Consequences of nitrogen enrichment on lake plankton

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Summary

Human alterations of the earth nitrogen cycle are an important environmental problem. Due to human activities the production of reactive nitrogen compounds (e.g. fertilizer production) increased during the last decades. Consequently also the release of reactive nitrogen to the environment increases. Of major concern are increasing concentrations of reactive nitrogen in the atmosphere, which are deposited via precipitation into terrestrial and aquatic ecosystems. Nitrogen is an important nutrient that is known to limit primary production globally. The accumulation of nitrogen can cause a variety of negative consequences such as eutrophication, acidification or species loss. Thus, effects of increasing nitrogen loads are various and might not only concern nitrogen limited ecosystem.

In my thesis, I studied the effects of increased reactive nitrogen availability on aquatic ecosystems that are primarily phosphorus limited. Additionally, I investigated consequences of varying supply ratios of the two most important reactive nitrogen forms, ammonium and nitrate.

To perform these investigations large field experiments with natural plankton communities enclosed in suspended mesocosms and laboratory experiments with microcosms with artificial food chains of reduced complexity were established. Experimental manipulations include enrichment with reactive nitrogen in form of ammonium and nitrate based on natural occurring wet deposition concentrations of these nitrogen sources.

In lakes mainly phytoplankton is responsible for the direct uptake of nutrients and drives dynamics, representing the base of plankton food webs. I performed detailed analyses on phytoplankton abundances and chemical composition. As expected, most phytoplankton groups did not respond to the nitrogen enrichment in the phosphorus deficient environment. However, I observed an increase of mixotroph algae. Mixotroph algae are phytoplankton species that besides performing photosynthesis also feed on small particles, mostly bacteria. Additionally other bacterial consumers such as heterotrophic ciliates and flagellates increased with increasing nitrogen enrichment. These findings indicate that the additional nitrogen availability in phosphorus deficient lakes can positively influence the microbial activity and thereby the microbial food web (microbial loop) in the plankton of lakes, redirecting the flow of energy and matter towards heterotrophic food web components.

Even within a small regional scale lakes vary largely in their characteristics, such as nutrient availability. Effects of nitrogen enrichment might therefore vary between lakes. I performed nitrogen enrichment experiments in three lakes to compare responses of plankton

communities to nitrogen addition. Indeed, I observed varying responses of phytoplankton and zooplankton from different lakes to the same nitrogen enrichment gradient. The responses were dependent on the plankton composition which was different between lakes, however general mechanisms became clear. At high nitrogen enrichment zooplankton was reduced in all lake communities and a shift between zooplankton species was observed.

Besides the amount of available reactive nitrogen also the ratio between the different reactive nitrogen sources can be important. Predictions of future nitrogen deposition composition point towards an increased proportion of ammonium compared to nitrate. In a 2x2 factorial mesocosm experiment I investigated the response of a phytoplankton community to both increasing nitrogen availability and different nitrate to ammonium ratios. Total nitrogen had only minor effects on plankton dynamics whereas the different supply ratios of nitrate and ammonium resulted in much stronger consequences. High ammonium treatments resulted independent of the total nitrogen in higher chlorophyll *a* concentrations, higher abundances of small algae and a better resource use efficiency of phytoplankton for phosphorus. Thus, based on future deposition predictions substantial shifts in plankton dynamics can not only result from increasing nitrogen deposition but also from qualitative changes of future nitrogen deposition.

Despite the nutritional aspects of ammonium it has also to be considered as a toxin in aquatic ecosystems, especially at higher concentrations. In a laboratory experiment with an artificially assembled plankton community of reduced complexity I investigated the effects of increasing ammonium concentrations on different algae species and subsequent consequences for algae consumers, represented by *Daphnia*. Increasing ammonium concentrations had a negative impact on phytoplankton abundances but the effect sizes varied between algal species. Ammonium also affected the carbon to nutrient stoichiometry of algal biomass; increasing ammonium resulted in a better resource uptake efficiency of phytoplankton for phosphorus and thereby increased food quality for zooplankton. Growth rates of *Daphnia* fed with the algae exposed to ammonium as the only nitrogen source increased therefore with increasing ammonium concentrations. Such complex direct and indirect positive and negative effects of a nutrient on plankton dynamics will also result in complex food web effects which are clearly not only restricted to a growth enhancing effect of a nutrient.

My results show that predicted future deposition scenarios of reactive nitrogen have the potential to result in alterations of lake ecosystems even if the lake is phosphorus limited. Consequences can be seen on different lower trophic levels of plankton food webs and these

effects can be transferred to higher trophic levels up to fish. A coordinated nitrogen management managing the sources and deposition of reactive nitrogen is therefore also an important task to ensure a reliable ecosystem functioning of lakes in the future.

1

Introduction

1.1 Ecological background

1.1.1 Anthropogenic alterations of earth ecosystem processes

Since at least the onset of industrialization in the 19th century, anthropogenic influences are the main driver of complex processes affecting earth ecosystems. One of the most prominent examples is that human activities are causing climate change. However, there are far more biophysical and chemical processes that are seriously altered by human activities.

It is important to understand to which extent anthropogenic activities alter global ecosystems. Therefore Rockström et al. (2009) defined planetary boundaries to the nine most important human stressors affecting global ecosystems, whose stability is responsible for a well-being for humans on Earth. These stressors are: climate change, ocean acidification, stratospheric ozone depletion, biogeochemical flows, global freshwater use, changes in land use, biodiversity loss, atmospheric aerosol loading and chemical pollution. The defined boundaries for those stressors are recommendations, within which a sustainable live on our planet and stable earth system services seem possible.

An updated version (Steffen et al. 2015) of these planetary boundaries suggests that two stressors show an increasing risk of passing the calculated thresholds. The relevant stressors are 1) climate change; measured as atmospheric CO_2 concentration and radiative forcing, and 2) land system change; measured as changes in the proportion of forested land (Figure 1). Even more important is that two of these earth system stressors seem to have already crossed the calculated borders. These are 1) changes in genetic diversity; measured by species extinction rates, and 2) changes in biochemical flows, wherein both the nitrogen (N) cycle and the phosphorus (P) cycle crossed critical borders (Figure 1). As measure for alterations in N cycles, the amount of anthropogenic fixed N was used and for P the phosphorus flow from freshwater systems into the ocean and from fertilizers to soils.

By revealing the most critical anthropogenic alterations of global processes, the N cycle is pointed out as being one of the biggest environmental problems humans have to face (Steffen et al. 2015). This opinion is supported by frequent reports of severe human influences on global N cycle (Vitousek et al. 1997; Galloway et al. 2008; Canfield et al. 2010). Thus, it is important to fully understand the complexity of the global N cycle and to investigate consequences that arise from its anthropogenic alterations.

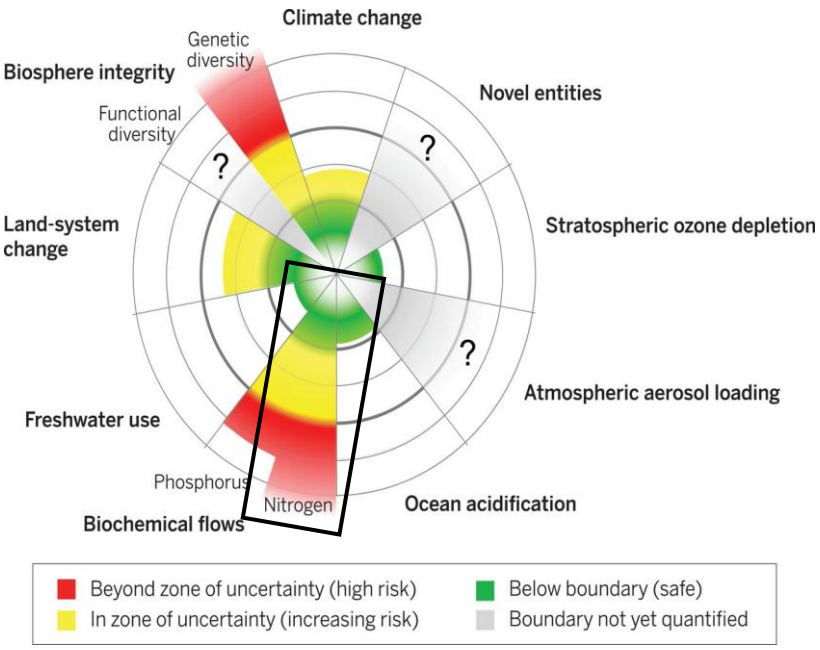

Figure 1: The current status of the most important earth processes responsible for a well working life on earth. The green area represents the so called "safe operating space" within which values should sustain. The yellow area represents an area of increasing risk for processes to cross borders. Of major concern (highlighted in red) are the loss of genetic diversity and biochemical flows. Within biochemical flows the nitrogen cycle (black rectangle) is highly altered. (modified after Steffen et al. 2015)

1.1.2 Anthropogenic alterations of the nitrogen cycle

On Earth most of N is present in its molecular form N_2 (around 78% of the atmosphere). A lot of energy is needed to transform molecular N into reactive N that can be used for biochemical processes (e.g. NO_x and NH_y, reactive nitrogen here after), such as protein synthesis and the production of biomass.

In nature there are two different ways of nitrogen fixation occurring, abiotic and biotic. Abiotic fixation can happen occasionally through lightings, biotic fixation can transform molecular N into reactive N by enzymatic driven biological processes (Figure 2). However, only few species of bacteria and archaea are capable of conducting biotic N fixation. This limitation in N fixation leads to N being the main limiting factor for primary production on a global scale (Vitousek and Howarth 1991; Le Bauer and Treseder 2008), although the element itself would be available in sufficient amounts. Consequently, a shift towards higher availability of reactive N can lead to severe changes in ecosystems by increasing a nutrient so far limiting primary production.

Especially since the mid of the 20th century humans change to a large extend the availability of reactive N (Galloway et al. 2003). Thereby, human's interventions with the earth N cycle can be divided in two parts: a) increasing fixation of airborne, molecular N and b) the release of reactive N into the atmosphere and into ecosystems.

a) Nitrogen fixation

The best known and also most important way of anthropogenic N fixation is the N-fertilizer production via the Haber-Bosch process that was invented in 1913. This process allows converting molecular N into ammonium nitrate, which is the main component of fertilizers. This industrial N fixation was of great importance, allowing covering rising food demands in the 20th century. Since then, fertilizer production has been rising from year to year parallel to growing human population and the ever since growing food demand (Ciais et al. 2013). Thus, the amount of fixed N through the Haber-Bosch process rose up to around 120 Tg N per year in 2010 (Fowler 2013, Figure 2).

However, there are also other ways in which humans can indirectly cause the fixation of airborne N (Figure 2). Associated with rising food demands and resulting increasing agricultural activities the amount of the so called *agricultural biological nitrogen fixation* is growing too (Herridge 2008). This term describes the biological fixation of N e.g. by symbiotic bacteria that occurs in agricultural areas. The agricultural biological N fixation is estimated to be around 60 Tg of reactive N per year nowadays (Fowler et al. 2013, Figure 2). Other ways how airborne N_2 can be converted to reactive N are combustion in engines, heating systems and industrial processes (Figure 2).

Altogether the amount of anthropogenic fixed N is currently around the same magnitude as the amount of natural fixed N (Fowler et al. 2013, Figure 2).

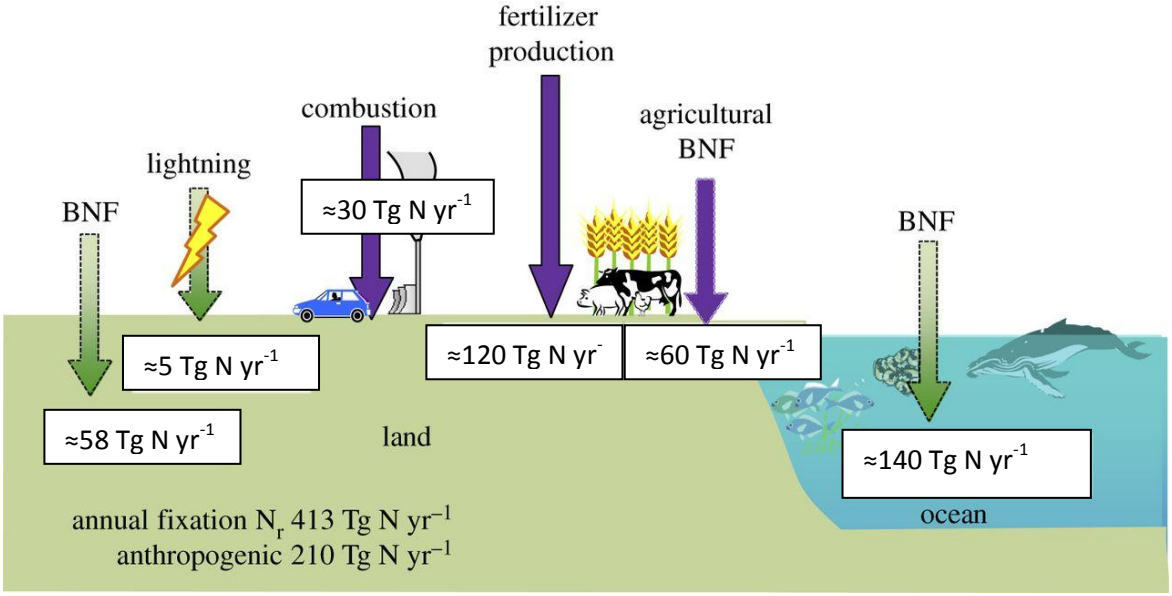

Figure 2: Global nitrogen fixation from atmospheric N_2 for the year 2010. In green natural ways of nitrogen fixation; these are: biological fixation (both on land and in the oceans) and fixation through lightning. In purple anthropogenic fixations are shown; this are: combustion, industrial fixation via Haber-Bosch process and agricultural biological fixation. (modified after Fowler et al. 2013).

b) Release of reactive nitrogen

Increasing anthropogenic N fixation is inevitable causing an increased supply of these N compounds to the environment, not only as desired by fertilizers but also through the atmosphere (Vitousek et al. 1997; Ciais et al. 2013). Main sources of airborne N are agriculture, the burning of fossil fuels and biomass and emissions from industrial plants (Galloway and Cowling 2002). The anthropogenic contribution to atmospheric reactive N is even 4-fold higher than the natural contribution such as by degassing from soils and creation through lightning (Ciais et al. 2013).

Reactive N in the atmosphere can easily be transported and distributed by wind, and gets deposited through precipitation and dry deposition in terrestrial and aquatic ecosystems (Lamarque et al. 2005; Elser et al. 2009a). The transport via atmosphere even leads to alterations of ecosystems, which do not experience the influence of other anthropogenic sources of N such as agricultural runoff (Elser et al. 2009b; Stevens et al. 2010a; Holtgrieve et al. 2011).

Although the amount of atmospheric N deposition is regionally distributed relatively uniform, its influence varies between different landscapes (Kaushal et al. 2011). The proportion of atmospheric N can vary between 10% of anthropogenic sources in agricultural regions up to representing the only anthropogenic N source in forest areas (Kaushal et al. 2011). The increasing deposition of reactive N via the atmosphere is within magnitudes that can cause severe changes to aquatic and terrestrial ecosystems (Dentener et al. 2006).

1.1.3 Nitrogen and ecosystem functioning

N is an important so called macronutrient which is needed in relative high demands for primary production. Due to the reasons mentioned before, N is the main limiting factor for primary production on a global scale. This accounts especially for land plants (Le Bauer and Treseder 2008). The most obvious consequence of increased N loads in N-limited systems is an increase of biomass of primary producers due to additional nutrient supply. A review by Le Bauer and Treseder (2008) indeed revealed an increased net primary production after N addition in a majority of terrestrial systems. Therefore, largest influences of increased anthropogenic N loads are expected in N-limited systems.

N addition to those systems has positive, fertilizing effects and result in a higher productivity. But, massive input of nutrients (especially of those naturally limiting primary production) are leading to eutrophication of ecosystems. Eutrophication is a well known problem caused by human interactions with nutrient cycles and can be observed in terrestrial as well as in aquatic ecosystems (Bouwman 2002; Smith 2003; Smith et al. 2009). Especially in coastal marine ecosystems, which are usually limited by N (Howarth and Marino 2006; Elser et al. 2007), increasing anthropogenic N input can have large influences on shifts in species composition, potentially resulting in harmful algal blooms accompanied by a reduction of water transparency and anoxia (especially in deep water layers) (Smith 2003).

Besides eutrophication, there are a variety of other potential negative consequences of increased N loads which can also affect ecosystems that are not N-limited in their primary production:

1) Toxic effects

Especially two forms of reactive nitrogen namely nitrite (NO_2^-) and NH_4^+ were shown to be toxic for a variety of organisms. On land, these include mainly plants (Dorland et al. 2003; Lucassen et al. 2003). In aquatic and marine ecosystems toxic effects of NO_2^- and NH_4^+ were shown throughout the whole food chain. Negative effects were for example shown in algae (Dai et al. 2008; Collos and Harrison 2014), invertebrates (Kohn et al. 1994; Xiang et al. 2010) and fish (Barbieri and Doi 2012; Dutra et al. 2016).

Toxic effects are mainly observed as short time response to high NO_2^- and NH_4^+ loads. However, the steady atmospheric depositions of reactive N are accumulating in aquatic ecosystems and lead to organisms being exposed to unnaturally high NO_2^- and NH_4^+ concentrations for a long time. Although these concentrations might not always lead to acute death they can cause chronic toxicity leading to decreases in biomass on various levels of the aquatic food chain.

2) Acidification

Another consequence is related to the acidification of ecosystems via the so called "acid rain" (Dillon and Molot 1990; Galloway 2001). Although deposition of SO_x and the resulting formation of sulfurous and sulfuric acids represent the main agent for

acidification, reactive N deposition also contributes with increasing importance (Singh and Agrawal 2007).

Negative consequences of acid deposition can occur on the level of primary producers and on animals (Singh and Agrawal 2007). Those consequences include for example reduced germination in woody plants (Fan and Wang 2000) or tissue damage in tropical tree species (Sant'Anna-Santos et al. 2006). In aquatic ecosystems acidification often leads to the reduction of species richness in both phytoplankton- and zooplankton communities and probably also to a reduction of zooplankton densities (Geelen 1986 and citations there in).

3) Species loss

A variety of studies indicate species loss in terrestrial ecosystems with increasing nitrogen deposition (e.g. Stevens et al. 2010a; Mayer et al. 2013; Roth et al. 2013). Mayer et al. (2013) for example propose chronicle N deposition as the driver of the lichens species decline in a remote alpine area. Also in aquatic ecosystems a connection between N load and species richness could be observed. In a study on 60 lakes in Poland and the UK, James et al. (2005) detected a relationship between low macrophyte species richness and high nitrate loads. Reasons for the species decline can include for example the promotion of nitrophilous species (species with high affinity to N), which can thereby outcompete other species (Bobbink et al. 1998; Stevens 2004). Stevens et al. (2010b) discussed the importance of several other N related factors leading to such potential species declines. They identified the eutrophication and acidification as driving forces but argued that other chemical changes may play a role in species decline too.

4) Sensitivity to other stressors

Further consequences of increasing N loads can be enhanced occurrence of stressors or increased susceptibility to existing stressors (Pardo et al. 2011). Examples for enhanced stressors are the N induced promotion of herbivore growth due to increased food quality (increased N concentration) of leaves, resulting in increasing grazing pressure on plants (Brunsting and Heil 1985; Power et al. 1998) and the promotion of competitive invasive species (Rao and Allen 2010). Signs for increased susceptibility to other, existing stressors with increasing N-fertilization were for example a reduced tolerance to low temperatures in montane red spruce (Schaberg et al. 2002) and a higher susceptibility to pathogens in tomato plants (Hoffland et al. 2000).

These above described examples do not represent nutritional consequences of increased N loads on primary producers. Thus, such processes can in theory also play an important role in non N-limited systems. Especially temperate lake ecosystems are usually rather P-limited or N-P-co-limited than N-limited (Schindler 1977; Elser et al. 2007). Within lakes especially the nutrient poor, oligotrophic ones seem to be rather P-limited (Sterner 2008).

By increasing one nutrient in an ecosystem, while other nutrients remain unaffected, the ratios between nutrients change, resulting potentially in far reaching consequences for ecosystems. Most important nutrients for phytoplankton growth are N, P and in the case of the algae groups of diatoms and some chrysophyceae species also silicon (Si). Although primary producers are in general flexible concerning nutrient ratios, there are optimal conditions for growth. The so called Redfield-Ratio defines optimal molar elemental stoichiometry of phytoplankton as C:N:P=106:16:1. Large deviations from this ratio within lake dissolved nutrients are considered to indicate potential carbon or nutrient limitation situations for phytoplankton growth. Measured dissolved nutrient ratios are therefore often used to detect possible signs of nutrient limitation.

Clear signals of strong shifts in N:P ratios being associated with atmospheric N deposition have been shown for large datasets of dissolved nutrients in lakes located in Norway, Sweden and the US (Bergström et al. 2005; Elser et al. 2009a). Both Elser et al. (2009a) and Bergström et al. (2005) pointed out that increasing atmospheric N deposition was resulting in a clear shift from N limitation to P limitation of phytoplankton. High atmospheric N deposition in these very remote lakes resulted in increased phytoplankton growth and eutrophication.

However, already before atmospheric N loads result in such extreme shifts of nutrient limitation patterns, their effects on ecosystems can be potentially detected. It has been shown, that changing nutrient ratios can influence phytoplankton species composition (Sommer 1994). Within different algal groups, species abundances can change due to different demands for N. It was shown, for example, that the contribution of different diatom species to total biovolume is changing along a N:Si ratio gradient (Sommer 1994). Shifts in nutrient availability ratios might even lead to a loss of those phytoplankton species that have reduced competitive success at these modified nutrient supply rates. Such shifts in community composition can result in changes in edibility of phytoplankton and

therefore change food availability for zooplankton and thereby the transfer efficiency of energy and matter in aquatic food webs.

However, not only the edibility, in terms of size and morphology, of phytoplankton can be altered. The described changes in community composition within phytoplankton and additionally within heterotrophic organisms such as bacteria can alter seston stoichiometry due to the varying stoichiometric composition of these organisms.

Higher trophic levels are usually much more constrained in their stoichiometry. Therefore those changes in seston stoichiometry can also drastically influence food quality for zooplankton. If food stoichiometry is very different from consumer stoichiometry a so called stoichiometric trophic mismatch can occur. Hence, beside food quantity also stoichiometric food quality can strongly determine the growth of different zooplankton species (Elser et al. 2001; Felpeto and Hairston 2013; Bullejos et al. 2014).

Effects of low phytoplankton nutritional quality can even be transported up the food chain to secondary consumers (Malzahn et al. 2007; Schoo et al. 2012). For example, phytoplankton C:P ratios above 300 can result in a P limitation of the growth of the most important zooplankton in lakes, *Daphnia* (water fleas) (Urabe and Watanabe 1992; DeMott et al. 2001). *Daphnia* has usually C:P ratios around 105, with very little variation. Facing food having C:P ratios larger 300 *Daphnia* is than not anymore constrained by the amount of food (carbon) but by the low amount of P within foods biomass (Hessen et al. 2004).

In order to investigate such possible effects of atmospheric nirogen deposition on P limited lake ecosystems, I performed mesocosm experiments investigating the spring bloom dynamics in two consecutive years.

The experiments were performed in three lakes in Upper-Bavaria. Lake Brunnensee is an oligotrophic lake with typical dissolved N:P molar ratios of >1000:1 during spring time; lake Klostersee is mesotrophic with N:P ratios around 150:1; and lake Thalersee can be characterized as meso-eutroph with N:P around 500:1 (values from regular lake monitoring). Thus, those lakes represent a variety of background nutritional status and provide different phytoplankton composition to investigate general effects of N supply in these systems.

As oligotrophic lakes are known to be strongly P-limited systems (Sterner 2008), I investigated the influence of nitrogen enrichment on the oligotrophic lake Brunnensee in

more detail. In the first field experiments I investigated the effects of N deposition on phytoplankton along a gradient of N enrichment. The N enrichment gradient was established by extrapolating measured natural atmospheric N inputs in nearby lakes. N was supplied in the form of ammonium and nitrate.

In following field experiments I further tested varying supply ratios of ammonium:nitrate to compare qualitative effects of different nitrogen sources on plankton responses. Additionally I performed laboratory experiments analyzing effects of ammonium on algae and subsequent consequences on herbivore consumers.

1.2 Research questions

My research questions, which I investigated with the above mentioned field and laboratory experiments, were as follows:

I *What are possible effects of nitrogen enrichment (within recent and predicted future atmospheric wet deposition ranges) on phytoplankton biomass and species composition in a phosphorus deficient system?*

Temperate lake ecosystems are often phosphorus-limited or nitrogen-phosphorus co-limited, rather than being solely nitrogen-limited (Elser et al. 2007). Nonetheless, an increased nitrogen supply might affect phytoplankton dynamics of temperate lakes through the different nutrient demands (Quigg et al. 2003) and usage by individual phytoplankton species (Domingues et al. 2011).

Additionally, nitrogen input also affects the relationship between different dissolved nutrients in aquatic ecosystems such as nitrogen:phosphorus (N:P) and nitrogen:silicon (N:Si) ratios (Elser et al. 2009a). It is well established that such changes can influence phytoplankton species composition (Sommer 1994; Roberts et al. 2003). In both laboratory and mesocosm experiments, changes in species abundance of diatoms were shown along a Si:N gradient (Sommer 1994), with higher diatom abundances and lower flagellate abundances resulting from increasing Si:N ratios (Sommer 1994; Roberts et al. 2003).

Long term dynamic shifts in nutrient ratios might even lead to a loss of species in phytoplankton (via competitive exclusion) due to a nutrient-ratio related decrease in competitive abilities. Such shifts in community composition might result in changes in

stoichiometry, biochemical composition and edibility of phytoplankton, and thus change the quality of food available for secondary consumers.

However, phytoplankton species composition is only one parameter describing a phytoplankton community that might be affected by increasing nitrogen loads. Phytoplankton biovolume, chlorophyll *a* content (representing the amount of the main photosynthetic pigment) and carbon content (an indicator for the food quantity of higher trophic levels) are other important parameters characterizing a phytoplankton community.

All of these factors are commonly used as phytoplankton biomass proxies (Bergström and Jansson 2006; Reynolds 2006; Berger et al. 2010). Each proxy represents a different aspect of the phytoplankton influencing pelagic food web dynamics. The dynamics of phytoplankton biomass proxies have been shown in field studies to be both spatially and temporally variable (Felip and Catalan 2000) and it has been shown in experimental studies that the biomass response to changes in nutrient limitation varied (Gilpin et al. 2004). The aim of this study was to investigate whether increased nitrogen input in multiple atmospheric amounts is able to influence phytoplankton community biomass, composition, and stoichiometry in a phosphorus deficient lake system.

I expected that nitrogen enrichment (within recent and future atmospheric wet deposition range) does not have a large effect on phytoplankton biomass in an otherwise phosphorus deficient system, but rather on phytoplankton species composition and stoichiometry.

I performed a mesocosm experiment in an oligotrophic lake with a usual spring dissolved inorganic nitrogen (DIN):total phosphorus (TP) molar ratio of >1000:1 (unpublished data from regular lake monitoring). A gradient of six nitrogen enrichments from 0 to 32 times the natural nitrogen wet deposition was applied, and the resulting effects on water chemistry, phytoplankton biomass, seston stoichiometry, amount of chlorophyll *a* and community composition were estimated.

II How variable are the responses of plankton to nitrogen addition in lakes with different availability of nutrients?

An increasing N supply is of biological importance since it leads to changes in the available nutrient ratios, and eventually, to excess N conditions and a P deficiency in

(terrestrial and aquatic) ecosystems (Bergström and Jansson 2006; Elser et al. 2009a, b; Crowley et al. 2012). Increasing N loads are of first and foremost concern for ecosystems in which the primary producers are limited by N, and thus, consequences of increasing biomass and primary production may arise through the additional fertilization (Tyrrell 1999; Reich et al. 2001; Elser et al. 2009b; Hessen 2013).

In addition, autotrophs, such as phytoplankton, have the flexibility to adapt their elemental (stoichiometric) composition according to the surrounding resource conditions (Sterner et al. 1997; Klausmeier et al. 2004). It has recently been shown that for predominantly N-limited lakes, in a gradient of atmospheric N deposition, the availability of higher dissolved N:P ratios can result in higher seston biomass N:P ratios (Elser et al. 2009a; Hessen 2013).

Amongst other factors, seston stoichiometry is an indicator of food quality for aquatic herbivores (Andersen and Hessen 1991; Hessen 1992), whose performance is related to both the producer food quantity and quality (Hessen et al. 2002). Food quality needs to meet the nutrient requirements of specific herbivores, which include seston stoichiometry in general (Andersen and Hessen 1991; Hessen 1992; Elser et al. 2000), fatty acid composition in particular (Müller-Navarra 1995; Müller-Navarra et al. 2000), as well as the edibility of algae (Sommer et al. 1986).

In terms of mineral nutrient limitation, a copepod's growth is limited by N-deficient food through the higher organismic demands of N (Hessen 1992; Sterner and Hessen 1994). In contrast, the growth and the reproduction of the abundant freshwater cladoceran *Daphnia* are commonly limited by the amount of available P (Sommer 1992; Sterner and Hessen 1994; Urabe et al. 1997). There is evidence from natural lake systems that an algal P limitation, expressed in high seston C:P ratios, is transferred to the trophic level of secondary producers and affects the growth of *Daphnia* (Elser et al. 2001; Berger et al. 2006).

Besides seston stoichiometry, the fatty acid content of phytoplankton is essential for herbivore growth and this may be reduced under an increased P limitation (Müller-Navarra 1995; Müller-Navarra et al. 2000). Hence, with an increasing N load in traditionally P deficient temperate lakes (Schindler 1977), it is conceivable that the growth and the reproduction of P-demanding herbivores should be derogated to a greater extent, and thus might lead to lower herbivore abundance under increasing N:P conditions.

In order to estimate the degree to which effects of an increased N load in P deficient lakes are transferred to the zooplankton trophic level, I experimentally investigated the consequences of N enrichment on P deficient plankton communities with mesocosm field experiments. I performed experiments in three lakes with different trophic conditions. Experiments were performed at the same time in all lakes and covered a broad N:P nutrient supply range. I hypothesised that the zooplankton groups with high P requirements would be the first to be negatively affected by an additional N enrichment due to the expected modified stoichiometry of their phytoplankton food.

III Do different supply ratios of nitrate and ammonium influence phytoplankton community dynamics?

Reactive N compounds concern both nitrous oxide (NO_x) and ammonia (NH_4^+), which were increasingly produced and deposited due to human activities in the last century compared to preindustrial reactive N contributions (Galloway et al. 2003). A recent analysis revealed that NO_x production is more or less confined nowadays, but NH_4^+ production is still increasing due to steadily increasing human food requirements (Ciais et al. 2013). Thus, future ecosystems do not only have to face the accumulation of N load in general but additionally a shift in reactive N sources from NO_x towards NH_4^+ dominance.

It has been agreed that nutrient management should include nitrate (NO_3^-) and NH_4^+ control (Lewis et al. 2011), since the quality of N compounds (NO_x or NH_4^+) exhibits a highly important role in terms of bioavailability for primary production. This was stressed especially in N limited coastal waters, where NH_4^+ stimulated higher biomass development than NO_3^-, being supplied in same quantities (Paerl 1997).

The attention on both NO_3^- and NH_4^+ compounds appears obvious in primarily N limited systems, where N is deficient available and since N is presumed to be globally a main limiting macronutrient (Elser et al. 2007). However, despite of nutrient limitation conditions also from an energetically point of view qualitative differences in the supply of NO_3^- and NH_4^+ compounds should influence primary production in ecosystems. NH_4^+ can be taken up directly by primary producers and does not require costly enzyme activity of NO_3^- reduction (Eppley et al. 1969b; Falkowski 1975). Additionally, phytoplankton physiological adjustments can suppress the synthesis and activity of NO_3^- reductase under NH_4^+ concentrations higher than 0.5-1 µmol L^{-1} (Eppley et al. 1969a).

Differential utilization of N compounds for phytoplankton was observed in various laboratory studies (Dortch 1990; Levasseur et al. 1993). Preference for NH_4^+ as N source for example was determined for algae groups like flagellates and green algae (Dortch 1990; Domingues et al. 2011; Donald et al. 2013), which have higher uptake affinities for NH_4^+ (Litchman et al. 2007). However, growth rates might be higher under NO_3^- supply, which accounts especially for a range of diatoms (Dortch 1990; Levasseur 1993; Litchman et al. 2007).

Also size or surface to volume ratio play an essential role for nutrient uptake as for example smaller sized algae are known to be good competitors for nutrients such as phosphorus (P) (Smith and Kalff 1982), which was also suggested for NO_3^- and NH_4^+ (Eppley et al. 1969b; Hein et al. 1995). On the other hand there is evidence that larger sized algae seem to perform better in NO_3^- uptake since large vacuoles reduce the inhibition of uptake rate by cytoplasmic accumulated nutrients (Stolte and Riegman 1995). This was shown for diatoms and dinoflagellates, were maximum uptake rates for NO_3^- on a per cell basis increases with cell size (Aksnes and Egge 1991; Litchman et al. 2007). Thus, since phytoplankton groups differ in traits regarding nutrient use, a shift in N resources might entail a shift in phytoplankton community composition through resource competition (Tilman 1977; Sommer 1985).

However, although freshwater field studies in terms of NO_3^- and NH_4^+ addition have been conducted, the outcome of a shift in N compound ratios in natural systems has not yet been investigated and remains unknown. Experiments with natural communities concerning N resource use are scarce (Axler and Reuter 1996; Twomey et al. 2005; Donald et al. 2013) and concern single or combined N compound fertilization in constant ratios.

For future investigations of interactions of the nitrogen cycle with carbon fixation (Gruber and Galloway 2008), it is most relevant to consider additionally the relative amounts of NO_3^- and NH_4^+ to approximate implications of human-induced changes of ecosystems. This is especially important, because it was observed that presence of NH_4^+ can inhibit the uptake of NO_3^-, reported thresholds for inhibition range from >0.1, 1, 4, up to 15 µmol L^{-1} (Dortch 1990; Dugdale et al. 2007). The degree of inhibition was shown to be influenced for example by the background N loads algae were grown in and adapted to, before applying N manipulations (Dortch and Conway 1984).

In order to investigate possible future implications of quantitative increasing N load combined with qualitatively two different NO_3^-:NH_4^+ supply ratios based on atmospheric N deposition scenarios, I performed a mesocosm experiment in an oligotrophic lake.

The supplied NH_4^+ amounts were relatively low (~1.5 µmol L^{-1} $fertilization^{-1}$in the highest NH_4^+ treatment), therefore I did not necessarily expect large inhibiting effects of NH_4^+ on NO_3^- uptake and production. Since dissolved NO_3^-:NH_4^+ molar ratios in spring usually are high in this lake (17:1), I rather expected shifts in phytoplankton community composition in response to experimental manipulations of N supply sources. According to previous studies investigating enrichment with NH_4^+ or NO_3^- (Dortch 1990; Sommer 1993; Donald et al. 2013) I expected that specific algal groups such as small flagellated algae or green algae would benefit from high NH_4^+ supply ratios.

IV How does ammonium affect algae from different taxonomic groups and are potential effects transfered to higher trophic levels?

Due to still growing food demands and increasing usage of combustion engines, future deposition scenarios show that a further increase of reactive nitrogen ($N_{r)}$ has to be expected (Lamarque et al. 2011). In the case of nitrogen oxide (NO_x), predictions are quite uncertain and both, a reduction and a further increase of deposition seem to be possible. For ammonium compounds (NH_x) a further increase of deposition is predicted (Galloway et al. 2004; Dentener et al. 2006; Lamarque et al. 2011; Glibert et al. 2016) and consequently increasing concentrations in ecosystems.

The steady input of ammonium can accumulate and cause a persisting rise of ammonium concentrations in ecosystems (Burkholder et al. 2006; Glibert et al. 2014). Bergström et al. (2006) for example could show a relationship between the amount of atmospheric N_r deposition and lake dissolved inorganic nitrogen (DIN) loads. A steady input of nitrogen compounds will consequently result in an increasing exposure of aquatic organisms to high ammonia concentrations.

NH_4^+ first of all serves as a nutrient for primary producers; however with increasing concentrations also negative effects can be seen. This effect was described as the paradox of NH_4^+ (Britto and Kronzucker 2002; Dugdale et al. 2012; Glibert 2016). Besides the acute toxicity due to high doses of NH_4^+ also chronic toxicity due to the exposition to rather moderate concentrations of NH_4^+ can affect a variety of aquatic organisms (e.g.

Allan et al. 1990; Miller et al. 1990; Ip et al. 2005). Thus, although accumulating NH_4^+ concentrations might not lead to acute death of organisms, they can result in lower growth and thereby for example in a decrease of phytoplankton biomass (Glibert et al. 2016).

Negative effects of high ammonium concentrations on algal growth were frequently observed (Collos and Harison 2014 and citation therein). Growth effects of NH_4^+ on algae vary largely between algal species; however, ambient conditions differed between the experiments, which are described in the above-mentioned review. Studies, which experimentally compare algal species with respect to their response to ammonium under similar conditions, are rare. Dai et al. (2012) for example compared the response of a variety of species to increasing NH_4^+ concentrations. They calculated for 18 algal species the EC_{50} value representing the ammonium concentration for which a decrease of the operational PSII quantum yield by 50% compared to the control was observed. This EC_{50} values varied largely between algae, starting from 0.27 mM NH_4^+ for *Tabellaria sp.* reaching up to a maximum dose of 18.25 mM for *Chlamydomonas microsphaera*.

Considering these varying responses of algae, changes in ammonia levels might also lead to changes in phytoplankton community composition due to both, the nutritional advantages of ammonium for certain algal groups and the exclusion of species less tolerant to high ammonia concentrations. Changes in community composition caused by ammonia have been shown in a nutrient enrichment mesocosm experiment by Domingues et al. (2011). They detected increased growth rates of green algae and cyanobacteria and decreased growth rates of diatoms and dinoflagellata when comparing ammonium enriched mesocosms with those without nutrient addition.

Ammonium has also a direct toxic effect on other aquatic organisms, e.g., zooplankton or different fish species (Thurston et al. 1981; Maltby 1995; Sarma et al. 2003; El-Shafai et al. 2004). For example, the crustacean model organism *Daphnia* was shown to be negatively affected by increasing NH_4^+ concentrations (Gerisch et al. 1986; Xiang et al. 2010; Yang et al. 2012; Lyu et al. 2013a). In a laboratory experiment, high NH_4^+ concentrations in the media caused delayed offspring production, reduced size of maternal mothers (size at first clutch) and a decrease in number of offspring compared to the no-NH_4^+ control in *Daphnia magna* (Yang et al. 2012). Similar reactions to increasing NH_4^+ concentrations were obtained by Lyu et al. (2013a) when analysing combined effects of hypoxia and ammonia on *Daphnia similis*.

However, NH_4^+ might have also indirect effects on consumers through bottom up driven effects when their diet was exposed to increased amounts of ammonia. First, as stated above ammonia can have negative effects on the quantity of consumer's diet. In cases were ammonia leads to an overall decrease of phytoplankton biovolume (Glibert et al. 2016), certainly the food availability for herbivorous zooplankton decreases. Second, ammonia can also cause qualitative effects on phytoplankton as food source by shifting community and or biomass composition or phytoplankton stoichiometry. Such effects might be caused by both nutritional and toxic consequences of NH_4^+.

Alterations in the community composition due to changes in N supply might result in a phytoplankton community representing a new food spectrum for zooplankton. One can expect that the nutritional quality might increase due to an increase of ammonia as for example chlorophyceae tolerate higher levels of NH_4^+ than cyanobacteria (Collos and Harrison 2014), which are known to be of low nutritional quality. For example the well edible green algae *Chlamydomonas* sp. is tolerating more than ten times higher ammonia levels then the largely inedible cyanobacteria *Microcystis* sp. (Dai et al. 2012).

Additionally, changes in ammonia concentrations might also lead to direct changes in phytoplankton stoichiometry. For land plants such an effect was already shown when plants treated with NH_4^+ had higher N content and lower C:N ratios than those treated with the same amount of NO_3^- (Tylova-Munzarova et al. 2005). Thus again qualitative food effects for zooplankton are possible as phytoplankton stoichiometry plays an important role for zooplankton nutrition (Elser et al. 2001; Hessen et al. 2002; Jensen et al. 2004). Thus, it is difficult to predict how possible alterations in the phytoplankton community (e.g. biovolume, community composition and stoichiometry) interact with each other and influence zooplankton growth.

Altogether effects of increased NH_4^+ on aquatic organisms are various and complex. Therefore, it is important to analyse effects of increased NH_4^+ concentrations under highly reduced complexity of the studied system and thus exclude side effects. In experiments investigating hole communities a distinction of direct effects of NH_4^+ on zooplankton and indirect through changes in algae quality and quantity is not possible. The same is true for laboratory experiments were the manipulation of NH_4^+ is applied on *Daphnia* grown ad libitum, as usually done in fitness essays, the NH_4^+ can also affect algae growth and thus direct toxic effects and inadvertent food manipulation cannot be distinguished. In

conclusion, it is important to experimentally uncouple effects. Especially possible effects of NH_4^+ on algae that change their biochemical content quality as food for *Daphnia* are a very important factor (Elser et al. 2001; Hessen et al. 2002).

To estimate the influence of NH_4^+ on different algal species and subsequent consequences on zooplankton I performed a laboratory experiment where I manipulated NH_4^+ availability for algae. I analysed the response of three common freshwater microalgae to a gradient of NH_4^+ concentrations. In my analyses I took into account effects on quantity (measured as chlorophyll *a*) and quality (stoichiometry) of the cultivated microalgae. The algae exposed to the NH_4^+ gradients were then used as a food source in a somatic growth experiment with *Daphnia magna*. Thereby I was able to analyse secondary, qualitative effects of increasing NH_4^+ exposure of algae for higher trophic levels separated from direct effects of NH_4^+ on the consumer.

2

Material and Methods

2.1 Mesocosm experiments 2013 (Research questions I and II)

Study site and experimental design

The mesocosm field experiments were performed during the spring of 2013 in three lakes with different trophic statuses (Lake Brunnensee 27.03.13 – 31.05.13, Lake Klostersee and Lake Thalersee 27.03.13 – 28.05.13) in Bavaria, Germany. The lakes were chosen for their different nutrient backgrounds (Table 1), in which the dissolved molar N:P ratios (nitrate and ammonium compared to total phosphorus concentrations) after winter mixing varied from 150:1 N:P (Lake Klostersee), 500:1 N:P (Lake Thalersee), to >1000:1 N:P (Lake Brunnensee). This was far higher than the classical Redfield ratio of 16:1 N:P. The total N supply of the lakes is continuously affected by atmospheric deposition and leaching, but also, the groundwater discharges and the surface runoffs contribute to the total of N in the lakes. Lake Brunnensee (18.6 m max. depth, 502 x10³ m³) is mainly groundwater fed, whereas Lake Klostersee (16 m max. depth, 2762 x10³ m³) and Lake Thalersee (7 m max. depth, 166 x10³ m³) have small streams running through them. All lakes are located close to the Limnological Research Station of the LMU Munich, where all water and plankton analyses were conducted.

Table 1 Background nutrient data (TP µg L^{-1}, NO_3^- mg L^{-1}, NH_4^+ µg L^{-1}) of the lakes Brunnensee, Klostersee and Thalersee.

	Brunnensee	**Klostersee**	**Thalersee**
TP [µg L^{-1}]	6.6	12	13
NO_3^- [mg L^{-1}]	17	1.3	12
NH_4^+ [µg L^{-1}]	56	304	29

Per lake, 12 enclosures (cylindrical bags made of white PE foil, with 150 µm thickness, Biofol Film GmbH, Germany; dimensions: 4 m deep, 0.95 m diameter) were filled with respective lake water by uplifting them from a depth of ~8 m (6 m for Lake Thalersee) to the water surface, thereby trapping a well-mixed subsample of the phytoplankton and zooplankton community of the lake. Enclosures were attached to anchored rafts in the lakes and were open to the atmosphere. The experimental setup was covered with a transparent foil roof to avoid external wet deposition of nutrients but still allowing gas exchange and penetration of the full light spectrum. This way, the experimental setup excludes input of

nitrogen through wet deposition as well as inputs from the surrounding water due to the impermeable plastic foil.

For nitrogen fertilization, calculations of natural input were based on 75 mg m^{-2} nitrate (NO_3^-) and 25 mg m^{-2} ammonium (NH_4^+) per week (data provided by the Bavarian Environment Agency) and a weekly precipitation of approximately 25 L m^{-2} (German Meteorological Survey). This resulted in a weekly nitrogen input of 60 mg NO_3^- and 20 mg NH_4^+ per enclosure surface as a simulated natural input. Treatments followed a replicated gradient design (Cottingham et al. 2005). Six increasing nitrogen enrichment treatments (replicated twice) were established, including sodium nitrate and ammonium chloride as a nitrogen source. The treatments consisted of 0-, 1-, 2-, 8-, 16- and 32- times the natural regional nitrogen wet deposition. The 0 treatment did therefore not receive any nitrogen input except ambient atmospheric dry deposition. From a stock solution with a concentration of 30 g L^{-1} NO_3^- and 10 mg L^{-1} NH_4^+ 0, 1, 2, 8, 16 and 32 mL were added to distilled water to a final volume of 1 L. Nitrogen enrichment was performed twice a week over a period of nine weeks. In order to ensure sufficient distribution of the nitrogen in the enclosures, they were mixed with a Secchi disc.

Sampling program and measurements

Nutrients: Water samples were taken once a week (two days after a nitrogen addition) using an integrated tubular water sampler with a volume of 2 L (KC DenmarkA/S Research Equipment, Denmark) from a depth of 1-3 m from each enclosure. The water was pre-filtered through a 250 µm gauze to exclude mesozooplankton. Nitrate and nitrite content was measured in an ion chromatograph system (Dionex ICS-1100 Basic Integrated IC System; Thermo Scientific, USA) after 0.45 µm filtration (CS 400 Syringe Filters Cellulose Acetate 0.45 m; Nalgene, USA). Ammonium was measured fluorometrically (Trilogy Labratory Fluorometer Module CDOM/NH_4; Turner Designs, USA) using a working reagent including orthophtalate, sodium sulfite and borate buffer (after Holmes et al. 1999).

Total phosphorus (TP) was measured spectrophotometrically (Shimadzu UV-1700, Shimadzu Cooperation, Germany) using the molybdenum blue method (Wetzel and Likens 1991). In weeks 4, 6, 8, and 10, biogenic silicate (Si) was analyzed by filtering between 50 ml and 200 ml of enclosure water onto cellulose-acetate filters (0.6 µm pore size, Satorius) and then frozen (-20°C) until further analyses. The filters were subsequently extracted in a water

bath (95° C, for 4 h, in 0.2 mol NaOH) (Ragueneau and Tréguer 1994) and measured spectrophotometrically using the molybdenum blue method.

Phytoplankton and heterotrophic protists: Water samples were taken twice a week as described above. Chlorophyll *a* concentration, based on multispectral fluorescence analyses, was measured *in vivo* with an AlgaeLabAnalyser (bbe Moldaenke GmbH, Germany). Samples were dark adapted (20 minutes) prior to measurement. Additionally to total chlorophyll *a* concentrations, the AlgaeLabAnalyser provides information about abundances of phytoplankton groups according to their pigment contents. The four identified spectral phytoplankton groups are green algae, diatoms and dinoflagellates, cryptophytes and cyanobacteria (Beutler et al. 2002).

For Lake Brunnensee once a week, phytoplankton samples were fixed in Lugol's iodine and later counted using an inverted microscope, following established methods (Utermöhl 1958). Sedimentation chambers were filled with 25-50 mL of the Lugol fixed sample and the composition and abundances of phytoplankton, ciliates and heterotrophic nanoflagellates (HNF) were counted. Phytoplankton and HNF biovolume was then calculated from abundance data by multiplying species cell counts by individual phytoplankton cell volume. For all species, besides bacillariophyceae (diatoms), cell volumes were obtained from existing data of our own monitoring programmes and measurements of phytoplankton of the lake Brunnensee (shape estimates of Hillebrand et al. 1999). For the highly variable bacillariophyceae, cell volumes were calculated for each sampling day using own measurements based on geometric shape estimates of Hillebrand et al. (1999). Relevant dimensions of at least 100 individuals per diatom species were measured using analySIS software (Pro 2.11.006, Soft-Imaging Software GmbH).

Zooplankton: Once a week, zooplankton was sampled by net hauls (105µm) through the 4 m water column of each enclosure. Zooplankton sampling started on day 22 as I did not want to disturb zooplankton dynamics at the initially very low densities. Zooplankton samples were fixed with 70% Ethanol and counted at 25x magnification using a Wild M3Z stereomicroscope.

Seston stoichiometry: For weekly measurements of particulate organic carbon (POC), nitrogen (PN) and phosphate (PP) 150-400 mL water was filtered on glass fibre filters (GF/F). Filters were frozen at -20 C° until later analysis. POC and PN were measured in an elemental

analyzer (vario MicroCube, Elementar, Germany) and PP was measured spectrofluorometrically after molybdate reaction following sulphuric acid digestion (Wetzel and Likens 1991). Seston stoichiometric ratios of C:N:P were calculated accordingly.

Statistical analysis

Detailed analyses of Lake Brunnensee (research question I)

Statistical analyses were performed using Sigma Plot 11.0 (Systat Software 2008). Linear (lr) and quadratic (qr) regression models were applied with log (base 10) transformed data for biovolume, nitrogen and POC data to meet statistical assumptions (Sokal and Rohlf 1995). Averaged values are given as mean ± standard error. To determine possible chronological shifts with increasing nitrogen enrichment, the peak day for each of the three biomass proxies chlorophyll *a*, POC and biovolume was identified for each enclosure separately.

For statistical analyses of the phytoplankton succession the experiment was divided into two important phases, an initial growth phase (spring bloom) up to a peak and a descending phase after the peak resulting in low chlorophyll *a* levels (clear water phase). This classification is based on chlorophyll *a* data, due to the higher temporal resolution of the chlorophyll *a* measurements and was applied for all total biomass proxies, biovolume on phytoplankton group and species level, and seston stoichiometry data.

The following parameters in each phase were analyzed separately by calculating averages per enclosure: POC, chlorophyll a, total biovolume, biovolume on phytoplankton group and species level and stoichiometry data (C, N, P). Finally, the following ratios were calculated: a) chlorophyll *a*:algal biovolume, b) chlorophyll *a*:POC and c) algal biovolume:POC, to estimate possible nitrogen-dependent variations of the algal chlorophyll *a* content (a) and the contribution of algal pigments and algal biovolume to POC > 0.7 µm (b,c). For all ratio calculations (biomass proxies and stoichiometry) the ratios were first calculated for each time point and then averaged over time for each of the two phases. Additionally, initial exponential growth rates (based on biovolumes) were calculated from the start of the experiment up to the total biovolume peak of each enclosure. This was done for total biovolume and the biovolume of major phytoplankton groups. I refer to significance levels between 0.05 and 0.1 as trends.

Lake comparison of zooplankton and phytoplankton (research question II)

Following the logarithmic fertilization design, the statistical analyses were performed with regression models (linear or unimodal) of logarithmic transformed data using SigmaPlot 11.0 (Systat Software 2008) against the log-transformed N fertilization treatments or, ecologically meaningful, against the dissolved reactive N or dissolved N:P ratios. In the case of the phytoplankton, the maximum chlorophyll *a* values were defined as being the highest values after the growth phase.

The "peak phase" was defined according to the maximum of total chlorophyll *a* values in Lake Brunnensee and Lake Thalersee (chromophytes in the case of Lake Klostersee), including two measurements before and after the maximum chlorophyll *a* values. Before and after the peak phase, the exponential growth phase and the subsiding phase were determined. I statistically analyzed the total chlorophyll *a* levels and all of the spectral algal classes for their maximum and average chlorophyll *a* levels during the peak phase.

For zooplankton, the densities and biomass of copepods, cladocerans, and rotifers, together with the total zooplankton species, were analyzed. Calculations were performed for the total data set, the average, and the maximum reached ("peak") densities during the experimental period. For mesozooplankton carbon measurements, the total zooplankton carbon and the proportions of copepod and cladocerans to measured carbon estimated by their relative abundances were analysed. Multivariate analyses were performed as canonical correspondence analyses (CCA, Legendre and Legendre 1998) using PAST 2.17 Software (Hammer et al. 2001). Explanatory variables included the TP levels, the dissolved reactive N levels, and the dissolved N:P ratios. Log-transformed abundances of calanoid copepods, nauplii, cladocerans, and rotifers, were used as species variables.

2.2 Mesocosm experiment 2014 (Research question III)

In spring 2014 (11.03.-28.05.14) another mesocosm experiment was conducted in Lake Brunnensee.

The experimental setup, fertilization procedures and sampling (nutrients and phytoplankton) were the same as in the mesocosm experiments 2013.

To test effects of increasing N quantity and changing N quality (in terms of N-source NO_3^- and NH_4^+) a 2x2 factorial design (three times replicated) was applied, using sodium nitrate ($NaNO_3$) and ammonium chloride (NH_4Cl) as N source. The N addition in the experiment was based on a weekly, natural input of 2 mmol per enclosure surface (calculated based on rain samplers on the lake (Trommer, unpublished data) and data of the Bavarian Environment Agency and the German Meteorological Survey). Quantitative N effects were tested as the natural amount as well as the fourfold amount (8 mmol) of N input and qualitative N effects as extrapolated molar ratios of 4:1 and 1:4 NO_3^-:NH_4^+ (natural input 1:1 NO_3^-: NH_4^+). The 2x2 factorial design resulted in three different supply levels of NO_3^- and NH_4^+ (0.4, 1.6 and 6.4 mmol week-1). Over a period of eleven weeks N addition was performed twice a week by adding 1 L of distilled water enriched with the according N addition (Table 2), freshly prepared from a stock solution.

Table 2 Overview of experimental N fertilization scheme per mesocosm (~2100 L). The according amounts of NO_3^- and NH_4^+ were added within one liter fertilization solution two times a week.

Treatment	**N addition [mmol]** per fertilization		**N addition [mg]** per fertilization	
	NO_3^-	**NH_4^+**	**NO_3^-**	**NH_4^+**
1-4:1	0.8	0.2	49.6	3.6
1-1:4	0.2	0.8	12.4	14.4
4-4:1	3.2	0.8	198	14.4
4-1:4	0.8	3.2	49.6	57.6

The chlorophyll *a* data were additionally used to calculate the resource use efficiency ($RUE_{Chl\ a}$, see Ptacnik et al. 2008) of phytoplankton. For each individual mesocosm, $RUE_{Chl\ a}$ was calculated by dividing the maximal reached chlorophyll *a* concentration through the TP value at the beginning of the experiment.

To determine the size spectrum of phytoplankton communities, samples fixed in Lugols Iodine were measured with a particle counter (Casy 1, Schärfe Systems, Germany). Particles of 2.5-37.5 µm diameter were determined and subsequently sorted according to equivalent spherical diameter (ESD). The measured particles represent microplankton, which is well edible food for herbivores (Hansen et al. 1994). They were grouped in 14 size classes in 2.5 µm steps and were categorized by their mean ESD (1.25 µm – 36.25 µm). Accordingly, biovolume of each size class was calculated (Biovolume=4/3 * $(ESD/2)^3$ * π * no. counts

within ESD) and statistically analyzed for the peak period of phytoplankton biomass (day 49 – 63).

The data were analyzed with Sigma Plot 11.0 (Systat Software 2008) via two way ANOVAs (twA) with N amount and NO_3^-:NH_4^+ ratio as fixed factors and with linear regression (lr) along the NO_3^- and NH4 fertilization gradient per week (PostHoc test: Holms Sidak). In order to test for normality Shapiro-Wilk tests were performed. If necessary, data were 1/x transformed.

2.3 Ammonium enrichment (Research question IV)

1 Algal NH_4^+ growth experiment

A laboratory experiment was performed with cultures of the algal species *Chlamydomonas reinhardtii* (chlorophyta), *Chroococcus minutus* (cyanobacteria) and *Dinobryon sp.* (crysophyta) grown semi-continuously in a gradient of five NH_4^+ concentrations (Table 3). Each treatment was replicated three times (45 experimental units). In the presented experiment I chose NH_4^+ concentrations (Table 3) which were lower than tested concentrations of NH_4^+ where cultures showed no growth or algae died completely (data not shown). All algal species originated from the algae collection of the LMU Munich and have been cultured in WC-medium since several years (standard growth medium, Guillard and Lorenzen 1972).

Table 3 NH_4^+ concentrations [mmol L^{-1}] for the three algae species.

NH_4^+ concentrations / **Species**	**I**	**II**	**III**	**IV**	**V**
C. reinhardtii	0	0.5	2	8	32
C. minutus	0	0.5	2	8	32
Dinobryon sp.	0	0.1	0.2	0.4	0.8

For each algal species a stock culture containing 2 mg particular carbon biomass per L was prepared 200 mL of each stock culture were sampled for all initial measurements. The rest of the stock culture was divided in five equal portions. To each of these five cultures, dissolved ammonium chloride (1 mol L^{-1}) was added in the appropriate amount to establish the desired experimental gradient of NH_4^+ (Table 3). Finally, the cultures were split into three

replicates (200 mL) per ammonium treatment (200 mL culture flasks). Algae were grown for four weeks in a temperature controlled climate chamber with a temperature of 20 ± 2 °C and a 12h:12h light:dark cycle with a light intensity of 60 µmol photons m^{-2} s^{-1}.

Twice a week 12.5 % of the algae cultures (25 mL) were sampled and replaced by fresh WC-medium (including ammonium in the according concentration of each treatment). In vivo chlorophyll *a* concentrations of all samples were measured with an Algae LabAnalyzer (bbe Moldaenke GmbH, Germany) after 20 minutes of dark adaptation.

Chemical analyses: At the beginning (day 1), as well as at the end of the experiment (day 28) following analyses were performed: NH_4^+ was measured by fluorometry (Trilogy Laboratory Fluorometer Module CDOM/NH_4; Turner Designs, USA) using an orthophtalate method (Holmes et al., 1999). For particulate C, N and P analyses, between 5 and 50 mL of each algae culture (depending on density) were filtered onto a pre-combusted and acid-washed GF/F filter (Whatman, England) and frozen until further analyses. Measurements for particulate organic carbon (POC) and particulate N were done with an elemental analyser (varioMicrocube, Elementar, Germany). Particulate P (PP) was measured spectrophotometrically using an established method based on wet oxidation and the molybdenum blue method (Wetzel and Likens, 1991).

The ammonium uptake by algae was predicted as expected concentration (ammonium input) minus the measured concentration at the end of the experiment (day 28). This value was first normalized for different biomasses in the treatments by integrating the chlorophyll *a* concentrations of the relevant treatments over time and then dividing ammonium uptake through that total chlorophyll *a* biomass. Then the per day uptake of ammonium per µg chlorophyll *a* was calculated and analysed.

2 Daphnia magna somatic growth experiment

In order to detect effects of NH_4^+ on algal food quality a growth experiment with the crustacean species *Daphnia magna* as phytoplankton consumer was performed. Prior to the experiment *Daphnia* were grown for several generations under experimental conditions (20 ± 2 °C and a 12 h:12 h light:dark cycle) in order to exclude maternal effects. *Daphnia* mothers were synchronised and neonates of the third clutch (all born within 8 h) were used for the experiment. Three *Daphnia* for each algae culture of the NH_4^+ growth test were placed together in a jar containing semi-artificial *Daphnia* growth medium (SSS; Jeschke and Tollrian 2000). Additionally, five *Daphnia* were fixed in 4 % sugar-formol (Haney and Hall

1973) at the beginning of the experiment. *Daphnia* were fed with algae from the different NH_4^+ treatments. After three days *Daphnia* were fixed in 4 % sugar-formol. Afterwards the length (distance from upper end of the eye to the base of the spine) of all fixed *Daphnia* was measured using a binocular together with the DinoCapture Imaging Software 2.0 (AnMo Electronics Cooperation). The growth rate (r) of *Daphnia* was then calculated using the mean length of the five initially fixed *Daphnia* as starting value.

$$r = \ln\left(\frac{lenght_{end}[mm]}{length_{start}[mm]}\right) * d^{-1}$$

Food preparation: In order to exclude direct effects of the NH_4^+ in the algae medium, algal cultures were centrifuged (1137 rpm for 6 minutes) and the algal pellet was resuspended in water. Colonies of *Dinobryon sp.* are difficult to ingest for *Daphnia*, therefore cultures were sonicated for 5 minutes (microscopic analyses were done to check successful separation of colonies). After preparation of the food, algae were measured with a cell counter (Casy 1, Schärfe Systems, Germany) in order to calculate the volume needed to reach a food concentration of 2 mg C L^{-1} for *C. reinhadrtii* and *C. minutus*. Due to the low concentrations of all *Dinobryon sp*. cultures for this algae only 1 mg C L^{-1} were used.

All data analyses were performed with Sigmaplot 11.0 (Systat Software 2008, Germany). Data were analysed using different regression models (lr: linear regression; hi: hyperbolic increase; hd: hyperbolic decrease; ei: exponential increase to a maximum). Data was analysed with the three models and the best fitting one was chosen according to the Akaike information criterion (AIC). I refer to significance levels between 0.05 and 0.1 as trends.

3

Results

I Detailed observation of phytoplankton responses

Chemical analyses

Dissolved NH_4^+ concentrations in the enclosures showed a steady increase over time following the continuous nitrogen enrichment in the nitrogen enrichment treatments (Figure 3). Dissolved NO_3^- concentrations fluctuated over time showing a slow increase and interim peaks on days 21 and 49 (Figure 3). NO_3^- and NH_4^+ concentrations were 16.9 mg L^{-1} and 0.058 mg L^{-1} at the beginning of the experiment and rose up to 25.3 mg L^{-1} and 2.1 mg L^{-1} by the end of the experiment in the highest nitrogen treatment. Final concentrations of dissolved nitrogen compounds (NH_4^+ and NO_3^-) correlated significantly with nitrogen enrichment treatments (NH_4^+: $p<0.01$, $R^2=0.98$; NO_3^-: $p<0.01$, $R^2=0.89$). Dissolved nitrite was rarely detectable and concentrations were on average below 0.04 mg L^{-1} throughout the experiment. TP declined from 6.7 ± 0.18 µg L^{-1} to 3.0±0.20 µg L^{-1} over time and was unaffected by nitrogen enrichment.

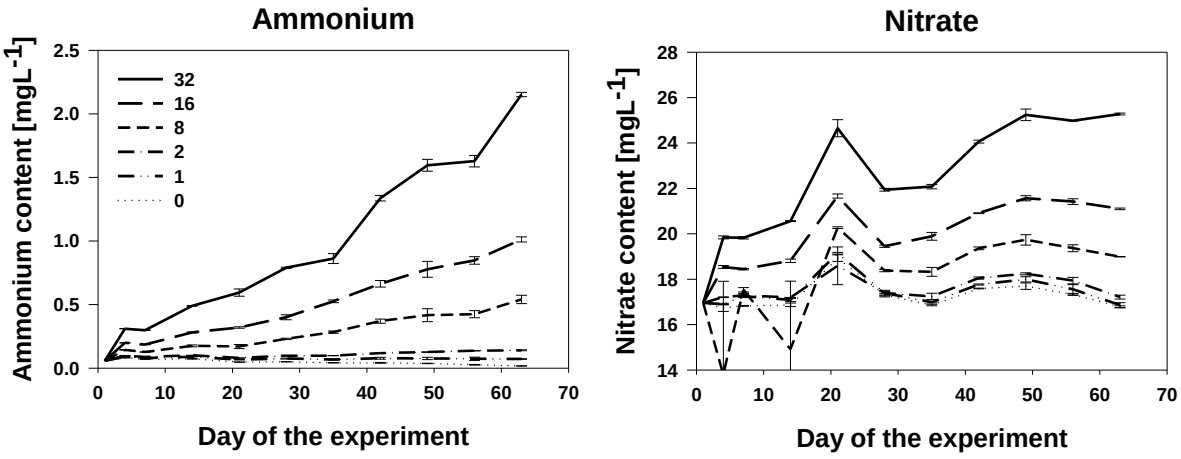

Figure 3 Nitrate (left) and ammonium (right) content over time. The lines show mean values from the two respective enclosures per treatment and the whiskers show standard errors.

Seston stoichiometry

PP declined from 3.6 ± 0.08 µg L^{-1} at the beginning of the experiment to 2.8 ± 0.13 µg L^{-1} at the end, following the general TP trend (data not shown). At the beginning of the experiment, POC concentrations were on average 324.92 ± 12.18 µg L^{-1} (Figure 4a). An increase of POC was observed for all enclosures followed by a decline (starting from day 35) to an average of 287.29 ± 14.85 µg L^{-1} at the end of the experiment (Figure 4a). PN showed

large fluctuations with a general declining trend; starting on average with values around 60.92 ± 1.65 µg L^{-1} and end concentrations of 37.92 ± 1.94 µg L^{-1}. Seston stoichiometric ratios were in the range of 477.36 ± 1.98 and 63.5 ± 0.24 for C:P and N:P, indicative of typical phosphorus-limitation signatures. Seston C:N ratios increased from on average 6.21 ± 0.33 to 8.87 ± 0.52 at the end of the experiment.

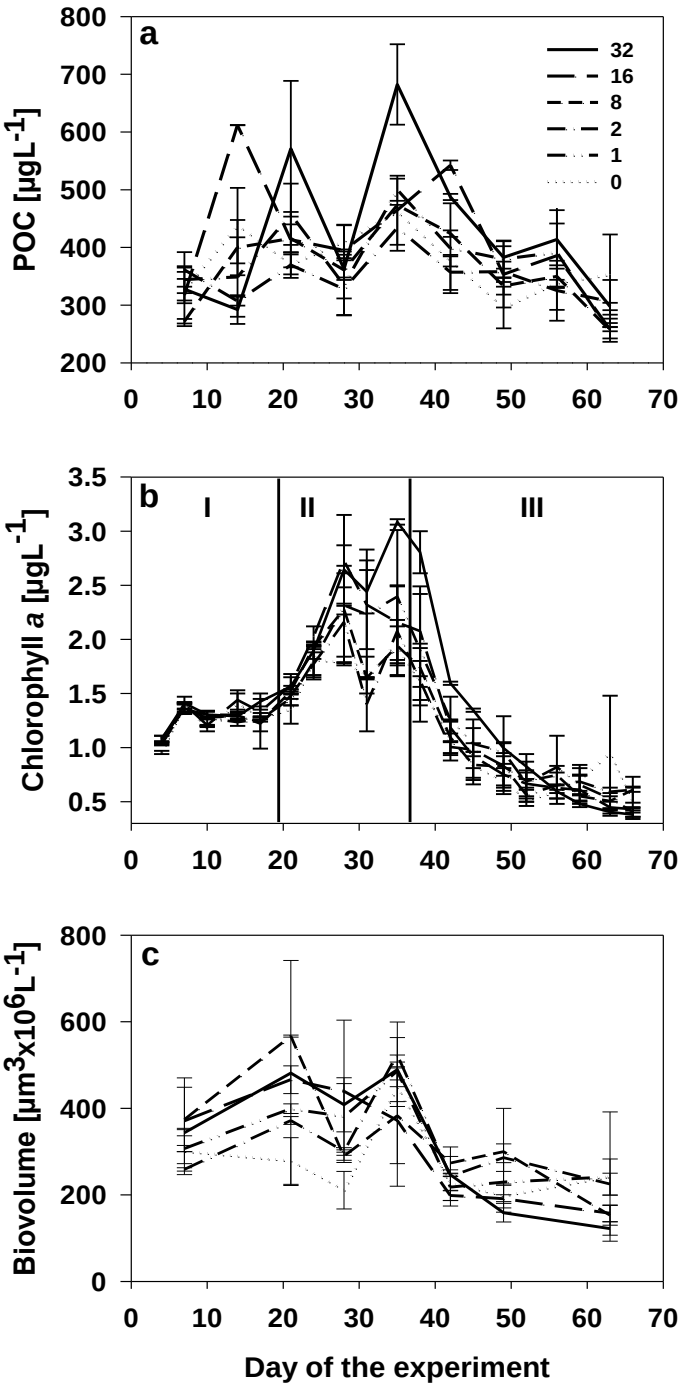

Figure 4 Time course of the three phytoplankton biomass proxies: (a) POC [µg L^{-1}], (b) *In vivo* chlorophyll *a* [µg L^{-1}] (lines divide the time course in: (I) initializing, (II) growth phase and (III) descending phase), (c) Biovolume [μm^3 10^6 L^{-1}]. Lines plot mean values from the two respective enclosures per treatment and whiskers show standard errors.

Statistical analyses of seston stoichiometry data revealed a significant effect of nitrogen enrichment for seston C:P ratios (Figure 5b; lr: p=0.01, R^2=0.47) and N:P ratios (Figure 5c; lr: p<0.01, R^2=0.57) for the growth phase. However, no trend was observed for C:N ratios during this phase (Figure 5a; lr: p= 0.28, R^2=0.12). On the contrary, during the descending phase only seston C:N ratio showed a positive response with increasing nitrogen enrichment (Figure 5d; lr: p=0.03, R^2=0.40) and no patterns where observed in seston C:P and N:P ratios (Figure 5e-f).

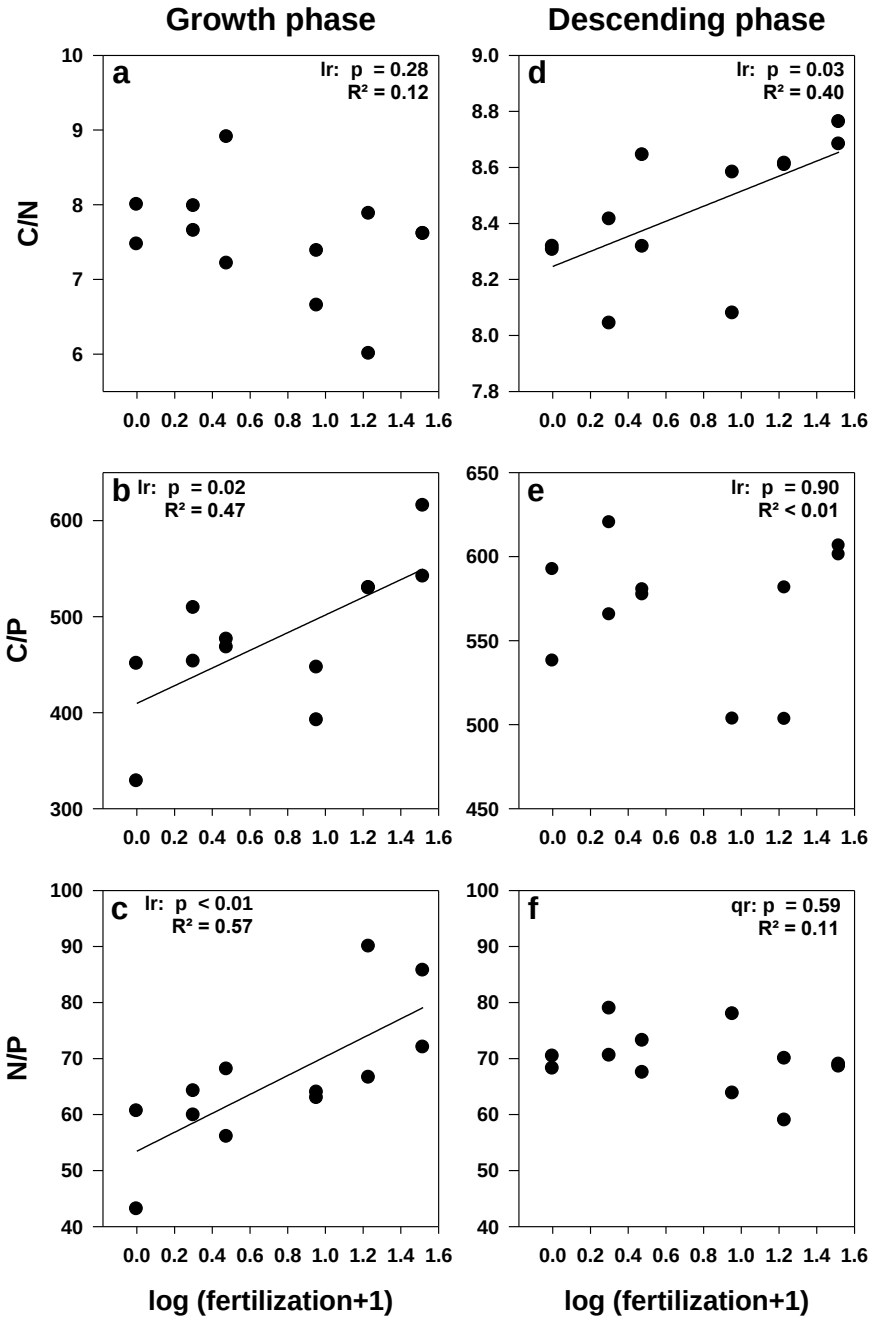

Figure 5 Responses of stoichiometry ratios to nitrogen enrichment. The left column presents data from the growth phase and the right column presents data from the descending phase: (a,d) C:N, (b,e) C:P, (c,f) N:P. Regression lines show significant responses with p<0.05.

Phytoplankton biomass and community composition

Chlorophyll a and POC

In the phytoplankton development of all treatments a peak was observed, representing the typical seasonal spring development for oligotrophic temperate lakes (Sommer *et al.* 1986). Chlorophyll *a* increased from on average 1.03 ± 0.01 µg L^{-1} to 2.27 ± 0.20 µg L^{-1} on day 28, when six of the enclosures reached their chlorophyll *a* maximum and an average of 2.61 ± 0.22 µg L^{-1} on day 35 for the remaining six enclosures (Figure 4b). A continuous and rapid decrease in chlorophyll *a* concentrations of all enclosures was observed after day 35 (Figure 4b). From the mean chlorophyll *a* values, I defined that the overall growth phase began on day 17 (excluding the initial lack phase when no growth was observed) and ended on day 35. The descending phase began on day 38 and ended on day 63. The seasonal development of the chlorophyll *a* concentration did not differ greatly between the treatments, and the timing of the day on which the chlorophyll *a*-peak occurred in each enclosure was not influenced by nitrogen enrichment (lr: p=0.72, R^2=0.01).

Chlorophyll *a* showed a trend to higher concentrations with increasing nitrogen enrichment for the growth phase (Figure 6b; lr: p=0.06, R^2=0.31). For the descending phase, no effect of nitrogen enrichments on chlorophyll *a* could be observed (Figure 6e; lr: p=0.28, R^2=0.12).

POC showed a quadratic response to increasing nitrogen enrichment during the growth phase (Figure 6a; qr: p=0.02, R^2=0.59) with the lowest values found in the two-fold nitrogen increase treatment. During the descending phase, there was a trend of increasing POC with increasing nitrogen enrichment (Figure 6d; lr: p=0.08, R^2=0.27). The timing of the day on which the POC-peak occurred was not influenced by nitrogen enrichment (lr: p=0.90, R^2<0.00)

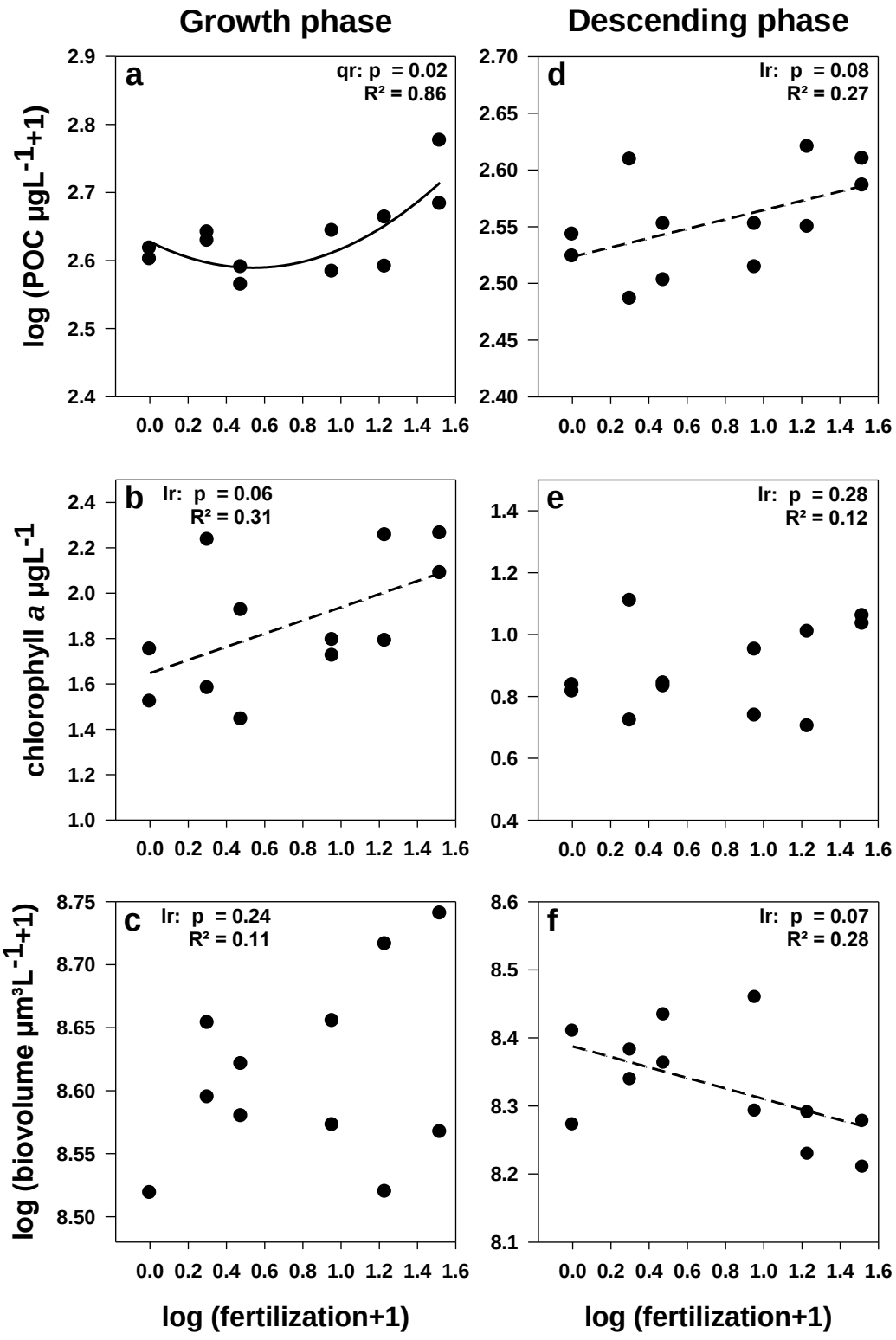

Figure 6 Responses of the three phytoplankton biomass proxies to nitrogen enrichment. In the left column for the growth phase and in the right column for the descending phase: (a,d) POC, (b,e) chlorophyll *a*, (c,f) biovolume. Regression lines are solid for significant responses with $p<0.05$ and dashed for trends with $0.05<p<0.1$.

Microscopic analyses of phytoplankton biovolume

Throughout the whole experiment, the phytoplankton community was dominated by bacillariophyceae, dinoflagellates, chrysophyceae and cryptophyceae. Only a few individuals of chlorophyceae and cyanobacteria (mainly *Anabaena sp*.) were present. The most abundant species within the bacillariophyceae were *Asterionella formosa, Cyclotella sp.* and *Fragilaria crotonenis*. Other highly abundant species were the chrysophyceae species *Dinobryon divergens* and the dinoflagellate *Ceratium hirudinella*.

Peak phytoplankton biovolume was reached in the enclosures between day 21 (highest average biovolume of $451.6*10^6 \pm 11.7*10^6$ $\mu m^3 L^{-1}$) and day 35 (Figure 4c) and occurred earlier with increasing nitrogen enrichment (Figure 7a; lt-. $y=36.2-9.5*x$; $p<0.01$, $R^2=0.64$). Total phytoplankton growth rates (based on biovolumes) estimated up to the phytoplankton peak in each enclosure, did show a significant response to increasing nitrogen enrichment (Figure 4b; lr: $p=0.02$, $R^2=0.46$).

However, growth rates of individual phytoplankton groups showed different responses to the N enrichment. Whereas initial growth rates (up to the total phytoplankton peak) of mixotrophic chrysophyceae, esp. *Dinobryon sp.*, and dinoflagellates showed a clear increase with increasing N (chrysophyceae: Figure 7c; lr: $y=0.12+0.05*x$; $p<0.01$, $R^2=0.77$; dinoflagellates: Figure 7d; lr: $y=0.04+0.04*x$; $p<0.01$, $R^2=0.51$) growth rates of more autotrophic groups, such as chlorophyceae or bacillariophyceae, did not respond in a significant directed way to nitrogen addition.

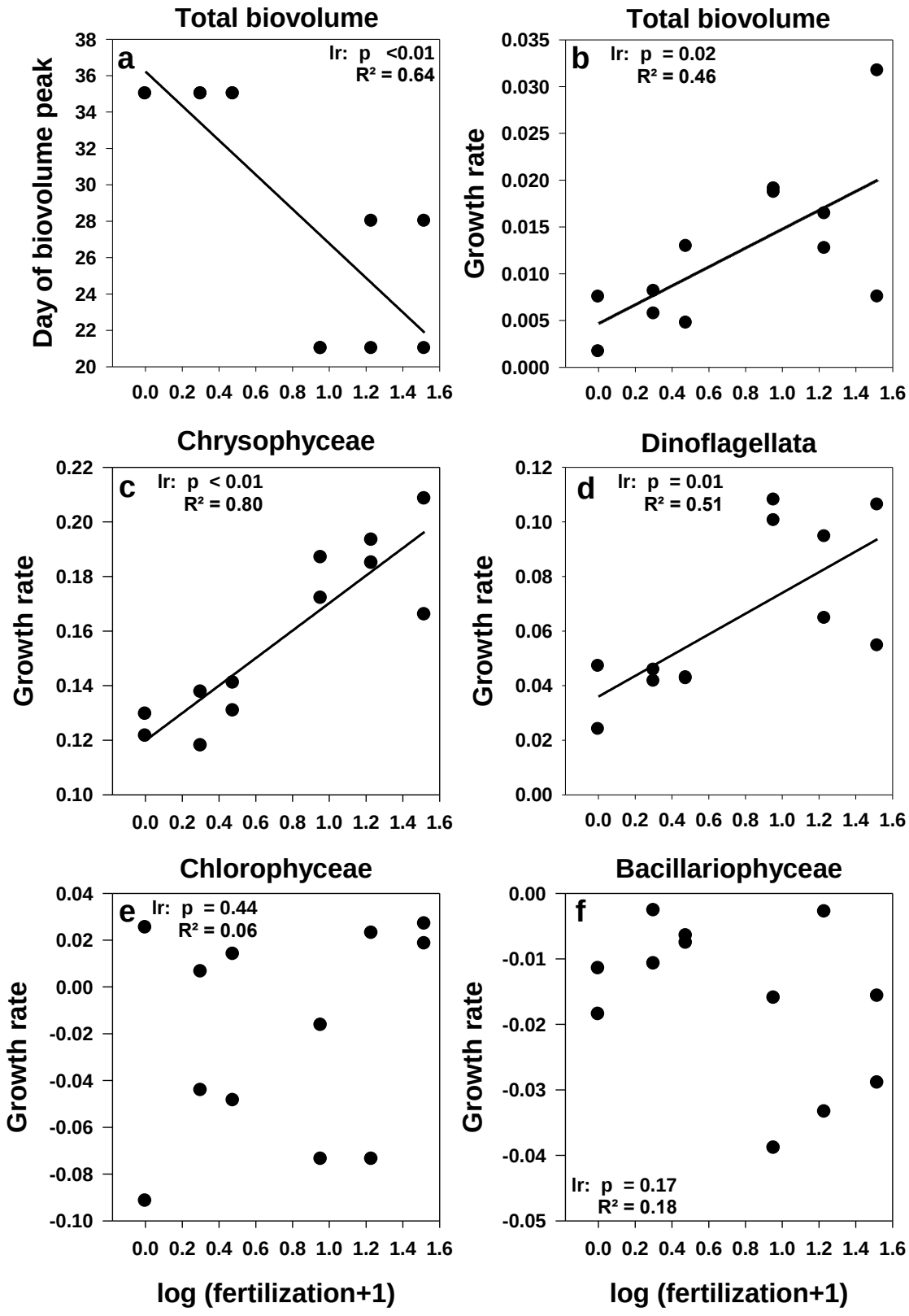

Figure 7 Response of the timing of the biovolume peak (a) and growth rates of major phytoplankton groups to nitrogen enrichment. (b) total biovolume, (c) chrysophyceae, (d) dinoflagellata, (e) chlorophyceae, (f) bacillariophyceae. Regression lines show a significant responses with $p<0.05$

Within the phytoplankton groups, a clear peak in biomass development could be observed for chlorophyceae (peak on day 21, $34.0*10^6 \pm 8.6*10^6$ µm³ L^{-1}), dinoflagellates (day 21, $142.6*10^6 \pm 37.2*10^6$ µm³ L^{-1}) and chrysophyceae (day 35, $71.4*10^6 \pm 7.0*10^6$ µm³ L^{-1}) (Figure 8a, b, d). Within the dinoflagellates, *C. hirudinella* reached peak densities on day 28 ($21.0*10^6 \pm 2.0*10^6$ µm³ L^{-1}); *D. divergens*, representing the majority of chrysophyceae found, reached its peak on day 35 ($20.1*10^6 \pm 1.6*10^6$ µm³ L^{-1}) (Figure 8f, i). A fluctuation over time with no obvious biovolume peak development was observed in cryptophyceae ($72.8*10^6 \pm 6.6*10^6$ µm³ L^{-1}) and bacillariophyceae ($103.3*10^6 \pm 4.1*10^6$ µm³ L^{-1}) (Figure 8c, e). However, within the bacillariophyceae, different species showed succession patterns with varying time courses. At the beginning of the experiment, bacillariophyceae were mainly represented by *A. formosa* ($119.9*10^6 \pm 5.06*10^6$ µm³ L^{-1}), which subsequently decreased over time and were almost absent after day 49 (Figure 8g). *Cyclotella sp.* showed the reverse pattern, having a classical peak development, with an increase until day 42 ($85.4*10^6 \pm 7.0*10^6$ µm³ L^{-1}) and a decrease afterwards (Figure 8j). Finally, *F. crotonenis* started to increase after day 42 and continued to increase until the end of the experiment ($21.1*10^6 \pm 5.3*10^6$ µm³ L^{-1}) (Figure 8h). Even though *F. crotonensis* increased over time, the maximum abundance (biovolume) at the end of the experiment was low compared to the abundances (biovolumes) of the other bacillariophyceae species.

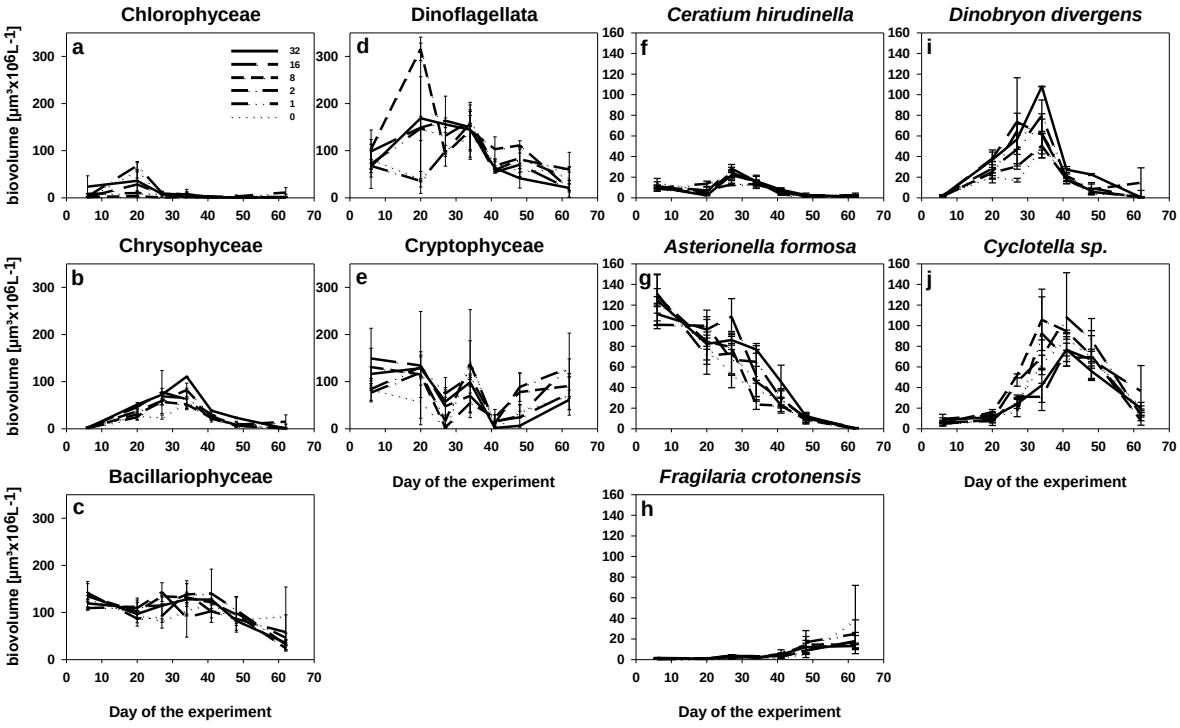

Figure 8 Biovolume developments over time for the most abundant algae groups and species. The left two columns show group data and the right two columns show species data.

Total algal biovolume did not show a response to increased nitrogen enrichment in the growth phase (Figure 6c; lr: p=0.24, R^2=0.11). On group level, only chrysophyceae biovolume showed an increase with increasing nitrogen enrichment (Figure 9b; lr: p=0.05, R^2=0.33) when comparing treatments during the growth phase. In other algal groups, no significant effects of nitrogen on biovolume could be observed when comparing treatments during growth phase (Figure 9a-f, column 1).

When considering the dynamics of individual species (Figure 9 n-r, column 3), I found that the chrysophyceae *D. divergens* biovolume showed a trend to increase with increasing nitrogen enrichment (Figure 9o; lr: p=0.09, R^2=0.27). Within bacillariophceae, *A. formosa* declined in all treatments over time (Fig 8 g) but showed a trend to increase with increasing nitrogen enrichment when comparing treatments during growth phase (Figure 9p; lr: p=0.06, R^2=0.32), *Cyclotella sp.* showed a trend towards a quadratic relationship, with nitrogen peaking just after the 4-fold natural nitrogen input (Figure 9q; qr: p=0.10, R^2=0.40).

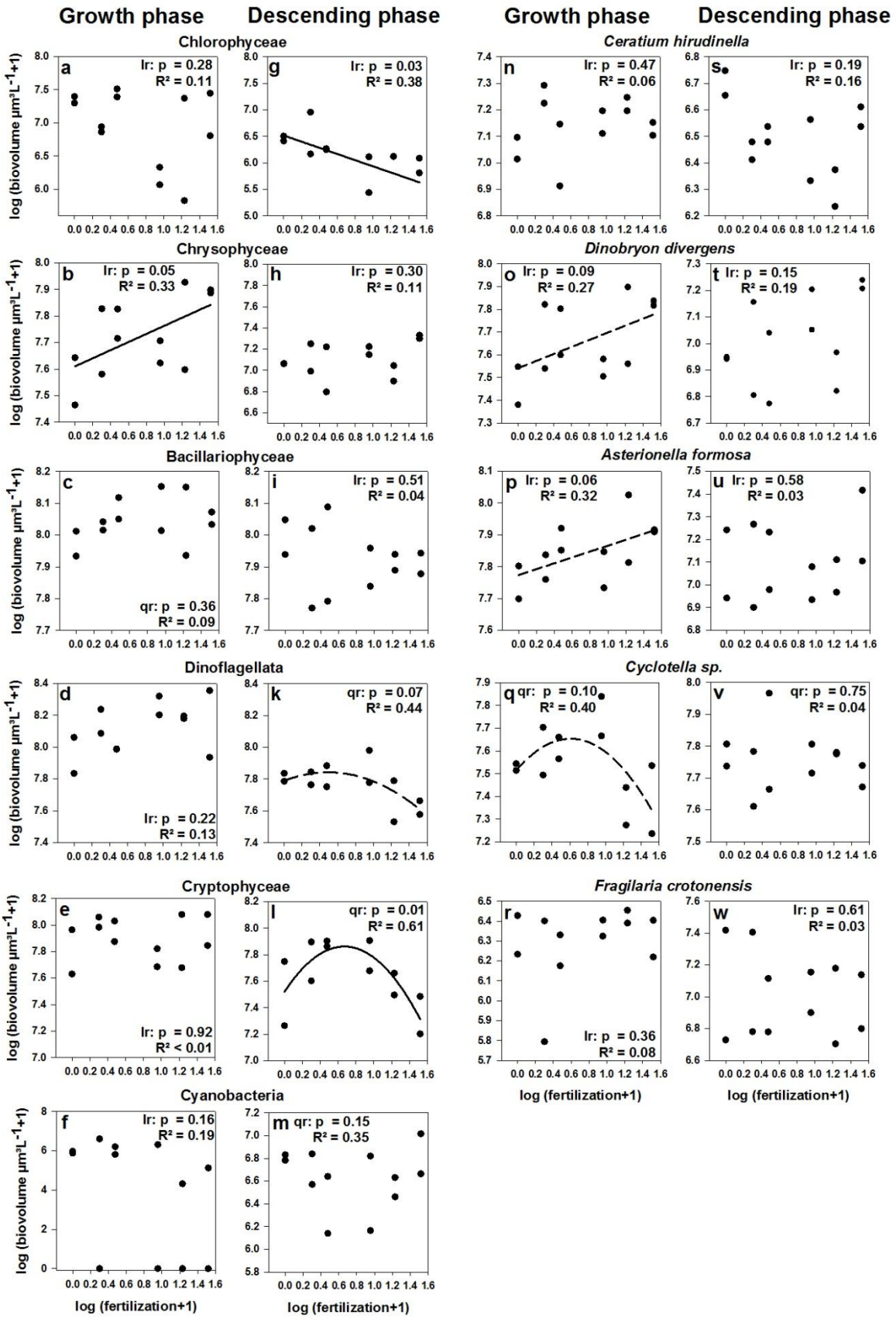

Figure 9 Biovolume versus nitrogen enrichment for algae groups (left two columns) and most abundant species (right two columns): (a-f) algae groups in the growth phase (days 21 till 35), (g-m) algae groups in the descending phase (days 42 till 63), (n-r) species in the growth phase, (s-w) species in the descending phase. Regression lines are solid for significant responses with $p<0.05$ and dashed for trends with $0.05<p<0.1$.

When comparing treatments in the descending phase total algal biovolumes showed a trend to decrease with increasing nitrogen enrichment (Figure 6f; lr: p=0.07, R^2=0.28). A significant decrease with nitrogen enrichment, when comparing treatments in the descending phase, was found for the chlorophyceae (Figure 9g; lr: p=0.03, R^2=0.38). Additionally, a quadratic relationship (trend) was found for the dinoflagellates, with a peak between the 2- and 8-fold nitrogen enrichment (Figure 9k; lr: p=0.07, R^2=0.44). Cryptophyceae showed a similar pattern with increasing nitrogen enrichment (Figure 9l; qr: p=0.01, R^2=0.61). In the other groups and species no significant responses could be observed (Figure 9 g-m and s-w, column 2 and 4).

Comparing relationships between the different biomass proxies (chlorophyll *a*:biovolume, chlorophyll *a*:POC, biovolume:POC ratios) revealed a quadratic response of biovolume:POC ratio (Figure 10c; qr: p=0.05, R^2=0.48) to nitrogen enrichment during growth phase and no response for the other two proxies (Figure 10a-b). However, a significant influence of increasing nitrogen enrichment could be observed for the chlorophyll *a*:biovolume ratio (Figure 10d; qr: p<0.01, R^2=0.74) and biovolume:POC ratio (Figure 10f; qr: p<0.01, R^2=0.69) in the descending phase. While chlorophyll *a*:biovolume ratio was lowest around the twofold nitrogen enrichment treatment, biovolume:POC ratio was highest at that treatment. For the chlorophyll *a*:POC ratio in the descending phase, a declining trend with increasing nitrogen enrichment could be observed (Figure 10e; lr: p=0.09, R^2=0.26).

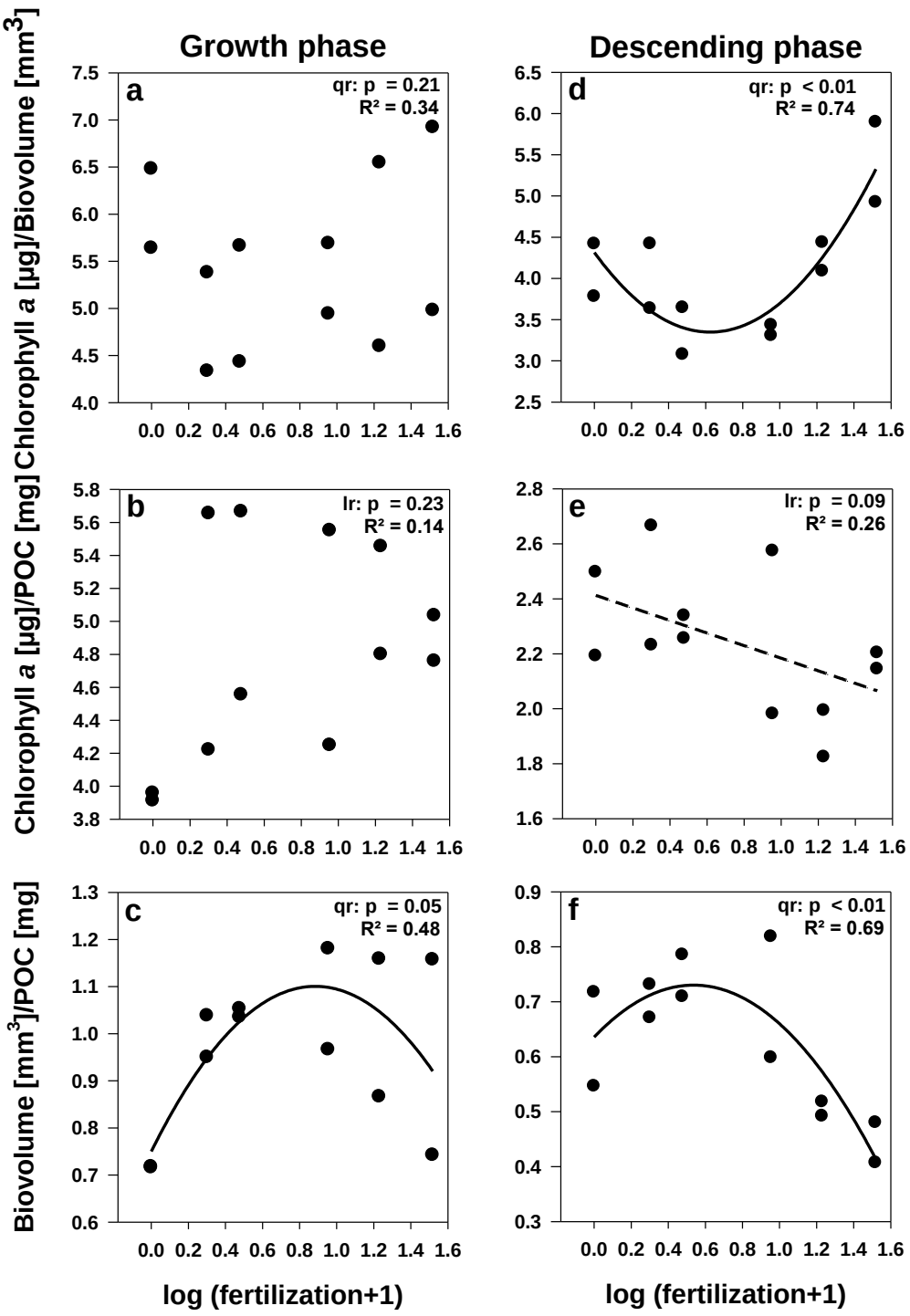

Figure 10 Ratios between biomass proxies versus nitrogen enrichment. The left column shows the growth phase and the right column shows the descending phase: (a,d) chlorophyll *a*/biovolume, (b,e) chlorophyll *a*/POC, (c,f) biovolume/POC. Regression lines show significant responses with $p<0.05$, dotted lines show trends with $0.05<p<0.1$.

Protist and zooplankton development

Nanoflagellates showed a peak development with highest biovolumes of $6.5*10^7 \pm 3.2*10^7$ µm³ L^{-1} on day 35 (Figure 11a). In the phytoplankton growth phase, they slightly increased with increasing nitrogen enrichment (lr: p=0.08, R^2=0.28) and in the descending phase no response was observed (lr: p=0.12, R^2=0.23).

Ciliates decreased during the course of the experiment from $14.71*10^3 \pm 2.04*10^3$ individuals L^{-1} to $2.12*10^3 \pm 0.30*10^3$ individuals L^{-1} (Figure 11b). During the initial phytoplankton growth phase, ciliate abundances were positively correlated with nitrogen enrichment (lr: p= 0.04, R^2=0.36). During the descending phase, no effect of nitrogen enrichment on ciliate abundances could be observed (lr: p=0.52, R^2=0.04).

Crustacean mesozooplankton (cladocera and copepods) increased over time from 2.98 ± 0.22 individuals L^{-1} to 10.72 ± 0.98 individuals L^{-1} and very rapidly between days 29 and 36 (Figure 11c). Neither during the phytoplankton growth phase, nor during their descending phase, a response of mesozooplankton to the nitrogen enrichment could be observed (phytoplankton growth phase: qr: p=0.16, R^2=0.33; phytoplankton descending phase: qr: p=0.20, R^2=0.30).

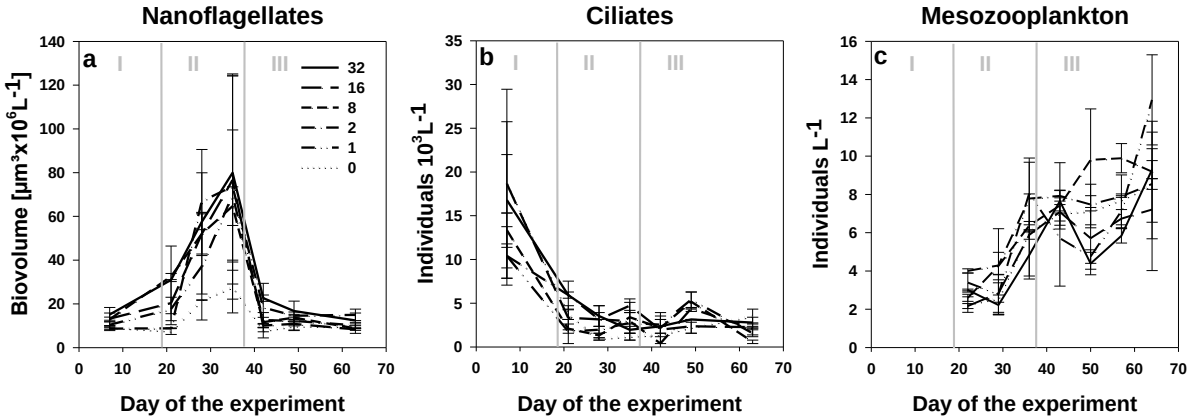

Figure 11 Development of nanoflagellates, ciliates and mesozooplankton over time. Lines plot mean values from the two respective enclosures per treatment and whiskers show standard errors. Grey lines divide the time course in phytoplankton growth phases: I) initial phase, II) phytoplankton growth phase, III) phytoplankton descending phase.

II Variable responses to nitrogen addition in different lakes

Nitrogen enrichment

In all of the three lakes, the required nitrogen gradient was successfully established in the mesocosms (linear regression over all of the lakes versus N fertilization by the end of the experiment: NO_3^-: R^2= 0.69, F= 316, p < 0.001; NH_4^+: R^2= 0.25, F= 23.7, p < 0.001). It varied between 3.8 mg – 5.7 mg L^{-1} NO_3-N (0.01 mg – 1.7 mg L^{-1} NH_4^+-N) in Lake Brunnensee, 0.3 mg – 2 mg L^{-1} NO_3-N (0.14 mg – 1.8 mg L^{-1} NH_4^+-N) in Lake Klostersee, and 2.8 mg – 4.9 mg L^{-1} NO_3-N (0.004 mg – 1.7 mg L^{-1} NH_4^+-N) in Lake Thalersee. In contrast, the TP concentrations in the mesocosms did not follow any trend in terms of the N fertilization (R^2 = 0.01, F= 1.99, p = 0.16) and ranged between 3–8 µg L^{-1} in Lake Brunnensee, 6–15 µg L^{-1} in Lake Klostersee, and 6–17 µg L^{-1} in Lake Thalersee.

When comparing the fertilization treatments at the end of the experiments and equating the observed versus the predicted concentrations of the theoretically accumulated NH_4^+, the achieved NH_4^+ concentrations were consistently below the predicted concentrations in all of the N treatments (negative intercepts of linear regressions, Table 4). The lower than predicted NH_4^+ concentrations indicated a significant NH_4^+ conversion into biomass or other oxidized molecular forms in all of the lakes. For Lake Thalersee, the observed NO_3^- concentrations for the higher N treatments were higher than what would be expected from the fertilization alone (slope >1, Table 4), whereas in Lake Klostersee, an increasing NO_3^- removal could be observed in the applied 8 – 32-fold N treatments (slope <1, Table 4). In Lake Brunnensee, the NO_3^- removal occurred only in the lower N treatments (0–2-fold) but this effect was reduced in the 8 – 32-fold N treatments (slope >1, Table 4).

Table 4 Linear regression results of predicted versus observed NH_4^+ and NO_3^- concentrations versus the nitrogen fertilization (all R^2>0.985). p-values in parentheses

	NH_4^+ mg L^{-1}			**NO_3^- mg L^{-1}**		
	Mean uptake	**Intercept**	**Slope**	**Mean uptake**	**Intercept**	**Slope**
All lakes	0.10	-0.08 **(<0.001)**	0.98 (0.18)	0.06	-0.32 **(<0.05)**	1.02 **(<0.05)**
Brunnensee	0.04	-0.06 **(<0.001)**	1.02 **(<0.05)**	0.14	-0.79 **(<0.01)**	1.03 **(<0.01)**
Klostersee	0.16	-0.14 **(<0.001)**	0.97 (0.08)	0.34	0.18 **(<0.05)**	0.87 **(<0.001)**
Thalersee	0.09	-0.08 **(<0.05)**	0.99 (0.36)	-0.29	-2.18 **(<0.001)**	1.17 (<**0.001)**

Phytoplankton

In terms of phytoplankton growth, mesocoms in oligotrophic Lake Brunnensee reached the highest chlorophyll *a* values between days 28 and 35 (Figure 12a). Enclosures in Lake Thalersee had already reached the highest chlorophyll *a* values between days 7 and 10 (Figure 12c) and it lacked an exponential growth phase. Over the entire experimental duration, the fertilized phytoplankton communities in Lake Thalersee consisted equally of green algae and the spectral group of diatoms and dinoflagellates, and in Lake Brunnensee, it was mainly the spectral group of diatoms and dinoflagellates. The experiment in Lake Klostersee was dominated by high green algae concentrations during the first three weeks, after which the adjoining "peak phase" of the spring bloom between the days 31 and 35 (Figure 12b) was primarily achieved by the spectral group of diatoms and dinoflagelates. In Lake Klostersee, the highest chlorophyll *a* concentration levels after the growth phase of the spectral group of diatoms and dinoflagellates were lower than at the start of the experiment in some of the mesocosms.

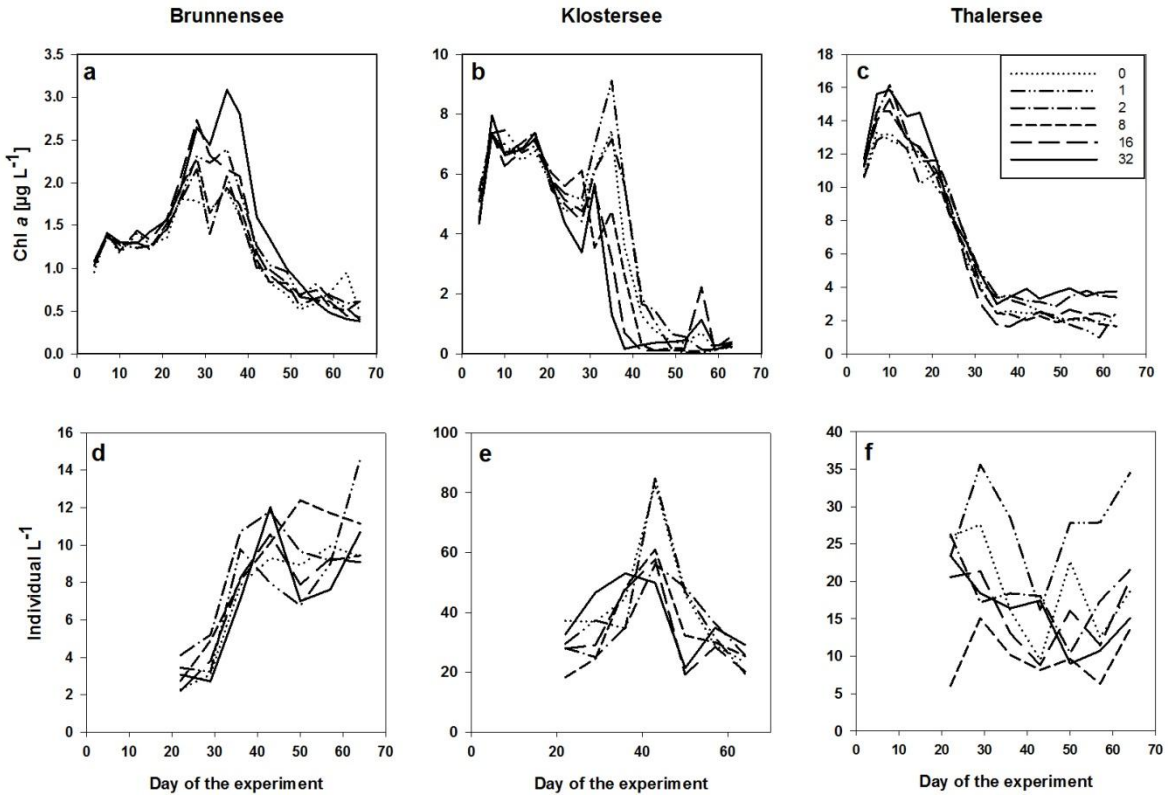

Figure 12 Development of the chlorophyll *a* [µg L^{-1}] (**a**, **b**, **c**) and the zooplankton densities (Individuals L^{-1}) (**d**, **e**, **f**) over the experimental duration. Displayed are the average concentrations (n = 2) of each of the six N fertilization treatments for lakes Brunnensee (left), Klostersee (middle) and Thalersee (right).

In terms of the N fertilization, different responses of the chlorophyll *a* concentration levels could be observed between the lakes. In two of the lakes (Brunnensee and Thalersee), the chlorophyll *a* levels increased with the increasing N fertilization during the peak phase (Table 5). In Lake Brunnensee, there was a positive relationship between the maximally reached chlorophyll *a* concentrations and the N fertilization (Table 5), as well as with the average chlorophyll *a* concentrations of the peak phase (Table 5, Figure 13a). This trend was mainly driven by the spectral group of diatoms and dinoflagellates, which made up >95% of the total chlorophyll *a* concentration levels, and therefore, this phytoplankton group also showed a positive relationship of the chlorophyll *a* peaks with an N fertilization ($R^2 = 0.41$, $F = 6.82$, $p < 0.05$). In Lake Thalersee, the average chlorophyll *a* concentrations increased with increasing N fertilization during the peak phase (Table 5, Figure 13c). In contrast to Lake Brunnensee and Lake Thalersee, in Lake Klostersee the average chlorophyll *a* concentrations decreased during the peak phase with increasing N fertilization. The regression analyses revealed a significant negative relationship of maximum and average chlorophyll *a* levels with the increment of the N fertilization (Table 5, Figure 13b).

Table 5 Linear regression (slope, R^2, p-value) results of the lakes Brunnensee, Klostersee and Thalersee for the maximum total chlorophyll *a* values and the average chlorophyll *a* values of the peak phase versus N fertilization (log-transformed data)

	Brunnensee	**Klostersee**	**Thalersee**
Maximum total chl *a*	0.61, 0.42, **<0.05**	-0.55, 0.14, 0,23	1.5, 0.29, 0.07
Average chl *a* peak phase	0.44, 0.38, **<0.05**	-1.72, 0.45, **<0.05**	1.09, 0.43, **<0.05**

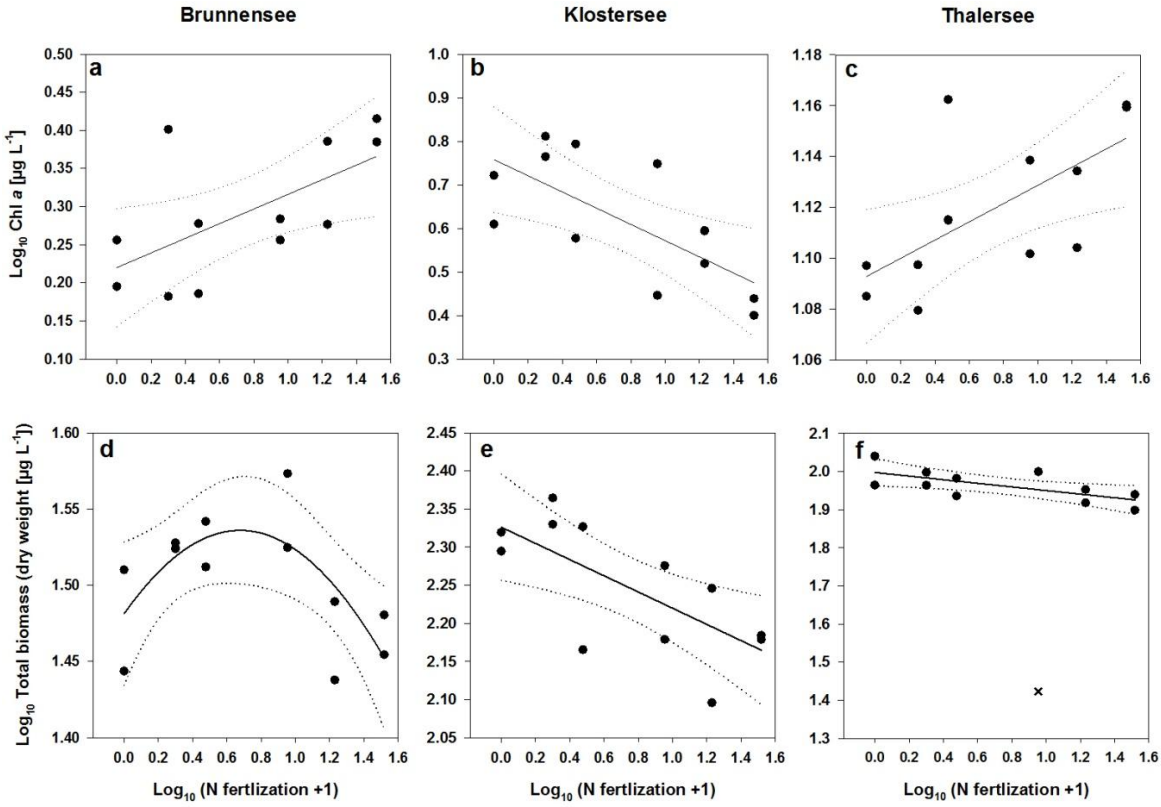

Figure 13 Responses of phytoplankton and zooplankton to nitrogen enrichment in the lakes Brunnensee (left), Klostersee (middle) and Thalersee (right): the average chlorophyll *a* concentrations [µg L^{-1}] of the peak phase (**a**, **b**, **c**) and the zooplankton biomass (**d**: average densities, **e, f**: peak densities). Displayed are the significant regression curves (solid, $P < 0.05$) and the 95% confidence bands (dotted); in **f** without the outlier (cross)

In terms of the seston stoichiometry, significant changes in the experimental enclosures in Lake Brunnensee and Lake Thalersee due to the N fertilization could be observed, but none in Lake Klostersee (Table 6). For Lake Brunnensee, particulate C ($R^2 = 0.04$, $F = 4.79$, $p < 0.05$), particulate N ($R^2 = 0.04$, $F = 3.97$, $p < 0.05$) as well as the seston N:Si ratio (Table 6) increased with N fertilization.

A higher seston stoichiometric P limitation with an increasing N fertilization was observed at the time of the chlorophyll *a* peak, where the seston C:P ratios (day 28: $R^2 = 0.42$, $F = 7.38$, $p < 0.05$; day 35: $R^2 = 0.49$, $F = 7.58$, $p < 0.05$), as well as the seston N:P ratios (day 35: $R^2 = 0.42$, $F = 5.81$, $p < 0.05$), significantly increased with the N fertilization. In Lake Brunnensee, the seston C:P ratios over the entire experimental duration correlated significantly with the dissolved N:P ratios ($R^2 = 0.05$, $F = 5.37$, $p < 0.05$). In Lake Thalersee, I observed decreasing seston C:N ratios with the N fertilization (Table 6), which might indicate a higher N uptake in the highly fertilized treatments – but there were no changes in the seston C:P, in the N:P, or in the N:Si ratios, when increasing the N load.

Over all of the lakes and all of the experimental treatments, a general response of the seston stoichiometry was observed for the seston C:P and N:P ratios, which increased with the dissolved reactive N and the dissolved N:P ratios (Table 7, Figure 14a).

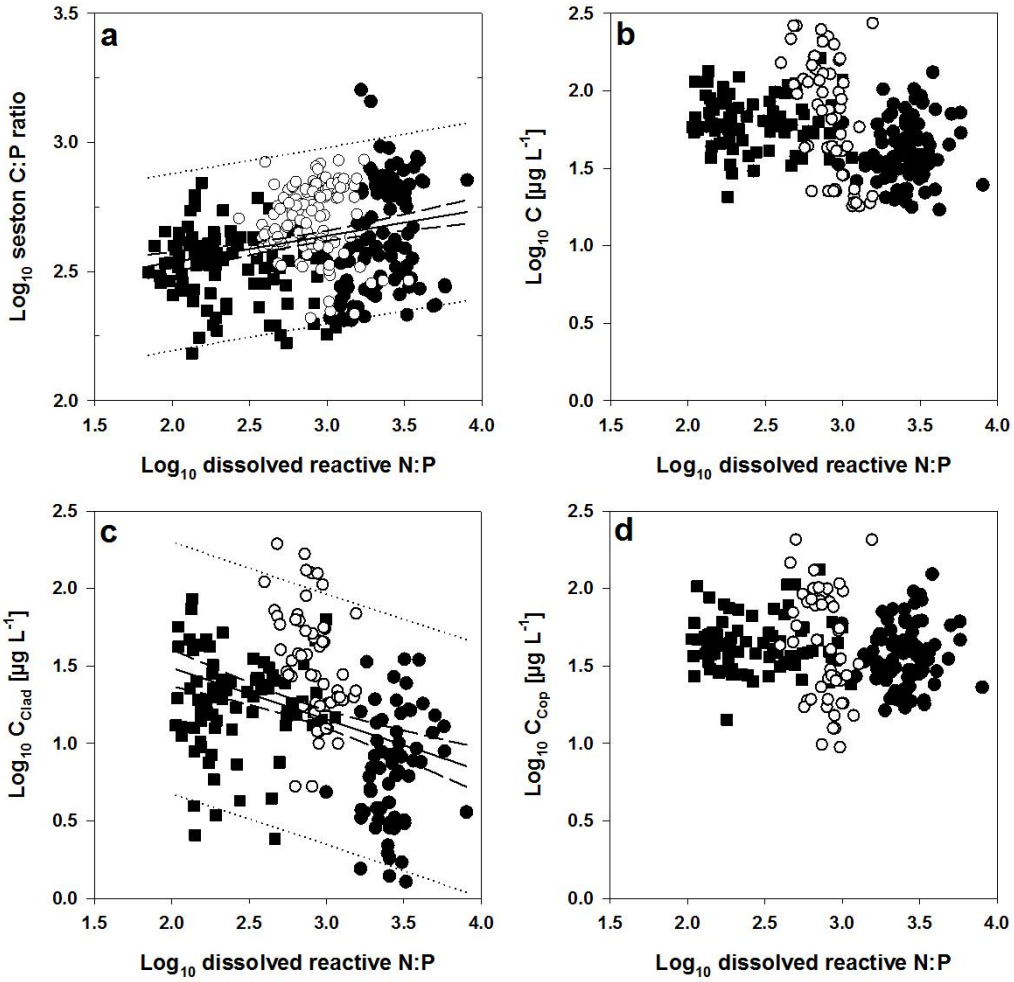

Figure 14 Regression analyses of (a) the seston C:P ratios, (b) the total zooplankton biomass (C µg L^{-1}), (c) the cladoceran biomass (C µg L^{-1}), and the copepod biomass (C µg L^{-1}) versus the dissolved reactive N:P ratios in the mesocosms. Linear regressions: (a) n=321, $R^2 = 0.07$, $p < 0.001$; (b) n= 225, $R^2 = 0.11$, $p < 0.001$; (c) n=202, $R^2=0.13$, $p < 0.001$. Black squares: Lake Klostersee, circles: Lake Thalersee, dots: Lake Brunnensee

Table 6 Linear regression results of the seston stoichiometry data versus the log-transformed N fertilization of the lakes Brunnensee, Klostersee and Thalersee. Given are the sample size (n), the average values ± standard deviation and the p-values (significant results bold and with leading sign of slopes)

	Brunnensee			**Klostersee**			**Thalersee**		
	n	Av±Stdev	p	n	Av±Stdev	p	n	Av±Stdev	p
C:N	105	7.5±1.5	0.89	84	8.0±1.2	0.71	108	8.2±1.0	**- <0.05**
C:P	107	498±253	0.49	108	348±102	0.55	108	526±154	0.95
N:P	106	63±26	0.42	84	44±16	0.49	108	65±21	0.33
N:Si	46	2.5±0.9	**+ <0.01**	48	2.3±1.9	0.49	48	0.6±0.3	0.54

Table 7 Linear regression results of the log-transformed seston stoichiometry data over all the lakes (Brunnensee, Klostersee and Thalersee) versus the dissolved inorganic N (DIN mmol L^{-1}) and the log-transformed dissolved N:P ratios. Given are the sample size (n), R^2 and the p-values (bold: significant)

All lakes	**n**	**R^2**	**p**
C:P vs. N	323	0.09	**<0.001**
C:P vs. N:P	321	0.07	**<0.001**
C:N vs. N	297	0.01	0.14
C:N vs. N:P	295	0.00	0.33
N:P vs. N	297	0.10	**<0.001**
N:P vs. N:P	295	0.07	**<0.001**

Zooplankton

The community composition in all of the lakes included copepods, cladocerans, and rotifers. In the mesococms of Lake Brunnensee, the copepods were on average the most abundant taxonomical group and were followed by the cladocerans (Table 8). On the contrary, in Lake Klostersee and Lake Thalersee, the cladocerans were the most abundant group, followed by the copepods. In all of the lakes, the rotifers were the least abundant taxonomical group. In the lakes Brunnensee and Klostersee, the zooplankton populations increased in all of the mesocosm treatments over the course of the experiment and they reached peak densities after the chlorophyll *a* maxima between the days 35 and 45 (Figure 12d, e).

In Lake Brunnensee, the absolute zooplankton densities remained low compared to the other two lakes, but the cladoceran densities increased until the end of the experiment. In Lake Thalersee, no clear growth phase was observed and the zooplankton numbers showed a high variability and fluctuated between the treatments over the experimental duration (Figure 12f).

The CCA analysis of the data revealed that 80.2% of the variance in the entire data set could be explained by the first axis ($p < 0.01$), which was first correlated with the TP ($R = 0.54$) and then with the dissolved N:P ratios ($R = - 0.16$) (Figure 15). The remaining 19.2% of the variance in the data set was explained by the second axis, which was correlated with the highest of the dissolved reactive N ($p = - 0.25$), followed by the dissolved N:P ratios ($R = - 0.19$) and the TP ($R = 0.14$). The analysis supports that samples from TP-rich and low N:P environments, as in Lake Thalersee, were generally characterised by the presence of cladocerans, whereas the copepods (copepodids and adult copepods) were associated with the higher dissolved N:P ratios, as found in Lake Brunnensee (Figure 15). The nauplii held an intermediate position and since the Thalersee nauplii started to grow at the end of the

experiment under the highest treatment N concentrations, they plot together with the high dissolved reactive N.

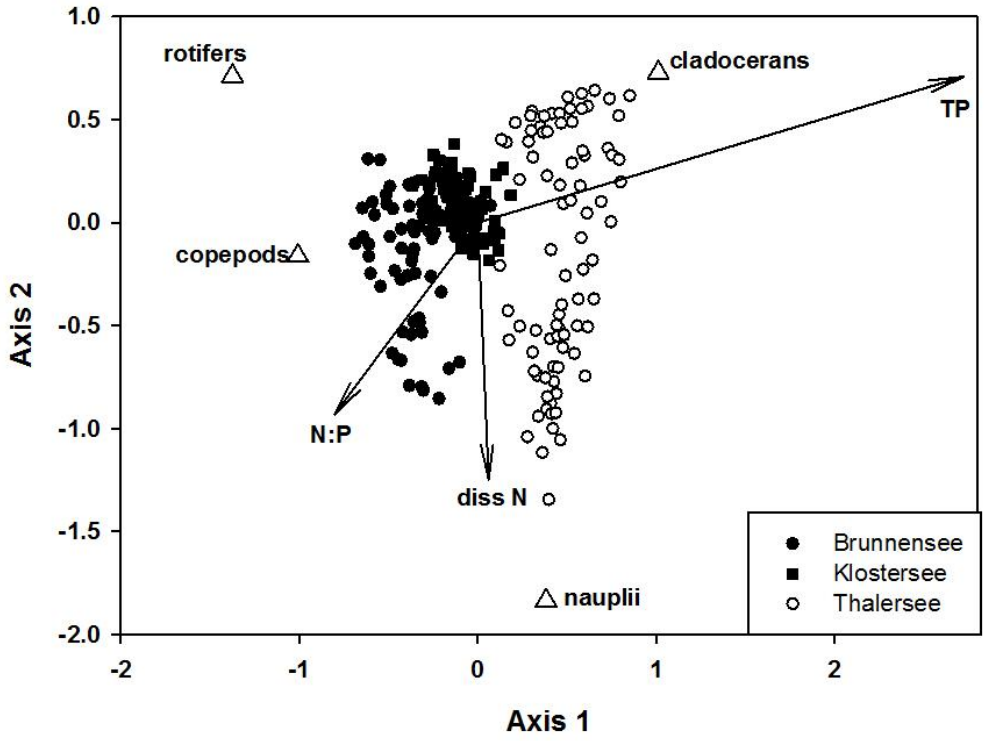

Figure 15 Canonical correspondence analysis of the log transformed absolute zooplankton abundances (Ind L^{-1}) (calanoid copepods, cladocerans, rotifers, nauplii) and the explanatory variables (TP, dissolved N:TP ratios, dissolved reactive N) over the entire experimental duration (n = 252). Black squares: Lake Klostersee, circles: Lake Thalersee, dots: Lake Brunnensee

Table 8 Composition of the zooplankton community of the lakes Brunnensee, Klostersee and Thalersee. Given are percentages of most abundant species and taxonomical groups. Rotifers comprised mainly of the species *Kellicottia sp.*, *Filinia longiseta*, *Keratella sp.*, and *Asplanchna sp*.

	Brunnensee	**Klostersee**	**Thalersee**
Calanoid copepods	30.3 %	16.1 %	3.7 %
Cyclopoid copepods	11.7 %	6 %	14.7 %
Nauplii	16 %	8.4 %	29.6 %
Daphnia sp.	14.6 %	14 %	6.3 %
Bosmina longirostris	3.8 %	36.4 %	40.9 %
Ceriodaphnia sp.	3.9 %	3.4 %	2 %
Pseudochydorus sp.	-	1.2 %	0.6 %
Rotifers	19.7 %	14.5 %	2.2 %

As common trend in all of the lakes zooplankton densities and their biomasses decreased with increasing N fertilization. However, specific zooplankton parameters differed between the lakes. In Lake Klostersee and Lake Thalersee, the peak zooplankton biomass was linearly decreasing over the N fertilization gradient (Table 9, Figure 13e, f). In Lake Brunnensee, the declining average biomass was observed only in the N treatments higher than eight times the natural wet deposition due to a unimodal relationship (Figure 13d, Table 9). For Lake Brunnensee, the unimodal trend was found in the nauplii (Table 9) and the cladoceran carbon biomass (Table 10). In Lake Klostersee, the nauplii and the cladocerans (Table 9, and the carbon biomass average: $R^2 = 0.38$, $F = 6.14$, $p < 0.05$, and peak: $R^2 = 0.59$, $F = 14.46$, $p < 0.01$) were negatively affected and showed significant decreasing biomass with the N fertilization. In Lake Thalersee, only the cladocerans were negatively affected by the N fertilization (Table 8) and their biomass strongly declined with increasing dissolved N:P ratios (Table 10).

Interestingly, in Lake Brunnensee and in Lake Klostersee, the rotifer biomass showed some positive relationship with the increased N fertilization (Table 9). The analyses of the zooplankton biomass as well as the carbon biomass data over all of the lakes demonstrated a declining relationship with increasing reactive N in a wider range of dissolved N:P ratios than experimentally achieved (Table 10, Figure 14b, densities Ind l^{-1} versus dissolved N:P: $R^2 = 0.49$, $p < 0.0001$). This overarching pattern was observed in the carbon proportion of cladocerans (Figure 14c) and not in the copepods (Figure 14d).

Table 9 Summary of the regression analyses of the zooplankton biomass (µg L^{-1}) of the lakes Brunnensee, Klostersee and Thalersee versus the N fertilization treatments (log-transformed). Applied regression models are linear (1) or unimodal Gauss fits (2). Slopes are indicated for the linear regression models as negative (-) or positive (+) trends. Significant p-values are highlighted ($p < 0.05$) and indicated for the data set: a: entire data set (n=84), b: average densities (n=12) and c: peak densities (n=12)

	Brunnensee			**Klostersee**			**Thalersee**		
Log Ind L^{-1}	Model	Slope	p	Model	Slope	p	Model	Slope	p
Total	2		* [b]	1	-	* [c]	1	-	* [c]
Cal. Copepoda	1	-		1	-		1	-	<0.1 [c]
Nauplii	2		* [a]	1	-	* [a, b]	1	-	
Cladocera	1	-		1	-	* [a,b,c]	1	-	* [a,c]
Rotifera	1	+	* [c]	1	+	* [a,b,c]	1	+	

Table 10 Linear regression results of the mesozooplankton biomass data over all the lakes (Brunnensee, Klostersee and Thalersee) versus the dissolved inorganic N (DIN mmol L^{-1}) and the dissolved N:P ratios (log-transformed). Given are the sample size (n), R^2 and the p-values (bold: significant with Power p< 0.05)

C µg L^{-1}	**N**			**N:P**		
	n	**R^2**	**p**	**n**	**R^2**	**p**
All Mesozoo	225	0.07	**<0.001**	224	0.11	**<0.001**
All Copepoda	213	0.00	0.56	212	0.02	0.06
All Cladocera	203	0.08	**<0.001**	202	0.13	**<0.001**
BS Mesozoo	84	0.00	0.17	82	0.01	0.37
BS Copepoda	81	0.04	0.08	80	0.00	0.54
BS Cladocera	63	0.00	0.60	63	0.14	**<0.05**[b]
KS Mesozoo	84	0.00	0.62	84	0.00	0.72
KS Copepoda	81	0.00	0.98	81	0.00	0.83
KS Cladocera	81	0.00	0.68	81	0.00	0.86
TS Mesozoo	58	0.02	0.12	58	0.30	**<0.001**
TS Copepoda	49	0.12	**<0.05**[a]	0.05	0.08	0.08
TS Cladocera	57	0.00	0.65	57	0.15	**<0.01**

[a]**:** positively related; [b]**:** a*exp(-0,5*(ln(x/x0)/b)^2)/x

III Consequences of changing nitrate to ammonium supply ratios

The experimental design was applied successfully and significant differences were established between the treatments (twA: N amount*ratio (day 77) NH_4^+: $F_{1,8}$=975.2, NO_3^-: $F_{1,8}$=74.1; p<0.001 for NH_4^+ and NO_3^-). The difference between the potential maximum dissolved nutrient concentrations (initial dissolved nutrient concentration plus the sum of fertilizations) and the dissolved nutrient concentrations measured at the end of the experiment is the amount of removed nutrients, which were larger for NH_4^+ than for NO_3^- (ΔNH_4^+: 9.7±1.7 µmol L^{-1} and ΔNO_3^-: 9.0±1.6 µmol L^{-1}). The removal was proportional higher for NH_4^+ than for NO_3^- (on average 78.3±5.4% NH_4^+ and 2.66±0.48% NO_3^-). The removal of dissolved NH_4^+ increased with increasing NH_4^+ enrichment (Figure 16; lr: y=0.41x+0.06, R^2=0.95, p<0.001) but not with increasing NO_3^- enrichment (lr: R^2=0.01, p=0.78). The removal of dissolved NO_3^- did not correlate with NO_3^- enrichment (Figure 16; lr: R^2=0.01, p=0.77) (Figure 16) but decreased with increasing NH_4^+ enrichment (lr: y=-0.09x+0.78, R^2=0.34, p<0.05).

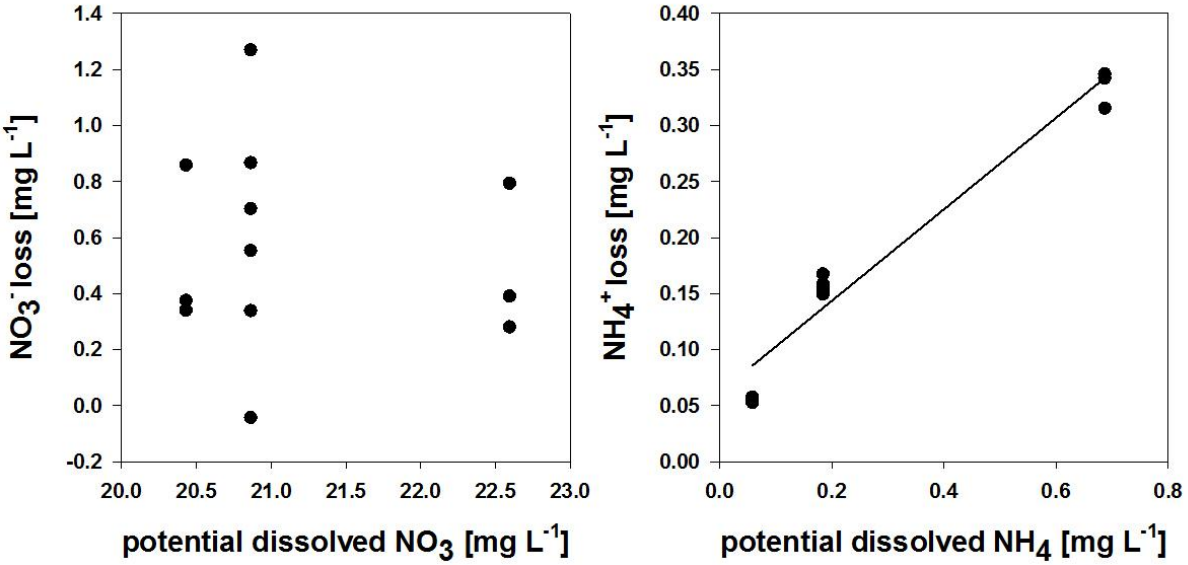

Figure 16 NO_3^- and NH_4^+ loss versus the potential NO_3^- and NH_4^+ concentrations. Potential dissolved concentrations are the initial dissolved concentrations plus fertilizations. Loss is calculated as the difference between those potential dissolved concentrations and dissolved end concentrations.

Seston stoichiometry did show a trend towards higher seston N:P ratios in treatments receiving 4-times higher N supply (twA: N ratio $F_{1,128}$=3.11; p=0.08). No differences were observed in seston C:P ratios. At the day of the chlorophyll *a* peak (day 49, 29.04.) lower seston C:N ratios were observed in the 4-times N treatments (twA: N amount $F_{1,8}$=6.05, p=0.04). Calculated chlorophyll *a* to POC ratios, showed lower values during the chlorophyll *a* peak in the surplus NO_3^- treatment (4:1 NO_3^-:NH_4^+ ratio) (day 49, 29.04., 06.05. twA: N ratio, p=0.03), indicating less chlorophyll *a* built up per unit carbon.

Total chlorophyll *a* concentrations reached maximum values of 2-6 µg L^{-1} in early May between days 49 and 59 (Figure 17a). These maximum concentrations were mainly established by the spectral group of diatoms and dinoflagellata, which made up >85% of the total chlorophyll *a* concentrations followed by the group of green algae (Figure 17b, c). However, during the first month of the experiment, also cryptophytes showed a bloom dynamic with maximum chlorophyll *a* values of 1 µg L^{-1} (Figure 17d). In terms of treatment effects, significant differences in chlorophyll *a* concentrations were revealed between the applied N ratio treatments for the peak chlorophyll *a* values for total chlorophyll *a* (Figure 18) as well as for green algae and the spectral group of diatoms and dinoflagellates (Table 11). For cryptophytes, differences between N ratios were marginally reached between values at the day of the chlorophyll *a* peak (Table 11), but were significant specifically on day 21 when cryptophytes themselves showed high abundances (twA: N ratio $F_{1,8}$=7.57, p=0.03). In all cases, the surplus NH_4^+ treatment (1:4 NO_3^-:NH_4^+ ratio) reached significant higher

chlorophyll *a* concentrations. The $RUE_{Chl\ a}$ of phytoplankton communities showed significantly higher values for both surplus NH_4^+ treatments (1:4 NO_3^-:NH_4^+ ratio) (twA: $F_{1,8}$=18.32, p<0.01) compared to the 4:1 NO_3^-:NH_4^+ treatments (Figure 19).

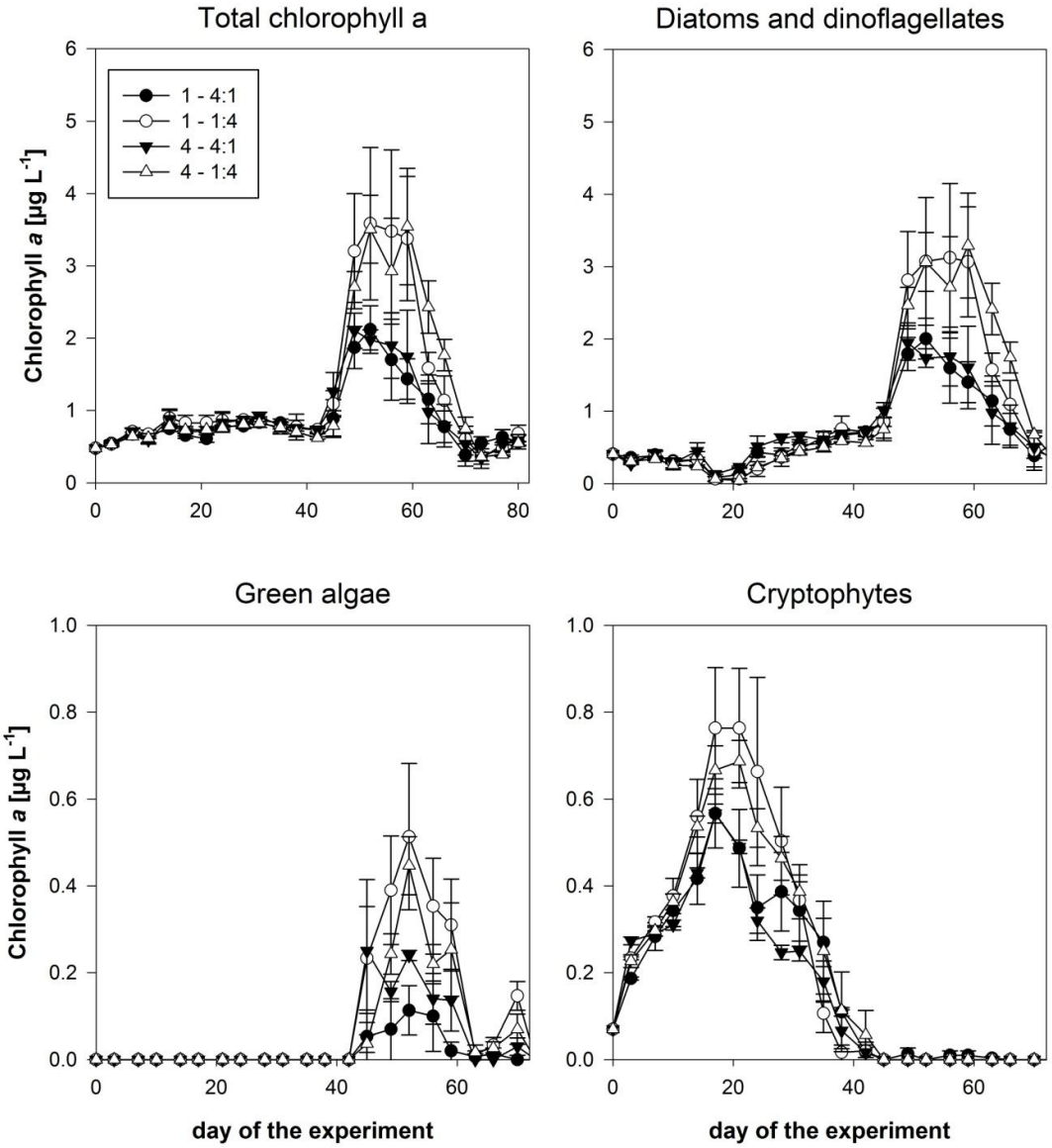

Figure 17 Time course of chlorophyll *a* concentrations over the entire experimental duration: total chlorophyll *a* and chlorophyll *a* of the spectral groups: diatoms and dinoflagellata, green algae and cryptophytes. Shown are mean values of the three respective enclosures, error bars represent standard deviation.

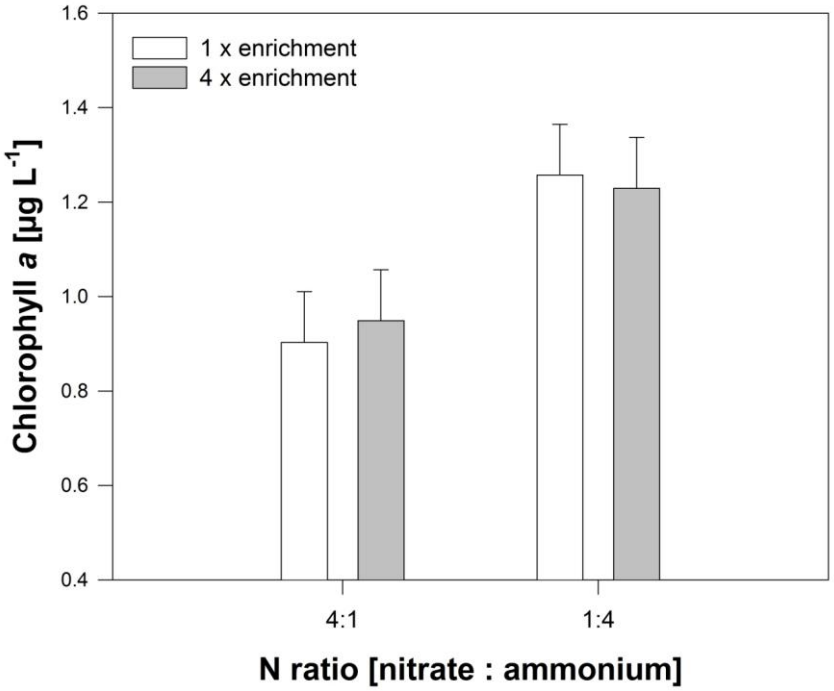

Figure 18 Bar plot of total chlorophyll *a* concentrations (µg L^{-1}) at the individual chlorophyll *a* peak versus NO_3^-:NH_4^+ ratio treatments (4:1 and 1:4). White bars (1): natural wet deposition enrichment, grey bars (4): 4-times natural N wet deposition enrichment. Chlorophyll *a* concentrations of NO_3^-:NH_4^+ supply ratios are significant different (F=18.3, p<0.01).

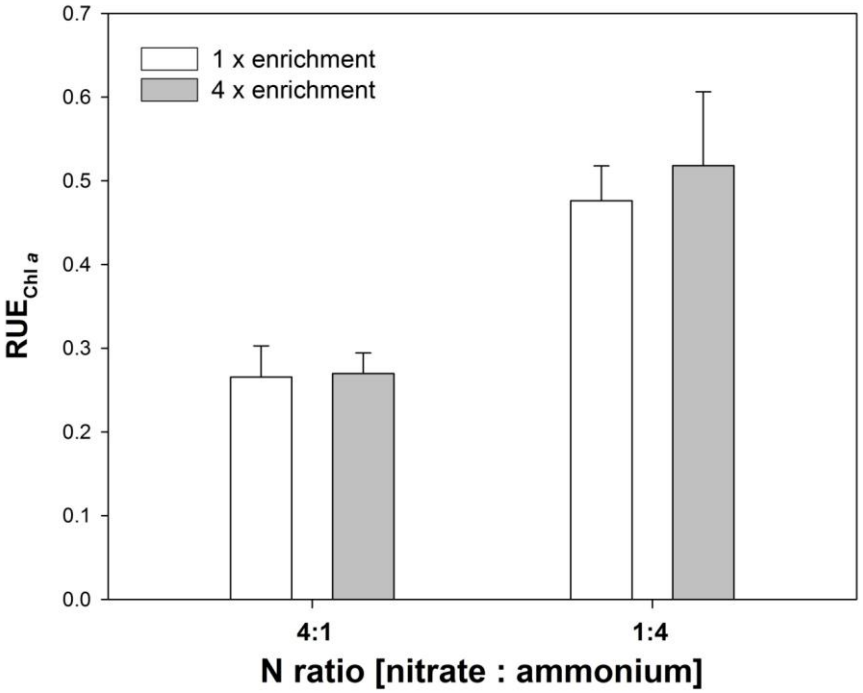

Figure 19 Bar plot of total $RUE_{Chl\ a}$ at the individual chlorophyll *a* peak versus NO_3^-:NH_4^+ ratio treatments (4:1 and 1:4). White bars (1): natural wet deposition enrichment, grey bars (4): 4-times natural N wet deposition enrichment. Chlorophyll *a* concentrations of NO_3^-:NH_4^+ supply ratios are significant different (F=18.3, p<0.01).

Table 11 Two way ANOVA results for chlorophyll a data with total N amount and NH_4^+:NO_3^- supply ratio as fixed factors for total chlorophyll a, diatoms and dinoflagellata, green algae, cryptophytes and cyanobacteria. Results are shown for maximum reached concentrations. Bold: p<0.05, italic: p<0.1.

	Total chl *a*	Diatoms and dinoflagellata	Green algae	Cryptophytes	Cyanobacteria
Amount *p*	0.73	0.79	0.85	0.76	0.87
$F_{1,8}$	0.12	0.08	0.04	0.34	0.02
Ratio *p*	**0.003**	**0.004**	**0.02**	***0.10***	0.43
$F_{1,8}$	18.32	15.52	7.69	3.48	0.69
Amount x Ratio *p*	0.68	0.815	0.22	0.65	0.43
$F_{1,8}$	0.19	0.06	1.8	0.22	0.69

Microscopic counting of phytoplankton communities at the chlorophyll *a* peak phase (days 49-63) showed the highest contribution to total phytoplankton biovolume by green algae with on average 67%, followed by diatoms (29 %) and other taxonomical groups. Treatment differences according to N fertilization revealed effects of the applied N ratio on total phytoplankton biomass (Table 12). This was determined by green algae, which reached higher biomass in the 1:4 NO_3^-:NH_4^+ ratio treatments. Furthermore, the N amount had a significant effect on the total cyanobacteria biovolume (Table 12), which represent however only 1% of the total algal biovolume. On species level *Chlamydomonas sp*. (the main abundant species with on average 67%) was affected by the N ratio with higher biovolumes in the surplus NH_4^+ treatments (1:4 NO_3^-:NH_4^+ ratio) (twA: N ratio $F_{1,8}$=7.87, p=0.023).

Table 12 Two way ANOVA results for microscopic analysis with total N enrichment and NO_3^-:NH_4^+ ratios as fixed factors. Results are shown for biovolumes averaged over the chlorophyll *a* peak phase (days 49-63). Bold: p<0.05.

	Total biovolume	Diatoms	Chryso-phytes	Dino-flagellates	Green algae	Crypto-phytes	Cyano-bacteria
Amount *p*	0.36	0.75	0.32	0.31	0.30	0.71	**0.01**
$F_{1,8}$	0.96	0.11	1.10	1.17	1.22	0.15	10.8
Ratio *p*	**0.02**	0.95	0.60	0.40	**0.02**	0.57	0.86
$F_{1,8}$	8.09	<0.01	0.29	0.80	7.87	0.34	0.03
Amount x Ratio *p*	0.86	0.66	0.16	0.84	1.00	0.50	0.37
$F_{1,8}$	0.04	0.22	2.38	0.05	<0.01	0.50	0.92

Particle size measurements revealed highest biovolume contribution of the size class with equivalent spherical diameter (ESD) 2.51-5.00 µm, followed by 5.01-7.50 µm and larger size classes during days 49-63 (Kruskal Wallis test n=36, H=220, p<0.001). A trend to higher total particle biovolume in the 1:4 NO_3^-:NH_4^+ ratio treatments was observed (twA: $F_{1,8}$=3.19, p=0.08). The observed trend to higher biovolumes in the 1:4 NO_3^-:NH_4^+ ratio treatments for the size class 2.51-5.00 µm ESD and to lower biovolumes in the 27.51-30.00 µm ESD size class (Table 13). The size class of 2.51-5.00 µm ESD contributed on average with 28±6 % to the total biovolume, with 25±6 % in the 4:1 NO_3^-:NH_4^+ ratio treatments and 31±3 % in the 1:4 NO_3^-:NH_4^+ ratio treatments.

Table 13 Two way ANOVA results for particle measurements with total N amount and NO_3^-:NH_4^+ supply ratios as fixed factors (days 49-63). Particles were grouped by the equivalent spherical diameter (ESD) and averaged over the peak time. Bold: p<0.05, italic 0.05<p≤0.1.

ESD (µm)	2.51-5.00	5.01-7.50	7.51-10.00	10.01-12.50	12.51-15.00	15.01-17.50	17.51-20.00
Amount *p*	0.67	0.21	0.51	0.34	0.14	0.57	0.24
$F_{1,8}$	0.19	1.85	0.47	1.03	2.69	0.35	1.63
Ratio *p*	***0.06***	0.89	0.77	0.48	0.19	0.90	0.24
$F_{1,8}$	4.96	0.02	0.09	0.54	2.07	0.02	1.63
Amount x Ratio *p*	0.93	0.80	0.31	0.26	0.19	0.69	***0.06***
$F_{1,8}$	0.01	0.07	1.15	1.47	2.07	0.17	4.93

ESD (µm)	20.01-22.50	22.51-25.00	25.01-27.50	27.51-30.00	30.01-32.50	32.51-35.00	35.01-37.50
Amount *p*	0.43	0.78	0.66	0.20	0.60	0.14	0.37
$F_{1,8}$	0.704	0.08	0.21	2.00	0.30	2.67	0.90
Ratio *p*	0.43	0.11	0.16	***0.05***	0.86	1.00	0.37
$F_{1,8}$	0.70	3.25	2.39	5.12	0.03	0.00	0.90
Amount x Ratio *p*	0.87	**0.025**	0.93	0.42	0.24	0.44	0.76
$F_{1,8}$	0.03	7.58	0.01	0.72	1.63	0.67	0.10

IV Effects of chronic ammonium toxicity

1 Algae NH_4^+ growth experiment

The chlorophyll *a* concentrations in the different NH_4^+ treatments of all three algae species showed a negative response to increasing ammonium concentrations. For *C. reinhardtti* a significant hyperbolic decrease of chlorophyll *a* concentrations with increasing ammonium concentrations could be observed from day 14 on (Table 14). For *C. minutus* a significant decrease of chlorophyll *a* with increasing ammonium concentrations could be observed from the third day on with exception of day 22 where only a trend was detected (Table 14). The chlorophyll *a* concentrations of *Dinobryon* sp. treatments showed a significant decrease with increasing ammonium concentrations throughout the whole experiment (Table 14). *Dinobryon sp*. showed a hyperbolic response to increasing ammonium concentrations on day 3 and a linear decrease on the following days (Table 14). The differences between treatments get more and more pronounced with time (increasing negative slope, Table 14). The responses of the different species to increasing ammonium concentrations are shown exemplary for day 28 as on that day samples for stoichiometry analyses were taken (Figure 20).

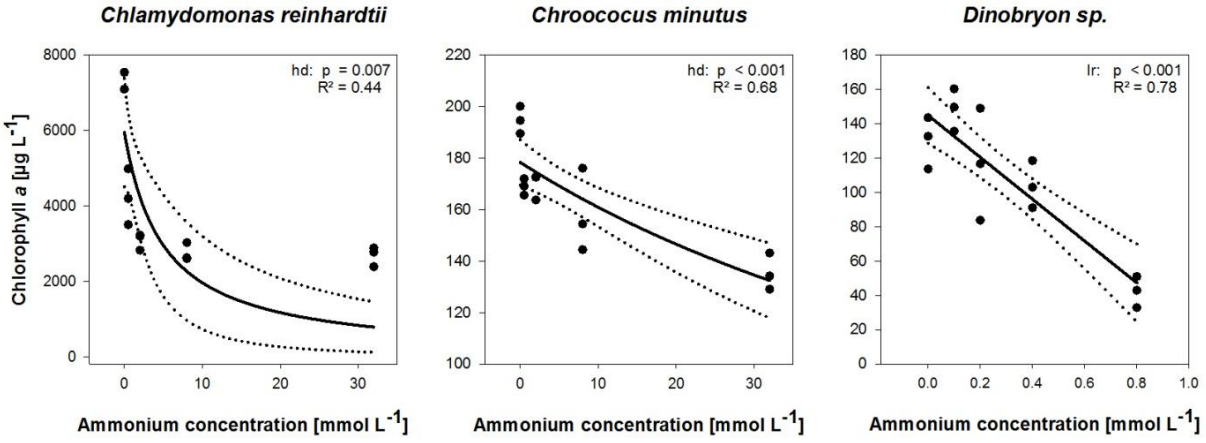

Figure 20 Response of chlorophyll *a* concentrations of the three algal species (day 28) to ammonium concentration. Regression lines are shown solid and dotted lines represent the 95% confidence band.

Table 14 Statistical analyses of algae biomass (measured as chlorophyll *a*) to increasing ammonium concentrations. Shown are : the statistical model (m; lr: linear regression; hd: hyperbolic decay), in case of linear models the slope, the R^2 and the p value. Significant p values ($p \leq 0.05$) are bold and p values representing a trend in italics ($0.05<p<0.1$). On day 30 (*) samples serving as food for *Daphnia* growth experiments were taken.

Day	*C. reinhardtii*				*C. minutus*				*Dinobryon sp.*			
	m	slope	R^2	p	m	slope	R^2	p	m	slope	R^2	p
3	hd		0.06	0.36	lr	-0.25	0.97	**<0.001**	hd		0.59	**<0.001**
7	hd		0.12	0.20	hd		0.70	**<0.001**	lr	-13.1	0.88	**<0.001**
10	hd		0.25	*0.06*	hd		0.83	**<0.001**	lr	-32.9	0.75	**<0.001**
14	hd		0.27	**0.05**	hd		0.56	**0.001**	lr	-77.4	0.86	**<0.001**
17	hd		0.64	**<0.001**	hd		0.98	**<0.001**	lr	-94.5	0.89	**<0.001**
22	hd		0.56	**0.001**	hd		0.24	*0.07*	lr	-92.7	0.61	**<0.001**
25	hd		0.45	**0.006**	hd		0.86	**<0.001**	lr	-103	0.68	**<0.001**
28	hd		0.44	**0.007**	hd		0.68	**<0.001**	lr	-122	0.78	**<0.001**
30*	hd		0.42	**0.01**	hd		0.83	**<0.001**	lr	-131	0.83	**<0.001**

The daily removal of dissolved ammonium (ammonium input minus final concentration) per chlorophyll *a* increased significantly (lr: $p<0.001$ for *C. reinhardtii* and *C. minutus* and hi: $p<0.001$ for *Dinobryon sp.*) with increasing ammonium concentrations (Figure 21).

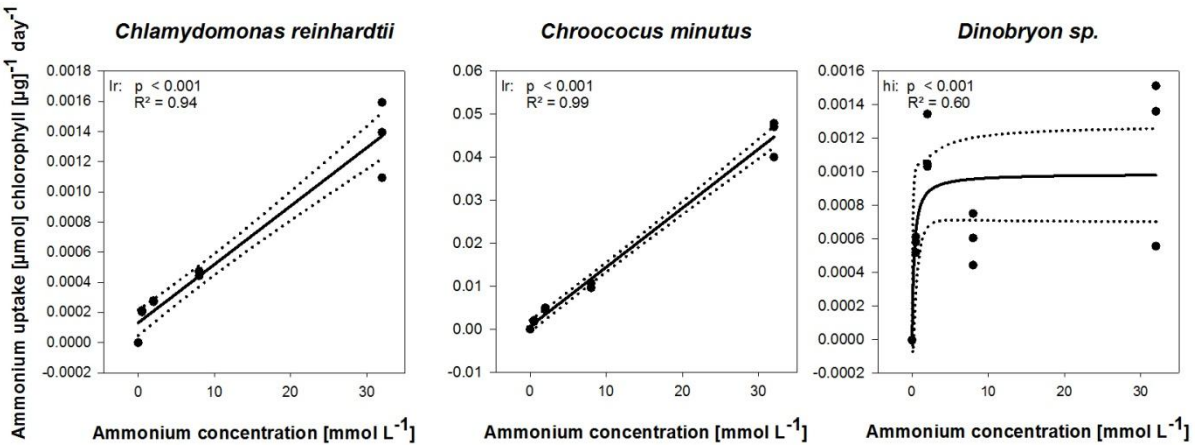

Figure 21 Daily ammonium removal from the dissolved phase (normalized for the respective chlorophyll *a* concentrations integrated over time) in relation to ammonium concentrations. Regression lines are shown solid and dotted lines represent the 95% confidence band.

Concerning phytoplankton stoichiometry, as a measure of food quality, I observed, together with a decrease of chlorophyll *a* with increasing NH_4^+ concentrations, a decrease in both particulate organic carbon (POC) and particulate nitrogen (PN) in *C. reinhardtii* and *Dinobryon sp.* and a decrease in POC in *C. minutes* (Table 15). In *C. minutes* additionally the concentration of particulate phosphorus (PP) decreased (Table 15). In the algae species

C. reinhardtii and *Dinobryon sp.* on the contrary there were no differences of PP content between NH_4^+ treatments (Table 15).

These differing particle concentrations resulted in the following differences of nutrient ratios. An hyperbolic decrease of C:N ratios with increasing NH_4^+ concentrations was observed in both *C. reinhardtii* (trend) and *C. minutus*, while for *Dinobryon sp.* no response could be observed (Table 15). The C:P ratio of *C. reinhardtii* as well as that of *Dinobryon sp.* decreased with NH_4^+ concentrations and no response could be observed for *C. minutes* (Table 15). The N:P ratios of *C. reinhardtii* and *Dinobryon sp.* showed a negative response to increasing NH_4^+ concentrations and those of *C. minutus* a positive response (Figure 22, Table 15).

Table 15 Statistical analyses of seston composition and elemental ratios to increasing ammonium concentrations. Shown are the statistical model (m; lr: linear regression; hd: hyperbolic decay), in case of linear models the slope, the R^2 and the p value. Significant p values ($p \leq 0.05$) are bold and p values representing a trend in italics ($0.05 < p < 0.1$).

	C. reinhardtii				***C. minutus***				***Dinobryon sp.***			
	m	slope	R^2	p	m	slope	R^2	p	m	slope	R^2	p
POC	hd		0.39	**0.01**	hd		0.60	**<0.001**	lr	-307	0.83	**<0.001**
PN	hd		0.35	**0.02**	lr	0.84	0.03	0.53	hd		0.25	*0.06*
PP	hd		0.04	0.48	lr	-0.15	0.82	**<0.001**	lr	0.68	0.16	0.14
C:N	hd		0.23	*0.07*	hd		0.41	**0.01**	lr	-1.19	0.10	0.26
C:P	hd		0.40	**0.01**	hd		0.00	0.86	lr	-107	0.71	**<0.001**
N:P	hd		0.33	**0.03**	lr	0.35	0.46	**0.008**	hd		0.26	**0.05**

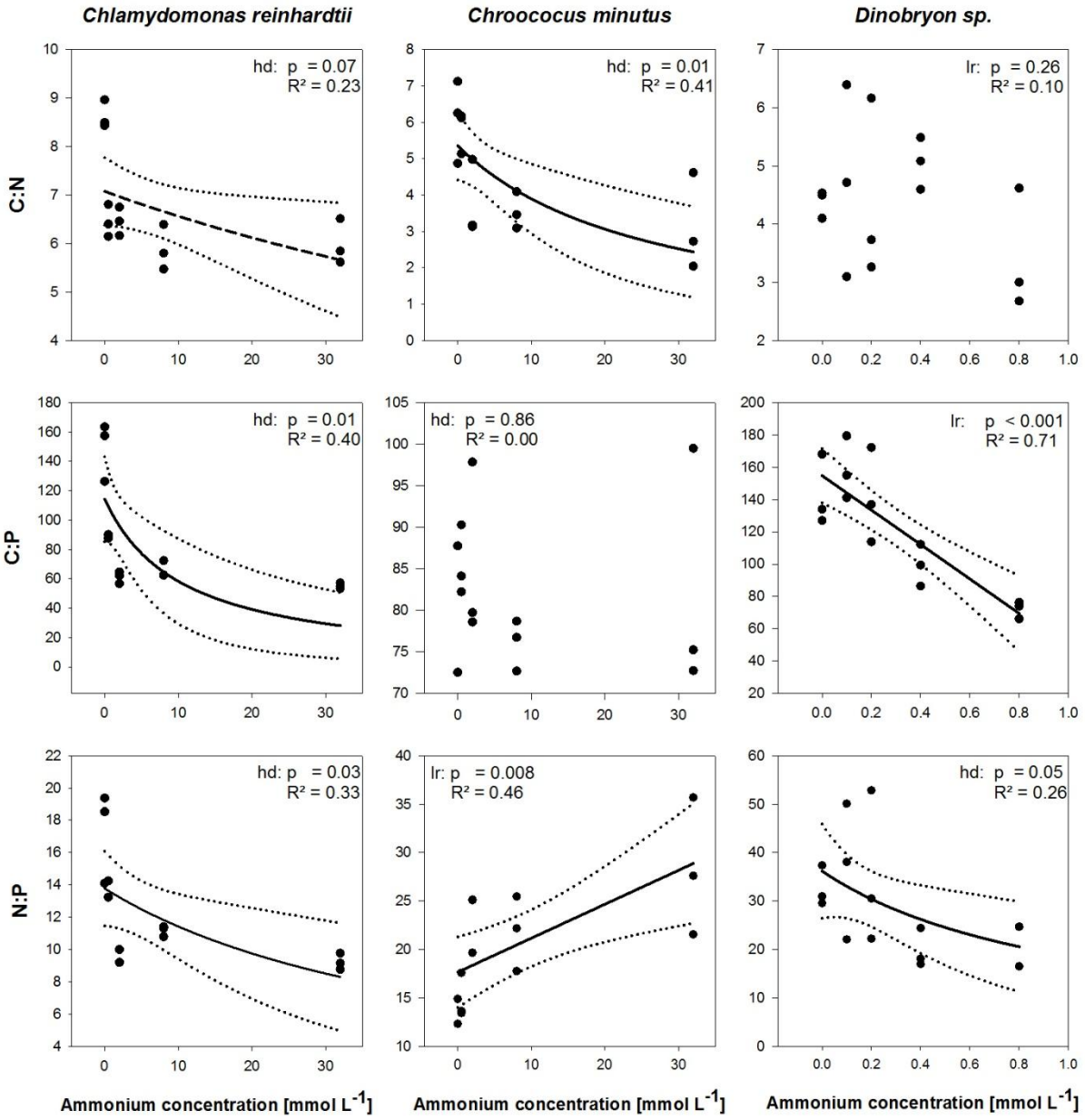

Figure 22 Biomass N:P ratios in response to ammonium concentrations. Regression lines are shown solid, when significant. Dotted lines represent the 95% confidence band.

2 Daphnia growth experiments

Growth rates of *Daphnia* fed with *C. reinhardtii* showed an exponential increase with increasing NH_4^+ concentrations of the cultivation medium in which algae were grown (ei: Figure 23a; R^2=0.34, p<0.001). Growth rates of *Daphnia* fed with *Dinobryon sp*. showed a linear increase with increasing NH_4^+ concentrations (lr: Figure 23c; R^2=0.11, p=0.026) and growth rates of *Daphnia* fed with *C. minutus* did not show a response (Figure 23b).

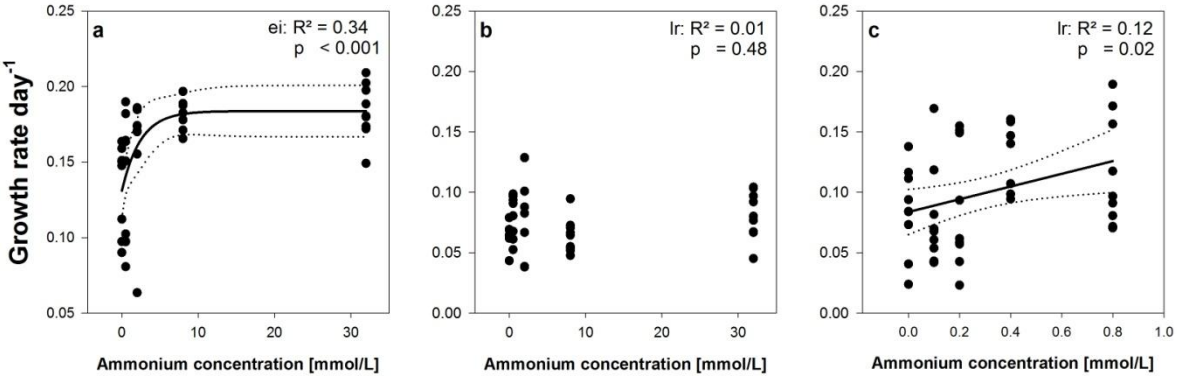

Figure 23 Response of *Daphnia* growth rates fed with the algae from the growth experiments with different ammonium concentrations. Regression lines are shown solid and dotted lines represent the 95% confidence band.

4

Discussion

Environmental nitrogen loads were steadily increasing in the past decades and are expected to do so in future. Thus, also atmospheric nitrogen deposition on aquatic ecosystems is increasing. Therefore I investigated effects of both increasing nitrogen loads and changing nitrate to ammonium ratios (within reasonable future wet deposition range) on natural plankton communities of phosphorus deficient lakes (Lake Brunnensee, Lake Klostersee and lake Thalersee). Additionally I performed an experiment in which I investigated different ammonium loads in their effects on assembled laboratory food chains.

Two answer my first two research questions I analysed the effects of increasing atmospheric nitrogen deposition, with constant supply ratios of the two major atmospheric reactive nitrogen (N_r) compounds nitrate (NO_3^-) and ammonium (NH_4^+), on natural plankton communities. The applied nitrogen gradient ranged from 0 to 32 times the natural wet deposition. In contrast to previous studies (Donald et al. 2013) the nitrogen enrichment was rather moderate (each enclosure received approximately 17 µg N L^{-1} as highest weekly enrichment, compared to 6 mg L^{-1} in Donald et al. (2013)). The resulting nitrogen concentrations in the enclosures were within the range that can already be observed in Scandinavian lakes exposed to increased nitrogen deposition (Elser et al. 2009).

I What are possible effects of nitrogen enrichment (within recent and predicted future atmospheric wet deposition ranges) on phytoplankton biomass and species composition in a phosphorus deficient system?

I investigated the effects of increased nitrogen deposition on phytoplankton biomass, community composition and seston stoichiometry in an oligotrophic lake. The lake is characterised by high DIN:TP ratios, suggesting severe P-limitation. Several studies have shown that high N:P ratios are indicators of P-limitation in plankton communities, with DIN:TP values being one of the best predictors for nutrient-limitation (Morris and Lewis Jr 1988; Bergström 2010). DIN:TP molar ratios of higher than 1.5 (as calculated from Bergström 2010) are considered to be indicators of P-limitation. Since I found an average molar ratio of DIN:TP of >1000:1 in my study lake, I expected the phytoplankton to be heavily phosphorus-limited. This is indicated by seston C:P and N:P molar ratios, which had average values of 232 ± 26 C:P and 37 ± 2 N:P at the beginning of the experiment.

For the three common phytoplankton biomass proxies, algal biovolume represents the most direct estimate for *in situ* biomass, while chlorophyll *a* represents the main

photosynthetic pigment within algae, and POC also includes other non-planktonic suspended particles < 250 µm. In my study, the chlorophyll *a* measurements showed a clear peak development, while POC and phytoplankton biovolume levels showed less pronounced patterns. Slight differences in the timing of the algal biomass peaks were found, depending on which biomass proxy was used (biovolume: between days 21 and 35, chlorophyll *a*: day 28 and 35 and POC: between days 14 and 35, Figure 4). Temporal differences between seasonal dynamics of in biovolume and chlorophyll *a* have been shown before in an oligotrophic lake over a 14 months monitoring project (Felip and Catalan 2000). In my study, temporal differences between the peaks of different proxies were independent of the applied nitrogen enrichment treatments.

Differences between the seasonal dynamics of chlorophyll *a* and POC among enclosures were also independent of nitrogen enrichment. However, differences in the timing of the phytoplankton biovolume peak were nitrogen dependent, with higher nitrogen enrichment resulting in earlier phytoplankton biovolume peaks. It is likely that this was due to bottom up effects, as phytoplankton growth rates were positively linked to nitrogen fertilization. This was mainly due to the positive response of primarily mixotroph chrysophytes and dinoflagellates growth rates to nitrogen enrichment. It is possible that N enrichment resulted in an increase of bacterial prey promoting the growth of mixotrophs. In support of this, algal species not combining auto- and phagotrophy, such as chlorophytes and bacillariophyceae, did not show a positive response in their initial growth rates to N enrichment.

Additionally, there was no direct relationship between the timing of the biovolume peak and mesozooplankton densities which would otherwise explain the observed time shift by grazing. Nevertheless, ciliate abundances showed - simliar to chrysophytes - a positive relationship with increasing nitrogen enrichment during the phytoplankton growth phase. This was probably also due to higher availability of small prey (Berninger et al. 1993) such as bacteria promoted by nitrogen enrichment (Roberts et al. 2003).

While the timing of phytoplankton peaks in terms of biovolume showed a clear response to nitrogen enrichment, probably due to enhanced initial mixotroph growth rates, total algal biovolume during the growth phase (days 17-35) did not differ between treatments. This indicates that the planktonic community was indeed not nitrogen-limited as the additional nitrogen could not support higher biovolumes. However, the effects of nitrogen enrichment on algal biomass dynamics were detectable after the peak phase (days 38-66), when zooplankton started to grow to larger densities. The observed decrease of total algal biovolume with

increasing nitrogen supply in the descending phase (see Figure 6f) suggests that interactions with the zooplankton might have been affected, and that indirect effects of variable grazing patterns along the nitrogen enrichment gradient lead to changes in phytoplankton biovolume. Although I could not detect direct responses of mesozooplankton grazers on phytoplankton biovolume during the descending phase, top down effects are still possible as interactions are complex and not only defined by abundances.

Using the phytoplankton proxies POC and chlorophyll *a* instead of algal biovolume for the analysis, phytoplankton dynamics showed different patterns. I observed the highest values of chlorophyll *a* (growth phase) and of POC (both phases) at the highest nitrogen enrichment treatment (see Figure 6). The increasing POC values must not necessarily imply higher phytoplankton contribution. Besides photoautotrophic organisms, my POC measurements included all other seston particles in the range of approximately 0.7-250 µm, including heterotrophic organisms. The increase of POC with increasing nitrogen enrichment could therefore also be caused by increasing abundances of heterotrophic organisms, which is supported by higher ciliate and nanoflagellate abundances with increasing nitrogen enrichment during the initial growth phase.

A mesocosm experiment comparing two different ratios of N:Si showed a shift to higher abundances of heterotrophic species (including dinoflagellates, microflagellates and ciliates) with N – enrichment (high N:Si ratios) (Roberts et al. 2003). My N enrichment experiment also resulted in N:Si ratio shifts as Si concentrations were the same in all treatments. The observed increase in POC with increasing nitrogen enrichment may therefore partly result from increasing abundances of heterotrophic organisms, additionally indicating higher microbial food web activities.

Collecting data on the responses of the three biomass proxies of phytoplankton to the nitrogen enrichment also allowed us to examine their relationships along the nitrogen gradient. Nitrogen enrichment had a significant effect on the chlorophyll *a*:biovolume ratio, the chlorophyll *a*:POC ratio, as well as on the biovolume:POC ratio within algal communities in the descending phase.

This could either result from different algal communities developing along the nitrogen enrichment gradient, or from direct effects of nitrogen on chlorophyll *a* synthesis and carbon fixation. Obtaining lower phytoplankton biovolume:POC and chlorophyll *a*:POC ratios with

increasing nitrogen enrichment supports my findings of a larger contribution of heterotrophic organisms to seston POC <250 µm.

For chlorophyll *a*:biovolume ratios, the lowest values were observed for intermediate nitrogen enrichment and highest values for high nitrogen enrichment. In the descending phase, cryptophyceae and dinoflagellate biovolumes were highest at intermediate nitrogen enrichment (see Figure 9k and l). These two groups, representing 12-29 % and 18-33 % of the algae community in terms of biovolume, might have been the drivers of the response in the chlorophyll *a*:biovolume ratio. The relative chlorophyll *a* content of dinoflagellates is low compared to other algae groups, indicated for example by decreasing chlorophyll *a*:biovolume ratios with increasing dinoflagellate abundances (Felip and Catalan 2000).

The co-occurrence of the chlorophyll *a* peak and that of *D. divergens* (together with the absence of a total biovolume peak on that day) additionally stresses the importance of community structure on chlorophyll *a*:biovolume ratios. A temporal overlap of chlorophyll *a* with high abundances of *D. divergens* has been observed before (Fee 1976) and is probably due to comparable high chlorophyll *a* contents of *D. divergens*. It can be concluded that changes in chlorophyll *a*:biovolume ratios, as well as in biovolume:POC ratios, appear to be driven by changes in the community structure rather than by direct effects of nitrogen on the chlorophyll *a* synthesis or the photosynthetic carbon fixation.

Instead of large phytoplankton biomass changes, I rather expected to find changes in phytoplankton community composition and/or seston stoichiometry. Stoichiometric differences in terms of POC:nutrient ratios were detected during the phytoplankton growth phase (see Figure 5). Both, seston C:P and N:P ratios, went up with increasing nitrogen enrichment and drift further away from the classical Redfield ratio of 106:1 (C:P) and 16:1 (N:P). Within the growth phase, ratios increased from around 400 (C:P) and 50 (N:P) in the lowest nitrogen enrichment treatment, up to values around 600 (C:P) and 80 (N:P) in highest nitrogen enrichment treatments (see Figure 5b and c).

However, I did not observe any decrease in seston C:N ratios with nitrogen enrichment within the growth phase. The increase in C:P ratios indicates an increase of phosphorus-limitation of the phytoplankton with increasing nitrogen enrichment. The observed shifts in C:P ratios with nitrogen enrichment could have further effects on zooplankton growth. P limitation of zooplankton growth can already be expected in the low nitrogen treatments,

since seston C:P ratios above 300 have been frequently reported to result in a decrease of the growth of daphniids (Urabe and Watanabe 1992; DeMott et al. 2001).

During the initial growth phase, algal communities shifted towards a higher contribution of chrysophyceae at higher nitrogen enrichment levels. A large number of species of that group are known to be mixotrophs that are able to take up nutrients from particulate sources such as bacteria (Holen and Boraas 1995).

In my experiment, the main species within that group was *D. divergens*, which is known to be strongly mixotrophic (Jones 2000 and citations therein). Similar to Roberts et al. (2003), nitrogen enrichment could additionally have promoted bacterial growth in my experiment, as indicated by both increasing POC and increasing POC:biovolume ratios with increasing nitrogen enrichment. Therefore, increased food availability for mixotrophic species could have been a potential driver of the observed increasing chrysophyceae abundances.

A higher proportion of mixotrophic species within phytoplankton would have far reaching consequences for food web dynamics. Mixotroph algal species direct carbon and nutrient flows from bacteria towards phytoplankton, and as a consequence the total carbon budgets of pelagic systems would become more heterotrophic. Accordingly, the fuelling of pelagic carbon flows with allochthonous dissolved organic carbon (DOC) would become more important.

Additionally, chrysophytes are well known to be a lower quality food source for herbivores, thereby reducing food web transfer efficiencies (Katechakis et al. 2005; Taipale et al. 2013). Altogether, with higher abundances of mixotrophic chrysophyceae, ciliates and heterotrophic nanoflagellates, within the initial growth phase, I observed strong signs of increasing nitrogen enrichment boosting the microbial loop within a phosphorus limited system. Microbial food webs represent crucial components of aquatic food webs and play an important role in the carbon and nutrient turnover in aquatic systems (Sherr and Sherr 2002; Löder et al. 2011). Therefore, changes in the microbial food web, resulting from nitrogen enrichment may, on a long term perspective, have far reaching consequences for food web dynamics, even in strongly P limited systems.

II How variable are the responses of plankton to nitrogen addition in lakes with different availability of nutrients?

To analyse general trends in responses of phytoplankton and zooplankton to nitrogen enrichment, the results of mesocosm experiments in three different lakes were compared. The main aspect for the selection of experimental sites was in this case differences in the N:P ratio of lakes. In Lake Brunnensee typical dissolved N:P molar ratios are higher than 1000:1 during spring time; in Lake Thalersee N:P is around 500:1 and in Lake Klostersee N:P ratios are around 150:1 (values from regular lake monitoring). Thus, plankton of those lakes is adapted to a variety of nutritional backgrounds and consequently might react different to manipulations of nutrients. However, the molar DIN:TP ratios of all three lakes are indicating a phosphorus limitation of phytoplankton (Morris and Lewis Jr 1988; Bergström 2010).

Due to the above mentioned indication of P limitation the phytoplankton biomass was not expected to change with N fertilization. However I observed an increase of chlorophyll *a* with increasing fertilization in two of the lakes (namely lakes Brunnensee and Thalersee). However, the chlorophyll *a* content per cell can be variable (Paasche 1971; Levasseur et al. 1993; Poxleitner et al. 2016), and chlorophyll *a* per phytoplankton biomass does vary with the community composition (Felip and Catalan 2000; Schindler et al. 2008).

Besides the indirect responses of phytoplankton to herbivore grazing pressure (Sommer et al. 1986), the addition of NH_4^+ in my fertilization solution might have promoted the growth of certain algal taxa (Donald et al. 2013; Glibert et al. 2016) or the chlorophyll *a* content per cell (Collos and Harrison 2014). As the utilization of NH_4^+ and NO_3^- is highly species specific it is possible that my N enrichment with the two N sources NH_4^+ and NO_3^- has directly influenced certain taxa. NH_4^+ is the mainly preferred N form; however diatoms for example prefer NO_3^- (Dortch 1990; Levasseur 1993; Litchman et al. 2007). As described above the detailed analyses of phytoplankton in lake Brunnensee revealed an increase of chrysophytes and dinoflagellates which are typically mixotroph species.

It was not necessarily expected that under the already high environmental N:P conditions effects of N enrichment on the zooplankton would be measurable. However, I observed declines of zooplankton at high fertilization levels in all lakes, and changes which had characteristic patterns in each of the lakes. The qualitative relationships of N load and zooplankton were markedly lake-specific (linear or unimodal) and restricted to distinct zooplankton parameters (average or peak biomass).

Additionally, a negative correlation of the zooplankton biomass with dissolved N:P ratios was seen over the entire data set including all treatments from the three lakes. Despite the experimental variability, the observed relationship of N load and zooplankton also seems to occur across all three investigated lakes. This general relationship supports the notion that trophic transfer of increasing nitrogen load to the zooplankton community follows an overarching trend but does so via lake-specific mechanisms.

Mesozooplankton was assigned to the groups cladocera (filter feeders), nauplii (copepod first larval stages which feed non-selectively on microalgae) and copepods (copepodits and adult copepods feeding highly selectively). A negative effect of the N enrichment was primarily expected for cladocerans and nauplii, which have typically higher P requirements, but to a lesser degree for copepodits and adult copepods with typically higher N requirements (Sterner and Hessen 1994, Carillo et al. 2001). Indeed, a negative relationship between N load (and dissolved N:P ratios) and cladocerans existed over all of the lakes but was expressed in different magnitudes in lakes Klostersee and Thalersee. Strongest effects of N enrichment on total cladoceran dry weights were observed in the peak biomass of the mesotrophic Lake Klostersee (Table 5).

The generally supposed constraints of the N and P requirements of cladocerans compared to copepodids and adult copepods are also supported by the multivariate analysis. In the data set over all three lakes, the cladocerans clearly correlate to a low N:P ratio and the copepods to higher N:P conditions (Figure 14). Subsequently, with increasing N:P ratios, the cladocerans are expected to be the first zooplankton group to be negatively affected. This is indeed true for Lake Thalersee and Lake Klostersee, which were dominated by cladocerans.

On the contrary, Lake Brunnensee is dominated by copepodids and adult copepods with only few cladocerans, and nauplii were more sensitive to increased N load. There is evidence for commonalities between the cladocerans and the nauplii, which both have low N:P body stoichiometry (<12:1), indicating high P demands (Andersen and Hessen 1991; Carrillo et al. 2001; Sterner and Elser 2002; Meunier et al. 2015), and similar optimal food size spectra (Hansen et al. 1994). The seston N:P ratios in this study were >44:1 (Table 6) indicating non-favourable food conditions for filter feeders with low biomass N:P ratios. Therefore, by increasing the P deficiency due to N enrichment, it is conceivable that this directly affects these two zooplankton groups, although it remains to be verified if the copepod egg production and/or the hatching success indirectly affect the nauplii abundances.

A possible link between nutrient supply and zooplankton densities would be seston (< 250 µm) stoichiometry (especially the N:P ratio), indicating food quality for zooplankton (Hessen et al. 2013). However, I did not observe any overarching responses to N enrichment in seston N:P ratios (increase in Brunnensee, decrease in Thalersee and no reaction in Klostersee). In lake Brunnensee the observed increase in seston C:P ratios together with increasing dissolved N:P ratios might explain declining zooplankton densities with high dissolved N.

However, previous investigations of other lakes also revealed that higher dissolved N:P resource supply ratios, together with an increased P deficiency, were not necessarily reflected in a significantly higher seston N:P stoichiometry (Elser et al. 2009b). Similarly, it has been shown that zooplankton nutrient recycling can diminish the seston stoichiometric signatures despite having very different initial nutrient supply ratios (Trommer et al. 2012). Therefore, the absence of clear responses of seston stoichiometry to an N enrichment within the lakes Klostersee and Thalersee, does not compulsorily mean that trophic food quality effects were not present.

The role of seston stoichiometry as an underlying ecological mechanism for the observed overarching zooplankton response in my study was supported by the increasing C:P ratios in Lake Brunnensee, together with the increase of dissolved N:P ratios over all of the lakes (Table 7, Figure 14a). The negative effects of seston C:P ratios > 300:1 (Urabe et al. 1997) and across a range of similar seston C: P ratios of natural lakes (Brett et al. 2000) have already been observed for the growth of the daphnid species.

Other than the seston stoichiometry, the food quality transfer to the higher trophic levels can also be related to other energetic pathways. It is for example known that the fatty acid composition of algae plays an essential role for food quality and can significantly influence the growth of *Daphnia sp.* (Müller-Navarra et al. 2000; Wacker and Elert 2001). There are indications that the abundance of fatty acids correlate with the relative P availability for algae to some extent, since some species grown in P-limited media produce less essential fatty acids than in P saturated media (Müller-Navarra 1995).

Additionally, *Daphnia* growth is seasonally stronger correlated to specific fatty acid concentrations than seston C:P ratios (Wacker and Elert 2001), which might also be related to physiological adaptations to high energy and low nutrient environments (Mulder and Bowden 2007). Higher metabolic costs from higher feeding activity (Plath and Boersma 2001), higher respiration rates (Darchambeau et al. 2003) or higher alkaline phosphatase activity (Elser et

al. 2010; McCarthy et al. 2010) could also have contributed to an energetic mismatch for cladocerans in the lakes Klostersee and Thalersee through an increasing P deficiency.

The immediate causes for unfavorable growth conditions for the zooplankton under a high N fertilization might also be related to the toxic effects of high N concentrations. However, the highest NO_3^- concentrations (5.7 mg L^{-1} NO_3-N in the Lake Brunnensee) did not reach critical values for the cladocerans (>14 mg L^{-1} NO_3-N, Camargo et al. 2005). In terms of an NH_4^+ toxicity, concentrations >0.6 mg L^{-1} ammonia nitrogen (NH_3-N) can result in chronic toxicity for *Daphnia magna* (Gersich and Hopkins 1986). These are higher than the NH_3-N concentrations in my experiments (<0.2 mg L^{-1} NH_3-N at 1.7 mg L^{-1} NH_4-N, pH 8, <20° C).

I could show that N enrichment can affect zooplankton dynamics (mainly cladocerancs) even in P deficient systems. The observed specific responses of the different taxa would imply that N enrichment could result in community composition changes, towards a more herbivorous copepod and rotifer dominated community, containing fewer cladocerans. Since the cladocerans typically represent the preferred food source for the planktivorous fish (Brooks 1968; Vanni et al. 1987), the progress of a phosphorus limitation in ecosystems (Vitousek et al. 2010; Goll et al. 2012) might cause negative effects on fish nutrition.

III Do different supply ratios of nitrate and ammonium influence phytoplankton community dynamics?

I could show in my experiments that an increase in Nr deposition, within realistic future deposition rates, can have an effect on phytoplankton and zooplankton communities even in phosphorus limited lakes. However, besides the amount of dissolved N available for primary production also the N source plays an important role for phytoplankton.

Predictions for future nitrogen deposition reveal that there will be a shift in the ratio of nitrogen compounds (Dentener et al. 2006; Lamarque et al. 2011; van Vuuren et al. 2011). It is assumed that the proportion of ammonium will increase (Lamarque et al. 2011). Such a change can have large influence on lake ecosystems as the utilization of NH_4^+ and NO_3^- is highly species specific. That was repeatedly shown in laboratory single species comparisons (e.g. Herndon 2007; Kok et al. 2015) as well as in nitrogen enrichment experiments with natural plankton communities (e.g. Domingues et al. 2011; Donald et al. 2013).

The majority of studies compare N sources as single additions in varying concentrations (Herndon 2007; Donald et al. 2013; Kok et al. 2015). In contrast to that, I applied the two N sources NH_4^+ and NO_3^- simultaneously and performed a mesocosm experiment in lake Brunnensee investigating both an increase of nitrogen deposition and a change of N source ratios.

The applied factorial experimental N design with two N fertilization steps and two NO_3^-:NH_4^+ ratios showed clear effects of the supplied NO_3^-:NH_4^+ ratios on the phytoplankton community. Most phytoplankton responses to NO_3^- and NH_4^+ supply have only been investigated in rather N limited systems (Bergström and Jansson 2006; Duce et al. 2008; Donald et al. 2013). Nevertheless, in Lake Brunnensee with dissolved molar N:P ratios of >1000:1 (spring mixing), significant higher phytoplankton biomass was observed in treatments with higher NH_4^+ fertilization, indicating advantageous growth conditions under higher NH_4^+ supply. This was clearly demonstrated by chlorophyll *a* measurements and total biovolume from microscopic counting both showing higher concentrations under higher NH_4^+ supply.

In terms of nitrogen budgets, removal of N from the dissolved phase was higher for NH_4^+ than for NO_3^-. NH_4^+ removal increased with increasing NH_4^+ supply (Figure 16) whereas NO_3^- removal from the dissolved phase decreased with increasing NH_4^+ supply. This removal does not only include the N uptake by algae but also potential uptake and conversion by bacteria. However, the differences in the proportion of nutrients removed from the dissolved phase were quite large (almost 80% for NH_4^+ compared to 3% for NO_3^-).

Thus, those results together with higher biomass in surplus NH_4^+ treatments point towards NH_4^+ as the preferable used N source for the algae community in my experiment. Similar effects were known from N limited systems, in which significantly higher phytoplankton biomass was achieved with NH_4^+ rather than NO_3^- fertilization in a P-rich lake (Donald et al. 2013) and decreasing NO_3^- consumption was observed with increasing NH_4^+ availability in an estuary (Domingues et al. 2011).

NH_4^+ supply may have positive effects on taxa like green algae and other flagellates but can additionally promote NO_3^- uptake inhibition for other dominant taxa like large diatoms as preferred NO_3^- users (Eppley et al. 1969b; Sommer 1993; Glibert 2016). My observed decrease in NO_3^- removal can indicate inhibition of NO_3^- uptake through NH_4^+. It is known

that NH_4^+ can influence and inhibit NO_3^- uptake depending on the species composition and nitrogen condition, beginning from various NH_4^+ concentrations of higher than 1-15 µmol L^{-1} (Dortch 1990).

In my data set, NH_4^+ concentrations of above 1 µmol L^{-1} were continuously present in all but the 1 time 4:1 NO_3^-:NH_4^+ ratio treatment, with highest average concentrations of 14 µmol L^{-1} in the 4 times 1:4 NO_3^-:NH_4^+ treatment. The observed decrease in NO_3^- removal and an increase in NH_4^+ removal with increasing NH_4^+ fertilization point towards NH_4^+ preference and/or NO_3^- uptake inhibition. It has been observed in laboratory studies, that NO_3^- uptake of algae can be suppressed by NH_4^+ (Eppley et al. 1969a; Cochlan et al. 1991; Hii et al. 2011).

Overall, NH_4^+ seemed to be the preferred N source in the experiment and appeared to promote biomass growth. However, if absolute NH_4^+ availability would limit phytoplankton production in the investigated lake, highest phytoplankton biomass development could have been expected under highest NH_4^+ supply (4-times 1:4 NO_3^-:NH_4^+ ratio treatment, N fertilization per week: 6.4 mmol/enclosure or 2.9 µmol L^{-1}). However, increasing N fertilization to 4 fold amounts did not show any additional effects on phytoplankton biomass compared to the 1 times enrichment treatments in either of the NO_3^-:NH_4^+ supply ratios of this study, pointing towards P limitation of the system. However, it seems that the maximum reachable biomass was higher at high NH_4^+ supply than at high NO_3^- supply. Phytoplankton resource use efficiency ($RUE_{Chl\ a}$) was higher when more NH_4^+ was available compared to NO_3^- (at otherwise identical resource conditions) which suggests that the ratio between the two N sources can affect how efficient the available P is transformed into new biomass.

Results of particle size distributions indicate that especially smaller sized algae were positively affected by increasing NH_4^+ availability. Particles of the size class between 2.5 and 5 µm ESD contributed in general in highest proportions of all investigated size classes to total biovolume. They increased significantly in the treatments with surplus NH_4^+ (1:4 NO_3^-:NH_4^+ ratio), whereas no effect of the absolute N amount was observed.

Attributed to the advantageous high surface area to volume ratio, it is known that smaller phytoplankton has higher specific uptake and growth rates under nutrient scarcity, which was shown for P (Smith and Kalff 1982), and for NO_3^- and NH_4^+ (Eppley et al. 1969b; Dortch 1990; Hein et al. 1995). On the contrary, it was suggested that larger algae tend to have an advantage under fluctuating nutrient supply (Stolte and Riegman 1995; 1996) and high

nutrient and light availability (Parsons and Takahashi 1973; Litchman et al. 2007). My results support previous studies that smaller algae have higher competitive abilities under high dissolved N:P ratios compared to large ones (Edwards et al. 2011) and are in accordance to predictions that a shift to higher availability of NH_4^+ in comparison to NO_3^- lead to a shift towards small algae species (Glibert et al. 2016). Due to their higher rates of nutrient uptake and growth under low P (Smith and Kalff 1982) and low NH_4^+ concentrations (Hein et al. 1995), such as observed in the investigated lake, even small NH_4^+ supply rates seem sufficient to promote growth of small algae. It was postulated that N deficiency would increase the preference for NH_4^+ as N source (Dortch 1990) but my data show that this seems also to account for N excess and P deficient environments.

The spectral group of diatoms and dinoflagellates (determined by flourometric measurements) showed higher chlorophyll *a* values under higher NH_4^+ supply (1:4 NO_3^-:NH_4^+ ratio). However this effect was not present in biovolume data as neither diatoms nor dinoflagellates showed a response to changing N supply. Thus the higher NH_4^+ supply seems to have promoted chlorophyll *a* built up rather than biovolume built up in the case of diatoms and dinoflagellates.

The main abundant diatom species in my experiment was *Cyclotella sp.*. Representing 87±1.4% of the diatom biomass (average over all microscopically analysed samples), this species is mainly driving the observed total diatom responses. Laboratory studies on another small centric diatom species (*Thalassiosira pseudonana*) observed higher chlorophyll *a* levels for the algae grown only on NH_4^+ than only on NO_3^- (Levasseur et al. 1993).

Additionally, experiments in marine batch culture experiments with natural phytoplankton communities showed that chlorophyll *a* increased at higher rates than cell numbers under NH_4^+ supply (Eppley et al. 1971). These results are similar to findings in my experiment, in which stronger effects of NH_4^+ supply on chlorophyll *a* than on biovolume were observed (comparison of Table 11 and 12) as well as higher chlorophyll *a* production per unit carbon in the high NH_4^+ supply treatments. It seems that NH_4^+ has a direct positive effect on chlorophyll *a* production and that the N-rich molecule of chlorophyll *a* profits from NH_4^+ being the energetically more efficient N source (Wetzel et al. 1991).

In the case of green algae the microscopic results of higher green algae biovolumes with high NH_4^+ availability (1:4 NO_3^-:NH_4^+ ratio) coincide largely with data from spectral algae

groups, indicating that green algae showed higher chlorophyll *a* values under higher NH_4^+ supply (Table 11 and 12). These results about green algae are in principal agreement with earlier studies showing that green algae or flagellated species have higher uptake affinities and growth rates with NH_4^+ as a N source (Dortch 1990; Raven et al. 1992; Litchman et al. 2007) and thus profiting from high NH_4^+ supply (Domingues et al. 2011).

Within a seasonal succession in Lake Plußsee, Sommer (1993) found also significant correlations of phytoplankton species with dissolved NO_3^-:NH_4^+ ratios in a P-limited system. Comparable to my experimental results, Sommer observed a positive correlation of the abundance of three green algae species with decreasing NO_3^-:NH_4^+ ratios. However, in observational studies it is hard to clearly link observed changes to a single factor as at the same time a variety of factors differ between samplings. Thus, my experimental manipulation of ratios between N sources with otherwise unchanged conditions support observed responses of phytoplankton species to changing NO_3^-:NH_4^+ ratios in a more causal manner.

The main abundant alga in my experiment was the green algae flagellate *Chlamydomonas sp.*. Representing on average 67% of the algae biomass its response to my N enrichment (higher biovolumes at the surplus NH_4^+ treatments) can be expected to drive the overall trend. As most flagellates (Raven 1997) *Chlamydomonas sp.* is able to take up P from particulate sources to meet high P demands for motility. Higher abundances of *Chlamydomonas sp.* in the 1:4 NO_3^-:NH_4^+ ratios can thus partly explain the better $RUE_{Chl\ a}$ at those treatments. In a previous experiment I already observed increasing abundances of mixotroph species with increasing N concentrations (given as increasing NO_3^- and NH_4^+ load in same ratios) suggesting interactions between N fertilization and bacteria consumption (Poxleitner et al. 2016).

Observing higher biovolume of *Chlamydomonas sp.* in surplus NH_4^+ treatments (1:4 NO_3^-:NH_4^+ ratio) indicates that the energetically less costly NH_4^+ uptake (Eppley et al. 1969b; Falkowski 1975) leads to flagellated species being potentially able to compensate the high energy demands of motility and make better use of their nutritional advantage (compared to autotroph species) to take up PP. However, motility is energetically very costly and thus requires high P loads for ATP. The nutritionally advantage of taking up particulate P is thereby lowered by requirements for motility. As NH_4^+ uptake is energetically less costly than NO_3^- uptake (Eppley et al. 1969b; Falkowski 1975) less energy (and thus P) is needed for N uptake

in surplus NH_4^+ treatments (1:4 NO_3^-:NH_4^+ ratio). Consequently *Chlamydomonas sp.* can probably use a larger proportion of the available P for growth in those treatments.

My data suggest that already small changes in the ratio of supplied NH_4^+ to NO_3^- may lead to detectable changes in biomass, community composition and chlorophyll *a* content of phytoplankton in P limited lakes. With regard to future N deposition scenarios, even a restriction of N production and emission amounts would possibly entail potential consequences for lake dynamics if the deposited N form would shift towards relatively higher NH_4^+ deposition. The potential shift to higher NH_4^+ ratios possible leads to a better resource use efficiency (RUE) of phytoplankton and a shift towards higher proportions of small green algae and especially flagellated, mixotroph species (as indicated by an ammonium induced increase of *Chlamydomonas sp.* in my experiment).

In contrast a decrease of diatoms can be expected due to their preference of NO_3^- (Dortch 1990; Levasseur 1993; Litchman et al. 2007). Such community changes at the base of the food web can have large influence on higher trophic levels due to feeding preferences of zooplankton. Therefore, changes in the supply ratio of N sources can lead to changes in aquatic food webs even in ecosystems were N is not per se a limiting factor. Consequently N management as part of conservation strategies ensuring sustainable ecosystem functioning and services should not only be restricted to often N limited terrestrial ecosystems but also should include largely P limited freshwater lakes.

IV How does ammonium affect algae from different taxonomic groups and are potential effects transfered to higher trophic levels?

I investigated the effects of increasing NH_4^+ fertilization on three common freshwater algal species and analysed bottom up effects via nutritional quality for *Daphnia*. In my experiment, the chrysophyceae species *Dinobryon* sp. was the least tolerant to ammonium. I detected a reduction of growth of almost 70% at the maximum ammonium concentration of 0.8 mmol L^{-1} compared to the control. Especially, for the crysophyte species *Dinobryon* sp., NH_4^+ is a chronic toxicant, which can be clearly shown by its influence on growth which is more and more pronounced with time (increasing differences between chlorophyll *a* concentrations of the treatments).

However, the other two species in my experiment, *C. minutus* and *C. reinhardtii*, were more tolerant and still grew at about 20 times higher ammonium concentrations. Even at the highest concentration of NH_4^+ I observed differences between those two algae species. At this high concentration, the cyanobacterial species *C. minutus* tolerated ammonium better (only 30% growth reduction) compared to the green algae *C. reinhardtii* which showed a growth reduction of 64% compared to controls. These varying responses of different algae species to NH_4^+ enrichment can lead to community composition changes in aquatic ecosystems by reducing or even excluding sensitive algae species while others are still able to grow.

As ammonium is not only a toxin but also serves as a resource for algal growth (Britto and Kronzucker 2002; Dugdale et al. 2012; Glibert 2016), I analysed the removal of ammonium from the dissolved phase in relation to the increased ammonium supply. However, the removal of dissolved ammonium includes not only uptake by algae, but as well that of bacteria (non axenic conditions) and other chemical processes in the water. Thus the measured ammonium removal is not solely due to algal growth. Though, due to the high algae densities in my experiments, algae were probably the main driver of the observed NH_4^+ dynamics. Despite the observed, negative toxic effects of NH_4^+ on algae, the relative N removal (per chlorophyll *a*) increased with increased NH_4^+ input. An increase in nitrogen uptake with increasing NH_4^+ fertilization is also indicated by a simultaneous decrease in the algal C:N ratios, indicating an increase in the biomass N content per unit carbon.

Despite lower algae concentrations (indicated by both decreasing POC and chlorophyll *a*) with increasing NH_4^+ supply the total PP concentrations in cultures has not changed for the algae species *Dinobryon* sp. and *C. reinhardtii*. Thus, more P was accumulated per single algae and consequently the overall N:P and C:P ratios decreased with increasing NH_4^+ supply concentrations due to the decrease of total PN and POC (caused by reduced algae concentrations). This effect of increasing NH_4^+ concentrations becomes especially important as the relative P content is a major aspect determining food quality for zooplankton (Urabe and Watanabe 1992; DeMott et al. 2001; Elser et al. 2001). This finding of increased relative biomass P concentrations with increasing dissolved NH_4^+ concentrations strengthens the importance of separating indirect effects of increased NH_4^+ on zooplankton (via nutrition) from direct toxic effects.

Direct toxic effects of NH_4^+ have been experimentally analyzed before in several *Daphnia* species (Gerisch et al. 1986; Xiang et al. 2010; Yang et al. 2012; Lyu et al. 2013b). For example, effects are the suppression of the citric acid cycle or the reduction of internal Na^+

(Camargo and Alonso 2006). However, observed changes in live history traits, like a decrease of size at different developmental stages or a reduced number of offspring with increasing NH_4^+ supply (Yang et al. 2012; Lyu et al. 2013b) could also be connected to changes in the consumed algae due to the increased NH_4^+ concentration.

Experiments that analyse the effects of increased NH_4^+ concentrations on plankton , as those mentioned above, are performed by adding the NH_4^+ into an assembly of primary producers and consumers. Thus, indirect bottom up driven effects due to changing food quality cannot be separated from direct effects of the NH_4^+ on consumers or from effects on algae quantity.

Besides the direct effect of decreased food quantity on zooplankton due to underfeeding, there are also interacting effects of food concentrations and reaction of *Daphnia* to NH_4^+ (Mangas-Ramirez et al. 2001). For the species *Daphnia pulex* Mangas-Ramirez and colleges (2001) showed that lower supplied food concentrations lead to higher susceptibility to increasing NH_4^+ concentrations (measured as decreasing population size). This finding again highlights the complexity of interactions in food webs when confronted with toxins and the importance to uncouple effects of NH_4^+ on different trophic levels and analyze them isolated.

To the best of my knowledge my experiment was the first one to separate bottom up effects via food quality from other aspects. I excluded direct toxic effects of NH_4^+ on *Daphnia* by removing the algae medium before feeding *Daphnia* with the cultivated algae. Thus, the varying NH_4^+ concentrations in which algae were grown were removed and only indirect bottom up effects on *Daphnia* growth remained.

Additionally, quantitative effects of NH_4^+ on algae (in high NH_4^+ concentrations most probably lower algae concentrations) were excluded by feeding defined amounts of algae. Thereby both decreased food availability and interacting effects with NH_4^+ toxicity (Mangas-Ramirez et al. 2001) were excluded. Thus, responses of *Daphnia* growth rates in my experiment can be linked to consequences of increasing NH_4^+ concentrations on qualitative food aspects of the cultivated algae species.

Daphnia growth rates with *C. minutu*s as food source were quite low in all treatments as cyanobacteria have generally low nutritional quality for *Daphnia* (DeMott and Müller-Navarra 1997; von Elert et al. 2003; Martin-Creuzburg et al. 2008). Consequently, changes in *C. minutus* stoichiometry with increasing NH_4^+ concentrations did not affect *Daphnia* growth.

As other factors were excluded in my experiment the observed increase of growth rates with increasing NH_4^+ concentrations in *Daphnia* fed with *C. reinhardtii* and *Dinobryon sp*. can be linked to better nutritional quality of the respective algae. One important qualitative aspect of algae as *Daphnia* food is the relative phosphorus content as *Daphnia* have high P requirements to their food (Sommer 1992; Sterner and Hessen 1994; Boersma 2000). As I could show, the relative P content of algae (compared to C and N) increased with increasing NH_4^+ concentrations in algae medium. Thus, it is probably the qualitative effect of increasing NH_4^+ concentrations in algae that had a positive effect on *Daphnia* growth.

These qualitative positive effects can counteract in natural conditions with potential negative effects of NH_4^+ on phytoplankton abundance (quantitative effects). Additionally, they counteract with the negative direct effects of NH_4^+ on *Daphnia*. While previous studies showed a decrease in *Daphnia* body size as a direct consequence of increasing NH_4^+ concentration (Yang et al. 2012; Lyu et al. 2013a; Lyu et al. 2013b) my results show that indirect bottom up driven effects can lead to an increase in *Daphnia* body size.

The observed, varying changes of algae growth due to increasing NH_4^+ concentrations in different algae species can potentially lead to changes in phytoplankton communities in ecosystems. Additionally, besides direct toxic effects of NH_4^+ on zooplankton, bottom up driven effects are possible due to the NH_4^+ induced changes in phytoplankton. First, expected changes in community composition can change food composition for zooplankton (possible leading to higher proportions of low food quality cyanobacteria). Second, observed changes (reductions) in biovolume affect the food quantity for zooplankton (lower growth rates in all observed species). Third, observed changes in algae stoichiometry change the food quality for zooplankton (higher nutritional value due to higher P content per cell/carbon).

Thus, effects of elevated NH_4^+ concentrations on algae can be transported across trophic levels and seem to have both positive and negative aspects. As I could show for the effects of changing food quality on *Daphnia* growth rates it is important to reduce the complexity of the analysed system to understand interacting effects of elevated NH_4^+ concentrations. Analysing single effects of NH_4^+ under highly reduced complexity can help to understand the outcome of complex interactions.

Conclusion

So far, studies of increased atmospheric nitrogen addition were mainly focused on nitrogen limited systems (Paerl 1997; Bergström et al. 2006; Meunier et al. 2015). However, summarizing the results of my mesocosm experiments, I could show that future changes in atmospheric nitrogen deposition within a reasonable range can influence even systems that are primary phosphorus limited.

In two subsequent years I found influences of experimentally modified nitrogen loads on phytoplankton communities of an oligotrophic lake. I revealed the importance of mixotroph species for observed effects of increasing N loads and changes in N source ratios. In both years the main drivers of increasing chlorophyll *a* levels (either with increasing N or in surplus ammonium treatments) were mixotroph species. The increasing abundance of those species seems to result from a combination of the nutritional advantage of mixotroph algae being able to take up phosphorus not only from dissolved but also from particulate sources and the changes in nitrogen source availability and energetic differences in uptake of either ammonium or nitrate. Altogether my results point towards ammonium being the main driver of the increasing abundance of mixotroph species. As ammonium uptake is energetically less costly than nitrate uptake (Eppley et al. 1969b; Falkowski 1975) less energy (and thus phosphorus) is needed for N uptake when sufficient ammonium is present. Consequently algae can probably use a larger proportion of the available phosphorus for growth in those treatments.

Thus an important effect of changing atmospheric nitrogen input is the boosting of the microbial loop. Microbial food webs represent crucial components of aquatic food webs and play an important role in the carbon and nutrient turnover in aquatic systems (Sherr and Sherr 2002; Löder et al. 2011). Therefore, changes in the microbial food web, resulting from either nitrogen enrichment or changes in nitrogen source ratios may, on a long term perspective, have far reaching consequences for food web dynamics; even in primary phosphorus limited systems.

Beside changes in community composition the modification of nitrogen loads can also cause changes in seston stoichiometry. An enrichment with nitrogen resulted in a decrease of relative phosphorus content. Thus, in the nitrogen enrichment experiments the consequence was a decrease of zooplankton species with high phosphorus requirements like *Daphnia* (Sommer 1992; Sterner and Hessen 1994; Boersma 2000). Zooplankton species with lower

phosphorus demands, like herbivorous copepods (Sterner and Hessen 1994, Elser et al. 1996) thus have a competition advantage and a shift in zooplankton composition could be expected.

In contrast to that the laboratory experiment with single algae species revealed an increase of the relative biomass phosphorus content of mixotroph algae with increasing ammonium availability. Consequently *Daphnia* indirectly profited from increased ammonium addition through the better nutritional quality of their food represented solely by a mixotroph algae. The predicted shift to a higher proportion of ammonium in atmospheric N deposition (Ciais et al. 2013; Glibert et al. 2016) might on longer term lead to higher abundances of mixotroph algae. Consequently future phytoplankton stoichiometry will be influenced by P-rich mixotroph algae and the C:P ratio of phytoplankton biomass could thereby also increase.

Altogether I could show effects of changing nitrogen availability on phytoplankton stoichiometry and community composition of both phytoplankton and zooplankton of lakes as well as on artificially assembled plankton communities in laboratory experiments. However, interactions between the individual components of lake ecosystems are complex and thus clear predictions of the consequences of changing N loads on lakes are difficult to make. More knowledge is needed to understand the complexity of species specific reactions to nitrogen enrichment but also about the resulting direct and indirect, bottom up driven food web effects.

5

Outlook

Mixotrophy and boosting of the microbial food web

My microscopic analyses of phytoplankton revealed the importance of mixotrophic algae for responses to N enrichment in P limited lakes. This was reflected on the one hand by increasing abundances of *Dinobryon divergens* with increasing N amounts (Experiment 1) and on the other hand by increasing concentrations of *Chlamydomonas reinhardtii* with surplus ammonium (Experiment 3). Both species are able to take up nutrients from bacteria and other particulate sources. Thus, emphasis should be laid on mixotrophic species, especially as their importance for aquatic food webs was revealed in recent studies (Mitra et al. 2014, Unrein et al. 2014, Fischer et al. 2016). Due to the importance of mixotrophic species, measurements of bacteria and dissolved organic compounds like dissolved organic carbon should be performed in future mesocosm experiments analysing natural phytoplankton communities.

However, it is also important to understand basic species specific interactions before analysing complex food webs. Thus, I recently performed first laboratory experiments in order to investigate bottom up driven effects on a single species scale. I compared a phototrophic and a mixotrophic algae under P limitation, both with and without bacteria addition. In this experiment I could show that the addition of a natural bacteria community increased the growth of the mixotrophic algae, but decreased the phototrophic algae growth rate. The decrease in growth of the phototrophic algae was probably due to competition for nutrients with the bacteria. I could show that a laboratory strain of *Dinobryon sp.* is able to make use of the supplied natural bacteria community. Based on this experiment, further experiments can be performed along an additional N gradient and comparing the growth of the two algae species in presence and absence of bacteria.

Furthermore, the response of bacterial growth to N enrichment without the presence of bacterial consumers should be analysed. Thus one can separate possible direct effects of increased N on bacteria from interacting effects like the recycling of nutrients in an algae-bacteria-loop.

Bottom up effects of high ammonium concentrations

Direct toxicity of NH_4^+ on *Daphnia* was shown to affect the body size and different parameters of fecundity of *Daphnia* (Yang et al. 2012, Lyu et al. 2013b). I could show a

strong effect of NH_4^+ on the body size of *Daphnia* mediated by effects of algal quality (Experiment 4). One can expect also such indirect effects of NH_4^+ on *Daphnia* fecundity. Thus, long term life history experiments with *Daphnia*, continuously fed with algae, grown in a gradient of NH_4^+ concentrations, should be performed to analyse further indirect consequences.

I expect the altered biomass N:P ratio of algae, (due to the experimentally increased NH_4^+ concentration in the growth medium), to be responsible for the growth effects of NH_4^+ on *Daphnia*. Thus it would also be interesting to analyse the response of zooplankton species with low P requirements such as copepods to NH_4^+ related changes in food quality.

Nitrogen management

Probably due to the general assumption that lake ecosystems are mainly P limited and terrestrial ecosystems mainly N limited research about effects of increasing N loads was so far focused on terrestrial ecosystems. Also governmental actions regarding N loads (such as monitoring programs and estimations of critical loads) are mostly missing for lakes. The "German Federal Committee for Air Pollution" published 2012 critical N loads for different types of ecosystems and the potential consequences when critical loads are exceeded (read in: Bayerisches Landesamt für Umwelt "Bavarian Environment Agency", 2013). With my experiments I could clearly show that effects of increased N input can also be expected in lakes which are primary P limited.

My findings strengthen the importance of taking action in controlling N inputs by nitrogen management strategies. As N input is mainly a problem of non point pollution via the atmosphere or groundwater (in contrast to pointed P pollution e.g. from wastewater treatment plants) inputs are much harder to control. Thus, focus needs to be laid on reduction of non point emissions from sources like farming, industry or burning of fossil fuels. Nitrogen budgets quantifying the loss of nitrogen from all kind of applications using nitrogen as a fertilizer could be a first step towards a more efficient use and thereby reduction of nitrogen deposition. Additionally the development and improvement of filter systems for industry and vehicles should be promoted in order to reduce emissions of nitrogen.

6

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7 Personal notes

7.1 Curriculum vitae

Personal Data

Name	Monika Poxleitner
Date of birth	06.07.1987
Place of birth	Freyung

Education

since 10. 2012	**Ludwig-Maximilians-University Munich** Dissertation: "Dynamik und Auswirkung von Stickstoff auf Seeökosysteme" Supervisor: Prof. Dr. Herwig Stibor
10. 2010 – 08. 2012	**Ludwig-Maximilians- University Munich** Master of Science (in Evolution, Ecology and Systematics) Thesis: "Can a hybrid outcompete its parental species? - A study on a "super clone" of *Daphnia* hybrid" Supervisor: PD Dr. Justyna Wolinska
10.2007 – 08.2010	**Ludwig-Maximilians- University Munich** Bachelor of Science (in Biology) Thesis: "Adaption to cold in *Drosophila ananassae*" Supervisor: Prof. Dr. John Parsch
09. 1998 – 06.2007	**Gymnasium, Freyung**

Work experience

08.2016 – 12.2016	**Ludwig-Maximilians-University Munich** Research assistant at the Aquatic Ecology Group, LMU, Prof. Dr. Herwig Stibor
Since 05.2016	**Eurofins BioPharma Product Testing Munich GmbH** Assistant in the quality assurance section
12.2012 – 07.2013	**Ludwig-Maximilians-University Munich** Research assistant at the Aquatic Ecology Group, LMU, Prof. Dr. Herwig Stibor

04.2011 – 09.2011	**Ludwig-Maximilians-University Munich** Individual Research Training: „Quantitative analysis of *Daphnia longispina* in new freshwater habitats in and around Munich“ Supervisor: Dr. Justyna Wolinska
04.2011 – 08.2011	**Ludwig-Maximilians-University Munich** Tutor in “Statistic for biologists”, LMU, Prof. Dr. Dirk Metzler
10.2010 – 03.2011	**Ludwig-Maximilians-University Munich** Individual Research Training: „Visualizing sequence data to detect selection under various demographic scenarios“ Supervisor: Prof. Dr. Dirk Metzler

Prizes and grants

08.2016	Travel-grant for the participation at the SIL conference in Turin, Italy; provided by the "Società Metropolitana Acque Torino"
08.2013-07.2016	governmental scholarship; provided by Cusanuswerk
02.2015	Travel-grant for the participation at the ASLO conference in Granada, Spain; provided by Cusanuswerk
10.2012	“EES Young Researcher Price in Evolution, Ecology and Systematics“, for the best master thesis presentation

Additional skills

Languages	German – first language English - fluent French - basics
Computing	Microsoft Word, Excel, PowerPoint Analysis, ArcGIS, DinoCapture, Dynamics Solver, msms, Populus, R-project, SigmaPlot
Workshops	Multivariate statistics, 2015, LMU und Wassercluster Lunz EES-Summerschool Aquatic ecology, 2013, LMU Leading and Promoting Discussions, 2010, Sprachraum, LMU

7.2 Publications

7.2.1 Publications and manuscripts connected to my dissertation

Poxleitner, M., G. Trommer, and H. Stibor (2016). "Effects of increased atmospheric nitrogen load on phytoplankton in a phosphorus limited lake." *Freshwater Biology*, **61**, 1966-1980

> Dealing with research question 1: What are possible effects of nitrogen enrichment (within recent and predicted future atmospheric wet deposition ranges) on phytoplankton biomass and species composition in a phosphorus deficient system?

Trommer, G., **M. Poxleitner**, P. Lorenz, E. Bitzilekis, A. Gogaladze, S. Schultes, and H. Stibor "Altered food-web dynamics under increased atmospheric nitrogen deposition in primary phosphorus-limited lakes." *Aquatic Sciences*, in revision

> Dealing with research question 2: How variable are the responses of plankton to nitrogen addition in lakes with different availability of nutrients?

Trommer, G., **M. Poxleitner**, and H. Stibor "Lake phytoplankton communities in a changing nitrogen (nitrate : ammonium) world." Ready for submission to *Journal of Plankton Research*

> Dealing with research question 3: Do different supply ratios of ammonium and nitrate influence phytoplankton community dynamics?

Poxleitner, M., M. Stockenreiter, H. Stibor "The effect of varying ammonium concentrations on three common freshwater algae species and subsequent effects on food quality." Ready for submission to *Journal of Plankton Research*

> Dealing with research question 4: How does ammonium affect algae from different taxonomic groups and are potential effects transfered to higher trophic levels?

7.2.2 Additional publications and manuscripts

Peer reviewed

Griebel, J., S. Gießler, **M. Poxleitner**, A.Navas Faria, M. Yin, and J. Wolinska (2015). "Extreme environments facilitate hybrid superiority – The story of a successful *Daphnia galeata×longispina* hybrid clone." *PloS one* **10**.10: e0140275.

Non peer reviewed

Poxleitner, M., G. Trommer, H. and Stibor (2015). "Influences of increased nitrogen load on the phytoplankton community in a phosphorus limited lake." Proceedings of the DGL meeting 2014 (Magdeburg), Hardegsen 2015

7.3 Presentations

Talks

Poxleitner, M., G. Trommer, M. Stockenreiter, and H. Stibor (2016). "The advantage of mixotrophy under phosphorus limitation." SIL meeting, Turin, Italy

Poxleitner, M., G. Trommer, and H. Stibor (2015). "Effects of increased atmospheric nitrogen load on phytoplankton in a primary phosphorus limited lake." ASLO aquatic science meeting, Granada, Spain

Trommer, G., **M. Poxleitner**, P. Lorenz, and H. Stibor (2015). "Increased atmospheric nitrogen deposition causes biomass changes in plankton communities even in phosphorus limited lake ecosystems." ASLO aquatic science meeting, Granada, Spain

Poxleitner, M., G. Trommer, and H. Stibor (2015). "Effects of increased atmospheric nitrogen load on phytoplankton in a primary phosphorus limited lake." Fresh Blood for Fresh Water Meeting, Mondsee, Austria

Poxleitner, M., G. Trommer, and H. Stibor (2014). "Auswirkungen von atmosphärischem Stickstoffeintrag auf die Phytoplanktongemeinschaft eines Phosphor limitierten Sees." DGL meeting, Magdeburg, Germany

Poxleitner, M., J. Griebel, S. Gießler, A. Navas Faria, M. Yin, J. and Wolinska (2014). "Can a hybrid outcompete its parental species? - A study on a "super clone" (*Daphnia galeata×longispina* hybrid)." Volkswagen Foundation's symposium on "The Evolution Of German Evolutionary Biology", Hannover, Germany. **INVITED TALK**

Trommer, G., **M. Poxleitner**, and H. Stibor (2013). "Einfluss von erhöhter Stickstoffzufuhr auf Seeökosysteme – ein experimenteller Ansatz." Conference, German Limnological Society, Potsdam, Germany

Griebel, J., **M. Poxleitner**, S. Gießler, and J. Wolinska (2012). "Can a hybrid outcompete its parental species? - a competition experiment with a "super clone" (*Daphnia galeata×longispina* hybrid)." Conference, German Zoological Society, Konstanz, Germany

Poxleitner, M., J. Griebel, S. Gießler, A. Navas Faria, M. Yin, and J. Wolinska (2012). "Can a hybrid outcompete its parental species? - A study on a "super clone" (*Daphnia galeata×longispina* hybrid)." Conference, Evolution, Ecology and Systematics – Masterprogram, Munich, Germany

Posters

Poxleitner, M., G. Trommer, and H. Stibor (2014). "Effects of increased atmospheric nitrogen load on phytoplankton in a phosphorus limited lake." Meeting, Societas Internationalis Limnologiae Austria, Lunz, Austria

Trommer, G., **M. Poxleitner**, E. Bitzilekis, A. Gogaladze, P. Lorenz, and H. Stibor (2014). "Nitrogen deposition on phosphorus limited lakes: Effects of increasing phosphorus limitation." Meeting, Societas Internationalis Limnologiae Austria, Lunz, Austria

Poxleitner, M., J. Griebel, S. Gießler, and J. Wolinska (2012). "Can a hybrid outcompete its parental species? -a life-history experiment with a "super clone" *(Daphnia galeata* × *longispina* hybrid)." Conference, German Zoological Society, Konstanz, Germany

Poxleitner, M. and J. Wolinska (2011). "How much life is in Munich's quarry lakes?" Conferece, Evolution, Ecology and Systematics –Masterprogram, Munich, Germany

Grath, S., **M. Poxleitner, M**., and J. Parsch (2010). "Phenotypic analysis of cold tolerance in *Drosophila ananassae* populations." International Conference "Stress and Evolution", German Zoological Society, Münster, Germany

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9 Declaration

Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt ist.

München, den 16.2.2017 Monika Poxleitner

(Unterschrift)

Erklärung

Hiermit erkläre ich, *

☐ dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist.

☐ dass ich mich anderweitig einer Doktorprüfung ohne Erfolg **nicht** unterzogen habe.

☐ dass ich mich mit Erfolg der Doktorprüfung im Hauptfach .....

und in den Nebenfächern .....

bei der Fakultät für ..... der .....

(Hochschule/Universität)

unterzogen habe.

☐ dass ich ohne Erfolg versucht habe, eine Dissertation einzureichen oder mich der Doktorprüfung zu unterziehen.

München, den 16.2.2017 Monika Poxleitner

(Unterschrift)

*) Nichtzutreffendes streichen