Effects of inorganic nitrogen and organic carbon on pelagic food webs in boreal lakes

Anne Deininger
To my family,
especially A., M. and M. Deininger
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List of papers

This thesis is a summary of the following papers that are referred to in the text by their Roman numerals:

I. Faithfull, C., Deininger, A. & Bergström A.-K.
Food quantity and quality in unproductive clear water and humic lakes and consequences for pelagic mesozooplankton
*Manuscript*

II. Deininger, A., Faithfull, C., Karlsson J., Klaus, M. & Bergström A.-K.
Pelagic food web response to whole lake N fertilization
*Manuscript accepted in Limnology and Oceanography*

III. Deininger, A., Faithfull, C. & Bergström A.-K.
Phytoplankton response to whole lake inorganic N fertilization along a gradient in dissolved organic carbon
*Manuscript in Review*

IV. Deininger, A., Faithfull, C. & Bergström A.-K.
Nitrogen effects on aquatic food web efficiency in the pelagic zone of unproductive lakes along a gradient of dissolved organic carbon
*Manuscript in Review*
Author contributions

Paper I
CLF wrote the paper with contributions from AD, and AKB. AKB conceived the study. CLF, AKB and AD performed the fieldwork and CLF and AD performed the microscopic counting. CLF analyzed the data with contributions from AD.

Paper II
AD wrote the paper with contributions from all authors. AKB conceived the study with contributions from JK. AD and MK performed the field work. AD performed the stable isotope analysis, as well as other data analysis.

Paper III
AD wrote the paper with contributions from AKB and CLF. AKB conceived the study. AD performed the fieldwork. AD and CLF conducted the microscopic counting. AD analyzed the data.

Paper IV
AD wrote the paper with contributions from CLF and AKB. AKB conceived the study with contributions from CLF. AD performed the fieldwork. CLF and AKB conducted the mesocosm experiment in 2011, AD in 2013. AD and CLF conducted the microscopic counting. AD analyzed the data with contributions from CLF.

Authors: AD: Anne Deininger, AKB: Ann-Kristin Bergström, CLF: Carolyn L. Faithfull, JK: Jan Karlsson, MK: Marcus Klaus
Abbreviations and glossary

**Boreal ecosystems** — Ecosystems with a subarctic climate in the Northern Hemisphere, roughly between latitude 50° to 70° N

**Pelagic zone** — Open water column in a lake or seas, i.e. water that is neither close to the bottom nor near the shore

**Autochthonous carbon** — Internally produced organic carbon in lakes; i.e. produced via photosynthesis in the water column

**Allochthonous carbon** — Externally derived organic carbon in lakes; i.e. produced via photosynthesis in the terrestrial catchments surrounding lakes

**BP (or BP<sub>tot</sub>)** — Bacterial production; based on both *autochthonous* and *allochthonous* organic carbon

**BP<sub>allo</sub>** — Bacterial production based on only *allochthonous* organic carbon

**PP** — Primary production; organic carbon synthesized from inorganic compounds and light energy. In aquatic systems: e.g. via algae; in terrestrial systems: e.g. via plants

**PEM** — Pelagic energy mobilization; all energy mobilized by basal producers in the open water column (phytoplankton and bacteria, PP + BP<sub>allo</sub>)

**FWE** — Food web efficiency; zooplankton production per total basal pelagic energy mobilized (PEM)

**C** — Carbon

**N** — Nitrogen

**DIN** — Dissolved inorganic nitrogen: sum of NH<sub>4</sub>+-N, NO<sub>3</sub>–-N and NO<sub>2</sub>–-N

**DOC** — Dissolved organic carbon: operational classification of any organic molecules smaller than 0.45 µm

**Seston** — Particulate matter suspended in water bodies (e.g. lakes and seas). It includes both living organisms (such as plankton and nekton) and non-living matter (such as plant debris or suspended soil particles) smaller than 50 µm
Sammanfattning

Abstract

Anthropogenic activities are increasing inorganic nitrogen (N) loadings to lakes in the northern hemisphere. In many boreal lakes phytoplankton are N limited, therefore enhanced N input may affect the productivity of pelagic food webs. Simultaneously, global change causes increased inflows of terrestrial dissolved organic carbon (DOC) to boreal lakes. Between clear and humic lakes, whole lake primary and consumer production naturally differs. However, research is inconclusive as to what controls pelagic production in these lakes. Further, it is unclear how DOC affects the response of the pelagic food web to enhanced inorganic N availability. The overarching goal of this thesis was to study the effects of inorganic N and organic C for pelagic food webs in boreal lakes. In the thesis, I first identified the main drivers of pelagic production during summer in eight non-manipulated Swedish boreal lakes with naturally low or high DOC. Then I investigated how increased N availability affects the pelagic food chain, and how the response differs with DOC. Therefore, whole lake inorganic N fertilization experiments were conducted in six Swedish boreal lakes across a DOC gradient (low, medium, high) divided into three lake pairs (control, N enriched) with one reference and two impact years. In each lake, I also investigated the response of zooplankton growth using in situ mesocosm experiments excluding planktivores. I found that humic boreal lakes had lower phytoplankton production and biomass than clear water lakes. Further, phytoplankton community composition and food quality differed with DOC. However, high DOC did not reduce pelagic energy mobilization or zooplankton biomass, but promoted a higher dominance of cladoceran relative to copepod species. N addition clearly enhanced phytoplankton biomass and production in the experimental lakes. However, this stimulating N effect decreased with DOC as caused by light limitation. Further, the newly available phytoplankton energy derived from N addition was not efficiently transferred to zooplankton, which indicates a mismatch between producer energy supply and consumer energy use. Indeed, the mesocosm experiment revealed that decreased food quality of phytoplankton in response to N addition resulted in reduced food web performance, especially in clearer lakes. In humic lakes, zooplankton production and food web efficiency were clearly more resilient to N addition. In summary, my thesis suggests that any change in the landscape that enhances inorganic N availability will especially affect pelagic food webs in clear water lakes. In contrast, brownification will result in more lakes being resilient to eutrophication caused by enhanced N deposition.

Key words: autochthony, basal production, boreal, global change, dissolved organic carbon, food web efficiency, N deposition, phytoplankton, seston stoichiometry, whole lake enrichment, zooplankton
Background

The highest concentration of water bodies worldwide can be found at boreal and arctic latitudes (45 - 75° N), with a major contribution of small lakes (Downing et al. 2006; Verpoorter et al. 2014). Small lakes provide the largest land-lake interface globally. Changing natural balances in these lakes will therefore have large consequences for global aquatic elemental flux, biodiversity, and productivity (Verpoorter et al. 2014).

At present small unproductive boreal lakes are experiencing two drastic changes induced by humans: increased inorganic nitrogen (N) availability caused by N deposition, and increased input of terrestrial dissolved organic carbon (DOC) caused by global change (Rockström et al. 2009; Greaver et al. 2016). These changes should have large consequences for aquatic food webs and energy transfer in the open water column (pelagic zone) of small unproductive boreal lakes.

Nitrogen deposition

The worldwide increase of inorganic nitrogen (N) deposition mediated by fossil fuel combustion, fertilization, and forestry (Vitousek et al. 1997; Galloway et al. 2008; Kreutzweiser et al. 2008), has been intervening drastically with the Earths’ natural N cycle (Rockström et al. 2009). By altering natural nutrient balances, the anthropogenic release of inorganic N has far reaching consequences for aquatic ecosystems (Bergström and Jansson 2006; Elser et al. 2009a). Especially in unproductive lakes, increased inorganic N availability has been shown to play an important role for phytoplankton biomass development (Fig. 1) (Bergström and Jansson 2006). Hence, in areas with low N deposition, phytoplankton are naturally N limited, wherefore enhanced inorganic N availability may increase primary production (PP) and phytoplankton biomass (Bergström and Jansson 2006; Elser et al. 2009a).

However, there has been a paucity of whole lake ecosystem studies addressing the effects of elevated N supply in unproductive lakes in areas with low N deposition, and especially studies which include consumers (Schindler et al. 2008; Pace et al. 2015). At present, most whole lake ecosystem studies have addressed nutrient limitation in comparatively nutrient rich lakes, or in nutrient enriched lakes after whole lake N and P fertilization (Schindler 1977; Matthews et al. 2013), where N limited conditions have been shown to be alleviated either by N deposition (Bergström and Jansson 2006; Elser et al. 2009a) or N₂ fixing cyanobacteria (Schindler et al. 2008). Additionally, bioassay experiments with N and P demonstrating N limitation of phytoplankton in unproductive boreal lakes (Bergström et al. 2008; Elser et al. 2009b) have shortcomings for addressing
whole lake conditions due to their short time frames and exclusion of both atmosphere and benthic habitats. Consequently, whole lake experiments demonstrating the consequences of increased N availability for ecosystem scale productivity and resource use by consumer communities in unproductive lakes with low N deposition are clearly needed.

Fig. 1. Conceptual model illustrating the basal components of boreal pelagic food chains. 

DOC = dissolved organic carbon, DIC = dissolved inorganic carbon, 
DIN = dissolved inorganic nitrogen, P = phosphorus

Dissolved organic carbon and global change
Global change mediated by warming, increased precipitation and reduced atmospheric sulfate deposition is predicted to enhance terrestrial dissolved organic carbon (DOC) loadings to aquatic systems (Monteith et al. 2007; Finstad et al. 2016). This may have consequences for food web processes. DOC may constrain pelagic PP through shading and light absorption (Thrane et al. 2014; Seekell et al. 2015), but may also stimulate basal production by enhancing nutrient availability, as DOC serves as an important carrier of N and phosphorus (P) to lakes (Jansson et al. 2001; Lennon and Pfaff 2005; Jones et al. 2012). Therefore, increased terrestrial inputs of DOC to small unproductive boreal lakes might potentially have large effects on phytoplankton biomass, production, and community composition (Jones 1992; Klug 2002; Drakare et al. 2003). Further, increasing terrestrial carbon subsidies supplied by DOC might benefit potentially competitive organisms to phytoplankton such as bacteria, but also heterotrophs and mixotrophic phytoplankton, via grazing on bacteria (Fig. 1). Thus, due to different abiotic and biotic conditions created by varying lake DOC concentrations, natural
phytoplankton communities in boreal, unproductive lakes have been observed to typically shift from a dominance of non-flagellated autotrophs in clear water lakes, to flagellated autotrophs in humic lakes (Klug 2002; Drakare et al. 2003).

Despite the accumulating evidence that phytoplankton community composition and biomass production in boreal lakes are altered by increased inorganic N availability (Jansson et al. 2001; Bergström and Jansson 2006; Elser et al. 2009a), only one whole lake inorganic N enrichment experiment has been conducted in an N limited unproductive lake, and that is at high DOC concentration (Jansson et al. 2001). Whole lake ecosystem scale studies examining the net effects of increased N availability in lakes of different DOC concentrations on phytoplankton development and community composition are clearly absent. However, addressing net effects in whole ecosystem studies is essential in order to make realistic predictions about responses in phytoplankton development to future changes in N deposition and with brownification (Klug and Cottingham 2001; Greaver et al. 2016).

**Pelagic food webs in boreal lakes**

The above explained changes in phytoplankton production and biomass in unproductive ecosystems such as small boreal lakes are likely to directly affect consumers; especially since the growth of zooplankton consumers in these lakes is often resource limited (Persson et al. 2007). However, it is unclear how efficiently any additional PP following N addition will be transferred to zooplankton consumers, and whether differences in DOC concentration will affect potential consumer responses to increased N input. As zooplankton provide an important link between basal producers and higher consumers such as fish, these are crucial questions to address in order to understand the consequences of both increased N deposition and DOC input to the functioning of boreal lake ecosystems.

In small boreal unproductive lakes, the pelagic energy available for zooplankton is mobilized by both autotrophic and heterotrophic basal producers (Fig. 1) (Jones 1992; Jansson et al. 2007). The sum of PP and bacterial production based on terrestrial or allochthonous organic sources (BP_{allo}) can be used to classify pelagic energy mobilization (PEM), which in turn is a direct measure of the amount of energy mobilized and available to consumers in the pelagic zone (Jansson et al. 2003). PEM has been shown to be similar in N limited boreal clear water and humic lakes; however the contribution of BP_{allo} to PEM increases with increasing DOC concentrations, whereas the contribution of PP to PEM decreases due to DOC induced light limitation (Faithfull et al. 2015). The mobilized energy can reach zooplankton consumers via two pathways depending on its origin: The shorter, and more energy efficient pathway where zooplankton directly graze on phytoplankton and bacteria, and the longer, more inefficient bacteria-
bacterivorous pathway with additional intermediate trophic levels, due to the small size of bacteria (i.e. via phagotrophic microorganisms, Fig. 1) (Berglund et al. 2007). The ratio of PP to total bacterial production (BP$_{tot}$) can therefore be used as an indicator of food chain length with higher ratios indicating more efficient energy transfer via PP than lower ratios (Sommer and Sommer 2006; Jansson et al. 2007). As more BP$_{tot}$ (and BP$_{allo}$) relative to PP is promoted with increasing DOC, an increase in DOC promotes longer food chains (Tranvik 1988; Kritzberg et al. 2006; Berglund et al. 2007). Increased N input to N limited boreal lakes should therefore increase the relative contribution of PP to PEM, resulting not only in increased food quantity, but also a promotion of the shorter and more energy efficient pathway via PP.

In consequence, we might expect that the increased availability of phytoplankton (autochthonous) resources following elevated N input should have major effects on resource use by herbivore consumers in boreal lakes (i.e. zooplankton autochthony). However, the use of different resources (autochthonous versus allochthonous carbon) has been shown to be influenced by species specific feeding modes of crustacean zooplankton (Barnett et al. 2007; Berggren et al. 2014). The diet of unselective filter feeding cladocerans (Sommer and Sommer 2006; Barnett et al. 2007) typically reflects the relative availability of autochthonous versus allochthonous resources (Pace et al. 2004; Cole et al. 2011; Karlsson et al. 2012), suggesting that cladoceran autochthony might increase with enhanced PP:BP ratios. The diet of selective feeding copepods, on the other hand, differs between cyclopoids, which are specialized omnivores (Hopp et al. 1997), and calanoids, which feed more selectively on phytoplankton (Demott 1988; Tiselius and Jonsson 1990). Thus, it is not clear whether increased availability of phytoplankton food resources following higher inorganic N availability will increase autochthony in crustacean consumers in general, or whether there will be differences between taxa in accordance with their specific feeding modes.

Food web efficiency (FWE = Zooplankton production per PEM), denotes how efficiently energy is transferred from basal trophic levels (phytoplankton and bacteria) to zooplankton (Berglund et al. 2007). FWE depends both on the length of the food chain (the PP:BP ratio) and on how efficient energy is transferred between each trophic link. The latter can be affected by organism stoichiometry, i.e. changes in the stoichiometry of phytoplankton (i.e. N:P, carbon (C):P ratios). Phytoplankton stoichiometry is determined by both light and nutrient availability (Sterner et al. 1998; Elser et al. 2009a). Enhanced N availability in N limited lakes should therefore stimulate growth (C production) and promote phytoplankton of high C:P and N:P ratios resulting in poor quality food for consumers with high P demands (Sterner et al. 1998; Elser et al. 2009a). However, it is still not clear to what
extent reduced phytoplankton food quality (i.e. increased C:P and N:P) may counteract the stimulating effect of increased N availability on food quantity (PEM) and the promotion of shorter food chains (increased PP:BP) for zooplankton. Even more difficult to predict is how responses of transfer efficiencies to increased N availability will differ between lakes with different DOC concentrations, despite these being timely questions (Hessen 2013; Solomon et al. 2015; Greaver et al. 2016).

**Aims of this thesis**

This thesis consists of four papers (I–IV below) which focus on the effects of nitrate enrichment on basal producers (phytoplankton, bacteria), and the subsequent consequences for zooplankton consumers in pelagic food webs of boreal unproductive lakes with differing DOC concentrations. The aims of the four papers are as follows:

**Paper I.** To (A) compare the main factors controlling food quantity and quality in four clear water and four humic boreal unproductive lakes without N addition

To (B) relate how the identified factors controlling food quantity and quality under non-manipulated conditions influence zooplankton community composition and biomass

**Paper II.** To assess the effects of increased inorganic N availability on pelagic autochthonous resource supply and consumer resource use (degree of autochthony) in six unproductive boreal lakes with low N deposition

**Paper III.** To investigate how increased availability of inorganic N affects phytoplankton production, biomass and community composition in six boreal lakes with low N deposition along a gradient of DOC concentration

**Paper IV.** To examine the effects of increased inorganic N availability on pelagic energy mobilization and the consequences for zooplankton growth and food web efficiency in six boreal lakes with low N deposition along a gradient of DOC concentration
Materials and methods

Whole lake monitoring was conducted in four humic and four clear water boreal unproductive lakes (paper I). Whole lake fertilization experiments were conducted in six boreal unproductive lakes divided into three lake pairs (control, N enriched) across a DOC gradient (low, medium, high) (papers II, III, IV) (Fig. 2). Additional in situ mesocosm experiments excluding predators of zooplankton were conducted in all lakes being part of the whole lake fertilization experiment (paper IV) (Fig. 3). Lastly, to examine whether zooplankton community composition was associated with DOC concentrations at a wider scale we used lake data from the Swedish national lake monitoring program hosted by the Swedish Agricultural University (SLU) (www.miljodata.slu.se/mvm) (paper I). Since we were only interested in boreal N limited unproductive lakes in paper I, we selected lakes found north of 60° N from this dataset (Bergström et al. 2005; Sponseller et al. 2014).

Study area

The experimental lakes are located in boreal, northern Sweden (64.12 to 64.25° N, 18.76 to 18.80° E). N deposition in this area is low (wet dissolved inorganic N, i.e. DIN deposition < 200 kg km⁻² yr⁻¹) (Bergström et al. 2008), and previously conducted bioassay experiments with N, P and N+P addition have indicated strict N limitation of primary producers through the summer season (Bergström et al. 2008). Except for forestry, anthropogenic influences on the lakes are negligible.

All experimental lakes were chosen to be as similar as possible in climatic conditions, morphometry and catchment characteristics. However, lakes were chosen to differ in DOC concentrations, in order to represent the typical variety in DOC concentration found in unproductive lakes in the boreal landscape (Downing et al. 2006; Sobek et al. 2007). The lakes’ drainage areas consist of coniferous forests and open Sphagnum dominated mires; the lake’s littoral zones were small. The lakes covered a range in water retention times (WRT), with longer WRT (800 days) in clear lakes and shorter WRT (50 days) in humic (brown) lakes. The lakes are typically ice covered from early November to early May. Thermal stratification develops from mid to late May until mid to late September.

Whole lake experiments

In paper I, we studied eight lakes in the study area. These lakes were not manipulated, but naturally differed in DOC concentrations. The lakes consisted of two sets, clear and humic (brown) lakes, at a DOC concentration
of 15 mg L⁻¹, based on clear differences in phytoplankton community composition at this threshold. Sampling took place over a two year period from 2011 to 2012, with part of the lakes also used in the whole lake fertilization experiment as non-manipulated lakes (cf. below). All humic lakes had fish present, whereas half of the clear water lakes had fish present and half were fishless.

In papers II, III, and IV, six lakes were selected at three DOC levels (Low DOC ~7 mg L⁻¹, Medium DOC ~11 mg L⁻¹, High DOC ~20 mg L⁻¹), with one lake pair at each level (Fig. 2). For each DOC level, one lake served as a control lake (‘Control lakes’) and the other lake as the enrichment lake (‘N lakes’). The reference year was 2011 (Before; all lakes), and 2012 and 2013 were impact years (After, with N enrichment in 3 of 6 lakes). Fish communities were similar within each lake pair; the low DOC lakes were fishless, the medium DOC lakes had stunted perch populations, and the high DOC lakes had bimodally distributed perch populations.

Fig. 2. Location of experimental lakes that were part of the N enrichment experiment in Northern Sweden. Blue lines indicate lake areas, green and orange lines catchment areas in control, and N-Lakes (+N) along the DOC gradient (Low, Medium, High), respectively. (Source: Lantmäteriet 2012).

In order to investigate and quantify responses of basal producers (bacteria and phytoplankton), and zooplankton consumers under control conditions in lakes with differing DOC concentrations (paper I), and following whole-lake N fertilization (paper II, III, IV), samples for biological, but also physical, and chemical parameters were taken biweekly from the epilimnion.
at the deepest point of each lake from June to early September. For physical parameters we measured temperature, oxygen, and light intensity in situ (all papers). For chemical parameters we collected samples for analysis of dissolved organic and inorganic carbon, ammonium, nitrite+nitrate, total nitrogen and total phosphorus. Further, we measured chlorophyll-a, and determined elemental ratios (N:P, C:P) of edible seston (< 50µm) (all papers). For basal pelagic production, PP and BP were measured using the \(^{14}\text{C}\) (rate of C production via photosynthesis) and leucine (protein synthesis in bacteria) isotope methods, respectively (all papers). Biomass of phytoplankton (paper I, III) taxa was determined using the Utermöhl settling technique and standard counting methods. In paper III, phytoplankton were grouped into the functional groups: non-flagellated autotrophs, flagellated autotrophs, mixotrophic flagellates and heterotrophic flagellates as described in Jansson et al. (1996), with the exception for cryptophytes which were classified as autotrophic flagellates (Isaksson et al. 1999; Jansson et al. 2001). Further, for paper I, we calculated phytoplankton edibility according to Dickman et al. (2008) by assigning a value between 0–2 for each phytoplankton taxa, with higher values representing higher food quality. Cyanobacteria and all taxa with a greatest axial length dimension longer than 30 µm were considered poor quality food and assigned 0. Cryptophytes and diatoms were considered high quality food and were assigned 2. Chlorophytes and other groups were assigned intermediate values (i.e. 1.24 for chlorophytes). For investigation of zooplankton, biomass of crustacean taxa was quantified using the Utermöhl settling technique and standard counting methods (all papers). For paper II, we further determined zooplankton resource use of phytoplankton derived (autochthonous) resources. Here, we collected and measured stable isotope samples for water (explained in more depth below), terrestrial material (peat, spruce, pine soils; allochthonous organic matter), periphyton (autochthonous organic matter), and the three crustacean zooplankton groups Calanoids (Eudiaptomus sp.), Cyclopoids (Cyclops sp.), and Cladocerans (mainly Ceriodaphnia sp., Daphnia sp., Bosmina sp, Diaphanosoma brachyurum, Holopedium gibberum, and Sida sp.). For investigation of zooplankton production and food web efficiency (paper IV), we conducted in situ mesocosm experiments in each experimental lake before and after N fertilization to explicitly assess how inorganic N enrichment affects energy transfer to consumers excluding any predatory effects (see below: Mesocosm experiment).

N fertilization
Nitrogen in the form of dissolved potassium nitrate (14 M nitrogen as KNO\(_3\)) in 2012 and concentrated nitric acid (14 M nitrogen as HNO\(_3\)) in 2013 were added equally across the surface of the N fertilized lakes. Different sources of
N were used in 2012 and 2013 due to practical reasons, as HNO$_3$ turned out to be easier to dilute in lake water than KNO$_3$. Nitrate (NO$_3^-$) was used, as NO$_3^-$ leakage from the catchment is the most typical N form that enters boreal lakes originating from atmospheric N deposition and forest clear cutting (Moldan et al. 2006; Kreutzweiser et al. 2008) as ammonia (NH$_4^+$) is efficiently taken up by terrestrial vegetation (Molot and Dillon 1993; Hessen 2013). As leaching events typically follow high catchment runoff during winter and spring (Bergström et al. 2008), we tried to mimic the seasonal variation in external DIN loading by fertilizing the mixed water column once through evenly distributed holes drilled in the ice cover in both 2012 (late March), and 2013 (early April). Thereafter, nitrate was added at the onset of stratification in late May (2013) or early June (2012) until late August. Nitrate was added in order to increase the DIN in the whole lake (during ice off) or in the epilimnion by 100 µg N L$^{-1}$, to mimic DIN inputs for lakes in southwest Sweden with high N deposition (Bergström et al. 2008). The amount of fertilizer added to each N lake was estimated depending on the lake volume, stratification depth and the calculated water residence time. During stratification, fertilization occurred every second week in all lakes in 2012. In 2013, fertilization was performed every second week in the low and medium DOC N lakes, whereas in the high DOC N lake, fertilization occurred every week due to its shorter water residence time.

**Stable isotopes**

In paper II, we used stable hydrogen isotopes (i.e. deuterium; δ$^2$H) (Estep and Dabrowski 1980) to estimate use of phytoplankton derived (autochthonous) versus terrestrial derived (allochthonous) resources by crustacean zooplankton consumers. We calculated the proportion of autochthonous carbon to consumer biomass (i.e. zooplankton autochthony %) using an autochthonous-allochthonous δ$^2$H mixing model based on the δ$^2$H signal of zooplankton samples (δ$^2$H$_{\text{consumer}}$), the allochthonous source (δ$^2$H$_{\text{allo}}$), the autochthonous source (δ$^2$H$_{\text{auto}}$), and lastly water (δ$^2$H$_{\text{water}}$), in order to correct for dietary water contribution (Solomon et al. 2009). The autochthony of the total crustacean zooplankton community (i.e. community autochthony) was estimated by calculating the biomass weighted mean autochthony of the different zooplankton groups (Berggren et al. 2015).

For autochthony assessment, alternative isotopes (e.g. δ$^{13}$C) have been used in other lake studies (Cole et al. 2011). However, for boreal low productive lakes algebraic 2-source δ$^2$H mixing models have been shown to be as precise as advanced multi isotopes models in estimating autochthony (Berggren et al. 2014). Further, δ$^2$H signals of the investigated end members tend to be more different than δ$^{13}$C signals (Karlsson et al. 2012), wherefore δ$^2$H potentially gives better estimates of autochthony in our case (δ$^{13}$C: Terrestrial C = -26 to -28 ‰; Phyto C = -35 to -45 ‰; δ$^2$H: Terrestrial C = -
134 to -152‰; Phyto C = -218 to -233‰). Therefore, autochthony assessment was based on the simpler δ²H mixing model in our study.

**Mesocosm experiment**

In order to measure zooplankton growth rates and food web efficiency independent of fish predation (paper IV), we constructed mesh net cubic mesocosms (1 m³) which allowed a natural flow of water into and out of the mesocosms while keeping fish and invertebrate predators out (Fig. 3).

Three mesocosms were deployed on the surface of each lake for one summer month both in the reference year (2011), and one impact year (2013). Crustacean mesozooplankton were added to each mesocosm at ambient lake densities, after removing zooplankton predators. Simultaneously, we measured background physical and chemical conditions, as well as pelagic energy mobilized by primary producers, and bacteria, phytoplankton biomass, seston stoichiometry, zooplankton biomass, and community composition (see above). Zooplankton production for each major crustacean taxon (6 major cladocera taxa, calanoids, cyclopoids) was calculated by using changes in biomass of adults, egg counts per female and the density of different development stages (i.e. copepod nauplii and copepodites) over time, and correcting development times for temperature effects (Bottrell et al. 1976; Mason and Abdulhussein 1991; Dickman et al. 2008).

![Fig. 3. Mesocosm experiment. (a) Running experiment, (b) Mesocosm construction, (c) Mesocosm transport.](image)

**Data analysis and statistics**

In this thesis a variety of different statistical approaches were used. To account for natural between year variation, data from 2011 and 2012 (lake monitoring), and 2012 and 2013 (fertilization experiment) was pooled and
treated as one experimental period. To analyse effects of DOC (papers I, III, VI) and fish (paper I) on response variables, only data from non-manipulated lakes was used. We performed linear mixed effect model (LME) analysis with lake as random factor (paper I, VI), or Person’s correlation analysis (paper III). The effects of fish, however, could only be tested in clear lakes, as all humic lakes had fish present. To further determine relationships between response variables and environmental variables in non-manipulated lakes (paper I), we used general linear models (GLM) with lake as a random factor and time as a correlation structure to negate the effects of autocorrelation between multiple sampling occasions in the same lakes. Here, multiple correlation structures were tested, and the optimal chosen based on the model with the lowest Akaike information criterion (AIC). The final model was chosen based on the most parsimonious model with the lowest AIC, as non-significant terms were removed step-wise from the model using backward selection. To test the effect of N fertilization (papers II, III, IV) and its interaction effects with DOC (papers III, IV) on response variables, we performed LME analysis with ‘N enrichment’, ‘DOC’, and their interaction as factorial explanatory variables. To account for natural seasonal variation, analysis was performed on the net responses (Δ After = After - Before) of control lakes versus N lakes. Further, ‘date’ was included in the LME model as a random effect to correct for pseudoreplication over time. Standardized effect sizes (ES) were calculated for the effect of DOC and fish (paper I), and DOC and N addition (papers III, IV) using Cohens d, where |d| < 0.2 is considered a weak effect and |d| > 0.8 is considered a strong effect. To explore whether DOC (papers I, III, IV), N fertilization, or their interaction (paper III, IV) affected phytoplankton or zooplankton community composition, we applied permutational multivariate analysis of variance (PERMANOVA) and non-metric multidimensional scaling (NMDS) to the 90% most abundant phytoplankton taxa (log(x+1) transformed), and present zooplankton taxa. Distances among the samples were computed as Bray-Curtis dissimilarities. LME, and multivariate analysis were performed in the statistical program R (R Development Core Team; version 3.1.2), using the packages “nlme” (Pinheiro et al. 2009), and "vegan” (Oksanen et al. 2016). Pearson’s correlation analysis was performed using the program Sigma Plot (Version 12.5, Systat Software, Inc). Lastly, we performed sensitivity analysis of consumer autochthony to test the influence of the potential errors introduced by uncertainty in model parameters (ω, δ²Hallo, τ) on the final estimate of consumer resource use using the program MATLAB (R2016a).
Major results and discussion

My thesis aimed to advance our knowledge about the response of boreal unproductive lakes to human induced increases in inorganic N and organic carbon and specifically the response of the pelagic food chain. A recent review by Greaver et al. (2016) summarizing “how global change alters key processes in terrestrial and freshwater ecosystems related to nitrogen cycling and availability” comes to the conclusion that we lack an integrated understanding of these environmental stressors. Further, the review points out that in our time of increasing computer capacities especially field experiments gathering empirical data are crucial to build predictive frameworks and models to diagnose when, where and how ecosystems will respond to global change and N deposition. Developing such models is important, as it will greatly aid our ability to manage future global change. My thesis provides an important step towards an integrated understanding of pelagic key processes in N-limited boreal lakes related to enhanced inorganic N availability in lakes with different organic carbon concentrations. The major results of each paper are presented and briefly discussed below.

**Food quantity and quality for zooplankton in boreal lakes**

**Paper I** identified the main factors controlling food quantity and quality in four non-manipulated clear water and humic boreal unproductive lakes, respectively. Further, it related how these factors influence zooplankton community composition and biomass. High DOC concentrations (>15 mg L⁻¹) were associated with lower phytoplankton production and biomass, in agreement with earlier studies (Carpenter et al. 1998; Nürnberg and Shaw 1998; Jansson et al. 2003). Lower PP in humic lakes was related to light rather than nutrient availability, with light: TP ratio (TP = total phosphorus) and photosynthetically available radiation (PAR) explaining nearly 50% of the variability in PP. The evidence of light rather than nutrient limitation of PP in humic lakes was supported by twice as high concentrations of bioavailable nutrients in the form of dissolved inorganic nitrogen and soluble reactive phosphorus in humic compared to clear water lakes (Jansson et al. 2001). Coupled with our findings that nitrogen and phosphorus resource use efficiency by phytoplankton were much lower in humic lakes, it appears that in humic lakes phytoplankton were unable to use these nutrients effectively, most likely due to light limitation of PP (Jansson et al. 2001; Thrane et al. 2014). In clear water lakes, PP was positively related to total N concentration and temperature rather than light availability, supporting that at this latitude in Sweden, PP is primarily N-limited or N- and P-co-limited due to low levels of N-deposition and low anthropogenic influence (Bergström et al. 2001).
However, the total amount of energy mobilized for higher consumers in the pelagic zone (PEM = PP + BP_{allo}) and the total amount of food available as seston C, were not affected by DOC concentration. Thus, in humic lakes lower phytoplankton production and biomass were compensated by increased BP_{allo} and seston C from detritus (i.e. non-living phytoplankton or terrestrial particulate organic C), which resulted in similar total amounts of mobilized basal energy (PEM) and biomass (seston C concentrations) available for higher consumers in the pelagic zone. Indeed, total crustacean mesozooplankton abundance did not differ with respect to DOC concentration, and was not related to phytoplankton production or biomass. Further, total zooplankton biomass was not related to estimates of food availability (phytoplankton biomass, seston C) or to estimates of food quality (edibility of phytoplankton, seston C: nutrient ratios). Indeed, it was unlikely that stoichiometric food quality would have affected zooplankton, as median C: P ratios were 252; this number is in the lower range of the C: P threshold elemental ratios of 150–400, which have been suggested to indicate P limitation in aquatic crustaceans (Sterner and Elser 2002; Frost et al. 2006).

However, although the overall biomass of zooplankton in the pelagic zone was not affected by DOC concentration individual zooplankton taxa showed different preferences concerning DOC concentration and the cladoceran: copepod ratio was five times higher in the humic lakes. This was confirmed when analyzing the subset of lakes from northern Sweden where the cladoceran: copepod ratio was positively correlated with DOC concentration. This may reflect the greater ability of cladoceran taxa to graze directly on bacteria and t-POC (Pace et al. 1983; Bergggren et al. 2015). Further, the change in cladoceran: copepod ratio in our study lakes was primarily driven by *Ceriodaphnia* biomass, which was 11 fold higher in humic compared to clear water lakes. *Ceriodaphnia* are common in humic lakes (Rask et al. 1986) and are efficient bacterial consumers able to survive and grow on a bacterioplankton diet (Pace et al. 1983). Hence, the direct bacterial grazing strategy that many cladoceran taxa display (e.g. *Bosmina* spp., *Daphnia* sp., *Ceriodaphnia* spp., *H. gibberum*) (Pace et al. 1983; Hwang and Heath 1999; Faithfull et al. 2012) seems to be an advantage in DOC rich lakes.

In summary, paper I suggests that total zooplankton biomass is not controlled *per se* by the proportion of food available as phytoplankton in these unproductive lakes, but by the total amount of energy available from both autochthonous and allochthonous sources. Further, our study indicates that in the pelagic zone of unproductive clear water and humic lakes energy availability for secondary and even tertiary consumers such as planktivorous fish is similar.
Pelagic food web and N fertilization

**Paper II** aimed to assess the effects of increased inorganic N availability on pelagic autochthonous resource supply and consumer resource use (degree of autochthony) in six unproductive boreal lakes with low N deposition. Inorganic N fertilization significantly increased phytoplankton biomass, PP, and PP:BP ratios. This finding indicates that bioavailable pools of P were not used to their full potential under natural (i.e. control) conditions (Jansson et al. 2001). Further, our whole lake inorganic N fertilization experiments clearly demonstrates that phytoplankton are N limited in areas with low N deposition, which is in line with results from earlier bioassays and the few whole lake inorganic N fertilization experiments (Jansson et al. 2001; Bergström et al. 2008; Elser et al. 2009a). **Paper II** and the aforementioned studies show that N$_2$ fixation by cyanobacteria (Schindler et al. 2008) is not a worldwide response to compensate for N limitation (Jansson et al. 2001). Possibly, in unproductive boreal lakes the development of cyanobacteria might be constrained (separately or in combination) by naturally low nutrient levels (of especially P) (Downing et al. 2001), low water temperatures (Butterwick et al. 2005), and low ferric iron concentrations (Molot et al. 2014). Subsequently, if boreal unproductive lakes in areas with low N deposition were to be exposed to higher inorganic N loadings, for example from atmospheric deposition or forest fertilization (Hole and Engardt 2008; Kreutzweiser et al. 2008), our results suggest that phytoplankton would become an increasingly dominant energy source for the zooplankton community. However, the net responses in autochthony were modest with autochthony only increasing in one of the three major zooplankton groups (15% increase in autochthony in Cladocera) and with no effect on zooplankton biomass. Here, differing effects of N fertilization on resource use in different crustacean zooplankton groups were most likely attributed to differences in species specific feeding modes (Barnett et al. 2007; Berggren et al. 2014). Unselective filter feeding cladocrans were more affected by the relative availability of autochthonous and allochthonous food resources (i.e. PP:BP ratio) than selective feeding copepods (Pace et al. 2004; Cole et al. 2011; Karlsson et al. 2012).

Summarizing the findings in **Paper II** indicate that the additional phytoplankton production induced by N fertilization was not as efficiently transferred up the food chain to zooplankton consumers as expected. Thus, the findings in **Paper II** suggests that the mismatch in producer energy supply and consumer energy usage could, at least in the short term (i.e. two years), potentially result in an accumulation of unconsumed primary producer biomass, potentially increasing transport of dead cells to the lake benthos. Alternatively, it is possible that the response time of crustacean
consumer autochthony to N fertilization is longer than our experimental time frame (2 years of N fertilization).

**Phytoplankton response to N enrichment**

**Paper III** aimed to investigate how increased availability of inorganic N affects phytoplankton production, biomass and community composition in six boreal lakes with low N deposition along a gradient of DOC concentration. Under non-manipulated conditions, PP and phytoplankton biomass were significantly lower in lakes with high DOC (as in **Paper I**). These findings support the view that although the availability of nutrients increases with increasing lake DOC concentrations, phytoplankton development becomes increasingly constrained by light availability (Jones 1992; Thrane et al. 2014; Faithfull et al. 2015). Further, phytoplankton community composition strongly differed between lakes with different DOC concentrations, and shifted from a dominance of non-flagellated autotrophs in low DOC lakes to subsequent higher proportions of flagellated autotrophs with increasing lake DOC concentration (Klug and Cottingham 2001; Drakare et al. 2003). We hypothesize that the observed shift in community composition is primarily caused by adaptations to the different abiotic conditions related to the lake DOC. The presence of a flagella likely provides flagellated autotrophs with a competitive advantage over non-flagellated autotrophs as it allows them both to actively move to deeper depths for compensational uptake of essential limiting nutrients, and to stay in the comparably shallow photic zone in DOC rich lakes (Wetzel 2001). Non motile autotrophs, on the other hand, would sink out quickly from the photic zone (Jones 1992; Wetzel 2001). We also discovered that at the high DOC level autotrophic flagellates mainly consisted of *Cryptomonas* spp. This taxa is known to be adapted to low light conditions and it is capable of surviving during prolonged periods of darkness (Gervais 1997); further, *Cryptomonas* has been observed to show signs of bacterivory under extremely low light conditions (Tranvik 1989; Marshall and Laybourn-Parry 2002). Hence, in addition to constraining PP and phytoplankton biomass development, light also seems to have an important structural impact on the community composition of phytoplankton.

N fertilization stimulated PP and phytoplankton biomass development in all lakes (see also **Paper II**). In addition, **paper III** clearly illustrates that the net effect of N fertilization on phytoplankton production and biomass declined with increasing lake DOC (Fig. 4), i.e. simultaneous to the decrease in light:TP ratio. Hence, at low DOC concentrations N limited phytoplankton have more light available per unit TP (high light:TP ratio), wherefore phytoplankton can increase in response to N addition until theoretically becoming P limited. The subsequent weaker response of PP and phytoplankton biomass to N fertilization with higher lake DOC and lower
light:TP ratios illustrates that as light becomes increasingly constrained for PP, phytoplankton are unable to respond to N addition to the same extent. Thus, although TP concentrations are highest in the high DOC lakes, light availability sets the upper threshold to what extent the limiting nutrient, inorganic N, can be utilized. Thus, the nutrient versus light model earlier illustrated for whole lake PP (pelagic and benthic) (Seekell et al. 2015), seems to be true also for pelagic PP (Paper III, Fig. 4).

Fig. 4. Response of phytoplankton to N addition in lakes along a DOC concentration gradient. (a) Conceptual model illustrating the N-effect along the DOC gradient of phytoplankton biomass and productivity in non-fertilized lakes (thin dashed line, \( n = 12 \)), and fertilized lakes (thick dashed line, \( n = 6 \)), (b) phytoplankton biomass and (c) primary production (PP) response in our whole lake experiment. Details on linear regression lines: see Paper III.
Interestingly, phytoplankton community composition did not change with N addition since mainly the dominant functional groups increased. Thus, distinct differences in community compositions along the lake DOC gradient were not affected by N addition (i.e. low DOC: non-flagellated autotrophs; medium, high DOC: flagellated autotrophs; Fig. 5). Therefore, paper III suggests that the lake DOC concentration has a stronger structural impact on the phytoplankton community composition (cf. above) than enhanced inorganic N availability. The adaptation of the phytoplankton community to the environmental conditions present at each specific lake DOC level seems to overrule any community structure effects that the comparably short term increase in N availability from fertilization may have induced.

![Fig. 5. Relative biomass of phytoplankton community shown as functional group composition in control and N-Lakes in the before period (B as 2011) and after period (A1 = 2012; A2 = 2013) for each DOC level (Low DOC, Medium DOC, High DOC).](image)

In summary, paper III indicates that browning will reduce phytoplankton production and biomass and influence phytoplankton community composition, whereas increased inorganic N loadings from deposition, forestry or other land use will primarily enhance phytoplankton biomass and production. However, with increasing DOC concentration, N effects on phytoplankton biomass and production will be reduced due to decreased light availability per nutrient availability.
N effects on zooplankton growth and food web efficiency
The aim of Paper IV was to examine the effects of increased N availability on pelagic energy mobilization and the consequences for zooplankton growth and food web efficiency (FWE) in six boreal lakes with low N deposition across a gradient of DOC concentration. We found that although PP and PEM increased following N fertilization, the responses of zooplankton production and FWE to N fertilization were dependent on the background lake DOC concentration. Zooplankton growth and FWE were negatively affected by N fertilization in low and medium DOC lakes, although the net increase in phytoplankton food quantity (%PEM\textsubscript{PP}) was highest in these lakes and potentially resulted in a shortened pelagic food web (increased PP:BP ratios) (Berglund et al. 2007; Jansson et al. 2007). The decrease in FWE in low and medium DOC lakes was associated with a decrease in phytoplankton quality (higher seston C:P and N:P ratios), which seemed to offset any increase in zooplankton production in response to increased food availability with N fertilization (Fig. 6).

![Fig. 6. Seston (a) N:P, and (b) C:P ratios in non-fertilized (white, n = 12), and fertilized (black, n = 6) lakes across the DOC continuum during the whole growing season with linear regression lines (solid: fertilized lakes, striped: non-manipulated lakes). Relationships between total zooplankton production (Tot. Zoopl. Production) and (c) seston N:P and (d) C:P, and food web efficiency (FWE) and (e) seston N:P and (f) C:P during the time frame of the mesocosm experiment. Details on regression lines: see Paper IV. Note log scale.](image)
The threshold elemental C:P and N:P ratios for *Daphnia* and calanoid copepods have earlier been found to range between 200-300 and ca. 12-18 (Baudouin and Scoppa 1975; Hessen and Lyche 1991; Sterner and Elser 2002). Our observed doubling of the seston N:P ratio, and the exceedance of the P limitation C:P threshold of 300 over the whole experimental period in response to N fertilization suggest that consumers grazing on phytoplankton became severely P limited in our experiment (Sterner et al. 1998; Elser et al. 2010; Hessen 2013). Supporting this hypothesis we found that zooplankton growth and FWE decreased most in the lake where the availability of poor quality food increased most, the low DOC lake (Fig. 6). However, with enhanced DOC and higher P- and reduced light availability, the N:P ratios, but not the C:P ratios in phytoplankton were enhanced to the same extent as at low DOC. As a consequence, zooplankton growth and FWE in the high DOC lake were slightly enhanced after N fertilization despite the relatively small increase in %PEM and PEM, whereas FWE in the medium DOC lake still decreased, but not to the same extent as in the low DOC lake.

Interestingly, we observed different responses among zooplankton species to N addition along the lake DOC gradient, likely linked to their different feeding strategies, and somatic P requirements. Responses to changes in food quantity and quality (Sterner et al. 1998; Elser et al. 2010; Hessen 2013) were especially apparent for selectively feeding calanoid copepods (Demott 1988; Tiselius and Jonsson 1990). Calanoids typically specialize on phytoplankton predation during summer (Berggren et al. 2015), wherefore changes in phytoplankton quantity and quality should be mirrored most in this zooplankton species. Indeed, growth of calanoids was severely reduced in the low DOC lake, when seston N:P and C:P ratios were enhanced (> 30 and > 400, respectively), implying that this species got severely P limited at this DOC level (Kibby 1971; Hessen and Lyche 1991). Thus, although phytoplankton quantity increased at low DOC, decreased food quality sufficiently reduced the fitness of calanoids (Sterner et al. 1998; Elser et al. 2010; Hessen 2013). In the high DOC lake, however, growth of calanoid copepods was slightly enhanced by N addition since the change in food quality (especially seston C:P) was low compared to the lakes with lower DOC concentrations (Fig. 6), although phytoplankton quantity only increased modestly at this DOC level. Since calanoid copepods were the main group contributing to the overall change in total zooplankton growth after N fertilization, we hypothesize that food web performances in lakes dominated by calanoids will be most affected by changes in N deposition. In contrast, N fertilization did not affect the two dominant cladoceran species *Ceriodaphnia* and *Bosmina*, which were most abundant at high DOC levels. Most likely grazing on P rich bacteria (Pace et al. 1983; Sterner and Elser 2002) enabled these filtering feeding zooplankton taxa to compensate for fertilization induced P limitation, especially at high DOC where bacterial
abundance was highest. This result suggests that lakes dominated by filter feeding cladocerans are likely to be less sensitive to stoichiometric changes in phytoplankton than lakes dominated by copepods, and especially selectively feeding calanoid copepods.

In summary, the results from paper IV clearly illustrate that effects of N deposition on FWE and zooplankton production in boreal unproductive lakes will differ depending on background DOC concentrations. The positive effects of N deposition such as increased food quantity and shortening of the pelagic food chain caused by elevated PP will be outweighed by its negative effects, namely the increased availability of poor quality phytoplankton with increased N:P, C:P stoichiometry. As a consequence, zooplankton growth and FWE will be impaired. However, with increasing DOC, this outcome will disappear since both present P rich bacteria and phytoplankton prey of higher stoichiometric quality will increase. Thus, allochthonous DOC will in the end determine the net effect of increased inorganic N for the food web performance of boreal unproductive lakes: firstly, by influencing light availability and thus, phytoplankton production, community composition and stoichiometry, and secondly by providing an external energy source for bacteria and enhancing P availability.

Conclusions and future questions

Paper I reveals that increased DOC concentrations in northern boreal lakes will cause significant differences in how basal energy is partitioned between phytoplankton, bacteria and detritus. However, this is unlikely to affect total zooplankton biomass, although enhanced DOC concentrations will potentially promote a shift in zooplankton community composition towards a higher proportion of cladocerans compared to copepods. Consequently, the total food availability for pelagic planktivorous consumers, such as young-of-the-year fish, is unlikely to differ between clear water and humic lakes.

Although we did not find an effect of fish presence on total zooplankton biomass, top-down grazing played a role in shaping zooplankton community composition. Cyclopoid copepods and Bosmina spp. were more abundant in clear water lakes with fish. Unfortunately, we could only determine the effects of fish in clear water lakes, as all humic lakes had fish present. A previous study found that in humic lakes predation pressure by planktivorous fish such as Eurasian perch may be lower than in clear lakes. In contrary, predation pressure by Roach and invertebrate predators such as Chaoborus may be the same or higher in humic lakes (Wissel et al. 2003; Estlander et al. 2012). This was indicated
by a shift from smaller zooplankton taxa in clear lakes to larger species in humic lakes (Estlander et al. 2012). Additionally, a study investigating the effects of DOC on winter mortality in fish suggested that reduced visibility caused by increased DOC reduces search efficiency of visual feeding fish during winter. However, it is unclear whether this potential release of zooplankton from top down predation has consequences for zooplankton community composition or biomass (Hedström et al. 2016). In our study, conducted during summer, we saw no differences in zooplankton size structure between clear and humic lakes with fish. However, further work examining the interaction effects between fish predation and DOC concentration, especially in winter, would provide valuable insights as to how fish predation on zooplankton is affected by light availability, water colour and terrestrial particulate matter.

Paper II illustrates that increased inorganic N availability will cause major changes in the pelagic energy pathways of boreal unproductive lakes by increasing autochthonous resources. However, the increased availability of autochthonous resources will only contribute to a modest increase in autochthonous resource use (autochthony) in zooplankton consumers. Therefore, newly available bottom up energy by phytoplankton following N fertilization will not be efficiently transferred up the pelagic food chain to crustacean zooplankton. Predicted increased inorganic N loads from anthropogenic activities may therefore potentially contribute to unconsumed primary producer biomass with associated changes in water quality.

Given these finding it would be interesting to test whether increased degradation of dead phytoplankton cells in the lake benthos promoted anoxia in the lake sediments, the hypolimnion, or as a consequence activated anaerobic processes such as denitrification, or methanogenesis (Groffman and Rosi-Marshal 2013).

Further, it would have been really interesting to have had more zooplankton samples for autochthony analysis during summer. Firstly, this would have improved our statistical outcome, but secondly also enabled us to better follow changes of zooplankton resource use during the whole summer season. It could be expected that the timing of increased zooplankton autochthony would match the timing of increased resource availability (potentially with lag time); however this has not been tested, since generally only one study has to our knowledge looked at the seasonal variability of zooplankton autochthony (Berggren et al. 2015). Additional winter sampling would have further provided us with interesting insights about feeding strategies of crustacean zooplankton under the ice.

Paper III provides important whole lake insights concerning the consequences of brownification and increased N availability on pelagic food
webs, and phytoplankton specifically. With increasing DOC, phytoplankton production and biomass will decrease, and phytoplankton communities may change, shifting towards a dominance of high DOC adapted phytoplankton species such as autotrophic flagellates. Further, the whole lake N fertilization experiment shows that enhanced inorganic N availability due to N deposition, forestry or other land use change will promote phytoplankton biomass production in these unproductive lake ecosystems without affecting phytoplankton communities. Thus, the potential change in phytoplankton community composition with brownification towards a dominance of high DOC adapted species will persist even with increased N fertilization. Together, the production and biomass declines with brownification will be counteracted by any change in the landscape that enhanced inorganic N availability; however, this counteracting effect will be much weaker and even neutralized in browner lakes as caused by light limitation. This suggests that high DOC lakes may be more resilient to eutrophication caused by increased N deposition or land use change, whereas clearer lakes may be more susceptible to increased N input. The findings in paper III highlight the importance of light in predicting how global change (i.e. brownification, N deposition) will affect both the productivity, but also the phytoplankton community composition in the pelagic food web of boreal lakes creating different food conditions for higher trophic levels potentially affecting their growth and reproduction.

Clearly, we only investigated three DOC levels, not a true DOC continuum. Further, our data set did not include lakes with a DOC concentration below 7 mg L⁻¹. Future studies including data on more lakes are needed to verify our findings, especially for lakes with very low (< 7 mg L⁻¹) and very high DOC concentrations (> 21 mg L⁻¹). Additionally, this would help to answer the question whether PP and phytoplankton biomass really follow a linear relationship with increasing DOC concentration or rather a hump-shaped (or quadratic) relationship as found in other studies (Hanson et al. 2003; Ask et al. 2009; Solomon et al. 2015).

Paper IV provides first important insights how consumers in unproductive boreal lakes will be affected by both future N deposition and DOC input. Our results clearly show that increased N deposition will decrease food web performance in clear lakes caused by mismatches in food quality demand and supply. In humic lakes this mismatch will not occur, wherefore zooplankton production and FWE will increase slightly following enhanced N deposition. Lastly, communities dominated by cladocerans will be more resilient to eutrophication caused by increased N deposition, whereas communities dominated by copepods will be more sensitive depending on the quality change of available food items.
Our study was the first of its kind. Therefore, any study investigating the consequences of DOC x N interactions on zooplankton production and community composition will provide valuable insights. Further, I observed that most other zooplankton species than Daphnia and Calanoids are comparably under-represented in laboratory experiments addressing consequences of food quality on zooplankton growth. Therefore, additional laboratory/field experiments with other crustacean taxa will be very helpful to better understand the role of food quality for pelagic food webs in boreal unproductive lakes.

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References


Oksanen, J., F. G. Blanchet, R. Kindt and others 2016. vegan: Community Ecology Package. R.


Thank you

“And so I sit, poor silly one  
No wiser now than when I began”  

“Da steh ich nun, ich armer Tor!  
Und bin so klug als wie zuvor“

Faust - Johan W. Goethe (Lines 357-356)

Oh happy I am that this quotation is no longer true for me - It used to be my favorite one, now it’s time for an update ;) Not only have I (hopefully) become wiser in science, I have become richer in friends, a new culture, new languages and loads of life experience that will serve me great in whatever is there to come; all this is thanks to all you people that have made these past years in Sweden a wonderful time.

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Kram, Umarmung, Abrazo, Hugs, Je vous embrasse, Abbraccio, 保重！

Anne

“As you set out for Ithaka
hope the voyage is a long one,
full of adventure, full of discovery.”

Ithaka - C.P. Cavafy (Lines 1-3)