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„Is *Arthrospira fusiformis* able to fix molecular nitrogen?“

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Anna-Maria Gschwandner, BSc

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1. Allgemeine Einleitung

Arthrospira fusiformis zählt zu den Algen. Algen sind als funktionelle Gruppe aufzufassen und umfassen Organismen unterschiedlichster stammesgeschichtlicher Herkunft. Zu den Algen werden Eukaryoten und Prokaryoten gezählt, die zumeist oxygene Photosynthese betreiben, aber im Gegensatz zu den Gefäßpflanzen keine Gefäßbündel ausbilden und nicht in Blatt, Stamm und Wurzel untergliedert sind. *A. fusiformis* gehört zu den Cyanobakterien; diese Gruppe war die erste, die vor rund 3,6 Milliarden Jahren erstmals Photosynthese betrieb, bei der als Nebenprodukt O_2 anfällt. Cyanobakterien sind somit für die Entstehung der O_2-haltigen Atmosphäre verantwortlich und stellen damit den Grundstein für komplexes Leben dar (Graham et al. 2009; Catling & Claire 2005).

Unerlässlich für das Überleben von *A. fusiformis* sind Phosphor und Stickstoff. Obwohl 78% unserer Atmosphäre aus Stickstoff bestehen, ist N_2 chemisch schwer zu nutzen, da dieses Gasmolekül sehr stabil ist. Während alle anderen Algengruppen auf reduzierte (Ammonium), oxydierte (Nitrat, Nitrit) oder organische N-Verbindungen angewiesen sind, können viele Cyanobakterien den inerten molekularen Stickstoff nutzen, indem sie ihn über das Enzym Nitrogenase reduzieren und ihn in weiterer Folge in zelleigene Strukturen einbauen. Diese Eigenschaft ist äußerst wichtig, da so bereits reduzierter Stickstoff in Systeme gebracht werden kann, sei es in Böden oder in aquatische Systeme. Cyanobakterien dienen hier als entscheidendes Bindeglied innerhalb des Stickstoffkreislaufs.

Der Prozess der N_2-Fixierung ist allerdings enorm energieaufwendig (Raymond 2004). Zusätzlich erschwerend wirkt sich die Tatsache aus, dass die Nitrogenase extrem

sauerstoff-empfindlich ist; bereits ab geringen O_2-Konzentrationen in der Zelle stellt die Nitrogenase ihre Aktivität ein. Im Lauf der Evolution haben sich zwei Möglichkeiten entwickelt, wie Organismen mit dieser Problematik umgehen: (1) eine räumliche Trennung zwischen Photosynthese und Stickstofffixierung und (2) eine zeitliche Trennung.

Zur räumlichen Trennung der N-Fixierung (1) wurde ein eigener Zelltyp entwickelt, die sogenannte Heterocyte. Diese Zellen haben eine besonders dicke Zellwand, um das Eindiffundieren von Gasen, vor allem von O_2, zu erschweren. Zusätzlich besitzen diese Zellen kein Photosystem II, welches für die O_2-Freisetzung verantwortlich ist und sie zeigen erhöhte Respirationsaktivität (Lee 2008; Graham et al. 2009).

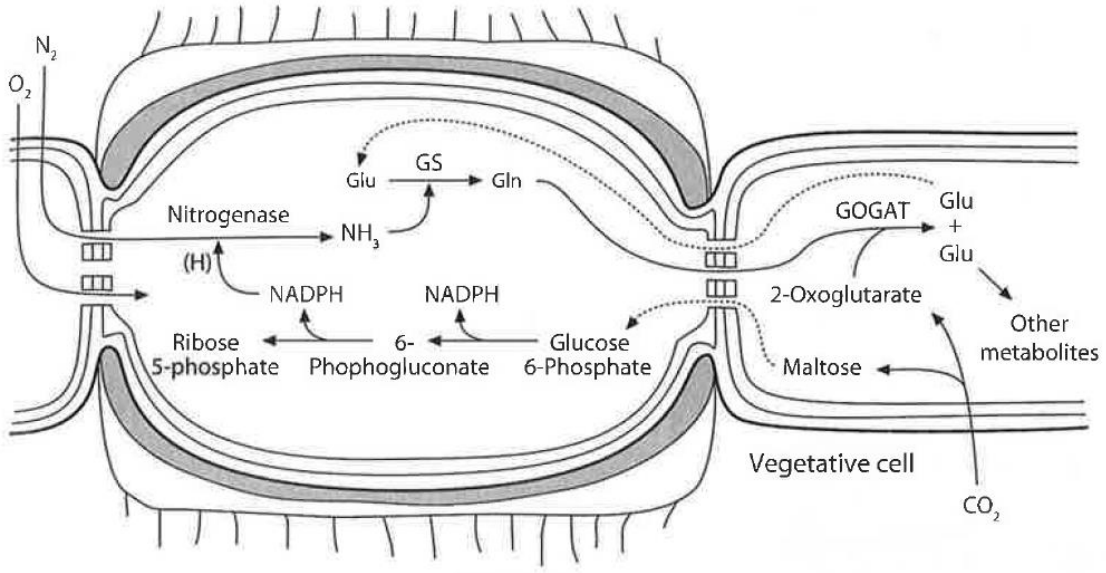

Abbildung 2.1.: Schematische Darstellung einer Heterocyte mit ihrer Funktion (Haselkorn 1978)

Dabei wird N_2 durch die Nitrogenase reduziert und diese Aminogruppe weiter auf Glutamat transferiert, wodurch dieses zu Glutamin wird. In dieser Form wird der fixierte Stickstoff an Nachbarzellen weitergegeben, wo das Enzym „Glutamin-Oxyglutarat-Aminotransferase“ (GOGAT) Glutamat regeneriert, d.h. eine Aminogruppe von Glutamin

auf 2-Oxoglutarat übertragen wird (**Abb. 2.1.**). Das Resultat sind zwei Glutamat-Moleküle, wobei eines davon wieder zurück in die Heterocyte transportiert wird, um neuerlich eine Aminogruppe aufnehmen zu können. Das andere Glutamat-Molekül verbleibt in der vegetativen Zelle und wird für diverse weitere metabolische Prozesse verwendet (Graham et al. 2009; Haselkorn 1978). Diese Variante wird von vielen fädigen Cyanobakterien genutzt, sie wandeln dafür in regelmäßigen Intervallen eine „normale“ vegetative Zelle in eine Heterocyte um (Kulasooriya et al. 1972). Im Vergleich zu ausreichender N-Versorgung wird bei N-limitierung häufig eine erhöhte Heterocyten-Frequenz gefunden (Fogg 1944; Mickelson et al. 1967).

Die zeitliche Trennung zwischen Photosynthese und Stickstofffixierung (2) ist häufig bei einzelligen Cyanobakterien zu finden. Untertags betreibt die Zelle Photosynthese und während der Dunkelperiode fixiert dieselbe Zelle molekularen Stickstoff (Stal 1995; Steppe et al. 2001; Graham et al. 2009). Diese Methode ist recht aufwendig, da in einer Zelle sämtliche Strukturen für beide Stoffwechselwege vorhanden sein müssen.

Daneben gibt es noch „Speziallösungen“ zum Umgang mit der O_2-Sensitivität der Nitrogenase. Die einzellige *Cyanothece* besitzt die Möglichkeit, das Photosystem II, welches O_2 während der Photosynthese produziert, „auszuschalten“, sobald Stickstoff benötigt und fixiert wird (Meunier et al. 1998). Ein weiteres erwähnenswertes Beispiel ist *Trichodesmium*, welches zwar ein filamentöses, koloniebildendes Cyanobakterium ist, aber keine Heterocyten ausbildet, sondern in den mittleren Zellen ihres Fadenbündels Stickstoff fixiert, während die Zellen an den Außenseiten nur Photosynthese betreiben (Berman-Frank et al. 2001; Fredriksson & Bergman 1997).

Arthrospira fusiformis ist eine filamentöse Blaualge ohne Heterocyten, weswegen die Möglichkeit besteht, dass dieses Taxon eine zeitliche Trennung zwischen Photosynthese und Stickstofffixierung nutzt. *A. fusiformis* tritt als alkaliphile Alge in Sodaseen als dominante Art auf und führt zu extremen Algenblüten (Whitton & Potts 2002; Kaggwa et al. 2013). Binnensalzgewässer sind fast immer Seen ohne Abfluss und werden auch endorheische Seen genannt (Vareschi 1987). Die ganze Fracht an gelösten Stoffen und Salzen verbleibt im System, welches mit dem Wasser oder durch Luft aus dem Einzugsgebiet in die Seen transportiert wird. Wasser verdunstet und im Laufe der Zeit versalzt das System zunehmend (Burgis & Morris 1987). Binnensalzgewässer sind extreme Standorte, sie weisen häufig sehr hohe Salzkonzentrationen auf, die um ein Mehrfaches höher als Meerwasser sind (Schagerl 2016). Zusätzlich haben sie eine sehr unterschiedliche Ionenzusammensetzung und werden je nach Haupt-Anionenanteil als Chlorid-, Sulfat-, oder Sodaseen bezeichnet (Hammer & Dumont 1986). Was all diese Binnensalzgewässer gemein haben, sind starke Schwankungen in der Salzkonzentration, pH-Wert und Wasserstand (Williams 1972). Die Unterschiede hängen mit den Niederschlägen und der Verdunstungsrate zusammen, wodurch sich in jedem Salzsee, je nach seiner geographischen Lage, den damit einhergehenden Witterungsverhältnissen und dem Einzugsgebiet ein unterschiedlicher und ganz individueller Rhythmus der Salz-konzentrationsschwankung einstellt. Teilweise trocknen diese Seen auch vollständig aus und lassen dicke Salzkrusten zurück (Hammer & Dumont 1986). Salzstandorte sind zusätzlich sehr gefährdete Lebensräume, da sie nicht nur durch die Klimaveränderung aus ihrer sensiblen Balance gebracht werden, sondern auch durch das direkte und aktive Eingreifen des Menschen (Burgis & Morris 1987).

Im Ostafrikanischen Grabenbruch finden sich durch die spezielle Geologie und durch abflusslose Becken zahlreiche Sodaseen (Schagerl 2016). Sodaseen haben einen sehr hohen Anteil an Karbonaten (HCO_3 und $NaHCO_3$) und können dadurch pH-Werte bis 11 erreichen. Des Weiteren zeigen sie sehr niedrige Mg^{2+} und Ca^{2+} Konzentrationen, während ihr SO_4^{2-} in aller Regel recht hoch ist (Schagerl 2016; Vonshak et al. 1982; Whitton 2000). Bei mittleren Salzkonzentrationen dominiert in diesen Seen *A. fusiformis* als alkaliphile Art (Iltis 1969). Nimmt die Konzentration etwa auf Grund von häufigen Niederschlägen ab, wird *A. fusiformis* von anderen Phytoplanktern oder gar benthischen Algen abgelöst (Whitton 2000). Auch bei sehr hohen Salzkonzentrationen wird *A. fusiformis* durch Picoplankton abgelöst (Schagerl 2016).

A. fusifomis ist auf Grund ihres sehr hohen Protein-, Vitamin- und Nährstoffgehaltes nicht nur für die Industrie als Nahrungsergänzungsmittel interessant (Sotiroudis & Sotiroudis 2013), sondern stellt die Nahrungsgrundlage des Zwergflamingos *Phoenicopterus minor* und auch des Fisches *Alcolapia grahami* dar (Coe 1966; Bergman et al. 2003). Der Zwergflamingo erhält seine typisch rosarote Färbung durch Carotinoide, welche in *A. fusiformis* enthalten sind (Fox 1976). Sodaseen haben in aller Regel sehr hohe Phosphatgehalte und stellen damit extrem produktive Systeme dar, allerdings können sie stickstofflimitiert sein. Aus diesem Grund wurde in der vorliegenden Arbeit *A. fusiformis* auf ihre Fähigkeit zur N_2-fixierung hin untersucht. Um ein breites Spektrum abzudecken, wurden insgesamt neun Klone aus verschiedenen Sodaseen des Ostafrikanischen Grabenbruches untersucht.

Der experimentelle Ansatz setzt sich aus drei Teilen zusammen. (1) *A. fusiformis* wurde genetisch auf das *nifH* Gen und dessen Exprimierung hin untersucht. *Nif*H ist eines der

Gene, welches das Enzym Nitrogenase kodiert und hat sich in den letzten Jahren als genetischer Marker zur Identifizierung potenzieller stickstofffixierender Organismen durchgesetzt. (2) Wachstumstests in Medien ohne ausreichende anorganische Stickstoff-Quelle (NO_3). Im Falle von N_2-Fixierung kann der Organismus wachsen. (3) Inkubation mit $^{15}N_2$ -Gas. ^{15}N ist das schwerere stabile Isotop des häufigeren ^{14}N und kommt in einer Konzentration von < 1 % im Gesamtstickstoff unserer Atmosphäre vor. Diese Methode erlaubt es, zu messen, ob ein Organismus N_2 fixiert und in seiner Zelle einlagert oder nicht, sogar dann noch, wenn die N_2-Fixierungsrate nicht ausreichen sollte, um ein Überleben im Labor dauerhaft zu gewährleisten.

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2. Is *Arthrospira fusiformis* able to fix molecular nitrogen?

2.1. Abstract

East African soda lakes (EASL), some of them known as Flamingo lakes, range amongst the most productive aquatic ecosystems worldwide. Compared to phosphorus and carbon availability, these lakes show reduced nitrogen concentrations. The filamentous cyanoprokaryote *Arthrospira fusiformis* (formerly *Spirulina platensis*) is on the base of the food web and might be able to fix molecular nitrogen, although this taxon lacks heterocytes. We cultivated nine strains of *Arthrospira fusiformis* originating from various EASL under nitrate- rich and nitrate- depleted conditions to study the potential of molecular nitrogen fixation. We found the *nifH* gene in all strains; six out of these strains also express this gene, five of them under both high and low nitrate- supply, and one strain under only nitrate-limiting conditions. ^{15}N incubation showed slightly enriched $\delta^{15}N$ values of some strains, but the enrichment was very small compared to controls such as *Anabaena*. Under laboratory conditions, all strains faded during nitrate-depleted growth after around three weeks. Our data suggest that although the *nifH* genes were expressed in many strains, nitrogenase activity is not sufficient to ensure constant growth at offered growth conditions.

Keywords: non-heterocytous cyanobacterium, diazotrophic, blue-green algae, East African Soda lakes, *Spirulina platensis*

2.2. Introduction

Arthrospira fusiformis (Voronichin) Komárek and Lund, sometimes still referred to as *Spirulina platensis* (Komárek & Lund 1990), is a filamentous and helically coiled cyanobacterium, prospering in East African Soda Lakes (EASL) (Kebede 1997; M. N. Kaggwa et al. 2013). The water temperatures of these lakes are about 25°C, but especially in shallow areas, temperatures may rise to 40°C due to high insolation coupled with dark sediments (Whitton 2000). Soda lakes commonly have pH values up to 11 and may contain up to 14 g L^{-1} Cl^- (Oduor & Schagerl 2007a; Vonshak et al. 1982). Their salinity ranges from slightly brackish to hyper-saline (Hammer & Dumont 1986), and most of these lakes have very low concentrations of Mg^{2+} and Ca^{2+}. Such environments are not only found in East Africa, but also in North America and in the continental interiors of Asia and Europe (Grant et al. 1990).

Despite extreme environments, these water bodies are amongst the most productive aquatic ecosystems worldwide due to the high availability of carbon dioxide (Oduor & Schagerl 2007b). Also phosphorus is available in excess (Schagerl & Oduor 2008), but N-depletion may be one growth-limiting factor for photoautotrophs in soda lakes. Especially shallow lakes release N_2 into the atmosphere through denitrification processes (Bootsma et al. 2003), which are boosted by high temperature and anoxic conditions in near-bottom zones. In surface waters, high amounts of N_2 are dissolved because of its equilibrium with the atmosphere. Once inorganic N such as NH_4^+ and NO_3^- is depleted, organisms capable of N_2-fixation are promoted (Graham et al. 2009). N_2-fixation is an energetically costly process, requiring 16 molecules of ATP per fixed N_2 (Raymond 2004). Thus, it is a highly regulated process on both a transcriptional (Bothe et al. 2006; Dixon & Kahn 2004) and

post-translational level (Kim et al. 1999). The nitrogenase complex is composed of two enzymes, dinitrogenase and dinitrogenase-reductase (Howard & Rees 1994) encoded by the group of *nif* genes which are highly conserved regions. The dinitrogenase, responsible for N_2-reduction, is build up by two subunits alpha and beta that are encoded by *nifD* and *nifK* genes, respectively. The dinitrogenase-reductase is encoded by the *nifH* gene (Latysheva et al. 2012). In general, there are about 16 different *nif* genes known from cyanobacteria, eight of them are closely related to the core N_2-fixation-process (Ruvkun & Ausubel 1980). However, the *nif* gene-cluster varies between different diazotophs (Dai et al. 2014).

The nitrogenase enzyme complex is highly sensitive to O_2. Therefore, photoautotrophic diazotrophs must separate photosynthesis either temporally or spatially from N_2-fixation (Belnap 2001; Chen et al. 1998). Altogether, two major types of N_2-fixing cyanobacteria are known. First, heterocytous cyanobacteria like *Anabaena, Nostoc, Nodularia,* and *Calothrix* form heterocytes as a spatial separation between oxygenic photosynthesis and N_2-fixation. Heterocytes are specialized cells with reduced photosynthetic activity, increased respiration, and thicker cell walls to prevent oxygen diffusion and therefore, organisms are able to fix N_2 during daytime. The second group is formed by non-heterocytous cyanobacteria which fix N_2 under anaerobic or very low oxygen conditions only, for instance, *Synechococcus* sp.*, Plectonema boryanum* Gomont, and *Oscillatoria limnetica* Lemmermann (Stal 1995). The mechanism behind is a temporal separation from photosynthesis: these organisms mainly fix N_2 during night-time when respiration is prevailing, so oxygen that otherwise may inactivate nitrogenase is only found at minor concentration.

It remains unclear how *A. fusiformis* can reach such high densities in EASL between 200 up to 1430 mg DM L^{-1} (M. Kaggwa et al. 2013; Vareschi 1978; Vareschi 1982; Tuite 1981) since the lakes are limited in N availability (Schagerl & Oduor 2008; Talling & Talling 1965; Melack et al. 1982) compared to their available phosphate and carbonate. One possible option to cope with the low N availability is fixation of molecular N. Although *A. fusiformis* is a filamentous cyanobacterium, it does not develop heterocytes. From some non-heterocytous cyanobacteria, specific ways of avoiding oxygen within the cell during N_2-fixation are already known. *Trichodesmium* and *Katagnymene* are examples of filamentous cyanobacteria that are capable to fix N_2 in the light without forming heterocytes (Bergman & Carpenter 1991). N_2 fixing cells are located in the central parts of filaments while the cells at the edges perform photosynthesis (Fredriksson & Bergman 1997; Berman-Frank et al. 2001). Furthermore, *Trichodesmium* and *Katagnymene* occur in tropical seas with relatively low dissolved oxygen concentration which may support the maintenance of anaerobic microenvironments during N_2 fixation (Staal et al. 2003). Another example is the unicellular cyanobacterium *Cyanothece* which down-regulates PS II for a short time during N_2-fixation. This process is possible even during continuous light (Meunier et al. 1998). *A. fusiformis* may use similar mechanisms; a few studies including the genus *Arthrospira* revealed its potential of N_2-fixation. According to Lefort et al. (2014) *A. palentsis* (Parca) is equipped with a typical nitrogenase. In *A. maxima* (CS 328), *nifJ* and *nifS* genes were found by Latysheva et al. (2012), but about the presence of *nifH*, *– K* and *–D*, they made no clear statement. In a study based on 16S rRNA-analyses, Highway (2001) suggested that *A. fusiformis* and *A. maxima* should be treated as the same species. This was however scrutinized by Daadhech et al. (2010), who studied various strains from different

continents and found two distinct clades: saline-alkaline lakes of the paleotropics are inhabited by *A. fusiformis*, water bodies from the neotropics are dominated by *A. maxima*.

We hypothesize that A. fusiformis is capable of molecular nitrogen fixation to reach its tremendous high biomass in EASL despite the fact of nitrate limitation within these ecosystems. In this survey, we tried to proof this hypothesis through investigation (1) if *A. fusiformis* holds the *nifH* gene that is generally used as genetic marker to identify diazotrophs. (2) Further, we examined if this gene might be expressed under nitrate- rich and/or depleted conditions. (3) We compared growth of *A. fusiformis* at nitrate-limiting conditions with a control group supplied with nitrate. (4) We labelled culture medium with $^{15}N_2$ gas in order to test if it will be incorporated into biomass which would be a further indication for N_2-fixation.

2.3. Material and Methods

We studied nine strains of *A. fusiformis* originating from different lakes of the EASL. We labelled the strains according to their origins (**Tab. 1**). All strains were grown on shakers with 130 rpm at 12:12 light-dark -cycle (50 µmol photons m^{-2} s^{-1}, fluorescent tubes) at 28 ± 2 °C in both full Zarrouk medium (Zarrouk 1966) as control group and N-depleted medium without adding $NaNO_3$. Methods for testing N_2-fiaxtion were (1) genetic analysis, (2) growth experiments in different media, and (3) ^{15}N-incubation experiments.

(1) For DNA-based analyses, we used triplicates harvested during the exponential growth phase by vacuum filtration at MGC 47 mm Ø and then immediately frozen at – 80°C. DNA and RNA extraction were conducted according to the modified protocol of Feinstein et al. (2009) and Henckel et al. (1999). Ahead cDNA synthesis, purification of RNA and DNA digestion was performed by using GeneJET RNA Cleanup and Concentration Mircro Kit (Thermo Scientific™). cDNA strands were synthesised from pure RNA using Invitrogen's SuperScript™ III reagents. *nifH*-PCR was conducted with DNA, RNA, and cDNA of each strain using the primer pair IGK3 (5'-GCIWTHTAYGGIAARGGIGGIATHGGIAA-3') and DVV (5'-ATIGCRAAICCICCRCAIACIACRTC-3'). The PCR amplification was carried out after the following thermocycler program: initial 4 min at 94 °C, following by 35 cycles of 0.45 min at 52 °C, 0.30 min at 72°C, 0.30 min at 94°and a final step for 0.45 min at 52°C and a cooling down phase at 4°C. For amplification of the 16S rRNA the general bacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3') were used. Clone libraries were created by using Invitrogen's TOPO TA cloning kit and both fragments *nifH* and 16s rRNA were sequenced by Sanger sequencing.

Sequence editing was conducted in Geneious v.4.8.5 and aligned in MAFFT v.7. For analyses of *nifH* and 16S rRNA fragments, nucleotide substitution models were calculated by using MEGA v.6 (**Tab. 2**) and for the final editing of the trees, FigTree v.1.4.2 was used. For phylogenetic analysis, the 16S rRNA fragments were not partitioned. The 16S rRNA tree was a combination of sequences obtained in this study and those provided by the database SILVA (**Tab. 3**). For the *nifH*- tree additional sequences were provided by NCBI's Genbank (**Tab. 4**). Both are maximum-likelihood trees calculated with a bootstrap value of 1000.

(2) For the growth experiments, we used quadruples of 1 L SCHOTT-flasks filled with 700 ml media and inoculum. The growth experiments lasted for 15 days, and several parameters were evaluated in regular intervals. Every second day, optical density (OD) and in vivo chlorophyll *a* (chl *a*) were measured as proxy for biomass. Additional parameters were obtained three times (day 1, 9 and 15): DM, spectrophotometrical chl *a*, total carotenoids and phycobilins analyses, and C/N analyses of the biomass. Moreover, nitrate concentration was analysed from the filtrate.

OD was measured photometrically (Hitachi U-200) at 750 nm to minimize any interference with photosynthetic pigments. In-vivo chl *a* was measured spectrofluorometrically (Shimadzu RF-530-PC) with an excitation wavelength of 410 nm and emission of 670 nm (Gregor & Marsálek 2004). Based on these two parameters, growth rate μ [d^{-1}] = ($\ln N_t - \ln N_0$)/t was calculated. Furthermore the minimal doubling time was calculated by the following formula: T_d = (ln2-ln1)/μ (Graham et al. 2009) with μ=specific growth rate, t_d=doubling time, N=biomass (we used chl *a* and OD as a surrogate), t=time between harvests in days. Finally, we used chl *a* in vivo measurements for calculating doubling times

as this is a more accurate parameter compared to OD which is a general measure on turbidity also including other particles.

For pigment analysis, DM, and C/N-analysis, a defined volume of the culture was filtrated onto Munktell GF/C 47 mm Ø filters. Filters for pigment analysis were frozen at -20° for later extraction while the filters for DM and C/N-analysis were dried at 60°C and then stored in a desiccator until analysis.

For analysis of lipophilic pigments (chl *a* and total carotenoids), filters were homogenized with an ultrasonicator (Branson Sonifier 250) for 15-20 seconds, extracted in 5 ml 90% acetone in the dark for 12h at 4°, and then completed by centrifugation. The supernatant was measured spectrophotometrically at 663, 480, and 520 nm; chl *a* was calculated according to Lorenzen (1967) and the total carotenoids content according to Strickland & Parsons (1972).

For phycobilin analyses, filters were homogenised with an ultrasonicator (Branson Sonifier 250) for 15-20 seconds, extracted for 24 h in 5 ml phosphate buffer (16,73% 0,1 m NaH_2PO_4 and 83,27 % 0,1 m Na_2HPO_4) at 4°C, followed by centrifugation. Afterwards, the supernatant was measured spectrometrically at 564 nm, 618 nm, and 730 nm. Finally, concentrations of phycoerythrin and phycocyanin were calculated according to the formula described by Sampath-Wiley & Neefus (2007).

For cellular C/N-contents, filters were first treated with 10 % HCl for 10 s and washed with MiliQ to remove all inorganic carbon. After drying at 60°C, the filters were packed in Zn-foil and C/N detected with the elemental analyser Vario MICRO Cube. The nitrate content from the filtrate was analysed by means of ion chromatography (OENORM EN ISO 10304-

1:2012-06-01). Furthermore, all strains were observed microscopically during the growth experiment.

(3) For $^{15}N_2$ Incubation experiments, N-depleted Zarrouk medium was degassed at ≤ 200 mbar for 30 min with a magnetic stirrer and then filled in one vial until overflow. In a first step to prepare $^{15}N_2$-enriched media, 1 ml $^{15}N_2$ gas was added per 26 ml into the vial with the degassed medium followed by shaking to ensure that all $^{15}N_2$ was equally dissolved. Second, each 14 ml vial in which the incubation was conducted were filled with 12.6 ml of medium already pre-inoculated with *A. fusiformis;* the remaining 1.4 ml were filled with $^{15}N_2$ enriched medium (Mohr et al. 2010). In a preliminary test run, these vials were exposed to a light:dark circle of 12:12 h at 24°C. The first sample of each inoculated strain was harvested after 24 h, the second after 80 h together with the negative control that represents the natural abundance of the heavier $^{15}N_2$ isotope. This preliminary incubation experiment was run with the intention to investigate how long the strains should be incubated and to test the incubation conditions like the duration of the light-cycle.

In the main $^{15}N_2$-incubation experiment, we used triplicates of both $^{15}N_2$ labelled and unlabelled *A. fusiformis* which were exposed to a light: dark cycle of 8:16 h at 24°C for 5 d. The prolonged darkness was chosen to minimize interferences of the nitrogenase activity with oxygen (oxygen measurements proved that oxygen concentration already dropped after 5 h of darkness below 1 mg L^{-1}). We used *Nostoc* and *Anabaena* as positive controls, as they are known diazotrophs. Harvesting was done by vacuum filtration followed by freeze-drying of the material. Finally, 0.3- 0.8 mg of dried biomass were packed into stannic tons and analysed by Isotope-Ratio Mass Spectrometry (IRMS) to detect the ratio of ^{15}N to

^{14}N which further allows to conclude if the cells incorporated $^{15}N_2$ during the incubation-experiment.

All statistics were conducted with a significance level of 0.05 with the software Statistica version 7 (StatSoft). Anova with repeated measurement design (rm-ANOVA) was used to calculate significant differences for phycobilins, chl *a*, and total carotenoids content during the experiment. Variance homogeneity was tested with Cochran C, Hartley and Bartlett tests; Tukey's HSD for unequal n was used as post-hoc test.

2.4. Results

The genetic analysis revealed that all nine examined *A. fusiformis* strains carry the *nifH* gene. Five of them (Arenguade, Sonachi, Simbi, Big Momella, Abijata) express the *nifH* gene under both N supplying and limiting conditions. L.B. Momella, Oloidien, and Chitu generally do not express the *nifH* gene, independent of N/no N supply. Finally, Nakuru expresses the *nifH* gene only under N depleted conditions (**Tab. 5**)**.**

The 16S rRNA tree (**Fig. 1**), containing both strains of this study and those from the SILVA database, showed a rather homogeneous group of all *A. fusiformis* clones. The *nifH* tree (**Fig. 2**) did not reveal any differences*,* but a small variation between the *nifH* gene of *A. fusifomis* and that of other diazotrophs could be recognized.

All cultures developed from pale green to an intense blue-green colour during 15 days of growth (**Fig. 3**). Most strains showed comparable growth under nitrate- rich and limiting conditions. Exceptions were Sonachi, Simbi, and L.B. Momella, which did not perform well under nitrate- depletion (**Figs. 5 and 6**). Based on the minimum doubling times (**Tab. 6**), we did not find significant differences between the treatments (**Fig. 4**). Simbi showed the fastest mean doubling time with 1,6 d (days) followed by Arenguade with 1,7 d (both in nitrate- supplied medium).

Concerning the phycobilins analysis, both phycocyanin and phycoerythrin showed a very similar pattern (**Figs. 7, 8**); the same applies to chl *a* and total carotenoids (**Figs. 9, 10**). The Phycocyanin contents varied from around 30 mg g^{-1} DM for Arenguade at the beginning to 100 mg g^{-1} DM for L.B. Momella at the end of the growth experiment. For phycoerythrin, we found contents ranges between 1.5 mg g^{-1} DM for Big Momella at the start and 8.0 mg

g^{-1} DM for L.B. Momella at the end of the experiment. The conducted rm- Anova ($p < 0,05$, $n = 4$) which is summarized in **Tab. 7** revealed as main effect that Arenguade, Sonachi, and L.B. Momella showed significant differences between nitrate-supply and nitrate-limitation in both phycobilins and chl *a* per unit DM.

Under nitrate-limiting growth Sonachi and L.B. Momella showed a significant decrease of phycobilins, chl *a*, and total carotenoids per unit DM over time. Chitu showed a significant decrease of phycobilins, while Big Momella even showed significant increased phycocyanin contents per unit DM over time. Only the Nakuru strain had significantly increased values of all studied pigments under nitrate-depletion.

Under nitrate-rich conditions, Sonachi, Simbi, and Abijata had stable pigment contents over time per unit biomass (DM). Nakuru and Big Momella significantly increased photosynthetic pigment contents over time per unit DM; all other strains did not show a clear pattern under nitrate- supply.

When comparing total N between initial cultures and the end of the experiment, no notable changes for most of the cultures were detected (**Fig. 11**). Exceptions were Simbi and Big Momella: Simbi showed a significant decrease and Big Momella a significant increase. Particulate N of most cultures showed a significant increase over time (Mann-Whitney-U-Test). Exceptions were Simbi and L.B. Momella with a significant decrease between the start and end of growth. In accordance, dissolved N was significantly lowered at the end of the experiment for Arenguade, Sonachi, Nakuru, Chitu, Big Momella, Abijata, and Oloidien. Simibi showed increased NO_3-N in the medium, while L.B. Momella had no significant changes. The N per unit DM showed a significant increase during the growth

experiment for Arenguade from 29,6 to 44,3 mg g^{-1} DM, Nakuru from 31,1 to 45,2 mg g^{-1} DM, and Big Momella from 25,1 to 46,5 mg g^{-1} DM, while Sonachi, Chitu, Abijata, and Oloidien showed a significant decrease concerning their N content per unit DM. (**Tab. 8**).

Arenguade, Nakuru, and Abijata had slightly enriched $\delta^{15}N$-values compared to their natural abundance (**Fig. 12**). A conducted Kruskal Wallis H-Test showed significantly enriched $\delta^{15}N$-values for both positive control groups *Anabaena* and *Nostoc*, while L.B. Momella showed a significantly decreased $\delta^{15}N$-value. For the remaining strains, no significant changes of $\delta^{15}N$-values could be obtained.

2.5. Discussion

It is a challenge to obtain information exactly matching *A. fusiformis*, as the nomenclature of this species is extremely confusing. Taxonomy of *Arthrospira/Spirulina* often changed because of its complexity (Vonshak & Tomaselli 2000). Further, information on the origin of clones is often missing, which hampers comparisons and conclusions concerning distribution patterns of species. The genus *Arthrospira* comprises two planktonic species: *A. fusiformis*, which occurs in Africa and Asia, is often confusingly referred to as *A. platensis* (Nordstedt) Gomont or *Spirulina platensis* (Gomont) Geitler and *A. maxima* Setchell et. Gardner which occurs in America (Komárek & Lund 1990; Vonshak & Tomaselli 2000; Schagerl 2016). However, this distinct delineation is rarely found in literature. All the raised points impede clear information of *A. fusiformis*.

Various *Arthrospira* strains were sequenced in genomic projects accomplished by different workgroups (Fujisawa et al. 2010; Cheevadhanarak et al. 2012; Janssen et al. 2010; Lefort et al. 2014). Fujisawa et al. (2010) performed an almost complete genome sequence of *Arthrospira (Spirulina) platensis* (NIES-39) originating from Lake Chad. The authors assumed that this clone is not able of fixing molecular N, as they could not detect nitrogenase genes. Interestingly, they detected nif-related genes together with heterocyte coding genes (Fujisawa et al. 2010) but did not further explain this fact. The whole genome of *Arthrospira platensis* C1 (*Arthrospira* sp. PCC 9438) was sequenced by Cheevadhanarak et al. (2012). The authors compared its genome with other cyanobacteria and therefore concluded that this strain is not able to fix N_2 without going into any more detail (Cheevadhanarak et al. 2012). Clone PCC 9438 was isolated from a Lake near Mogadishu in Somalia and most probably belongs to *A. fusiformis* (Prof. Avigad Vonshak, pers. comm.).

Sequencing of *Arthrospira* sp. (PCC 8005) revealed that essential nif-genes are missing, but they also did not explain this fact any further or give any information about exactly which nif genes were missing or if they even found any (Janssen et al. 2010). The origin of this strain is not known, as the information was lost according to Pasteur Culture Collection. However, investigations of its ITS-region suggested that this strain may originate from India (Genoscope - Centre National de Séquençage). Contrarily to these studies, we found the *nifH* gene in all examined strains, in most cases it was also expressed. With only this region available, we however are not able to prove that the whole nitrogenase enzyme complex is synthetized/active. Also Lefort et al. (2014) mentioned that *Arthrospira platensis* originating from Peru is equipped with the typical nitrogenase complex and they also identified some nitrogenase genes, but they also did not give detailed information about which nif-gene they found exactly or how they were looking for nitrogenase. The *Arthrospira* clones PCC 9438 and NIES-39 were included in our 16S rRNA tree, both of them clustered closely together with our strains. All the examined *nifH* genes clustered together and are closely related to *nifH* genes of other diazotrophs (**Fig. 2, Tab. 4**). The 16S rRNA tree clearly revealed that all examined *A. fusiformis* are closely related (based only on their 16S rRNA) to other *Arthrospira* strains taken from SILVA database (**Fig. 1**). From these data, we assume that a frequent transposition of *A. fusiformis* takes place between the lakes, and that most probably Lesser Flamingos (*Phoenicopterus minor*) act as vectors (Childress et al. 2007; Vareschi 1978; Tuite 2000).

Arenguade, Chitu, Abijata, and Oloidien showed characteristic sigmoid growth curves under both nitrate-supply and nitrate-depletion (**Figs. 5,6**). Growth rates differed between the clones; especially Nakuru and Big Momella were still in the exponential phase at the

end of the experiment in both approaches. Other strains such as Sonachi, Simbi, and L.B. Momella showed almost no growth at nitrate- depletion.

The strains Arenguade, Nakuru, Chitu, Big Momella, Abijata, and Oloidien showed almost identical growth under both nitrate-depletion and nitrate-supply, although only Arenguade, Nakuru, Big Momella, and Abijata expressed the *nifH* gene under nitrate-limiting conditions. The correlation between chl *a* in vivo and OD seen in **Fig. 13** shows a package effect for these strains, as OD is still rising while the chl *a* in vivo kept stable. This can be explained by increased self-shading due to high algal biomass which might lead to a reduction of internal pigment content (Agustí & Phlips 1992). In general, we found that the actual strain condition is represented in more detail in phycobilins than it is in chl *a* or carotenoid content per unit biomass, which remain mostly stable.

A reason for this similar growing behaviour could be internal N storage that may allow maintaining high growth rates even under nitrate-limiting conditions. A substantial amount of N is found in phycobilisomes which are light harvesting complexes mainly connected to PS II. Phycobilisomes consist of phycobiliproteids which mainly absorb light energy between 550 to 650 nm; they contain up to 25 % of cellular N (Vonshak 1997). Furthermore, cyanobacteria have the possibility to store N during N-rich conditions as cyanophycin (Whitton 2000), which is a polymer consisting of arginine and aspartate (Kromkamp 1987). Also phycobilinsomes are known to act as N storage (Boussiba & Richmond 1980). Chitu, Big Momella, Abijata, and Oloidien showed a fast decrease of their phycobilin contents per unit DM between the ninth an fifteenth day of the experiment,

which suggests that these clones used their internal N-depot to maintain their growth. Consequently, the cells are still dividing, but every daughter cell receives a smaller amount of phycobilins. Arenguade and Nakuru also showed some tendency towards lower phycobilins contents per unit DM. It is quite striking that the studied strains showed such big variations in phycobilin contents per DM, although they belong to the same species with only small genetic differences of the considered sequences and identical growth conditions. The ranges we found are in accordance to other studies: Cheng et al. (1996) found phycocyanin contents of 130 ± 10 mg g^{-1} for *Spirulina platensis* UTEX 1926 isolated from Del Mar slough/California. Sotiroudis & Sotiroudis (2013) mentioned that *Arthrospira* (*Spirulina) platensis* contains around 15-25 % phycocyanin based on DM, while Oliveira et al. (2007) stated that 16 % of DM is ascribed to phycocyanin. Concerning phycoerythrin, Tarko et al. (2012) found 1.1 - 2.2 mg g-1 DM. Since it is in most cases not possible to reconstruct if this mentioned *A. platensis* matches *A. fusiformis*, we had to assume that this comparison of pigment content is not within the same species but at least within the same genera.

L.B. Momella, Sonachi, and Simbi generally only showed little growth under nitrate-depleted conditions, despite the expression of the *nifH* gene in the latter two strains. We assume that offered growth conditions were suboptimal to maintain N_2 fixation. Growth pattern of L.B. Momella corresponded with the non-expression of its *nifH* gene during N limitation (high growth under nitrate-supply and almost no growth under nitrate-limiting conditions). Based on the calculated doubling times we did not find any significant differences, although all strains tended to grow slightly faster under nitrate-supply and

showed smaller variations in the growth parameters compared to nitrate-depleted cultures.

When comparing dissolved N and particulate N, a clear transfer of N from the media into the cells is visible (**Fig. 11**). All examined strains except for Simbi and L.B. Momella (generally poor growth) showed a significant increase of particulate N with time. Simbi showed a significant increase of nitrate in the medium which can be explained by cell lysis which was also proved via microscopical observations.

Arenguade, Nakuru, and Big Momella which showed a significant increase of N per unit DM. Out of these Big Momella was the only clone showing a significant increase of total N which points toward N_2 fixation. However, no significant $^{15}N_2$ enrichment could be detected, this difference results might be explainable due to different experimental setups, as the growing experiment lasted for a longer time period compared to the incubation-experiment. Furthermore, the ^{15}N-experiment was conducted with only 14 ml of culture, maybe a higher volume or a longer incubation time would be necessary to see a distinct ^{15}N enrichment. For all other strains, comparable total N content from the start until the end of the growth experiments was observed. We therefore conclude that under the given growth conditions, N_2-fixation was only marginal. Concerning the growth experiment, it also has to be considered that too much nitrate was introduced into the nitrate-limiting treatment from the inoculum. The ^{15}N-incubation experiment showed slightly enriched $\delta^{15}N$-values for Arenguade, Nakuru, and Abijata indicating nitrogenase activity in these strains. In addition, they also expressed the *nifH* gene during the genetic experiment. For L.B. Momella we definitely excluded N_2-fixation under the conditions provided, as (1) it did not express the *nifH* gene, (2) it did not grow under nitrate-depletion,

and (3) the $\delta^{15}N$-value was significant depleted to its natural abundance which could be explained through cell lyses during incubation followed by filtration which distort the ratio of ^{15}N:^{14}N.

Based on our results, we assume that most *A. fusiformis* strains might be capable of fixing N_2, however, under the given conditions the fixation is only marginal. This is supported by the fact that most strains expressed the *nifH* gene and that some clones also showed ^{15}N-incorporation. Small fixation rates can partly be explained by the culture conditions which were not comparable to the natural environment of *A. fusiformis*. The soda lakes are extremely turbid systems (Oduor & Schagerl 2007b; Melack & Kilham 1974; Schagerl 2016) which is caused by high phytoplankton densities, high sediments loads through re-suspendation (shallow lakes) (Oduor & Schagerl 2007a) and very high amounts of humic substances (Jirsa et al. 2013). High light attenuation and high respiration go along with oxygen consumption deeper zones (Vareschi 1982; Melack & Kilham 1974; Talling & Talling 1965; Ogato et al. 2015). Lake Bogoria, for example, shows anoxic layers even during daytime (Oduor & Schagerl 2007b), as does Lake Arenguade below a depth of 4 m (Lanzén et al. 2013). *A. fusiformis* has gas vesicles and is therefore capable to move from surface layers through deeper, oxygen-depleted zones through its ability of buoyancy (Lee 2008; Vonshak & Tomaselli 2002) where it may fix molecular N_2. Another possible way of N_2 fixation may be during night time, fostered by low oxygen concentrations in the water column; Vareschi (1982) described that lake Nakuru is anoxic close to the sediment in the second half of the night, although it shows oxygen oversaturation of 300% during day time (Oduor & Schagerl 2007b). In addition, bacteria might be associated on the surface of *Arthrospira*-filaments thus accelerating microanaerobic zones around or inside the spirals.

For ^{15}N-incubation, we proved anoxic conditions at least for some hours, but for the long-lasting growth experiments, we needed to shake the flasks for homogenous nutrient supply. A next step to explain the enigma of extremely high biomass in the lakes under low N-supply needs to include culture conditions mimicking the natural environment with fast changing light supply and strong gradients of oxygen availability.

2.6. List of tables

2.7. Tables

Table 1:

Origin of all examined strains

Strains	**Lake**	**Country**
Arenguade	Arenguade	Ethiopia
Sonachi	Sonachi	Kenya
Simbi	Simbi	Kenya
L. B. Momella	Momella lakes	Tanzania
Nakuru	Nakuru	Kenya
Chitu	Chitu	Ethiopia
Big Momella	Big Momella	Tanzania
Abijata	Abijata	Ethiopia
Oloidien	Oloidien	Kenya

Table 2:

Substitution models used for calculating the *nifH*-tree and the 16S rRNA- tree, respectively. JC=Jukes Cantor, KHY+G+I=Hasegawa-Kishino-Yano

Fragment	Unpartitioned	Codon position 1	Codon position 2	Codon position 3
nifH	JC	JC	JC	JC
16S rRNA	HKY+G+I	-	-	-

Table 3:

List of accession numbers of all sequences used from SILVA database for phylogenetic analysis.

Accession number	ID
JN831261.1.1482	*Arthrospira erdosensis* 'Inner Mongolia'
AY575930.1.1385	*Arthrospira indica* PD1998/pus
AY575931.1.1405	*Arthrospira indica* PD1997/ram
AY575932.1.1405	*Arthrospira indica* PD2002/ana
AF260509.1.1293	*Arthrospira maxima*
ABYK01000016.29330.30811	*Arthrospira maxima* CS-328
FJ826622.1.1458	*Arthrospira maxima* FACHB-438
GQ206141.1.1335	*Arthrospira maxima* BJ 2000
KC217543.1.1300	*Arthrospira maxima* CY-023
AF260510.1.1294	*Arthrospira fusiformis*
AY575923.1.1405	*Arthrospira fusiformis* AB2002/01
AY575924.1.1375	*Arthrospira fusiformis* AB2002/02
AY575925.1.1385	*Arthrospira fusiformis* AB2002/03
AY575926.1.1405	*Arthrospira fusiformis* AB2002/04
AY575927.1.1405	*Arthrospira fusiformis* AB2002/05
AY575928.1.1405	*Arthrospira fusiformis* AB2002/10
AY575929.1.1384	*Arthrospira fusiformis* AB2002/11
AY672715.1.1355	*Arthrospira fusiformis*
AY672718.1.1355	*Arthrospira fusiformis*

AY672720.1.1355	*Arthrospira fusiformis*
AY672721.1.1355	*Arthrospira fusiformis*
AY672723.1.1355	*Arthrospira fusiformis*
AY672725.1.1355	*Arthrospira fusiformis*
AFXD01000008.93166.94642	*Arthrospira platensis* C1
AP011615.2583379.2584861	*Arthrospira platensis* NIES-39
AP011615.3508130.3509612	*Arthrospira platensis* NIES-39
AY672713.1.1355	*Arthrospira platensis*
DQ279767.1.1483	*Arthrospira platensis*
DQ279768.1.1482	*Arthrospira platensis*
DQ279769.1.1484	*Arthrospira platensis*
DQ279770.1.1482	*Arthrospira platensis*
JQ769115.1.1445	*Phormidium autumnale* 3A3
JQ769122.1.1443	*Phormidium autumnale* A28
JQ769129.1.1461	*Phormidium autumnale* A10
JQ769131.1.1461	*Phormidium autumnale* A25
AB251863.1.1444	*Nostoc commune*
AB721392.1.1444	*Nostoc commune*
AB933329.1.1444	*Nostoc commune*
FJ234887.1.1188	*Anabaena bergii* ANA360B
FJ234889.1.1203	*Anabaena bergii* ANA283B
FJ234892.1.1183	*Anabaena bergii* ANA283D
FJ234897.1.1204	*Anabaena bergii* ANA283A

Table 4:

List of accession numbers of all sequences used for the *nifH*- tree provided from NCBI's Genbank.

Accession number	**ID**
DQ439648.1	*Anabaena sphaerica*
AB557944.2	*Cyanothece sp.*
KT074973.1	*Nostoc commune*
DQ531670.1	*Nostoc commune* clone 1
DQ531672.1	*Nostoc commune* clone 2
AY221815.1	*Oscillatoria sancta*
AY768417.1	*Oscillatoria sp.*
AF227927.1	*Phormidium sp.* AD1
L00689.1	*Trichodesmium erythraeum*
L00688.1	*Trichodesmium sp.*

Table 5:

Summary of *nifH*- PCR results;

Legend:

"+"… the gene was found or in the case of cDNA expressed

"–" … the *nifH* gene was not expressed

		DNA (N-)	cDNA (N-)	DNA (N+)	cDNA (N+)
I	Arenguade	+	+	+	+
II	Sonachi	+	+	+	+
III	Simbi	+	+	+	+
IV	L.B. Momella	+	-	+	-
V	Nakuru	+	+	+	-
VI	Chitu	+	-	+	-
VIII	Big Momella	+	+	+	+
IX	Abijata	+	+	+	+
X	L. Oloidien	+	-	+	-

Table 6:

Overview of mean and standard deviation (SD) of all different strains of both treatments concerning the minimal doubling times in days based von chl *a* in vivo measurements.

	N-			N+		
	mean	SD	n	mean	SD	n
Arenguade	2,000	0,748	22	1,781	0,759	23
Sonachi	2,363	0,564	10	2,118	0,597	17
Simbi	2,601	1,429	9	1,684	0,723	15
L.B.Momella	2,884	0,886	8	2,117	0,914	18
Nakuru	2,827	1,043	16	2,347	0,892	16
Chitu	2,834	1,348	21	2,084	0,746	18
Big Momella	2,160	1,161	19	2,011	0,853	18
Abijata	2,044	0,616	19	2,034	0,793	23
Oloidien	2,251	0,961	21	2,081	0,734	22

Table 7:

Summary of all results from ANOVA with repeated measurement design which was conducted for all pigments per unit DM.

Legend:

"="… no significant change in pigment concentration over time,

"↑"… significant increase of this pigment content during the growth experiment,

"↓"… significant decrease of this pigment content during the experiment,

"*"… there is a general significant difference between N depleted and N rich grown cultures regarding this certain pigment per unit DM; $p < 0,05$ n=4

	phycocyanin	N	phycoerythrin	N	chl *a*	N	total carotenoids	N
Arenguade N-	=	*	=	*	↑	*	↑	
Arenguade N+	=		=		↑		↑	
Sonachi N-	↓	*	↓	*	↓	*	↓	*
Sonachi N+	=		=		=		=	
Simbi N-	=	*	=		=		=	
Simbi N+	=		=		=		=	
L.B. Momella N-	↓	*	↓	*	↓	*	↓	*
L.B. Momella N+	↑		=		=		=	
Nakuru N-	↑		=		↑		↑	
Nakuru N+	↑		↑		↑		↑	
Chitu N-	↓		↓		=		=	
Chitu N+	↑		=		=		=	
Big Momella N-	↑	*	=		=		=	
Big Momella N+	↑		↑		↑		↑	
Abijata N-	=		=		=		=	
Abijata N+	=		=		=		=	
Oloidien N-	=		=		=		=	
Oloidien N+	↑		↑		=		=	

Table 8:

Summary of all results from Mann-Whitney-U-Test between the start and the end of the growth experiment concerning particulate, dissolved, and total nitrogen as well as N per unit DM.

Legend:

"="… no significant difference between the start and end of the experiment.

"↑"… significant increase from the starting conditions to the end,

"↓"… significant decrease, $p < 0,05$ n=4

	particulate N	Dissolved N	Total N	N per unit DM
Arenguade N-	↑	↓	=	↑
Sonachi N-	↑	↓	=	↓
Simbi N-	↓	↑	↓	=
L.B. Momella N-	↓	=	=	=
Nakuru N-	↑	↓	=	↑
Chitu N-	↑	↓	=	↓
Big Momella N-	↑	↓	↑	↑
Abijata N-	↑	↓	=	↓
Oloidien N-	↑	↓	=	↓
Nakuru N+	↑	↓	=	↑

2.8. List of figures

2.9. Figures

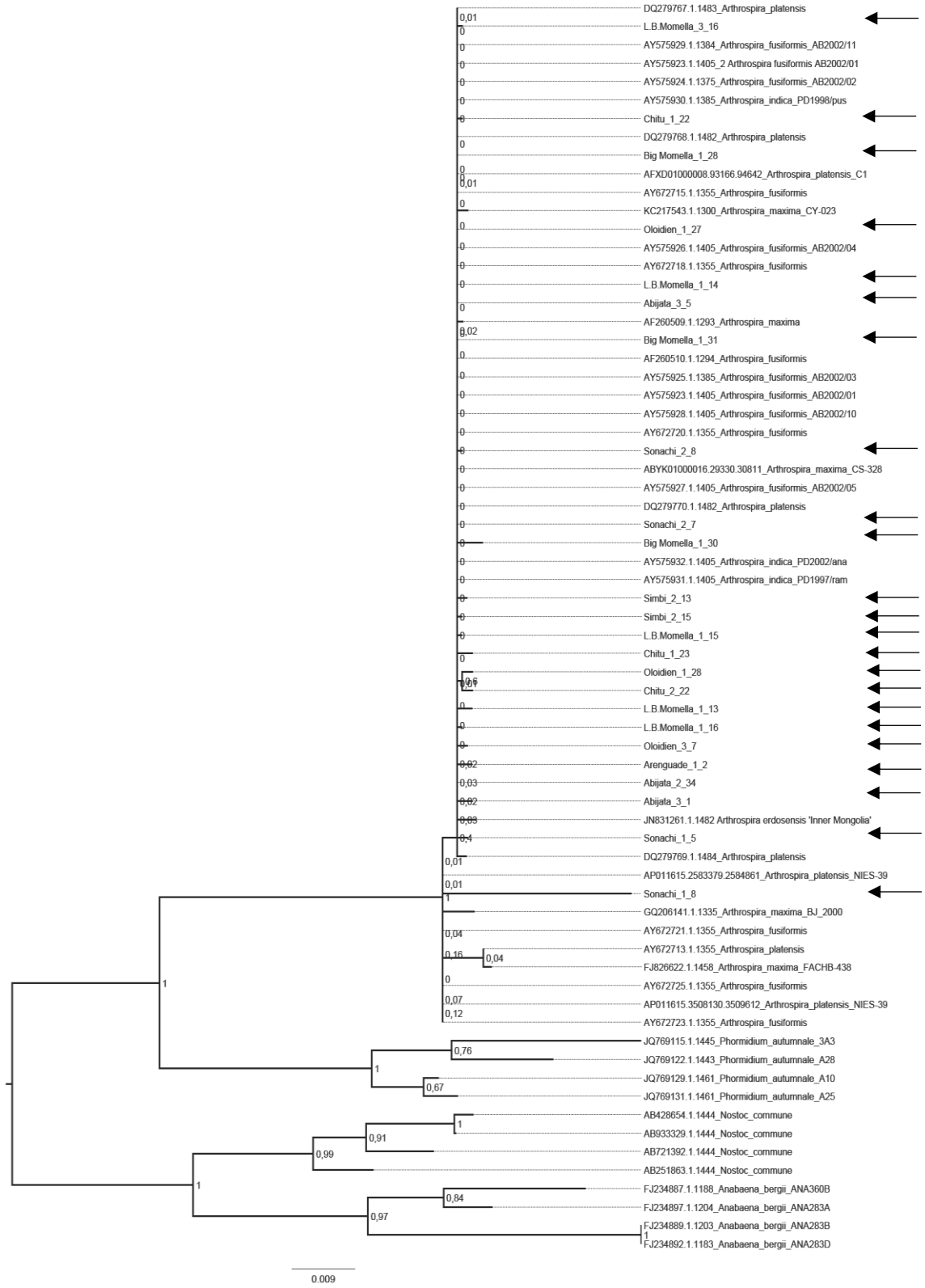

Figure 1: 16S rRNA- tree based on sequences from this study and sequences provided by SILVA; number at branches indicates the bootstrap support

Figure 2: Maximum-likelihood tree based on the *nifH* sequences conducted in this study and sequences provided by NCBI's Genbank

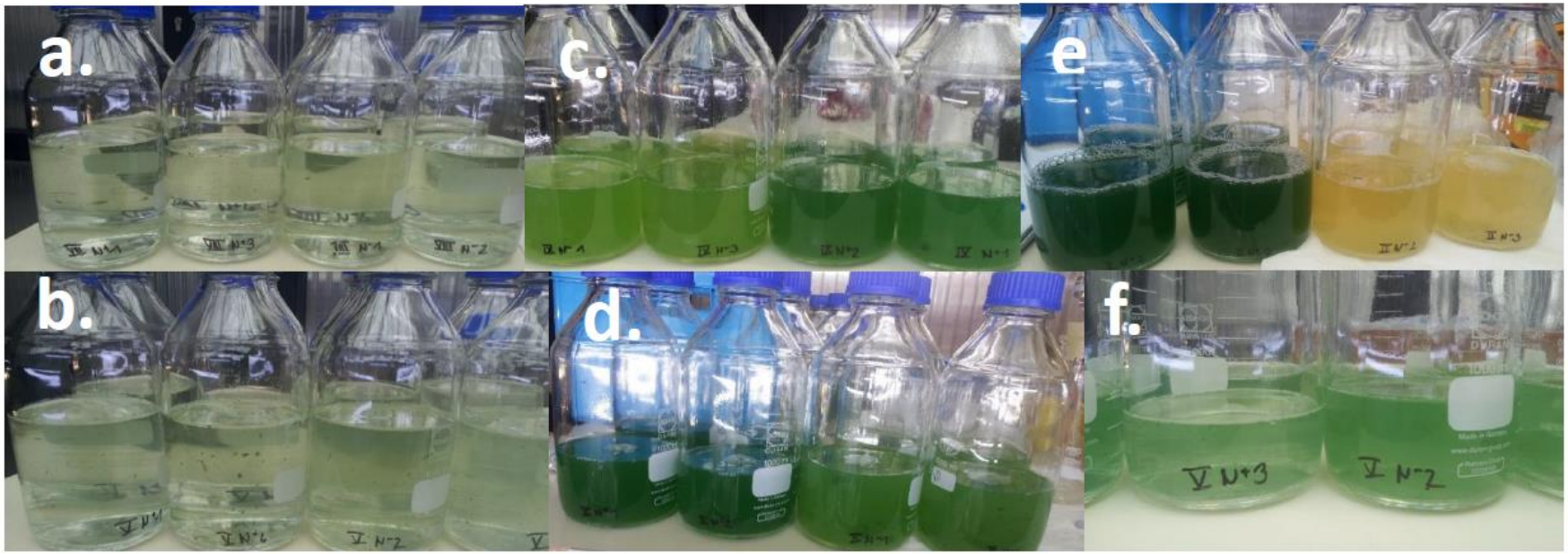

Figure 3: Strains at different times during the growth experiment. (a, b) An example for the coloration at the beginning of the growth experiment (c) Abijata: shows slightly different coloration between nitrate- rich (at the right site) and limited growth (left side) at the end of the experiment. (d) Oloidien: also shows only a slide difference between nitrate- rich (left side of the picture) and limited growing conditions. (e) Sonachi: a distinct different coloration between the different growth conditions is noticeable. (f) Nakuru: here it is the other way around, already at the ninth day of the experiment the nitrate- limited cultures showed a slightly greener coloration than the control group

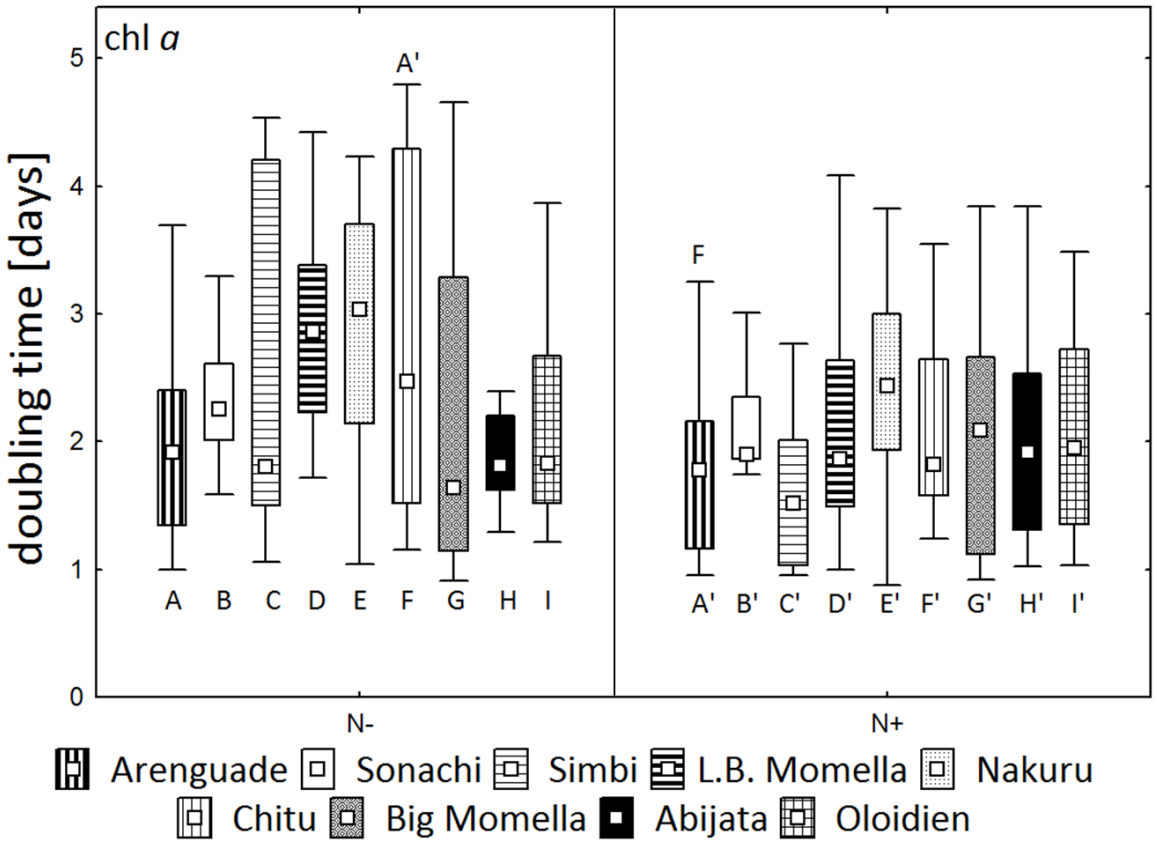

Figure 4: minimum doubling time based on the chl *a* in vivo measurements of all examined strains under nitrate- depleted conditions (left) and under nitrate- rich conditions (right)

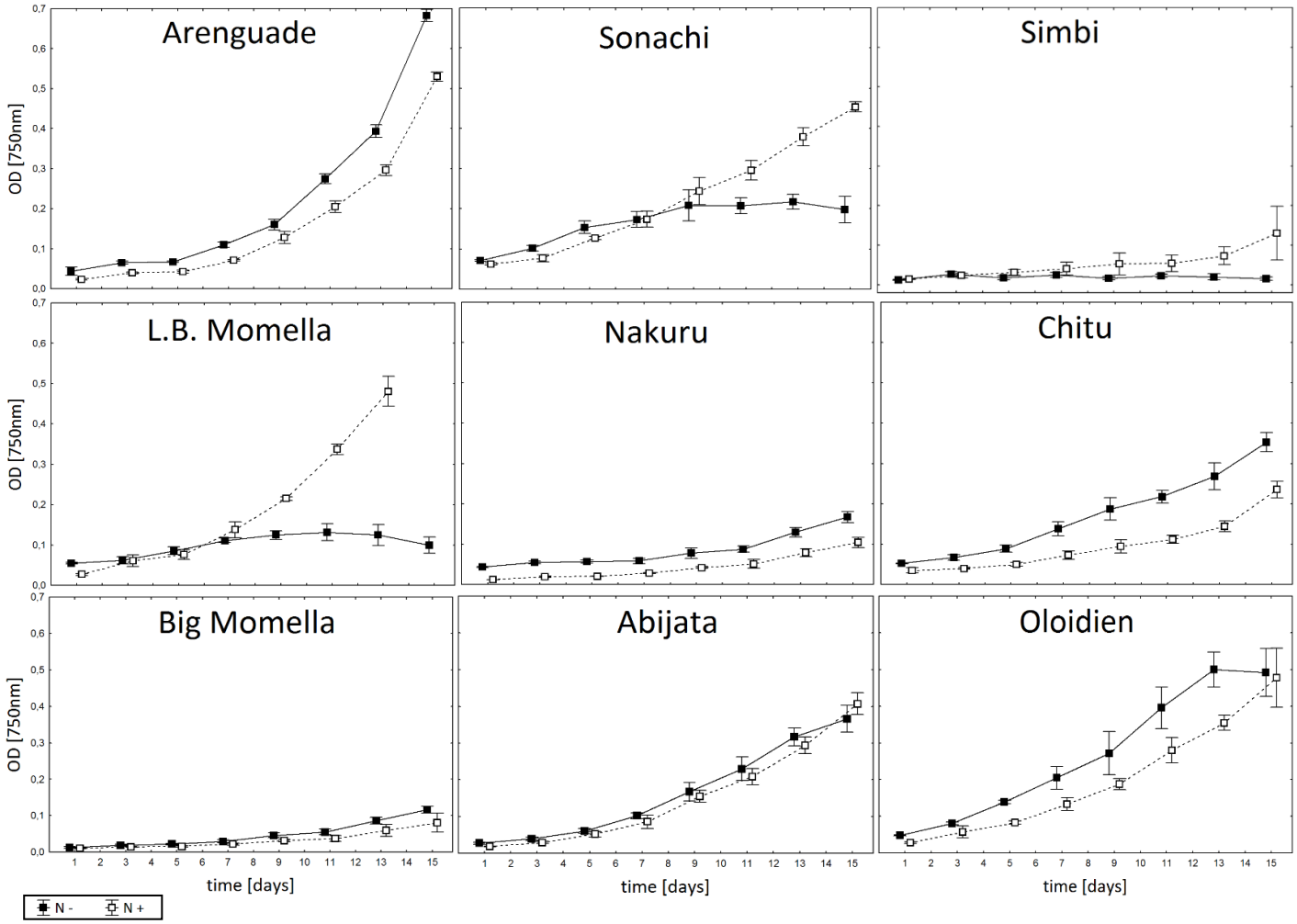

Figure 5: Growth curves of all examined strains based on OD measured every second day during the whole experiment; error bars = SD

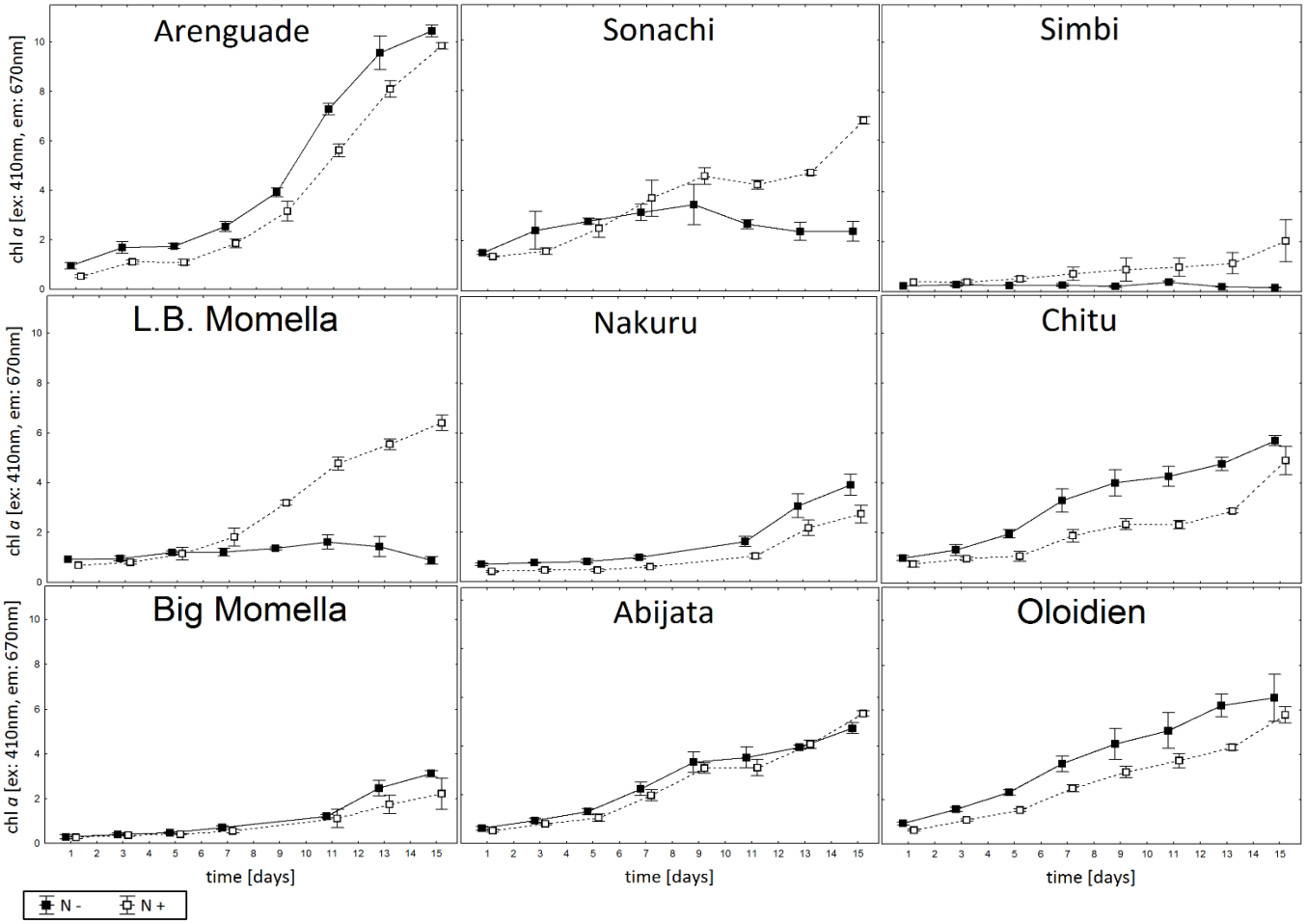

Figure 6*:* Growth curves of all examined strains based on the chl *a* in vivo measurements every second day during the whole growth experiment; error bars = SD

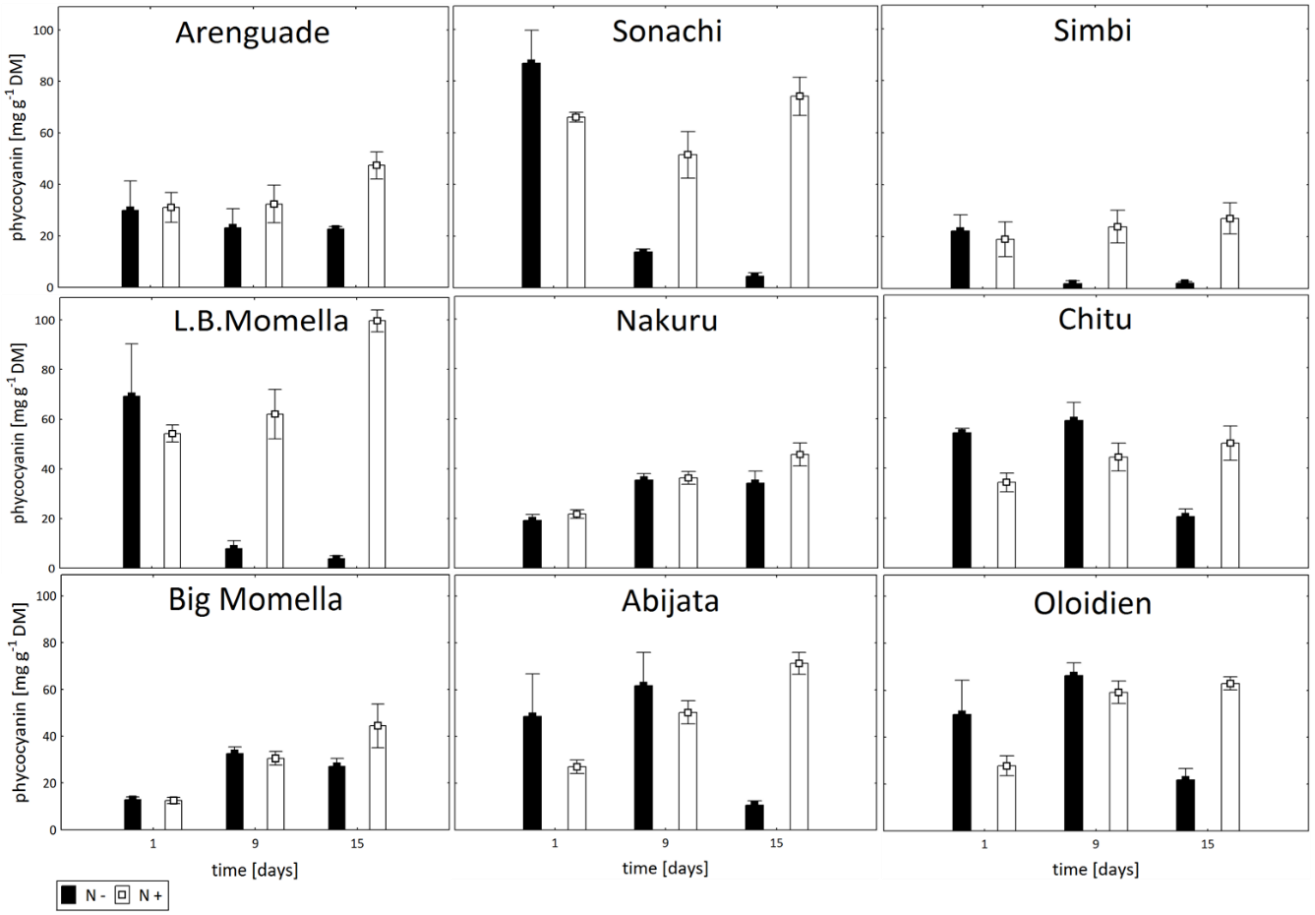

Figure 7: phycocyanin content of all examined strains at the first, the ninth, and the fifteenth day of the growth experiment, whereby the white bars represent the cultures under nitrate- rich growing conditions and the black ones under limiting conditions;

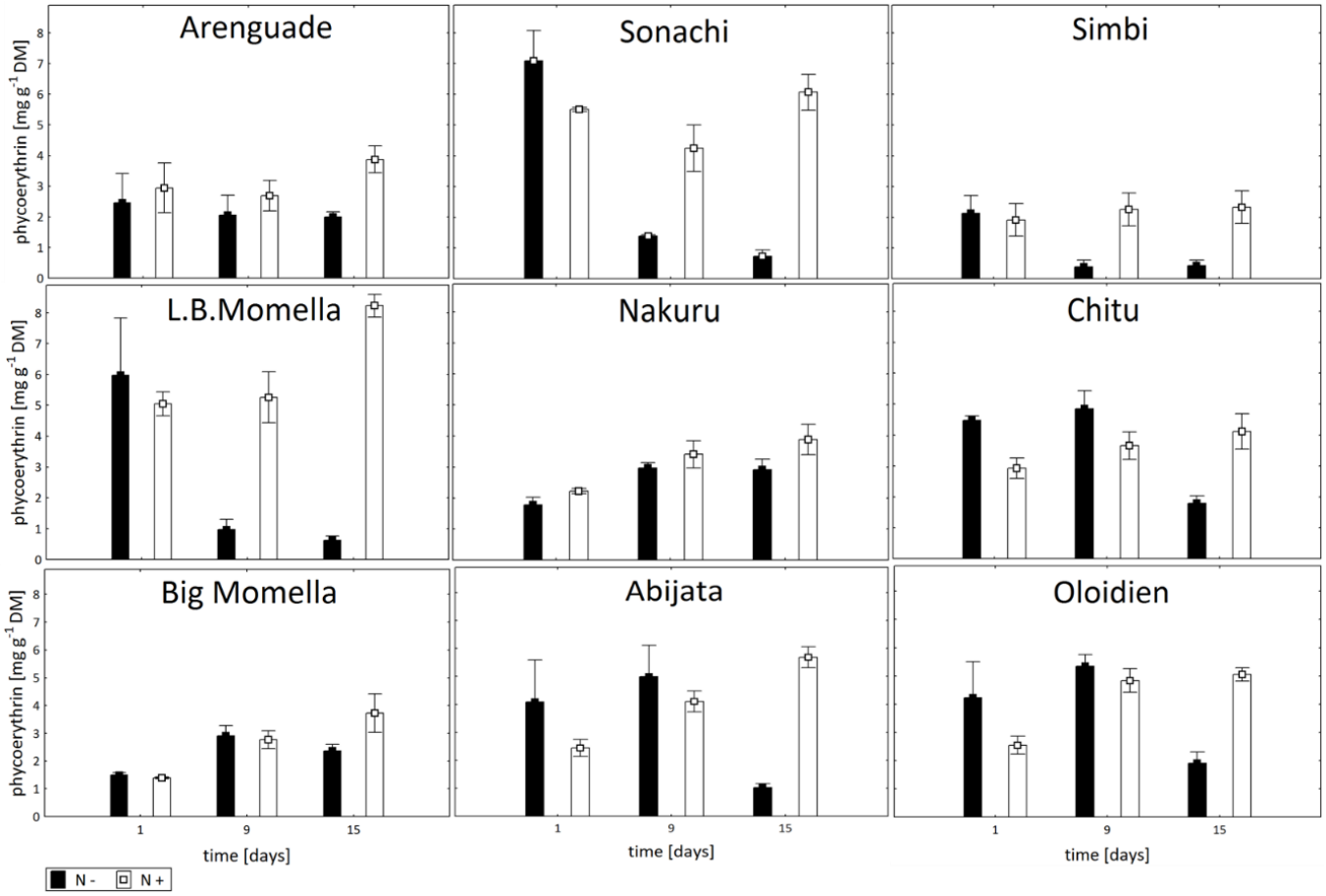

Figure 8: phycoerythrin content of all examined strains at the first, the ninth, and the fifteenth day of the growth experiment, whereby the white bars represent the cultures under nitrate- rich growing conditions and the black ones under limiting conditions; error bars= SD

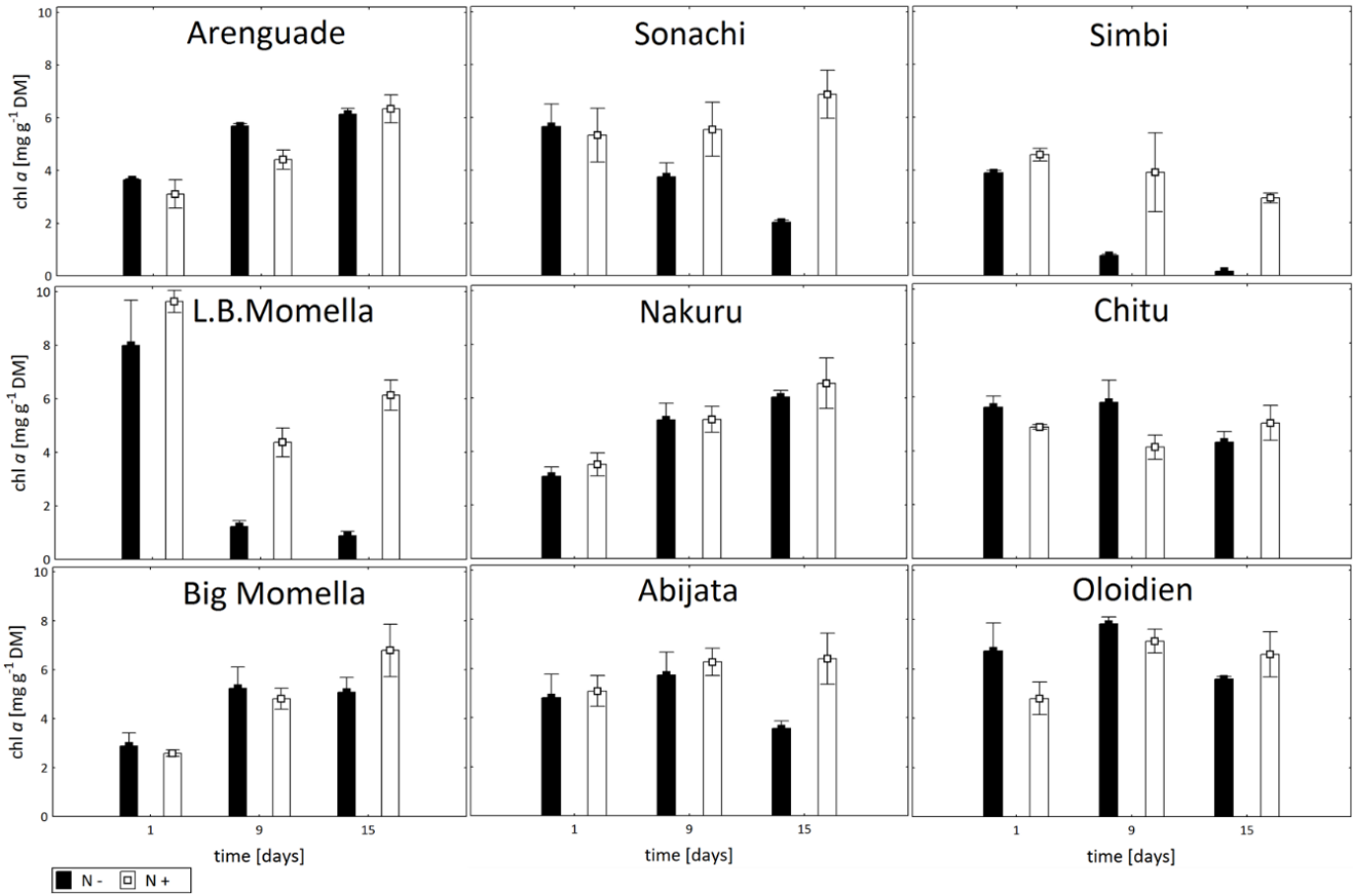

Figure 9: chl *a* content of all examined strains at the first, the ninth, and the fifteenth day of the growth experiment, whereby the white bars represent the cultures under nitrate- rich growing conditions and the black ones under limiting conditions; error bars= SD

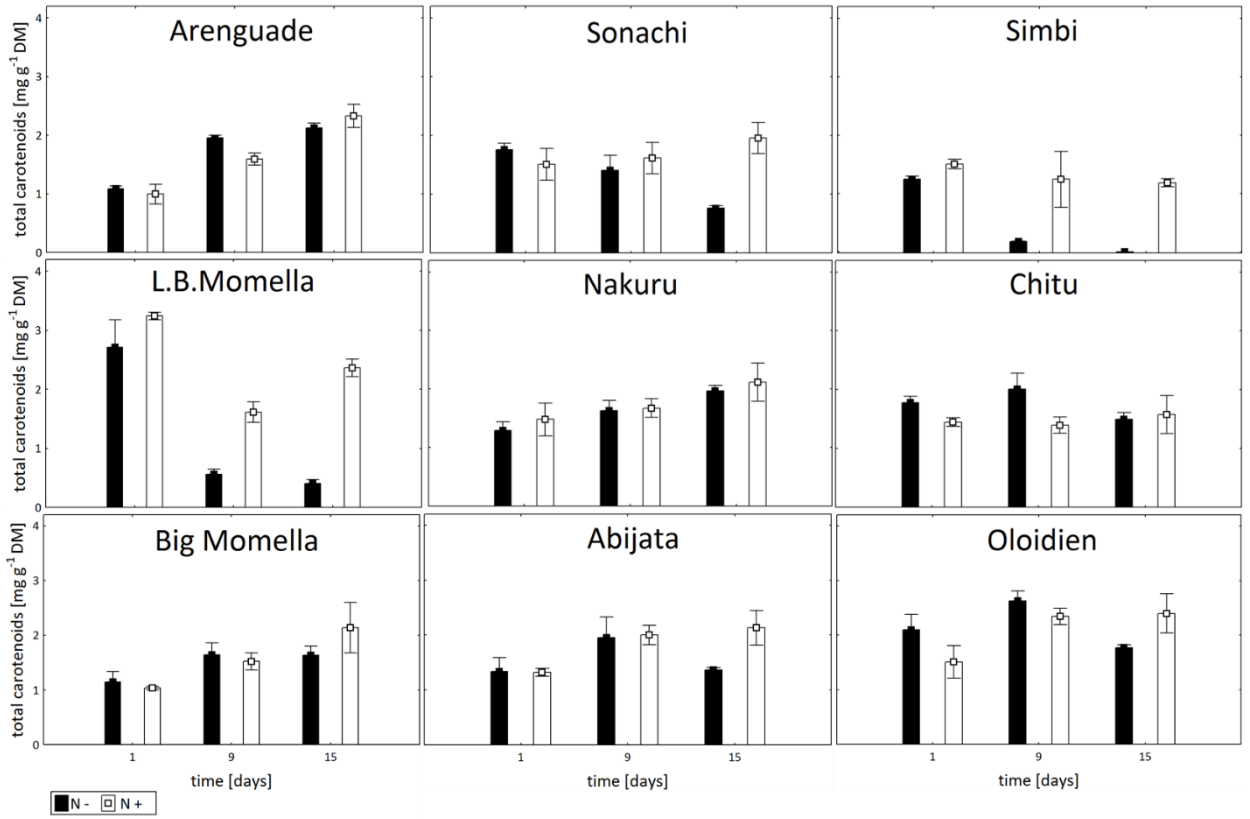

Figure 10: total carotenoids content of all examined strains at the first, the ninth, and the fifteenth day of the growth experiment, whereby the white bars are representing the cultures under nitrate- rich growth conditions and the black ones under limiting conditions; error bars= SD

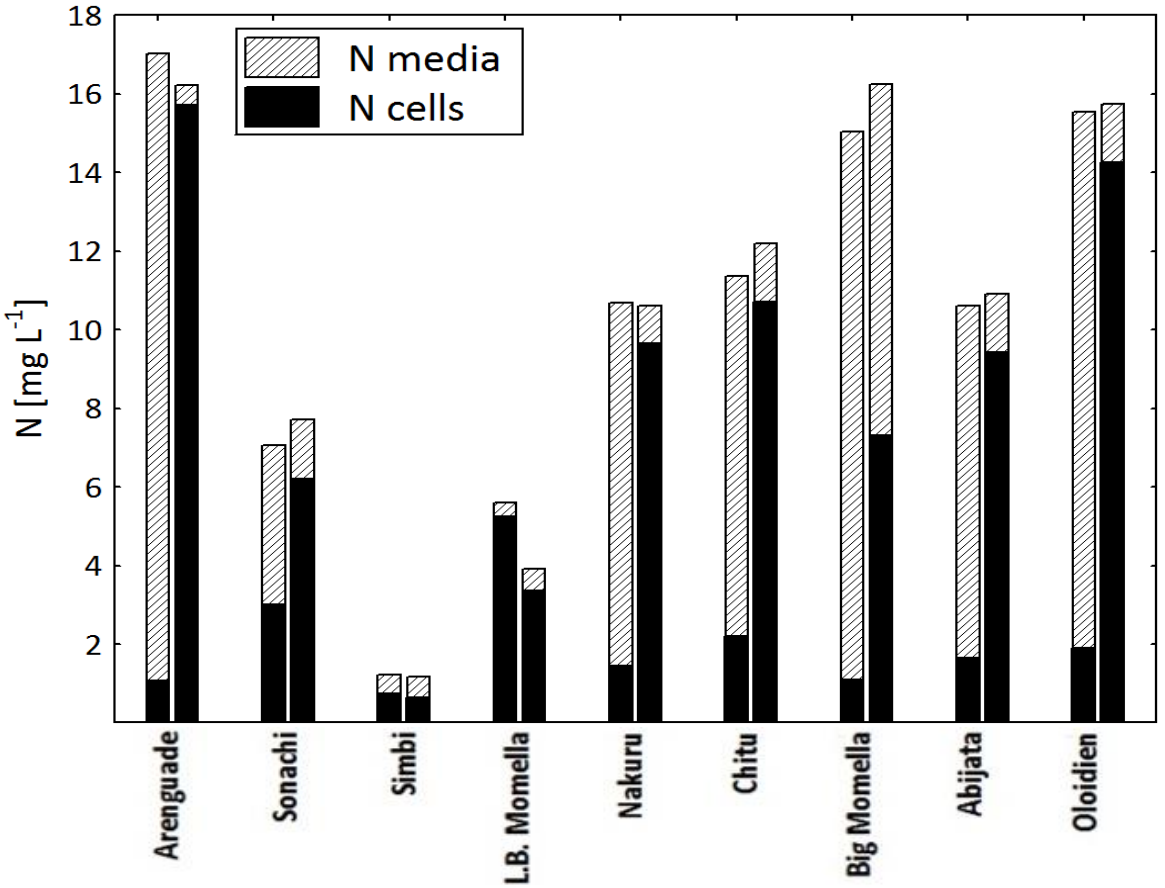

Figure 11: the total N content, composing of particulate N and dissolved N, at the beginning of the growth experiment (first bar) and at the end (second bar) for each examined strain under nitrate- limited growing conditions.

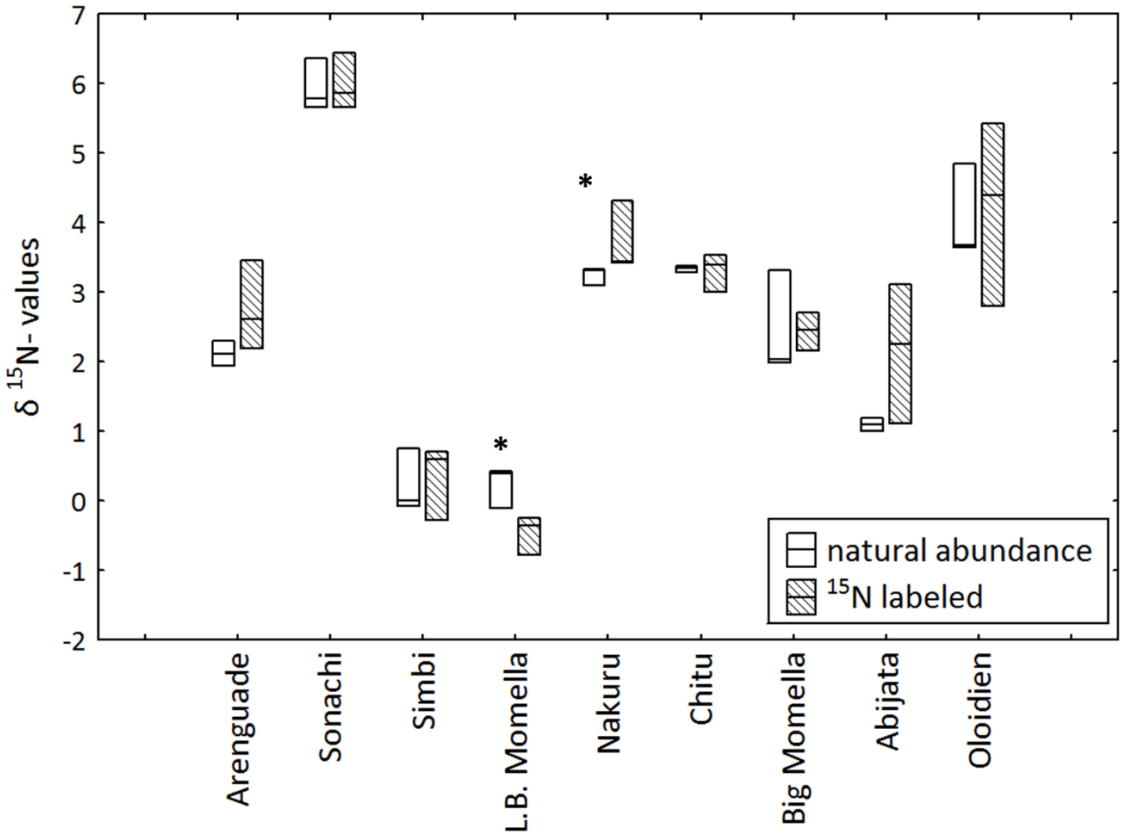

Figure 12: $\delta^{15}N$-values of all examined strains whereby the first (white) bar represents the natural abundance while the second (striped) one represents the ^{15}N labelled samples; * shows significant difference between natural abundance and ^{15}N labelled samples in accordance to a conducted Kruskal Wallis H-test; $p < 0,05$, n=3

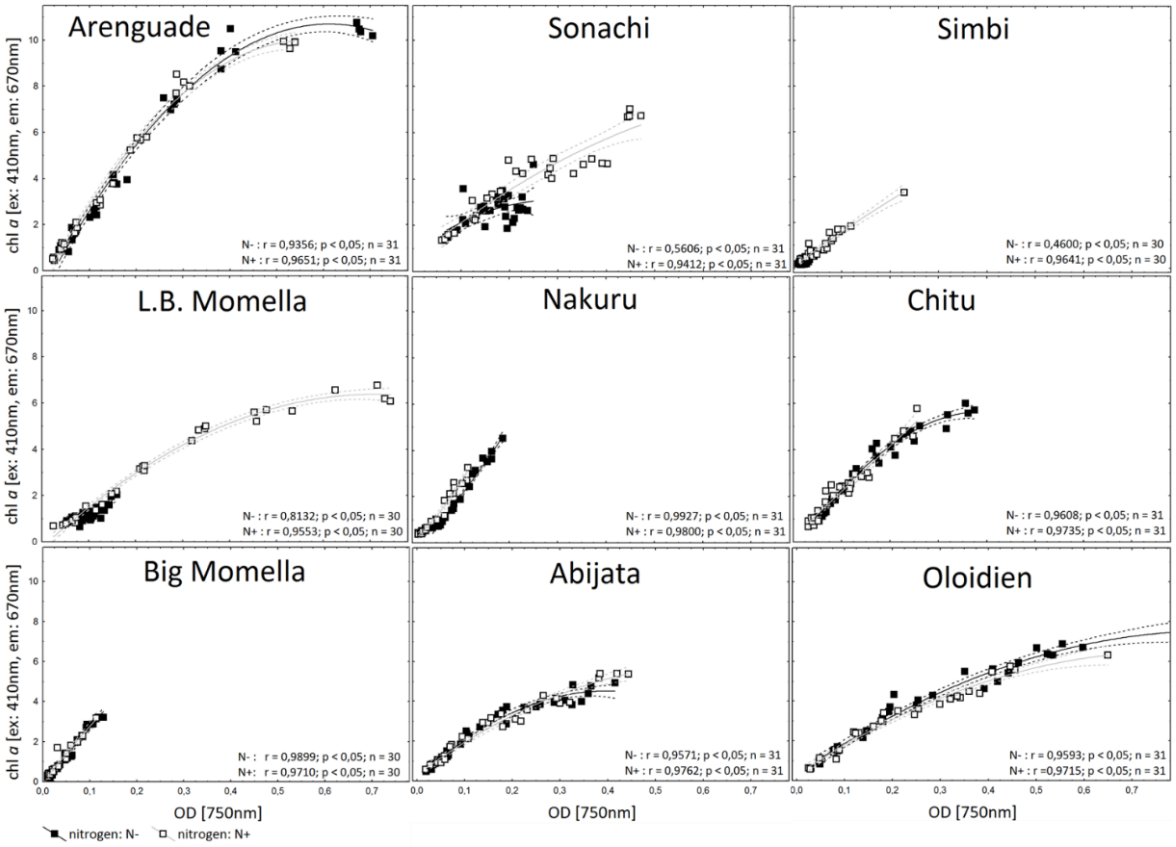

Figure 13: Correlation between OD and chl *a* in vivo, both were measured every second day during the growth experiment; the white squares are representing the nitrate- rich growing cultures while the black ones representing the nitrate- limited treatments, dashed lines= confidence interval

2.10. References

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3. Appendix

3.1. Additional information to genetic analysis

Detailed genetic protocols:

Total Nucleic Acids Extraction

The frozen filter together with 275µl PB, 125µl TNC, and 300µl phenol were processed in the bead beater for 30 seconds at 6,5 m s^{-1}. After chill on ice for 10 s they were centrifuged at 14 000 rpm for 3 minutes, followed by the transfer of the supernatant to a fresh tube. Adding 800µl phenol-chloroform-isoamylalcohol and centrifuging again for 3 minutes, this step we repeated twice to receive a better result (as Arthrospira has lot of polysaccharides).

In ne next step the supernatant was transferred again and 1 vol. of chloroform-isoamylalcohol was added bevor centrifuging for 3minutes.

Supernatant was transferred to non-sticky silicone tubes followed by adding 2 µl glycogen and 1ml PEG-precipitation solution and additional centrifuged for 60 minutes.

Next, as much as possible was removed from the supernatant and 75% EtOH was added and mixed until resuspending the pellet. Followed by centrifuging for 20 minutes and repeated removing of the supernatant. Tubes were left open at room temperature until remaining EtOH was evaporated.

All centrifugation steps were carried out at 4° with 14000 rpm.

Finally, this pellet was resuspended in 200µl low TE buffer, frozen at -80°, while a working solution was diluted 1:10 with lTe, and stored at -4°C.

RNA purification:

We used 20 µl of the TNA extract adding 5 µl 10x Buffer, 1 µl RNase inhibitor, 1 µl 0,1M DTT, 4 µl Turbo DNase and filled it up to 50 µl with RNase free water. Followed by an incubation step at 37° for 30 minutes.

Afterwards, 250 µl binding buffer and 300 µl 96-100 % ethanol were added to this mixture followed by transferring to a Gene JET RNA Purification Micro Column and additional centrifuging for 1 minutes at 14 000rpm. The flow-through was discarded; 700µl wash buffer 1 added to this microcolumn, centrifuged again for 1 minute and again discarded the flow-through. 700 µl wash buffer 2 was added, centrifuged for 1 minute, and discarded the flow through, this step was repeated twice. Finally, the spin column was placed in a new collection tube and 20 µl RSS was added to the top of the filter and centrifuged for 1 minute.

RNA stock solution was stored at -80°C while the working solution was diluted with RSS 2:10 and stored at -4°C.

cDNA synthesis:

We used 1 µl RNA template and added 1 µl random hexamer primers (0,5 µg μl^{-1}) and filled it up to 10,5 µl with RNase free water. This mixture was incubated at 65°C for 5 min in a thermocycler and then chilled on ice for 1 minute.

Following mixture was added to each tube: 4 µl 5x Reaction buffer, 2 µl 10mM dNTP mix, 1 µl 0,1 M DTT, 1 µl RNaseOUT, 0,5 µl BSA, and 1 µl SuprScript III RT (reverse Transcriptase).

The thermocycler programm for cDNA synthesis: for 5 minutes at 25°C, for 2 h at 50°C, and for 15 minutes at 70°C for 15 minutes. cDNA was stored at -20°C.

nifH-PCR:

In a preliminary *nifH*-PCR run the primer pair 19F and R6 leads to an unspecific result in contrast to the primers IGK3 and DVV. Therefore, we decided to use IGK3 and DVV for all PCRs.

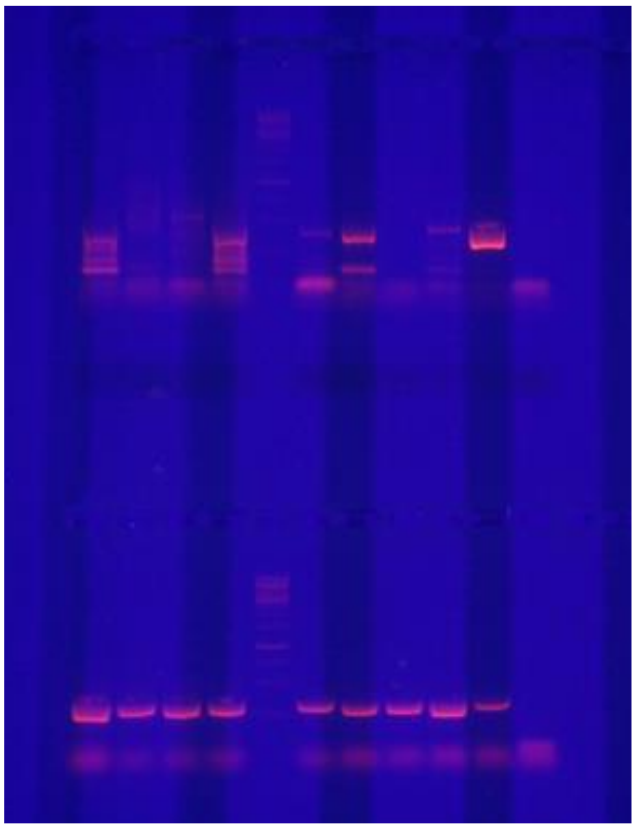

Figure: in the first row the primers 19F and 6R were used, while in the second row DVV and IGK3

For the amplification of *nifH* we used 1 µl template and added 2,5 µl DreamTaq Green Buffer, 2,5 µl dNTP, 0,2 µl BSA, 2 µl F-primer, 2 µl R-primer, 0,123 taq polymerase, and 14, 675 H_2O.

Blast summary results: the marked clones were used for further analyses like calculating the *nifH* tree

strain	clone	*nifH* nucbest hit	sequenzID	query cover	%ident
Arenguade	S-1 nifH 1 1	Roseibacterium elongatum DSM 19469, complete genome	gb|CP004372.1|	98	87
Arenguade	S-1 nifH 1 2	Roseibacterium elongatum DSM 19469, complete genome	gb|CP004372.1|	100	84
Arenguade	S-1 nifH 1 3	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Arenguade	S-1 nifH 1 4	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Sonachi	S-2 nifH 1 5	Uncultured bacterium clone F02 protochlorophyllide reductase-like gene, partial sequence	gb|EF599074.1|	87	82
Sonachi	S-2 nifH 1 6	Uncultured bacterium clone F02 protochlorophyllide reductase-like gene, partial sequence	gb|EF599074.1|	87	83
Sonachi	S-2 nifH 1 7	Uncultured bacterium clone F02 protochlorophyllide reductase-like gene, partial sequence	gb|EF599074.1|	87	83
Sonachi	S-2 nifH 1 8	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Simbi	S-3 nifH 1 9	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Simbi	S-3 nifH 1 10	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Simbi	S-3 nifH 1 11	Uncultured bacterium clone F02 protochlorophyllide reductase-like gene, partial sequence	gb|EF599074.1|	87	83
Simbi	S-3 nifH 1 12	no sig similarities found			
L.B.Momella	S-4 nifH 1 13	no sig similarities found			
L.B.Momella	S-4 nifH 1 14	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
L.B.Momella	S-4 nifH 1 15	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
L.B.Momella	S-4 nifH 1 16	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Nakuru	S-5 nifH 1 18	Uncultured bacterium clone F02 protochlorophyllide reductase-like gene, partial sequence	gb|EF599074.1|	87	83
Nakuru	S-5 nifH 1 19	Uncultured microorganism clone LostN-nifH-447 nifH-like protein (nifH) gene, partial cds	gb|KJ647137.1|	82	79
Nakuru	S-5 nifH 1 20	Uncultured microorganism clone LostN-nifH-447 nifH-like protein (nifH) gene, partial cds	gb|KJ647137.1|	82	79
Nakuru	S-5 nifH 1 21	Uncultured microorganism clone LostK-nifH-331 nifH-like protein (nifH) gene, partial cds	gb|KJ647067.1|	99	90

Chitu	S-6 nifH 1 23	Uncultured microorganism clone LostN-nifH-447 nifH-like protein (nifH) gene, partial cds	gb|KJ647137.1|	82	78
Chitu	S-6 nifH 1 24	Uncultured microorganism clone LostN-nifH-447 nifH-like protein (nifH) gene, partial cds	gb|KJ647137.1|	82	79
Chitu	S-6 nifH 1 25	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Chitu	S-6 nifH 1 26	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Big Momella	S-8 nifH 2 26	Uncultured bacterium clone F02 protochlorophyllide reductase-like gene, partial sequence	gb|EF599074.1|	87	83
Big Momella	S-8 nifH 2 27	Dinoroseobacter shibae DFL 12, complete genome	gb|CP000830.1|	98	87
Big Momella	S-8 nifH 2 28	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Big Momella	S-8 nifH 2 29	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Big Momella	S-8 nifH 2 30	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Abijata	S-9 nifH 1 33	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Abijata	S-9 nifH 1 34	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Abijata	S-9 nifH 2 33	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Abijata	S-9 nifH 2 34	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Oloidien	S-10 nifH 2 27	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Oloidien	S-10 nifH 2 28	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99
Oloidien	S-10 nifH 2 29	Sphingomonas sp. WHSC-8, complete genome	gb|CP010836.1|	98	80
Oloidien	S-10 nifH 2 30	Arthrospira sp. str. PCC 8005 chromosome, complete genome	emb|FO818640.1|	100	99

Blast summary results: the sequences of the blue marked clones were used for further analyses like calculating the 16S rRNA tree.

Strain	clone	16S besthits	sequenzID	query cover	%ident
Arenguade	S-1 16S 1 2	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99
Arenguade	S-1 16S 1 3	Uncultured CFB group bacterium clone ML310M-34 16S ribosomal RNA gene, partial sequence	gb|AF449773.1|	100	94
Arenguade	S-1 16S 2 1	Uncultured Beggiatoa sp. partial 16S rRNA gene, clone M8C_clone196	emb|FR687033.1|	75	97

Arenguade	S-1 16S 2 4	Uncultured CFB group bacterium clone ML310M-34 16S ribosomal RNA gene, partial sequence	gb|AF449773.1|	100	95
Arenguade	S-1 16S 2 6	Uncultured bacterium clone Y1-5 16S ribosomal RNA gene, partial sequence	gb|KF912963.1|	100	99
Sonachi	S-2 16S 1 5	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99
Sonachi	S-2 16S 1 7	Uncultured Flavobacteriales bacterium clone Clip 94 16S ribosomal RNA gene, partial sequence	gb|HQ692013.1|	95	96
Sonachi	S-2 16S 1 8	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	94	99
Sonachi	S-2 16S 2 7	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	100
Sonachi	S-2 16S 2 8	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99
Sonachi	S-2 16S 2 9	Uncultured Flavobacteriales bacterium clone Clip 94 16S ribosomal RNA gene, partial sequence	gb|HQ692013.1|	95	96
Sonachi	S-2 16S 2 10	Uncultured Flavobacteriales bacterium clone Clip 94 16S ribosomal RNA gene, partial sequence	gb|HQ692013.1|	95	96
Simbi	S-3 16S 1 9	Roseinatronobacter sp. LC05P24 16S ribosomal RNA gene, partial sequence	gb|JX945790.1|	97	99
Simbi	S-3 16S 1 10	Bacteroidetes bacterium enrichment culture clone OCL_P2D11 16S ribosomal RNA gene, partial sequence	gb|KJ004404.1|	100	97
Simbi	S-3 16S 1 11	Uncultured organism clone SBYS_5156 16S ribosomal RNA gene, partial sequence	gb|JN475865.1|	86	98
Simbi	S-3 16S 1 12	Roseinatronobacter sp. LC05P24 16S ribosomal RNA gene, partial sequence	gb|JX945790.1|	97	99
Simbi	S-3 16S 2 13	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99
Simbi	S-3 16S 2 14	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	99	96
Simbi	S-3 16S 2 15	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99
L.B. Momella	S-4 16S 1 13	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99

L.B. Momella	S-4 16S 1 14	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	100
L.B. Momella	S-4 16S 1 15	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99
L.B. Momella	S-4 16S 1 16	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99
L.B. Momella	S-4 16S 2 17	Uncultured bacterium clone SINP414 16S ribosomal RNA gene, partial sequence	gb|HM127437.1|	99	99
L.B. Momella	S-4 16S 2 18	Uncultured bacterium clone Y1-5 16S ribosomal RNA gene, partial sequence	gb|KF912963.1|	100	99
L.B. Momella	S-4 16S 3 16	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99
Nakuru	S-5 16S 1 17	Uncultured bacterium clone SINP414 16S ribosomal RNA gene, partial sequence	gb|HM127437.1|	99	96
Nakuru	S-5 16S 1 18	Uncultured bacterium clone SINP414 16S ribosomal RNA gene, partial sequence	gb|HM127437.1|	99	99
Nakuru	S-5 16S 1 19	Uncultured bacterium clone SINP414 16S ribosomal RNA gene, partial sequence	gb|HM127437.1|	99	99
Nakuru	S-5 16S 1 20	Uncultured bacterium clone Y1-5 16S ribosomal RNA gene, partial sequence	gb|KF912963.1|	100	98
Nakuru	S-5 16S 2 19	Uncultured bacterium clone SINP414 16S ribosomal RNA gene, partial sequence	gb|HM127437.1|	99	99
Nakuru	S-5 16S 2 20	Uncultured bacterium clone SINP414 16S ribosomal RNA gene, partial sequence	gb|HM127437.1|	99	99
Nakuru	S-5 16S 3 14	Glycocaulis abyssi strain MCS 33 16S ribosomal RNA gene, partial sequence	ref|NR_108136.1|	100	99
Chitu	S-6 16S 1 21	Uncultured bacterium clone Y1-36 16S ribosomal RNA gene, partial sequence	gb|KF912987.1|	100	97
Chitu	S-6 16S 1 22	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99
Chitu	S-6 16S 1 23	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	99	99
Chitu	S-6 16S 1 24	Uncultured bacterium clone Y1-36 16S ribosomal RNA gene, partial sequence	gb|KF912987.1|	100	97
Chitu	S-6 16S 2 22	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99

Chitu	S-6 16S 2 23	Uncultured CFB group bacterium clone ML310M-34 16S ribosomal RNA gene, partial sequence	gb|AF449773.1|	100	94
Chitu	S-6 16S 2 24	Uncultured bacterium clone Y1-36 16S ribosomal RNA gene, partial sequence	gb|KF912987.1|	88	93
Big Momella	S-8 16S 1 28	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	100
Big Momella	S-8 16S 1 29	Uncultured organism clone SBXY_1705 16S ribosomal RNA gene, partial sequence	gb|JN427607.1|	97	94
Big Momella	S-8 16S 1 30	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99
Big Momella	S-8 16S 1 31	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99
Big Momella	S-8 16S 1 32	Roseinatronobacter sp. LC05P24 16S ribosomal RNA gene, partial sequence	gb|JX945790.1|	96	96
Abijata	S-9 16S 2 33	Uncultured bacterium gene for 16S ribosomal RNA, partial sequence, clone: CG23	dbj|AB491721.1|	94	93
Abijata	S-9 16S 2 34	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	94	93
Abijata	S-9 16S 3 1	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99
Abijata	S-9 16S 3 2	Uncultured CFB group bacterium clone ML310M-34 16S ribosomal RNA gene, partial sequence	gb|AF449773.1|	100	94
Abijata	S-9 16S 3 3	Uncultured CFB group bacterium clone ML310M-34 16S ribosomal RNA gene, partial sequence	gb|AF449773.1|	100	94
Abijata	S-9 16S 3 4	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	94	96
Abijata	S-9 16S 3 5	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	100
Oloidien	S-10 16S 1 27	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	100
Oloidien	S-10 16S 1 28	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99
Oloidien	S-10 16S 1 29	Uncultured bacterium gene for 16S ribosomal RNA, partial sequence, clone: CG23	dbj|AB491721.1|	98	98
Oloidien	S-10 16S 3 6	Uncultured CFB group bacterium clone ML310M-34 16S ribosomal RNA gene, partial sequence	gb|AF449773.1|	99	94

Oloidien	S-10 16S 3 7	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	100	99
Oloidien	S-10 16S 3 8	Arthrospira platensis CHM 16S ribosomal RNA gene, partial sequence	gb|KJ463625.1|	86	99
Oloidien	S-10 16S 3 9	Uncultured bacterium clone EV818CFSSAHH36 16S ribosomal RNA gene, partial sequence	gb|DQ336987.1|	100	91

3.2. Additional information to the growth experiment

Zarrouk Medium (Zarrouk 1966)**:**

	Composition [g L^{-1}]
K_2HPO_4	0,5
K_2SO_4	1
NaCl	1
$NaNO_3$	2,5
$MgSO_4 \cdot 7\ H_2O$	0,2
$CaCl_2 \cdot 2\ H_2O$	0,04
$FeSO_4 \cdot 7\ H_2O$	0,01
EDTA	0,08
Micronutrient solution	1 ml
$NaHCO_3$ (after autoclaving)	16,8

Micronutrient solution:	Composition [g L^{-1}]
H_3BO_3	2,86
$MnCl_2 \cdot 4\ H_2O$	1,81
$ZnSO_4 \cdot 4\ H_2O$	0,2222
Na_2MoO_4	0,0177
$CuSO_4 \cdot 5\ H_2O$	0,079

For N-depleted media no $NaNO_3$ was added.

BG 11 medium for cyanobacteria (Rippka & Herdman 1992)**:**

	Composition [g L^{-1}]
$NaNO_3$	1,5
$K_2HPO_4 \cdot 3\ H_2O$	0,04
$MgSO_4 \cdot 7\ H_2O$	0,075
$CaCl_2 \cdot 2\ H_2O$	0,036
Citric acid	0,006
Ferric ammonium citrate	0,006
EDTA	0,001
Na_2CO_3	0,02
Micronutrient solution	1 ml

Micronutrient solution (Kuhl & Lorenzen 1964):	Composition [mg L^{-1}]
H_3BO_3	61
$MnSO_4 \cdot H_20$	169
$ZnSO_4 \cdot 7\ H_20$	287
$CuSO_4 \cdot 5\ H_2O$	2,5
$(NH_4)_6Mo_7O_{24}$	12,5

For N-depleted media, the BG-11 Medium was prepared without adding $NaNO_3$

3.3. P-values

Exact p-values of rm- ANOVA's main effects between nitrate- rich and limited treatment:

N^-*N^+ p- values	phycocyanin	phycoerythrin	chl *a*	total carotenoids
Arenguade	**0,001861**	**0,038718**	**0,006852**	0,071114
Sonachi	**0,014605**	**0,017761**	**0,015412**	**0,00883**
Simbi	**0,042795**	0,062197	0,296602	0,254645
L.B. Momella	**0,012723**	**0,015413**	**0,030832**	**0,020063**
Nakuru	0,130028	0,064914	0,318757	0,41987
Chitu	0,339123	0,330501	0,612407	0,101993
Big Momella	**0,034924**	0,093944	0,237554	0,15342
Abijata	0,121565	0,146618	0,216001	0,380311
Oloidien	0,675007	0,686854	0,184901	0,387929

Exact p-values of Anova with repeated measurement design for all pigments:

Phycocyanin

Arenguade	nitrate	measurement	1	2	3	4	5	6
1	N-	day 1		1,000	1,000	1,000	0,762	0,262
2	N-	day 9	1,000		0,998	1,000	0,479	0,203
3	N-	day 15	1,000	0,998		1,000	0,892	0,267
4	N+	day 1	1,000	1,000	1,000		0,848	0,393
5	N+	day 9	0,762	0,479	0,892	0,848		0,935
6	N+	day 15	0,262	0,203	0,267	0,393	0,935	

Sonachi	nitrate	measurement	1	2	3	4	5	6
1	N-	day 1		0,004	0,003	0,241	0,046	0,225
2	N-	day 9	0,004		0,793	0,004	0,067	0,003
3	N-	day 15	0,003	0,793		0,002	0,003	0,031
4	N+	day 1	0,241	0,004	0,002		0,840	1,000
5	N+	day 9	0,046	0,067	0,003	0,840		0,735
6	N+	day 15	0,225	0,003	0,031	1,000	0,735	

Simbi	nitrate	measurement	1	2	3	4	5	6
1	N-	day 1		0,190	0,202	0,984	0,992	0,980
2	N-	day 9	0,190		1,000	0,204	0,169	0,058
3	N-	day 15	0,202	1,000		0,222	0,072	0,165
4	N+	day 1	0,984	0,204	0,222		0,906	0,864
5	N+	day 9	0,992	0,169	0,072	0,906		1,000
6	N+	day 15	0,980	0,058	0,165	0,864	1,000	

L.B. Momella	nitrate	measurement	1	2	3	4	5	6
1	N-	day 1		0,022	0,015	0,621	0,875	0,096
2	N-	day 9	0,022		0,973	0,025	0,104	0,001
3	N-	day 15	0,015	0,973		0,013	0,008	0,028
4	N+	day 1	0,621	0,025	0,013		0,994	0,049

5	N+	day 9	0,875	0,104	0,008	0,994		0,069
6	N+	day 15	0,096	0,001	0,028	0,049	0,069	

Nakuru	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,033	0,024	0,962	0,018	0,001
2	N-	day 9	0,033		0,991	0,051	1,000	0,036
3	N-	day 15	0,024	0,991		0,031	1,000	0,256
4	N+	day 1	0,962	0,051	0,031		0,049	0,005
5	N+	day 9	0,018	1,000	1,000	0,049		0,059
6	N+	day 15	0,001	0,036	0,256	0,005	0,059	

Chitu	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,288	0,009	0,315	0,208	0,998
2	N-	day 9	0,288		0,003	0,014	0,212	0,285
3	N-	day 15	0,009	0,003		0,284	0,105	0,153
4	N+	day 1	0,315	0,014	0,284		0,801	0,075
5	N+	day 9	0,208	0,212	0,105	0,801		0,206
6	N+	day 15	0,998	0,285	0,153	0,075	0,206	

Big Momella	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,016	0,028	1,000	0,004	0,000
2	N-	day 9	0,016		0,920	0,004	1,000	0,004
3	N-	day 15	0,028	0,920		0,008	0,958	0,040
4	N+	day 1	1,000	0,004	0,008		0,017	0,001
5	N+	day 9	0,004	1,000	0,958	0,017		0,015
6	N+	day 15	0,000	0,004	0,040	0,001	0,015	

Abijata	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,891	0,351	0,155	1,000	0,507
2	N-	day 9	0,891		0,146	0,208	0,211	0,978
3	N-	day 15	0,351	0,146		0,846	0,259	0,022
4	N+	day 1	0,155	0,208	0,846		0,802	0,204
5	N+	day 9	1,000	0,211	0,259	0,802		0,589
6	N+	day 15	0,507	0,978	0,022	0,204	0,589	

Oloidien	nitrate	measurement	1	2	3	4	5	6
1	N-	day 1		0,211	0,062	0,519	0,669	0,726
2	N-	day 9	0,211		0,011	0,042	0,996	0,975
3	N-	day 15	0,062	0,011		0,999	0,049	0,266
4	N+	day 1	0,519	0,042	0,999		0,021	0,023
5	N+	day 9	0,669	0,996	0,049	0,021		1,000
6	N+	day 15	0,726	0,975	0,266	0,023	1,000	

phycoerythrin

Arenguade	nitrate	measurement	1	2	3	4	5	6
1	N-	day 1		0,931	0,997	0,841	0,996	0,849
2	N-	day 9	0,931		0,994	0,667	0,233	0,385
3	N-	day 15	0,997	0,994		0,912	0,923	0,205
4	N+	day 1	0,841	0,667	0,912		1,000	0,990
5	N+	day 9	0,996	0,233	0,923	1,000		0,987
6	N+	day 15	0,849	0,385	0,205	0,990	0,987	

Sonachi	nitrate	measurement	1	2	3	4	5	6
1	N-	day 1		0,004	0,003	0,298	0,041	0,214
2	N-	day 9	0,004		0,854	0,003	0,080	0,003
3	N-	day 15	0,003	0,854		0,001	0,003	0,039
4	N+	day 1	0,298	0,003	0,001		0,757	1,000
5	N+	day 9	0,041	0,080	0,003	0,757		0,698
6	N+	day 15	0,214	0,003	0,039	1,000	0,698	

Simbi	nitrate	measurement	1	2	3	4	5	6
1	N-	day 1		0,131	0,163	0,997	0,988	1,000
2	N-	day 9	0,131		1,000	0,148	0,211	0,074
3	N-	day 15	0,163	1,000		0,197	0,067	0,276
4	N+	day 1	0,997	0,148	0,197		0,896	0,980
5	N+	day 9	0,988	0,211	0,067	0,896		0,999

6	N+	day 15	1,000	0,074	0,276	0,980	0,999	

L.B. Momella	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,029	0,019	0,814	0,845	0,178
2	N-	day 9	0,029		0,961	0,027	0,133	0,001
3	N-	day 15	0,019	0,961		0,013	0,013	0,035
4	N+	day 1	0,814	0,027	0,013		1,000	0,099
5	N+	day 9	0,845	0,133	0,013	1,000		0,097
6	N+	day 15	0,178	0,001	0,035	0,099	0,097	

Nakuru	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,162	0,119	0,728	0,027	0,003
2	N-	day 9	0,162		0,998	0,443	0,752	0,060
3	N-	day 15	0,119	0,998		0,297	0,935	0,223
4	N+	day 1	0,728	0,443	0,297		0,170	0,028
5	N+	day 9	0,027	0,752	0,935	0,170		0,314
6	N+	day 15	0,003	0,060	0,223	0,028	0,314	

Chitu	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,298	0,009	0,331	0,193	0,996
2	N-	day 9	0,298		0,003	0,016	0,209	0,275
3	N-	day 15	0,009	0,003		0,271	0,121	0,159
4	N+	day 1	0,331	0,016	0,271		0,896	0,089
5	N+	day 9	0,193	0,209	0,121	0,896		0,203
6	N+	day 15	0,996	0,275	0,159	0,089	0,203	

Big Momella	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,020	0,077	0,996	0,017	0,000
2	N-	day 9	0,020		0,433	0,005	0,851	0,023
3	N-	day 15	0,077	0,433		0,028	0,917	0,074
4	N+	day 1	0,996	0,005	0,028		0,030	0,002

5	N+	day 9	0,017	0,851	0,917	0,030		0,024
6	N+	day 15	0,000	0,023	0,074	0,002	0,024	

Abijata	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,930	0,347	0,196	1,000	0,601
2	N-	day 9	0,930		0,160	0,259	0,259	0,985
3	N-	day 15	0,347	0,160		0,807	0,281	0,028
4	N+	day 1	0,196	0,259	0,807		0,864	0,252
5	N+	day 9	1,000	0,259	0,281	0,864		0,630
6	N+	day 15	0,601	0,985	0,028	0,252	0,630	

Oloidien	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,378	0,069	0,582	0,793	0,919
2	N-	day 9	0,378		0,016	0,071	0,999	0,973
3	N-	day 15	0,069	0,016		0,989	0,061	0,322
4	N+	day 1	0,582	0,071	0,989		0,035	0,048
5	N+	day 9	0,793	0,999	0,061	0,035		0,996
6	N+	day 15	0,919	0,973	0,322	0,048	0,996	

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Arenguade	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,057	0,022	0,129	0,619	0,014
2	N-	day 9	0,057		0,689	0,011	0,021	0,896
3	N-	day 15	0,022	0,689		0,005	0,024	0,360
4	N+	day 1	0,129	0,011	0,005		0,282	0,016
5	N+	day 9	0,619	0,021	0,024	0,282		0,084
6	N+	day 15	0,014	0,896	0,360	0,016	0,084	

Sonachi	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,193	0,042	0,970	1,000	0,553
2	N-	day 9	0,193		0,468	0,187	0,149	0,021

3	N-	day 15	0,042	0,468		0,022	0,013	0,036
4	N+	day 1	0,970	0,187	0,022		0,991	0,452
5	N+	day 9	1,000	0,149	0,013	0,991		0,685
6	N+	day 15	0,553	0,021	0,036	0,452	0,685	

Simbi	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,610	0,511	0,997	1,000	0,989
2	N-	day 9	0,610		0,999	0,492	0,747	0,866
3	N-	day 15	0,511	0,999		0,401	0,514	0,824
4	N+	day 1	0,997	0,492	0,401		0,987	0,727
5	N+	day 9	1,000	0,747	0,514	0,987		0,918
6	N+	day 15	0,989	0,866	0,824	0,727	0,918	

L.B. Momella	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,006	0,004	0,668	0,036	0,436
2	N-	day 9	0,006		0,932	0,001	0,363	0,013
3	N-	day 15	0,004	0,932		0,001	0,056	0,114
4	N+	day 1	0,668	0,001	0,001		0,011	0,061
5	N+	day 9	0,036	0,363	0,056	0,011		0,234
6	N+	day 15	0,436	0,013	0,114	0,061	0,234	

Nakuru	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,097	0,017	0,991	0,106	0,017
2	N-	day 9	0,097		0,264	0,424	0,975	0,122
3	N-	day 15	0,017	0,264		0,081	0,887	0,837
4	N+	day 1	0,991	0,424	0,081		0,078	0,009
5	N+	day 9	0,106	0,975	0,887	0,078		0,116
6	N+	day 15	0,017	0,122	0,837	0,009	0,116	

Chitu	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,417	0,086	0,891	0,161	0,994
2	N-	day 9	0,417		0,021	0,283	0,294	0,609

3	N-	day 15	0,086	0,021		0,958	0,801	0,827
4	N+	day 1	0,891	0,283	0,958		0,125	0,539
5	N+	day 9	0,161	0,294	0,801	0,125		0,033
6	N+	day 15	0,994	0,609	0,827	0,539	0,033	

Big Momella	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,077	0,081	0,990	0,067	0,002
2	N-	day 9	0,077		1,000	0,024	0,983	0,070
3	N-	day 15	0,081	1,000		0,025	0,995	0,194
4	N+	day 1	0,990	0,024	0,025		0,082	0,007
5	N+	day 9	0,067	0,983	0,995	0,082		0,079
6	N+	day 15	0,002	0,070	0,194	0,007	0,079	

Abijata	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,949	0,917	0,999	0,859	0,765
2	N-	day 9	0,949		0,567	0,986	0,999	0,996
3	N-	day 15	0,917	0,567		0,814	0,396	0,392
4	N+	day 1	0,999	0,986	0,814		0,945	0,884
5	N+	day 9	0,859	0,999	0,396	0,945		1,000
6	N+	day 15	0,765	0,996	0,392	0,884	1,000	

Oloidien	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,509	0,558	0,301	0,999	0,979
2	N-	day 9	0,509		0,112	0,018	0,728	0,249
3	N-	day 15	0,558	0,112		0,718	0,394	0,879
4	N+	day 1	0,301	0,018	0,718		0,119	0,293
5	N+	day 9	0,999	0,728	0,394	0,119		0,877
6	N+	day 15	0,979	0,249	0,879	0,293	0,877	

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Arenguade	nitrate	measurement	1	2	3	4	5	6
1	N-	day 1		0,035	0,014	0,781	0,104	0,004
2	N-	day 9	0,035		0,667	0,008	0,124	0,481
3	N-	day 15	0,014	0,667		0,003	0,047	1,000
4	N+	day 1	0,781	0,008	0,003		0,118	0,010
5	N+	day 9	0,104	0,124	0,047	0,118		0,092
6	N+	day 15	0,004	0,481	1,000	0,010	0,092	

Sonachi	nitrate	measurement	1	2	3	4	5	6
1	N-	day 1		0,193	0,024	0,251	0,993	0,913
2	N-	day 9	0,193		0,219	0,524	0,092	0,046
3	N-	day 15	0,024	0,219		0,026	0,012	0,015
4	N+	day 1	0,251	0,524	0,026		0,893	0,369
5	N+	day 9	0,993	0,092	0,012	0,893		0,794
6	N+	day 15	0,913	0,046	0,015	0,369	0,794	

Simbi	nitrate	measurement	1	2	3	4	5	6
1	N-	day 1		0,550	0,455	0,994	1,000	1,000
2	N-	day 9	0,550		0,998	0,432	0,719	0,651
3	N-	day 15	0,455	0,998		0,352	0,450	0,709
4	N+	day 1	0,994	0,432	0,352		0,985	0,899
5	N+	day 9	1,000	0,719	0,450	0,985		0,996
6	N+	day 15	1,000	0,651	0,709	0,899	0,996	

L.B. Momella	nitrate	measurement	1	2	3	4	5	6
1	N-	day 1		0,003	0,002	0,577	0,024	0,626
2	N-	day 9	0,003		0,710	0,001	0,275	0,004
3	N-	day 15	0,002	0,710		0,000	0,020	0,070
4	N+	day 1	0,577	0,001	0,000		0,006	0,058
5	N+	day 9	0,024	0,275	0,020	0,006		0,088

6	N+	day 15	0,626	0,004	0,070	0,058	0,088	

Nakuru	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,485	0,028	0,991	0,537	0,119
2	N-	day 9	0,485		0,111	1,000	0,987	0,230
3	N-	day 15	0,028	0,111		0,421	0,852	0,963
4	N+	day 1	0,991	1,000	0,421		0,437	0,019
5	N+	day 9	0,537	0,987	0,852	0,437		0,075
6	N+	day 15	0,119	0,230	0,963	0,019	0,075	

Chitu	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,096	0,427	0,645	0,146	1,000
2	N-	day 9	0,096		0,023	0,037	0,165	0,211
3	N-	day 15	0,427	0,023		0,956	0,553	0,878
4	N+	day 1	0,645	0,037	0,956		0,743	0,186
5	N+	day 9	0,146	0,165	0,553	0,743		0,062
6	N+	day 15	1,000	0,211	0,878	0,186	0,062	

Big Momella	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,253	0,180	0,970	0,202	0,003
2	N-	day 9	0,253		0,998	0,092	1,000	0,028
3	N-	day 15	0,180	0,998		0,059	0,993	0,145
4	N+	day 1	0,970	0,092	0,059		0,171	0,009
5	N+	day 9	0,202	1,000	0,993	0,171		0,061
6	N+	day 15	0,003	0,028	0,145	0,009	0,061	

Abijata	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,613	1,000	1,000	0,644	0,432
2	N-	day 9	0,613		0,660	0,528	1,000	1,000
3	N-	day 15	1,000	0,660		1,000	0,698	0,472
4	N+	day 1	1,000	0,528	1,000		0,675	0,490
5	N+	day 9	0,644	1,000	0,698	0,675		0,997

6	N+	day 15	0,432	1,000	0,472	0,490	0,997	

Oloidien	**nitrate**	**measurement**	**1**	**2**	**3**	**4**	**5**	**6**
1	N-	day 1		0,196	0,660	0,495	0,980	0,946
2	N-	day 9	0,196		0,057	0,022	0,713	0,595
3	N-	day 15	0,660	0,057		0,740	0,465	0,630
4	N+	day 1	0,495	0,022	0,740		0,088	0,074
5	N+	day 9	0,980	0,713	0,465	0,088		1,000
6	N+	day 15	0,946	0,595	0,630	0,074	1,000	

4. Zusammenfassung

Bei *Arthrospira fusiformis*, im Handel auch als Spirulina bekannt, handelt es sich um ein filamentöses Cyanobakterium. Es ist die dominante Alge in Sodaseen des ostafrikanischen Grabenbruchs. Diese Sonderstandorte sind - im Vergleich zu ihrer Phosphat- und Kohlenstoffverfügbarkeit - in aller Regel stickstofflimitiert. In der vorliegenden Studie wurde deswegen *A. fusiformis* auf ihre mögliche Fähigkeit zur Stickstofffixierung hin untersucht, da dies eine Erklärung für ihre enorme Wachstumsfähigkeit trotz stickstofflimitierenden Bedingungen wäre.

Dabei wurde *A. fusiformis* in einem dreiteiligen experimentellen Setup, bestehend aus einem Wachstumsversuch, genetischen Analysen sowie $^{15}N_2$-Inkubation, aus insgesamt neun verschiedenen Standorten entlang des Ostafrikanischen Grabenbruchs untersucht.

Durch die genetischen Analysen konnte eindeutig gezeigt werden, dass *A. fusiformis* das *nifH* Gen besitzt und dieses teilweise auch exprimiert. Dieses Gen wird als genetischer Marker für diazotophe Organismen verwendet. Allerdings konnte im Wachstumsversuch die volle Funktionsfähigkeit der Nitrogenase nicht bestätigt werden, da *A. fusiformis* ohne Zugabe von Nitrat im Nährmedium nach einigen Wochen nicht mehr lebensfähig war. Wahrscheinlich verhinderten die Laborbedingungen eine voll funktionierende N-Fixierung. Dieses Ergebnis wird auch von der $^{15}N_2$-Inkubation unterstützt, da die IRMS Ergebnisse eine geringe ^{15}N Anreicherung bestätigen, wobei diese geringe N-Fixierung nicht ausreichend ist, um dauerhaftes Zellwachstum – zumindest unter den verwendeten Kultivierungsbedingungen – zu gewährleisten.