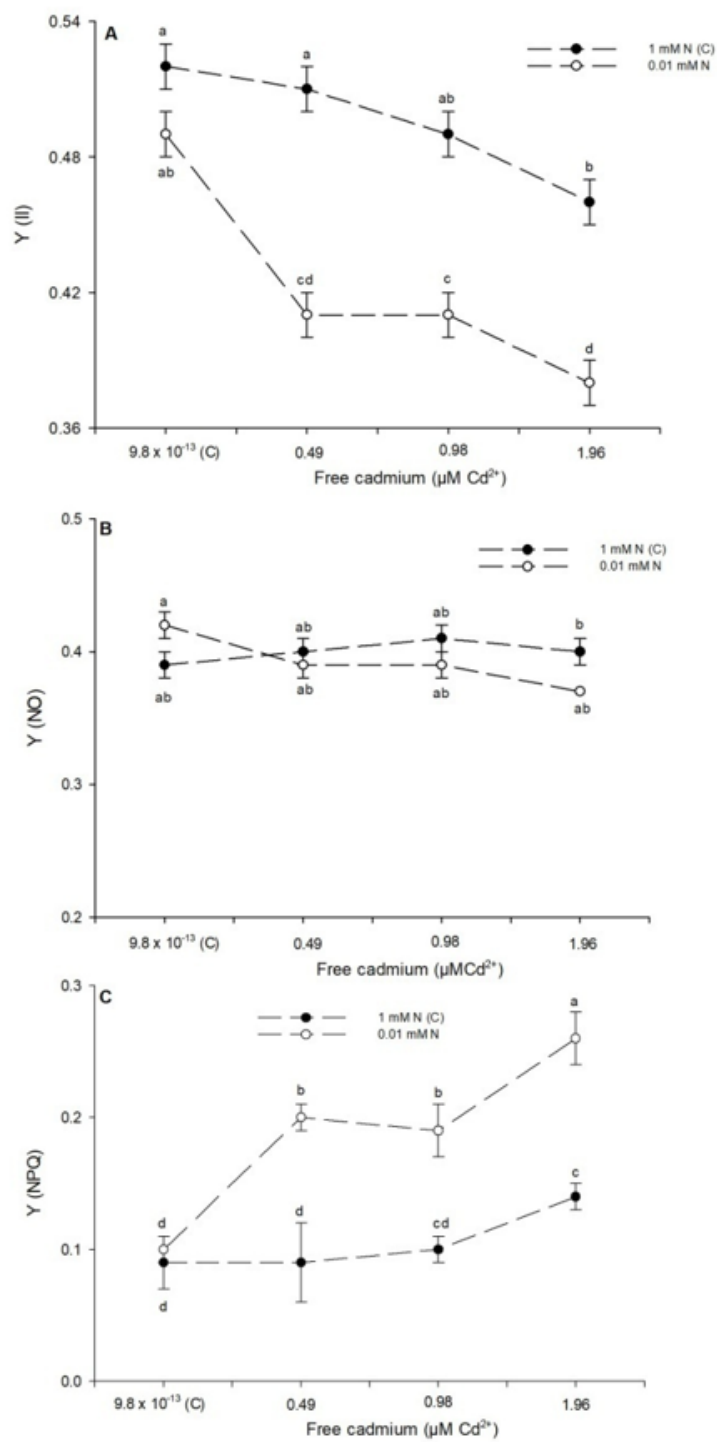


Figure 2

Effective quantum yield - Y (II) (A); energy dissipated passively - Y (NO) (B); and energy dissipated via photoprotective mechanisms - Y (NPQ) (C) of *Ankistrodesmus densus* at 72 h exposed to different Cd and N concentrations.

Supplementary Files

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