Belo Horizonte, Brazil, April 2018

Dear Editor,

Please find the revised version of our manuscript entitled "Distinctive effects of allochthonous and autochthonous organic matter on CDOM spectra in a tropical lake", with a detailed response to the reviewers' comments in the public forum discussion. We thank the reviewers for providing constructive comments. We have considered all comments and revised the manuscript accordingly. You will find below our point-by-point response to each of these comments. All the additions we have made to the text are highlighted in yellow (in the revised manuscript and here) in order to facilitate understanding. We hope that this version will be satisfactory and thank you for your time in this matter.

Best Regards

Luciana Brandão and collaborators

<u>Comments from Referee #3:</u>

- **Comment: In chapter "2.4 Data analysis" lines 178 to 180 you use a lot of acronyms which must be explained beforehand; i.e. NUT stands for nutrient addition or NUTSH stands for nutrient addition and shadowing experiments.**

Answer: We included the sentence explaining the meanings of the acronyms after mentioning in the text the different treatments performed: (line 175) "Treatments with addition of organic matter have an acronym OM, those with nutrients addition have an acronym NUT and those shaded are called SH. Thereby different combinations with these acronyms give rise to the names of the different treatments as shown in figure 1 (for example OMNUT are the treatments with additions of organic matter and nutrients, and OMNUTSH are those with additions of organic matter, nutrients and shade)."

<u>Comments from Referee #2:</u>

- **Comment: lines 54-59: How does TSS or turbidity differ in tropical lakes? Greater terrestrial productivity and its subsequent leaching to the aquatic system should increase the turbidity of the water and potentially offset the impact of the higher light availability. Let's make sure greater light availability means "in water" rather than just on surface.**

Answer: Considering that even with high turbidity, the aquatic systems of this region are not limited by light, we replaced "light availability" by "water light availability" (line 71).

- **Comment: line 60: Reverse the order of the sentence so that tropical environments are mentioned first.**

Answer: The sentence was rewritten by mentioning tropics first (line 76-79). "The drivers of DOM dynamics in tropical environments are different from those in temperate, because in the first one the seasonality of rainfall is the main driver for allochthonous contribution to the aquatic system (Suhett et al., 2006) and in temperate systems it is related to changes in temperature (such as by the flow of the melting water in the surroundings or by the destratification of lakes; Lindell et al., 2000)."

- **Comment: Line 89: Maybe ": : :ways" to "... pathways"**

Answer: Ways was replaced by pathways (line 116).

- **Comment: Line 100 and method 2.2: Leachate from leaves are known to produce lots of labile DOM that have protein-like optical properties. Maybe add a sentence to show that the week long incubation should remove most labile DOM from the leaves and detritus.**

Answer: We included the sentence at lines 192-193: "One week of incubation of this allochthonous material is time enough for most of the existing labile DOM from the leaves and detritus to be degraded before being added to the mesocosms."

- **Comment: Line 206: Temperature of the mesocosms should be in the method section (or at a different place in the results section).**

Answer: The sentence about temperature was removed from the Results section and included in the Methods section (Line 205). Sentence "During the experiment, the water temperature of the mesocosms ranged between 28.4 and 31.3 ºC (average 30.8 ºC)."

- **Comment: Line 215: Missing a ")" somewhere.**

Answer: Removed "(".

- **Comment: Line 259: Rather than starting with how this study confirms previous findings, start with something that is unique to this study.**

Answer: We included one new sentence (lines 324-325) showing our main result at the beginning of the Discussion section. Sentence "Our results showed a pronounced sensitivity of the composition of DOM in a tropical lake on artificial alterations in environmental parameters as light and nutrients availability, and allochthonous matter inputs."

- **Comment: line 267: Redfield ratio was not based on freshwater phytoplankton. Could you cite a freshwater reference?**

Answer: We cited a freshwater reference about molar ratio N:P. The reference was included in the reference list at the end. Lines 335-338: "However, the molar ratio (DIN:DIP) was always higher than 10:1 suggesting limitation by phosphorus (molar ratios between 21.3 and 55.3) over the days of experiment and only becoming limited by nitrogen following day twelve (molar ratio 12.2) (Hecky and Kilham, 1988)."

- **Comment: 270: Photodegradation of terrestrial DOM can also increase the amount of labile DOM. Look at works by Rose Cory. If sunlight is indeed stronger in the tropics, photostimulated respiration or release of labile substances from photodegradation would also be an explanation for what's happening here.**

Answer: Certainly phytoplankton may be benefiting from photodegradation products. We included this explanation in this section with the sentence (line 345): "The increase of phytoplankton in these treatments therefore suggests that algal growth was stimulated by a combination of increases in nutrient availability due to degradation of the OM added (Hessen and Tranvik, 1998) and possibly due to release of labile substances derived from photodegradation (Cory et al., 2014)." The new reference (Cory et al., 2014) was included in the reference list at the end.

- **Comment: 339: Implications – Most of the findings in this study is not unique but supports previous findings. Thus, there should be a greater emphasis on why this study matters in the context of this ecosystem. Aside from the land use change in surrounding forest, why should readers care about tropical lakes in the first place? Are they a great contributor to atmospheric CO2, source for fishery product in the region, source for drinking water in areas with growing population, or transient storage for terrestrial DOM to the oceans? It only would take a few sentences in the introduction, discussion, and implications to make sure that the readers are fully aware of the importance of tropical lakes.**

Answer: The Implications section has been reformulated with an emphasis on the impacts and importance of our findings for updating tropical lakes and for the region.

We also included in the Introduction (lines 86-88 and 116-119) and in the Discussion (341-349) a few sentences highlighting such importance.

<u>Others minor revisions:</u>

- Two addresses of authors have been updated
- Abstract: some sentences highlighted in yellow were slightly reformulated in order to make it clearer.
- Introduction: Some words and expressions are highlighted in yellow to improve English and understanding. These modifications were more frequent in the last paragraph of the Introduction.
- Line 357: a new reference was cited. Also in the reference list (line 497)
- Line 415: Improved English.

Distinctive effects of allochthonous and autochthonous organic matter on CDOM spectra in a tropical lake

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Abstract

Despite the increasing understanding about differences in carbon cycling between temperate and tropical freshwater systems, our knowledge on the importance of organic matter (OM) pools on light absorption properties in tropical lakes is very scarce. We performed a factorial mesocosm experiment in a tropical lake (Minas Gerais, Brazil) to evaluate the effects of increased concentrations of allochthonous and autochthonous OM, and differences in light availability on the light absorption characteristics of colored dissolved organic matter (CDOM). Autochthonous OM deriving from phytoplankton (~Chl-*a*) was stimulated by addition of nutrients, while OM from degradation of terrestrial leaves increased allochthonous OM, and neutral shading was used to manipulate light availability. Effects of the additions and shading on DOC, Chl-*a*, nutrients, total suspended solid concentrations (TSM) and spectral CDOM absorption were monitored every three days. CDOM quality was characterized by spectral indices ($S_{250\text{-}450}$, $S_{275\text{-}295}$, $S_{350\text{-}450}$, S_R and $SUVA_{254}$). Effects of carbon sources and shading on the spectral CDOM absorption was investigated through principal component (PCA) and redundancy (RDA) analyses. The two different OM sources affected CDOM quality very differently and shading had minor effects on OM levels, but significant effects on OM quality, especially in combination with nutrient additions. Spectral indices ($S_{250\text{-}450}$ and S_R) were mostly affected by allochthonous OM addition. The PCA showed that enrichment by allochthonous carbon had a strong effect on

the CDOM spectra in the range between 300 and 400 nm, while the increase of autochthonous carbon increased absorption at wavelengths below 350 nm. Our study shows that small inputs of allochthonous OM can have large effects on the spectral light absorption compared to large production of autochthonous OM, with important implications for carbon cycling in tropical lakes.

Keywords: dissolved organic matter; mesocosm; carbon cycling; tropical lake

1 Introduction

Organic matter (OM) consists of particulate organic matter (POM; organic compounds represented by aquatic communities and detritus), and dissolved organic matter (DOM – in most of DOM studies it is the compounds smaller than 0.2 or 0.7 µm) which is by far the largest pool of organic carbon in aquatic systems (Hedges, 1992). A better understanding of carbon cycling in aquatic environments and its regional and global importance therefore requires knowledge of the biogeochemical processes involved in the transformation, production and mineralization of DOM (Bertilsson and Tranvik, 2000; Johannessen et al., 2007; Tranvik et al., 2009).

The main sources of DOM in aquatic ecosystems can be divided into two main categories: the allochthonous pool, which comes from terrestrial vegetation and soil sources (Kieber et al., 2006; Miller et al., 2009), and the autochthonous pool which is produced by aquatic primary producers (Kritzberg et al., 2004; Guillemette and Del Giorgio, 2012). These two DOM pools have fundamental differences in their optical and chemical characteristics which in turn influence the mechanisms by which DOM is degraded (Wetzel et al., 1995; Bertilsson and Tranvik, 2000). The allochthonous DOM is considered to be more susceptible to photodegradation because it contains relatively large molecules with high numbers of aromatic compounds which strongly absorb UV light (Amon and Benner, 1994; McKnight et al., 1994; Benner, 2002; Helms et al., 2008). The autochthonous DOM originating from phytoplankton mainly consists of simple molecules (carbohydrates, proteins, amino acids) of low molecular weight and is typically more labile for microbial community (Farjalla et al., 2009; Fonte et al., 2013).

The rate of photodegradation depends on a combination of available sunlight and the chemical characteristics of DOM (Benner, 2002), whereas the microbial degradation rate depends on the inherent DOM bioavailability and the utilization efficiency of the bacterial community (Catalán et al., 2013; Asmala et al., 2014), and both are important processes that transform and remove DOM in aquatic ecosystems (Roland et al., 2010; Mopper et al., 2015). Photodegradation is also known to transform DOM to ammonia and other highly bioavailable compounds (Aarnos et al., 2012) which can be an important nutrient supply for both phytoplankton (Hessen and Tranvik 1998) and heterotrophic bacterial communities

(Kieber et al., 1989; Miller et al., 2002; Lønborg et al., 2010). The microbial uptake of DOM by heterotrophic organisms converts it to POM, which in turn can be assimilated by protozooplankton through the microbial loop (Azam et al., 1983). Additionally, biodegradation of DOM can be stimulated by inorganic nutrients, mainly nitrogen and phosphorus, which increase the bacterial growth efficiency (Zweifel et al., 1995; Asmala et al., 2013) by reducing the energetic cost of substrate acquisition (Hopkinson et al., 1998). In tropical lakes, aquatic processes including mineralization of organic compounds occur more rapidly than in temperate lakes due to high temperatures and water light availability throughout the year (Marotta et al., 2010). However, there are only few studies on the photochemical (Teixeira et al., 2013; Bittar et al., 2015) and bacterial (Farjalla et al., 2002; 2009; Roland et al., 2010) degradation of DOM in tropical environments compared to temperate freshwater systems and estuaries (Bertilsson and Tranvik, 2000; Anesio and Granéli, 2003; Boreen et al., 2008; Asmala et al., 2014; Attermeyer et al., 2015).

The drivers of DOM dynamics in tropical environments are different from those in temperate, because in the tropics the seasonality of rainfall is the main driver for allochthonous contribution to the aquatic system (Suhett et al., 2006) and in temperate systems it is related to changes in temperature (such as by the flow of the melting water in the surroundings or by the destratification of lakes; Lindell et al., 2000). Brazil has a variety of complex freshwater systems that behave in different ways regarding the temporal dynamics of DOM. For example, in most tropical rivers and lakes, the seasonal allochthonous contribution occurs via runoff in the rainy season (between September and April), raising humic carbon concentrations and water color (Farjalla et al., 2002). In some regions, such as the complexes of Brazilian rivers and Amazonian lakes, the contribution of allochthonous material is related to the hydrological pulse, which raises the level of the water invading the surrounding forests (Amado et al., 2006).

The lake system of the Middle Rio Doce is composed of about 300 natural lakes and is among the three most important in Brazil, behind the Amazonian and Pantanal basins (Maillard et al., 2012). Many of these lakes are used by the local population both for water supply for human consumption and for water use in economic activities such as agriculture, livestock and large mining companies present in the region. Fishing is also an important activity in this region. Recent studies in this region have shown that the marked seasonality in the inputs of allochthonous material (nutrients and organic matter) during periods of rain (thermal stratification period, summer) plays a key role in the pattern observed for the optical characteristics of lakes, for example the transparency to photosynthetically active radiation (PAR) and ultraviolet (UV) (Gagliardi, 2015; Brandão et al., 2016). Contrary to expectations, greater transparency of these lakes is observed during the rainy season, since the allochthonous material remains trapped in the hypolimnium by temperature difference, until the water mixture (dry season, winter) redistributes it throughout the water column (Reynolds, 2009; Brandão et al., 2016). In this context, higher net phytoplankton production rates occur in the mixing periods, with lower solar radiation incidence and lower transparency (Brighenti et al., 2015). Bezerra-Neto et al. (2006) showed a strong negative influence on the concentration of CDOM (chromophoric dissolved organic matter) and transparency to the PAR radiation in a set of lakes of this lacustrine system, which emphasizes the importance of the chromophoric carbon from allochthonous origin for the physical and chemical conditions of the lakes, and consequently seasonal dynamics of phytoplankton and aquatic metabolism. In this way, the physical, chemical and ecological balance of these lakes is strongly affected by inputs of nutrients and organic matter from the catchment during the rainy season. However, the frequency and intensity of precipitation events in this region has changed over the last decade (Roland et al., 2012). In addition, the Atlantic Forest is a threatened and extremely devastated biome (Myers et al., 2000) and most of the lakes have already had the surrounding forest replaced by eucalyptus and pasture plantations. Land use transformation and the disruption of biogeochemical cycles may change the amount and quality of the inputs of dissolved nutrients (such as phosphate and nitrogen compounds) and organic matter into the lake (Vitousek et al., 1997; Pinheiro et al., 2015), changing the balance between allochthonous and autochthonous DOM sources in the systems and consequently DOM degradation pathways. Besides that tropical lakes are considered hot spots for biodiversity and greenhouse gas cycles, and furthermore they are believed to be highly sensitive to climate changes causing profound changes of lake physical (ie volume, area, stratification) and chemical (ie dissolved oxygen, nutrients, organic matter) conditions with ultimate effects on regional carbon cycling.

Some recent studies have demonstrated that DOM transformations (such as the the effect of photodegradation and biodegradation on the absorption properties of CDOM) was not constant over the spectral range, thus influencing the shape of the absorption curve (Helms et al., 2013; Reader et al., 2015). As modifications in the spectral shape reflect underlying changes in the carbon compounds at the molecular level, studies on biological and chemical effects on CDOM spectra allows a better understanding of the DOM transformations and how this links to overall carbon cycling in aquatic ecosystems (Stubbins et al., 2014).

To investigate how the optical properties of the lakes change due to more common anthropogenic impacts (such as eutrophication, land use change) and recent regional changes in rainfall, we performed a mesocosm experiment in a local tropical lake, manipulating nutrients, OM and light conditions. We expected that addition of nutrients would stimulate algal growth and increase the production of autochthonous DOM (Schindler, 1977; Lean and Pick, 1981) until nutrients became exhausted. For comparison, addition of extracted OM from leaves of the native forest mimicked effects of increasing allochthonous DOM concentrations. We hypothesized that OM from different sources would change the CDOM absorption spectra and associated indices differently over time. We also investigated the effect of the two interacting OM sources on the optical characteristics of DOM. Finally, we applied a shading filter to investigate expectations of a high importance of light availability on both autochthonous OM production and variable levels of photodegradation for different pools of OM.

2 Methods

2.1 Study area

This study was conducted in Carioca Lake (19°45'26.0"S; 42°37'06.2"W), one of the approximately 300 natural lakes of the Middle Rio Doce lacustrine system, the third most important of Brazil. It is located in the southern part of the State Park of Rio Doce (PERD, Minas Gerais, Brazil) which is the largest remnant of the Atlantic Forest in Minas Gerais (36000 ha forest) with lakes occupying 9.8% (3530 ha) of its total area. Carioca Lake is surrounded by secondary Atlantic Forest and is a warm-monomictic lake with a mixing period during the dry winter (May to August) and thermal stratification during the rest of the year (September to April). It is a mesotrophic lake (total phosphorus: 5.6-21.4 $\mu g\ L^{-1}$, epilimnion annual average 13.9 $\mu g\ L^{-1}$; chlorophyll-*a*: 1.3-16.6 $\mu g\ L^{-1}$, epilimnion annual average 7.7 $\mu g\ L^{-1}$) (Petrucio et al., 2006; Brighenti, 2014), with 1718.6 m of perimeter, 14.1 ha, $671 \times 10^3\ m^3$, and maximum depth of 11.8 m and average depth of 4.8 m (Bezerra-Neto et al., 2010). Carioca is one of the lakes that have been monitored for water quality and aquatic biota since 2000 through the Brasil-LTER Programme (PELD-CNPq Proc. 403698/2012-0). It is known that the input of DOM and nutrients in Carioca Lake occurs via runoff during the rainy season, but most of them remain below the thermocline due to temperature differences (Reynolds, 2009). During the mixing period, DOC, CDOM and nutrients become distributed in the water column, increasing their concentrations and availability in the surface layer, which in turn contributes to increased primary production and respiration rates (Brighenti et al., 2015).

2.2 Experimental design and measurements

To test the effect of organic matter inputs, sunlight, and nutrients on DOM degradation, we conducted an *in situ* experiment using a total of 16 cylindrical mesocosms (diameter 1.3 m, height 1.5 m and volume 2 m^3) with eight different combinations (two replicates for each combination). The sampling was carried out between January 20th and February 1st 2015 with daily measurements occurring between 10:00 am and 12:00 pm. Water samples (3L at 0.5 m from the surface) from mesocosms were collected every three days. The mesocosms setup was based on a 2 x 2 x 2 factorial design as follows: 1) with and without addition of organic matter extracted from leaves surrounding the lake, 2) with and without addition of nutrients ($NaNO_3$, K_2HPO_4, NH_4Cl) and with and without 50% of shading of solar radiation (Fig. 1). Treatments with addition of organic matter have an acronym OM, those with nutrients addition have an acronym NUT and those shaded are called SH. Thereby different combinations with these acronyms give rise to the names of the different treatments as shown in figure 1 (for example OMNUT are the treatments with additions of organic matter and nutrients, and OMNUTSH are those with additions of organic matter, nutrients and shade).

The organic matter added to the mesocosms was a mixture of leaves, plant detritus and soil particles adhered to this material from the ground around the lake (4 cylinders, ca. 20 L each). The material was placed in buckets with distilled water (60 L) for decomposition and stored in the laboratory under room temperature (ca. 25 °C). After one week the water was filtered with 20 µm mesh and 7.5 L of the filtrate was added to each of the 8 units in order to increase allochthonous organic matter availability in these mesocosms. One week of incubation of this allochthonous material is time enough for most of the existing labile DOM from the leaves and detritus to be degraded before being added to the mesocosms. The initial organic matter concentration in each of the 8 carbon amended cylinders was 8.6 ± 0.1 mg L^{-1} (DOC, mean ± standard deviation), 21.44 ± 0.52 m^{-1} ($a_{CDOM254}$, mean ± standard deviation) and 0.66 ± 0.04 m^{-1} ($a_{CDOM440}$, mean ± standard deviation). In the 8 units without organic matter addition the initial organic matter was 8.0 ± 0.4 mg L-1 (DOC, mean ± standard deviation), 17.11 ± 0.43 m^{-1} ($a_{CDOM254}$, mean ± standard deviation) and 0.43 ± 0.04 m^{-1} ($a_{CDOM440}$, mean ± standard deviation). We performed a pilot mesocosm experiment in July 2014 to study the suitable OM addition levels for the experiment. In this pilot study, we noticed that this methodology and volume of extract was enough to modify the quality of organic matter and also light attenuation levels in the organic matter added treatment (with added OM: $a_{CDOM254}$ 22.31 m^{-1}, Kd_{PAR} 1.68 m^{-1}; without OM addition: $a_{CDOM254}$ 16.67 m^{-1}, Kd_{PAR} 0.99 m^{-1}).

Mesocosms were submerged on the surface of the lake and filled with lake water. Mesocosms with reduced light availability (SH) were shaded with spectrally neutral shading screens (50%) and only opened for quick samplings and measurements. Every day, the mesocosms were gently stirred and measured for water temperature using a probe Hydrolab DS5 (Hach Inc.). During the experiment, the water temperature of the mesocosms ranged between 28.4 and 31.3 °C (average 30.8 °C).

Water samples were filtered immediately after sampling for Chl-*a* and nutrients (0.47 µm filter) and TSM (AP040 filter). The filters were kept frozen until analysis. Water samples were also filtered for analysis of DOC and CDOM (0.22 µm Millipore filter) and stored in amber glass bottles (pre-washed with distilled water and hydrochloric acid 10%) at 4°C in the dark. The Chl-*a* concentration corrected by pheophytin (µg L^{-1}) was obtained by acetone extraction (90%) measured in a spectrophotometer (UV-VIS Shimadzu) at 665 and 750 nm and calculated using the protocol provided in APHA (1998). The TSM (mg L^{-1}) were determined by the gravimetric method, considering the difference between the dry weights of AP40 Millipore filters (105 °C for 2 hours) before and after the filtration of water samples (APHA 1998). The DOC concentration (mg L^{-1}) was obtained by catalytic oxidation method of high temperature using TOC Analyzer (Shimadzu TOC – 5000A).

Filtered water samples were taken for dissolved nutrients (ammonia, nitrate, nitrite and phosphate; µg L^{-1}) and frozen until analyzes with an auto-analyzer (Metrohm 8000 IC-Plus).

2.3 CDOM optical properties

Absorption spectra of CDOM were obtained between 250 and 700 nm at 1 nm intervals with a spectrophotometer (UV-VIS Shimadzu) using a 5 cm quartz cuvette and a Milli-Q water sample as blank reference. The absorption spectra of each sample were measured in replicate (standard deviation < 0.01). The absorption coefficients ($a_{\mathrm{CDOM}}(\lambda)$; m^{-1}) were derived from absorbance measurements according to the equation $a_{\mathrm{CDOM}}(\lambda) = 2.303A(\lambda)L^{-1}$, where A(λ) is the absorbance measured at wavelength λ and L is the optical path of the cuvette (in meters). Absorption coefficients were corrected for backscattering by subtracting the value of the coefficient at 700 nm. The absorption coefficient at 254 nm (a_{CDOM254}) was used as an index of CDOM UV-absorption and at 440 nm (a_{CDOM440}) as a CDOM PAR-absorption.

We used a simple exponential curve to model the decrease in absorption with increasing wavelength using the equation (Jerlov, 1968; Bricaud et al., 1981; Stedmon and Markager, 2001): $a_{\mathrm{CDOM}}(\lambda) = a_{\mathrm{CDOM}}(\lambda 0)\ e^{-S(\lambda - \lambda 0)} + K$,where a_{CDOM} is the absorption coefficient (m^{-1}), λ is the wavelength (nm), λ0 is a reference wavelength (nm), K is a background constant (m^{-1}) accounting for scatter in the cuvette and drift of the instrument and S is the spectral slope (nm^{-1}) that describes the approximate exponential rate of decreasing absorption with increasing wavelength. Furthermore, we calculated the spectral slope between 275-295 nm ($S_{275\text{-}295}$) and 350-400 nm ($S_{350\text{-}400}$). The slope ratio (S_R, Helms et al., 2008) was obtained by dividing $S_{275\text{-}295\mathrm{nm}}$ by $S_{350\text{-}400\mathrm{nm}}$. These metrics were calculated using the *cdom* R package (Massicotte, 2016). We also calculated $S_{250\text{-}450}$ as a proxy to measure changes in the entire spectrum including UV and PAR-absorbing (we limit to 450 nm because at higher wavelengths the signal to noise ratio was high). This metric was further used in the principal component analysis. The specific UV absorbance ($SUVA_{254}$) (m^2 mg^{-1} C) was calculated dividing the value of the absorption coefficient at 254 nm (m^{-1}) by the concentration of DOC (mg L^{-1}) (Weishaar et al., 2003).

2.4 Data analysis

The mesocosms were grouped in the figures as follows in order to show the differences between the two different sources of OM (allochthonous versus autochthonous source): the group "OM addition" includes the OM, OMNUT, OMSH and OMNUTSH treatments combined. The group "nutrients addition" includes the NUT, OMNUT, NUTSH, OMNUTSH treatments and the group "with shade" include the SH, OMSH, NUTSH and OMNUTSH. The last three groups "without OM addition", "without NUT addition" and "full light" include the remaining four treatments, totaling six different groups (see Table S1 in the Supplementary Material). In this context, the group "OM addition" represents the allochthonous source, while the group "nutrients addition" represents the autochthonous OM source.

The relative changes (%) of the parameters over time were calculated dividing the value measured at the end of the experiment by the value at the beginning (day 0) of the experiment, after subtracting this result from 1 and multiplying by 100 [(1 - end/start) x 100]. Negative relative change values indicate decrease and positive values indicate increase compared to initial values in the beginning of the experiment.

We performed a three-way ANOVA plus the second order interactions, in order to verify the effect of each factor (nutrients and OM additions and shade) on the response variables (quantity [a_{CDOM254}, a_{CDOM440} and DOC] and quality [$SUVA_{254}$ and spectral slopes] of DOM and phytoplankton biomass [measured by Chl-*a* concentration]). We also partitioned the coefficient of determination of each ANOVA in terms of its factors and interactions in order to estimate the relative importance of each driver on the results for DOM optical properties (Lindeman et al., 1980). The interactions that were worth to mention we compute the least-squares means, plus the 95% confidence interval, for specified factors combinations. All statistical analysis were performed in software R (R Core Team, 2017), plus packages "lsmeans" (Lenth, 2016) and "relaimpo" (Grömping, 2006). A threshold significance level of 5% was considered.

A principal component analysis (PCA) was carried out using CDOM absorption spectra (with 1 nm interval between 250 and 450 nm) on a $n \times p$ matrix where n is the number of observations in the dataset (n = 80) and p is the wavelength number ($250 \leq p \leq 450$). The PCA was performed on scaled data (zero mean and unit variance) as suggested by Borcard et al., (2011). This approach was used to summarize absorption spectra (Reader et al., 2015), which are difficult to summarize into a single value observation such as other variables like Chl-*a*. Based on the Kaiser-Guttman criterion (Kaiser, 1960), the first two principal components were kept for subsequent analyzes. PCA scores of first two axes (PC1 and PC2) were correlated against environmental variables (categorical variables shading, nutrients and OM additions and the continuous variables Chl-*a*, TSM, DOC, S_R and $SUVA_{254}$) using a redundancy analysis (RDA) to help to understand their interpretation. All statistical analyzes were performed using R Software (R core development team 2011).

3 Results

3.1 Characteristics of the treatments

The results obtained by Three Way Analysis of Variance (Table 1) showed that Chl-*a* levels were significantly higher for treatments with addition of nutrients (average 11.9 µg L^{-1}), and also with addition of OM (average 9.2 µg L^{-1}) (Fig. 2A-B).

Concentrations of DOC and absorption coefficients of $a_{CDOM254}$ and $a_{CDOM440}$ were significantly higher in all the treatments with addition of OM (averages DOC – 8.6 mg L-¹; $a_{CDOM254}$ – 21.9 m-¹; $a_{CDOM440}$ – 0.7 m-¹). Lower $S_{275-295}$ (average 0.02 nm-¹) and higher $SUVA_{254}$ (average 2.5 m² mg-¹C) were observed also in the treatments with OM added. Treatments shaded showed lower DOC concentrations (average 8.2 mg L-¹), higher $SUVA_{254}$ (average 2.4 m² mg -¹ C) and lower $S_{275-295}$ (average 0.02 nm-¹). The results also showed significant effect for the interaction between nutrients and shade for $a_{CDOM254}$ and $a_{CDOM440}$ (Table 1 and Fig. S1 and S2 in the Supplementary Material).

The partitioned coefficient of determination for $a_{CDOM254}$, $a_{CDOM440}$, $SUVA_{254}$ and $S_{275-295}$ were very high (>75%), indicating that the most important drivers for these variables were included in the experiment. For DOC and Chl-*a* the coefficient of determination showed intermediate values (~50%), indicating that other variables not considered in the experiment also played an important role. The difference between treatments with and without OM played the biggest role in explaing the variation of all response variables evaluated in the experiment, except Chl-*a*. As expected, the difference in nutrients had the biggest effect over Chl-*a* concentrations.

3.2 Temporal changes in the mesocosms

Average phytoplankton biomass (Chl-*a*) increased over time only in the treatments with addition of nutrients (from average of 3.9 on day 0 to 19.1 µg L-¹ on the 12th day) and OM (from minimum average 3.4 on day 0 to maximum of 12.1 µg L-¹ on 9th day) (Fig. 2A-B). Average DOC increased in the experimental units with added nutrients until day 9 (maximum 8.7 mg L^{-1}). In treatments with addition of OM, DOC was higher as expected with a minimum of 8.3 mg L^{-1} at day 3 and maximum of 8.9 mg L^{-1} on the 9th day, while in the treatments without addition of OM the DOC had a maximum of 8.2 mg L-¹ in 6th day (Fig. 2C-D). $S_{250-450}$ and slope ratio (S_R) had the same pattern in the treatments with and without nutrients addition, but decreased consistently in treatments with OM addition (Fig. 2 E-H).

The relative changes in CDOM absorption along the spectral range were different for each sampling day (Fig. 3A-E). On the initial day, only treatments with and without addition of OM had distinct absorption curves, especially in the UV range below 400 nm, and the absorption spectra for each treatment group on day 0 are shown in Fig. 3A. To evaluate treatment effects we determined the change in light absorption spectra for the other sampling days relative to the initial day (Fig. 3B-E). On day 3, treatments with and without nutrients added were quite similar, while those with and without OM and with and without shading showed opposing changes. Loss of absorption occurred only in treatments with full light (less than 5% between 300-420 nm) and in those without OM addition (the loss of absorption increased with the increase of wavelength) (Fig. 3B). On day 6 all treatments showed an increased absorption especially after 350 nm (higher increase with shade: ~

40% at 450 nm), except the treatments with full light that still had a loss of absorption (Fig. 3C). On day 9 and 12, all treatments had a loss of CDOM absorption with increasing wavelengths, especially for the full light treatment (Fig. 3D-E).

The concentrations of DIP and DIN (µg L^{-1}) and the molar ratio between them (DIN:DIP) were higher in treatments with added nutrients as expected (Fig. 4A-C). In the treatments without nutrients addition, DIP concentrations ranged between 3 and 9 µg L^{-1} and DIN between 1.7 and 100.4 µg L^{-1}, with DIN:DIP ratios ranging between 7.6 (day 12) and 21.3 (day 0) and below 13.9 after day 3 suggesting that phytoplankton community were mostly limited by nitrogen in these treatments (Reynolds, 1999). All the dissolved nutrients decreased over the course of the experiment in all treatments with nutrients addition (DIP decreased from an average of 153 to 59 µg L^{-1} and DIN from 2600 to 400 µg L^{-1}). The DIN:DIP molar ratio ranged from 55.3 (day 3) to 12.2 (day 12) in the mesocosms with addition of nutrients.

3.3 Principal component analysis results

The first principal component of the redundancy analysis (Fig. 5A) was mostly associated with availability of OM. Samples presenting high scores on the first principal component furthermore tended to have high values of DOC and $SUVA_{254}$ but low values of S_R. High scores in the second principal component correlated positively with Chl-*a*, nutrients and TSM and negatively with shading.

Exploration of spectral PCA loadings (Fig. 5B-C) revealed that principal component 1 (PC1) had the strongest effect on the shape of CDOM absorbance between 300 and 400 nm. Principal component 2 (PC2) loadings showed a quasi-linear decrease with increasing wavelength suggesting that phytoplankton enrichment had a stronger effect at lower wavelengths. Furthermore, loading values were negative after ~340 nm, indicating that phytoplankton was on average lowering CDOM absorption after this threshold. Based on the redundancy analysis, PC1 was renamed “allochthonous carbon enrichment” whereas PC2 was renamed “autochthonous carbon enrichment”.

4 Discussion

4.1 Nutrients, allochthonous matter and shade responses in DOM and Chl-a

Our results showed a pronounced sensitivity of the composition of DOM in a tropical lake on artificial alterations in environmental parameters as light and nutrients availability, and allochthonous matter inputs. As expected, the addition of allochthonous matter results in a DOM pool which is dominated by more aromatic carbon with higher molecular weight (Bertilsson and Tranvik, 2000; Benner,2002) and lower spectral slopes (Helms et al., 2008; Fig. 2F, 2H). Addition of nutrients also affected DOM quantity and quality related to autochthonous production by phytoplankton growth which can be an important source of DOM (Zhang et al., 2009; Zhang et al., 2013; Brandão et al., 2016). In the treatments without addition of nutrients, the phytoplankton community was limited by nitrogen since the beginning of the experiment (DIN < 100 $\mu g\ L^{-1}$; Reynolds, 1999). The DIN:DIP molar ratio in these treatments indicates that nitrogen was consumed very fast and was limited after day 3 (ratio below 13.9; Hecky and Kilham, 1988). With the addition of nutrients, the concentrations of DIN and DIP were higher than considered to be limiting (Reynolds,1999). However, the molar ratio (DIN:DIP) was always higher than 10:1 suggesting limitation by phosphorus (molar ratios between 21.3 and 55.3) over the days of experiment and only becoming limited by nitrogen following day twelve (molar ratio 12.2) (Hecky and Kilham, 1988). Moreover, these apparent limitations did not restrict the phytoplankton growth in mesocosms with addition of nutrients. Mesocosms which did not receive nutrients, but which received allochthonous OM also experienced an increase in Chl-*a* after 3 days (Fig. 2B). The light attenuation caused by enhanced light absorption from added allochthonous OM (Kirk, 1994), favored growth of phytoplankton by reducing photoinhibition (Brighenti et al. in press), commonly occurring during summer in Lake Carioca (Brighenti et al., 2015). The increase of phytoplankton in these treatments therefore suggests that algal growth was stimulated by a combination of increases in nutrient availability due to degradation of the OM added (Hessen and Tranvik, 1998) and possibly due to release of labile substances derived from photodegradation (Cory et al., 2014). Our study shows that in addition to providing a source of nutrients, allochthonous inputs to tropical lakes, may strongly affect the seasonal dynamics of phytoplankton by reducing of photoinhibition through elevated UV absorption. These results corroborates previous studies that where lake ecosystem metabolism are indirectly controlled by terrestrial material during periods of higher rainfall (Brighenti et al., 2015; Brandão et al., 2016).

Although additions of allochthonous OM and nutrients both contributed to higher DOC concentrations, divergent effects of these additions were evident in the quality of carbon assessed by optical indices ($S_{250\text{-}450}$ and S_R). $S_{250\text{-}450}$ and S_R decreased significantly after addition of allochthonous OM (Fig. 2F, H). The decrease in the slope $S_{250\text{-}450}$ was related to the increase of a higher molecular weight carbon, which lowered the values of $S_{275\text{-}295}$ and consequently of S_R (Helms et al., 2008). This shows that small changes in the amount and quality of allochthonous contribution to lakes, either due to changes in rainfall or land use change, may cause considerable changes in the optical quality of the aquatic systems, as alterations in the water transparency by changes in the UV and PAR absorbing. This corroborates other studies that claim that tropical lakes are highly sensitive to climate changes (Adrian et al., 2009), causing serious modifications of lake physical and chemical conditions (Jeppesen et al., 2014), with ultimate effects on lake productivity for example (O'Reilly et al., 2003).

Addition of nutrients, however, had little effect on these metrics, which we interpreted as a consequence of autochthonous production of DOM. This is likely because these indices are derived from slope intervals in the ultraviolet range (250-400 nm) known to be influenced by carbon with higher molecular weight and aromatic compounds capable of absorbing energy at shorter wavelengths (Bertilsson and Tranvik, 2000; Benner, 2002; Helms et al., 2008). Moreover, nutrient additions only increased $a_{CDOM254}$ and $a_{CDOM440}$ in the shaded treatments (Fig. S1 and S2 in the Supplementary Material), suggesting an increase of PAR and UV absorbance due to less photodegradation and less photoinhibition for the phytoplankton in the shaded treatments.

Manipulations of nutrients, allochthonous OM and light availability caused distinctive changes in the spectral curves of CDOM over the sampling days (Fig. 3). Several studies have shown that aromatic organic carbon, typically of terrestrial origin, has relatively higher absorption in the ultraviolet range (Bertilsson and Tranvik, 2000; Benner, 2002; Helms et al., 2008). This can explain the initial (day 0) effects of allochthonous OM addition on elevated CDOM absorption primarily below 350 nm (Fig. 3A). We interpret the following increase in the CDOM absorption (days 3 and 6, especially above 350 nm) for most treatments to result from autochthonous DOM related to phytoplankton growth, as increases in absorption in the PAR range (Fig. 3B-C) is known to be related to increases in carbon of simple structures from algal origin (Amon and Benner, 1994; McKnight et al., 1994; Benner, 2002; Helms et al., 2008). After day 9 (Fig. 3D-E) the absorption loss was larger than the gain by the autochthonous production in all treatments, and such spectral changes with larger absorption decreases in higher wavelengths are likely due to biological degradation of CDOM (Asmala et al., 2014). However, it is important to note that the relative changes in the spectral curves shown in Fig. 3 reflect the net change from two counteracting processes: autochthonous production and loss of absorption by photodegradation and/or biodegradation. Treatments that were exposed to full light (orange solid lines) and the shaded treatments (orange dashed lines) presented notable differences between each other in the relative changes in the CDOM absorption spectrum. This corroborates to our results of the more aromatic carbon with larger molecular size (higher $SUVA_{254}$ and lower $S_{275-295}$, Table 1) observed in shaded mesocosms, suggesting lower photodegradation in these units.

4.2 Effect of allochthonous and autochthonous DOM on CDOM spectra

The PCA and redundancy analysis showed that the increase of allochthonous OM increased absorption between the wavelengths 300 and 400 nm (PC1, Fig. 5B). Several studies have shown that photodegradation is more pronounced at shorter wavelengths (300-400 nm) due to absorption of aromatic carbon compounds (Helms et al., 2008; Helms et al., 2013) typically related to degradation of either terrestrial vegetation (Bertilsson and Tranvik, 2000; Benner, 2002; Helms et al., 2008) or aquatic macrophytes (Catalán et al., 2013). We noticed a decrease in the CDOM absorption below 300 nm, suggesting a greater degradation by photodegradation in these compounds from allochthonous origin affecting the absorption at shorter wavelengths and increasing the absorption between 300 and 400 nm. In contrast, the increase of autochthonous

OM from the phytoplankton growth is likely to have resulted in an increase of absorption in the UV range and a loss of absorption at wavelengths beyond 350 nm (PC2; Fig. 5C). The loss of absorption above 350 nm indicates degradation by microorganisms which have greater impact on the PAR absorption. Substances that absorb in this range are typically non-aromatic compounds originating from algal sources with high lability for bacterial degradation (Baines and Pace, 1990; Berggren et al., 2009).

5 Conclusions

Additions of terrestrial OM and inorganic nutrients to a tropical lake mesocosm caused fast changes in the production and transformation of OM pools as well as distinct changes in the absorption spectra of CDOM. Increased production of autochthonous OM caused an increase of CDOM absorption in the UV range. However, we found that CDOM absorption was reduced in the PAR range, indicating bacterial degradation of highly labile algal material (Baines and Pace, 1990; Berggren et al., 2009). In contrast, the additions of allochthonous OM caused increased absorption of CDOM, especially between 300 and 400 nm. $S_{250\text{-}450}$ was an effective tool to evaluate the spectral changes in general from short to long wavelengths, not restricting spectrum ranges where we can only perceive changes in some regions of UV-absorbing ($S_{275\text{-}295}$, $S_{350\text{-}400}$). Although the non-shaded treatments showed apparent effects of photodegradation, changes in CDOM absorption curves after day 9 suggest that biodegradation was overall responsible for the largest percentage of OM degradation in these experiments. The 50% reduction of sunlight in some mesocosms accordingly had minor effects on overall changes in OM concentration, but the effect of shading was significant to OM quality and was important for the effect of nutrients to the absorbance at 254 and 440 nm.

5.1 Implications

Our experiment adds knowledge on how input of terrestrial OM and nutrients, related to water column mixing and rains, influence carbon cycling in tropical lakes (Brighenti et al., 2015; Brandão et al., 2016). Even small inputs of allochthonous OM can have much larger effects on the spectral characteristics on the lake CDOM, compared to large production of autochthonous OM. Local changes in land use, through replacement of Atlantic forest, will likely alter the spectral light quality. Such changes can affect a range of chemical (concentration and quality of DOM), physical (e.g upper layer mixing due to changes in the absorption of PAR and UV radiation (Read and Rose, 2013) and biological conditions (vertical distribution and productivity of aquatic organisms (Gagliardi et al., in press). Recent reductions in regional precipitation (Roland et al., 2012) and observed lake volumes provide further evidence of a strong control by allochthonous OM on the physical, chemical and biological conditions in the Rio Doce region. As draughts are expected to become more common

(IPCC 2013), allochthonous OM will likely have an increasing control on aquatic metabolism, DOM dynamics and water transparency in these tropical lakes (Gagliardi, 2015; Brighenti et al., 2015; Brandão, 2016). These impacts certainly will alter the water quality for consumption and for water use in the economic activities in the region with ultimate effects on regional carbon cycling.

Author contribution

Luciana Brandão designed the experiment, participated in the field work, laboratory analysis and writing the manuscript.

Ludmila Brighenti designed the experiment, participated in the field work and laboratory analysis, and reviewed the manuscript.

Peter Anton Staehr designed the experiment, participated in the field work, writing and revision of the manuscript.

Eero Asmala participated in the writing and revision of the manuscript.

Philippe Massicotte participated in the writing and revision of the manuscript and the statistical analyzes.

Denise Tonetta participated in the field work and revision of the manuscript.

Francisco Barbosa designed the experiment, participated in the field work, and revision of the manuscript.

Diego Pujoni participated in the statistical analyzes, figures and revision of the manuscript.

José Fernandes Bezerra-Neto designed the experiment, participated in the field work, writing and revision of the manuscript.

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Figure Captions

Fig. 1 Squematic figure of the factorial design of mesocosms experiments

Fig. 2 Temporal variation in the mesocosms units with and without nutrients (left panel) and organic matter additions (right panel) for Chl-*a* (A,B), DOC (C,D), $S_{250\text{-}450}$ (E,F), S_R (G,H) (center line-median, 5th/95th percentiles, 95% confidence level, black circles-outlier)

Fig. 3 Spectral absorption curves of CDOM in the different mesocosms units for the initial day (A) and the relative changes for each sampling day compared with the initial day (3rd day – B, 6th day – C, 9th day – D, 12th day – E)

Fig. 4 Temporal variation in the mesocosms units with and without nutrients additions for dissolved inorganic phosphorus-DIP (A), dissolved inorganic nitrogen-DIN (B) and the molar ratio DIN/DIP (C) (center line-median, 5th/95th percentiles, 95% confidence level, black circles-outlier)

Fig. 5 Results of redundancy analysis-RDA (A) and the first two principal components obtained from PCA analysis, PC1 (B) and PC2 (C), plotted against wavelengths. Gray symbols represents the treatments without organic matter addition and the black ones those with organic matter. Triangles represent those without added nutrients and balls those with nutrients addition.

Table 1 - Results of the Three Way Analysis of Variance. The coefficient of determination of the analysis of variance partition is represented in the last column by %R2.

Parameters	Source of variation	DF	SS	MS	F	P	%R2
a254 (m^{-1})	OM	1	419.88	419.88	1427.44	<0.001	93.3%
	NUT	1	6.51	6.51	22.13	<0.001	1.4%
	SH	1	0.01	0.01	0.04	0.841	0.0%
	OM x NUT	1	0.52	0.52	1.76	0.189	0.1%
	OM x SH	1	0.47	0.47	1.59	0.211	0.1%
	NUT x SH	1	1.31	1.31	4.47	0.038	0.3%
	Residuals	73	21.47	0.29			0.0%
	Total	79	450.17				95.2%
a440 (m^{-1})	OM	1	1.33	1.33	230.38	<0.001	73.5%
	NUT	1	0.01	0.01	1.69	0.198	0.5%
	SH	1	0.02	0.02	2.99	0.088	1.0%
	OM x NUT	1	0.01	0.01	0.97	0.329	0.3%
	OM x SH	1	7.37E-07	7.37E-07	1.28E-04	0.991	0.0%
	NUT x SH	1	0.03	0.03	4.37	0.040	1.4%
	Residuals	73	0.42	0.01			0.0%
	Total	79	1.81				76.7%
DOC (mg L^{-1})	OM	1	7.07	7.07	61.63	<0.001	42.8%
	NUT	1	0.38	0.38	3.30	0.073	2.3%
	SH	1	0.70	0.70	6.07	0.016	4.2%
	OM x NUT	1	1.32E-03	1.32E-03	0.01	0.915	0.0%
	OM x SH	1	3.74E-04	3.74E-04	3.26E-03	0.955	0.0%
	NUT x SH	1	1.97E-03	1.97E-03	0.02	0.896	0.0%
	Residuals	73	8.38	0.11			0.0%
	Total	79	16.53				49.3%
$SUVA_{254}$ (m^2 mg^{-1} C)	OM	1	2.85	2.85	296.38	<0.001	78.0%
	NUT	1	0.02	0.02	2.37	0.128	0.7%
	SH	1	0.05	0.05	5.60	0.021	1.4%
	OM x NUT	1	0.01	0.01	0.98	0.325	0.3%
	OM x SH	1	0.01	0.01	0.67	0.417	0.2%
	NUT x SH	1	0.02	0.02	1.90	0.172	0.5%
	Residuals	72	0.69	0.01			0.0%
	Total	78	3.65				81.0%
$S_{275-295}$ (nm^{-1})	OM	1	1.89E-04	1.89E-04	287.91	<0.001	76.4%
	NUT	1	2.00E-06	2.00E-06	3.05	0.085	0.8%
	SH	1	7.93E-06	7.93E-06	12.08	0.001	3.2%
	OM x NUT	1	2.95E-09	2.95E-09	4.50E-03	0.947	0.0%
	OM x SH	1	3.58E-08	3.58E-08	0.05	0.816	0.0%
	NUT x SH	1	4.11E-07	4.11E-07	0.63	0.431	0.2%
	Residuals	73	4.79E-05	6.56E-07			0.0%
	Total	79	2.47E-04				80.6%
Chl-a (µg L^{-1})	OM	1	92.60	92.60	4.46	0.038	3.3%
	NUT	1	1172.94	1172.94	56.53	<0.001	41.4%
	SH	1	6.61	6.61	0.32	0.574	0.2%
	OM x NUT	1	32.24	32.24	1.55	0.217	1.1%
	OM x SH	1	12.44	12.44	0.60	0.441	0.4%
	NUT x SH	1	0.29	0.29	0.01	0.906	0.0%
	Residuals	73	1514.56	20.75			0.0%
	Total	79	2831.67				46.5%

593 OM = organic matter NUT = nutrients SH = shade

Fig 1

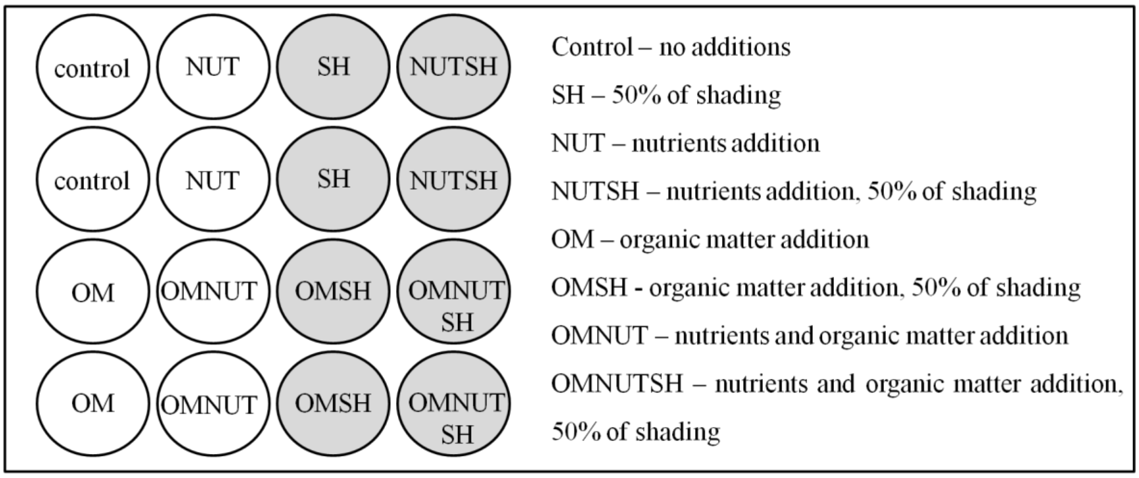

control
NUT
SH
NUTSH
control
NUT
SH
NUTSH
OM
OMNUT
OMSH
OMNUT SH
OM
OMNUT
OMSH
OMNUT SH
Control – no additions
SH – 50% of shading
NUT – nutrients addition
NUTSH – nutrients addition, 50% of shading
OM – organic matter addition
OMSH - organic matter addition, 50% of shading
OMNUT – nutrients and organic matter addition
OMNUTSH – nutrients and organic matter addition, 50% of shading

Fig 2

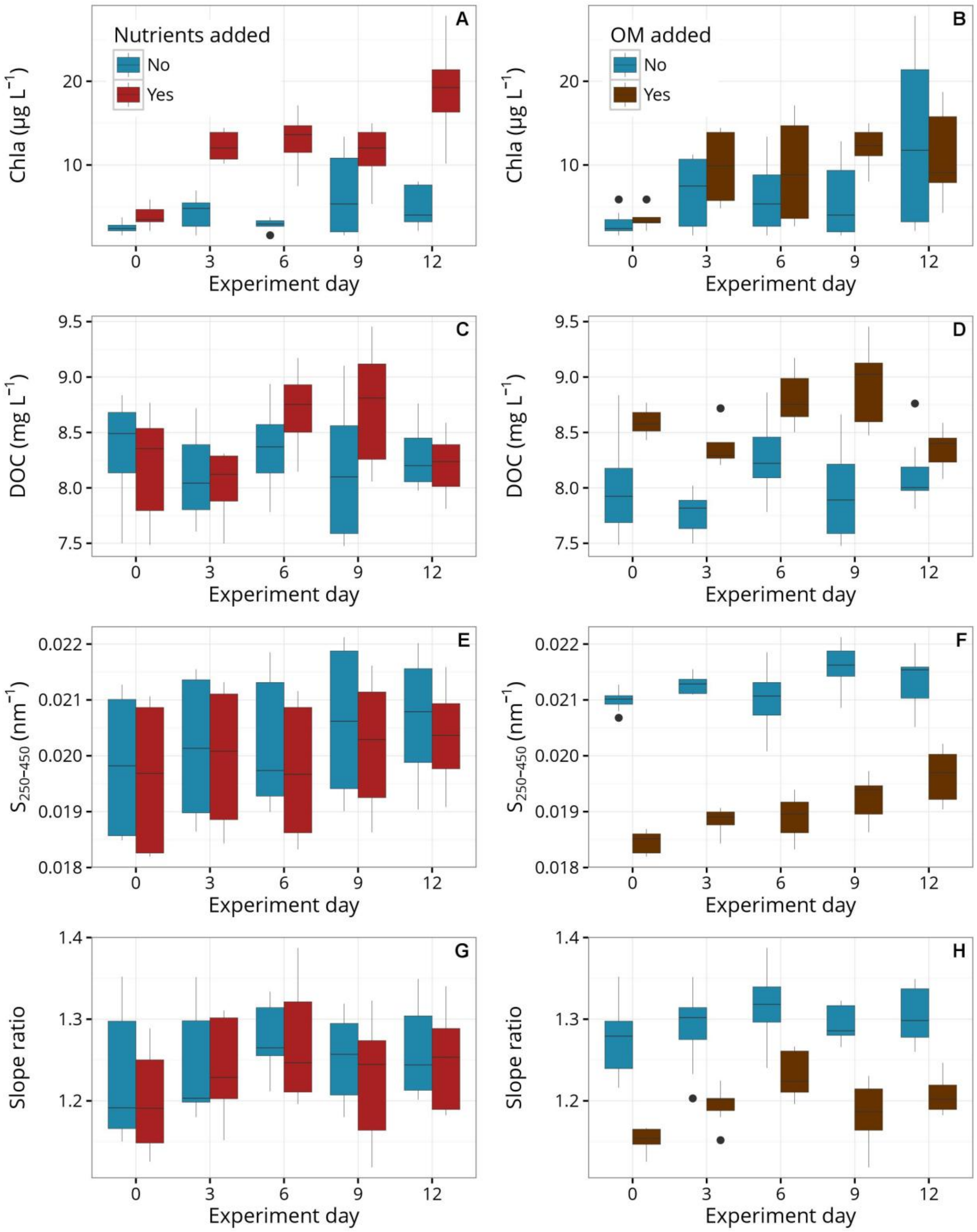
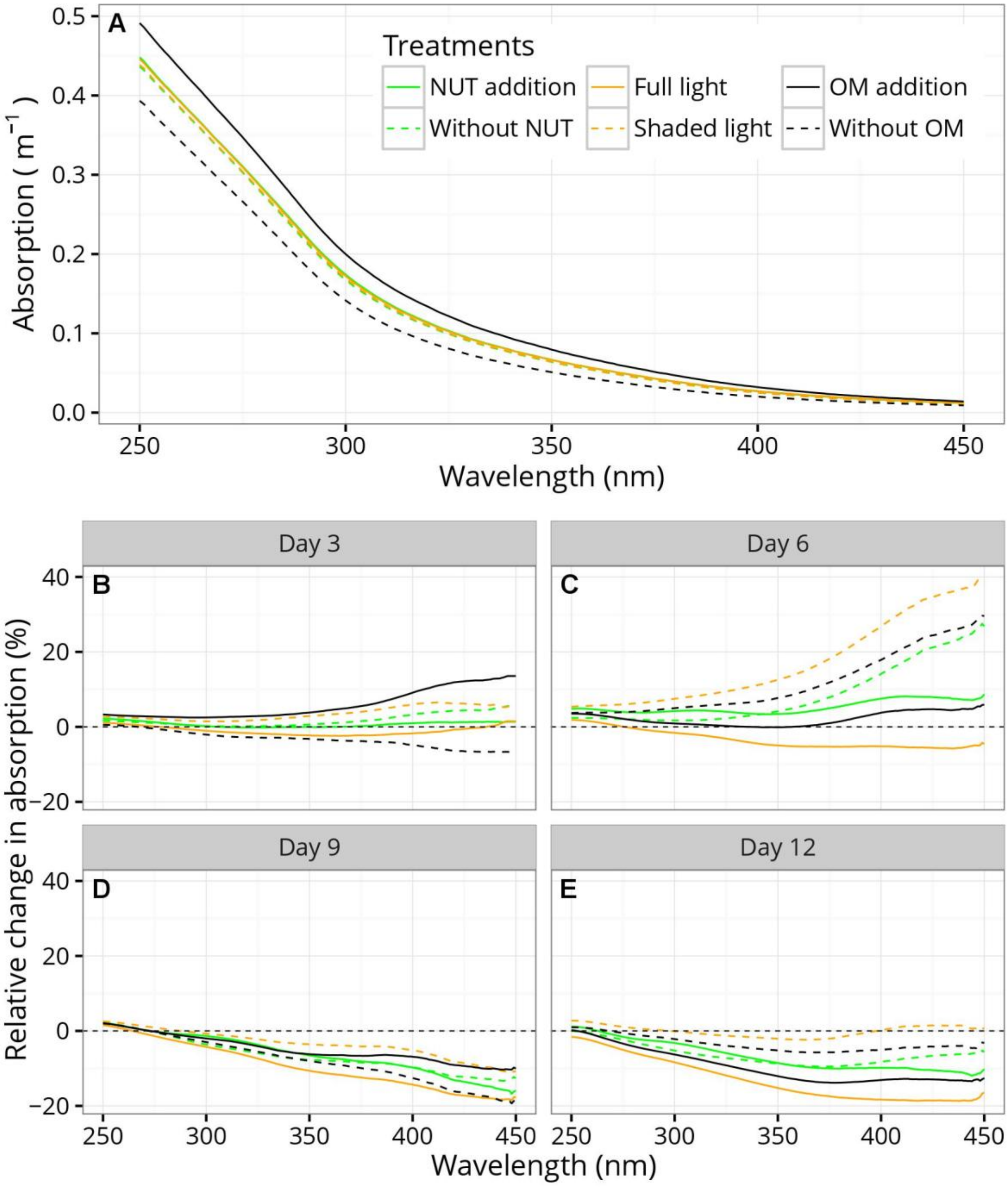

A
Nutrients added
No
Yes
Chla (µg L⁻¹)
Experiment day
B
OM added
No
Yes
Chla (µg L⁻¹)
Experiment day
C
DOC (mg L⁻¹)
Experiment day
D
DOC (mg L⁻¹)
Experiment day
E
S250–450 (nm⁻¹)
Experiment day
F
S250–450 (nm⁻¹)
Experiment day
G
Slope ratio
Experiment day
H
Slope ratio
Experiment day

Fig 3



A
Treatments
NUT addition
Without NUT
Full light
Shaded light
OM addition
Without OM
Absorption (m^{-1})
0.5
0.4
0.3
0.2
0.1
0.0
250
300
350
400
450
Wavelength (nm)
Day 3
Day 6
Day 9
Day 12
B
C
D
E
Relative change in absorption (%)
40
20
0
−20
Wavelength (nm)

Fig 4

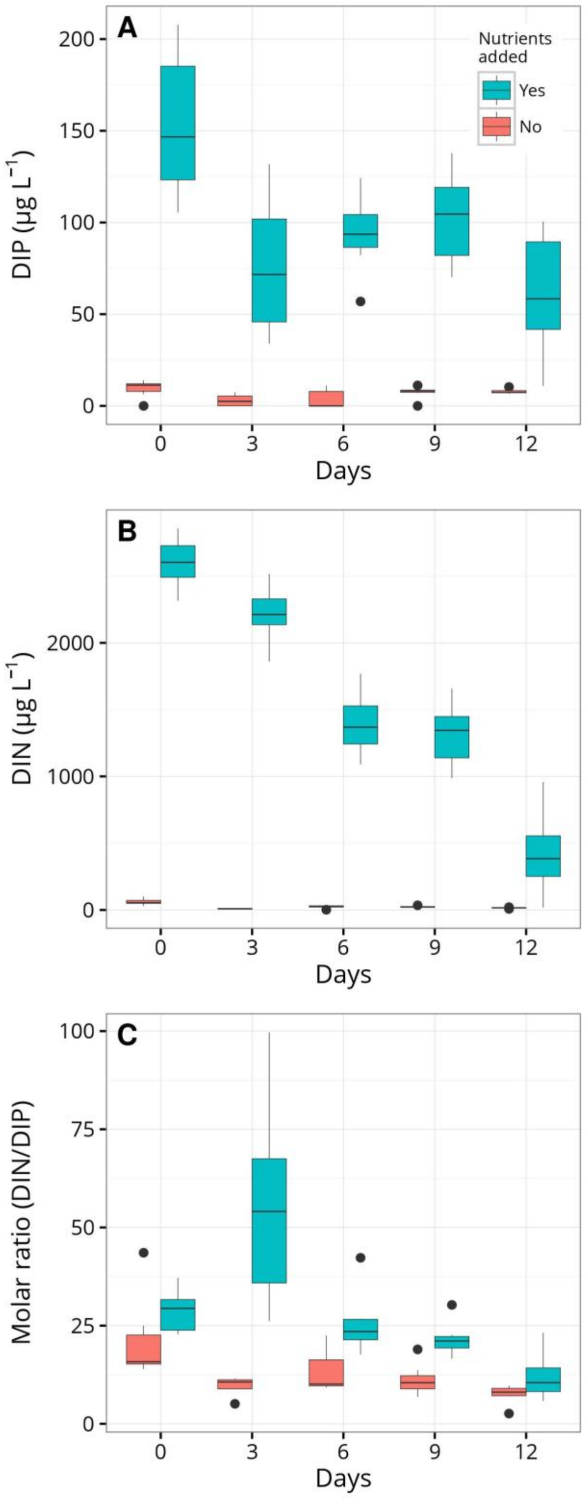
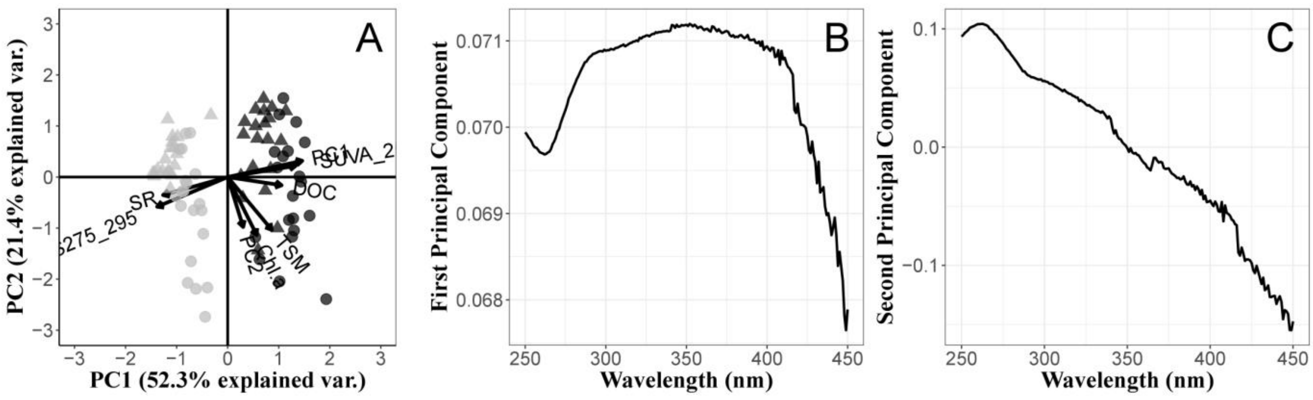

A
Nutrients added
Yes
No
DIP (µg L^{-1})
200
150
100
50
0
0
3
6
9
12
Days
B
DIN (µg L^{-1})
2000
1000
0
0
3
6
9
12
Days
C
Molar ratio (DIN/DIP)
100
75
50
25
0
0
3
6
9
12
Days

Fig 5


A
PC2 (21.4% explained var.)
PC1 (52.3% explained var.)
PC1
SUVA_2
DOC
SR
S275_295
TSM
PC2
Chl.a
B
First Principal Component
Wavelength (nm)
C
Second Principal Component
Wavelength (nm)