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Utilization and Uptake of Dissolved Organic Nitrogen by Freshwater Phytoplankton

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Master's thesis

Berlin, February 2014

Abstract

The contribution of dissolves organic nitrogen (DON) to algal growth was examined in batch cultures of freshwater algae (*Chlamydomonas spec.*, *Monoraphidium contortum*, *Cyclotella meneghiniana*, *Fragilaria ulna*, *Microcystis aeruginosa* and *Anabaena flos-aquae*). DON compounds tested were urea (U), humic substances (HS), dissolved combined amino acids (DCAA) and dissolved free amino acids (DFAA). They were either available as an exclusive N source or with dissolved inorganic nitrogen (DIN) in form of nitrate as a second nitrogen (N) source. All studied phytoplankton species could grow with the DON compounds U, DCAA and DFAA under both conditions. Although HS was used by some species, they showed no or even an inhibiting effect on the growth of *Fragilaria ulna* and *Microcystis aeruginosa*.

^{15}N short-term assimilation experiments revealed that DCAA was assimilated in high quantity, while the uptake of U-^{15}N was comparatively slow. Furthermore, changes in pigment composition were detected with a High-performance liquid chromatography (HPLC)-analysis of *Chlamydomonas spec.* demonstrating that the *chlorophyll a* to *chlorophyll b* ratio rises when the culture grows well on the DON compound. Pigment analysis with delayed fluorescence (DF) excitation spectroscopy illustrates the degradation (or decreased synthesis respectively) of phycobilisomes (PB) by *Anabaena flos-aquae* especially in the DCAA treatments.

Even when nitrate was used most efficiently for growth, the study supports the growing opinion that DON is accessible and utilized by freshwater algae species. U was used more efficient than DCAA and DFFA, whereas HS had the lowest effect on growth in relation to the available N. The impact of DON on freshwater ecosystems should not be underestimated and further research on the contribution of DON compounds to algae growth is recommended. For instance, the ability of algae to grow on natural concentrations of DON should be tested. Furthermore, bacteria are a key factor in the aquatic ecosystems N-cycle and their role in algae nutrition should always be included in research.

Keywords: Phytoplankton, dissolved organic nitrogen, nitrate, stable isotopes, pigments

Zusammenfassung

In Batch-Kulturen wurde der Einfluss von gelöstem organischen Stickstoffs (DON) auf das Wachstum von Süßwasseralgen (*Chlamydomonas spec.*, *Monoraphidium contortum*, *Cyclotella meneghiniana*, *Fragilaria ulna*, *Microcystis aeruginosa* and *Anabaena flos-aquae*) untersucht. Es wurden Urea (U), Huminstoffe (HS), gebundene gelöste Aminosäuren (DCAA) und frei gelöste Aminosäuren (DFAA) getestet. Diese waren entweder als einzige Stickstoffquelle vorhanden oder mit gelöstem anorganischem Stickstoff (DIN) in Form von Nitrat (N) als weitere Quelle. Alle untersuchten Phytoplanktonarten konnten unter beiden Bedingungen mit den DON-Verbindungen U, DCAA und DFAA wachsen. Auch wenn HS von einigen Arten verwendet wurde, zeigten sie keinen oder sogar einen hemmenden Effekt auf das Wachstum von *Fragilaria ulna* und *Microcystis aeruginosa*.

^{15}N Kurzzeit-Aufnahmeexperimente zeigten, dass DCAA in großen Mengen assimiliert wurde, wohingegen die Aufnahme von U-^{15}N vergleichsweise langsam war. Weiterhin wurden Veränderungen der Pigmentzusammensetzung in *Chlamydomonas spec.*-Kulturen mit der Hochflüssigkeitschromatographie (HPLC) analysiert welche zeigte, dass das Verhältnis von *Chlorophyll a* zu *Chlorophyll b* zunimmt, je besser die Algen auf der jeweiligen DON-Verbindung wachsen. Eine Pigmentanalyse mit verzögerter Fluoreszenzspektroskopie (DF) verdeutlicht den Abbau (bzw. die verringerte Synthese) von Phycobilisomen (PB) in *Anabaena flos-aquae*–Kulturen besonders in den DCAA-Ansätzen.

Auch wenn Nitrat am effizientesten für das Wachstum genutzt wurde unterstütz diese Untersuchung die wachsende Meinung, dass DON für Süßwasseralgen zugänglich ist und für das Wachstum verwendet werden kann. U wurde effizienter als DCAA und DFAA verwendet, wohingegen HS in Bezug auf den verfügbaren Stickstoff den geringsten Effekt auf das Wachstum hatte. Der Einfluss von DON auf die Ökosysteme der Binnengewässer sollte nicht unterschätz werden und weitere Forschung bezüglich des Einflusses von DON auf das Algenwachstum ist zu empfehlen. Beispielsweise sollte die Fähigkeit der Algen bei natürlichen DON-Konzentrationen zu wachsen untersucht werden. Weiterhin sind Bakterien ein Schlüsselfaktor im Stickstoffkreislauf aquatischer Ökosysteme und ihre Rolle in der Algenernährung sollte in Untersuchungen stets miteinbezogen werden.

Stichwörter: Phytoplankton, gelöster organischer Stickstoff, Nitrat, stabile Isotope, Pigmente

Preface

This thesis was conducted within the NITROLIMIT project of the Federal Ministry of Education and Research (BMBF). The study was part of the PhD Thesis of Dorothea Fiedler at the Leibniz-Institute of Freshwater Ecology and Inland Fishery (IGB) in the department of ecosystem research.

I am happy to thank everyone who was supporting me during my time at the IGB:

Foremost, my thanks apply to Prof. Dr. Markus Weiler and Prof. Dr. PD Hans-Peter Grossart for the examination of my thesis. I want to thank Prof. Dr. PD Hans-Peter Grossart and Dr. Jan Köhler for the constructive reviews and comments on my thesis. Thanks also to Dorothea Fiedler to give me the possibility to give me the possibility to contribute to her research project.

I want to thank Dr. rer. nat. Wolfgang Pritzkow from the Federal Institute for Materials Research and Testing (BAM) for the kind assistance and the valuable technical support and his expertise. Furthermore, I am grateful to Barbara Meinck, Grit Siegert, Helgard Täuscher, Katrin Preuß and Elke Zwirnmann for their technical assistance. Special thanks apply to Franziska Leunert and Dr. rer. Nat. Falk Eigemann for their positive attitude and the interesting and helpful input.

Berta Ortiz Crespo and Maria Badrian, I not only want to thank you for your help in the laboratory, but also for your cheerful minds at work and during free time. Laughing together with you saved me not only once.

Last but not least, I want to thank my German and Belgian family and my friends for the support during the last years, especially my parents. My studies would not have been possible without you. Floris, I am very lucky to know you Floris. Thank you for your friendship and unlimited optimism.

Anja Nohe

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List of Abbreviations

AA	Amino acid
AAc	Ammonium acetate solution
Al	Alanine
$AlK(SO_4)_2$	Aluminium potassium sulphate
APC	Allophycocyanin
Apx.	Appendix
Asp	Aspartic acid
BAM	Federal Institute for Materials Research and Testing
BMBF	Federal Ministry of Education and Research
C	Carbon or Control in figures
$CaCl_2$	Calcium chloride
$CaSO_4$	Calcium sulphate
chl a	*Chlorophyll a*
chl b	*Chlorophyll b*
chl c	*Chlorophyll c*
CO_2	Carbon dioxide
$CuSO_4$	Copper sulphate
d	Days
DCAA	Dissolved combined amino acid
DF	Delayed Fluorescence
DFAA	Dissolved free amino acid
DIN	Dissolved inorganic nitrogen
DMF	Dimethylformamid
DNA	Deoxyribonucleic acid
DON	Dissolved organic nitrogen
EDTA	Ethylenediamine tetraacetic acid

F_0	Fluorescence after dark adaption
$F_{\Delta t}$	Fluorescence after cultivation time t
F_t	Start fluorescence for µ
Fig.	Figure
Glu	Glutamic acid
Gly	Glycine
h	Hours
H_3BO_3	Boric acid
HC	Heterocysts
HCl	Hydrogen chloride
HMW	High molecular weight
HPLC	High-performance liquid chromatography
HS	Humic substances
I	Ion current [nA]
IGB	Leibniz Institute of Freshwater Ecology and Inland Fishery
iPAM	IMAGING-PAM Chlorophyll Fluorometer
IRMS	Isotope ratio mass spectrometry
KCl	Potassium chloride
KH_2PO_4	Potassium hydrogen phosphate
LMW	Low molecular weight
µ	Growth rate [d^{-1}]
magn.	magnification
$MgSO_4$	Magnesium sulphate
M III KS	Growth medium
$MnSO_4$	Manganese sulphate
n	Total number of replicates
N	Nitrogen
$NaHCO_3$	Sodium bicarbonate
Na_2MoO_4	Sodium molybdate

$NaNO_3$	Sodium nitrate
Na_2SiO_3	Sodium silicate
Orn	Ornithine
p	Statistical significance
PBS	Phycobilisome
PC	Phycocyanin
PE	Phycoerythrin
PS I	Photosystem I
PS II	Photosystem II
ρ	Spearmans's rho
RNA	Ribonucleic acid
rpms	rotations per minute
Ser	Serine
SIMS	Secondary ion mass spectrometry
Tab.	Table
Tau	Taurine
U	Urea
Val	Valine
$ZnSO_4$	Zinc sulphate

1 Introduction

Through anthropogenic activity today's environment is facing increased nutrient loads, particularly a high nitrogen supply. Humans altered and alter the nitrogen cycle especially by the combustion of fossil fuels and the extensive use of nitrogen fertilizer in agriculture. Not only the availability, but also the mobility of nitrogen dramatically increased, which results in considerable environmental consequences. Many terrestrial, marine and freshwater ecosystems are changed in their species composition, diversity and their dynamics (Vitousek et al. 1997). Especially the sensitive freshwater ecosystems are harmed by the increased nitrogen input. Anyway, the expensive reduction of dissolved inorganic nitrogen (DIN) loads into freshwaters could have a low effect when instead dissolved organic nitrogen (DON) or N_2 are used as a nitrogen source. This explains the importance of research on the utilization of dissolved organic nitrogen (DON) in aquatic environments.

Nitrogen (N) is a major element in living organisms. Proteins, enzymes, nucleic acids (DNA and RNA) and structural tissues contain N. Not only dissolved inorganic nitrogen compounds serve as nitrogen pools, but also a set of dissolved organic nitrogen compounds (JØrgensen 2009). The importance of DIN in the nutrition cycle of algae never seemed to be doubted, whereas the impact of dissolved organic nitrogen compounds on freshwater algae and the dynamic utilization of DON compounds by phytoplankton have been largely neglected. Because some DON compounds are quite resistant to degradation, it was believed that DON is more or less unavailable as a N source for phytoplankton nutrition. More recently, it has been shown that the turnover rates of DON in natural environments are much more rapid than previously assumed. During the last decades, several studies demonstrate the importance of DON in phytoplankton nitrogen cycle. However, there is still a great need for a better understanding of the ecological role of DON, especially in freshwater environments (Berman and Bronk 2003).

1.1 Dissolved organic nitrogen

In freshwaters DON often has very high turnover rates (JØrgensen 2009) and exceeds the amount of DIN which underlies seasonal processes (Berman and Bronk 2003). Today it is known that algae, cyanobacteria and bacteria represent a sink for DON and that these organic compounds play an active role in algae nutrition. Furthermore, the availability of different DON can influence the phytoplankton species composition (Berman and Bronk 2003).

Some DON compounds consist of urea, ureide, amino acids, nucleotides, amino sugars and humic substances. They can be grouped into high molecular components (HMW > 1kDa) and low molecular components (LMW) (Berman and Bronk 2003). The HMW components include proteins like en-

zymes, dissolved combined amino acids, bacterial cell wall proteins, nucleic acids (DNA, RNA) and humic substances. The group of LMW components comprises urea, peptides, amino sugars, dissolved free amino acids, pyrimidine, purines, pteridines, amides, polyamines and some others (Berman and Bronk 2003).

The composition of DON in the aquatic environment differs strongly in space and time. Sources for DON in lakes, rivers and the ocean can be autochthonous which means that the DON comes from soil by terrestrial runoff, by a release from sediments or through atmospheric deposition. Another origin is the DON contribution through allochthonous sources such as the release through cell death or lysis of algae, bacteria and macrophytes, or through excretion from micro- and macrozooplankton or waterfowl. A lot of DON transported through rivers into lakes and estuaries come from anthropogenic influenced environments (Berman and Bronk 2003).

1.1.1 Humic substances

Humic substances (HS) are generated during the decomposition of organic material (Schwoerbel and Brendelberger 2005) and are often an important fraction of the DON pool (Berman and Bronk 2003). Main components are the fulvic acids, humic acids and humins which differ in their size and solubility properties (Schwoerbel and Brendelberger 2005). Some components are loosely attached to each other like amino acids (Berman and Bronk 2003) which can be released from humic matter (JØrgensen 2009). Other compounds are part of the core structure (Berman and Bronk 2003). In waterbodies humic substances play an important role as chelating agent and cation exchanger (Schwoerbel and Brendelberger 2005).

Even when the portion of HS in natural environments can be quite high, there is a lack of knowledge to which extent humic substances contribute to phytoplankton N nutrition. In some experiments the growth of some algae species was stimulated by the addition of humic substances, even when their N content is low (Berman and Bronk 2003).

1.1.2 Amino Acids

One distinguishes between dissolved free amino acids (DFAA) and dissolved combined amino acids (DCAA) (Berman and Bronk 2003). Frequently occurring DFAA from proteins are alanine (Al), aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), serine (Ser) and valine (Val). Non-protein amino acids that are commonly found in lakes are, e.g., ornithine (Orn) and taurine (Tau) (JØrgensen 2009). DCAA can consist of proteins and oligo- and polypeptides (Berman and Bronk 2003). DCAA can also be attached to other inorganic or organic material. Because DCAA must first be hydrolysed by microorganisms into free amino acids, the concentration of DCAA is typically higher than the concentration of DFAA (JØrgensen 2009).

In general the natural occurring amino acid concentration is mostly low and contributes a few percentages of the whole DON pool. Amino acids which derive from the hydrolysis of proteins and peptides can be taken up directly by bacteria and phytoplankton (Berman and Bronk 2003). DFAA are mainly transported by active transport in the algae cells (Antia et al.1991). Many algae can grow on amino acids as a N source, but the ability to grow on it as the single nitrogen source is limited. There is a discrepancy between the ability to transport amino acids into the cell and the capability to metabolize them (Kohl and Nicklisch 1988). Amino acids are hydrolysed through the surface enzyme amino-oxidase and transported into the cell as NH_4^+ (Berman and Bronk 2003). There are at least three different carrier systems to transport either alkaline, acid or neutral amino acids into the algal cell (Kohl and Nicklisch 1988).

A part of the DCAA fraction is rapidly turned over in the environment while other DCAA are quite resistant to microbial degradation (Berman and Bronk 2003). DCAA and DFAA are released from living algae, but also from autolysis or viral lysis. Other sources are zooplankton and solving from organic seston. For estuaries and coastal waters it was found that DFAA are used rather than DCAA, and it did not matter if the concentration of DFAA was high or low. In other environments, the opposite situation occurred (Berman and Bronk 2003). In lab experiments with axenic cultures it could be shown that they can use amino acids when there is a high initial concentration offered. Yet, it remains unclear whether they are also able to use lower concentrations (Berman and Bronk 2003).

1.1.3 Urea

Urea (U) is a degradation product of purines (JØrgensen 2009) and arginine removed by bacteria (Berman and Bronk 2003), but also an immediate waste product of aquatic organisms (JØrgensen 2009). It is excreted by freshwater crabs, fish, zooplankton and many other organisms. Rainfall, water runoff and atmospheric deposition lead to a further increase in urea concentration in freshwaters. In addition, U contributes about 40% of the N fertilizer used worldwide (Berman and Bronk 2003).

U is a valuable nitrogen source to phytoplankton (JØrgensen 2009) and can be taken up directly by phytoplankton. Also the degradation products NH_4^+ and the nitrified NO_3^- can be assimilated directly. In lab experiments the uptake of urea was exceeded by the uptake rate of NH_4^+, but the uptake rate of urea was higher than the one of NO_3^-. An explanation for this is that the small sized NH_4^+ can easier pass the cell membrane while urea uptake is dependent on an energy-rich transport system. Furthermore, the plankton needs to synthesis the urease to metabolize urea within the cell (Berman and Bronk 2003).

Urea is a mayor N source for some marine and freshwater cyanobacteria. It is known that axenic cultures of algae can use urea at high amounts. Yet, it is unclear if they are able to use much lower concentrations. In some studies, algae were able to decompose urea faster than the present bacteria (Berman and Bronk 2003).

1.2 Nitrogen use by Bacteria

DON is an important N source not only for phytoplankton, but also for bacteria (Sanderson et al. 2008). The degradation through bacteria seems to be the main sink for DON (Berman and Bronk 2003). Bacteria are strong competitors for available N. Especially urea is used by them as a N source. In microcosm experiments with urea and AA, urea made up to 88 % of the DON-N nutrition (Sanderson et al. 2008). In other studies it was found that urea uptake is very variable and lowers when also DFAA and NH_4^+ is available (JØrgensen 2003). Furthermore, bacteria are considered to be the main consumer of the DFAA fraction (Berman and Bronk 2003). In addition, DCAA appears to be an important substrate for planktonic bacterial growth (Rosenstock and Simon 1993), especially when there is a lack of DFAA and NH_4^+ in the water (Middelboe et al. 1995). DCAA-uptake appears to be is non-specific (Coffin 1989).

It is known that cyanobacteria and their associated bacteria benefit from each other. Associated bacteria are capable to assimilate a variety of organic substances. Moreover, without the presence of bacteria some genera cannot grow in laboratory experiments. Through experiments with axenic and non-axenic *Anabaena flos-aquae* strains it was shown that bacteria-free cultures have lower N_2-uptake rates (Paerl 1988). The effect of bacteria was excluded in our study, but their importance for DON degradation should not be forgotten when interpreting the result.

1.3 Aim of the study

The aim of the present study is to contribute to a profound knowledge on the relevance of DON in freshwater phytoplankton nutrition. Growth and DON uptake experiments were performed with the green algae *Chlamydomonas spec.* and *Monoraphidium contortum*, the diatoms *Cyclotella meneghiniana* and *Fragilaria ulna*, and the cyanobacteria *Microcystis aeruginosa* and *Anabaena flos-aquae*. Selected dissolved organic nitrogen compounds were used: urea (U), dissolved combined amino acids (DCAA), dissolved free amino acids (DFAA) and humic substances (HS). All were tested under two conditions. On the one hand, they served as the sole nitrogen source and on the other hand, they were supplied with a low amount of DIN in form of nitrate.

Growth rates were determined either through measurements of fluorescence with an IMAGING-PAM fluorometer or through measurements of the total *chlorophyll a* content with a PHYTO-PAM fluorometer. The DON compounds, except the humic substances, were marked with ^{15}N isotopes. Thus, the ^{15}N uptake could be analysed using isotope ratio mass spectrometry (IRMS).

When starving from nitrogen phytoplankton can undergo morphological changes for instance a change in pigment composition (Schwoerbel and Brendelberger 2005). Therefore, several analyses of pigment composition were performed. Via high pressure liquid chromatography (HPLC) the *chlorophyll a* (*chl a*) and *chlorophyll b* (*chl b*) content *of Chlamydomonas spec.* was analysed.

The phycobiliprotein phycoerythrin (PE) appears to be an important part of the internal algal nitrogen pool and serves as a source for nitrogen when starving from it (Bird et al. 2004). To answer the question to which extend they are degraded under different DON treatments they were detected via delayed fluorescence (DF) excitation spectroscopy. The C:N ratio was analysed for *Chlamydomonas spec.* which also changes through nitrogen starvation. Furthermore, the N_2-fixing cyanobacteria respond to a lack of nitrogen with the synthesis of heterocysts (Bird et al. 2004). For that reason the cyanobacterium *Anabaena flos-aquae* was microscopically analysed on the change in heterocysts to biovolume ratio.

With our study the question should be answered if and to which extend different algae groups can use specific DON compounds under laboratory conditions and how they influence algal physiology and morphology.

2 Material and Methods

2.1 Organisms

In this study non-axenic algae cultures isolated from the Müggelsee, Germany, in 2011 were analysed. Six algae species were used: the green algae *Chlamydomonas spec.* and *Monoraphidium contortum*, the two diatom species *Cyclotella meneghiniana* and *Fragilaria ulna*, as well as the cyanobacteria *Microcystis aeruginosa* and the N_2-fixing *Anabaena flos-aquae*.

2.2 Cultivation

The cultures were kept in M III KS nutrient solution, which was especially developed for diatoms (Nicklisch 1992). To guarantee the same conditions for all algae, this medium was used for each of the three analysed groups green algae, diatoms and cyanobacteria. The medium consists of macro elements calcium sulphate ($CaSO_4$), calcium chloride ($CaCl_2$), magnesium sulphate ($MgSO_4$), sodium nitrate ($NaNO_3$), potassium hydrogen phosphate (KH_2PO_4), potassium chloride (KCl) and sodium silicate (Na_2SiO_3), as well as hydrogen chloride (HCl) which were added to distilled water. The medium needed to be autoclaved. Afterwards, a basis fertiliser solution was added, which contained different micronutrients such as boric acid (H_3BO_3), manganese sulphate ($MnSO_4$), zinc sulphate ($ZnSO_4$), sodium molybdate (Na_2MoO_4), copper sulphate ($CuSO_4$) and aluminium potassium sulphate ($AlK(SO_4)_2$). Also sodium bicarbonate ($NaHCO_3$) and ethylenediamine tetraacetic acid EDTA were added. Finally, different vitamins were added to the medium and the pH was measured, and adjusted to a pH of 8.3.

The cultures were cultivated continuously in four to five 1 litre Erlenmeyer flasks which contained about 400 ml of medium. They were stored in a climate-controlled chamber (BINDER) at 16 °C with illumination simulating a 14 h:10 h light:dark photoperiod on a rotary shaker at 100 rpms. The cultures were exposed to ca. 90 $\mu Em^{-2}s^{-1}$ (measured with a spherical light sensor of Biospherical Instruments Inc.). New medium was added regularly every ten days until enough algae biomass could be harvested.

2.3 Experimental design and culture conditions

The growth experiments and the experiments for the stable isotope analysis were both performed in a series of 500 ml Erlenmeyer flasks. Half of them contained 200 ml N-free medium, the other half medium with NO_3^- (Fig. 1). Additionally, the following dissolved organic nitrogen compounds were add-

ed: humic substances (HS), which originated from marsh land close to Lake Stechlin, Germany, dissolved combined amino acids (SIGMA-ALDRICH Algal crude protein extract-^{15}N, 98 + atom% ^{15}N), dissolved free amino acids (SIGMA-ALDRICH Algal amino acid mixture-^{15}N, 98 + atom% ^{15}N) and urea (ISOTEC Urea-$^{15}N_2$, 98 + atom% ^{15}N). The added amount of organic N supplements were adjusted to five times the natural average concentration (Tab. 1) (data from Chemistry Laboratory of the IGB). Treatments with nitrate additionally contained 50 µg L^{-1} NO_3^-–N. For every treatment four replicates were used.

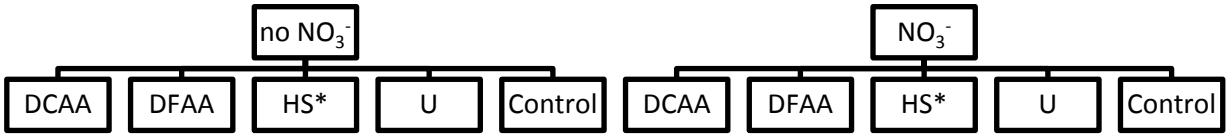

Fig. 1 Experimental design. *Note that HS was not analysed with mass spectrometry.

In total, 40 Erlenmeyer flasks were incubated. In principle, the experiments were carried out under the same illumination and temperature conditions as used for algae cultivation (see 2.2). A modification was used for the flasks without humic substances. They were stored under an acrylic glass box which contained the same concentration of humic substances as the cultures. Thereby, the difference in light absorption in the flasks with humic substances was compensated.

Tab. 1 Used start concentrations of nitrogen. HS and NO_3^- were not labelled with ^{15}N.

Treatment	total N [mg L^{-1}]	^{15}N [mg L^{-1}]
HS	1.93	-
DCAA	0.50	0.49
DFAA	0.07	0.07
U	0.51	0.50
Control	0.00	-
NO_3^-/HS	1.98	-
NO_3^-/DCAA	0.55	0.54
NO_3^-/DFAA	0.12	0.12
NO_3^-/U	0.56	0.55
NO_3^-/- (control)	0.05	-

Furthermore, air was bubbled through the culture flask though a cannula inserted in the closing plug to guarantee that no CO_2 limitation occurred during cultivation. To avoid contamination of the culture, a filter was put on top of the cannula before the air entered the bottle. Through a second cannula the air could leak again from the flask.

2.4 Culture preparation

To concentrate the cultures, the algae were filled in Falcon Tubes and centrifuged for 5 minutes at 3000 rpms. Afterwards they were washed three times at 2500 to 3000 rpms with modified nitrogen

free M III KS nutrient solution. The concentrated and washed algae were then transferred for 24 hours to a flask with about 300 to 400 ml of nitrogen free M III KS medium.

After 24 hours the cell number of the two green algae species and the two diatoms was determined by counting them under the microscope. For the two cyanobacteria a dilution series was made to determine the amount of culture to be used. The initial abundance in growth experiments should lay around 30.000 cells per ml which corresponded to the algae density of blooms in the Müggelsee (data of IGB's department of ecosystem research). Due to the detection limit of the stable isotope method a ten times higher amount of cells was used for these experiments.

2.5 Measurement and determination of growth rate

The cultures of the growth experiments were incubated for 8 to 10 days. Samples were taken at one day intervals. Through the examination of the fluorescence with the IMAGING-PAM Chlorophyll Fluorometer (iPAM) (Heinz Walz GmbH, Effeltrich, Germany) temporal changes in algal biomass could be monitored. Due to the non-functional camera of the iPAM the *Anabaena flos-aquae* experiment was measured with an available PHYTO-PAM fluorometer (Heinz Walz GmbH, Effeltrich, Germany). Both, the iPAM and the PHYTO-PAM, measure the fluorescence F_0. Out of the determined F_0 –value the PHYTO-PAM calculates the *chl a* concentration of the sample using a preset parameter. Due to the close relation of fluorescence and *chl a* with the biomass, both units were taken as a reliable basis to measure the change in biomass.

In the growth experiments measured with the iPAM triplicate subsamples of 1 ml each were taken. For the measurement with the PHYTO-PAM only one sample was taken. The samples needed to be dark adapted for 20 minutes before measurement. The HS samples of *Anabaena flos-aquae* were diluted 1:1 and measured in a single-sided mirrored-cuvette.

During the measurement with the PHYTO-PAM the background fluorescence was subtracted with the zero-offset function. Therefore, the culture medium was filtered to retain organic material. The fluorescence of the nutrient solution and the soluble organic compounds were measured (Chang et al. 2012). For iPAM measurements no correction of the background fluorescence was made (see 4.1).

As biomass is correlated to the fluorescence, the specific growth rate μ was calculated via the change in fluorescence, and accordingly *chl a*, over time (Δt):

$$\mu = \ln\left(\frac{F_{\Delta t}}{F_t}\right) \times \left(\frac{1}{\Delta t}\right) \qquad (1)$$

where $F_{\Delta t}$ is the fluorescence after cultivation time t, F_t is the start fluorescence and Δt the time until the end of the experiment (Mehnert et al. 2010).

For every species the growth rate μ was calculated by taking a F_0 and an $F_{\Delta t}$ value of a growth phase as close as possible to an exponential curve. Due to the different length of the lag and growth phases,

these F_0 and $F_{\Delta t}$ were taken from different days for every species. To make a statistical comparison possible, the same time points were chosen for every treatment within an experiment of a single species.

For *Chlamydomonas spec.* the growth rates were calculated from the third until the fifth day, for *Monoraphidium contortum* and *Fragilaria ulna* from the first until the third day of the experiment and for C*yclotella meneghiniana* from the first until the fifth. The growth rates of *Microcystis aeruginosa* were calculated from day zero until the last day of the experiment (day seven) and for *Anabaena flos-aquae* µ was calculated from day five until day eight.

2.6 Pigment Analysis

2.6.1 High-performance liquid chromatography (HPLC)

Phytoplankton uses a range of light harvesting pigments for photosynthesis. The main pigment used is *chlorophyll a* (*chl a*). Further pigment compounds are *chlorophyll b* (*chl b*) and *c* (*chl c*), carotenoids and phycobiliproteins. High-performance liquid chromatography (HPLC) is an excellent tool to identify these pigments and their degradation products, to differentiate the algal groups in a water sample or to determine their biomass and productivity (Bidigare et al. 2005).

Whatman GF/F glass fibre filters were used to concentrate the algae from every sample. To assess a more reliable statistical analyse, all replicates were filtered twice. The amount of water filtered varied between 20 to 100 ml. The filters were folded and stored at -85 °C in frost-resistant Eppendorf reaction vessels.

Before each measurement, samples were freeze-dried. By dimmed light and in the freezer, a mixture of glass beads and 1 ml dimethylformamide (DMF) were added into the Eppendorf reaction vessels. Afterwards the samples were shaken at maximum frequency. 0.1 ml ammonium acetate solution (Aac) solution was added and the samples were shaken for 45 minutes. 200 µl of the extract were transferred to plastic vials and transferred to the cooled auto sampler (Shatwell et al. 2012).

A volume of 60 µl was injected with a separation module (WATERS Alliance HPLC 2695). Samples were measured at two wavelengths, 440 nm and 410 nm (WATERS Photodiode Array Detector 2996). The spectra of 440 nm were analysed for the pigments *chl a* and *chl a* related pigments, *chl b* and *chl c* and their degradation products, echinenon, zeaxanthin and their derivates. For the 410 nm spectra only *chl a* and the related pigments were analysed. Pigments were identified by their chromatographic curve, as well as their retention time. To quantify the amount of pigment, the area under the peak curve was integrated and compared to spectra from the literature (Jeffrey et al. 1997). For the analyses of *Chlamydomonas spec.* the *chl a*:*chl b* ratio was calculated.

2.6.2 Delayed Fluorescence (DF) Excitation Spectroscopy

The delayed fluorescence (DF) excitation spectroscopy is used to detect algal composition by the use of information about their photosynthetic active pigments (Bodemer 2004). The resulting excitation spectra allow to distinguish between the accessory pigments phycocyanin (PC), phycoerythrin (PE) and allophycocyanin (APC) and *chl a*. In this study the method was applied for *Microcystis aeruginosa* and *Anabaena flos-aquae* in order to gain information about the amount of phycobiliproteins in proportion to the *chl a* content and additionally to compare the phycobiliproteins with each other.

The four replicates of one treatment were pooled and dark-adapted for at least 20 minutes. The phytoplankton samples were excited with monochromatic light between 400 to 730 nm. The excitation light results in an energy transfer and a following charge separation at the reaction centres of the PSI and PSII (delay time 0.2 - 1 s). By photon counting the delayed fluorescence was detected, which resulted from an electron-hole at PSII and the caused light-dark transition (Bodemer 2004). Every pooled treatment was measured twice to gain a more reliable result.

For the measured emitted wavelengths, fits were produced. The resulting chromatograms were analysed with *Origin 8.5G* (Microcal Software, Inc.) and the *Multiple Peak Fit* function. A Gauss curve fit was applied to the resulting spectra in order to determine the contribution to charge separation of the single accessory pigments (Bodemer 2004). The peak of PE was set to around 550 nm, the peak of PC at around 610 nm, the peak of APC at 650 nm and the *chl a* peak at 668 nm. The bandwidth was fixed to 25 nm. Due to PE poor cultures and a non-optimal Gauss curve fit of this protein, the integrals of the PC and PE curves were merged. A (PE+PC):APC:*chl a*, as well as a (PE+PC):APC ratios were calculated.

2.7 Carbon Nitrogen Elementary Analysis

The CN-elementary analysis is a method to determine the carbon and nitrogen amount of a sample (Kohl and Nicklisch 1988) where the C:N ratio represents the relative proportion of the two elements. The analysis was done for the green algae species *Chlamydomonas spec.* Whatman GF/F glass fibre filters were washed with around 100 ml distilled water. They were put in a heating cabinet at around 105 °C until they were dry. Afterwards the filters were put overnight in a muffle furnace at 450 °C in order to burn all organic substances which were possibly left on the filter. Then every filter was weighed and stored in an exsiccator until further analysis.

For the CN-analysis there were filtered twice 50 ml per replicate. Afterwards the filters were dried again (see above) and the resulting dry weight was determined. Subtracting the two weights from each other gave information about the present cell material on the filter. Subsequently they were packed in aluminium.

The CN-analysis was carried out with an elementary analyser (elementar vario EL, Elementar Analysensysteme GmbH). The glass fibre filters with the sample were fold, packed in tin foil and pressed free from air. The filters and samples were burned under oxygen with a mixed catalyst at 980 °C. The flue gases were reduced and C and N measured as CO_2 and N_2 with a heat conductivity detector (Zwirnmann et al. 2007). The C:N ratio for every sample was analysed.

2.8 Stable Isotope Analysis

The uptake of specific DON compounds was measured by using ^{15}N -labelled molecules. The experiment was conducted for *Chlamydomonas spec.*, *Cyclotella meneghiniana* and *Microcystis aeruginosa*. The preparation (see 2.4) as well as the experimental setup (see 2.3) was the same as for all growth experiments with the exception that the samples of the HS treatment were not analysed at the end.

In order to survey the difference in uptake velocity, samples were collected after 1, 6, 24 and 72 hours. The samples collected after 72 hours were only analysed for *Cyclotella meneghiniana*. Aside from that, there were only triplicates in the experiment with *Cyclotella meneghiniana*, whereas in experiments with *Chlamydomonas spec.* and *Microcystis aeruginosa* the number of replicates was increased to four.

For each sample, 50 ml of culture were transferred into a Falcon Tube and the algae were concentrated by centrifugation (10 minutes, 4500 rpms). The pellet was transferred to a culture vessel and washed three times for ten minutes with N-free medium. At the end, the spared medium was removed. The remaining pellet was stored at -80 °C. Before the samples were analysed, they were freeze-dried and stored at -4 °C. The measurements were executed at the Federal Institute for Materials Research and Testing (BAM) in Berlin.

At the BAM the samples needed to be weighted first and packed into tin boats. Before the measuring process started, standards were measured first. The resulting ion current I was set to ca. 6 nA. The material in each sample creates an ion flux similar to the reference. In the elemental analyser (elementar vario EL III, Elementar Analysensysteme GmbH) the samples were burned and the resulting gas was first entering an oxidation, afterwards a reduction pipe and then an adsorption column. The resulting N_2 gas entered the sector mass spectrometer (GV Instruments IsoPrime) in which the ions were deviated through a magnetic and an electrical field. Through a different mass to charge ratio the resulting radius was bigger for heavier isotopes. Two Faraday cups detected the striking ^{15}N and ^{14}N ions. By using equation (2):

$$\delta^{15}\mathrm{N} = \frac{\left(\frac{^{15}\mathrm{N}}{^{14}\mathrm{N}}\right)_{\mathrm{sample}} - \left(\frac{^{15}\mathrm{N}}{^{14}\mathrm{N}}\right)_{\mathrm{standard}}}{\left(\frac{^{15}\mathrm{N}}{^{14}\mathrm{N}}\right)_{\mathrm{standard}}} \times 1000 \qquad (2)$$

$\delta^{15}N$ values were determined which represented the ^{15}N to ^{14}N isotope ratio. Alternately a standard N_2 gas and a sample were measured. In addition, at every sixteenth sample a standard was measured (personal communication with Dr. rer. nat. Wolfgang Pritzkow).

2.9 Microscopic analysis

Anabaena flos-aquae cultures were fixed with acidic Lugol's solution on the last day of the growth experiment. The number of heterocysts per biovolume was determined in Utermöhl sedimentation chambers and with the help of an inverted phase contrast microscope (Nikon Diaphot 300, 200x magn.). The settling time was about four hours. For every sample 30 filaments were measured in length and width and the number of heterocysts was counted. The heterocyst to biomass ratio was calculated and the change for the different treatments was investigated.

2.10 Statistical data analysis

The statistical analyses were carried out with the programming language *RStudio* (Version 0.97.551, © 2009-2012 RStudio Inc.). The means $\bar{x}$ were computed using the following equation (3):

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} \qquad (3)$$

where n is the total number of replicates in the set and x_i represents the individual values for a particular parameter.

The standard deviations (sd) were calculated using equation 4:

$$sd = \sqrt{\frac{\sum_{i=1}^{n} (\bar{x} - x_i)^2}{n-1}} \qquad (4)$$

The variance analysis of the growth rates (for calculation see 2.5) was performed by using the non-parametrical Wilcox test. Due to the ties, the package *exactRankTest* for the Wilcox exact test was applied. The growth rates were compared among one species experiment. There was no comparison made between the species, because the growth rates were calculated for different periods. The treatments were compared with each other and with the corresponding control. Also the treatments of the nitrate containing and the nitrate free experiments with the same DON compound available were compared.

Due to the small number of replicates (n=3), there was no variance analysis for the uptake experiment with *Cyclotella meneghiniana* made. The improved experimental design with four replicates per treatment in the conducted experiments with *Chlamydomonas spec*. and *Microcystis aeruginos*a made a variance analysis with the non-parametrical Wilcox test possible. The test was used to determine the statistical differences of the $\delta^{15}N$-values of each treatment and sampling time (1, 6, 24 hours) with the

corresponding control, as well as between the treatments with the same DON compounds (with and without NO_3^-).

Furthermore, the *chl a*:*chl b* ratio deriving from the HPLC-aided pigment analysis, the C:N ratios and the heterocysts counting were statistical analysed with the Wilcox test. Additionally to the comparison of the treatments with the corresponding controls, and between the nitrate free and the nitrate containing experiment, there was also a variance analysis between each treatment and the reference made. The reference sample was taken from the culture before the experiment started.

In order to give a distinct overview of the variance statistics, results were grouped as follows:

[+] statistical significant difference ($p \leq 0.05$)

[~] trend ($0.05 < p \leq 0.058$)

[-] no statistical significant difference ($p > 0.058$)

The correlation of different *chl a* proxy measurements were evaluated. To summarize, in our study four methods were used to make an assumption of the *chl a* content in a culture: (i) F_0 measurements with the iPAM, (ii) *chl a* detection with the PHYTO-PAM, (iii) measurement of the *chl a* concentration through HPLC and (iv) *chl a* estimation with DF. These methods were compared when two or more methods were applied in the same experiment. This was three times the case:

(i) in the experiment with *Chlamydomonas spec.* the *chl a* concentration was determined with HPLC and the F_0-values were detected with the iPAM,

(ii) in the experiment with *Microcystis aeruginosa* the F_0-values were measured with the iPAM and the *chl a* amount was determined through the integration of the excitation spectrometry of the delayed fluorescence,

(iii) in the experiment with *Anabaena flos-aquae chl a* was detected with the PHYTO-PAM and the *chl a* concentration was determined through the integration of the delayed fluorescence intensity detected with excitation spectrometry.

The correlation of the mean values determined with the two measuring modes was tested with the Spearman's rank correlation coefficient (Spearman's rho).

3 Results

3.1 Green Algae

3.1.1 *Chlamydomonas spec.*

The nitrate free experiment with the green algae *Chlamydomonas spec.* (Fig. 2A) showed an initial lag period of two days with the two DON sources HS and U. In the DCAA and DFAA treatments this lag phase lasted 3 days. Growth on HS and U started on the second day and lasted until the fifth day. Growth on U reached a plateau which was more or less stable until the seventh and last day of the experiment, while algal growth on HS decreased between the fifth and sixth day. Growth on DCAA and DFAA started on day three, but already stopped on the fourth day. From day four to five there was no change in fluorescence in both treatments. The DON free control increased from day 0 until day 7.

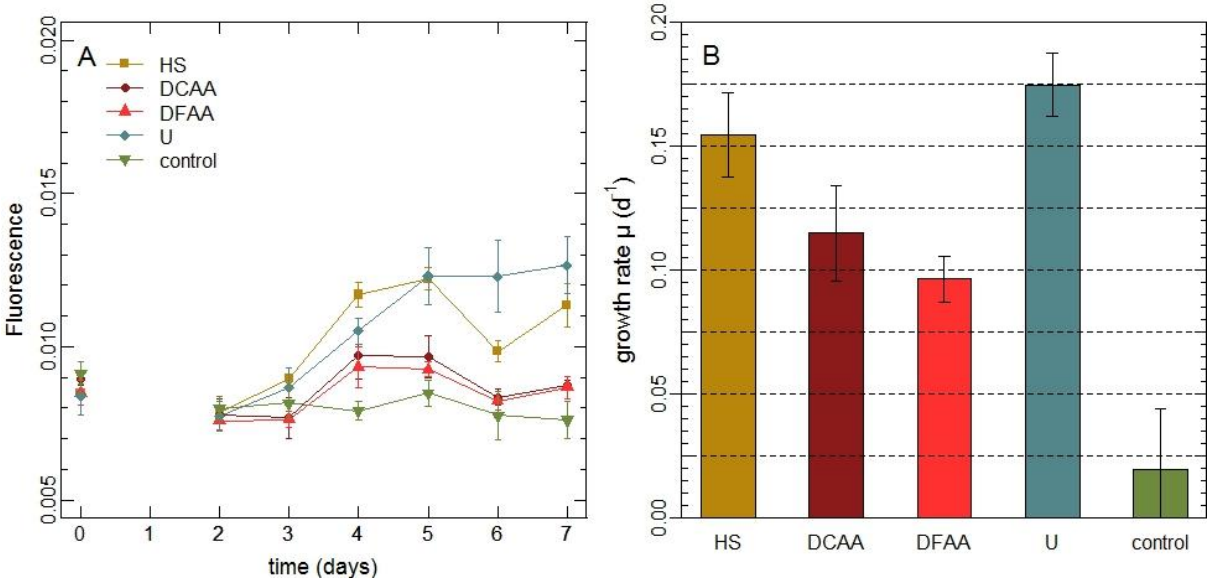

Fig. 2 Nitrate free growth experiment with *Chlamydomonas spec.* A: Fluorescence values, B: Growth rates μ (d^{-1}) between day 3 and 5. The experiment was performed for seven days (n=4). Error bars show standard deviation.

Growth rates (Fig. 2B and Apx. A Tab. 1) were calculated from day three until day five. Algae growing on U had the highest growth rate (μ = 0.175 ± 0.013 d^{-1}), followed by algae in the HS treatment (μ = 0.154 ± 0.017 d^{-1}), in the DCAA treatment (μ = 0.115 ± 0.019 d^{-1}) and the DFAA treatment (μ = 0.096 ± 0.009 d^{-1}). The control also had a little gain in fluorescence (μ = 0.02 ± 0.025 d^{-1}) between day three and five, but increased overall from the first until the last day of the experiment. The comparison of growth rates showed, that any treatment was statistical significantly different from the control (Apx. B Tab. 1). The two treatments DCAA and U which had a similar total N content were significantly different from each other.

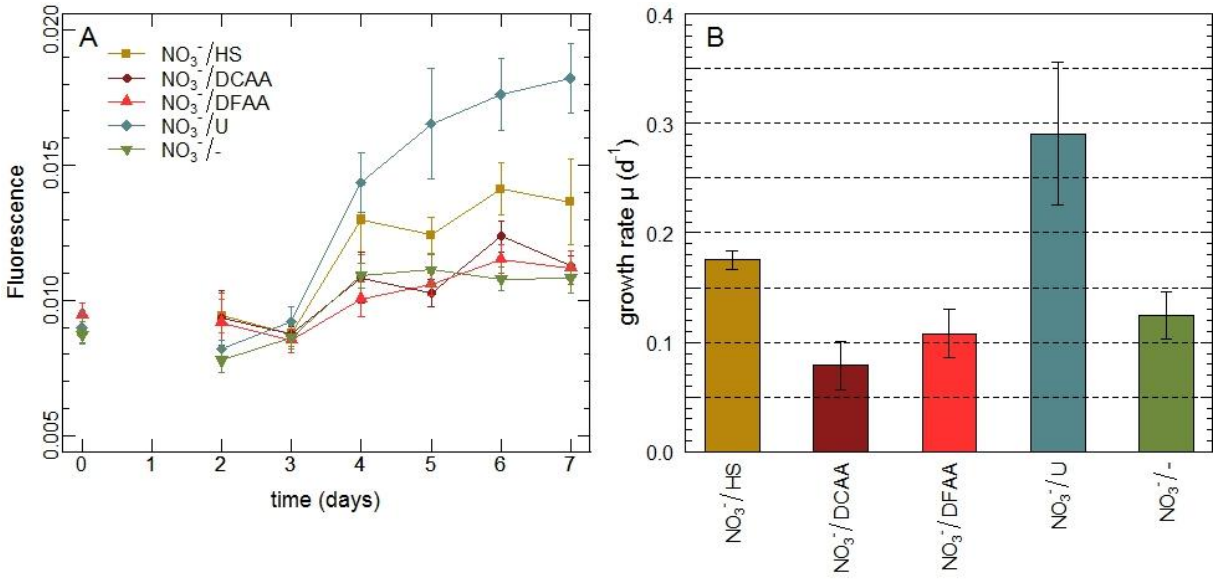

Fig. 3 Nitrate containing growth experiment with *Chlamydomonas spec.* A: Fluorescence values, B: Growth rates μ (d^{-1}) between day 3 and 5. The experiment was performed for seven days (n=4). Error bars show standard deviation.

The DON/DIN treatments (Fig. 3A) showed a similar order of the growth curves, but increases in fluorescence were generally higher after nitrate addition. The lag phase of algae growing on NO_3^-/U and NO_3^-/- (control) lasted the first two days, while the lag phase of algae growing on NO_3^-/HS, NO_3^-/DCAA and NO_3^-/DFAA lasted one day longer. In these three treatments, thereafter, algae grew until day six. Solely, in the NO_3^-/U treatment algae grew until the seventh day, which is equivalent to a growth phase of five days in total. In contrast, in the NO_3^-/- control algae grew until day four and thereafter reached the steady-state phase which lasted until the end of the experiment.

Growth pattern and calculated growth rates (Fig. 3B and Apx. A Tab. 1) revealed that algae in the NO_3^-/U treatment had the highest growth rate ($\mu = 0.291 \pm 0.065\ d^{-1}$), followed by the algae growing on NO_3^-/HS treatment ($\mu = 0.175 \pm 0.008\ d^{-1}$). NO_3^-/DFAA treatment ($\mu = 0.108 \pm 0.022\ d^{-1}$) and NO_3^-/DCAA treatment ($\mu = 0.079 \pm 0.022\ d^{-1}$) showed even lower growth between day three and five than the control NO_3^-/- ($\mu = 0.125 \pm 0.022\ d^{-1}$). The growth rates of the algae growing on NO_3^-/HS and NO_3^-/U were significantly different from the control, while treatments NO_3^-/DCAA and NO_3^-/DFAA were not (Apx. B Tab. 1). Drawing a comparison of NO_3^-/DCAA and NO_3^-/U, the result was a significant difference between the growth rates of the two treatments.

When comparing the NO_3^--free treatments with those where NO_3^- was added, algal growth rates in the treatments DCAA and NO_3^-/DCAA, as well as DFAA and NO_3^-/DFAA did not differ significantly from each other. Treatments HS and NO_3^-/HS were not significantly different from each other, but its p-value almost reached 0.05. Treatment NO_3^-/U and U, as well as both controls significantly differed from each other.

The ^{15}N short-term assimilation experiment with *Chlamydomonas spec.* (Fig. 4) revealed that already after one hour the enrichment of ^{15}N was significantly different from the control (Apx. B Tab. 2). Both DCAA treatments showed the highest enrichment of ^{15}N. In the course of the experiment the enrich-

ment was always highest in the NO_3^-/DCAA treatment than the one with only DCAA. In both U treatments highest accumulation of ^{15}N occurred between 6 and 24 hours. Even when the accumulation of ^{15}N was higher in the single treatment with U, there was never a significant difference in the enrichments. The algae growing on DFAA showed the lowest values of $\delta^{15}N$, but were slightly higher than the controls.

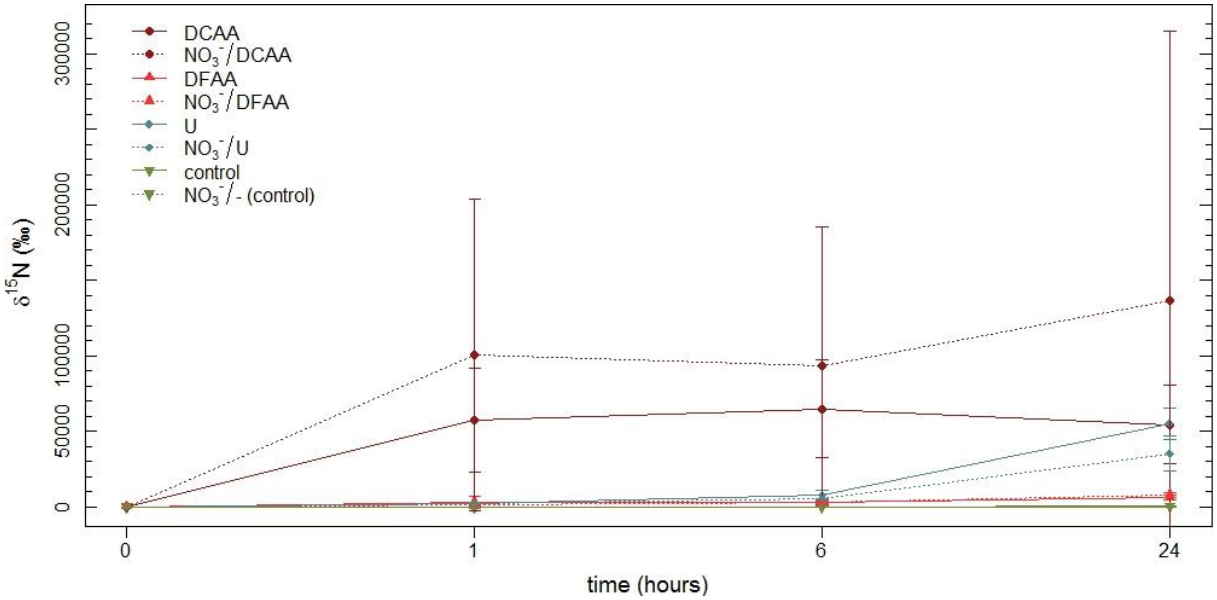

Fig. 4 ^{15}N assimilation experiment with *Chlamydomonas spec*. DCAA, DFAA and U were labelled with ^{15}N. NO_3^- was not labelled with ^{15}N . Samples were taken after one, six and 24 hours (n=4). Error bars show standard deviation.

HPLC analysis of the *Chlamydomonas spec.* experiment (Fig. 5) was performed for pigments *chl a* and *chl b*, as well as related pigments and their degradation products. Neither degradation products, nor related pigments were detectable. The calculated *chl a*:*chl b* ratio, however, changed for the different treatments (Fig. 5 and Apx. A Tab. 3). Comparing the ratios with each other revealed that the two highest values in the nitrate free experiment belonged to DCAA (6.64 ± 0.83) and the control (6.53 ± 0.61). In the treatments with nitrate the control NO_3^-/- (6.78 ± 0.73) and NO_3^-/U (5.48 ± 0.09) had the highest *chl a*: *chl b* ratio. HS (5.32 ± 0.11) and U (5.35 ± 0.09) had similar small values. However, in the nitrate containing experiment NO_3^-/DFAA (4.84 ± 1.19) and NO_3^-/HS (5.07 ± 0.7) showed the lowest *chl a*:*chl b* ratio. In the nitrate containing experiment each treatment was significantly different from their control NO_3^-/- (Apx. B Tab. 3). In contrast, DCAA and DFAA treatments did not significantly differ from the control.

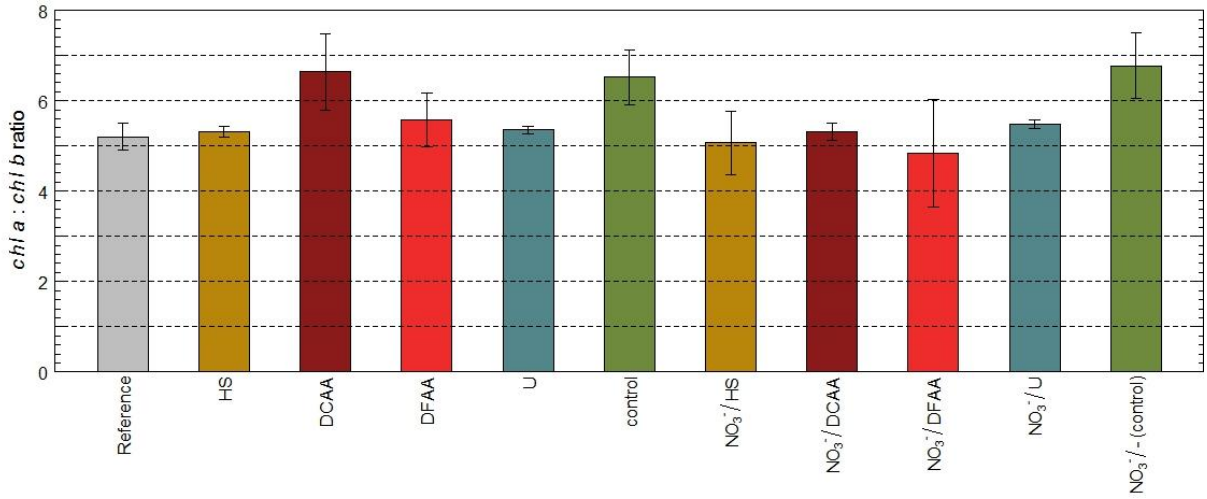

Fig. 5 *Chl a:chl b* ratio of *Chlamydomonas spec*. The reference bar represents the *chl a*:*chl b* ratio of the strain before the experiment. Samples were taken on day 7 (n=4). Error bars show standard deviation.

CN-analysis of *Chlamydomonas spec.* after seven days of incubation (Fig. 6) revealed a change in the C:N ratio of the different treatments. Within nitrate free and nitrate containing treatments, the pattern in C:N ratio was the same. Highest values occurred in the controls (27.87 ± 2.43 without NO_3^- and 25.02 ± 1.9 with NO_3^-), followed by DFAA (23.66 ± 2.32 without NO_3^- and 24.34 ± 2.34 with NO_3^-), U (20.35 ± 2.89 without NO_3^- and 22.07 ± 1.72 with NO_3^-), HS (15.49 ± 0.94 without NO_3^- and 15.29 ± 1.08 with NO_3^-) and finally DCAA (13.18 ± 1.03 without NO_3^- and 12.36 ± 0.44 with NO_3^-) treatments (Apx. A Tab. 4).

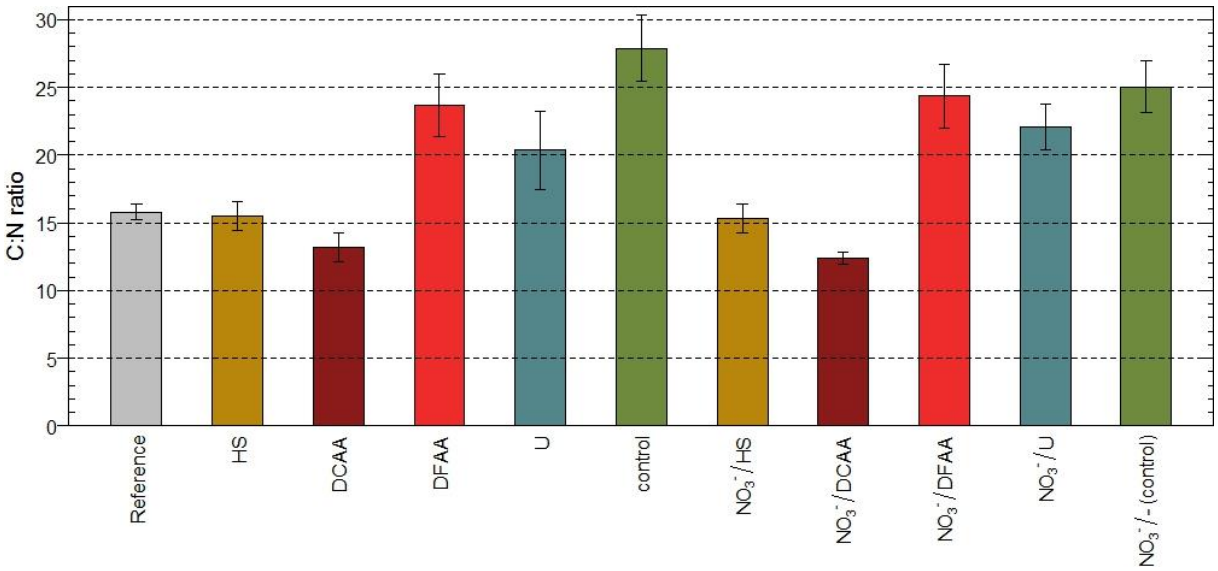

Fig. 6 C:N ratios of *Chlamydomonas spec.* Samples were taken on day seven. The reference bar represents the C:N ratio of the strain before the experiment (n=4). Error bars show standard deviation.

Without nitrate each treatment, except the one with DFAA, was statistically significant different from the control (Apx. A Tab. 4) . When NO_3^- was added, solely NO_3^-/HS and NO_3^-/DCAA treatments were significantly different from the control. Furthermore, the significant difference between DCAA and U treatments, as well as between NO_3^-/DCAA and NO_3^-/U could be shown. Comparison of nitrate free

with NO_3^- containing treatments revealed no significant difference when the same DON compound was added.

3.1.2 *Monoraphidium contortum*

The second green algae species analysed was *Monoraphidium contortum* (Fig. 7 and Fig. 8). The nitrate free treatments (Fig. 7) had lag phases between one (DCAA and U) and two days (HS, DFAA and control). The algae in the HS and DCAA treatments began to grow on the second day until the fifth, while U treatment showed a decline in algal growth on the fourth day. Algae in the DCAA treatment grew only between day one and three, but the fluorescence decreased until the fourth day and thereafter remained in steady-state until the last day of the experiment. Algae in the U treatment already started to grow on day one until day seven. From day five on, fluorescence of the HS and DFAA treatments and of the control stayed stable or slightly increased in fluorescence.

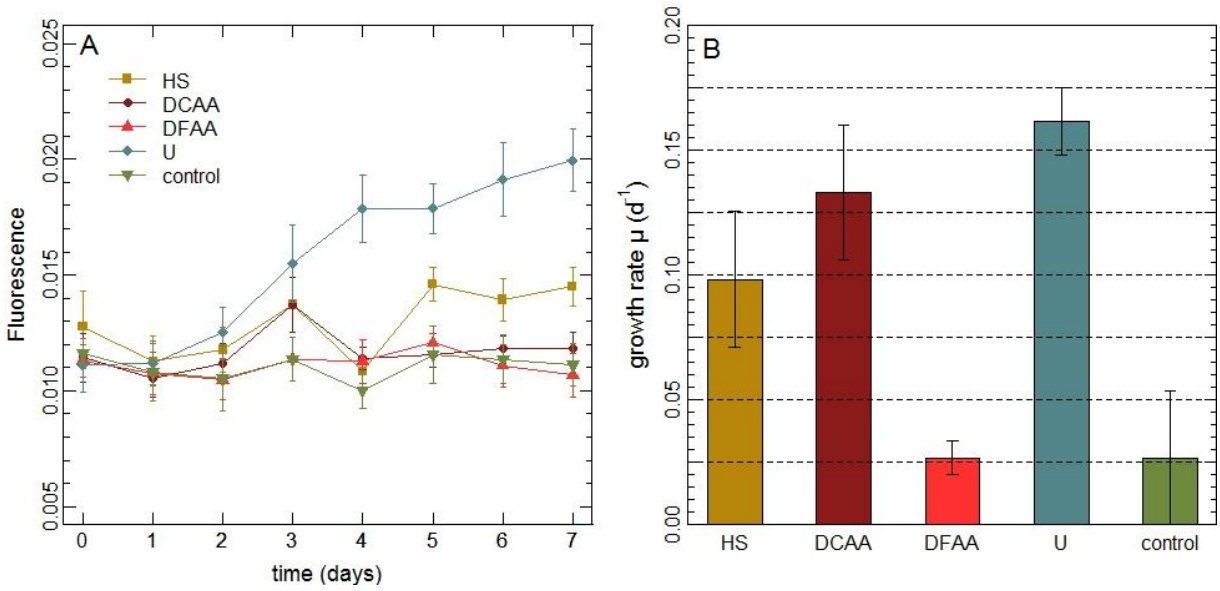

Fig. 7 Nitrate free growth experiment with *Monoraphidium contortum*. A: Fluorescence values, B: Growth rates μ (d^{-1}) between day 1 and 3. The experiment was performed for seven days (n=4). Error bars show standard deviation.

Growth rates of these treatments (Fig. 7B and Apx. A Tab. 1) were calculated between day one and three. Algae in the U treatment showed the highest growth rate (μ = 0.161 ± 0.013 d^{-1}), followed by the DCAA (μ = 0.133 ± 0.027 d^{-1}) and HS (μ = 0.098 ± 0.027 d^{-1}) treatments. Algae in the DFAA treatment (μ = 0.027 ± 0.007 d^{-1}) and control (μ = 0.027 ± 0.027 d^{-1}) showed the same growth rate. Each growth rate (except the one of DFAA) was significantly different from the control (Apx. B Tab. 5).

The NO_3^-/DCAA and NO_3^-/DFAA treatments (Fig. 8A) showed a lag phase of 2 days, while the other treatments revealed shorter or no lag phases. Algae in the NO_3^-/HS treatment immediately started to grow until day 3 and growth was more or less constant until day 7. Algae in the NO_3^-/DCAA and NO_3^-/DFAA treatments grew between the second and fourth day. Algae of the NO_3^-/U treatment showed

the longest growth period (from day one to five). The control (NO_3^-/-) showed almost no change in fluorescence.

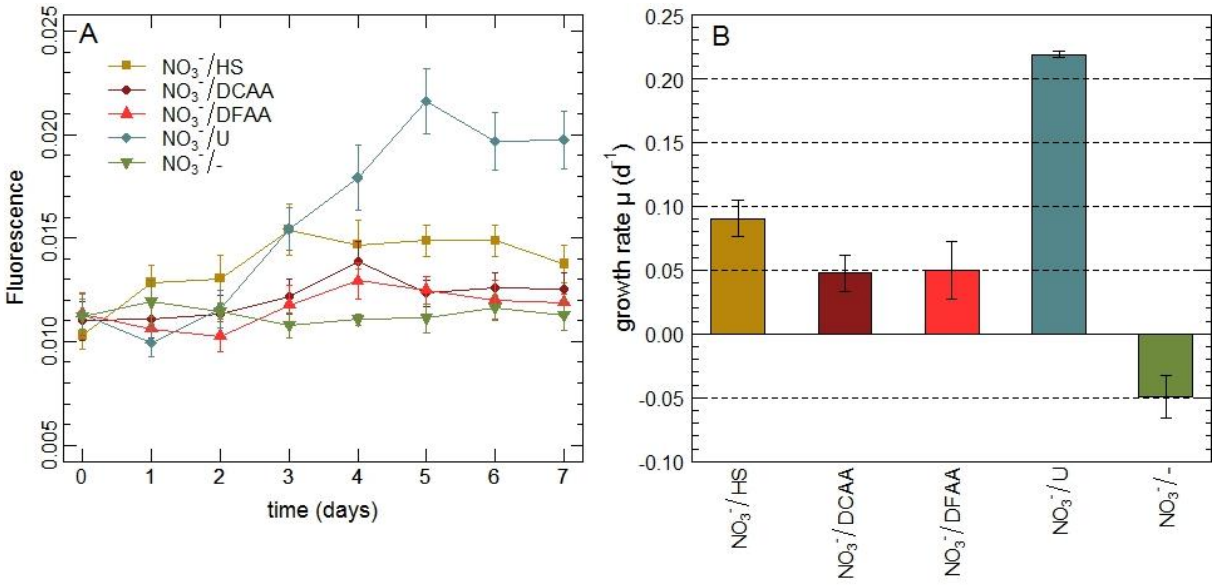

Fig. 8 Nitrate containing growth experiment with *Monoraphidium contortum*. A: Fluorescence values, B: Growth rates μ (d^{-1}) between day 1 and 3. The experiment was performed for seven days (n=4). Error bars show standard deviation.

Algal growth rate (Fig. 8B and Apx. A Tab. 1) between the first and third day was highest for the NO_3^-/U treatment (μ= 0.219 ± 0.003 d^{-1}). Algal growth rate in the NO_3^-/HS treatment was the second highest (μ = 0.09 ± 0.014 d^{-1}). For the NO_3^-/DCAA and NO_3^-/DFAA treatments growth rates were almost the same (μ = 0.048 ± 0.014 d^{-1} and 0.05 ± 0.023 d^{-1}, respectively). The control (NO_3^-/-) showed a negative growth rate (μ = -0.049 ± 0.016 d^{-1}). The comparison between growth rates (Apx. B Tab. 5) in treatment NO_3^-/DFAA and the control (NO_3^-/-) did not show any significant differences. Statistical comparison of algal growth rates between the control and the NO_3^-/DCAA treatment revealed a p value close to 0.05, and the NO_3^-/HS and NO_3^-/U treatments differed significantly from the control. Algal growth rates in the NO_3^-/DCAA and NO_3^-/U treatments were statistically significant different.

3.2 Diatoms

3.2.1 *Cyclotella meneghiniana*

Lag periods of the NO_3^- free treatments with the diatom *Cyclotella meneghiniana* (Fig. 9A) lasted mostly only for the first day. On the second day fluorescence increased in all treatments, but decreased until the third day again (DFAA treatment until day four). Growth on U and the control build an exception from this pattern. Algae began to grow and continued growing until the fifth day. The control reached a plateau from day two until day three, before biomass increased again until the fourth day. Algae in the HS treatment grew between the third and fifth day. Algae growing on DCAA on the other

hand stayed stable and began to grow again on day four until day five, which also did the culture of the DFAA treatment and the control.

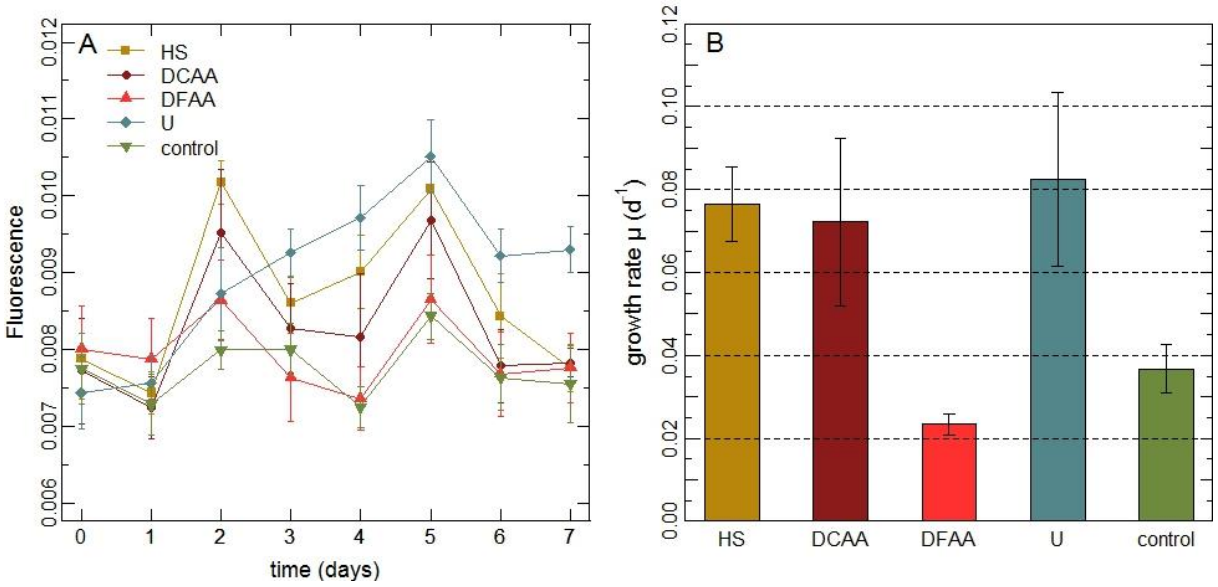

Fig. 9 Nitrate free growth experiment with *Cyclotella meneghenianа*. A: Fluorescence values, B: Growth rates μ (d^{-1}) between day 1 and 5. The experiment was performed for seven days (n=4). Error bars show standard deviation.

Growth rates were calculated between day one and five (Fig. 9B and Apx. A Tab. 1) with the result that algae growing on U had the highest average growth rate (μ = 0.082 ± 0.021 d^{-1}). Algae growing on HS had the second highest growth rate (μ = 0.076 ± 0.009 d^{-1}), but was similar to growth rate of algae in the DCAA treatment (μ = 0.072 ± 0.02 d^{-1}). The control showed even a little higher algal growth rate (μ = 0.037 ± 0.006 d^{-1}) than the algae growing on DFAA (μ = 0.024 ± 0.003 d^{-1}). The algal growth rates of each treatment were significantly different from the control (Apx. B Tab. 6). In the experiment with *Cyclotella meneghiniana* there was no significant difference found between growth rates of algae growing on DCAA and U.

In the experiments with DIN and DON (Fig. 10) algal growth revealed a lag phase of one day when growing on NO_3^-/HS, NO_3^-/DCAA and NO_3^-/DFAA, while algae in the NO_3^-/U treatment and the control NO_3^-/- began immediately to grow until the sixth day. Increase in fluorescence lasted for all treatments until day six and declined on the last day of the experiment.

As in the NO_3^- free experiment the highest growth rate (Fig. 10B and Apx. A Tab. 1) was reached by algae in NO_3^-/U treatment (μ = 0.096 ± 0.006 d^{-1}). This was followed by algae growing on NO_3^-/DFAA (μ = 0.076 ± 0.02 d^{-1}) and NO_3^-/DCAA (μ = 0.062 ± 0.013 d^{-1}). Algal growth on NO_3^-/HS (μ = 0.048 ± 0.011 d^{-1}) and NO_3^-/- (control) (μ = 0.042 ± 0.007 d^{-1}) revealed growth rates, which did not differ a lot from each other. Only algal growth on NO_3^-/U was significantly different from the control NO_3^-/- (Apx. B Tab. 6). Algal growth rates of the NO_3^-/DCAA treatment and NO_3^-/DFAA treatment revealed at least a trend. However, growth rates of algae growing on NO_3^-/HS did not. The treatments with a similar content of total N with each other, NO_3^-/DCAA and NO_3^-/U, were significantly different from each other.

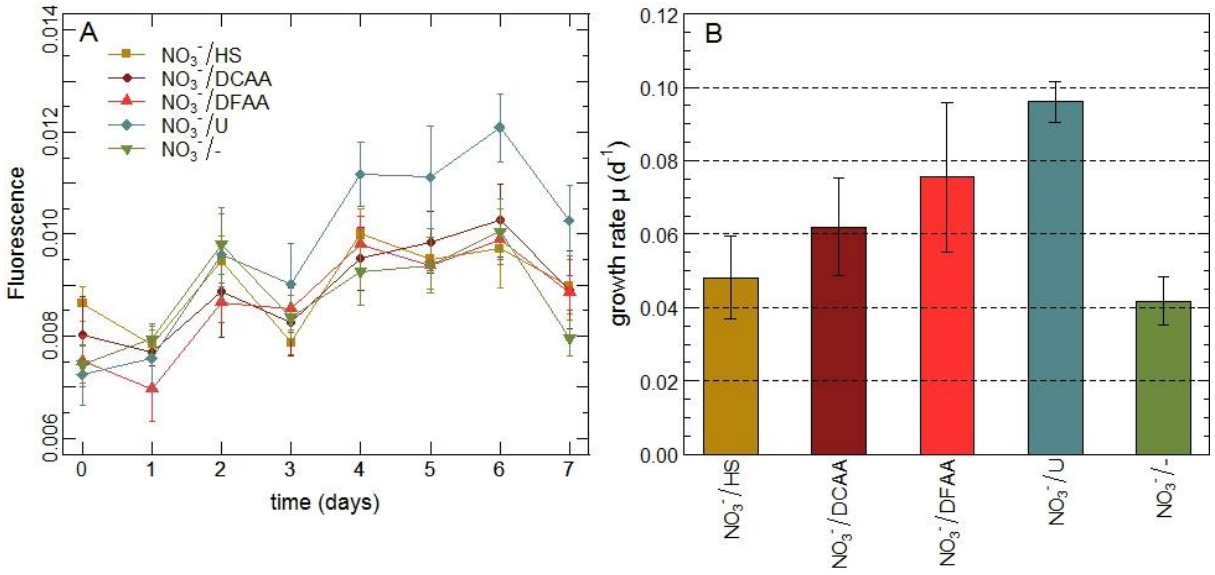

Fig. 10 Nitrate containing growth experiment with *Cyclotella menegheniana*. A: Fluorescence values, B: Growth rates μ (d^{-1}) between day 1 and 5. The experiment was performed for seven days (n=4). Error bars show standard deviation.

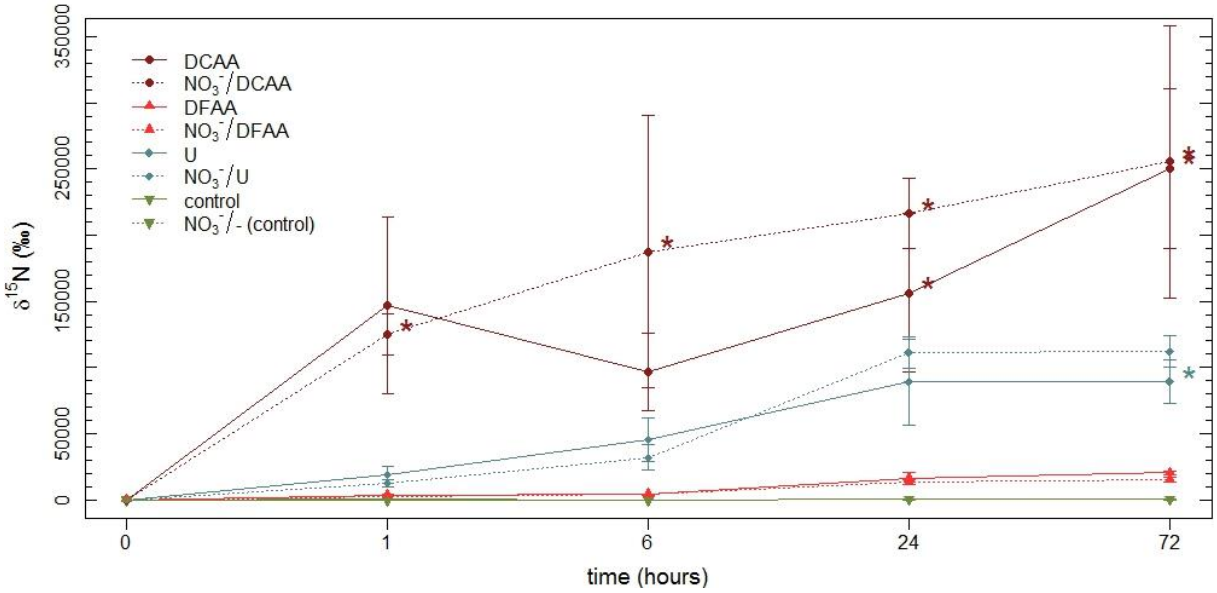

Fig. 11 ^{15}N assimilation experiment with C*yclotella meneghiniana*. DCAA, DFAA and U were labelled with ^{15}N . NO_3^- was not labelled with ^{15}N . Samples were taken after 1, 6, 24 and 72 hours (n=3). Error bars show standard deviation. *Note that the δ^{15}N values are underrepresented due to an incomplete recording of the ^{15}N-peak.

The ^{15}N short-term assimilation experiment (Fig. 11) showed that after one hour the enrichment was highest in the DCAA treatment as well as the NO_3^-/DCAA treatment. After six hours this concentration decreased, while DCAA increased further until the last measurement after 72 hours. For NO_3^-/DCAA followed again an accumulation until 72 hours. Also in the U treatment an enrichment of ^{15}N was found already after one hour. This development lasted for 24 hours. Afterwards almost no ^{15}N was assimilated anymore. The algae inNO_3^-/U treatment assimilated ^{15}N after one and six hours slower than the NO_3^- free treatment. However, after 24 hours the concentration of ^{15}N exceeded the concen-

tration in the U treatment. The amounts of ^{15}N originating from the free amino acids, increased in the single treatment with DFAA as well as with NO_3^-/DFAA. But the $\delta^{15}N$-values in the DFAA treatment were always slightly higher (Apx. A Tab. 5).

3.2.2 *Fragilaria ulna*

The second diatom analysed was *Fragilaria ulna* (Fig. 12 and Fig. 13). The lag phase of the DCAA, DFAA and U treatment, as well as for the control lasted from day zero to day one. The fluorescence of the algae stayed relatively stable, but overall the intensity increased a little during the seven days. Algae growing the best on U. Fluorescence of the DCAA treatment decreased over the whole course of the experiment. With fluctuations algae in the DFAA treatment grew between day one and seven. However, algae in the DFAA treatment decreased in fluorescence over the whole course of the experiment. The algae in the control grew with fluctuations between day one to six, but revealed also a lower fluorescence value on the last day of the experiment than on the first day.

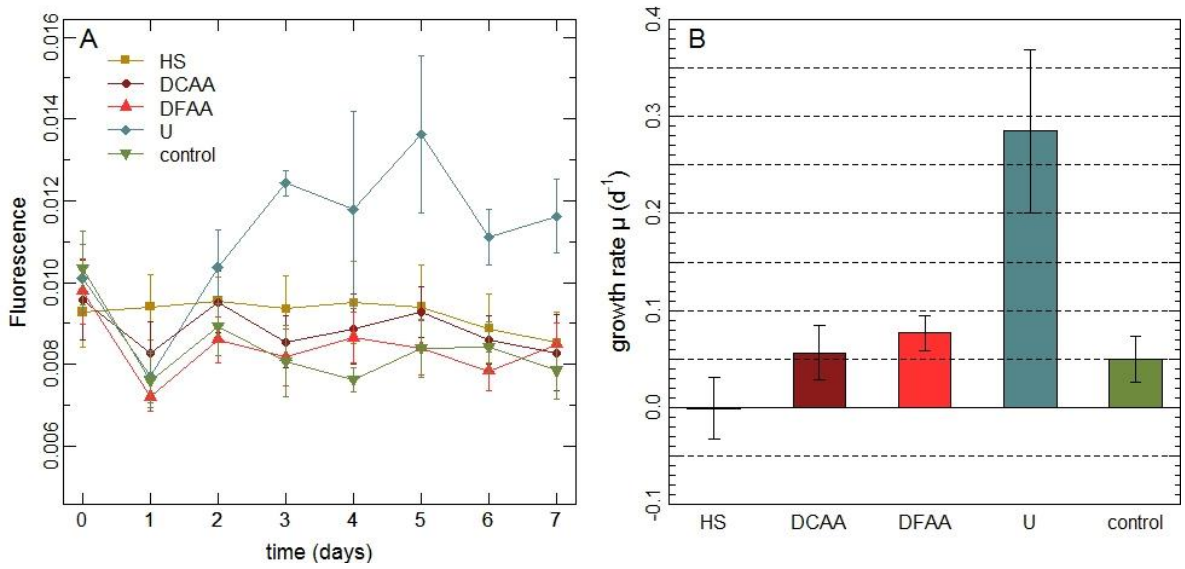

Fig. 12 Nitrate free growth experiment with *Fragilaria ulna*. A: Fluorescence values, B: Growth rates μ (d^{-1}) between day 1 and 3. The experiment was performed for seven days (n=4). Error bars show standard deviation.

Growth rates (Fig. 12B and Apx. A Tab. 1) were calculated between day one and three. Algal growth rate was highest in the U treatment (μ = 0.285 ± 0.084 d^{-1}) and lowest in the HS treatment (μ = -0.001 ± 0.032 d^{-1}). Growth rate of algae growing on DFAA (μ = 0.077 ± 0.018 d^{-1}) was higher than the one of algae growing on DCAA (μ = 0.057 ± 0.028 d^{-1}), which was similar to the one of the control (μ = 0.05 ± 0.024 d^{-1}). Significant difference was reached between algal growth rates of the DFAA treatment and the U treatment with the control (Apx. B Tab. 7). Algal growth rates of the HS treatment and the DCAA treatment were not significantly different from the control. DCAA and U were significantly different from each other.

The experiment with a combination of DIN and DON (Fig. 13B) revealed a lag phase of one day for the algae of the NO_3^-/DCAA and the NO_3^-/DFAA treatments. Algae growing on the other treatments began to grow already from day zero on. Algae growing on NO_3^-/U and NO_3^-/DCAA grew from day one to three. Afterwards the fluorescence of the NO_3^-/U treatment decreased. Algae growing on NO_3^-/DCAA increased from day five on. The fluorescence of algae growing on NO_3^-/HS, NO_3^-/DFAA and the control NO_3^-/- increased from the second day on, NO_3^-/HS and NO_3^-/DCAA until day four and the control until day three. Afterwards a stable value for the control or a little gain of fluorescence (in the NO_3^-/HS treatment) was measured. Between the sixth and seventh day algae growing on NO_3^-/DCAA increased again.

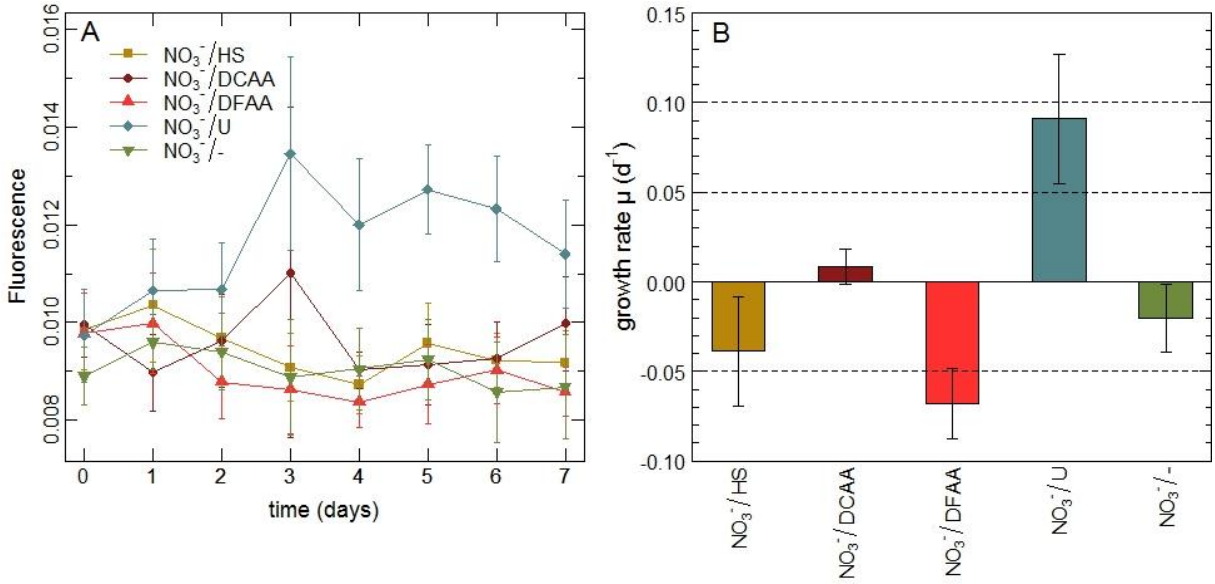

Fig. 13 Nitrate containing growth experiment with *Fragilaria ulna*. A: Fluorescence values, B: Growth rates μ (d^{-1}) between day 1 and 3. The experiment was performed for sevent days (n=4). Error bars show standard deviation.

Just like growth rates of the algae growing on NO_3^- free treatments, algae of the U treatment had the highest growth rate (μ = 0.091 ± 0.036 d^{-1}) (Fig. 13B and Apx. A Tab. 1). With a growth rate of 0.009 ± 0.01 d^{-1} algae in the NO_3^-/DCAA treatment had the second highest value. The algal growth rates for the treatments NO_3^-/- (control) (μ = -0.02 ± 0.019 d^{-1}), NO_3^-/HS (μ = -0.039 ± 0.03 d^{-1}) and NO_3^-/DFAA (μ = -0.068 ± 0.02 d^{-1}) were even negative. The NO_3^-/DCAA treatment and the NO_3^-/U treatment were significantly different from each other (Apx. B Tab. 7). Also the growth rates of the NO_3^-/DFAA treatment and the NO_3^-/U treatment differed from the control NO_3^-/-, while on the other hand the NO_3^-/DCAA treatment and the NO_3^-/DCAA treatment revealed no significant difference from it.

3.3 Cyanobacteria

3.3.1 *Microcystis aeruginosa*

The nitrate free experiment with the cyanobacterium *Microcystis aeruginosa* (Fig. 14A) can be divided in two phases. The first phase lasted from day zero until day four, the second one from day four until day seven. After the initial growth phase fluorescence declined between day three and five and subsequently algae seemed to enter another growth phase.

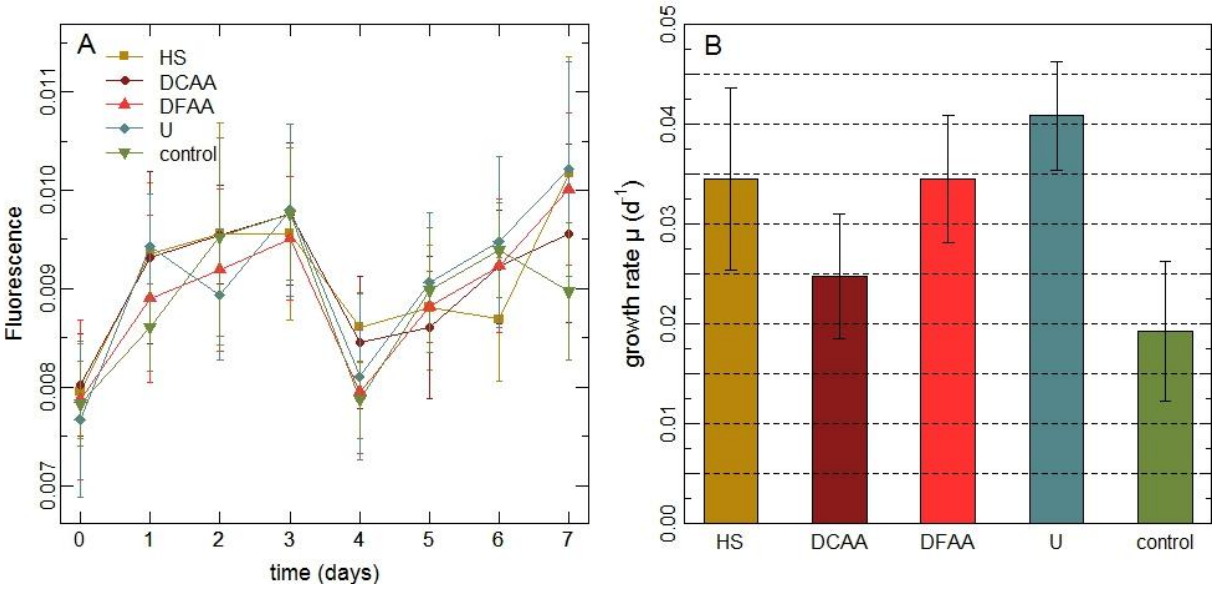

Fig. 14 Nitrate free growth experiment with *Microcystis aeruginosa*. A: Fluorescence values, B: Growth rates μ (d^{-1}) between day 0 and 7. The experiment was performed for seven days (n=4). Error bars show standard deviation.

Growth rates were calculated from day zero until day seven (Fig. 14B and Apx. A Tab. 1). Cyanobacteria growing on U had the highest growth rate (μ = 0.041 ± 0.005 d^{-1}). Cyanobacteria growing on HS and on DFAA showed almost the same growth rate (μ = 0.034 ± 0.009 d^{-1}, μ = 0.035 ± 0.006 d^{-1}). Algae growing on DCAA (μ = 0.025 ± 0.006 d^{-1}) had the fourth highest rate and the control the lowest (μ = 0.019 ± 0.007 d^{-1}). The growth rates of each culture, except the one growing on DCAA were significantly different from the control (Apx. B Tab. 8). Also the growth rates of the algae in the DCAA treatment and the U treatment were significantly different from each other.

Due to the little gain in fluorescence in the NO_3^--experiment (Fig. 15A) it is difficult to distinguish between the lag phase and the growth phase of the treatments. Overall, the fluorescence of the NO_3^-/DFAA treatment and the NO_3^-/U increased between day zero and day seven, but with fluctuations. Fluorescence of algae growing on NO_3^-/HS decreased from day zero to two. The fluorescence in the NO_3^-/DCAA treatment on the other hand decreased until day four and increased afterwards again. The control NO_3^-/- started to grow on day zero until day three and decreased than continuously in fluorescence until the last day of the experiment.

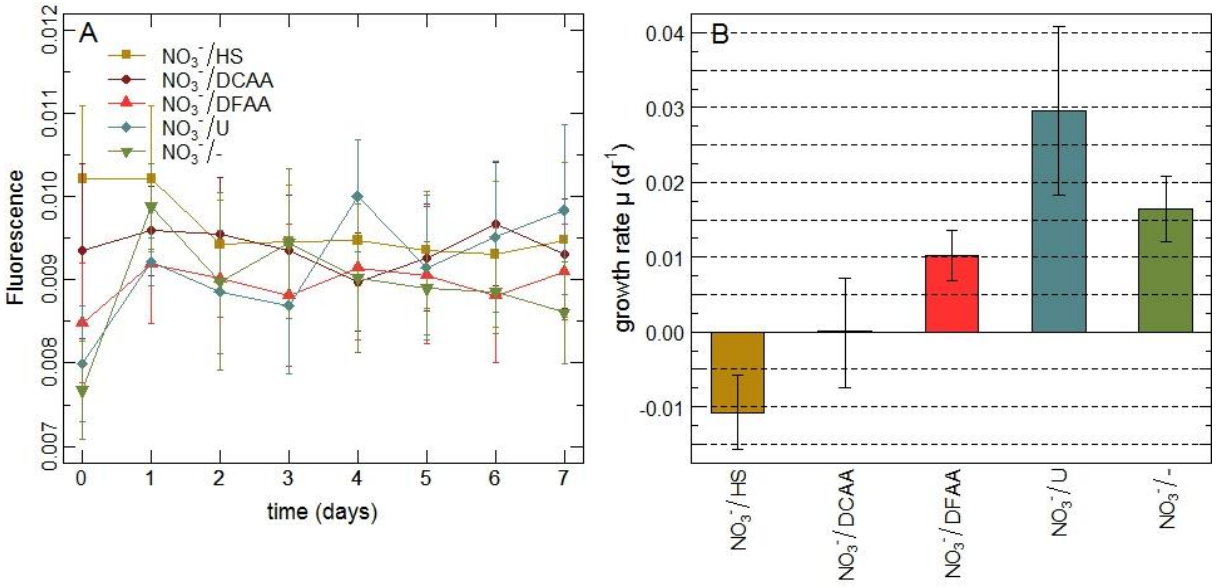

Fig. 15 Nitrate containing growth experiment *Microcystis aeruginosa*. A: Fluorescence values, B: Growth rates μ (d^{-1}) between day 0 and 7. The experiment was performed for seven days (n=4). Error bars show standard deviation.

Growth rates of *M. aeruginosa* growing on NO_3^- (Fig. 15B and Apx. A Tab. 1) were highest for NO_3^-/U (μ = 0.03 ± 0.011 d^{-1}). The second highest growth rate was the one of the control NO_3^-/- (μ = 0.016 ± 0.004 d^{-1}). These two were followed by NO_3^-/DFAA (μ = 0.01 ± 0.003 d^{-1}), NO_3^-/DCAA (μ = 0 ± 0.007 d^{-1}) and finally by NO_3^-/HS (μ = -0.011 ± 0.005 d^{-1}). Growth rates of NO_3^-/HS and NO_3^-/DCAA were significantly different from the control NO_3^-/- (Apx. B Tab. 8). On the other hand, NO_3^-/DFAA and NO_3^-/U were not significantly different. The two treatments which have approximately the same total N content in the beginning of the experiment (NO_3^-/DCAA and NO_3^-/U) were statistically different.

The ^{15}N assimilation short-term experiment (Fig. 16 and Apx. A Tab. 6) revealed that the enrichment of ^{15}N was highest when the algae had access to DCAA. The highest increase in $\delta^{15}N$ occurred already after the first hour. In the U treatment the increase was highest between the sixth and twenty-fourth hour. Even when a difference in DON accumulation is visible when NO_3^- is given as a second nitrogen source, there is never a significant difference between these two (Apx. B Tab. 9). With access to DCAA the ^{15}N accumulation after 24 hours was higher when there was also NO_3^- available. For the U treatment $\delta^{15}N$ was for all measured time points higher when there was also NO_3^- present.

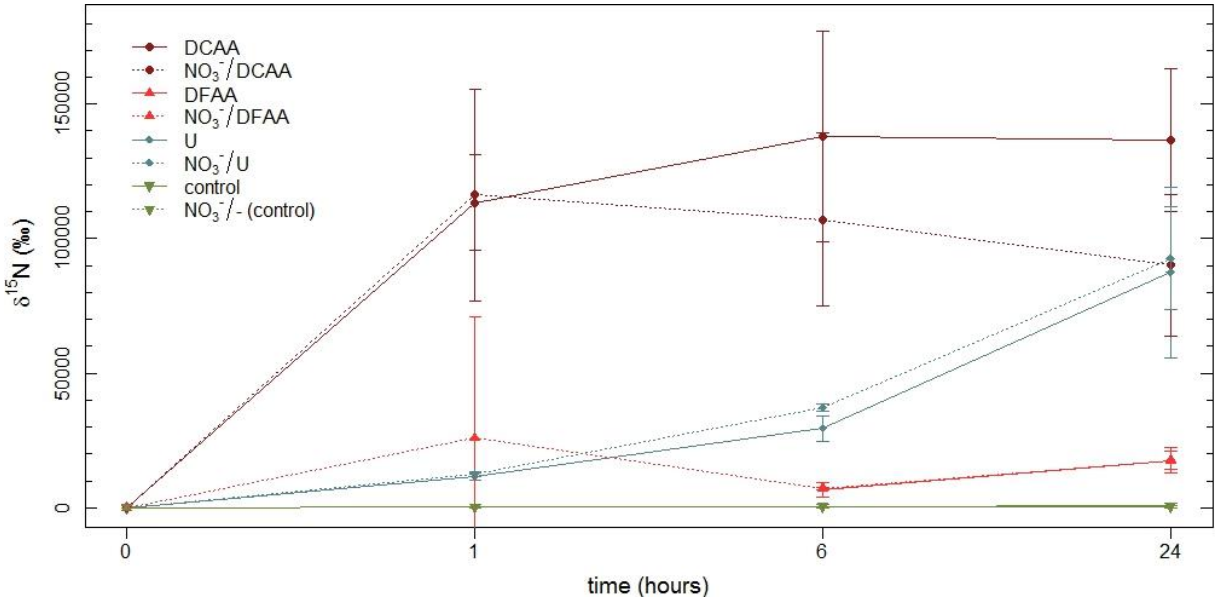

Fig. 16 ^{15}N assimilation experiment with *Microcystis aeruginosa*. DCAA, DFAA and U were labelled with ^{15}N . NO_3^- was not labelled with ^{15}N . Samples were taken after one, six and 24 hours (n=4). Error bars represent standard deviation.

Delayed fluorescence measurements illustrated that the ratio of the phycobiliproteins to *chl a* (Fig. 17) and the pigment composition (Apx. A Tab. 7) changed through the different treatments. The highest (PE+PC) content in relation to *chl a* occurred in the control, as well as the highest APC:*chl a* ratio. Both ratios were lowest in the U treatment. However, the highest (PE+PC):APC ratio was present in the U treatment. In the NO_3^- treatments, the control had the highest (PE+PC) amount in relation to *chl a* and APC. The NO_3^- /HS treatment showed the lowest proportion of (PE+PC) to *chl a* and also to APC.

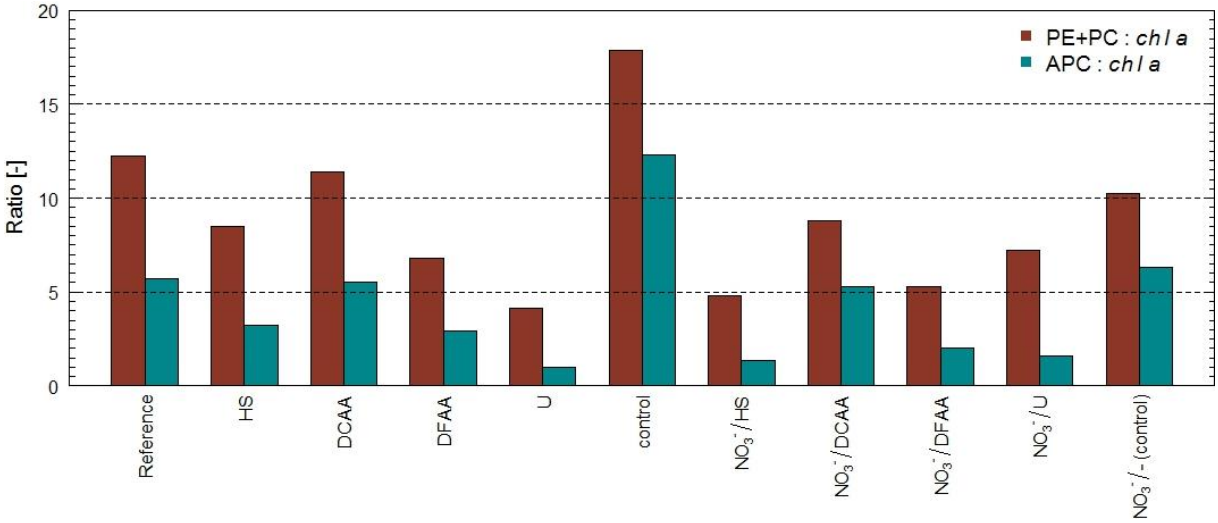

Fig. 17 Ratios of PB to *chl a* content of *Microcystis aeruginosa*. Samples were taken on day 7 (n=1). Reference bar shows the PB:*chl a* ratios of the strain before the experiment. *Chl a* was normalized to 1.

3.3.2 *Anabaena flos-aquae*

In the experiment with the cyanobacterium *Anabaena flos-aquae chl a* content increased with small fluctuations in all treatments until the last day of the experiment.

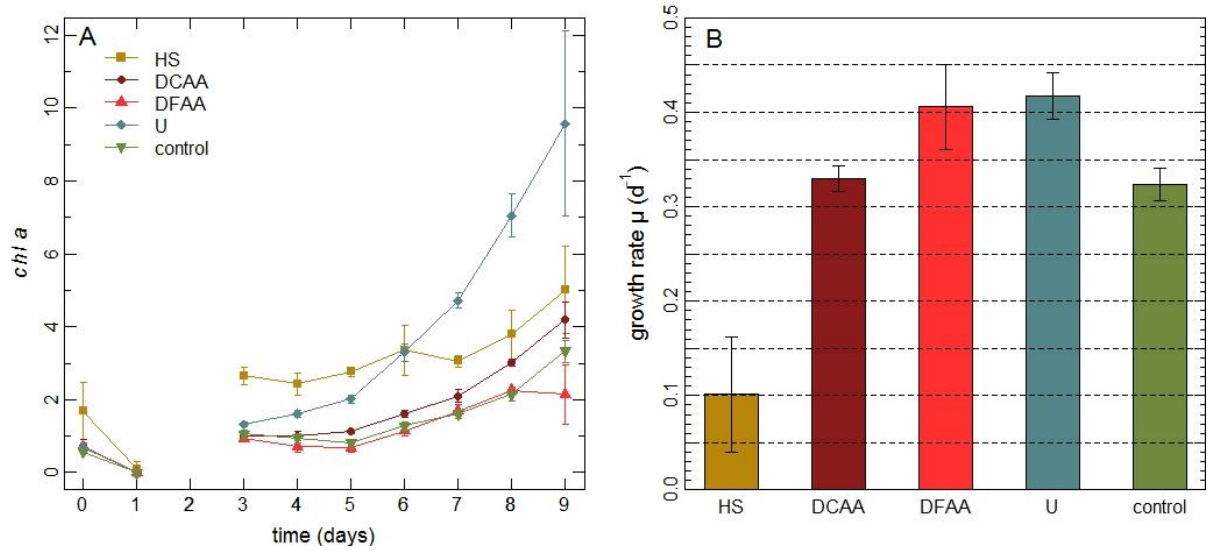

Fig. 18 Nitrate free growth experiment with *Anabaena flos-aquae*. A: Fluorescence values, B: Growth rates μ (d^{-1}) between day 5 and 8. The experiment was performed for nine days (n=4). Error bars show standard deviation.

The growth rate of *A. flos-aquae* growing on U (μ = 0.42 ± 0.02 d^{-1}) was the highest and the one of DFAA (μ = 0.41 ± 0.05 d^{-1}) the second highest (Fig. 18B and Apx. A Tab. 1). Growth rates of cyanobacteria growing on DCAA (μ = 0.33 ± 0.01 d^{-1}) and the control (μ = 0.32 ± 0.02 d^{-1}) were quite similar. The growth rate of HS (μ = 0.1 ± 0.06 d^{-1}) was the lowest. The statistical analysis of the *Anabaena flos-aquae* growth rates (Apx. B Tab. 10) revealed a significant difference between the control and the HS, DFAA and U treatment. This could not be shown for the treatment with DCAA. Comparing the DCAA treatment with the U treatment a significant difference was found.

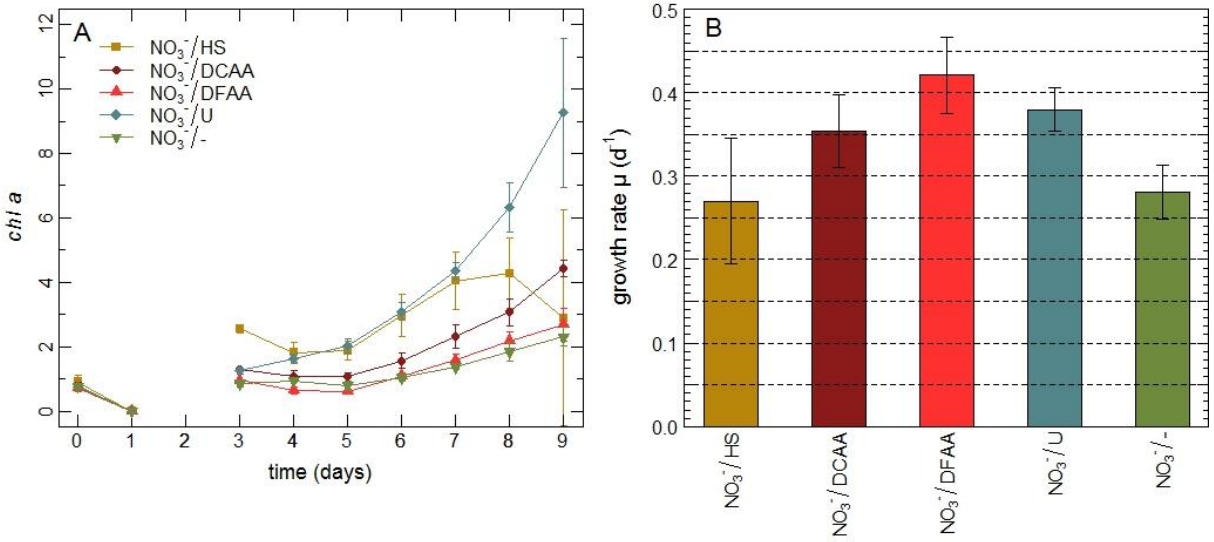

Fig. 19 Nitrate containing growth experiment with *Anabaena flos-aquae*. A: Fluorescence values, B: Growth rates μ (d^{-1}) between day 5 and 8. The experiment was performed for nine days (n=4). Error bars show standard deviation.

In the *Anabaena flos-aquae* NO_3^- containing experiment (Fig. 19A) the *chl a* content in the NO_3^-/DCAA treatment, the NO_3^-/DFAA treatment, the NO_3^-/U treatment and the control NO_3^-/- started and kept growing until day nine.

The ranking of the NO_3^- treatments growth rates (Fig. 19B and Apx. A Tab. 1) differed from the ones of the nitrate free treatments only in the rank of the NO_3^-/DFAA treatment and the NO_3^-/U treatment. The growth rate of the algae growing on NO_3^-/DFAA ($\mu = 0.42 \pm 0.05\ d^{-1}$) was the highest, NO_3^-/U ($\mu = 0.38 \pm 0.03\ d^{-1}$) was the second highest and algal growth rate of the NO_3^-/DCAA treatment ($\mu = 0.35 \pm 0.04\ d^{-1}$) was the third highest. The growth rate of the control NO_3^-/- ($\mu = 0.28 \pm 0.06\ d^{-1}$) was higher than the one of the algae growing on NO_3^-/HS ($\mu = 0.27 \pm 0.08\ d^{-1}$). The control NO_3^-/- was statistically different from the NO_3^-/DFAA treatment and the NO_3^-/U treatment. At least a significant trend was revealed by the NO_3^-/DCAA treatment (Apx. B Tab. 10). The NO_3^-/HS treatment and the NO_3^-/DCAA treatment were not significantly different from the control NO_3^-/-.

Composition of phycobiliproteins and *chl a* changed in the different treatments (Fig. 20) and Apx. A Tab. 8). The highest PE+PC content in relation to *chl a* occurred in the reference, whereas the APC value was the second smallest. Also the ratio of PE+PC to APC was highest in the reference. The PE+PC to *chl a* contribution was lowest in the two controls, while the APC:*chl a* ratio was highest in these. This was also shown by the low PE+PC:APC ratio.

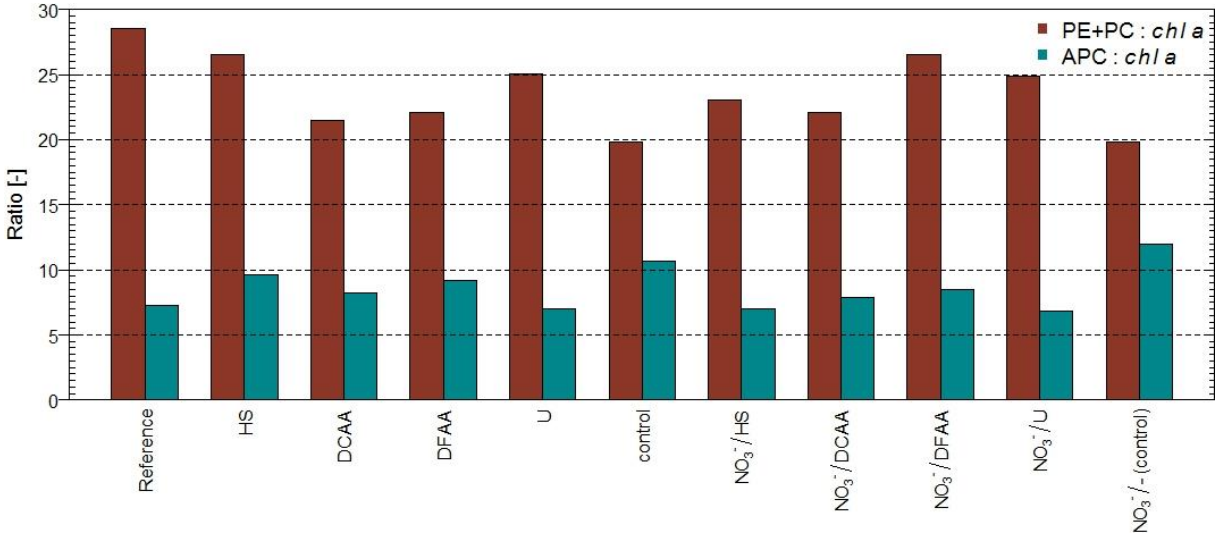

Fig. 20 Ratios of PB to *chl a* concentration of *Anabaena flos-aquae*. The reference bar shows the ratios of the strain before the experiment. Samples were taken on day 9 (n=1). *Chl a* was normalized to *chl a*=1.

The analysis of the heterocysts per biovolume revealed (Fig. 21 and Apx. A Tab. 9) that the ranking of the HC:biovolume ratio was the same for the treatments with and without nitrate. The ratio was highest for DFAA and DCAA treatments, followed by the control, U and HS. Only the HS and NO_3^-/HS treatments were significantly different from their controls (Apx. B Tab. 11). Comparing treatments with and without nitrate, there was never a significant difference between the treatments with the same DON source.

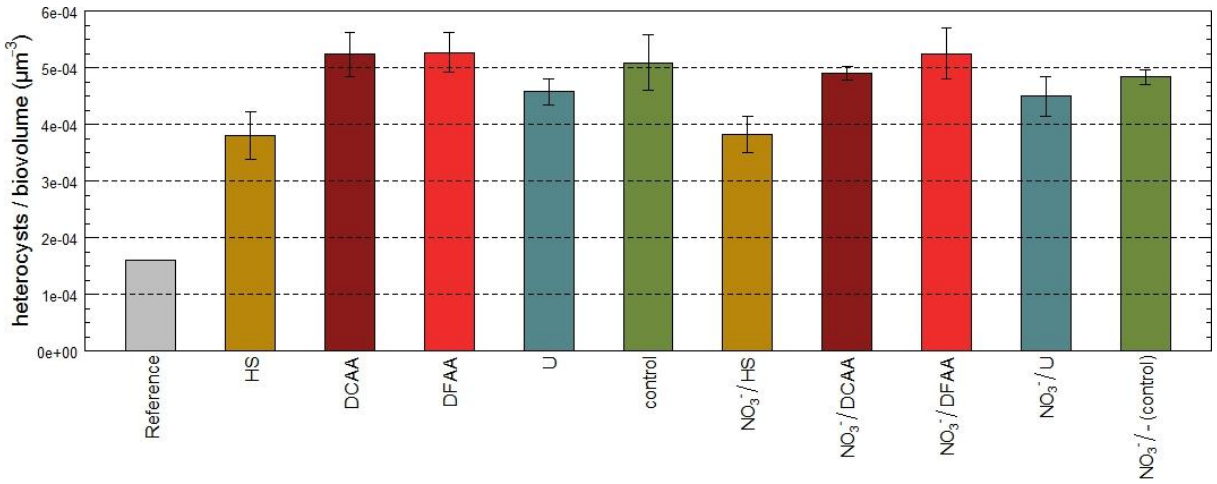

Fig. 21 Amount of heterocysts per biovolume of *Anabaena flos-aquae*. Reference bar shows the amount of HC per biovolume of the strain before the experiment. Samples were taken on day 9 (n=4). Error bars show standard deviation.

4 Discussion

4.1 Methods

Due to the high number of flasks the batch culture method was chosen for cultivation. This must be seen critical because there was no exchange of nutrients, which would have eventually resulted in a nutrient limitation in the long-term run. A lack of any nutrient limits the growth and would have made the proper interpretation of the data impossible, because the actually limitation factor could not have been assigned. However, this should not have been a severe problem in the short-time frame of our experiments.

In order to make a comparison between the treatments possible, it was chosen always the same period for the calculation of the algal growth rate. However, algal growing behaviour may have been much more complex than can be measured by fluorescence. Sources of errors are lag and growth phases of different length, fluctuations within the exponential growth phase and exclusion of the biomass development after the experiment has stopped. By neglecting these factors, the growth rates could lead to an over- or underestimation of the overall importance for growth of specific DON compounds. Thus, the determined growth rates only represent a minor part of algae development and do not allow any conclusion about the future growth trend. However, the calculated growth rate is still a good indicator for the detection of differences of algal growth behaviour and to find a general ability of the species to use a certain DON compound.

In contrast to the PHYTO-PAM, the iPAM should have been a tool to rapidly and easily measure the fluorescence in a sample. In the course of the study, however, it became obvious that the iPAM has several disadvantages. Actually, the iPAM should be able to simultaneously measure the fluorescence of samples in several wells, but measurements with the same concentration of culture were resulting in different fluorescence signals in different wells. In general, it can be stated that the resulting signals were highest in the centre of the well plate and decreased towards its edges. In order to avoid this effect, samples were only measured in wells in the centre of the microwell plate. By this, the discrepancy was reduced to a minimum. Furthermore, every sample was continuously measured in the same well. Thus, the systematic error was the same for each replicate and has no consequences on the comparability of the treatments. The results are still reliable. Nevertheless, the biggest advantage of the iPAM, namely measuring many samples simultaneously, was not given.

In the course of the study, it was also found that very dense algal cultures could influence the fluorescence readings of samples in the adjacent wells. This signal enhancement was most likely due to scat-

tered light. This should not be a problem with the low concentrations of alga used in our study, but it should be kept in mind when using this method for other research.

Another disadvantage of the iPAM is the lack of a z_{off} function to set an automatic baseline adjustment. Trying to subtract the background fluorescence of the medium from the measured fluorescence resulted occasionally in negative values. Therefore, we decided to no correct for the background fluorescence. To sum up, it is recommended to prefer the PHYTO-PAM over the iPAM method, even though the latter may safe some time.

For the results of the IRMS analysis, it turned out that the samples with the lowest δ^{15}N-values (smallest accumulation of ^{15}N) should be measured first. Unfortunately, our samples were not arranged like that. From the δ^{15}N-values of the standard (casein) could be seen how the high accumulation of ^{15}N-values adulterate the values of the standard. However, the deviations lay only at around 1‰ to 6 ‰ and are negligible for the high δ^{15}N-values of our samples.

Furthermore, there is the possibility that some of the ^{15}N measured was actually not taken up by the algae, but was accumulated in bacteria associated to the cell wall of the algae. No data of the amount of bacteria were collected, but one can assume that the bacterial biomass was much lower than the algae biomass and is therefore possibly negligible. In future studies it should be considered to use a Nano-Secondary Ion Mass Spectrometry (Nano-SIMS) in order to measure separately the algal and bacterial ^{15}N-uptake.

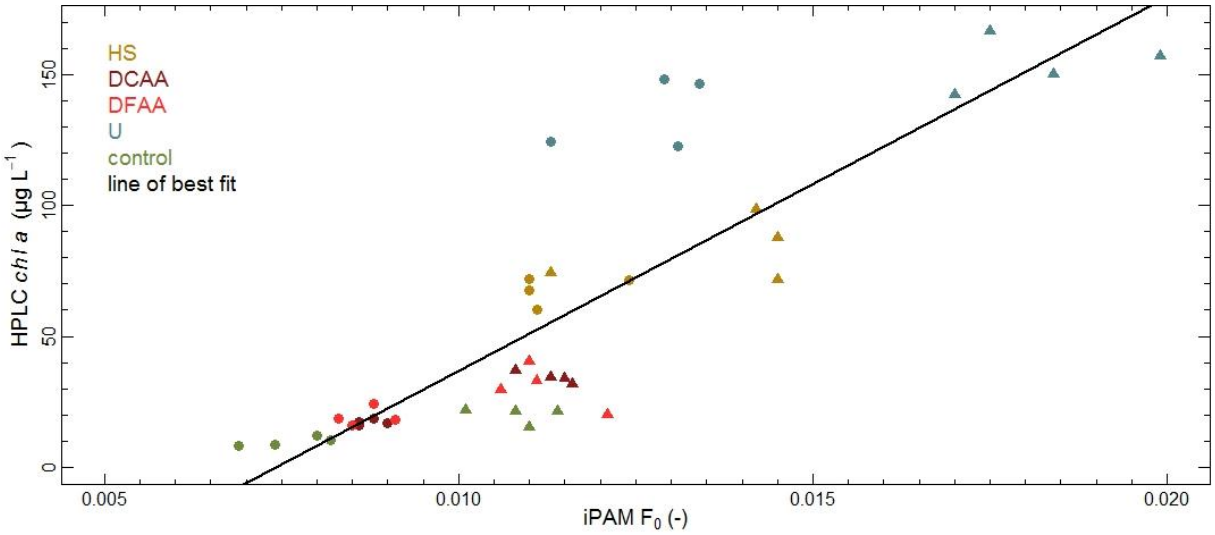

Fig. 22 Comparison of the *chl a* proxy F_0 measured with an iPAM and the *chl a* concentration determined with HPLC. Data derive from the experiment with *Chlamydomomnas spec.* ● represent the NO_3^--free treatments, ▲represent the NO_3^--containing treatments (n=40).

Three times it was the case that in the same experiment two measurement techniques were applied for *chl a* detection. Thereby it was possible to compare the results and test their statistical correlation. In the experiment with *Chlamydomonas spec.* iPAM measurements to detect the fluorescence F_0 and HPLC to measure the *chl a* concentration was applied (Fig. 22). With the Spearman's rank correlation analysis a strong positive correlation of these two methods was found ($S = 4$, $\rho = 0.98$, $p \leq 0.05$). The

HPLC technique is a reliable technique to get information about the pigment composition of a culture. Thus, it can be concluded that also the iPAM-technique (like it was used in our study) is a good method to detect F_0 as a reliable proxy for the *chl a* content of algae cultures.

The cyanobacterium *Microcystis aeruginosa* was analysed with the iPAM and delayed fluorescence excitation spectrometry (Fig. 23A). These two methods showed a positive correlation ($S = 52$, $\rho = 0.68$, $p \leq 0.05$). In the experiment with *Anabaena flos-aquae* (Fig. 23B) a strong positive correlation ($S = 28$, $\rho = 0.83$, $p \leq 0.05$) between the measurements with the PHYTO-PAM and the DF excitation spectrometry was found. It can be concluded that all measuring methods are a good indicator for the *chl a* development in an algae culture. But anyway, due to the change in the *chl a*:*chl b* ratio in the experiment with *Chlamydomonas spec.* one can suppose that *chl a* content is not necessarily a tool to precisely describe the algal growth in a culture.

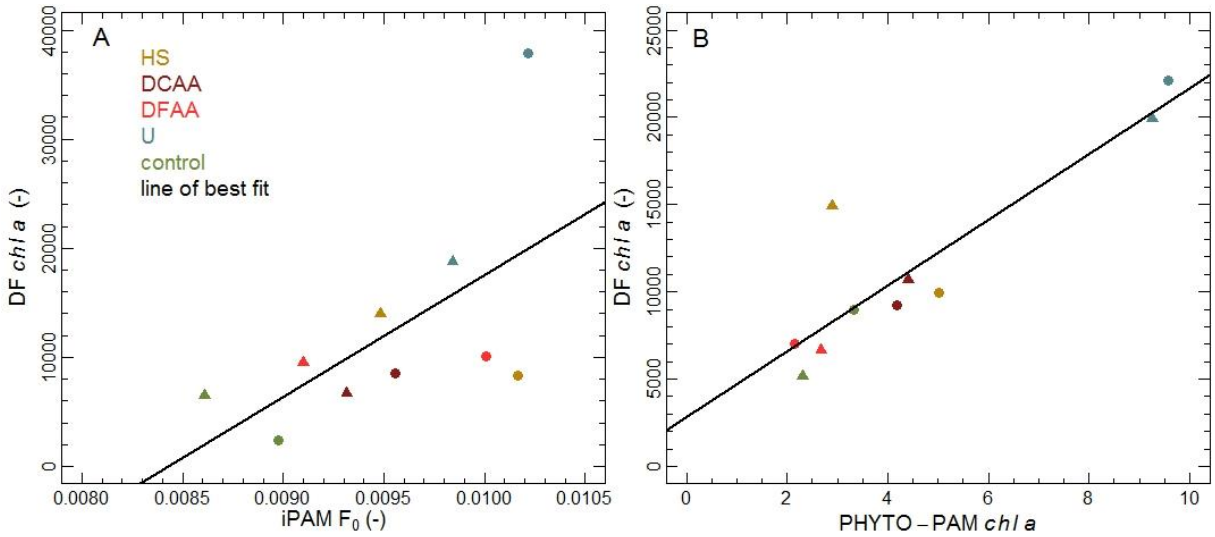

Fig. 23 Comparison of PAM measurements and determination of *chl a* with delayed fluorescence. A Data derive from the experiment with *Microcystis aeoginosa*. F_0 was measured with an iPAM (n=10). B In the experiment with *Anabaena flos-aquae chl a* was determined by PHYTO-PAM and DF (n=10). ● represent the NO_3^--free treatments, ▲represent the NO_3^--containing treatments.

The determination of the carbon and nitrogen content of the cultures enables to estimating the nutritional conditions of the algae (Wheeler et al. 1974). With an increasing deficit of N the intracellular N amount hyperbolically decreases. On the other hand, C amount of the algal biomass is independent of the N availability in the environment. Thus, the C:N ratio is a good indicator for algal N limitation (Kohl and Nicklisch 1988).

4.2 Results

Our results show that all six analysed phytoplankton species were able to grow on the provided DON compounds DCAA, DFAA and U, and mostly also on HS. When analysing our results it must be kept in mind that the total N content of the treatments differed. Merely, the DCAA and U treatments, as well as the NO_3^-/DCAA and NO_3^-/U treatments had similar total N contents and thus their growth rates

are well comparable. By analysing the change in fluorescence in relation to the total N amount added (Fig. 24) the efficiency to use a specific N source is illustrated. Optimal N use for growth was found for the NO_3^-/- treatment followed by the U treatments, which are commonly thought to be the most important nitrogen source for phytoplankton growth. Furthermore, DFAA can be used more efficiently than DCAA, which contributes to the idea that DFAA can be taken up faster by phytoplankton. The small gain in fluorescence per added nitrogen amount reveals the minor efficiency in HS-N utilization.

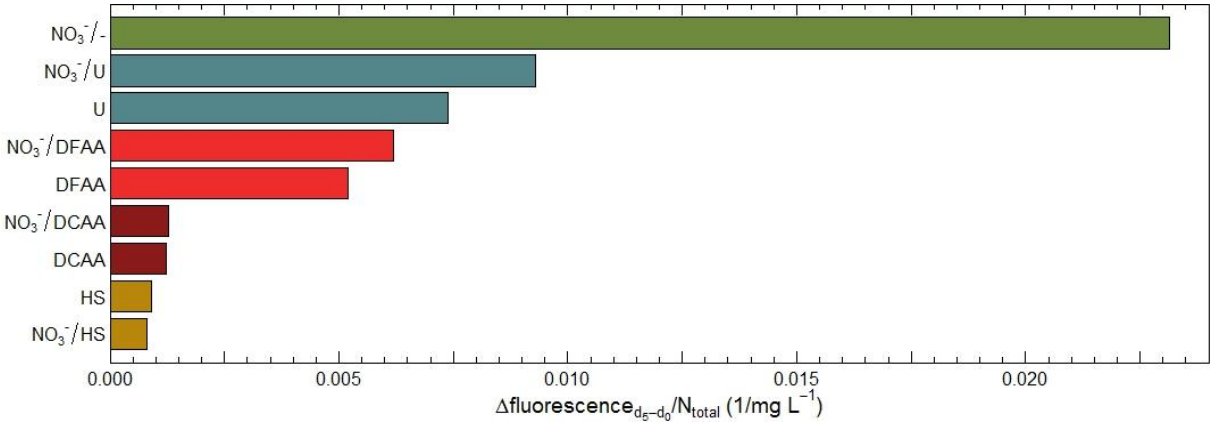

Fig. 24 Relation of the average gain in fluorescence to the added N amount. ∆fluorescence was determined between day 0 and 5. Only the experiments with *Chlamydomonas spec.*, *M. contortum*, *C. menegheniana*, *F. ulna* and *M. aeruginosa* were used in this analysis. Due to another measuring size the experiment with *A. flos-aquae* was excluded.

Although N content was ca. five times higher than the natural annual average in Müggelsee, algae treated with DFAA consistently showed low growth rates. It is very likely, that the DFAA mixture was depleted in some AA important for algal growth. As stated previously, algal AA transport systems do not differentiate between amino acids used for algal growth or others. Therefore, also AA are taken up which do not contribute to algal biomass formation (Antia et al. 1991).

Flynn and Butler (1986) wrote that other N sources do mostly not inhibit amino acid uptake. In the present study this seemed to be particularly true for *Chlamydomonas spec.* (Fig. 4) and *Microcystis aeruginosa* (Fig. 16). The intracellular amino acid pools may also store basic amino acids and cells may use them when depriving from other amino acid sources. This could explain why more ^{15}N was taken up into cells of the NO_3^- treatments. However, at no sampling point was a statistical significant different found for the $\delta^{15}N$-values of the nitrogen-containing and nitrogen-free treatments.

DFAA uptake is mainly an active process executed by three different transport systems (Antia et al. 1991). In general, DFAA metabolism of phytoplankton is quite fast and AA may be quickly excreted into the surrounding medium (Wheeler et al. 1974). It can be speculated that DFAA-^{15}N uptake has been underestimated due to a rapid release of nitrogen. Furthermore, AA transport systems are sensitive to nitrogen stress (Wheeler et al. 1974), which may negatively feed back to the AA uptake rates. This could explain a higher ^{15}N uptake in the experiment with *Chlamydomonas spec.* and *C. meneghiniana* when NO_3^- is available, yet this observed pattern is not statistically significant.

Algal growth rates on DCAA were consistently low for each algal species. This also applies for the NO_3^-/DCAA treatment. However, in the short-term experiments algae which had access to DCAA showed a high ^{15}N uptake. It was assumed that DCAA are of no direct use for phytoplankton due to the difficulty of transporting the relatively large peptides and proteins through the cell membrane. However, algae are able to use extracellular enzymes to hydrolyse proteins and peptides and furthermore synthesise intracellular endo- and exopeptides which play an important role in protein degradation (Berges and Mulholland 2008).

Anyway, it is likely that DCAA were first hydrolysed in large part by bacteria which successfully compete for DCAA-N. It is know that some bacteria species even prefer peptides over DFAA. In general, amino acids are rather used by bacteria than by phytoplankton, but the regenerated ammonium can be well used by algae (Flynn and Butler 1986). Therefore, the actual role of bacteria in the assimilation of DON should not be underestimated.

The high values of $\delta^{15}N$ could imply that more N in the DCAA short-term treatment was taken up than in the treatment with U. This raises the question why phytoplankton growth in the U treatments was always higher than in the DCAA experiments. It is very likely that ^{15}N uptake varies in the short- vs. the long-term run. This could be related to the facts that the urease need to be synthesised first (Berman and Bronk 2003) and that urea needs to be hydrolysed before its uptake into the cells. Thus, ^{15}N uptake via urea may be delayed. Furthermore, in the experiment with *Chlamydomonas spec.* the second urea uptake systems, the enzyme-complex ATP:urea amidolyase (UAL) which is only present in the chlorophyte algae, is inactivated during a lack of urea and a general lack of N (Kohl and Nicklisch 1988). It needs to be activated first, whereas the urease is constitutive active. Thus, the delay in uptake could also derive from the inactivated UAL. Presumably, U degradation products are very efficiently used as DON sources for phytoplankton growth, but because also bacteria have slow uptake systems for urea (JØrgensen 2003) the degradation products are only available with some time delay.

A high, but variable capacity to use U has been shown before in several experiments with algae (e.g. McCarthy 1972; Twomey et al. 2005; Sanderson et al. 2008) and bacteria (JØrgensen 2003). Algal growth rates in the U treatments were always higher than in the DCAA treatments, although incorporation of U may not be directly connected to algal growth (Flynn and Butler 1986). The higher ^{15}N-uptake of *Chlamydomonas spec.*, *Cyclotella meneghiniana* and *Microcystis aeruginosa* via DCAA-^{15}N than U-^{15}N points to the fact that N-uptake via urea degradation is slower in the short term-run. However, an efficient use of U in the long-term run is very probable.

HS is known to stimulate the growth of microorganism at low concentrations, while high concentrations may have inhibiting effects. HS is able to interact with electrons and therefore is supposed to influence the electron transport chain in the PSII (Steinberg et al. 2008). The review of Steinberg et al. (2008) summarises that HS effects on algal growth pattern even of closely related species may differ a lot. In general, HS negatively affect cyanobacteria more than other algae groups. In the conducted

experiments, however, growth of both green algae, the diatom *Cyclotella meneghiniana* and also the cyanobacterium *Anabaena flos-aquae* was enhanced by adding HS. Whereas *Microcystis aeruginosa* did grow less on HS and growth of *Fragilaria ulna* seemed to be suppressed. Since natural HS represent complex organic molecules with different amounts of associated organic and inorganic matter, it is rather speculative whether algal growth stimulation or inhibition just results from the N bound in the HS.

DIN in form of NO_3^- is an important nitrogen source for phytoplankton (Flynn and Butler 1986). In the NO_3^--amended DON treatments phytoplankton growth rates were generally higher than those in treatments without NO_3^- addition. It should be kept in mind that epigenetic modifications through nutrient supply history plays a role in the phytoplankton ability to take up DIN and DON compounds. In the full medium, phytoplankton cultures were adapted to use nitrate and, therefore, it is difficult to predict their growth behaviour on another medium (Flynn and Butler 1986).

In some studies, the presence of DIN can suppress DON uptake, in particular the suppression of U uptake by ammonium (Antia et al. 1991). In the present studies, such a co-inhibition did not occur when DIN was added in form of NO_3^-. Solely, in the NO_3^-/U treatment of *Chlamydomonas spec.* (Fig. 4) and in the NO_3^-/DCAA treatment of *Microcystis aeruginosa* (Fig. 16) the uptake of ^{15}N was lower than in the nitrate free treatments, but no significant difference was detected (Apx. B Tab. 2 and Apx. B Tab. 9).

The HPLC analysis of the pigment composition of *Chlamydomonas spec.* did not reveal decomposition products, but a change in the *chl a*:*chl b* ratio. Proportional to *chl b* there was more *chl a* present in the treatments with reduced growth. Consequently, the nitrogen containing and the nitrogen free control, as well as the DCAA treatment had the highest ratios of *chl a*:*chl b*. It can be concluded that the synthesis of these pigments is influenced by the nutrition statues of the algae culture and is not uniformly down or up regulated.

In general, the measured C:N ratios of *Chlamydomonas spec.* were quite high. At high nitrogen availability the C:N ratio is usually about 6-10, whereas already the on full medium growing reference had a ratio of more than 15. Theoretically, the highest C:N ratio should have occurred in the treatments with the highest incorporation of the DON compound. This however, also depends on the growth rates. The quite low value of the two DCAA treatments make us suppose that a quite high amount of this compound has been accumulated during the seven days of incubation. However, it seems that it was finally not used for growth.

In both cyanobacteria cultures PC contributed the highest portion to the DF excitation spectra. In the cultures of *Anabaena flos-aquae* the DF excitation spectra caused by the phycobiliproteins remained between 96 to 97 %. For *Microcystis aeruginosa* these portions varied stronger (between 86 to 93 %). Algae growing on U were an exception with only 76 %. In general, our results support findings in

other studies where the contribution to the charge separation caused by PB was about 90 % (Bodemer 2004).

Tab. 2 Ratios of phycobiliproteins and *chl a* in the experiment with *Microcystis aeruginosa*. Data derived from multiple Gauss Curve fits of the DF excitation spectra. A The phycobiliprotein:*chl a* ratios were normalized to *chl a*=1. B Phycobiliprotein ratios were normalized to APC=1.

A	PE+PC	APC	chl a	B	PE+PC	APC
Reference	12.2	5.7	1		2.6	1
HS	8.5	3.2	1		6.9	1
DCAA	11.4	5.5	1		11.0	1
DFAA	6.8	2.9	1		3.4	1
U	4.1	1.0	1		13.1	1
Control	17.9	12.3	1		8.6	1
NO_3^-/HS	4.8	1.4	1		4.1	1
NO_3^-/DCAA	8.8	5.3	1		6.9	1
NO_3^-/DFAA	5.3	2.0	1		4.6	1
NO_3^-/U	7.2	1.6	1		4.5	1
NO_3^-/- (control)	10.2	6.3	1		9.2	1

In the *Anabaena flos-aquae* culture ratios of (PE+PC) to *chl a* were lower than of the reference (Tab. 3A), which was kept in a nitrogen rich medium. In general, cultures growing less, had lower ratios of (PE+PC) to *chl a*, but higher ones for APC:*chl a*. Assuming that the *chl a* level stayed relatively stable in the culture like it was found in experiments with *Anacystis nidulans* (Grossmann et al. 1993), it can be concluded that the proteins of (PE+PC) were either degraded or synthesised in a lower amount, whereas APC synthesis raised. In the *Microcystis aeruginosa* experiment the pattern was the opposite where both controls and the DCAA treatment showed the highest ratios of (PE+PC) to *chl a* and also of APC:*chl a* (Tab. 2A). The gauss curve fits of *Microcystis aeruginosa* differed a lot, probably a consequence of the random noise of the chromatogram. Therefore, the results should be interpreted with caution.

Tab. 3 Ratios of phycobiliproteins and *chl a* in the experiment with *Anabaena aeruginosa*. Data derived from a multiple Gauss Curve fit of the DF excitation spectra. A The phycobiliprotein:*chl a* ratios were normalized to *chl a*=1. B The (PE+PC):APC ratios were normalized to APC=1.

A	PE+PC	APC	Chl a	B	PE+PC	APC
Reference	28.6	7.2	1		4.0	1
HS	26.6	9.7	1		2.7	1
DCAA	21.4	8.2	1		3.2	1
DFAA	22.1	9.2	1		2.6	1
U	25.0	7.0	1		3.6	1
control	19.8	10.7	1		2.1	1
NO_3^-/HS	23.0	7.0	1		3.4	1
NO_3^-/DCAA	22.1	7.9	1		2.8	1
NO_3^-/DFAA	26.5	8.5	1		3.1	1
NO_3^-/U	24.9	6.9	1		3.6	1
NO_3^-/- (control)	19.8	12.0	1		1.7	1

In former studies, PBS in algae cultures starving from nitrogen were rapidly decomposed and a reduction of the PC to APC ratio occurred since synthesis of PC in nitrogen poor environments was diminished (Grossmann et al. 1993). This could be also shown for our *Anabaena flos-aquae* treatments

where the ratio of (PE+PC) to APC decreased in treatments with no or only a little nitrogen available (Tab. 3B). However, *Microcystis aeruginosa* differed in its behaviour (Tab. 2B), and all treatments showed a higher ratio of (PE+PC) to APC than the reference. Cultures with higher growth in the HS, NO_3^-/HS and NO_3^-/U treatments always had lower ratios than their controls. Thus, the general consideration that in cyanobacteria amino acids are provided to the cell via degradation of PBS (Grossmann et al. 1993) is supported by our experiment with *Anabaena flos-aquae*.

The nitrogen stress of *Anabaena flos-aquae* was well indicated by the synthesis of heterocysts, e.g. the reference showed the lowest ratio of heterocysts to biovolume. Surprisingly, controls did not have the highest ratios, presumably due to the overall much lower growth. Comparing the HS treatment with the control and NO_3^-/HS with NO_3^-/- revealed a statistical significance. Apparently, also the well growing cyanobacteria which were provided with high amounts of total N in form of U synthesised quite high numbers of HC. This finding raises the question if *A. flos-aquae* grew so well because of the increased numbers of heterocysts or because they formed so many HC because N offered by U could not get assimilated easily. This important outcome requires a more severe testing.

5 Conclusion and Outlook

Species grew best on U, even when the nitrogen content was only around 0.51 mg L^{-1}. In comparison, there was lower growth with an equal concentration of N deriving from DCAA (0.5 mg N L^{-1}). Furthermore, it can be concluded that addition of in form of NO_3^-N stimulates phytoplankton growth and biomass build-up.

Because it is difficult to transfer the result of laboratory experiments to environmental conditions, it is of great interest to change our experiments to simulate more natural environmental conditions. For this purpose, it is recommended to carry out experiments with natural concentration of DON, because it is not yet known whether phytoplankton can grow on such low concentrations (Berman and Bronk 2003). Furthermore, application of semi-continuous cultures instead of batch-cultivation is strongly recommended.

It would be of common interest to carry out further experiments to gain more information about the role of bacteria for phytoplankton DON availability. One the one hand, there is an increasing competition for N with bacteria at low concentrations. And therefore, the high DON concentrations used in our study do not mean that low concentrations of DON compounds can be utilized by the phytoplankton (Wheeler et al. 1974). But on the other hand, bacteria can increase DON availability by hydrolysis Thus, studies with axenic algae should be carried out, to evaluate phytoplankton species-specific DON uptake rates. However, this would again relate to unnatural conditions. Another way to find out more about the role of bacteria in the DON cycle would be to separate bacteria from the algae and measure them separately with IRMS. This approach seems to be difficult since a high bacterial biomass will be needed for such stable isotope measurements. Yet, with a Nano-Secondary Ion Mass Spectrometry (Nano-SIMS) the ^{15}N-content in single algae and bacteria cells can be quantified.

In addition, more algal species should be tested in order to allow for more general statements regarding the different algae groups green algae, diatoms and cyanobacteria. Thereby, competition experiments with different algae species could give some more detailed information on the DON-uptake competitiveness of specific alga species in a mixed culture.

Further experiments with other DIN compounds such as ammonium and nitrite could be of interest, as well as DON compounds such as polyamines, amino-sugars, purines, pyrimidines, DNA and RNA. In the present study, only single DON compounds were used. However, the natural DON pool is much more complex and may vary greatly in space and time. In natural environments, for example, there is always a competition between several DON compounds, and thus our results cannot be directly transferred to natural conditions, but provide insights into the general capability of phytoplankton species to assimilated DON.

Aside of all the methodological and experimental improvements stated above, a general usage of the DON compounds DFAA, DCAA, U and HS by green algae species, diatoms and cyanobacteria has been well demonstrated. This important knowledge needs to be taken into account for improvement of management strategies to diminish nutrient, particularly N input into the environment. Our study should stimulate research for a more efficient reduction of DON (e.g. U, HS, DCAA and DFAA) inputs into the environment. Since urea contributes about 40 % of the N fertilizer used worldwide (Berman and Bronk 2003), it would be of general interest to significantly reduce its release into the environment to successfully combat future eutrophication. Furthermore, in most monitoring programs the urea concentration is not analysed. It is recommended to include urea measurements as a routine measurement.

Appendices

A. Summary Tables

Apx. A Tab. 1 Average growth rates per day.

	Chlamydomonas reinhardtii $\mu_{3\text{-}5}$ [d^{-1}]	*Monoraphidium contortum* $\mu_{1\text{-}3}$ [d^{-1}]	*Cyclotella meneghiniana* $\mu_{1\text{-}5}$ [d^{-1}]	*Fragilaria ulna* $\mu_{1\text{-}3}$ [d^{-1}]	*Microcystis aeruginosa* $\mu_{0\text{-}7}$ [d^{-1}]	*Anabaena flos-aquae* $\mu_{5\text{-}8}$ [d^{-1}]
HS	0.154 ± 0.017	0.098 ± 0.027	0.076 ± 0.009	-0.001 ± 0.032	0.034 ± 0.009	0.1 ± 0.06
DCAA	0.115 ± 0.019	0.133 ± 0.027	0.072 ± 0.02	0.057 ± 0.028	0.025 ± 0.006	0.33 ± 0.01
DFAA	0.096 ± 0.009	0.027 ± 0.007	0.024 ± 0.003	0.077 ± 0.018	0.035 ± 0.006	0.41 ± 0.05
U	0.175 ± 0.013	0.161 ± 0.013	0.082 ± 0.021	0.285 ± 0.084	0.041 ± 0.005	0.42 ± 0.02
control	0.02 ± 0.025	0.027 ± 0.027	0.037 ± 0.006	0.05 ± 0.024	0.019 ± 0.007	0.32 ± 0.02
NO_3^-/HS	0.175 ± 0.008	0.09 ± 0.014	0.048 ± 0.011	-0.039 ± 0.03	-0.011 ± 0.005	0.27 ± 0.08
NO_3^-/DCAA	0.079 ± 0.022	0.048 ± 0.014	0.062 ± 0.013	0.009 ± 0.01	0 ± 0.007	0.35 ± 0.04
NO_3^-/DFAA	0.108 ± 0.022	0.05 ± 0.023	0.076 ± 0.02	-0.068 ± 0.02	0.01 ± 0.003	0.42 ± 0.05
NO_3^-/U	0.291 ± 0.065	0.219 ± 0.003	0.096 ± 0.006	0.091 ± 0.036	0.03 ± 0.011	0.38 ± 0.03
NO_3^-/- (control)	0.125 ± 0.022	-0.049 ± 0.016	0.042 ± 0.007	-0.020 ± 0.019	0.016 ± 0.004	0.28 ± 0.06

Apx. A Tab. 2 Average δ^{15}N-values of the stable isotope analysis of *Chlamydomonas spec*. Samples were taken after 1, 6 and 24 hours (n=4).

Treatment	1 h	6 h	24 h
Reference	184.39		
DCAA	57614.83	65032.33	54401.53
DFAA	3139.77	2940.74	6429.13
U	2585.11	8061.31	55197.64
Control	127.12	214.11	447.75
NO_3^-/DCAA	100365.75	93245.09	136763.71
NO_3^-/DFAA	1464.14	3032.19	8104.76
NO_3^-/U	2180.80	5220.03	35354.45
NO_3^-/- (control)	54.28	105.47	137.51

Apx. A Tab. 3 Average *chl a* and *chl b* contents and *chl a*/*chl b* ratio of *Chlamydomonas spec.* Samples were taken on day seven (n=4).

	chl a [µg L^{-1}]	*chl b* [µg L^{-1}]	*chl a*/*chl b* [-]		*chl a* [µg L^{-1}]	*chl b* [µg L^{-1}]	*chl a*/*chl b* [-]
Reference	14.3 ± 2.56	2.75 ± 0.51	5.2 ± 0.3				
HS	67.86 ± 5.53	12.77 ± 1.16	5.32 ± 0.11	**NO_3^-/HS**	83.02 ± 12.55	16.49 ± 2.22	5.07 ± 0.7
DCAA	17.14 ± 1.06	2.62 ± 0.4	6.64 ± 0.83	**NO_3^-/DCAA**	34.14 ± 2.06	6.45 ± 0.6	5.31 ± 0.19
DFAA	19 ± 3.53	3.47 ± 0.95	5.58 ± 0.59	**NO_3^-/DFAA**	30.67 ± 8.51	6.33 ± 0.57	4.84 ± 1.19
U	135.54 ± 13.76	25.32 ± 2.47	5.35 ± 0.09	**NO_3^-/U**	154.07 ± 10.2	28.11 ± 1.76	5.48 ± 0.09
control	9.72 ± 1.76	1.51 ± 0.38	6.53 ± 0.61	**NO_3^-/-**	19.77 ± 3.09	2.95 ± 0.64	6.78 ± 0.73

Apx. A Tab. 4 Average values of the CN-analysis of *Chlamydomonas spec.* Samples were taken on day seven (n=4).

Treatment	C:N ratio [-]	Treatment	C:N ratio [-]
Reference	15.8 ± 0.6		
HS	15.49 ± 0.94	**NO_3^-/HS**	15.29 ± 1.08
DCAA	13.18 ± 1.03	**NO_3^-/DCAA**	12.36 ± 0.44
DFAA	23.66 ± 2.32	**NO_3^-/DFAA**	24.34 ± 2.34
U	20.35 ± 2.89	**NO_3^-/HS**	22.07 ± 1.72
control	27.87 ± 2.43	**NO_3^-/-**	25.02 ± 1.9

Apx. A Tab. 5 Average δ^{15}N-values of the stable isotope analysis of *Cyclotella meneghiniana*. Samples were taken after 1, 6, 24 and 72 hours (n=3). *note that the measurement range of the ^{15}N-isotope was exceeded, which leads to an underrepresentation of the δ^{15}N-value.

	1 h	6 h	24 h	72 h
Reference	13.14			
DCAA	146952.74	96710.48	156503.51*	250468.88*
DFAA	3307.57	4192.52	16076.49	20335.64
U	18902.00	45472.29	88935.68	89209.31*
Control	106.98	-279.89	232.60	450.83
NO3-/DCAA	125032.11*	187361.56*	216392.66*	255514.45*
NO3-/DFAA	2644.07	3896.17	13345.03	15046.17
NO3-/U	12275.08	31814.12	111170.00	112011.47
NO3-/- (control)	76.56	-172.54	203.21	226.08

Apx. A Tab. 6 Average δ^{15}N-values of the stable isotope analysis of *Microcystis aeruginosa*. Samples were taken after 1, 6 and 24 hours (n=4).

Treatment	1 h	6 h	24 h
Reference	53.44		
DCAA	113433.94	138020.40	136787.22
DFAA	3293.57	6842.50	17548.79
U	11766.54	29461.83	87527.82
control	296.92	610.73	569.90
NO_3^-/DCAA	116200.08	107122.06	90207.35
NO_3^-/DFAA	25943.80	7163.00	17622.53
NO_3^-/U	12735.27	37153.54	92649.30
NO_3^-/- (control)	230.16	231.40	794.32

Apx. A Tab. 7 Portion of the PB and *chl a* contributed to the DF (*Microcystis aeruginoa*).

	PE+PC	APC	*chl a*
Reference	0.66	0.27	0.07
HS	0.65	0.26	0.09
DCAA	0.63	0.28	0.09
DFAA	0.65	0.22	0.13
U	0.65	0.11	0.24
Control	0.57	0.40	0.03
NO_3^-/HS	0.67	0.18	0.14
NO_3^-/DCAA	0.60	0.32	0.08
NO_3^-/DFAA	0.64	0.24	0.12
NO_3^-/U	0.73	0.16	0.10
NO_3^-/- (control)	0.60	0.31	0.08

Apx. A Tab. 8 Portion of the PB and *chl a* contributed to the delayed fluorescence (*Anabaena flos-aquae*).

	PE+PC	APC	*chl a*
Reference	0.78	0.20	0.03
HS	0.71	0.26	0.03
DCAA	0.72	0.25	0.04
DFAA	0.69	0.28	0.03
U	0.76	0.21	0.03
Control	0.65	0.32	0.04
NO_3^-/HS	0.74	0.22	0.03
NO_3^-/DCAA	0.72	0.25	0.03
NO_3^-/DFAA	0.74	0.24	0.03
NO_3^-/U	0.76	0.21	0.03
NO_3^-/- (control)	0.60	0.37	0.03

Apx. A Tab. 9 Average values for the heterocysts per biovolume of *Anabaena flos-aquae*. Samples were taken on day nine (n=4).

Treatment	HC/biovolume [μm^{-3}]	Treatment	HC/biovolume [μm^{-3}]
Reference	$16.1*10^{-5}$		
HS	$38*10^{-5} \pm 4.2*10^{-5}$	**NO_3^-/HS**	$38.3*10^{-5} \pm 3.2*10^{-5}$
DCAA	$52.4*10^{-5} \pm 3.9*10^{-5}$	**NO_3^-/DCAA**	$49*10^{-5} \pm 1.2*10^{-5}$
DFAA	$52.7*10^{-5} \pm 3.4*10^{-5}$	**NO_3^-/DFAA**	$52.5*10^{-5} \pm 4.5*10^{-5}$
U	$45.7*10^{-5} \pm 2.4*10^{-5}$	**NO_3^-/HS**	$45*10^{-5} \pm 3.5*10^{-5}$
control	$50.9*10^{-5} \pm 4.9*10^{-5}$	**NO_3^-/-**	$48.3*10^{-5} \pm 1.4*10^{-5}$

B. Statistical results

Apx. B Tab. 1 Statistical significances of the *Chlamydomonas spec*. growth rates. Growth rates were calculated between day 3 and 5.

	HS	DCAA	DFAA	U	control	NO_3^-/HS	NO_3^-/DCAA	NO_3^-/DFAA	NO_3^-/U	NO_3^-/- (control)
HS		~	+	-	+					
DCAA			-	+	+					
DFAA				+	+					
U					+					
Control										
NO_3^-/HS	~						+	+	+	+
NO_3^-/DCAA		-						-	+	~
NO_3^-/DFAA			-						+	-
NO_3^-/U				+						+
NO_3^-/- (control)					+					

Apx. B Tab. 2 Statistical significances of the δ^{15}N-values deriving from the stable istope analysis of *Chlamydomonas spec*. Samples were taken after 1, 6 and 24 hours.

		1h/6h/24h						
		DCAA	DFAA	U	control	NO_3^-/DFAA	NO_3^-/U	NO_3^-/- (control)
1h	DCAA		+	+	+			
	DFAA			-	+			
	U				+			
	Control							
	NO_3^-/DCAA	-				+	+	+
	NO_3^-/DFAA		-				~	+
	NO_3^-/U			-				+
	NO_3^-/- (control)				-			
6h	DCAA		+	+	+			
	DFAA			+	+			
	U				+			
	Control							
	NO_3^-/DCAA	-				+	+	+
	NO_3^-/DFAA		-				+	+
	NO_3^-/U			~				+
	NO_3^-/- (control)				-			
24h	DCAA				+			
	DFAA				+			
	U				+			
	Control							
	NO_3^-/DCAA	-				+	-	+
	NO_3^-/DFAA		-				+	+
	NO_3^-/U			~				+
	NO_3^-/- (control)				-			

Apx. B Tab. 3 Statistical significances of the *chl a*:*chl b* ratio deriving from the HPLC-analysis of *Chlamydomonas spec.* Samples were taken on day seven of the experiment.

	Reference	HS	DCAA	DFAA	U	control	NO_3^-/HS	NO_3^-/DCAA	NO_3^-/DFAA	NO_3^-/U	NO_3^-/-
Reference											
HS	-		+	-	-	+					
DCAA	~			-	+	-					
DFAA	-				-	-					
U	-					+					
Control	~										
NO_3^-/HS	-	-						-	-	-	+
NO_3^-/DCAA	-		+						-	-	+
NO_3^-/DFAA	-			-						-	+
NO_3^-/U	-				-						+
NO_3^-/-	~					-					

Apx. B Tab. 4 Statistical significances of the C:N ratio deriving from the CN-analysis of *Chlamydomonas spec.* Samples were taken on day seven of the experiment.

	Reference	HS	DCAA	DFAA	U	control	NO_3^-/HS	NO_3^-/DCAA	NO_3^-/DFAA	NO_3^-/U	NO_3^-/-
Reference											
HS	-		~	+	+	+					
DCAA	~			+	+	+					
DFAA	~				-	-					
U	-					+					
Control	~										
NO_3^-/HS	-	-						+	+	+	+
NO_3^-/DCAA	~		-						+	+	+
NO_3^-/DFAA	~			-						-	-
NO_3^-/U	~				-						-
NO_3^-/- (control)	~					-					

Apx. B Tab. 5 Statistical significances of the *Monoraphidium contortum* growth rates. Growth rates were calculated between day 1 and 3.

	HS	DCAA	DFAA	U	Control	NO_3^-/HS	NO_3^-/DCAA	NO_3^-/DFAA	NO_3^-/U	NO_3^-/- (control)
HS		-	+	+	+					
DCAA			+	-	+					
DFAA				+	-					
U					+					
Control										
NO_3^-/HS	-						+	+	+	+
NO_3^-/DCAA		+						+	+	+
NO_3^-/DFAA			+						+	+
NO_3^-/U				+						+
NO_3^-/- (control)					+					

Apx. B Tab. 6 Statistical significance of the *Cyclotella meneghiniana* growth rates. Growth rates were calculated between day 1 and 5.

	HS	DCAA	DFAA	U	control	NO_3^-/HS	NO_3^-/DCAA	NO_3^-/DFAA	NO_3^-/U	NO_3^-/- (control)
HS		-	+	-	+					
DCAA			+	-	+					
DFAA				+	+					
U					+					
Control										
NO_3^-/HS	+						-	-	+	-
NO_3^-/DCAA		-						-	+	~
NO_3^-/DFAA			+						~	~
NO_3^-/U				-						+
NO_3^-/- (control)					-					

Apx. B Tab. 7 Statistical significances of the *Fragilaria ulna* growth rates. Growth rates were calculated between day 1 and 3.

	HS	DCAA	DFAA	U	control	NO_3^-/HS	NO_3^-/DCAA	NO_3^-/DFAA	NO_3^-/U	NO_3^-/- (control)
HS		+	+	+	~					
DCAA			-	+	-					
DFAA				+	+					
U					+					
Control										
NO_3^-/HS	-						~	-	+	-
NO_3^-/DCAA		+						+	+	~
NO_3^-/DFAA			+						+	+
NO_3^-/U				+						+
NO_3^-/- (control)					+					

Apx. B Tab. 8 Statistical significance of the *Microcystis aeruginosa* growth rates. Growth rates were calculated between day 0 and 7.

	HS	DCAA	DFAA	U	control	NO_3^-/HS	NO_3^-/DCAA	NO_3^-/DFAA	NO_3^-/U	NO_3^-/- (control)
HS		-	-	-	+					
DCAA			-	+	-					
DFAA				-	+					
U					+					
Control										
NO_3^-/HS	+						~	+	+	+
NO_3^-/DCAA		+						-	+	+
NO_3^-/DFAA			+						+	-
NO_3^-/U				-						-
NO_3^-/- (control)					-					

Apx. B Tab. 9 Statistical significances of the δ^{15}N-values deriving from the stable isotope analysis of *Microcystis aeruginosa*. Samples were taken after 1, 6 and 24 hours.

		1h/6h/24h						
		DCAA	DFAA	U	control	NO_3^-/DFAA	NO_3^-/U	NO_3^-/- (control)
1h	DCAA		~	+	+			
	DFAA			~	-			
	U				+			
	control							
	NO_3^-/DCAA	-				~	+	+
	NO_3^-/DFAA		-				-	+
	NO_3^-/U			-				+
	NO_3^-/- (control)				-			
6h	DCAA				+			
	DFAA				+			
	U				+			
	control							
	NO_3^-/DCAA	-				+	+	+
	NO_3^-/DFAA		-				+	+
	NO_3^-/U			-				+
	NO_3^-/- (control)				-			
24h	DCAA		+	+	+			
	DFAA			+	+			
	U				+			
	control							
	NO_3^-/DCAA	~				+	-	+
	NO_3^-/DFAA		-				+	+
	NO_3^-/U			-				+
	NO_3^-/- (control)				-			

Apx. B Tab. 10 Statistical significance of the *Anabaena flos-aquae* growth rates. Growth rates were calculated between day 5 and 8.

	HS	DCAA	DFAA	U	control	NO_3^-/HS	NO_3^-/DCAA	NO_3^-/DFAA	NO_3^-/U	NO_3^-/- (control)
HS		+	+	+	+					
DCAA			+	+	-					
DFAA				-	+					
U					+					
control										
NO_3^-/HS	+						-	+	+	-
NO_3^-/DCAA		-						-	-	~
NO_3^-/DFAA			-						-	+
NO_3^-/U				-						+
NO_3^-/- (control)					-					

Apx. B Tab. 11 Statistical significance of the heterocysts/biovolume ratio of *Anabaena flos-aquae*. Samples were taken on day nine.

	HS	DCAA	DFAA	U	control	NO_3^-/HS	NO_3^-/DCAA	NO_3^-/DFAA	NO_3^-/U	NO_3^-/- (control)
HS		+	+	+	+					
DCAA			-	-	-					
DFAA				~	-					
U					-					
Control										
NO_3^-/HS	-						+	+	~	+
NO_3^-/DCAA		-						-	~	-
NO_3^-/DFAA			-						-	-
NO_3^-/U				-						-
NO_3^-/- (control)					-					

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Statement of Affirmation

I affirm that I have written this Master's thesis on my own and that all cited resources have been listed in the references.

Place, Date Signature