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Characterization of the initial ammonium uptake in *Saccharina latissima*

Possible implications for cultivation in an
IMTA system with intensive salmon farming

Vegard Rønning Dahlen

Marine Coastal Development
Submission date: June 2018
Supervisor: Kjell Inge Reitan, IBI
Co-supervisor: Siv Anina Etter, IBI

Norwegian University of Science and Technology
Department of Biology

Abstract

Production of Atlantic salmon (*Salmo salar*) results in a release of ca. 39% of the total nitrogen content from the feed, which is mainly released as dissolved inorganic nitrogen (DIN), and mainly as the metabolic by product NH_4^+. At the same time, the most frequently limiting nutrient for macroalgae growth is DIN. Cultivation of the commercially attractive species *Saccharina latissima* in an integrated multi-trophic aquaculture system (IMTA) with intensive salmon farming, is therefore a suggested approach to increase the long-term sustainability of the aquaculture sector through bioremediation of released nutrients.

The aim of this thesis was to characterize the NH_4^+ uptake in *S. latissima* in a IMTA system. Two main studies were conducted: a control study to determine the uptake of NH_4^+ as the only available nitrogen source, and a preference study to investigate any interaction between effluent NH_4^+ and naturally occurring NO_3^-. Uptake was measured in a controlled laboratory experiment combining the commonly used multiple flask and perturbation methods. *S. latissima* of different nutritional histories were exposed to a gradient of NH_4^+ ranging between 0.25-16 µM, with and without 1 µM of NO_3^- available, over a 5-hour period. By following the depletion of substrate concentration, uptake rates (µM gDW^{-1} h^{-1}) of these "pulsed" availabilities were determined.

S. latissima appeared to efficiently adapt to new and different availabilities of NH_4^+, demonstrating rapid increases in uptake rates toward maximal rate, which was always observed within 50 minutes. Furthermore, the NH_4^+ uptake increased linearly with increased availability up to 16 µM, regardless of nutritional history and available nitrogen source. The rate of removal was the highest when NH_4^+ was the only available nitrogen source. The nutritional history of the *S. latissima* also appeared to affect the uptake, and nitrogen deficient specimens demonstrated a faster uptake of NH_4^+ than nitrogen sufficient specimens, with several significant differences. Upon exposure to new conditions, *S. latissima* demonstrated a brief initial induction period before uptake was evident. A consistent uptake of NO_3^- only appeared after a lag period of approximately 50 minutes, demonstrated a preferential uptake of the energetically favourable NH_4^+. When NO_3^- was available, the nutritional history appeared to have a smaller effect on uptake of both NO_3^- and NH_4^+, as there were observed relatively few and inconsistent significant differences between the samples with sufficient and deficient specimens.

Keywords: *Saccharina latissima*, ammonium uptake, DIN, IMTA.

Sammendrag

Produksjon av atlantisk laks (*Salmo salar*) i åpne sjømerder forårsaker et relativt stort utslipp av næringssalter til miljøet. Fra laksens fôr slippes omtrent 39% av det totale nitrogeninnholdet ut som løst uorganisk nitrogen (DIN), og hovedsakelig i form av det metabolske biproduktet NH_4^+. Samtidig er DIN det oftest begrensende næringssaltet for vekst av makroalger. Kultivering av den kommersielt attraktive arten *Saccharina latissima* i et integrert multitrofisk akvakultur system (IMTA) med intensiv produksjon av laks, er en foreslått tilnærming for å øke den langsiktige bæreevnen til akvakultursektoren gjennom en bioremediering av næringssalter.

Hensikten med denne oppgaven var å karakterisere NH_4^+ opptaket til *S. latissima* i et IMTA system. To hovedstudier ble gjennomført, med ett kontrollstudie der opptaket av NH_4^+ som eneste tilgjengelige nitrogenkilde ble bestemt, og ett preferansestudie der opptaket av NH_4^+ med tilgjengelig NO_3^-, som er naturlig forekommende, ble bestemt. Opptak ble målt i et kontrollert laboratorieeksperiment som kombinerte de ofte brukte "multiple flask" og "perturbation" teknikkene. *S. latissima* med ulik næringshistorikk ble eksponert for en gradient av NH_4^+ mellom 0.25-16 µM, med og uten 1 µM NO_3^-, i en periode på 5 timer. Ved å følge reduksjonen av substratkonsentrasjonen ble opptaksrate (µM gDW^{-1} h^{-1}) bestemt.

S. latissima viste seg å effektivt tilpasse seg til nye og ulike tilgjengeligheter av NH_4^+, og demonstrerte en rask økning i opptak mot den maksimale raten som alltid forekom innen de første 50 minuttene. Opptaket av NH_4^+ økte lineært med økende tilgjengelighet opp til 16 µM, uavhengig av næringshistorikk og tilgjengelige nitrogenkilder. Næringshistorikken til *S. latissima* påvirket opptak, og individ som var sultet for nitrogen hadde et høyere opptak enn individ som var mettet, med flere signifikante ulikheter. Ved eksponering av *S. latissima* til nye forhold, ble det observert en initial og kort induksjonsperiode uten opptak av NH_4^+. Et konsekvent opptak av NO_3^- begynte etter en periode på omtrent 50 minutter, som indikerer en preferanse for den mer energigunstige nitrogenkilden NH_4^+. Når NO_3^- var tilgjengelig ble det også observert relativt få og inkonsekvente signifikante forskjeller i opptak mellom prøvene med individ av ulik næringshistorikk.

Nøkkelord: *Saccharina latissima*, ammonium opptak, DIN, IMTA.

Acknowledgements

This thesis was written at the Department of Biology, Norwegian University of Science and Technology (NTNU), Trondheim 2018. The work was done in collaboration with the research project MACROSEA. The experiment in which this work was based upon were performed at Trondheim Biological Station (TBS).

I would especially like to express my gratitude and thank my supervisor Kjell Inge Reitan and my co-supervisor Ph.D. student Siv Anina Etter at the Department of Biology, NTNU. Thank you all for all your assistance and guidance throughout this project. I would also like to give a special thanks to Ph.D. student Silje Forbord at NTNU, Department of Biology, in the planning and running of the experimental work in collaboration with my co-supervisor Siv Anina Etter and myself.

To all fellow students and staff at NTNU Centre of Fisheries and Aquaculture (SeaLab) and at TBS, thank you for making these places a great place to study.

To my friends and partner, thank you for all your support, for all our wonderful times and for making my stay in Trondheim a special period I will cherish forever. And to my family, thank you for always supporting me no matter what and for encouraging me to always follow my interests

Trondheim, June 2018
Vegard Rønning Dahlen

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Abbreviations

AD	Ammonium study with deficient *S. latissima*
ANOVA	Analysis of variance
AS	Ammonium study with sufficient *S. latissima*
DIN	Dissolved inorganic nitrogen
DIP	Dissolved inorganic phosphorus
DW	Dry weight
IMTA	Integrated multi-trophic aquaculture
IQR	Interquartile range
NiR	Nitrite reductase
NR	Nitrate reductase
NS 4745	Norwegian Standard 4745: Determination of the sum of nitrite- and nitrate-nitrogen
NS 4746	Norwegian Standard 4756: Determination of ammonia-nitrogen
PD	Preference study with deficient *S. latissima*
PS	Preference study with sufficient *S. latissima*
rpm	Rounds per minute
SD	Standard deviation
SES	Seaweed Energy Solutions AS
TBS	Trondheim Biological Station

1 Introduction

1.1 Global seafood production

The current world population is 7.4 billion and is expected to reach 9.7 billion by 2050 (FAO, 2016a). This increase in world population is estimated to require a 60% increase in food production from the current 8.4 billion tonnes to 13.5 billion tonnes per year. This increase in food production is impossible without profound changes in today's food production systems, and depends on a transition to more sustainable production methods (FAO, 2014). Global net primary production in the ocean is similar to the production on land (Field, 1998). However, marine food contributes to only 2% of the human food supply (FAO, 2007), and therefore the production in the ocean has a great potential to contribute in the increasing food demand.

Capture fisheries has stabilized after the late 1980's at approximately 90 million tonnes per year, and many stocks have been, and are currently overexploited (FAO, 2016b). It has been suggested that the current harvest level of fisheries is at least twice the volume of that to ensure sustainable catch, and any significant increase in harvest from fisheries is unlikely for the future (Coll et al., 2008). However, aquaculture production of fish and aquatic plants combined has exceeded the production of fisheries by volume, and is expected to continue to grow to provide an additional 16-47 million tonnes of fish by 2030 (FAO, 2016b). Aquaculture fish production has experienced a rapid increase in global production, reaching approximately 74 million tonnes in 2014, with an annual growth of 7.2% per year in the period 1994-2004, and 5.8% in the period 2005-2014 (FAO, 2016b). In the same period of 1994-2004, aquaculture fish production therefore experienced a higher growth than population growth and growth in food production on land, with annual increases of 0.5% and 2.0%, respectively (Lutz et al., 2001; Duarte et al., 2009).

One of the major constraints for the future growth of aquaculture is the amount of feed available for the fed aquaculture species, which have experienced the greatest growth (FAO, 2016b). Cultivation of non-fed aquaculture, on the other hand, does not require input of feed, usually have less costly production and may contribute more positively to the environment and food security than cultivation of fed species (FAO, 2016b).

1.2 Aquaculture in Norway

Norway has become the world's leading producer of Atlantic salmon (*Salmo salar*) after a strong increase in the production since the technological breakthrough of the industry in the 1970's (Steinset, 2017). Today, Norway's total aquaculture production of fish is largely dominated by *S. salar*, accounting to more than 1.2 million tonnes per year (Statistisk Sentralbyrå – Statistics Norway, 2016). On-growing cultivation of salmon in Norway is mainly done in open sea pens, providing efficient production with low carbon footprint (Hognes et al., 2011). Open sea pens utilize relatively cheap technology, they are easy to move, clean and operate, and are exploiting the natural environment for great access to fresh seawater.

This open production system does on the other hand lead to several environmental challenges, limiting any further increase in production. The main challenge is the infestation of sea lice, while other challenges include eutrophication, escapees, diseases, use of pharmaceuticals and fish welfare (Rosten et al., 2011; Svåsand et al., 2017). Given solutions to these various problems, the industry believes an annual production of 5 million tonnes of salmon and trout can be accomplished by 2050 (Olafsen et al., 2012). The overall annual value produced by the aquaculture sector is also suggested to increase greatly, with a six-fold increase (Olafsen et al., 2012).

1.3 Nutrient discharge from intensive salmon farming

Aquaculture is the largest source of anthropogenic eutrophication to Norwegian coastal waters from Rogaland in the south, to Finnmark in the north (Svåsand et al., 2017). Nutrients are released to the surrounding water through feed loss, faeces and excretion. A discharge of 57% of nitrogen and 76% of phosphorus in salmon feed is estimated to be released to the environment (Wang et al., 2013). Out of total nitrogen and phosphorus in the feed, 39% and 24% is respectively released as dissolved inorganic nitrogen (DIN) and phosphorus (DIP) (Wang et al., 2013). Out of the DIN excreted by the salmon, NH_4^+ is the main excretion product, originating from protein metabolism (Fivelstad et al., 1990).

Dissolved nutrients from the sea pens are released in frequent pulses related to feeding (Wang et al., 2013). These nutrients are quickly diluted in the water mass, especially by strong currents, and may be readily taken up by phytoplankton (Price et al., 2015). Relatively low

concentrations are therefore found within 1-2 km from the sea pens depending on local conditions, fish biomass and season. Nitrogen is normally a limiting factor for primary production during the summer in Norway and an increased nitrogen load from salmon farming may result in an increase in primary production (Honkanen and Helminen, 2000; Robinson et al., 2005; Svåsand et al., 2017). NH_4^+ released may also be readily taken up by macroalgae and stimulate increased growth in fast-growing species that may outcompete slow-growing perennial species (Worm and Sommer, 2000). An increase in decomposition of algal biomass in deep water may also reduce available oxygen (Svåsand et al., 2017). These effects of an increase in primary production may therefore have the potential to reduce biodiversity. Phytoplankton production in areas with intensive aquaculture is expected to increase with an increased fish farming production in the future. An increase in salmon production to 3 million tonnes per year is estimated to increase algae production in Hordaland by 27% (one of the most intensive production areas in Norway; Svåsand et al, 2016).

1.4 Integrated multi-tropic aquaculture

Integrated multi-tropic aquaculture (IMTA) is the concept of which the excess resources of one species can be reused by another species at a lower tropic level to create biomass (Chopin et al., 2008). IMTA systems may include one fed species, such as *S. salar*, that release nutrients, which are available for organic and inorganic extractive species, such as mussels and seaweed (Figure 1.1). Feed is one of the main expenses in cultivation of salmon, accounting for approximately half the production cost (Guttormsen, 2002). This loss of nutrients is therefore a direct economic loss as well as being a threat to the environment through potential eutrophication. Hence, IMTA systems intend to increase the long-term sustainability, as well as profitability of the industry. As Norwegian production of salmon is expected to increase in the future, measures to limit possible negative effects must be introduced (Chopin et al., 2008).

Introducing seaweed farms in proximity to intensive salmon farming as a part of IMTA systems is a suggested approach to reduce the eutrophication to the local environment and utilize valuable nutrients that otherwise will be lost, as well as to replenish oxygen (Chopin et al., 2008). Macroalgae production does not require any input of fertilizers such as plant production on land does, but utilizes the nutrients available in the seawater. As intensive salmon farming in the ocean increases the availability of nutrients important for macroalgae growth, growing seaweed in the proximity to salmon farms can have a bioremediating effect, increasing both the

macroalgae yield as well as the utilization of the nutrient put in to the system to reduce the effect of eutrophication to the ecosystem and potential algal blooms (Chopin et al., 2008; Sanderson et al., 2012).

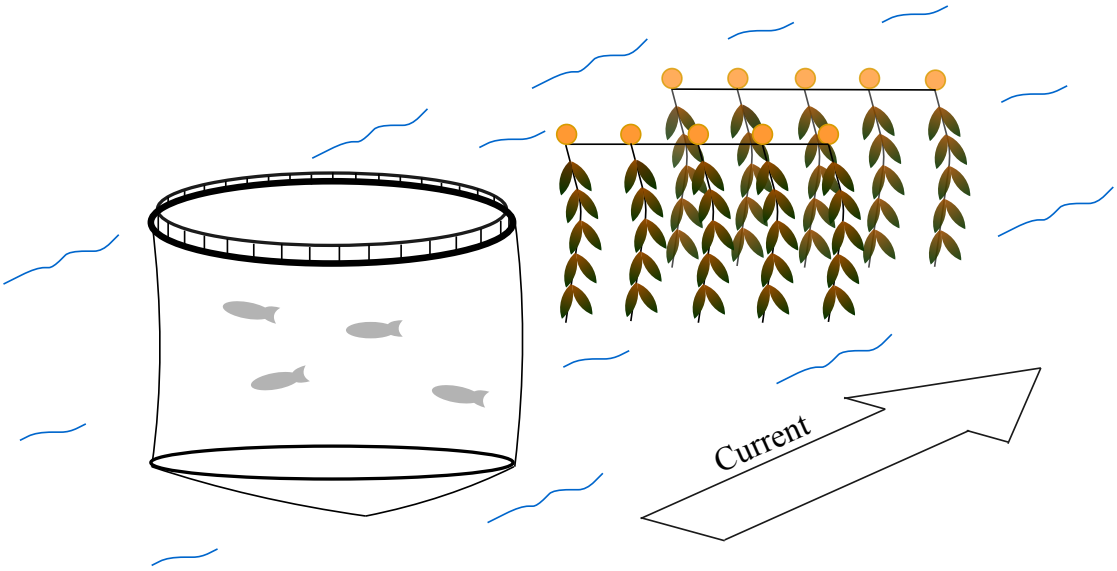

Figure 1.1: Illustration of cultivation of macroalgae in an IMTA system with open sea fish farming. Nutrients released by the fish may be taken up by macroalgae cultivated downstream of the fish farm.

1.5 Macroalgae

Macroalgae are traditionally classified as red (Rhodophyta), green (Chlorophyta) and brown (Phaeophyta) algae. Seaweed can be defined as macroscopic marine red, green and brown algae that at some stage in their life cycle form multicellular or siphonous macrothalli. Seaweeds are phototrophic and grow in the euphotic zone, using inorganic carbon, light, water and minerals for photosynthesis and growth (Hurd et al., 2014).

1.5.1 Cultivation of *Saccharina latissima*

Cultivation of macroalgae is the largest production sector within aquaculture measured by volume (Almås and Ratvik, 2017). In Norway, macroalgae have to a large degree been harvested from wild populations. However, in the recent years, cultivation of macroalgae have emerged as an industry with several producers. The combined value of cultivated and harvested macroalgae is suggested to greatly increase from todays' 1.2 billion NOK to 40 billion NOK by 2050 (Almås and Ratvik, 2017). Norwegian production of macroalgae is dominated by *S.*

latissima, amounting to 96% of total production volume (Stévant et al., 2017). The large focus on *S. latissima* as a cultivation species is mainly a result of its potential for offering a large biomass yield (Handå et al., 2013), high-quality nutritional content (Schiener et al., 2015), and broad market range (Stévant et al., 2017).

S. latissima is a brown alga belonging to the kelps, and is a cold-temperate water adapted species, growing in temperate climatic conditions in the northern hemisphere with a distribution from Spain to North Greenland (Wiencke et al., 1994; Bartsch et al., 2008). The natural growth sites are in clear and turbid coastal waters in sheltered and moderately exposed areas (Borum et al., 2002). They grow on rocks and hard substrate down to approximately 30 meters' depth (Bekkby and Moy, 2011). It is a perennial species and can grow up to four meters in length (Indergaard, 2010), and growth has been found to be optimal at temperatures between 10-15 ^{o}C (Fortes and Lüning, 1980; Bolton and Lüning, 1982). In the period from late winter to early spring, inorganic nitrogen is available and growth is rapid and a large biomass can be achieved in a relatively short period, being one of the fastest growing kelp species in European waters (Forbord et al., 2012). During summer, temperature and irradiance is high, nutrients are more limiting and epigrowth on the lamina are more abundant, lamina growth may therefore be reduced (Andersen, 2013).

Cultivation of *S. latissima* in proximity to intensive salmon farming have led to promising results in previous studies, proving effluent DIN from salmon farms may be taken up and increase the biomass yield (Ahn et al., 1998; Sanderson et al., 2012; Broch et al., 2013; Handå et al., 2013).

1.5.2 Nutrient availability for seaweed in seawater

The three major form of nitrogen sources available in seawater for seaweed are NO_3^-, NH_4^+, and urea (Phillips and Hurd, 2004). Different nitrogen sources are utilized differently by different species of seaweed, they are, however, unable to directly use nitrogen gas. Seaweed growth will be limited by the nutrient available at the smallest quantity with respect to its requirement (Hurd et al., 2014). The most frequently limiting nutrient is nitrogen, both globally and in Norwegian coastal waters, especially during the summer months after phytoplankton blooms (Hurd et al., 2014; Svåsand et al., 2017). Following nitrogen, phosphorus is often a limiting factor (Hurd et al., 2014), however, in Norwegian coastal waters phosphorus is rarely

a limiting factor, and will often not result in a direct response of phytoplankton production (Svåsand et al., 2017).

1.5.3 Nitrogen uptake in macroalgae

As seaweed are dispersed in water, ions are readily available to be taken up both passively and actively. Polar molecules require transport proteins to cross the cell membrane. Uncharged molecules and gases may diffuse through the membrane down a concentration gradient. Ions, on the other hand, are either repelled or immobilized by the membrane. Additionally, ions are often hydrophilic, reducing the rate of diffusion. Therefore, ions often require active uptake. Inorganic nutrients, such as NO_3^-, are typically found in the micromolar range in the external seawater and in the millimolar range within the seaweed, and therefore require active uptake. The cell wall does not generally inhibit ion entry as the plasmalemma does. Between these barriers there is an apparent free space that can experience an initial, passive, rapid uptake of nutrients after the algae is placed in a medium. The cell wall may attract some ions, especially cations, which consequently may not enter the cell (Hurd et al., 2014).

Nutrient-uptake rates in seaweed are affected by and vary considerably with variations in chemical, physical and biological factors (Harrison and Hurd, 2001):

- *Chemical factors* include the concentration of nutrients in their ionic or molecular form.
- *Physical factors* include irradiance, temperature, water motion and desiccation. Nutrient uptake is affected by light indirectly by regulating the photosynthetic activity, temperature by affecting the general cell metabolism, and water motion by affecting the movement of ions to the surface of the thallus by decreasing the thickness of the velocity- and concentration boundary layers (Hurd, 2000). Desiccation have demonstrated to increase the short-term nutrient uptake when re-submerged in seawater (Thomas, 1980; Thomas et al., 1987).
- *Biological factors* which influence the uptake rate of nutrients include the surface-area:volume ratio, type of tissue, age, nutritional history and inter-seaweed variability (Hurd et al., 2014). A large surface area to volume allows a larger part of the individual to take up nutrients (Wallentinus, 1984). The uptake capacity of different type of tissue vary greatly within and between species. Generally, the lamina takes up most the nutrients, while the stipes has a low activity. Young seaweeds and young tissue, may have a higher uptake rate of NH_4^+ and NO_3^- than older tissue, as they have a relatively high proportion of

metabolically active tissue with a high demand of nitrogen, while older individuals have tissue that do not actively require nitrogen (e.g. storage and support tissue). The nutritional history of the seaweed may also influence the uptake, and nutrient-limited individuals have shown to have a higher uptake rate than individuals with saturated internal storages (Pedersen, 1994).

As mentioned, the uptake characteristic of nutrients for macroalgae vary with species and its nutritional history. In many species, an ion's uptake may be saturated with increasing concentration, and may therefore be described by a rectangular hyperbola. In other cases, a saturation does not occur and a linear uptake may be experienced. Nutrient-limited (e.g. typically nitrogen-limited) macroalgae exposed to the limiting nutrient, may experience an uptake characterized by three phases (Figure 1.2). Firstly, there may be an initial and rapid "surge" uptake taking place as the internal storages are rapidly filled. When the storages are filled, the uptake will be limited by internal uptake mechanisms where nitrogen reducing enzymes limit any further uptake. Lastly, there is an externally controlled uptake where the availability of nutrients in the medium are becoming the limiting factor (Pedersen, 1994).

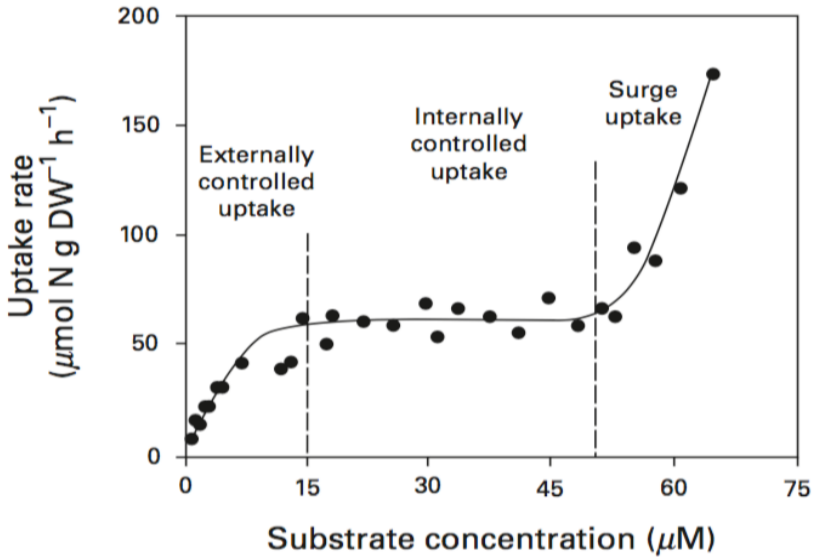

Figure 1.2: Nutrient limited algae may experience three phases of uptake rate when exposed to a high initial concentration of the nutrient under limitation. Algae with deficient internal storages may experience an initial "surge" uptake, followed by an internally controlled uptake, and a final reduced externally controlled uptake as the nutrients are depleting (Pedersen, 1994). In this example by Pedersen (1994), nitrogen depleted macroalgae were exposed to an initial high concentration, and the uptake rate was followed as the nutrients were depleted, demonstrating the three phases of uptake.

The processes for the seaweed to assimilate different sources of nitrogen vary and are represented in Figure 1.3. NH_4^+ can be taken up and directly converted into amino acids in the chloroplast via glutamine synthetase. NO_3^- and urea, on the other hand, must be reduced to NH_4^+ to be incorporated into amino acids (Syrett, 1981; Berges and Mulholland, 2008). NO_3^- is reduced intracellularly to NO_2^-, catalysed by nitrate reductase (NR) (Berges, 1997). NO_2^- is subsequently reduced to NH_4^+, catalysed by nitrite reductase (NiR). Accumulation of NO_2^- in intracellular pools may be toxic, and this is prevented by the enzymes, NR and NiR, being closely linked (Berges and Mulholland, 2008).

Internal nitrogen storage capabilities vary considerably between species. Some species may store enough to grow at maximal rate for several days without nitrogen sources (Lapointe and Ryther, 1979; Fujita, 1985), and other may have very limited storage capabilities (Lignell and Pedersen 1987). The difference in nitrogen-storage capabilities may be correlated to environmental adaptation. Species adapted to eutrophic environment may have low storage capabilities, whereas nitrogen-limited adapted species may have larger storage capabilities (Hurd et al., 2014). Correspondingly, high internal tissue concentration indicates nutrient storage and low internal tissue concentration may indicate nutrient deficiency (Hurd et al., 2014).

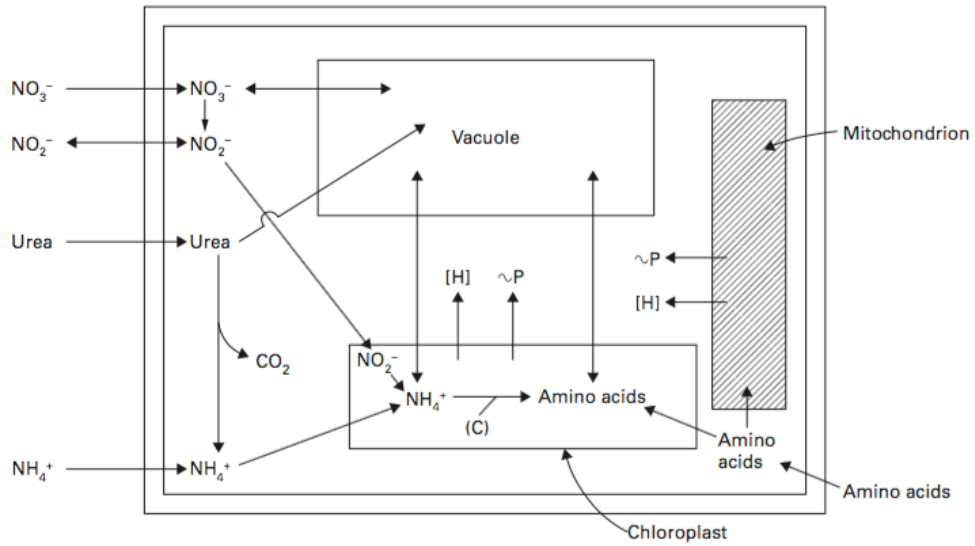

Figure 1.3: Main features of nitrogen uptake and assimilation in an algal cell. NH_4^+ may be directly taken up and incorporated into amino acids in the chloroplast. NO_3^-, NO_2^- and urea must be reduced into NH_4^+ to be incorporated (from Syrett, 1981).

1.5.4 Measurement of nutrient uptake

Laboratory measurements of nutrient uptake in seaweeds are normally done by adding epiphyte-free tissue discs or whole individuals to seawater with saturating levels of all nutrients, trace metals and vitamins, except the one under study. Four commonly used units to express nutrient uptake rates are used: surface area (μmol cm^{-2} h^{-1}), wet weight (μmol gWW^{-1} h^{-1}), dry weight (μmol gDW^{-1} h^{-1}), and a simplification of the nutrients' specific uptake rate expressed per hour (Hurd et al., 2014).

There are two main techniques for measuring nutrient uptake rates in the laboratory: the perturbation method and the multiple flask technique (Hurd et al., 2014). In the perturbation method, the algae are incubated in a medium, and the depletion of the nutrient under study is followed frequently until removed, with samples taken at short time intervals. With this technique, the uptake is consequently measured at many substrate concentrations with the same algae tissue that have changed its nutritional status. In the multiple flask technique, several initial substrate concentrations are used to incubate algae samples over a constant incubation period, and the substrate concentrations are measured before and after incubation. Measuring depletion is commonly done by either measuring radioactive or a stable isotopes' uptake (Glibert et al., 1982; Naldi and Wheeler, 2002), or by following the disappearance of the nutrient from the medium by collecting water samples, and measure the water samples colorimetrically (Harrison and Druhel, 1982; Harlin and Wheeler, 1995; Harrison et al., 1989). Nitrogen is most commonly measured following the disappearance of inorganic nitrogen from the medium using one of the two technique or a combination of both (Pedersen, 1994). A short incubation time (<10-15 min) is likely to yield an estimate of the gross uptake rate, whereas a long incubation time (>6 h) would give an estimate for the net uptake, taking the release of nutrients from the thallus into account (Hurd et al., 2014).

2 Aim of study

This study was a part of the MACROSEA project, aiming to investigate the potential of a successful and predictable industrial macroalgae production in Norway. The overall aim of this study was to increase the knowledge of NH_4^+ uptake rate in *S. latissima* by characterising the initial uptake of NH_4^+ in specimens with different nutritional histories. The focus of this thesis was to address the potential of utilizing effluent DIN from intensive salmon in the cultivation of *S. latissima* in an IMTA system.

The NH_4^+ characterisation was performed based on two objectives:

1) To describe the time- and concentration dependent changes of the initial NH_4^+ uptake by *S. latissima*.
2) To characterize the uptake of NH_4^+ with additional NO_3^- available at a potentially natural occurring concentration.

To characterize the NH_4^+ uptake in young *S. latissima*, two main experiments were conducted using a combination of the perturbation and multiple flask techniques. The experiments consisted of an ammonium study with only NH_4^+ available and a preference study with both NH_4^+ and NO_3^- available. Each study was conducted on *S. latissima* with both sufficient and deficient internal nitrogen storages to assess the effect of nutritional history on uptake. Uptake was determined by following the depletion of different initial substrate concentrations ranging between 0.25-16 μM over an incubation period of 5 hours.

Three hypotheses were formulated:

H_1: The NH_4^+ uptake rate will increase linearly with increased exposure concentration.

H_2: NH_4^+ is the preferred nitrogen source over NO_3^- for *S. latissima*.

H_3: The nutritional history is affecting the uptake, where *S. latissima* with deficient internal nitrogen storages will have a higher uptake rate than *S. latissima* with sufficient internal nitrogen storages.

3 Materials and methods

3.1 Collection and storage of *S. latissima*

S. latissima, deployed in December, was collected on the 21.03.2017 at Seaweed Energy Solution AS (SES) concession site at Taraskjæret, Frøya (63^{o} 42' N, 8^{o} 52' E). 75 meters of cultured rope was harvested from this location, and transported to Trondheim in wet plastic bags. The seaweed was stored in flow-through tanks at Seaweed Energy Solutions site overnight, supplied with filtrated deep water from 80 m depth. The next day, the seaweed was transported to Trondheim Biological Station (TBS).

3.2 Acclimation of *S. latissima* to saturated and depleted nitrogen conditions

Individuals of *S. latissima* for use in the experiment were chosen randomly from the sample delivered from SES, with a lamina length within the range 7-13 cm (Figure 3.1a). The *S. latissima* was then transferred to two different tanks: A) for acclimation to high nitrogen concentration (sufficient internal nitrogen pools), and B) for acclimation to low nitrogen concentrations (deficient internal nitrogen pools). Both tanks were provided artificial light sources set to a daily cycle of 12 hour of light and 12 hours of dark.

A) *Nitrogen sufficient conditions*: 750 individuals were transferred to a flow-through tank supplied with filtrated deep water from 100 m depth outside of TBS. The tank supplied with deep water had sufficient circulation of the water body, supplied by the inlet and outlet, for the individuals to circulate throughout the tank. *S. latissima* supplied with deep water were incubated for 2-3 days before the first uptake experiment.

B) *Nitrogen deficient conditions*: 750 individuals were transferred to another closed tank (e.g. no inlet and outlet) containing approximately 60 litres of artificial seawater enriched with modified f/2 medium without nitrogen and silicate (Guilard, 1975). Circulation was ensured by an aquarium flow pump, and air pumps with bubble stones provided aeration to the water. *S. latissima* stored in nitrogen depleted artificial seawater were incubated for 8 days before the first uptake experiment with deficient specimens. Approximately half of the water was changed 3 times over the incubation period, before used in the experiment to ensure depletion in the

seaweeds' internal nitrogen storages. Both degradation and growth of the lamina was observed over the incubation period (Figure 3.1b).

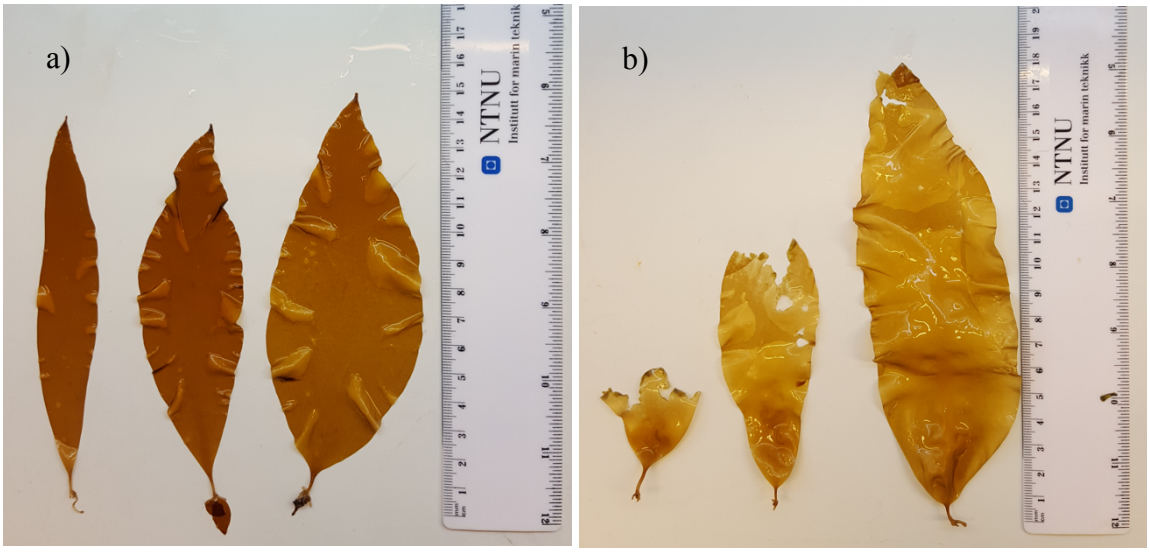

Figure 3.1: Individuals of *S. latissima* representing a sample of diffferent shapes available within equal length. No apparent correlation between length and width was observed. Seaweed were sorted based on lamina length only, within 7-13 cm. **a)** Different shapes available of *S. latissima* at equal length found at the beginning of the experiment. **b)** Difference in degree of degradation of the lamina observed for individuals incubated in nitrogen depleted conditions at the last day of the experiment. Some lamina had grown several cm (right: approx.18 cm) while other had degraded to almost half of the initial length (left: approx. 4 cm) after 9 days in nitrogen depleted conditions.

3.3 Creation of artificial seawater

The artificial seawater used in the experiment was created according to Kester et al., 1967. The artificial seawater was enriched with Guillard's f/2 growth medium (Guillard, 1975). This f/2 growth medium was modified to be depleted of nitrogen and silicate.

3.4 Experimental technique

The nitrogen-uptake studies were conducted with a combination of the perturbation- and the multiple flask techniques (Harrison and Druehl, 1982). The experiments were run with several perturbation experiments with *S. latissima* being exposed to multiple initial concentrations of NH_4^+ over a gradient. The decline in substrate was therefore frequently monitored for several different exposures over a constant incubation period of 5 hours.

3.5 Experimental setup

The nitrogen-uptake study was conducted with *S. latissima* coming from sufficient and deficient conditions of nitrogen. The *S. latissima* were placed in artificial seawater added modified f/2 medium (without nitrogen and silicate) and exposed to different concentrations of NH_4^+ and a combination of NH_4^+ and NO_3^- as nitrogen sources. Two uptake experiments were conducted with *S. latissima* initially deficient of and sufficient with internal nitrogen storages:

I. An ammonium study to investigate the uptake of NH_4^+ as the only available nitrogen source for sufficient (AS) and deficient (AD) *S. latissima*, added to a concentration gradient ranging from 0.25-16 μM. The setup of the study is presented in Table 3.1. In addition to 7 concentrations with 6 parallels each, two control samples without *S. latissima* were included corresponding to the lowest (0.25 μM) and the highest concentration (16 μM) in the gradient.

Table 3.1: Setup of the ammonium study, consisting of a gradient of 7 initial concentrations of NH_4^+ at 0.25, 0.50, 1, 2, 4, 8 and 16 μM. There were 6 replicates for each concentration (R1-R6). Two controls without *S. latissima* were included with one for the lowest concentration and one for the highest concentration in the gradient.

NH_4^+ gradient	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**Control**
0.25 μM	0.25	0.25	0.25	0.25	0.25	0.25	0.25
0.50 μM	0.50	0.50	0.50	0.50	0.50	0.50	-
1 μM	1	1	1	1	1	1	-
2 μM	2	2	2	2	2	2	-
4 μM	4	4	4	4	4	4	-
8 μM	8	8	8	8	8	8	-
16 μM	16	16	16	16	16	16	16

II. A preference study to investigate the uptake of NH_4^+ and NO_3^- with both NH_4^+ and NO_3^- as nitrogen sources for initially sufficient (PS) and deficient (PD) *S. latissima*. NH_4^+ was added in a concentration gradient ranging from 0.25-16 μM equal to experiment I. Each replicate was added an additional 1 μM NO_3^-. The setup of the preference study is presented in Table 3.2. In addition, two control samples without *S. latissima* were included corresponding to the lowest (0.25 μM) and the highest concentration (16 μM) of NH_4^+ in the gradient.

Table 3.2: Setup of the preference study, consisting of a gradient of 7 initial concentrations of NH_4^+ at 0.25, 0.50, 1, 2, 4, 8 and 16 µM. Each replicate was added an additional 1 µM NO_3^-. There were 6 replicates for each concentration (R1-R6). Two controls without *S. latissima* were included, with one at the lowest concentration and one at the highest concentration of NH_4^+ in the gradient.

Gradient		**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**Control**
0.25	NH_4^+	0.25	0.25	0.25	0.25	0.25	0.25	0.25
µM	NO_3^-	1	1	1	1	1	1	1
0.50	NH_4^+	0.50	0.50	0.50	0.50	0.50	0.50	-
µM	NO_3^-	1	1	1	1	1	1	-
1	NH_4^+	1	1	1	1	1	1	-
µM	NO_3^-	1	1	1	1	1	1	-
2	NH_4^+	2	2	2	2	2	2	-
µM	NO_3^-	1	1	1	1	1	1	-
4	NH_4^+	4	4	4	4	4	4	-
µM	NO_3^-	1	1	1	1	1	1	-
8	NH_4^+	8	8	8	8	8	8	-
µM	NO_3^-	1	1	1	1	1	1	-
16	NH_4^+	16	16	16	16	16	16	16
µM	NO_3^-	1	1	1	1	1	1	1

The two uptake experiments for the initially sufficient *S. latissima* (AS and PS) that were incubated in deep water, were run on two consecutive days. Similarly, the uptake experiments for the initially nitrogen deficient *S. latissima* (AD and PD) that were incubated under nitrogen deficient conditions for eight days were also run on two consecutive days. The ammonium study (I) was run first and the preference study (II) afterwards for both the experiments with sufficient and deficient *S. latissima*. In each experiment, new specimens were used.

Stock solutions of NH_4^+ and NO_3^- were created in advance of the experiment. These nitrogen stock solutions were used to create the NH_4^+ and NO_3^- gradients in the uptake experiments. The incubation and the experiments were conducted in a temperature regulated room at 10 ^oC.

The setup was identical for all uptake experiments, and is presented in Figure 3.2. The ammonium and the preference study's concentration gradients consisted of the same 7 concentrations of NH_4^+ (0.25 – 16 µM), and the preference study had an additional constant concentration of 1 µM NO_3^-. For each concentration, there were 6 replicates. Two control samples without seaweed were included, corresponding to lowest and highest concentration in the gradients (0.25 and 16 µM). NH_4^+ and NO_3^- stock solutions were made in advance. All replicate beakers were added nitrogen stock solution to create the wanted concentration, and

filled up to 250 mL with artificial seawater. All replicates were placed on stirring tables, and all replicates in the different concentration were set up equally. Artificial light sources were placed behind each stirring table to ensure sufficient photosynthetic activity.

Figure 3.2: Setup of the uptake experiment. In this picture, the NH_4^+ concentration in the gradient ranging from 0.25-2 µM are presented. All replicates were added the concentration of the nitrogen source wanted and filled up to 250 mL with artificial seawater with modified f/2 growth medium depleted of nitrogen and silicate. All flasks were placed on a stirring table with a light source illuminating from behind. Within each concentration, replicates were set up equally. The arrangement of the replicates is marked for the samples ranging between 0.25-2 µM in the gradient. Each flask was added 5 individuals of *S. latissima* at the start of the experiment. During the experiment, the stirring table was set to 100 rpm. A similar stirring table was used for remaining concentrations in the gradient (4-16 µM).

At the start of each experiment, 5 new specimens of *S. latissima* were randomly collected from the appropriate incubation tank and added to each replicate, except to the control flasks. The flasks were placed on a stirring table, and the stirring was set to 100 rounds per minute (rpm). At the addition of seaweed to the first beaker the time was recorded using a stopwatch. Water samples were collected from each beaker at set intervals using a pipette. 2 mL water samples were taken at 5, 10, 20, 30, 50, 90, 180 and 300 minutes after addition of seaweed. Samples from the controls were taken at first and last sampling point only. Samples were transferred to pre-marked 15 mL plastic tubes and frozen until analysis. After the uptake experiment, every *S. latissima* from each replicate was gently pat dry with paper towels and total biomass was weighed and recorded. Two individuals from each replicate were weighed individually, added

to pre-marked tinfoil sheets and dried in a drying cabinet at 80 ^{o}C for 24 hours. After drying, they were individually weighed and percent dry weight was calculated. From these dry weight percentages, a total dry weight estimate was determined for each flask.

The *S. latissima* used in the experiments with seaweed coming from the nitrogen deficient condition were, to the extent available, chosen within the set lamina size range of 7-13 cm. Due to both degradation and growth of the lamina under the deficient conditions, there were a lack of a few individuals with a full lamina and suitable size. Some smaller and some larger individuals were therefore chosen.

3.6 Chemical analysis of ammonium and nitrate in water samples

Prior to analysis, frozen water-samples from the uptake experiments were thawed at room temperature and filtrated using a 0.45 μm syringe filter to remove particles. Water samples were analysed photometrically for NH_4^+ and NO_3^- in a Flow Solution IV System, O. I. Analytical AutoAnalysator. NH_4^+-N was determined following Norwegian Standard 4746 (NSF, 1975a) and NO_3^--N was determined following Norwegian Standard 4745 (NSF, 1975b).

3.6.1 Norwegian standard 4746

NS4746 was used to analyse the concentration of NH_4^+-N in the water samples. The principle behind this analysis is to create the blue complex indophenol blue from NH_4^+, which is measured spectrophotometrically at a wavelength of 630 nm. In a weak alkaline solution (pH=10.8-11.4), NH_4^+ reacts with hypochlorite under the creation of monochloramine, which in the presence of phenol and an excess of hypochlorite creates indophenol blue. The reaction is catalysed by pentacyanonitrosylferrate. The lower detection limit is approximately 1 μg L^{-1} (NSF, 1975a).

3.6.2 Norwegian standard 4745

NS4745 was used to analyse the concentration of NO_3^--N in the water samples. The principle behind this analysis is to reduce NO_3^- to NO_2^-, and then transform the NO_2^- to an azo-colorant which is measured spectrophotometrically at a wavelength of 545 nm. NO_3^- was reduced to NO_2^- in a buffer solution (pH=8.0-8.5) using copper-coated cadmium. NO_2^- is then transferred

to a sour solution (pH=1.5-2.0), where it reacts with sulphanilamide to create a diazo-compound, which is then coupled with N-(1-napthyl)-etylendiamine to an azo colorant. The concentration of NO_3^- in the samples were determined by the measurement of the total amount of NO_2^- following the reduction of NO_3^- to NO_2^-. As NO_2^- in seawater is found at negligible concentrations, all measured NO_2^- was determined to be NO_3^-. The lower detection limit is approximately 1 $\mu g\ L^{-1}$ (NSF, 1975b).

3.7 Pilot study

Prior to the main experiment a small-scale pilot study was conducted to assess the methods. *S. latissima* and *Laminaria digitata* was collected in the low tide at Storsteinan, Trondheim. The individuals' lamina lengths were approximately 10 cm.

L. digitata were incubated in artificial seawater with modified f/2 medium without nitrogen and silicate over two weeks. The artificial seawater was replaced every second day and 3 individuals were removed and frozen until analysis. Individuals taken out over the two-week period were analysed for internal NO_3^- to follow depletion of the internal nitrogen storages.

An uptake experiment was run with a similar set-up as used in the main experiment. *S. latissima* was incubated in a concentration gradient of 0.25, 2 and 8 $\mu M\ NO_3^-$, and *L. digitata* was incubated in 0.25 and 8 $\mu M\ NO_3^-$. For each initial concentration, there were used 3 replicates. The replicates were added 250 mL of artificial nitrogen deficient seawater and added nitrogen corresponding to the exposure gradient, and 5 individuals. In addition, control samples were used without seaweed for the lowest and highest concentration (0.25 and 8 μM). 2 mL water samples for were taken out using a pipette at time intervals: 5, 10, 20, 50, 90, 180 and 300 min. Controls were sampled at first and last interval only. The water samples were analyzed for NO_3^--N in a Flow Solution IV System, O. I. Analytical AutoAnalysator, following Norwegian Standard 4745 (NSF, 1975b), see section 3.6.2.

After the uptake experiment, 2 individuals from each replicate were removed for determination of dry weight, and the remaining 3 individuals were frozen for analysis of internal NO_3^-. Dry weight was estimated by drying the seaweed in a drying cabinet at 80 ^oC for 24 hours. Internal NO_3^- was analysed for by thawing frozen individuals in room temperature, cutting up the

material and weighing out 0.12 g that was transferred to test tubes. To these test tubes, 12 mL of distilled water was added and the samples were boiled for 30 min. After boiling, the samples were cooled and filtrated with 0.45 μm syringe filters to remove particles. Internal NO_3^- was also analysed for in a Flow Solution IV System, O. I. Analytical AutoAnalysator, following Norwegian Standard 4745 (NSF, 1975b).

Based on the results from the pilot study, it was concluded that the setup of the experiment was suitable to follow the reduction of substrate concentration over time. An additional time interval for sampling was added at 30 min to better follow the initial uptake. It was also concluded that 7 days of incubation in modified nitrogen-deficient artificial seawater was sufficient for depletion of internal nitrogen storages in the *S. latissima*.

3.8 Calculation of uptake rate

Substrate concentrations of NH_4^+, and NO_3^- in the preference study, were measured at each sampling point to follow the change in concentration over time. The substrate concentrations were related to biomass (gram dry weight; gDW) of *S. latissima* in each flask and subsequently used to calculate uptake rates. The uptake rates were determined in each replicate flask between each sampling period according to equation 1 (Pedersen, 1994):

$$V = \frac{[(S_0 * vol_o) - (S_t * vol_t)]}{(t * B)} \quad (1)$$

Uptake rates (V) were based on changes of substrate concentration per gDW (μM gDW^{-1}) over time (h) and correspondingly expressed as μM gDW^{-1} h^{-1}. Calculations were based on the initial substrate concentration (S_0) and volume (vol_0) at first sample of the interval, change in substrate concentration (S_t) and volume (vol_t) at the end of the sampling interval, the time elapsed between the two samples (t) and the biomass (B) of *S. latissima* in dry weight.

3.9 Data treatment and statistical analysis

Large outlying substrate concentrations were determined to be removed from the data set as they were likely to represent contaminations or errors in the analysis. Samples that were higher or lower than 3 times the mean of the replicates at the sampling point were therefore removed from the data set. Discretion was used prior to removal, especially for low values in the samples with initially deficient *S. latissima*, were reduction was large and outlying values may represent rapid removal. Outlying values were removed from the data prior to analysis and plotting.

Microsoft Excel 2016 was used to calculate uptake rates for all experiments and to organize all data in tables. Testing for how one parameter (e.g. substrate concentration) was affected by another (e.g. time) was done by making linear regressions and one-way ANOVA tests for approximately normally distributed data. Data strongly deviating from normal distribution was analysed using the nonparametric Kruskal-Wallis test. Comparison of two groups (e.g. sufficient vs deficient *S. latissima*, or different initial concentrations in the gradient) was done using two-sample Student's t-test for approximately normally distributed data. Groups strongly deviating from normal distribution were compared using the nonparametric Mann-Whitney U test. All statistical analysis' are represented with a significance level $p \leq 0.05$. Normality in data was checked for by creating histograms of residual values and Shapiro-Wilk test. Homoscedasticity was tested for using Bartlett's test. All statistical analysis' and all plots were conducted and made in R Studio (RStudio, 2015).

All data were plotted in box plots. Box plots provide a useful way to represent the distributional characteristics of a set of grouped values (Figure 3.3). In a box plot, the dataset is placed within four quartiles representing the distribution, excluding the outlying values. The box represents the centre 50 % of the data, the interquartile range (IQR), and marks the median value. The whiskers represent the upper- (4^{th}) and lower (1^{st}) quartiles with the outer 25 % values. The peaks of the whiskers mark the maximum and minimum values within the quartiles. Outliers are defined as values more than 1.5 times the IQR in either direction.

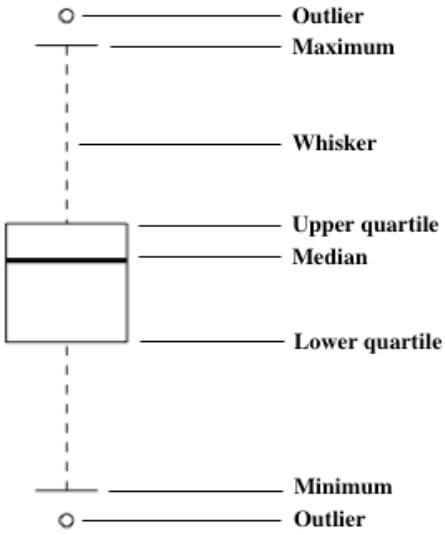

Figure 3.3: Example of a boxplot with the different characteristics of the plot marked.

4 Results

4.1 Biomass of *S. latissima*

The percent DW of the *S. latissima* was determined to be 10.9±1.5%, based on all specimens weighed after drying. From this %DW, the mean biomass (gDW) in the replicate flasks in the different experiments were 0.28±0.07g in experiment AS, 0.26±0.07g in AD, 0.26±0.06g in PS, and 0.27±0.07g in PD. Generally, no apparent differences in biomass were observed between all the different samples. However, the biomass in experiment AS was observed to be significantly lower than in experiment AD and PS in the 2 μM samples, and significantly higher than in experiments AD, PS and PD in the 4 μM samples.

4.2 Substrate concentration in the control samples

In the control samples without seaweed, no obvious changes in substrate concentration were observed over the course of the experimental period for most treatments. In the control sample in experiment AD exposed to 16 μM, an 81% increase in NH_4^+ concentration was observed from the first 5 min sample to the last sample at 5 hours. This is most likely due to a contamination. Any changes in substrate concentration with seaweed present was therefore regarded as due to the *S. latissima* only.

4.3 Reduction of ammonium substrate concentration

4.3.1 Reduction in ammonium substrate concentration in the ammonium study: AS and AD

Reduction of NH_4^+ substrate concentration per gram DW *S. latissima* for initially sufficient (AS) and deficient (AD) specimens are presented in Figure 4.1 a-g). In the figures, grey boxes represent experiment AS and white boxes represent experiment AD. Mean concentrations (μM gDW^{-1}) for each sampling point are given in Table A1 for AS and Table A2 for AD (Appendix A).

In experiment AS, average substrate concentrations (μM±SD) measured at the first sampling point for the gradient a) 0.25, b) 0.50, c) 1, d) 2, e) 4, f) 8 and g) 16 μM were similar to the initial exposure and found to be 0.90±1.75, 1.17±0.05, 1.49±0.78, 2.32±0.96, 4.22±0.89, 7.09±0.11 and 15.62±0.93 μM, respectively. A significant decrease in substrate concentration gDW^{-1} with time was observed for all treatments, and found after: a) 90, b) 90, c), 30, d), 30, e) 20, f) 50 min and g) 50 min from start (Table A1, Appendix A).

In experiment AD, the average substrate concentrations (μM±SD) measure at the first sampling point in the gradient a) 0.25, b) 0.50, c) 1, d) 2, e) 4, f) 8 and g) 16 μM were found to be 0.90±0.05, 0.56±0.06, 1.50±0.87, 2.12±0.95, 4.10±1.36, 7.67±2.66 and 13.27±3.18 μM, respectively. Also in this experiment, there were measured concentrations after 5 min that were corresponding to the initial substrate concentration for most samples. There were observed significant reductions in substrate concentration gDW^{-1} with time, and this was generally observed earlier for the deficient than for the sufficient *S. latissima*. A significant reduction was observed for the different exposures after a) 20, b) 30, c) 20, d) 20, e) 20, f) 30 and g) 20 min from start (Table A2, Appendix A). In this experiment, all NH_4^+ was removed from the substrate medium (e.g. taken up by the *S. latissima*) at a) 90 min, b) 90 min, c) 180 min, d) 90 min, e) 180 min, f) 300 min and g) 90 min. This was not observed in experiment AS.

A significant difference in substrate concentration gDW^{-1} was observed between experiments AS and AD at several sampling points (Table A5, Appendix A). The *S. latissima* coming from the deficient conditions were observed to have a significantly lower substrate concentration than when coming from the sufficient conditions at: 0.25 μM) all intervals, 0.50 μM) 5 min and 20-300 min, 1 μM) all intervals, 2 μM) all intervals, 4 μM) 180-300 min, 8 μM) 30-300 min and 16 μM) 20- 300 min.

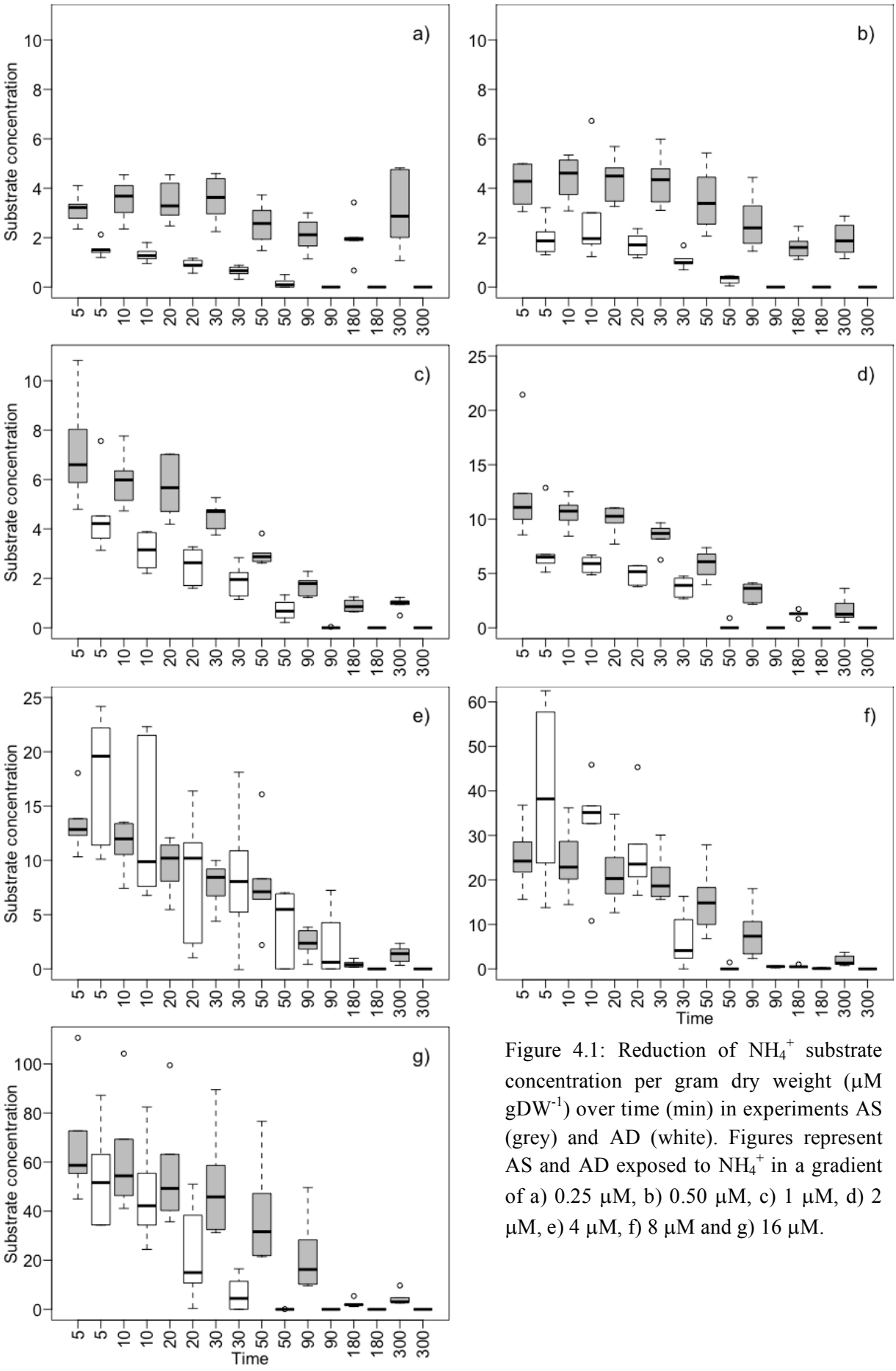

Figure 4.1: Reduction of NH_4^+ substrate concentration per gram dry weight (µM gDW^{-1}) over time (min) in experiments AS (grey) and AD (white). Figures represent AS and AD exposed to NH_4^+ in a gradient of a) 0.25 µM, b) 0.50 µM, c) 1 µM, d) 2 µM, e) 4 µM, f) 8 µM and g) 16 µM.

4.3.2 Reduction in ammonium substrate concentration with additional nitrate available in the preference study: PS and PD

Reduction of NH_4^+ substrate concentration gDW^{-1} with an additional availability of NO_3^- for initially sufficient (PS) and deficient (PD) *S. latissima* are presented in Figure 4.2 a-g). In the figures, grey boxes represent experiment PS and white boxes represent experiment PD. Mean concentrations (μM gDW^{-1}) for each sampling point are given in Table A3 for PS and Table A4 for PD (see Appendix A).

In experiment PS, average NH_4^+ substrate concentrations (μM±SD) measured at the first sampling point for the gradient a) 0.25, b) 0.50, c) 1, d) 2, e) 4, f) 8 and g) 16 μM were found to be 1.37±0.98, 1.80±1.21, 2.24±1.04, 2.99±1.29, 5.30±1.20, 9.92±1.19 and 19.70±0.99 μM, respectively. This shows that after 5 min there was measured corresponding concentrations to initial concentration for some samples, and several higher concentrations for other samples. A significant decrease in substrate concentration gDW^{-1} with time was observed after: a) 90, b) 180, c) 50, d) 30, e) 90, f) 90 and g) 50 min from start (Table A3, Appendix A).

In experiment PD, average substrate concentration (μM±SD) measure at the first sampling point in the gradient a) 0.25, b) 0.50, c) 1, d) 2, e) 4, f) 8 and g) 16 μM was found to be 1.57±1.04, 1.75±0.95, 2.24±0.96, 2.94±1.21, 5.65±1.39, 9.63±0.97 and 20.46±1.10 μM, respectively. Also in this experiment, there were measured concentrations were corresponding to the initial concentration for some samples, while there were several higher concentrations for other samples. There were observed significant reductions in substrate concentration gDW^{-1} with time, and this was generally observed earlier in the samples with initially deficient than in the sufficient *S. latissima*. A significant reduction was observed for the different exposure after: a) 180, b) 20, c), 20, d) 30, e) 20, f) 20 and g) 50 min from start (Table A4, Appendix A). Comparing the substrate concentration gDW^{-1} between the samples with sufficient and deficient *S. latissima*, it was observed earlier significant reductions in the 0.50, 1, 2, 4 and 8 μM samples in the gradient for the deficient than the sufficient *S. latissima*, similar at exposure to 16 μM and earlier for the sufficient than for the deficient *S. latissima* in the 0.25 μM sample.

A significant difference in substrate concentration gDW^{-1} at each sampling point between experiments PS and PD was observed at a few sampling points only (Table A6, Appendix A). There were observed significant differences where the deficient *S. latissima* had significantly

lower substrate concentration gDW^{-1} than the sufficient *S. latissima* at: 0.50 μM) 180-300 min, 2 μM) 300 min, 4 μM) 180-300 min, 8 μM) 90-300 min, and 16 μM) 90-300 min. A significantly higher concentration gDW^{-1} was observed in the samples initially exposed to 1 μM at 5 min only in the samples with seaweed coming from the sufficient conditions. No difference was observed for the initial exposure to 0.25 μM.

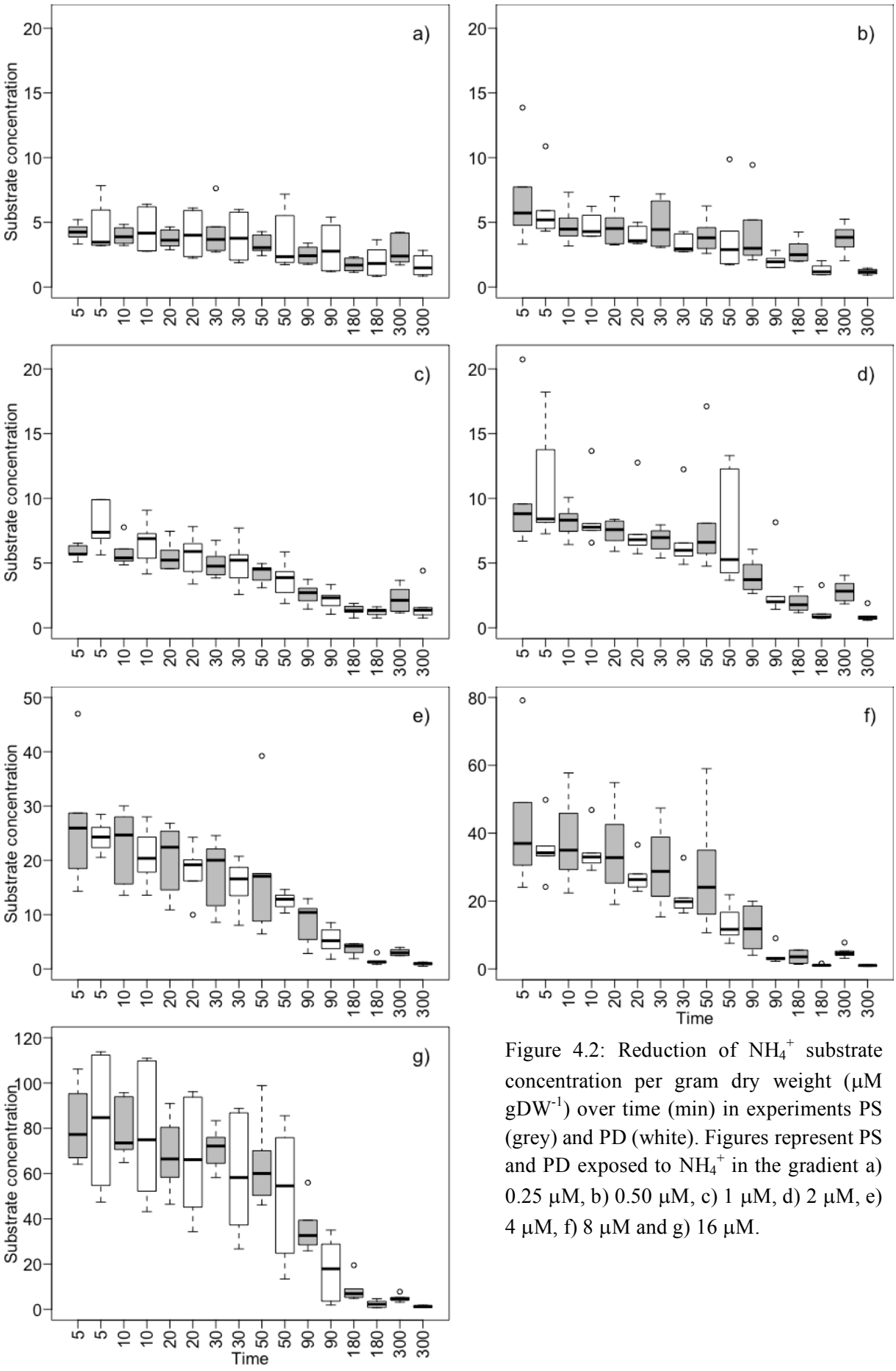

Figure 4.2: Reduction of NH_4^+ substrate concentration per gram dry weight (µM gDW^{-1}) over time (min) in experiments PS (grey) and PD (white). Figures represent PS and PD exposed to NH_4^+ in the gradient a) 0.25 µM, b) 0.50 µM, c) 1 µM, d) 2 µM, e) 4 µM, f) 8 µM and g) 16 µM.

4.3.3 Comparison of ammonium substrate concentration with and without additional nitrate available as substrate

There were observed significant differences in NH_4^+ substrate concentration (μM gDW^{-1}) between the ammonium study and the preference study at several sampling points within almost all the different samples in the concentration gradient. There were observed significantly lower substrate concentrations in experiment AS than in PS at: 0.25 μM) 5 min, 0.50 μM) 180-300 min, 1 μM) 50-300 min, 4 μM) all intervals except 50 min, 8 μM) 180 min, and 16 μM) 50 and 180 min. There were also observed significantly higher substrate concentration in experiment AS in the 2 μM samples between 10-30 min. For the samples with initially sufficient *S. latissima*, significant differences are given in Table A7 (Appendix A).

For *S. latissima* coming from nitrogen deficient conditions, there were observed higher rates of removal of NH_4^+ when NO_3^- was not present (AD) than when NO_3^- was present (PD) for all initial NH_4^+ exposures, and consequently there were observed more significant differences for these samples. All differences were due to a higher concentration in experiment PD than in AD, and the significant differences were observed at: 0.25 μM) all intervals, 0.50 μM) all intervals except 10 min, 1 μM) all intervals, 2 μM) all intervals except 5 min, 4 μM) all intervals except 10 and 90 min, 8 μM) 30-300 min and 16 μM) 20-300 min. The significant differences observed are given in Table A8 (Appendix A).

4.4 Reduction in nitrate substrate concentration with a gradient in availability of ammonium substrate

Change of NO_3^- substrate concentration gDW^{-1} in samples with *S. latissima* coming from sufficient and deficient conditions in experiments PS and PD, exposed to an initial concentration of 1 μM NO_3^- in addition to a) 0.25, b) 0.50, c) 1, d) 2, e) 4, f) 8 and g) 16 μM NH_4^+ are presented in Figure 4.3 a-g). In the figures, grey boxes represent experiment PS and white boxes represent experiment PD.

Change in substrate concentrations (μM gDW^{-1}) over time in the different samples in experiment PS, together with related p-values marking degree of change, are given in Table A9 (Appendix B). A significant decrease in NO_3^- substrate concentration gDW^{-1} in experiment PS

was observed after 180 min from start for all the initial NH_4^+ concentrations, except for the 1 μM samples, where a significant decrease was observed already after 90 min. Similarly, change in substrate concentrations (μM gDW^{-1}) over time in the different samples in experiment PD, together with related p-values marking degree of change, are given in Table A10 (Appendix B). A statistically significant decrease in NO_3^- substrate concentration gDW^{-1} in experiment PD was observed after 30 minutes in the 4 μM sample, 90 minutes in the 0.25, 0.50, 2 and 8 μM samples, 180 minutes in the 1 and 16 μM samples.

When comparing the NO_3^- substrate concentration (μM gDW^{-1}) per sampling point between these two experiments, some differences were observed. The substrate concentrations were significantly higher in experiment PS than in PD at 5-90 minutes in the 0.25 μM samples, at 90 minutes in the 0.50 μM samples, and 10-90 minutes in the 2 μM samples. In the 4 μM sample the substrate concentrations were significantly higher in experiment PD than in PS from 5-30 minutes. Overall, no apparent differences in concentration were observed for samples initially exposed to 1 μM, 8 μM and 16 μM. No differences were observed after 90 minutes. The significant differences observed are given in Table A11 (Appendix B).

In both experiments PS and PD, there were observed higher NO_3^- concentrations at the first sampling point than what was initially added. In experiment PS, average NO_3^- substrate concentrations (μM±SD) measure at the first sampling points in the samples in the gradient a) 0.25, b) 0.50, c) 1, d) 2, e) 4, f) 8 and g) 16 μM of NH_4^+ were found to be 2.86±0.46, 2.70±1.10, 2.55±0.56, 2.17±0.44, 1.90±0.27, 2.05±0.20 and 1.70±0.40 μM, respectively. Similarly, in experiment PD, average NO_3^- substrate concentrations (μM±SD) measure at the first sampling points in the samples in the gradient a) 0.25, b) 0.50, c) 1, d) 2, e) 4, f) 8 and g) 16 μM of NH_4^+ were found to be 1.76±0.45, 2.64±1.08, 1.95±0.34, 1.62±0.29, 3.23±0.55, 1.91±0.51 and 1.79±0.31 μM, respectively.

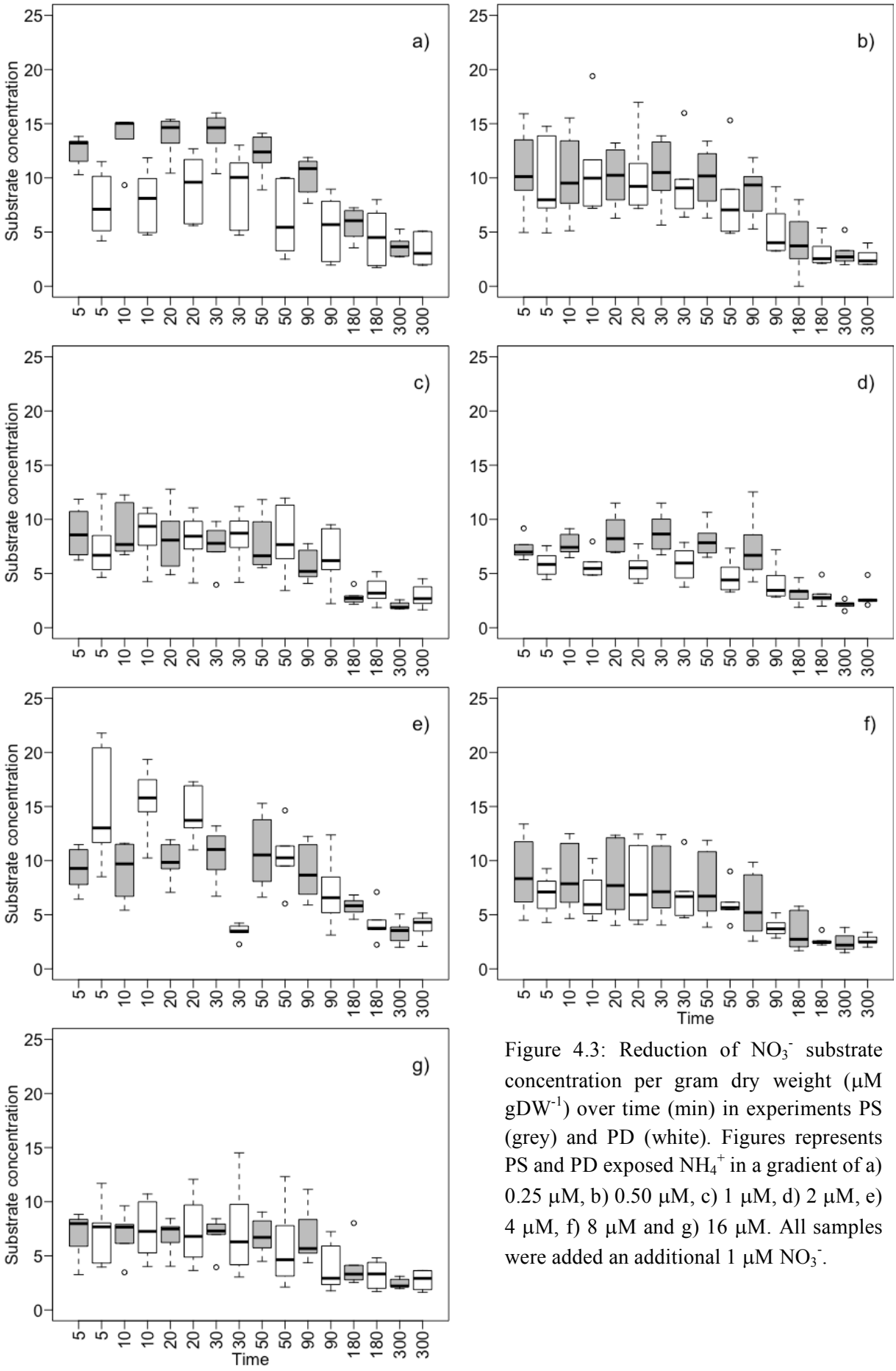

Figure 4.3: Reduction of NO_3^- substrate concentration per gram dry weight (µM gDW^{-1}) over time (min) in experiments PS (grey) and PD (white). Figures represents PS and PD exposed NH_4^+ in a gradient of a) 0.25 µM, b) 0.50 µM, c) 1 µM, d) 2 µM, e) 4 µM, f) 8 µM and g) 16 µM. All samples were added an additional 1 µM NO_3^-.

4.5 Ammonium uptake rate

4.5.1 Change in ammonium uptake rate in the ammonium study: AS and AD

Uptake rates of NH_4^+ (μM gDW^{-1} h^{-1}) between each sampling point between 10-300 min in experiments AS and AD initially exposed to a) 0.25, b) 0.50, c) 1, d) 2, e) 4, f) 8 and g) 16 μM NH_4^+, are presented in Figure 4.4 a-g). Grey boxes represent AS, and white boxes represent AD. Degree of significant change in uptake rate over time, determined from the peak in uptake rate, along with related mean values for replicates (±SD) for each exposure in the gradient are given in Table A12 for AS and Table A13 for AD (Appendix C). The peak in uptake rate was determined to be the point where the average highest uptake rate took place. This was also the point where the single highest rates were observed in all samples.

A summary of the mean uptake rates (±SD) over the 5-hour period for experiment AS is presented in Table 4.1 for all initial concentrations in the gradient, together with the highest uptake rate and the time at which this peak took place. The mean uptake rates over the course of the experiment were similar for the initial concentrations of 0.25 μM and 0.50 μM, and they were steadily increasing with an increase in initial concentration. The peaks in uptake rate in the different samples varied between 10-50 minutes. The peaks in uptake rate appeared to take place at earlier periods with an increase in concentration, ranging from 50 min for the 0.25 μM samples, to 10 min for the 16 μM samples. It was also observed similar peaks in uptake rates between initial concentrations of 0.25 μM and 0.50 μM, and the peaks were increasing with an increase in initial concentration. In the 0.25 μM, 0.50 μM and 2 μM samples, the peaks were relatively high, being significantly different to the other rates observed over time, whereas the rates were more steadily developing for the 1 μM, 4 μM and 8 μM samples, with significant reductions observed only toward the end, between 90-300 minutes. In the 16 μM sample, the peak in uptake rate observed at 10 min was significantly higher than almost all the following rates over time. The degree of significant change over time is presented in Table A12 (Appendix C). There was observed several negative uptake rates during the first 30 minutes for the 0.25 μM and 0.50 μM samples, a few for the 2 μM and 4 μM samples at 10 minutes, and no negative uptake rates for the 1, 8 and 16 μM samples.

Table 4.1: NH_4^+ uptake rates (μM gDW^{-1} h^{-1}) for the different initial concentrations in experiment AS given as mean (±SD) over the course of the experiment, and the peak in uptake rate (±SD) and what time (min) this peak was observed.

Gradient	**0.25 μM**	**0.50 μM**	**1 μM**	**2 μM**	**4 μM**	**8 μM**	**16 μM**
Mean	0.06	0.07	0.73	1.02	1.62	2.64	7.61
(±SD)	(0.41)	(0.58)	(0.87)	(0.97)	(1.95)	(1.94)	(6.70)
Peak	0.76	0.67	1.76	2.35	2.70	3.96	17.89
(±SD)	(0.21)	(0.33)	(1.30)	(0.47)	(4.44)	(1.21)	(9.67)
Time	50	50	30	30	10	20	10

A summary of the mean uptake rates (±SD) over the 5-hour period for experiment AD is presented in Table 4.2 for all initial concentrations in the gradient, together with the highest uptake rate and the time at which this peak took place. As in experiment AS, the mean uptake rate for the initial concentration of 0.25 μM and 0.50 μM were similar, and they were increasing steadily with increased initial concentration. Similarly, the peaks in uptake rate for the initial concentrations of 0.25 μM and 0.50 μM were also similar, and they were increasing with an increase in initial concentration. There was not observed any consistent trends in where the peaks in uptake rate were observed, and the time they were observed ranged between 10-50 minutes. The change in uptake rate over time in this experiment appeared to be larger than it was in experiment AS, so that the peaks had more significant differences from the rest of the rates observed at the other intervals (Table A13, Appendix C). There were also observed few negative uptake rates, and this only appeared in the 0.50 μM and 4 μM samples.

Table 4.2: NH_4^+ uptake rates (μM gDW^{-1} h^{-1}) for the different initial concentrations in experiment AD given as mean (±SD) over the course of the experiment, and the peak in uptake rate (±SD) at where the peak (±SD) was observed and what time this peak was observed.

Gradient	**0.25 μM**	**0.50 μM**	**1 μM**	**2 μM**	**4 μM**	**8 μM**	**16 μM**
Mean	0.47	0.49	0.97	1.33	3.23	9.11	18.97
(±SD)	(0.43)	(0.48)	(0.68)	(0.98)	(4.21)	(11.73)	(18.60)
Peak	1.07	1.01	2.10	2.68	9.80	27.91	36.88
(±SD)	(0.64)	(0.28)	(0.72)	(0.57)	(3.93)	(11.74)	(12.21)
Time	10	30	10	50	20	30	20

Significant difference in uptake rates between the same sampling points in experiments AS and AD were observed for all the different exposures (Table A16, Appendix). There was generally a trend toward the initially deficient *S. latissima* having a significantly higher uptake rate at the early intervals (10-30 min), and the initially sufficient *S. latissima* having a higher uptake rate at the later intervals (90-300 min). Significantly higher uptake rates were observed in the

samples with initially deficient *S. latissima* than in the initially sufficient *S. latissima* at: 0.25 μM) 10 and 30 min, 0.50 μM) 30 min, 1 μM) 10 min, 2 μM) 50 min, 4 μM) 20 and 300 min, 8 μM) 10, 20, 30 min, and 16 μM) 20 and 30 min. Significantly higher uptake rates in the samples with initially sufficient *S. latissima* were observed at: 0.25 μM) 50 and 90 min, 0.50 μM) 90 and 180 min, 1 μM) 90 and 180 min, 2 μM) 30, 90 and 180 min, 8 μM) 90 and 180 min, and 16 μM) 90 and 180 min.

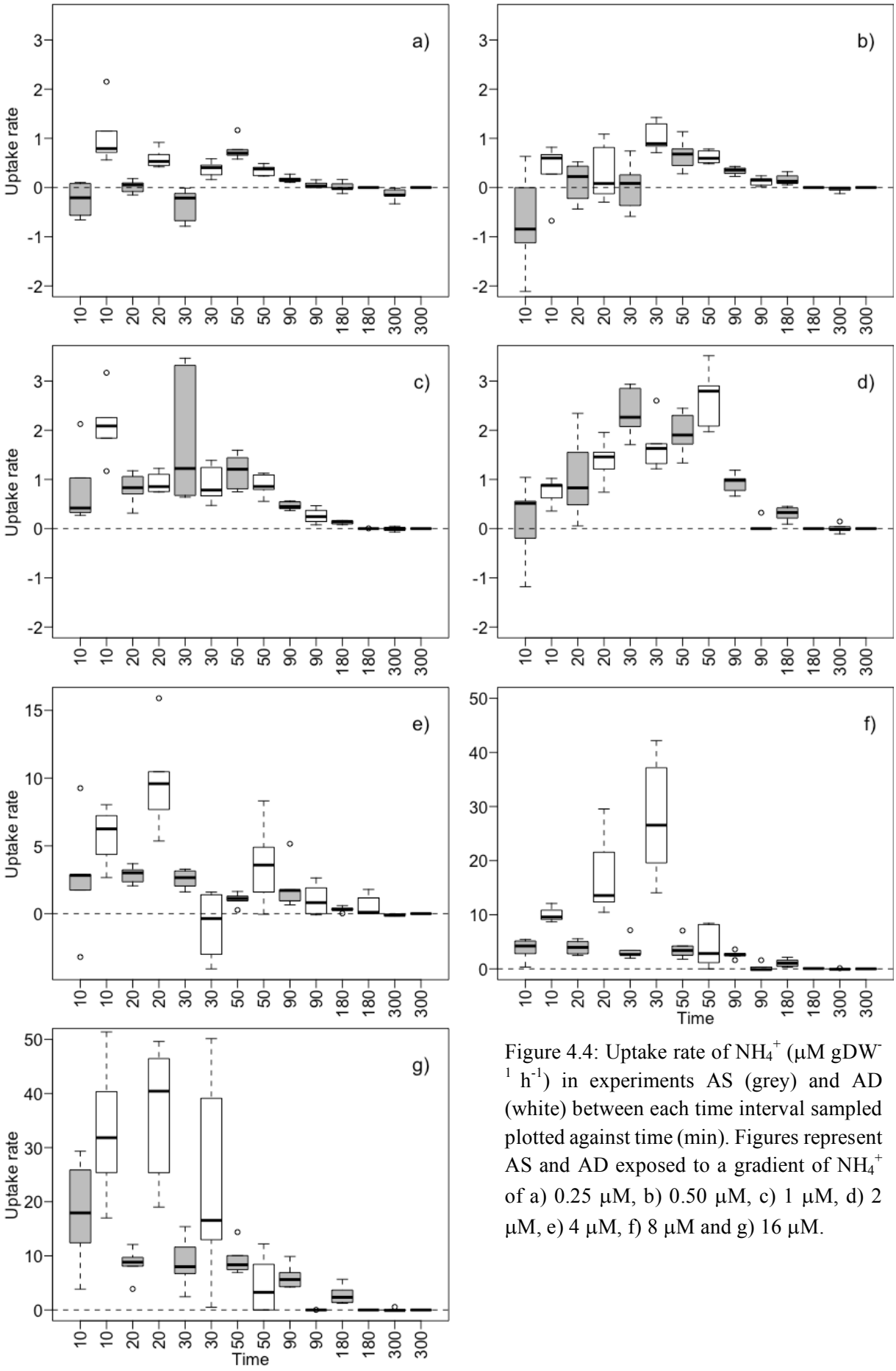

Figure 4.4: Uptake rate of NH_4^+ (µM gDW^{-1} h^{-1}) in experiments AS (grey) and AD (white) between each time interval sampled plotted against time (min). Figures represent AS and AD exposed to a gradient of NH_4^+ of a) 0.25 µM, b) 0.50 µM, c) 1 µM, d) 2 µM, e) 4 µM, f) 8 µM and g) 16 µM.

4.5.2 Change in ammonium uptake rate in the preference study: PS and PD

Uptake rates of NH_4^+ ($\mu M\ gDW^{-1}\ h^{-1}$) at each sampling point between 10-300 min in studies PS and PD exposed to a gradient of NH_4^+ of a) 0.25, b) 0.50, c) 1, d) 2, e) 4, f) 8 and g) 16, as well as an additional 1 μM NO_3^-, are presented in Figure 4.5 a-g). Grey boxes represent PS, and white boxes represent PD. Degree of statistically significant change in uptake rate over time, determine by the peak in uptake rate as well as single high values, along with related mean values between replicates (±SD) for each initial exposure are given in Table A14 for PS and Table A15 for PD (see Appendix C). The peak in uptake rate was determined to be the point where the average highest uptake rate took place. This was also the point where the single highest rates were observed in all samples.

A summary of the mean NH_4^+ uptake rates (±SD) over the 5-hour period for experiment PS is presented in Table 4.3 for all initial concentrations in the gradient, together with the highest uptake rate and the time at which this peak took place. The mean uptake rates over the course of the experiment were similar in the samples with the initial concentrations ranging between 0.25-2 μM. In the 4 μM, 8 μM and 16 μM samples, the rates were increasing with an increase in initial concentration. For all the samples, the peak in uptake rate was observed to take place between 10-20 minutes. The peaks in uptake rates showed similar trends as the mean uptake rates, and the samples with initial concentration ranging between 0.25-2 μM had similar uptake rates, whereas they were increasing with higher initial concentration. For the 4 μM, 8 μM and 16 μM samples, the peak in uptake rates were similar to the initial concentration they were exposed to, and they were approximately twice the average rate over the course of the experiment. For all samples, there were a general trend toward a decline in uptake rate from these early peaks, and at what time a significant reduction in uptake rate was observed ranged between 20-50 minutes for all samples. Generally, there was few negative uptake rates observed. In the 16 μM samples, there were observed several negative rates at 30 minutes.

A summary of the mean NH_4^+ uptake rates (±SD) over the 5-hour period for experiment PD is presented in Table 4.4 for all initial concentrations in the gradient, together with the highest uptake rate and the time at which this peak took place. The mean uptake rate over the course of the experiment only had a slight increase with an increase in initial concentration in the 0.25-2 μM samples, and a higher increase was observed with the higher initial concentrations.

Similarly, as in experiment PS for all the samples, the peaks were observed within 10-20 minutes. The peak in the 0.50 μM sample had the lowest peak in uptake rate, and for the 0.25 μM, 1 μM and 2 μM samples, the differences were low. For the higher initial concentrations, the peaks increased to a higher degree than what they did in experiment PS, and the peaks were consequently higher than the initial concentration they were exposed to. Also, for these samples, the peaks were approximately twice than the average rate over the course of the experiment. Only occasional negative uptake rates were observed.

Table 4.3: NH_4^+ uptake rates (μM gDW^{-1} h^{-1}) for the different initial concentrations in experiment PS given as mean (±SD) over the course of the experiment, and the peak in uptake rate (±SD) and what time (min) this peak was observed.

Gradient	**0.25 μM**	**0.50 μM**	**1 μM**	**2 μM**	**4 μM**	**8 μM**	**16 μM**
Mean	0.35	0.40	0.45	0.62	2.48	3.91	6.50
(±SD)	(0.50)	(0.67)	(0.39)	(0.75)	(1.94)	(2.89)	(8.80)
Peak	1.41	1.67	0.96	1.30	4.83	7.15	16.17
(±SD)	(0.60)	(0.96)	(0.32)	(0.84)	(2.68)	(2.96)	(10.69)
Time	10	10	10	20	10	10	20

Table 4.4: NH_4^+ uptake rates (μM gDW^{-1} h^{-1}) for the different initial concentrations in experiment PD given as mean (±SD) over the course of the experiment, and the peak in uptake rate (±SD) and what time (min) this peak was observed.

Gradient	**0.25 μM**	**0.50 μM**	**1 μM**	**2 μM**	**4 μM**	**8 μM**	**16 μM**
Mean	0.48	0.53	0.77	0.93	3.08	5.19	9.66
(±SD)	(0.87)	(0.64)	(0.62)	(0.71)	(2.90)	(4.47)	(8.54)
Peak	2.22	0.65	1.49	1.90	6.93	10.78	18.46
(±SD)	(1.42)	(1.31)	(0.35)	(0.77)	(4.79)	(2.48)	(12.66)
Time	10	10	20	10	10	20	10

Generally, the initially deficient *S. latissima* had higher uptake rates than the initially sufficient *S. latissima*, as seen from higher average rates and peaks. Significant differences in uptake rates between the same sample points between experiments PS and PD were observed for most of the different initial exposures (Table A17, Appendix C). Significantly higher uptake rates were observed in the initially deficient *S. latissima* than in the initially sufficient at: 0.25 μM) 300 min, 0.50 μM) 20, 30 and 300 min, 1 μM) 20, 50 and 300 min, 2 μM) 300 min, 8 μM) 20 min, and 16 μM) 30D min. A significantly higher uptake rate was observed in the initially sufficient *S. latissima* than in the initially deficient at 180 min in the 8 μM sample. No significant differences were observed for samples exposed to 4 μM.

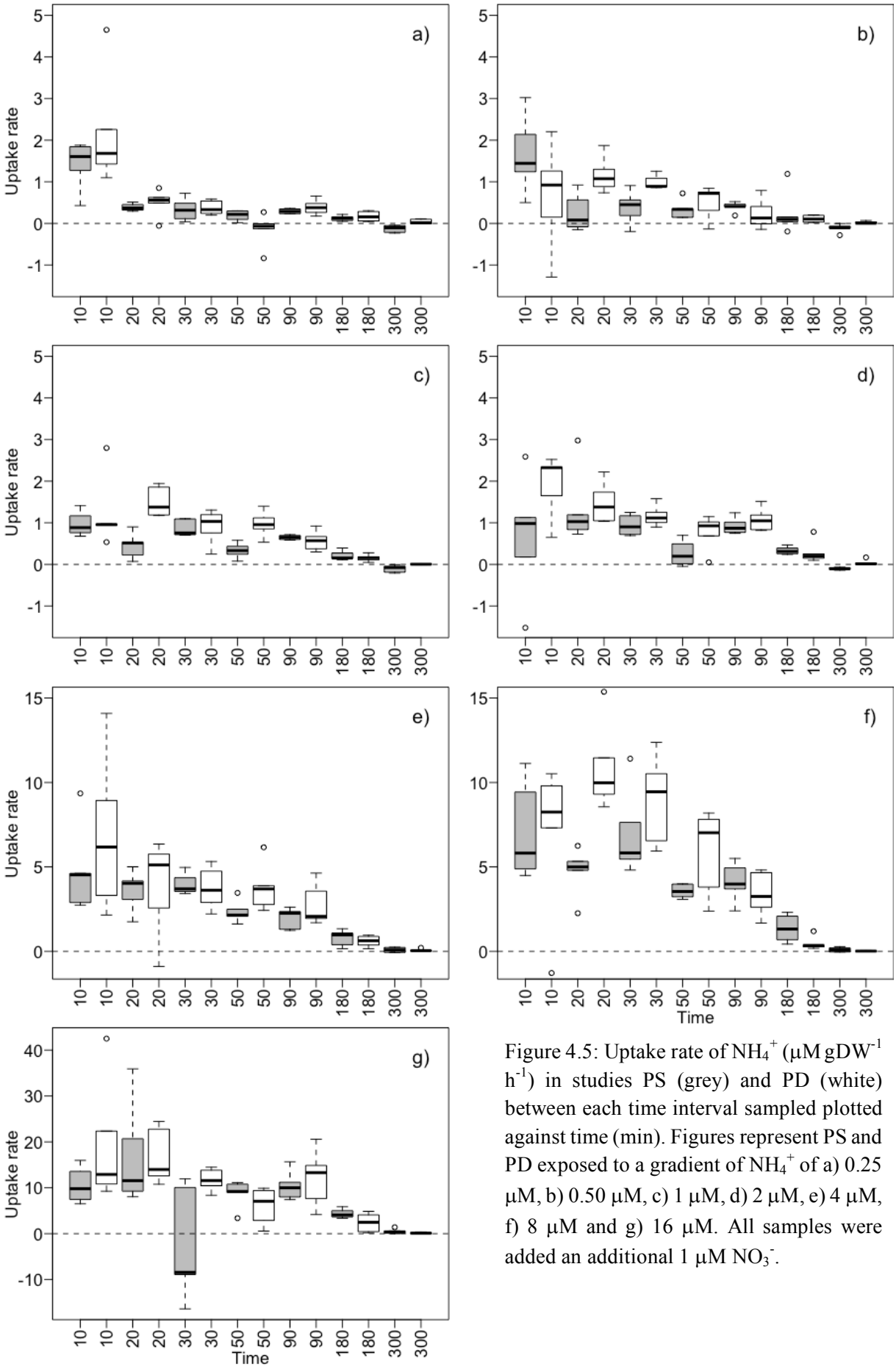

Figure 4.5: Uptake rate of NH_4^+ (µM gDW^{-1} h^{-1}) in studies PS (grey) and PD (white) between each time interval sampled plotted against time (min). Figures represent PS and PD exposed to a gradient of NH_4^+ of a) 0.25 µM, b) 0.50 µM, c) 1 µM, d) 2 µM, e) 4 µM, f) 8 µM and g) 16 µM. All samples were added an additional 1 µM NO_3^-.

4.5.3 Comparison of ammonium uptake rate over time with and without additional nitrate available as substrate

A comparison of NH_4^+ uptake rate between the ammonium study and the preference study in the different sample intervals for the samples with initially deficient *S. latissima* (AD and PD) and the samples with initially sufficient *S. latissima* (AS and PS) was conducted.

For *S. latissima* initially sufficient of nitrogen, there were observed significant differences in uptake rates between the different sample points in experiments AS and PS (Table A18, Appendix C). There were observed significantly higher uptake rates in experiment PS than in AS at: 0.25 μM) 10, 30, 90 and 180 min, 0.50 μM) 20 min, 4 μM) 30, 50, 180, 300 min, 8 μM) 30 and 300 min and 16 μM) 90 min. There were observed significantly higher uptake rates in experiment AS than in PS at: 0.25 μM) 50 min, 1 μM) 50 and 300 min, and 2 μM) 30 and 50 min.

For *S. latissima* initially deficient of nitrogen, there were also observed significant differences in uptake rate between at the different intervals (Table A19, Appendix C). There were observed significantly higher uptake rate in experiment PD than in AD at: 0.25 μM) 90 and 180 min, 0.50 μM) 20 and 180 min, 1 μM) 20, 90 and 180 min, 2 μM)10, 90 and 180 min, 4 μM) 30 and 90 min, 8 μM) 90 and 180 min, and 16 μM) 90 and 180 min. There were observed significantly higher uptake rates in experiment AD than in PD at: 0.25 μM) 50 min, 2 μM) 30 and 50 min, 4 μM) 20 min, 8 μM) 30 min, and 16 μM) 20 min.

4.6 Nitrate uptake rate in the preference study: PS and PD

Uptake rate of NO_3^- ($\mu M\ gDW^{-1}\ h^{-1}$) determined between each sampling point between 10-300 min for saturated (PS) and depleted *S. latissima* (PD) exposed to a gradient of NH_4^+ of a) 0.25, b) 0.50, c) 1, d) 2, e) 4, f) 8 and g) 16 µM in addition 1 µM NO_3^-, are presented in Figure 4.6 a-g). Grey boxes represent PS, and white boxes represent PD. Degree of statistically significant change in uptake rate over time, calculated from the peak in uptake rate, along with related mean values between replicates (±SD) for each concentration are given in Table A20 for PS and Table A21 for PD (Appendix D). The peak in uptake rate was determined to be the sampling point where the highest uptake was observed. This was also the point where the single highest rates were observed in all samples.

A summary of the mean NO_3^- uptake rates (±SD) over the 5-hour period for experiment PS is presented in Table 4.5 for all initial concentrations in the gradient, together with the sampling point with the highest uptake rate and the time at which this peak took place. For the general development of uptake rate throughout the experiment, there did not appear to be any consistent differences between the samples. The mean uptake rates over the course of the experiment in the different samples ranged from 0.00-0.51 $\mu M\ gDW^{-1}\ h^{-1}$. In general, the uptake rates during the first 50 minutes were fluctuating, showing both relatively large positive and negative values. After 50 minutes, the uptake rates appeared to be more consistently positive with a decline toward the end. For all the samples, except the 4 µM samples, the peaks in uptake rates were found between 10-20 minutes. In the 4 µM sample, the peak was found at 90 min. Few significant differences in uptake rate with time was observed (Table A20, Appendix D).

Table 4.5: NO_3^- uptake rates ($\mu M\ gDW^{-1}\ h^{-1}$) for the different initial concentrations in experiment PS given as mean (±SD) over the course of the experiment, and the peak in uptake rate (±SD) and what time (min) this peak was observed.

Gradient	**0.25 µM**	**0.50 µM**	**1 µM**	**2 µM**	**4 µM**	**8 µM**	**16 µM**
Mean	0.49	0.44	0.42	0.05	0.00	0.51	0.18
(±SD)	(1.16)	(1.09)	(1.30)	(1.07)	(0.94)	(0.68)	(0.82)
Peak	-0.69	1.67	0.97	-0.24	0.69	1.19	0.10
(±SD)	(2.89)	(1.89)	(2.24)	(1.65)	(0.39)	(1.34)	(1.56)
Time	10	10	20	10	90	10	10

A summary of the mean NO_3^- uptake rates (±SD) over the 5-hour period for experiment PS is presented Table 4.6 for all initial concentrations in the gradient, together with the sampling

point with the highest uptake rate and the time at which this peak took place. For the general development of uptake rate throughout the experiment, there did not appear to be any consistent differences between the samples. The mean uptake rate over the 5-hour period ranged between -0.04-2.07 μM gDW^{-1} h^{-1}. As in experiment PS, there were generally large fluctuations in uptake rates during the first 50 minutes with both positive and negative rates. This fluctuating appeared to be larger in this experiment than in experiment PS, having both higher positive and lower negative rates. After this first period, the rates were consistently positive and declining toward the end. The peak in uptake rate also appeared to vary more than in experiment PS. In the 4 μM sample, there was one interval with large deviating rates, with a mean rate of 15.52 μM gDW^{-1} h^{-1}. This rate is likely a result of some error in the analysis of substrate concentration, as the measured concentration experienced a large decline followed by a large increase, which resulted in these high rates. Few significant changes in uptake rate with time was observed, except for the 0.25 μM and 4 μM samples, where the peaks were significantly higher than the rest of the rates.

Table 4.6: NO_3^- uptake rates (μM gDW^{-1} h^{-1}) for the different initial concentrations in experiment PS given as mean (±SD) over the course of the experiment, and the peak in uptake rate (±SD) and what time (min) this peak was observed.

Gradient	**0.25 μM**	**0.50 μM**	**1 μM**	**2 μM**	**4 μM**	**8 μM**	**16 μM**
Mean	0.20	0.16	-0.04	0.25	2.07	-0.09	0.70
(±SD)	(1.37)	(2.10)	(2.15)	(0.77)	(6.70)	(1.56)	(1.22)
Peak	2.22	1.05	-1.73	0.45	15.52	-0.20	0.83
(±SD)	(1.74)	(0.93)	(6.02)	(1.44)	(2.77)	(2.57)	(3.51)
Time	50	30	10	10	30	10	10

There were observed significant differences in uptake rates of NO_3^- between experiments PS and PD at occasional sampling points (Table A22, Appendix D). No consistent trends were observed. There were observed significantly higher uptake rates in the initially sufficient *S. latissima* than in the initially deficient at: 0.25 μM) 20 and 180 min, 0.50 μM) 10 and 180 min, 2 μM) 90 and 300 min, and 4 μM) 50 and 300. There were observed significantly higher uptake rates for the initially deficient *S. latissima* than the initially sufficient at: 0.50 μM) 50 min, 4 μM) 20 and 30 min, and 16 μM) 50 min. No significant difference between PS and PD was observed for samples exposed to 1 μM and 8 μM.

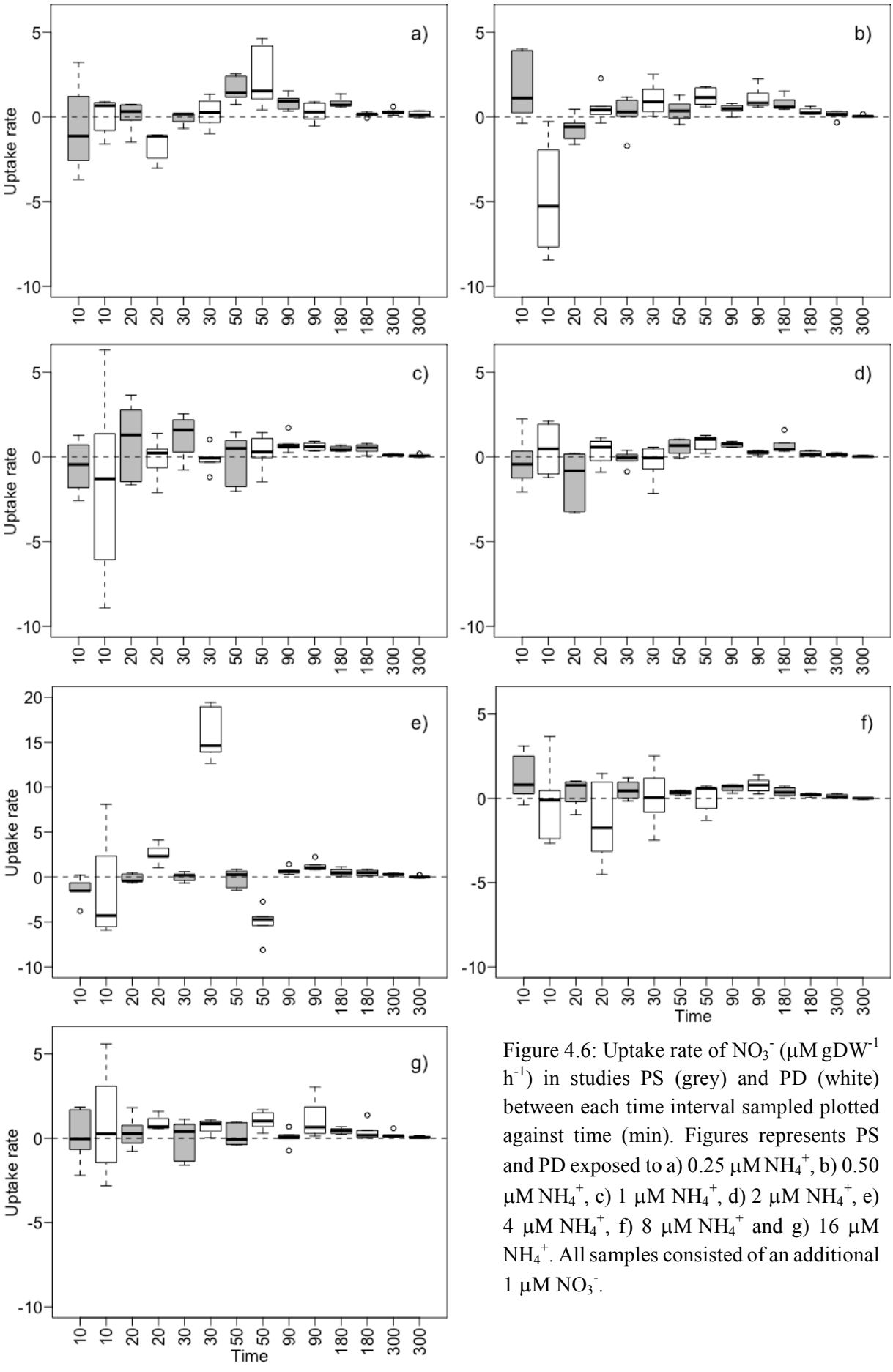

Figure 4.6: Uptake rate of NO_3^- ($\mu M\ gDW^{-1}\ h^{-1}$) in studies PS (grey) and PD (white) between each time interval sampled plotted against time (min). Figures represents PS and PD exposed to a) 0.25 $\mu M\ NH_4^+$, b) 0.50 $\mu M\ NH_4^+$, c) 1 $\mu M\ NH_4^+$, d) 2 $\mu M\ NH_4^+$, e) 4 $\mu M\ NH_4^+$, f) 8 $\mu M\ NH_4^+$ and g) 16 $\mu M\ NH_4^+$. All samples consisted of an additional 1 $\mu M\ NO_3^-$.

4.7 Correlation between ammonium substrate concentration and uptake rate

There was observed a linear correlation between the NH_4^+ substrate concentration (µM) in the medium in relation to the uptake rate (µM gDW^{-1} h^{-1}) for all experiments, as seen in Figure 4.7. The corresponding regression coefficients are given in Table 4.7. The increase in uptake rates in relation to substrate concentration was the steepest for experiment AD, very similar for experiments AS and PD, and the lowest in experiment PS.

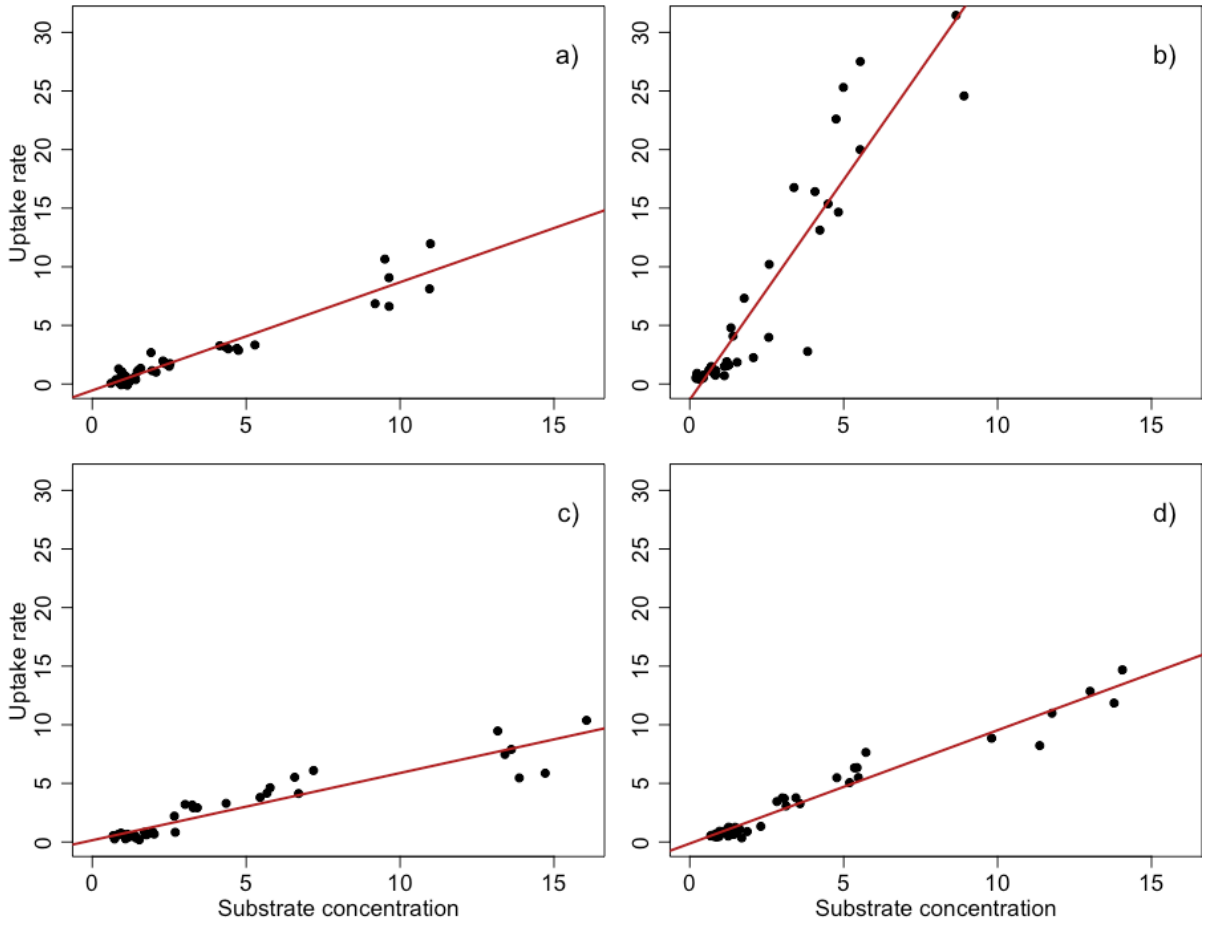

Figure 4.7: Correlation between NH_4^+ substrate concentration (µM) and uptake rate (µM gDW^{-1} h^{-1}) for the different experiments: **a)** AS, **b)** AD, **c)** PS and **d)** PD. The figures present the mean substrate concentration in relation to the mean uptake rate observed for all replicates, until the substrate concentration approximated 0 or had become negative.

Table 4.7: The regression coefficients (intercept, slope, R^2 and p-values) for the linear regression of uptake rate in relation to substrate concentration shown for the different experiments in Figure 4.7.

Experiment	Intercept (±SD)	Slope (±SD)	R^2	p-value
AS	-0.55 (0.18)	0.92 (0.04)	0.92	<0.001
AD	-1.34 (0.72)	3.75 (0.23)	0.86	<0.001
PS	0.15 (0.20)	0.57 (0.03)	0.88	<0.001
PD	-0.13 (0.17)	0.97 (0.03)	0.96	<0.001

5 Discussion

In this study, the initial uptake rate of NH_4^+ was investigated in relation to available nitrogen source and nutritional history of *S. latissima*. It was shown that NH_4^+ was the preferred nitrogen source over NO_3^- and that it was efficiently taken up. It was also shown that *S. latissima* with deficient internal nitrogen pools experienced a higher uptake than specimens with sufficient nitrogen pools. Uptake rate was shown to be directly related to the substrate concentration as there was observed a linear increase in uptake rate with an increase in available substrate concentration. Therefore, the depletion of substrate concentration is discussed first before uptake rates are discussed more thoroughly.

5.1 Reduction of ammonium from the substrate

It was evident that in all experiments, except in experiment AD, the first measurement of substrate concentration revealed a higher NH_4^+ concentration than what the samples were supposed to be initially added. This elevated concentration may have been a result of the *S. latissima* bringing nutrients with them into the beakers in experiments AS, PS and PD, or that the initial concentration created was inaccurate. The first measurement at 5 minutes corresponds to the control samples, suggesting a that the samples were created with a slightly high concentration. Controls were only used for the highest and lowest concentration in the gradient, for future studies, controls for all the samples in the gradient may be fitting. In experiment AD, only the lower 0.25, 0.50 and 1 µM samples contained a slightly higher concentration than the original addition, while the samples added a higher initial concentration consistently had a lower substrate concentration already after 5 minutes. This demonstrates a rapid removal of substrate from the medium in experiment AD that may have been a result of an initial diffusion into the apparent free space and apoplast (Harrison and Druehl, 1982). In experiment PD, the elevated initial substrate concentration suggests that the internal nitrogen pools in the *S. latissima* were not completely depleted. In these samples, the incubation period prior to the experiment was the longest and at the end of the incubation period a breakdown of the lamina was observed in most specimens, which may take place under nutrient limitation (Sjøtun, 1993), while a growth was observed in others. In addition, the medium appeared more turbid, and when filtrating the water samples the filters clogged more easily. This growth and higher initial concentration measured may have been related to this breakdown which potentially resulted in

a release of nutrients into the medium, and subsequently increased the tissue concentration within the remaining specimens.

When comparing the substrate concentration measured at each sampling point between the experiments, it was evident that the samples in experiment AD had significantly lower substrate concentrations than AS at several sampling points already after 5 minutes for several initial concentrations in the gradient. The greatest difference was observed for the low initial concentrations, e.g. 0.25-2 µM, while for the higher initial concentrations, e.g. 4-16 µM, a delay was observed before significant differences were evident. This large difference already at the first sampling point for the samples with low initial concentrations is likely a result of a small induction period where no apparent uptake took place (Harrison et al., 1986). The sufficient *S. latissima* in experiment AS may have brought a noticeable concentration of NH_4^+ into the 0.25-2 µM samples as there were often measured approximately 0.50 µM more NH_4^+ than the supposedly original addition at the first measurement at 5 minutes. While at the first measurement in the 0.25-1 µM samples in experiment AD with deficient *S. latissima*, no noticeable change of NH_4^+ concentration appeared. In the samples added the higher concentrations, the concentration was more often slightly lower than the original addition at this measurement in both experiment AS and AD, suggesting that high NH_4^+ availabilities may stimulate a more efficient uptake. Following this first period, the rate of removal appeared to be higher in the sample with the deficient *S. latissima* in all samples.

Also in the samples in the preference study there were observed elevated concentrations at the first sampling point. However, this was observed in almost all samples in both experiment PS and PD, which supports that the *S. latissima* in experiment PD was not depleted of internal nitrogen. These elevated concentrations suggest that there was an induction period also when NO_3^- was present and that this was taking place at the highest concentrations as well, suggesting a small initial inhibition of NO_3^- on NH_4^+, in contrast to a potential stimulation of NH_4^+ at high availabilities when it is the only available nitrogen source. This observation stand in contrast to previous reports, suggesting no interaction of NO_3^- on uptake of NH_4^+ (Subandar et al., 1993; Ahn et al., 1998). However, this potential inhibition appeared to be very limited, and were likely not observed in these previous studies due to methodological differences with less frequent samplings.

Typical depletion curves observed for the depletion of substrate concentration over time in the different experiments are presented in Figure 5.1. In experiments AS, PS and PD, the depletion followed the same trends with a non-linear uptake until the final sample point, as shown in Figure 5.1a. In these experiments, the three phases of uptake were followed with an initial "surge" uptake, followed by an internally controlled uptake and a final externally controlled uptake (Pedersen, 1994). The substrate concentration in these experiments was never fully removed, and a small, but measurable, concentration was observed before it often increased again at the final sampling in the samples with initially sufficient *S. latissima* in experiments AS and PS. In experiment AD, the depletion was following another trend where there was a larger "surge" uptake where the substrate concentration was rapidly removed, as the concentration became low, the depletion leveled off with an externally controlled uptake, as seen in Figure 5.1b. In addition, in experiment AD the NH_4^+ was always completely removed, which was not observed in any other samples. This full removal was likely a result of the internal nitrogen storages being completely depleted.

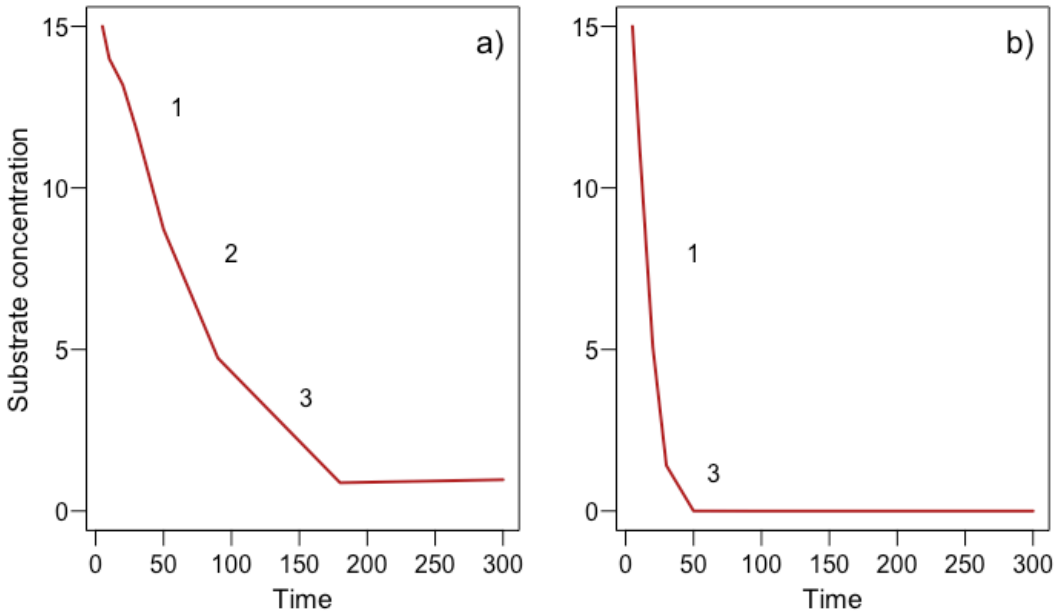

Figure 5.1: Typical depletion curves of NH_4^+ substrate concentration (µM) observed over time (min) for *S. latissima* observed for the different experiments in this study. **a)** Depletion of substrate concentration in experiments AS, PS and PD, showing an initial "surge" uptake (1), internally controlled assimilation (2) and an externally controlled uptake (3). **b)** Depletion in experiment AD, showing a very rapid initial uptake until depletion.

Throughout the 5-hour incubation period during the experiment, there were observed several significant differences in substrate concentration at each sampling point between the experiments in the ammonium study, and few significant difference in in the preference study.

This shows that the there was little difference in the rate of removal between the *S.* different nutritional in the preference study. The differences observed in the preference study was for the most part taking place at the end of the experiments, and appeared to be related to an increase in substrate concentration in several samples in experiment PS, which was only observed occasionally in experiment PD.

The increase in substrate concentration that was observed at the final sampling point in experiments PS and PD was also observed in experiment AS. This may be a result of an efflux of NH_4^+ from the cells back into the medium, which may take place if the cells have taken up more than they can metabolize and if the ambient concentration is low (Mulholland and Lomas, 2008). This suggests that the internal nitrogen storages were saturated. In addition, the final concentration measured was relatively similar between the different samples in these experiments, supporting that the uptake capacity of these final low concentrations may be limited due to internal uptake mechanisms. In addition, the removal of the final low concentrations may be reduced by the reduced number of molecules in the medium, reducing the uptake efficiency. Due to these final similarities regardless of initial concentration, the bioremediation capacity only increased with increased availability. Additionally, the uptake capacity appeared to be affected by the nutritional history, as the deficient specimens demonstrated faster removal of NH_4^+ than sufficient specimens. This was observed by earlier significant reductions in substrate concentration with time in experiments AD than in AS, and in PD than in PS. Within each experiment, there did not appear to be any consistent differences in when a significant reduction took place between the different initial concentration. Therefore, the point at which a significant removal of NH_4^+ from the substrate took place appeared to be more related to the nutritional status of the *S. latissima*, rather than the available concentration.

Comparing the NH_4^+ substrate concentrations between the ammonium and the preference studies, there were observed few differences between experiment AS and PS, suggesting that the availability of NO_3^- had limited effect on the rate of removal in *S. latissima* with sufficient internal nitrogen storages. In contrast, the differences between experiments AD and PD were large, demonstrating a higher uptake without available NO_3^-. However, as mentioned, this large difference is likely a result of the internal nitrogen storages not being fully depleted in experiment PD.

5.2 Reduction of nitrate from the substrate

The NO_3^- concentration in the substrate appeared to be relatively stable for the first 50-90 minutes for most samples in both experiment PD and PS. At the final sampling points (90-300 minutes), a reduction in concentration was clear towards the end in all samples. A significant reduction was observed at 90 minutes for most samples in experiment PD, while it was observed at 180 minutes for most samples in experiment PS. This suggests there was a lag period before NO_3^- uptake was evident, which may take place if there is a preferential uptake of NH_4^+ (Dortch, 1990). This shows that the initially deficient *S. latissima* experienced a shorter lag period than what the sufficient *S. latissima* did, suggesting that this NH_4^+ preference is less pronounced under limiting conditions, demonstrating a higher uptake capacity for both NO_3^- and NH_4^+. After this lag period a simultaneous uptake was observed, agreeing with previous reports (Subandar et al., 1993; Ahn et al., 1998). There were observed no consistent significant differences in NO_3^- substrate concentration between the two experiments per sampling points. These observations suggest that even though the NO_3^- was removed somewhat earlier in experiment PD, it was not depleted to a significantly lower concentration. This demonstrates that the nutritional status of the *S. latissima* has a limited effect on uptake of NO_3^- in this batch system with this low initial concentration. However, due to the shorter lag period observed for deficient specimens, a higher uptake capacity may be revealed under a continuous-flow system.

5.3 Ammonium uptake

By looking at the overall picture of NH_4^+ uptake in this study, there was observed a linear relationship between substrate concentration and uptake rate. This shows that there were not observed any saturation of uptake over the 5-hour period for the concentration gradient used. Similarly, by investigating this relationship during the first 50-minute period, where the highest uptake rates were observed, there were also observed a linear relationship between uptake rates and substrate concentrations. This demonstrates that there was a clear linear increase in uptake rate with increased initial concentration up to 16 µM for *S. latissima*, which supports previous observations for *S. latissima* (Ahn et al., 1998) and other macroalgae (Taylor et al., 1998; Phillips and Hurd, 2004). During the first 50 minutes of incubation, the mean uptake rates in experiments AS, PS and PD all equaled approximately 10 µM gDW^{-1} h^{-1}, which has previously been shown to be the saturating level of NH_4^+ uptake in *S. latissima* during the first hour of

incubation with higher initial concentrations (Ahn et al., 1998). Therefore, any further increase in substrate concentration may have resulted in a saturation.

In this study, the development of uptake rate over time for *S. latissima* followed two characteristic patterns as shown in Figure 5.2. In experiments AS, PS and PD the *S. latissima* typically followed the three phases of uptake (Figure 5.2a; Pedersen, 1994). For the *S. latissima* in experiment AD, the uptake rate typically followed a different pattern, where the internally controlled phase was skipped, and there was only an initial and large "surge" uptake, followed by an externally controlled phase as the NH_4^+ concentration was becoming limiting (Figure 5.2b). There were variations between the samples in how the uptake developed over time, and some samples in experiments AS, PS and PD did not demonstrate such a large difference between the "surge" uptake and the externally controlled phase as demonstrated in Figure 5.2a. In these experiments, some samples also followed the pattern demonstrated in Figure 5.2b. Similarly, the *S. latissima* in the experiment AD also demonstrated deviations from the typical pattern. Especially for the lower initial concentrations between 0.25-1 µM, where there were tendencies to observe a small stabilization of the rate between 30-50 minutes. During this period, the internally controlled phase appeared to take place. This phase was only observed occasionally and for a short period, and the externally controlled phase quickly took over.

Uptake rates varied between replicates which is likely a result of inter-seaweed variability (Hurd et al., 2014). Age and length were similar between the individuals used, however, the total surface area was varying to a large degree, changing the surface area:volume ratio, in addition, the varying incubation period before the different experiments affected the structure of the lamina. These factors may have affected the uptake capacity between the replicates.

From observing the mean uptake rate over the course of the experiment and the peaks in uptake rates in the experiment AS, there were observed no apparent differences between the 0.25 µM and 0.50 µM samples. With a further increase in initial substrate concentration, the uptake rates were also increasing. Similar trends were observed in experiment AD, although the rates were consistently higher than in experiment AS. These rates were also increasing to a higher degree with an increase in initial concentration. This shows that the deficient *S. latissima* had a higher capacity to take up NH_4^+ then what sufficient *S. latissima* did, and that this difference only increased with increased available concentration. This confirms the nutritional history

influences the NH_4^+ uptake for *S. latissima*, as is generally believed to the case for seaweed (Hurd et al., 2014).

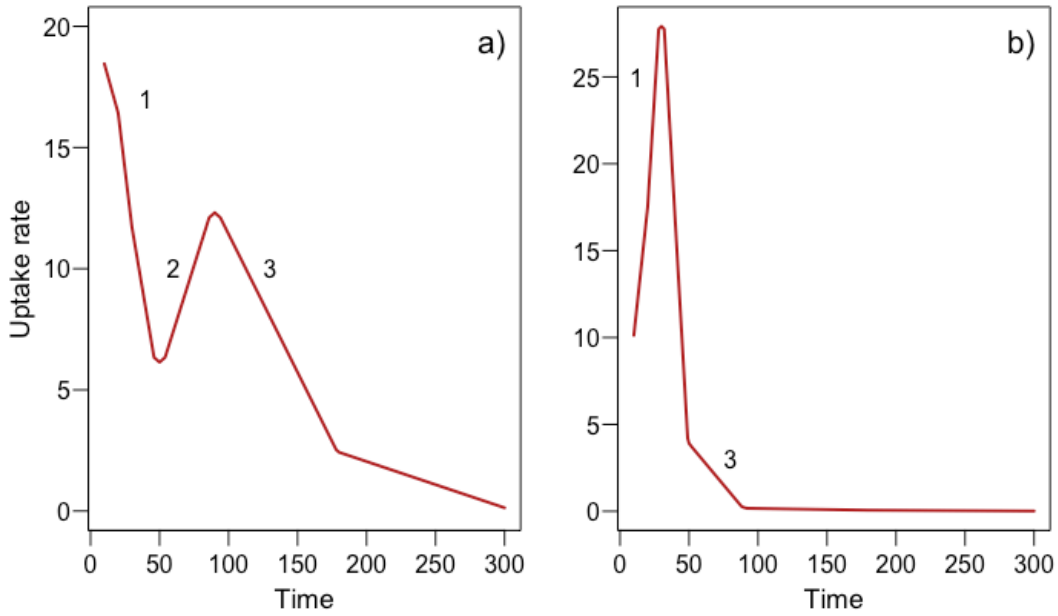

Figure 5.2: Typical development of NH_4^+ uptake rate (µM gDW^{-1} h^{-1}) observed over time (min) for S. latissima in the different experiments in this study. **a)** Typical development of uptake observed in experiments AS, PS and PD, showing (1) an initial "surge" uptake, (2) followed by an internally controlled uptake and (3) a final externally controlled uptake. **b)** Typical development of uptake observed in experiment AD, where (1) the initial "surge" uptake removed almost all substrate and (3) a final externally controlled uptake was evident until depletion.

From closer observations of the initial uptake rate of NH_4^+, it was apparent that all peaks were taking place within the first 50 minutes' period, either as a result of an initial "surge" uptake, or from an increasing internally controlled uptake. In experiment AS, the peaks in uptake rate appeared to take place at earlier time intervals with an increase in initial concentration, ranging between 10-50 minutes. The delayed peak in uptake rate observed in experiment AS for the initial low concentrations may be a result of the combination that the *S. latissima* did not experiencing an initial "surge" uptake as the internal pools were already filled to these concentrations. This was supported by several negative rates during this early period, suggesting that an efflux (e.g. diffusion) of NH_4^+ from the *S. latissima* to the medium took place as concentration in the pools were higher than in the medium. In experiment AD, there were for the most part observed a single large peak in uptake followed by a rapid decline, due to the "surge" uptake being so large that the NH_4^+ became so depleted before the phase was over, so that any further uptake was limited due to the external concentration. Due to this, the internally controlled phase was not observed in several samples, although it was apparent for a short

period in some samples, where the rate appeared to stabilize for a few minutes. There did not appear to be any consistent trends in where this peak in uptake rate was observed within the first 50 minutes between the different initial concentrations in experiment AD. The peak in uptake rates for the initially low concentrations that were found earlier in experiment AD than in experiment AS, is likely a result of the *S. latissima* having a "surge" uptake to fill up the depleted internal storages in the initially deficient *S. latissima*. The *S. latissima* in experiment AD also had few negative uptake rates, suggesting limited efflux as depleted internal storages were filling up.

In the preference study, the peaks in uptake rates consistently took place between 10-20 minutes, showing that the peaks were generally reached earlier in the preference study than in the ammonium study. The peaks for the samples in the gradient between 0.25-2 µM showed little difference within the two experiments. For the higher initial concentrations, the peaks in rate of uptake corresponded to the initial concentration added in experiment PS, whereas in experiment PD, the uptake rates were higher than what the initial concentrations were. Similarly, the mean uptake rate over the experiment varied relatively little between the 0.25-2 µM samples. In experiment PD, the mean uptake rate for the same low initial concentrations were slightly higher than in experiment PS, and the increase in uptake rate with an increase in concentration was also larger in this experiment. In general, there were observe few differences in when a significant reduction of uptake rate over time took place, and there were no consistent trends in significant difference between the two experiments per sample point. From these results, there appeared to be relatively little difference in uptake rate between experiment PS and PD in comparison to what was observed in the ammonium study. However, the rates in experiment PD were at almost all occasions slightly higher than in experiment PS, further supporting that the deficient *S. latissima* has a higher uptake capacity than sufficient *S. latissima*, also when NO_3^- is available. This difference was the lowest for the low initial concentrations and was increasing with an increased available concentration. As mentioned previously, there were indications that the internal nitrogen storages in the *S. latissima* in experiment PD were not completely depleted. If this was indeed the case, it may have affected the relatively low difference observed between experiments PS and PD. To be certain this is avoided, more frequent changes of incubation medium should have been done prior to the experiment. Analysis of internal NO_3^- could have verified these suspicions.

In both experiments with initially nitrogen sufficient *S. latissima*, there were generally observed little difference in uptake rate per sampling point. This suggests that there were limited interactions of NO_3^- on NH_4^+ uptake for the sufficient *S. latissima*. All peaks were found within the first 50 minutes, and there were no consistent patterns in which treatment resulted in the highest and lowest rates. From 90 minutes, the uptake rates between the two experiments were more similar, and generally, the rates in experiment AS were somewhat lower than they were in PS. In the experiments with initially nitrogen deficient *S. latissima*, the trends were different. For the high initial concentrations of 2-16 µM, there were generally observed higher uptake rates in experiment AD than in PD during the first 50 minutes. From 90 minutes in these samples, the rates in experiment AD were significantly reduced and generally lower than in PD with several significant differences, due to quickly reduced available substrate concentration. For the lower initial concentrations of 0.25-1 µM, the differences between the two experiments were lower, and they followed the same trends to a higher degree. This shows that the deficient *S. latissima* has a higher capacity to take up high available NH_4^+ concentrations when it is the only available nitrogen source.

When all experiments were compared, it became apparent that there were only spatial and no consistent differences in uptake rate between experiments AS, PS and PD. In addition, for the lower initial concentrations ranging between 0.25-1 µM, experiment AD was also similar to the other experiments. However, for the higher initial concentrations, the rates were higher in experiment AD and the initial "surge" uptake in this experiment was clearly the largest. Although the differences in uptake rate were small for the low initial concentrations, differences were apparent when observing substrate concentration, as previously mentioned. In experiment AD, the substrate concentrations were fully removed, proving that nutritional history and available nitrogen source do affect uptake even at these low concentrations.

These observations showed that there were no apparent differences in NH_4^+ uptake capacity with and without NO_3^- available for *S. latissima* coming from sufficient nutritional conditions. However, for *S. latissima* coming from deficient conditions, the availability of nitrogen do affect the uptake capacity. NH_4^+ uptake capacity in *S. latissima* is therefore suggested to be the greatest during pulses of high concentrations of NH_4^+ in otherwise deficient conditions.

5.4 Nitrate uptake

There were not observed any large differences in the trends for uptake rate of NO_3^- between experiments PS and PD. As observed from the substrate concentration, there was a relatively stable period in uptake for the first 50-90 minutes, before a decline was evident. During this relatively stable period of substrate concentrations, the uptake rates were fluctuating between relatively high positive and negative rates. The fluctuations in uptake rates appeared to be stabilizing at approximately 50 minutes. During the last period, the uptake rates were more consistently positive with a decline toward the end. There were observed few significant changes over time, suggesting that the rates were relatively stable throughout the experiment. There were also not observed any consistent differences between the two experiments per sampling point.

It has previously been demonstrated for marine primary producers that the presence of NH_4^+ can reduce the uptake of NO_3^-, and that this interaction varies greatly between species (Dorch et al., 1991; Collos et al., 2004). This depression of NO_3^- uptake has been suggested to be indirectly caused by feedback mechanisms from the accumulation of organic metabolites in intracellular pools, such as glutamine or the ratio of glutaime:glutamate and glutamine:alpha-ketoglutarate, which ultimately are related to the overall nitrogen status of the cells and consequently the enzymatic activity, which may inhibit NO_3^- transport (Flynn et al., 1989; Flynn, 1990; Page et al., 1999; Stephens et al., 2003; Flores and Herrero, 2005). Such an accumulation of amino acid and related regulatory compounds have been shown to increase more rapidly from DIN assimilation when NH_4^+ is available in phytoplankton (Wood and Flynn, 1995; Page et al., 1999). For *S. latissima* it has previously been demonstrated that relatively high concentrations of NH_4^+ and NO_3^- did not demonstrate any negative interaction (Ahn et al., 1998). However, in this study with a relatively low NH_4^+ concentration gradient and more frequent samplings, the interaction between NH_4^+ and NO_3^- appeared more complex. It was suggested that NO_3^- inhibited the first uptake of NH_4^+ at high concentrations, creating a brief induction period. In addition, it shown that NO_3^- uptake was suppressed by NH_4^+ during the first 50 minutes of NH_4^+ accumulation. These effects appeared to be reduced under nitrogen limitation, which corresponds to previous findings for phytoplankton and macroalgae (Conway, 1977; Thomas & Harrison, 1985).

There are two distinct processes that may explain the negative effect of NH_4^+ on the uptake of NO_3^- that was observed. These processes include an indirect interaction which is a preference for NH_4^+ over NO_3^-, or a direct interaction with an inhibitory effect by NH_4^+ on the uptake of NO_3^- (Dortch, 1990). A preference is evident if one source is more readily utilized by the other, and may be demonstrated in a variety of ways, including a higher maximum uptake rate observed in one source over the other, or a time lag before an uptake (Dortch, 1990). Inhibition on the other hand, takes place if the presence of one source prevents or reduces uptake of the other (Dortch, 1990). An inhibition is concentration dependent and may be quantified by comparing the uptake in the absence of the inhibitor to the uptake in the presence of it in a variety of concentrations (Dortch, 1990). In this study, there was a clear preference for NH_4^+ over NO_3^- as there was observed a time lag of approximately 50 minutes before NO_3^- uptake was evident, corresponding to what has been observed for *L. groenlandica* where a 30 minutes' suppression period was observed before a simultaneous uptake took place, possibly due to an induction period (Harrison et al., 1986). After this suppression period of 50 minutes, a near linear uptake was observed for both NO_3^- and NH_4^+, demonstrating a simultaneous uptake. As there were not observed any reduced NO_3^- uptake by an increased NH_4^+ concentration, an inhibitory effect of NH_4^+ on NO_3^- is suggested not to take place. Other interactions between NH_4^+ and NO_3^- that may be evident includes a stimulation of NH_4^+ uptake by NO_3^- (Dortch, 1990). In this study, such an effect was not observed.

5.5 Suitability of cultivating *S. latissima* in an IMTA system with intensive salmon farming in relation to initial ammonium uptake

The ability of *S. latissima* to remediate effluent NH_4^+ from an aquaculture system is critical for its potential for being used in an IMTA system. Nitrogen is the most frequent limiting nutrient along the Norwegian coast, and an increased availability due to effluent DIN from intensive salmon farming therefore has the potential to increase the biomass yield (Ahn et al., 1998; Sanderson et al., 2012; Broch et al., 2013; Handå et al., 2013). It has previously been suggested that cultivation of *S. latissima* using an area of the same size as a salmon farm, has a potential to utilize 10% of the effluent NH_4^+ (Broch et al., 2013).

Elevated concentrations in the seawater have been found at 200 meters away from fish farms (Ahn et al., 1998; Sanderson et al., 2008; Broch et al., 2013), and traces of nitrogen originating from salmon farming have been found at a distance of as much as 1 km away (Sanderson, 2006).

Seaweed cultivated in vicinity to fish farms have been demonstrated to assimilate effluent nutrients and to have increased internal nitrogen contents (Chopin et al., 2000; Abreu et al., 2009; Sanderson et al., 2012; Handå et al., 2013). However, effluent nutrients are rapidly diluted, therefore, to be able to maximize the utilization of the nutrients coming from a fish farm, it is important to have an optimal position of the *S. latissima* cultivation unit in relation to the currents and distance from the fish farm as practically possible (Broch et al., 2013). Cultivation too close to the salmon pens may have implications for the daily work, in addition, the lamina of the cultivated *S. latissima* may be covered by particulates, especially fish feces, under low current conditions. This may decrease the availability light and therefore reduce photosynthesis and nutrient uptake (Subandar et al., 1993).

This thesis demonstrated that NH_4^+ is efficiently taken up by young *S. latissima* as a preferred nitrogen source over NO_3^-. This corresponds to NH_4^+ being regarded as more energetically favourable as it can be directly incorporated into amino acids (Syrett, 1981; Berges and Mulholland, 2008). The NH_4^+ uptake was observed to increase linearly with increased availability in a gradient ranging between 0.25-16 µM, with a preferential uptake over NO_3^- during pulses of higher availabilities. Optimal growth for *S. latissima* has been found with ambient NO_3^- concentrations of approximately 10 µM (Chapman et al., 1978). The surface NO_3^- concentration found along the Norwegian coast is often below this concentration, with highest concentrations during early spring (coastal NO_3^- conditions from several studies are summed up in Svåsand et al., 2016). It is therefore suggested that an increase in the ratio of available NH_4^+ in relation to NO_3^- due to effluent release from a salmon farm enhance the protein syntheses and therefore the biomass yield as it is energetically favorable over NO_3^-. In addition, the internal nitrogen storages of *S. latissima* may be filled to a greater extent with additional NH_4^+ available, which may also contribute to the long-term growth (Chapman et al., 1978).

5.6 Further studies

To be able to fully understand the NH_4^+ uptake characteristics of *S. latissima* further research is recommended. This thesis give an insight into how nitrogen deficient and sufficient *S. latissima* responded to a gradient of different NH_4^+ concentrations in a controlled laboratory experiment. *In situ* field research to support these findings is recommended to gain a broader understanding of the NH_4^+ uptake characteristics in an IMTA system. Other parameters than the nutritional history is also of importance for uptake (e.g. chemical-, physical- and biological parameters), further research to characterize the effect of these on uptake is therefore also necessary to gain a broad understanding.

Preference and inhibition varies between species and environmental conditions, and may involve more than one step in the pathway for uptake and assimilation and involve both short- and long-term processes (Dortch, 1990). Further studies to characterize these processes are therefore suggested, including an investigation of any potential negative interactions of NO_3^- on NH_4^+ uptake, e.g. to incubate *S. latissima* with NH_4^+ in an increased gradient of NO_3^-. It is also relevant to investigate these interactions in a field study.

Additionally, further studies to investigate the uptake characteristics for individuals of different age is suggested, as it has previously been proven that young individuals may have a higher uptake than older individuals (Harrison et al., 1986).

6 Conclusion

The present study was conducted to investigate the uptake capacity of effluent NH_4^+ from intensive salmon farming by *S. latissima*, to address the potential of cultivating this species in an IMTA system. The initial NH_4^+ uptake was characterized in a controlled laboratory experiment combining the commonly used multiple flask- and perturbation techniques, using whole specimens of young *S. latissima*. The time and concentration dependent changes of initial NH_4^+ uptake was characterized with and without naturally occurring NO_3^- present.

It was hypothesised that (H_1) NH_4^+ uptake rate would experience a linear increase with increased availability, (H_2) that NH_4^+ was the preferred nitrogen source over NO_3^-, and (H_3) that the nutritional status of the cells affect uptake so that deficient *S. latissima* demonstrates a higher uptake capacity. In this study, these hypotheses were confirmed.

It was demonstrated that the NH_4^+ uptake rate increased linearly with increased concentrations $\leq$16 µM, regardless of nutritional history and available nitrogen source. The steepest increase of uptake rate was observed for the deficient *S. latissima* with only NH_4^+ available, while the lowest increase was observed for the sufficient *S. latissima* with additional NO_3^- available. There was also observed a preferential uptake of NH_4^+ over NO_3^-, as NO_3^- was only consistently taken up after a time lag of approximately 50 minutes. The nutritional status of the *S. latissima* was also proven to affect the uptake capacity, and a higher uptake was observed in deficient than in sufficient specimens. This was especially apparent when NH_4^+ was the only available nitrogen source. This additional capacity due to depleted storages appeared to be reduced when NO_3^- was available. However, there were indications that the *S. latissima* in the preference study did not have completely depleted internal nitrogen storages, and conclusions on any effect on NO_3^- on uptake of NH_4^+ should be made with caution.

This study contributes to the knowledge of the initial NH_4^+ uptake capacity of *S. latissima*, demonstrating that effluent NH_4^+ originating from intensive salmon farming is efficiently assimilated as uptake quickly increased with an increased availability. This study therefore contributes to previous studies that have suggested that *S. latissima* is a suitable species to be cultivated in an IMTA system in relation to its bioremediation capacity of NH_4^+.

7 References

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Appendix A – Ammonium substrate concentration per gDW

Change of ammonium substrate concentration per gDW over time in AS and AD

Table A1: NH_4^+ substrate concentrations (µM) in experiment AS, measured at each sampling point (5-300 min) for all initial exposure concentrations (0.25-16 µM). Substrate concentration is given as mean of all replicates (± SD). P-value is representing change of substrate concentration over time from first sampling point (5 min) to sampling point where the respective value is given. * marks statistically significant change over time.

AS: Gradient		5 min	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	Mean	3.16	3.56	3.45	3.57	2.56	2.11	1.99	3.06
	(SD)	(0.66)	(0.79)	(0.79)	(0.92)	(0.82)	(0.69)	(0.98)	(1.53)
	p-value	n/a	0.3894	0.6518	0.5391	0.1030	<0.0001*	<0.0001*	0.1459
0.50 µM	Mean	4.16	4.42	4.37	4.33	3.54	2.62	1.65	1.94
	(SD)	(0.81)	(0.87)	(0.90)	(1.14)	(1.28)	(1.13)	(0.49)	(0.71)
	p-value	n/a	0.5979	0.7203	0.8170	0.1799	<0.0001*	<0.0001*	<0.0001*
1 µM	Mean	7.12	5.60	5.72	4.54	2.98	1.71	0.90	0.97
	(SD)	(2.10)	(1.06)	(1.21)	(0.56)	(0.45)	(0.40)	(0.24)	(0.25)
	p-value	n/a	0.2684	0.1597	0.0035*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
2 µM	Mean	12.42	10.60	9.98	8.43	5.86	3.30	1.29	1.64
	(SD)	(4.61)	(1.39)	(1.24)	(1.18)	(1.25)	(0.89)	(0.32)	(1.13)
	p-value	n/a	0.3772	0.1852	0.0127*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
4 µM	Mean	13.37	11.47	9.57	7.86	7.87	2.39	0.44	1.34
	(SD)	(2.58)	(2.29)	(2.44)	(2.02)	(4.54)	(1.24)	(0.31)	(0.76)
	p-value	n/a	0.2087	0.0154*	0.0002*	0.0015*	<0.0001*	<0.0001*	<0.0001*
8 µM	Mean	25.20	24.21	21.67	20.36	15.44	8.19	0.59	1.84
	(SD)	(7.12)	(7.49)	(7.66)	(5.45)	(7.41)	(5.76)	(0.25)	(1.19)
	p-value	n/a	0.8189	0.3936	0.1727	0.0090*	<0.0001*	<0.0001*	<0.0001*
16 µM	Mean	66.86	61.62	56.20	50.60	38.41	21.71	2.43	4.31
	(SD)	(23.27)	(22.95)	(23.22)	(21.72)	(20.93)	(15.25)	(1.70)	(2.75)
	p-value	n/a	0.7032	0.4299	0.1897	0.0176*	<0.0001*	<0.0001*	<0.0001*

Table A2: NH_4^+ substrate concentrations (µM) in experiment AD, measured at each sampling point (5-300 min) for all initial exposure concentrations (0.25-16 µM). Substrate concentration is given as mean of all replicates (± SD). P-value is representing change of substrate concentration over time from first sampling point (5 min) to sampling point where the respective value is given. * marks statistically significant change over time.

AD: Gradient		5 min	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	Mean	1.55	1.32	0.90	0.64	0.16	0	0	0
	(SD)	(0.34)	(0.29)	(0.21)	(0.21)	(0.19)	(0)	(0)	(0)
	p-value	n/a	0.2471	0.0011 *	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
0.50 µM	Mean	1.99	2.77	1.72	1.07	0.31	0	0	0
	(SD)	(0.69)	(2.02)	(0.47)	(0.33)	(0.17)	(0)	(0)	(0)
	p-value	n/a	0.3891	0.5464	0.0477*	0.0003*	<0.0001*	<0.0001*	<0.0001*
1 µM	Mean	4.55	3.11	2.50	1.90	0.72	0.01	0	0
	(SD)	(1.56)	(0.70)	(0.71)	(0.65)	(0.41)	(0.02)	(0)	(0)
	p-value	n/a	0.0680	0.0082*	0.0002*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
2 µM	Mean	7.28	5.82	4.90	3.77	0.15	0	0	0
	(SD)	(2.81)	(0.73)	(0.89)	(0.87)	(0.37)	(0)	(0)	(0)
	p-value	n/a	0.2457	0.0365*	0.0006*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
4 µM	Mean	17.84	12.99	8.63	8.38	4.15	2.12	0	0
	(SD)	(5.77)	(7.03)	(5.90)	(6.11)	(3.34)	(3.00)	(0)	(0)
	p-value	n/a	0.2203	0.0217*	0.0110*	0.0003*	<0.0001*	<0.0001*	<0.0001*
8 µM	Mean	39.04	32.71	26.84	6.34	0.29	0.50	0.11	0
	(SD)	(18.82)	(11.67)	(11.16)	(6.16)	(0.66)	(0.14)	(0.10)	(0)
	p-value	n/a	0.5004	0.1814	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
16 µM	Mean	53.73	46.84	21.73	6.14	0.01	0	0	0
	(SD)	(20.07)	(20.26)	(19.12)	(6.69)	(0.02)	(0)	(0)	(0)
	p-value	n/a	0.5671	0.0085*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0002*

Change of ammonium substrate concentration per gDW over time in PS and PD

Table A3: NH_4^+ substrate concentrations (µM) in experiment PS, measured at each sampling point (5-300 min) for all initial exposure concentrations (0.25-16 µM). Substrate concentration is given as mean of all replicates (± SD). P-value is representing change of substrate concentration over time from first sampling point (5 min) to sampling point where the respective value is given. * marks statistically significant change over time.

PS: Gradient		5 min	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	Mean	4.26	3.96	3.73	4.20	3.28	2.49	1.73	2.81
	(SD)	(0.72)	(0.65)	(0.70)	(1.84)	(0.73)	(0.66)	(0.54)	(1.12)
	p-value	n/a	0.4930	0.2235	0.9455	0.1720	0.0012 *	<0.0001*	0.0005 *
0.50 µM	Mean	6.85	4.79	4.66	4.82	4.01	4.19	2.77	3.75
	(SD)	(3.73)	(1.43)	(1.39)	(1.75)	(1.31)	(2.79)	(0.92)	(1.12)
	p-value	n/a	0.2343	0.1903	0.2104	0.0729	0.1167	0.0072*	0.0224*
1 µM	Mean	5.87	5.78	5.50	4.96	4.18	2.63	1.36	2.22
	(SD)	(0.58)	(1.06)	(1.09)	(1.06)	(0.77)	(0.79)	(0.39)	(0.97)
	p-value	n/a	0.8680	0.5061	0.0915	0.0016 *	<0.0001*	<0.0001*	<0.0001*
2 µM	Mean	10.35	8.23	7.40	6.81	8.15	4.00	1.95	2.84
	(SD)	(5.21)	(1.27)	(0.10)	(0.96)	(4.55)	(1.28)	(0.76)	(0.86)
	p-value	n/a	0.3576	0.1441	0.0396*	0.3179	0.0025*	<0.0001*	<0.0001*
4 µM	Mean	26.73	22.76	20.41	17.82	17.70	8.86	3.76	3.06
	(SD)	(11.29)	(6.68)	(6.32)	(6.29)	(11.58)	(3.86)	(1.11)	(0.62)
	p-value	n/a	0.4755	0.2200	0.0542	0.0671	0.0003*	<0.0001*	<0.0001*
8 µM	Mean	42.80	37.55	34.56	30.09	28.16	12.01	3.54	4.88
	(SD)	(19.88)	(13.05)	(13.41)	(12.15)	(17.67)	(7.13)	(2.04)	(1.16)
	p-value	n/a	0.6008	0.3881	0.1370	0.0809	0.0002 *	<0.0001*	<0.0001*
16 µM	Mean	81.20	78.70	68.17	71.07	64.26	35.82	8.74	4.88
	(SD)	(16.41)	(12.94)	(15.87)	(8.87)	(19.12)	(11.09)	(5.52)	(1.61)
	p-value	n/a	0.7763	0.1268	0.1186	0.0400*	<0.0001*	<0.0001*	<0.0001*

Table A4: NH_4^+ substrate concentrations (µM) in experiment PD, measured at each sampling point (5-300 min) for all initial exposure concentrations (0.25-16 µM). Substrate concentration is given as mean of all replicates (± SD). P-value is representing change of substrate concentration over time from first sampling point (5 min) to sampling point where the respective value is given. * marks statistically significant change over time.

PD: Gradient		5 min	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	Mean	4.74	4.42	4.10	3.88	3.74	3.02	1.99	1.67
	(SD)	(2.08)	(1.79)	(1.93)	(2.00)	(2.47)	(1.98)	(1.24)	(0.85)
	p-value	n/a	0.7883	0.5884	0.4307	0.3805	0.1187	0.0051 *	0.0003 *
0.50 µM	Mean	6.00	4.70	3.94	3.29	3.92	2.00	1.32	1.19
	(SD)	(2.48)	(0.96)	(0.71)	(0.71)	(3.07)	(0.51)	(0.44)	(0.22)
	p-value	n/a	0.2597	0.0456*	0.0028*	0.0703	0.0008*	<0.0001*	<0.0001*
1 µM	Mean	7.85	6.61	5.64	5.04	3.76	2.21	1.24	1.74
	(SD)	(1.71)	(1.69)	(1.58)	(1.73)	(1.38)	(0.78)	(0.31)	(1.34)
	p-value	n/a	0.2358	0.0365*	0.0056*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
2 µM	Mean	10.70	8.56	7.62	6.87	7.34	2.99	1.25	0.95
	(SD)	(4.35)	(2.55)	(2.58)	(2.70)	(4.28)	(2.54)	(1.01)	(0.48)
	p-value	n/a	0.3230	0.1377	0.0447*	0.1111	0.0003*	<0.0001*	<0.0001*
4 µM	Mean	24.33	20.74	18.15	15.69	12.60	5.27	1.50	0.93
	(SD)	(2.80)	(5.06)	(4.81)	(4.56)	(1.55)	(2.44)	(0.78)	(0.30)
	p-value	n/a	0.1589	0.0279*	0.0017*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
8 µM	Mean	35.33	34.55	27.38	21.30	13.25	3.93	1.11	1.00
	(SD)	(8.28)	(6.31)	(4.86)	(5.83)	(5.23)	(2.54)	(0.26)	(0.20)
	p-value	n/a	0.8568	0.0372*	0.0002*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
16 µM	Mean	82.96	77.67	66.95	59.34	51.46	17.52	2.37	1.25
	(SD)	(29.29)	(29.12)	(26.06)	(26.10)	(29.47)	(14.89)	(1.67)	(0.49)
	p-value	n/a	0.7604	0.3163	0.1035	0.0285*	<0.0001*	<0.0001*	<0.0001*

Degree of significant difference in ammonium substrate concentration per gDW between experiments AS, AD, PS and PD

Table A5: Degree of significant difference between NH_4^+ substrate concentration ($\mu M\ gDW^{-1}$) in experiments AS and AD at the different sampling points. * marks statistically significant difference between the samples.

Gradient	5 min	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	0.0029 *	0.0004 *	0.0003 *	0.0004 *	0.0005 *	0.0007 *	0.0104 *	0.0044 *
0.50 µM	0.0005 *	0.1101	0.0002 *	0.0022 *	0.0014 *	0.0023 *	0.0004 *	0.0010 *
1 µM	0.0387 *	0.0004 *	0.0004 *	<0.0001*	<0.0001*	0.0001 *	0.0002 *	0.0002 *
2 µM	0.0471 *	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0002 *	0.0009 *	0.0164 *
4 µM	0.1268	0.6329	0.7301	0.8514	0.1400	0.8459	0.0165 *	0.0075 *
8 µM	0.1400	0.1691	0.4094	0.0020 *	0.0039 *	0.0221 *	0.0097 *	0.0127 *
16 µM	0.3205	0.2645	0.0192 *	0.0031 *	0.0064 *	0.0175 *	0.0331 *	0.0122 *

Table A6: Degree of significant difference between NH_4^+ substrate concentration ($\mu M\ gDW^{-1}$) in experiments PS and PD at the different sampling points. * marks statistically significant difference between the samples.

Gradient	5 min	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	0.6463	0.5782	0.6674	0.7815	0.7048	0.5511	0.6514	0.0773
0.50 µM	0.6523	0.9046	0.2916	0.0899	0.9481	0.1122	0.0098 *	0.0022 *
1 µM	0.0357 *	0.3330	0.8676	0.9228	0.5424	0.3757	0.5743	0.4988
2 µM	0.9011	0.7871	0.8564	0.9611	0.7576	0.4141	0.2052	0.0016 *
4 µM	0.6323	0.5689	0.5022	0.5180	0.3326	0.0887	0.0028 *	0.0001 *
8 µM	0.4250	0.6266	0.2617	0.1531	0.0959	0.0384 *	0.0326 *	0.0133 *
16 µM	0.9010	0.9390	0.9243	0.3366	0.3966	0.0383 *	0.0359 *	0.0020 *

Table A7: Degree of significant difference between NH_4^+ substrate concentration ($\mu M\ gDW^{-1}$) in experiments AS and PS at the different sampling points. * marks statistically significant difference between the samples.

Gradient	5 min	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	0.0368 *	0.3655	0.5365	0.4825	0.1436	0.3601	0.6125	0.7447
0.50 µM	0.1391	0.6055	0.6763	0.5919	0.5477	0.2439	0.0314 *	0.0097 *
1 µM	0.2121	0.7273	0.7580	0.4180	0.0211 *	0.0383 *	0.0370 *	0.0243 *
2 µM	0.4836	0.0118 *	0.0029 *	0.0269 *	0.281	0.3030	0.0933	0.0674
4 µM	0.0323 *	0.0075 *	0.0067 *	0.0100 *	0.0972	0.0079 *	0.0005 *	0.0017 *
8 µM	0.0852	0.0617	0.0753	0.1168	0.1499	0.3316	0.0161 *	0.2076
16 µM	0.2486	0.1515	0.3246	0.0720	0.0497 *	0.0995	0.0373 *	0.6699

Table A8: Degree of significant difference between NH_4^+ substrate concentration (μM gDW^{-1}) in experiments AD and PD at the different sampling points. * marks statistically significant difference between the samples.

Gradient	5 min	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 μM	0.0257 *	0.0076 *	0.0094 *	0.0106 *	0.0314 *	0.0135 *	0.0111 *	0.0049 *
0. 50 μM	0.0095 *	0.0716	0.0002 *	0.0002 *	0.0345 *	0.0002 *	0.0007 *	<0.0001*
1 μM	0.0059 *	0.0025 *	0.0030 *	0.0053 *	0.0022 *	0.0009 *	0.0002 *	0.0245 *
2 μM	0.1418	0.0460 *	0.0494 *	0.0365 *	0.0091 *	0.0345 *	0.0284 *	0.0049 *
4 μM	0.0412 *	0.0556	0.0126 *	0.0426 *	0.0008 *	0.0752	0.0052 *	0.0022 *
8 μM	0.6728	0.7441	0.9229	0.0015 *	0.0016 *	0.0209 *	<0.0001*	<0.0001*
16 μM	0.0751	0.0624	0.0073 *	0.0034 *	0.0079 *	0.0345 *	0.0178 *	0.0015 *

Appendix B – Nitrate substrate concentration per gDW

Change of nitrate substrate concentration per gDW in experiments PS and PD

Table A9: NO_3^- substrate concentrations (µM) in experiment PS, measured at each sampling point (5-300 min) for all initial exposure concentrations (0.25-16 µM). Substrate concentration is given as mean of all replicates (± SD). P-value is representing change of substrate concentration over time from first sampling point (5 min) to sampling point where the respective value is given. * marks statistically significant change over time.

PS: Gradient		5 min	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	Mean	12.45	13.63	13.87	13.95	12.11	10.13	5.68	3.71
	(SD)	(1.49)	(2.49)	(2.06)	(2.26)	(2.10)	(1.85)	(1.58)	(1.06)
	p-value	n/a	0.7593	0.7568	0.7864	0.6236	0.0925	<0.0001*	<0.0001*
0.50 µM	Mean	10.58	10.13	10.09	10.44	10.02	8.82	3.99	2.63
	(SD)	(3.85)	(3.80)	(2.68)	(3.03)	(2.69)	(2.35)	(2.76)	(1.15)
	p-value	n/a	0.8416	0.8252	0.9718	0.8489	0.3041	<0.0001*	<0.0001*
1 µM	Mean	8.78	8.83	8.23	7.55	7.70	5.68	2.82	2.02
	(SD)	(2.24)	(2.44)	(2.97)	(2.03)	(2.53)	(1.44)	(0.66)	(0.34)
	p-value	n/a	0.9741	0.6734	0.3029	0.2924	0.0083*	<0.0001*	<0.0001*
2 µM	Mean	7.30	7.68	8.65	8.80	8.08	7.35	3.22	2.14
	(SD)	(1.02)	(1.02)	(1.91)	(1.96)	(1.57)	(2.94)	(0.91)	(0.37)
	p-value	n/a	0.5337	0.0917	0.0578	0.3273	0.7178	<0.0001*	<0.0001*
4 µM	Mean	9.22	9.10	9.90	10.57	10.80	8.97	5.77	3.43
	(SD)	(1.93)	(2.56)	(1.74)	(2.42)	(3.35)	(2.54)	(0.79)	(1.06)
	p-value	n/a	0.9331	0.5244	0.1963	0.1419	0.9873	0.0015*	<0.0001*
8 µM	Mean	8.75	8.43	8.22	7.95	7.55	5.84	3.39	2.43
	(SD)	(3.36)	(3.06)	(3.44)	(3.31)	(3.20)	(3.05)	(1.77)	(0.89)
	p-value	n/a	0.8699	0.7888	0.6610	0.4832	0.0732	0.0003*	<0.0001*
16 µM	Mean	7.05	7.08	6.90	6.98	6.82	6.75	4.02	2.41
	(SD)	(2.11)	(2.09)	(1.57)	(1.58)	(1.68)	(2.54)	(2.04)	(0.45)
	p-value	n/a	0.9846	0.8735	0.9006	0.7925	0.7247	0.0022*	<0.0001*

Table A10: NO_3^- substrate concentrations (µM) in experiment PD, measured at each sampling point (5-300 min) for all initial exposure concentrations (0.25-16 µM). Substrate concentration is given as mean of all replicates (± SD). P-value is representing change of substrate concentration over time from first sampling point (5 min) to sampling point where the respective value is given. * marks statistically significant change over time.

PD: Gradient		5 min	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	Mean	7.53	7.95	9.16	9.06	6.10	5.40	4.56	3.36
	(SD)	(2.85)	(2.81)	(2.95)	(3.39)	(3.46)	(3.08)	(2.85)	(1.52)
	p-value	n/a	0.8007	0.3085	0.2894	0.4096	0.0492*	0.0055*	0.0001*
0.50 µM	Mean	9.46	10.93	10.24	9.59	8.05	5.08	3.07	2.63
	(SD)	(3.96)	(4.58)	(3.66)	(3.42)	(3.86)	(2.40)	(1.26)	(0.78)
	p-value	n/a	0.5636	0.8218	0.8820	0.2948	0.0042*	<0.0001*	<0.0001*
1 µM	Mean	7.37	8.69	8.20	8.34	8.07	6.43	3.40	2.92
	(SD)	(2.79)	(2.57)	(2.48)	(2.42)	(3.18)	(2.69)	(1.18)	(1.05)
	p-value	n/a	0.4139	0.6881	0.6788	0.8756	0.2439	0.0001*	<0.0001*
2 µM	Mean	5.88	5.78	5.60	5.87	4.76	4.11	3.03	2.85
	(SD)	(1.22)	(1.20)	(1.29)	(1.53)	(1.51)	(1.67)	(0.99)	(1.01)
	p-value	n/a	0.8904	0.6838	0.9573	0.1501	0.0079*	<0.0001*	<0.0001*
4 µM	Mean	14.74	15.53	14.28	3.47	10.34	7.05	4.17	4.00
	(SD)	(5.23)	(3.15)	(2.42)	(0.67)	(2.80)	(3.16)	(1.62)	(1.09)
	p-value	n/a	0.7566	0.7573	<0.0001*	0.0069*	0.0017*	<0.0001*	<0.0001*
8 µM	Mean	6.91	6.64	7.69	6.98	5.99	3.82	2.61	2.62
	(SD)	(1.78)	(2.22)	(3.49)	(2.54)	(1.66)	(0.81)	(0.50)	(0.48)
	p-value	n/a	0.8187	0.5397	0.7739	0.5046	0.0045*	<0.0001*	<0.0001*
16 µM	Mean	7.23	7.45	7.42	7.56	6.00	4.04	3.26	2.77
	(SD)	(2.84)	(2.90)	(3.46)	(4.66)	(4.13)	(2.40)	(1.40)	(0.94)
	p-value	n/a	0.9031	0.9271	0.8877	0.5412	0.0534	0.0030 *	0.0002 *

Degree of significant difference in nitrate substrate concentration in experiments PS and PD

Table A11: Degree of significant difference between NO_3^- substrate concentration ($\mu M\ gDW^{-1}$) in experiments PS and PD at the different sampling points. * marks statistically significant difference between the samples.

Gradient	5 min	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	0.0065 *	0.0062 *	0.0142 *	0.0196 *	0.0071 *	0.0132 *	0.4313	0.6697
0.50 µM	0.6291	0.7472	0.9379	0.6572	0.3317	0.0213 *	0.4817	0.4941
1 µM	0.3577	0.9275	0.9849	0.5524	0.8298	0.5656	0.3258	0.0917
2 µM	0.0556	0.0149 *	0.0106 *	0.0172 *	0.0039 *	0.0477 *	0.7391	0.1548
4 µM	0.0492 *	0.0033 *	0.0057 *	0.0005 *	0.8009	0.275	0.0640	0.3809
8 µM	0.2722	0.2736	0.7955	0.5839	0.3199	0.1713	0.3377	0.6589
16 µM	0.9036	0.8168	0.7663	0.8017	0.6950	0.1038	0.4749	0.4239

Appendix C – Ammonium uptake rate

Change in ammonium uptake rate over time

Table A12: Uptake rates (μM gDW^{-1} h^{-1}) of NH_4^+ per sample point from 10-300 min for experiment AS given in mean (± SD), for all initial exposure substrate concentration (0.25-16 μM). The time where the highest uptake rate was found is marked as bold. P-values represent degree of statistical significance from the point of highest uptake rate to/from the respective time. * marks statistically significant change with time.

AS: Gradient		10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 μM	Mean	-0.24	0.21	-0.36	**0.76**	0.17	0.01	-0.14
	(SD)	(0.38)	(0.45)	(0.35)	**(0.21)**	(0.06)	(0.11)	(0.12)
	p-value	0.0049*	0.0268*	<0.0001*	**n/a**	<0.0001*	0.0001*	<0.0001*
0.50 μM	Mean	-0.72	0.12	0.03	**0.67**	0.34	0.16	-0.03
	(SD)	(0.95)	(0.38)	(0.52)	**(0.33)**	(0.07)	(0.10)	(0.05)
	p-value	0.0028*	0.0376*	0.0497*	**n/a**	0.0417*	0.0015*	<0.0001*
1 μM	Mean	0.83	0.82	**1.76**	1.17	0.46	0.13	-0,01
	(SD)	(0.78)	(0.34)	**(1.30)**	(0.34)	(0.08)	(0.04)	(0.04)
	p-value	0.4019	0.1523	**n/a**	0.3075	0.0095*	0.0006*	<0.0001*
2 μM	Mean	0.15	1.01	**2.35**	1.93	0.93	0.30	0.01
	(SD)	(0.86)	(0.84)	**(0.47)**	(0.42)	(0.19)	(0.15)	(0.09)
	p-value	0.0001*	0.0070*	**n/a**	0.1383	<0.0001*	<0.0001*	<0.0001*
4 μM	Mean	**2.70**	2.89	2.56	1.05	1.99	0.31	-0.11
	(SD)	**(4.44)**	(0.60)	(0.64)	(0.51)	(1.62)	(0.19)	(0.06)
	p-value	**n/a**	0.9204	0.9157	0.1671	0.3293	0.0128 *	0.0004 *
8 μM	Mean	3.71	**3.96**	3.58	3.73	2.64	1.11	-0.06
	(SD)	(1.89)	**(1.21)**	(2.06)	(1.84)	(0.65)	(0.70)	(0.11)
	p-value	0.7827	**n/a**	0.7046	0.8562	0.1287	<0.0001*	<0.0001*
16 μM	Mean	**17.89**	8.58	8.70	9.25	6.01	2.77	0.02
	(SD)	**(9.68)**	(2.71)	(4.44)	(2.74)	(2.11)	(1.67)	(0.28)
	p-value	**n/a**	0.0465*	0.0278*	0.0637	0.0118*	0.0003*	<0.0001*

Table A13: Uptake rates (μM gDW^{-1} h^{-1}) of NH_4^+ per sample point from 10-300 min for experiment AD given in mean (± SD), for all initial exposure substrate concentration (0.25-16 μM). The time where the highest uptake rate was found is marked as bold. P-values represent degree of statistical significance from the point of highest uptake rate to/from the respective time. * marks statistically significant change with time.

AD: Gradient		10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 μM	Mean	**1.07**	0.59	0.38	0.36	0.05	0	0
	(SD)	**(0.64)**	(0.19)	(0.15)	(0.10)	(0.06)	(0)	(0)
	p-value	**n/a**	0.1063	0.0072*	0.0049*	<0.0001*	<0.0001*	<0.0001*
0.50 μM	Mean	0.34	0.31	**1.01**	0.62	0.13	0	0
	(SD)	(0.60)	(0.61)	**(0.28)**	(0.12)	(0.08)	(0)	(0)
	p-value	0.0439*	0.0323*	**n/a**	0.0109*	<0.0001*	<0.0001*	<0.0001*
1 μM	Mean	**2.10**	0.92	0.89	0.88	0.26	0.001	0
	(SD)	**(0.72)**	(0.12)	(0.35)	(0.21)	(0.14)	(0.002)	(0)
	p-value	**n/a**	0.0038*	0.0022*	0.0072*	<0.0001*	<0.0001*	0.0001*
2 μM	Mean	0.76	1.40	1.69	**2.68**	0.05	0	0
	(SD)	(0.27)	(0.40)	(0.49)	**(0.57)**	(0.13)	(0)	(0)
	p-value	<0.0001*	0.0002*	0.0093*	**n/a**	<0.0001*	0.0006*	0.0011*
4 μM	Mean	5.80	**9.80**	-0.80	3.65	1.01	0.52	0
	(SD)	(2.26)	**(3.93)**	(2.69)	(2.88)	(1.19)	(0.76)	(0)
	p-value	0.1159	**n/a**	0.0025 *	0.1742	0.0383*	0.0202*	0.0368*
8 μM	Mean	10.12	17.50	**27.91**	3.91	0.21	0.06	0.01
	(SD)	(1.76)	(7.95)	**(11.74)**	(3.64)	(0.71)	(0.02)	(0.01)
	p-value	0.0142*	0.1394	**n/a**	0.0009*	0.0009*	0.0041*	0.0050*
16 μM	Mean	33.18	**36.88**	22.64	4.53	0.003	0	0
	(SD)	(13.29)	**(12.21)**	(18.37)	(4.95)	(0.01)	(0)	(0)
	p-value	0.6422	**n/a**	0.1448	0.0005*	<0.0001*	<0.0001*	<0.0001*

Table A14: Uptake rates (µM gDW^{-1} h^{-1}) of NH_4^+ per sample point from 10-300 min for experiment PS given in mean (± SD), for all initial exposure substrate concentration (0.25-16 µM). The time where the highest uptake rate was found is marked as bold. P-values represent degree of statistical significance from the point of highest uptake rate to/from the respective time. * marks statistically significant change with time.

PS: Gradient		10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	Mean	**1.41**	0.39	0.34	0.19	0.29	0.12	-0.13
	(SD)	**(0.60)**	(0.09)	(0.28)	(0.13)	(0.06)	(0.06)	(0.08)
	p-value	**n/a**	0.0024*	0.0017*	0.0014*	0.0106*	0.0048*	<0.0001*
0.50 µM	Mean	**1.67**	0.24	0.38	0.34	0.40	0.04	-0.11
	(SD)	**(0.96)**	(0.42)	(0.41)	(0.24)	(0.13)	(0.14)	(0.09)
	p-value	**n/a**	0.0087*	0.0151*	0.0347*	0.0963	0.0222*	0.0019*
1 µM	Mean	**0.96**	0.46	0.85	0.33	0.65	0.20	-0.10
	(SD)	**(0.32)**	(0.29)	(0.19)	(0.19)	(0.06)	(0.11)	(0.08)
	p-value	**n/a**	0.0309*	0.8494	0.0347*	0.3284	0.0012*	<0.0001*
2 µM	Mean	0.67	**1.30**	0.94	0.27	0.93	0.33	-0.10
	(SD)	(1.50)	**(0.84)**	(0.24)	(0.32)	(0.20)	(0.09)	(0.03)
	p-value	0.4033	**n/a**	0.3376	0.0057*	0.3337	0.0164*	<0.0001*
4 µM	Mean	**4.82**	3.68	3.95	2.36	1.96	0.82	0.09
	(SD)	**(2.68)**	(1.12)	(0.60)	(0.69)	(0.64)	(0.45)	(0.14)
	p-value	**n/a**	0.3606	0.4062	0.0186*	0.0014*	<0.0001*	<0.0001*
8 µM	Mean	**7.15**	4.77	6.83	3.57	4.10	1.36	0.10
	(SD)	**(2.96)**	(1.34)	(2.43)	(0.42)	(1.20)	(0.82)	(0.13)
	p-value	**n/a**	0.1092	0.9072	0.0500*	0.0311*	<0.0001*	<0.0001*
16 µM	Mean	10.52	**16.17**	-3.36	8.69	10.38	4.34	0.46
	(SD)	(4.11)	**(10.69)**	(11.54)	(3.12)	(2.96)	(0.94)	(0.50)
	p-value	0.3938	**n/a**	0.0374*	0.0732*	0.1152	0.0129*	0.0013*

Table A15: Uptake rates (µM gDW^{-1} h^{-1}) of NH_4^+ per sample point from 10-300 min for experiment PD given in mean (± SD), for all initial exposure substrate concentration (0.25-16 µM). The time where the highest uptake rate was found is marked as bold. P-values represent degree of statistical significance from the point of highest uptake rate from the respective time. * marks statistically significant change with time.

PD: Gradient		10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	Mean	**2.22**	0.51	0.37	-0.15	0.39	0.17	0.04
	(SD)	**(1.42)**	(0.30)	(0.16)	(0.41)	(0.19)	(0.12)	(0.05)
	p-value	**n/a**	0.0173*	0.0035*	0.0006*	0.0290*	0.04153*	0.0190*
0.50 µM	Mean	**0.65**	1.16	0.98	0.50	0.24	0.11	0.02
	(SD)	**(1.31)**	(0.40)	(0.16)	(0.41)	(0.37)	(0.08)	(0.03)
	p-value	**n/a**	0.3875	0.5073	0.4729	0.0495*	0.0045*	0.0005*
1 µM	Mean	1.24	**1.49**	0.93	0.97	0.57	0.16	0.003
	(SD)	(0.89)	**(0.35)**	(0.38)	(0.29)	(0.23)	(0.08)	(0.01)
	p-value	0.5475	**n/a**	0.0250*	0.0618	0.0005*	<0.0001*	<0.0001*
2 µM	Mean	**1.90**	1.47	1.16	0.77	1.08	0.28	0.04
	(SD)	**(0.77)**	(0.45)	(0.24)	(0.43)	(0.29)	(0.25)	(0.06)
	p-value	**n/a**	0.2792	0.0283*	0.0011*	0.0217*	<0.0001*	<0.0001*
4 µM	Mean	**6.93**	4.00	3.74	3.79	2.66	0.61	0.07
	(SD)	**(4.79)**	(2.73)	(1.19)	(1.46)	(1.17)	(0.31)	(0.08)
	p-value	**n/a**	0.2331	0.1164	0.1550	0.0425*	0.0003*	<0.0001*
8 µM	Mean	6.92	**10.78**	9.05	6.04	3.38	0.45	0.02
	(SD)	(2.96)	**(2.48)**	(2.43)	(2.38)	(1.26)	(0.37)	(0.02)
	p-value	0.1165	**n/a**	0.2503	0.0028*	<0.0001*	<0.0001*	<0.0001*
16 µM	Mean	**18.46**	16.42	11.71	6.14	12.31	2.43	0.13
	(SD)	**(12.66)**	(5.72)	(2.24)	(3.81)	(5.80)	(2.12)	(0.15)
	p-value	**n/a**	0.7276	0.1582	0.0032*	0.1119	0.0005*	<0.0001*

Degree of significant difference in ammonium uptake rate in experiments AS, AD, PS and PD

Table A16: Degree of significant difference between NH_4^+ uptake rate ($\mu M\ gDW^{-1}\ h^{-1}$) in experiments AS and AD at the different sampling intervals. * marks statistically significant difference between the samples.

Gradient	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	0.0072 *	0.1142	0.0061 *	0.0034 *	0.0069 *	0.8092	0.0630
0.50 µM	0.0541	0.5680	0.0097 *	0.3511	0.0010 *	0.0139 *	0.1516
1 µM	0.0290 *	0.5616	0.1669	0.1161	0.0160 *	0.0004 *	0.6900
2 µM	0.1958	0.3504	0.0386 *	0.0301 *	<0.0001*	0.0107 *	0.8868
4 µM	0.2222	0.0164 *	0.0849	0.0777	0.2642	0.5378	0.0080 *
8 µM	0.0058 *	0.0183 *	0.0089 *	0.9194	0.0001 *	0.0145 *	0.1535
16 µM	0.0683	0.0019 *	0.0086 *	0.0764	0.0009 *	0.0096 *	0.8910

Table A17: Degree of significant difference between NH_4^+ uptake rate ($\mu M\ gDW^{-1}\ h^{-1}$) in experiments PS and PD at the different sampling intervals. * marks statistically significant difference between the samples.

Gradient	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	0.2862	0.3958	0.8119	0.1402	0.3135	0.4504	0.0026 *
0.50 µM	0.2007	0.0030 *	0.0295 *	0.4805	0.4026	0.3582	0.0175 *
1 µM	0.545	0.0003 *	0.6771	0.0019 *	0.4263	0.3957	0.0263*
2 µM	0.1562	0.6781	0.1390	0.0769	0.3666	0.6619	0.0020 *
4 µM	0.4224	0.7954	0.7061	0.0980	0.2387	0.3605	0.8201
8 µM	0.9290	0.0009 *	0.1449	0.0513	0.3541	0.0429 *	0.1815
16 µM	0.2001	0.9596	0.0232 *	0.2530	0.4889	0.0831	0.1768

Table A18: Degree of significant difference between NH_4^+ uptake rate ($\mu M\ gDW^{-1}\ h^{-1}$) in experiments AS and PS at the different sampling intervals. * marks statistically significant difference between the samples

Gradient	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	0.0017 *	0.3997	0.0086 *	0.0004 *	0.0053 *	0.0883 *	0.8029
0.50 µM	0.0028 *	0.6358	0.2679	0.1145	0.4172	0.1594	0.1025
1 µM	0.7493	0.0985	0.1323	0.0009 *	0.2684	0.1728	0.0415 *
2 µM	0.5234	0.5728	0.0003 *	<0.0001*	0.8686	0.2726	0.0571
4 µM	0.3916	0.1693	0.0032 *	0.0103 *	0.9666	0.0399 *	0.0162 *
8 µM	0.0617	0.2998	0.0397 *	0.8389	0.0509	0.5769	0.0448 *
16 µM	0.1406	0.2738	0.0512	0.7630	0.0178 *	0.0797	0.0955

Table A19: Degree of significant difference between NH_4^+ uptake rate (µM gDW^{-1} h^{-1}) in experiments AD and PD at the different sampling intervals. * marks statistically significant difference between the samples.

Gradient	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	0.1541	0.5916	0.9458	0.0489 *	0.0132 *	0.0205 *	0.1332
0.50 µM	0.6456	0.0330 *	0.8131	0.5635	0.5674	0.0193 *	0.2191
1 µM	0.1326	0.0092 *	0.8571	0.5559	0.0203 *	0.0043 *	0.4768
2 µM	0.0266 *	0.7809	0.0490 *	0.0001 *	0.0005 *	0.0411 *	0.2164
4 µM	0.6575	0.0273 *	0.0363 *	0.9236	0.0356 *	0.8131	0.1136
8 µM	0.2270	0.1332	0.0217 *	0.2615	0.0007 *	0.0487 *	0.8640
16 µM	0.0964	0.0073 *	0.2061	0.5431	0.0035 *	0.0379 *	0.07714

Appendix D – Nitrate uptake rate

Change of nitrate uptake rate over time

Table A20: Uptake rates (μM gDW^{-1} h^{-1}) of NO_3^- per sample point from 10-300 min for experiment PS given in mean (± SD). All samples were added 1 μM $NO3^-$, and a gradient of 0.25-16 μM NH_4^+. The time where the highest uptake rate was found is marked as bold. P-values represent degree of statistical significance from the point of highest uptake rate to/from the respective time. * marks statistically significant change with time.

PS: Gradient		10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 μM	Mean	**-0.69**	0.06	0.08	1.62	0.88	0.82	0.30
	(SD)	**(2.89)**	(0.86)	(0.39)	(0.71)	(0.43)	(0.28)	(0.17)
	p-value	**n/a**	0.5553	0.5860	0.0112*	0.0437*	0.1240	0.5885
0.50 μM	Mean	**1.67**	-0.68	-0.49	0.37	0.46	0.78	0.12
	(SD)	**(1.89)**	(0.81)	(1.14)	(0.65)	(0.29)	(0.41)	(0.24)
	p-value	**n/a**	0.0303*	0.1116	0.3482	0.6461	0.7385	0.6481
1 μM	Mean	-0.56	**0.97**	1.23	-0.07	0.75	0.46	0.10
	(SD)	(1.65)	**(2.24)**	(1.43)	(1.49)	(0.50)	(0.15)	(0.06)
	p-value	0.2783	**n/a**	0.8444	0.2755	0.6889	0.6209	0.2758
2 μM	Mean	**-0.24**	-1.31	-0.12	0.58	0.73	0.67	0.13
	(SD)	**(1.65)**	(1.59)	(0.44)	(0.45)	(0.15)	(0.49)	(0.07)
	p-value	**n/a**	0.3037	0.8191	0.0720	0.0148*	0.0159*	0.1226
4 μM	Mean	-1.46	-0.16	-0.002	-0.11	**0.69**	0.52	0.28
	(SD)	(1.49)	(0.51)	(0.51)	(0.98)	**(0.39)**	(0.41)	(0.14)
	p-value	0.0031*	0.0291*	0.0699	0.0956	**n/a**	0.4867	0.0456
8 μM	Mean	**1.19**	0.40	0.49	0.34	0.64	0.40	0.12
	(SD)	**(1.34)**	(0.81)	(0.59)	(0.13)	(0.20)	(0.24)	(0.11)
	p-value	**n/a**	0.2486	0.2276	0.1526	0.5113	0.3591	0.0679
16 μM	Mean	**0.10**	0.34	-0.04	0.16	0.05	0.44	0.19
	(SD)	**(1.56)**	(0.92)	(1.16)	(0.62)	(0.45)	(0.17)	(0.20)
	p-value	**n/a**	0.7496	0.8454	0.9476	0.8239	0.5459	0.6927

Table A21: Uptake rates (μM gDW^{-1} h^{-1}) of NO_3^- per sample point from 10-300 min for experiment PD given in mean (± SD). All samples were added 1 μM $NO3^-$, and a gradient of 0.25-16 μM NH_4^+. The time where the highest uptake rate was found is marked as bold. P-values represent degree of statistical significance from the point of highest uptake rate to/from the respective time. * marks statistically significant change with time.

PD: Gradient		10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 μM	Mean	-0.002	-1.65	0.25	**2.22**	0.27	0.14	0.15
	(SD)	(1.13)	(0.85)	(0.86)	**(1.74)**	(0.54)	(0.12)	(0.17)
	p-value	0.0015*	<0.0001*	0.0323*	**n/a**	0.0256*	0.0198*	0.0167*
0.50 μM	Mean	-4.81	0.62	**1.05**	1.19	1.09	0.32	0.05
	(SD)	(3.63)	(0.99)	**(0.93)**	(0.49)	(0.63)	(0.19)	(0.06)
	p-value	0.0024*	0.4790	**n/a**	0.7528	0.9689	0.0197*	0.0001*
1 μM	Mean	**-1.73**	-0.14	-0.12	0.25	0.61	0.49	0.06
	(SD)	**(6.02)**	(1.32)	(0.71)	(1.04)	(0.25)	(0.27)	(0.07)
	p-value	**n/a**	0.5813	0.4521	0.3325	0.2123	0.2450	0.4318
2 μM	Mean	**0.45**	0.33	-0.33	0.85	0.24	0.18	0.02
	(SD)	**(1.44)**	(0.77)	(1.01)	(0.43)	(0.11)	(0.14)	(0.03)
	p-value	**n/a**	0.8621	0.2275	0.5303	0.8747	0.8114	0.4327
4 μM	Mean	-1.95	2.39	**15.52**	-5.04	1.18	0.46	0.02
	(SD)	(6.42)	(1.15)	**(2.77)**	(1.76)	(0.54)	(0.30)	(0.12)
	p-value	0.0048*	0.0061*	**n/a**	0.0039*	0.0005*	<0.0001*	<0.0001*
8 μM	Mean	**-0.20**	-1.45	0.09	-0.003	0.79	0.20	0.001
	(SD)	**(2.57)**	(2.47)	(1.91)	(0.91)	(0.42)	(0.09)	(0.03)
	p-value	**n/a**	0.4343	0.8477	0.5667	0.1202	0.2405	0.4129
16 μM	Mean	**0.83**	0.89	0.71	1.04	1.11	0.38	0.06
	(SD)	**(3.51)**	(0.47)	(0.46)	(0.53)	(1.13)	(0.51)	(0.06)
	p-value	**n/a**	0.9742	0.9317	0.8274	0.6905	0.5213	0.1210

Degree of significant difference in nitrate uptake rate between experiment PS and PD

Table A22: Degree of significant difference between NO_3^- uptake rate ($\mu M\, gDW^{-1}\, h^{-1}$) in experiments PS and PD at the different sampling intervals. * marks statistically significant difference between the samples.

Gradient	10 min	20 min	30 min	50 min	90 min	180 min	300 min
0.25 µM	0.6782	0.0060 *	0.4276	0.4595	0.0590	0.0011 *	0.1496
0.50 µM	0.0293 *	0.0539	0.1966	0.0358 *	0.0626	0.0441 *	0.5684
1 µM	0.6963	0.3350	0.1548	0.6785	0.5705	0.8282	0.3384
2 µM	0.4804	0.0554	0.6530	0.3116	0.0004 *	0.0574	0.0137 *
4 µM	0.8900	0.0050 *	<0.0001*	0.0004 *	0.1051	0.7725	0.0068 *
8 µM	0.3181	0.1308	0.6715	0.4490	0.4383	0.1081	0.0554
16 µM	0.7185	0.2563	0.2030	0.0252 *	0.0723	0.7760	0.1689