Freshwater Biology

Freshwater Biology (2015) **60,** 646–662 doi:10.1111/fwb.12511

Factors influencing nitrogen processing in lakes: an experimental approach

SAARA OLSEN*,†, ERIK JEPPESEN*,†, BRIAN MOSS‡, KORHAN ÖZKAN*,§,¶, MERYEM BEKLIOĞLU§,**, HEIDRUN FEUCHTMAYR‡,††, MARÍA GONZÁLEZ SAGRARIO‡‡, LI WEI§§, SØREN LARSEN*, TORBEN S. LAURIDSEN*,† AND MARTIN SØNDERGAARD*

*Department of Bioscience, Aarhus University, Silkeborg, Denmark
†Sino-Danish Centre for Education and Research, Beijing, China
‡School of Environmental Sciences, University of Liverpool, Liverpool, U.K.
§Limnology Laboratory, Department of Biology, Middle East Technical University, Ankara, Turkey
¶Institute of Marine Sciences, Middle East Technical University, Mersin, Turkey
**Kemal Kurdaş Ecological Research and Training Stations, Lake Eymir, Middle East Technical University, Ankara, Turkey
††Centre for Ecology and Hydrology, Lancaster Environment Centre, Lancaster, U.K.
‡‡Institute of Marine and Coastal Research, CONICET, University of Mar del Plata, Mar del Plata, Buenos Aires, Argentina
§§Laboratory of Aquatic Plant Biology, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, China

SUMMARY

1. To help improve our understanding of the nitrogen cycle in lakes, particularly in the context of climate change, we analysed total nitrogen (TN) and nitrate (NO_3^--N) data from six mesocosm experiments (in Denmark, U.K., China and Turkey) covering different climatic regions. We assessed the effects of nitrogen (N) and phosphorus (P) loading, temperature, salinity and water level on N processing.
2. Water column N loss (defined as the nitrogen processed in and lost from the water column in units of net amount processed per unit area and per unit of time, or in relative terms as the percentage loss of the total pool in 2 weeks) was particularly sensitive to external nutrient loading to the mesocosms. Mean water column TN loss at high N loading varied from 111 to 250 mg m^{-2} day^{-1} and increased with N loading. High P loading resulted in increased water column N loss, possibly because of increased uptake into plants and attached algae and sedimentation of the increased algal crop. High salinity generally decreased water column TN loss; on average, 10% more TN was in the water column at 12‰ salinity than at 2‰ salinity, while no significant effect of water level was found.
3. Only weak relationships were observed between N processing and temperature, and mesocosms limited by P accumulated more nitrogen in their water columns than those with high P loadings. Our results suggest that N processing in lakes appears to be more sensitive to features of the catchment, such as hydrology and loading, than to climatic effects related to temperature, salinity and water level.

Keywords: climate change, eutrophication, nutrient cycling, salinification, shallow lake

Introduction

Increased nutrient loading has reduced lake water quality worldwide over the past century (Vitousek *et al*., 1997; Galloway, 1998; Karakoç, Ünlü Erkoç & Katırcıoğlu, 2003; Camargo & Alonso, 2006; Liu *et al*., 2011). While the role of phosphorus in eutrophication has long been recognised (Likens, 1972; Schindler, 1974), the effect of nitrogen has previously been dismissed, although interest has recently revived (Schindler *et al*., 2008; Moss, 2010; Lewis Jr, Wurtsbaugh & Paerl, 2011; Moss *et al*., 2013). High concentrations of N may exacerbate eutrophication (Jackson, 2003; Barker *et al*., 2008; Özkan *et al*., 2010; Lewis Jr *et al*., 2011) and cause changes in species composition (Qiu *et al*., 2001; González Sagrario *et al*., 2005; Özkan *et al*., 2010), eventually leading to a notable decrease in macrophyte biomass and species richness in shallow lakes (Barker *et al*., 2008).

Nitrogen concentrations in lakes are particularly dependent on external N loading, water residence time

Correspondence: Saara Olsen, Department of Bioscience, Aarhus University, Vejlsøvej 25, 8600 Silkeborg, Denmark E-mail: saol@dmu.dk

and denitrification (Keeney, 1973; Jensen, Kristensen & Jeppesen, 1990; Windolf *et al.*, 1996; Saunders & Kalff, 2001; Cook *et al.*, 2010). Lakes generally act as sinks for terrestrially derived nitrogen (Fleischer *et al.*, 1994; Harrison *et al.*, 2009), thereby reducing the N load downstream to the ocean (Seitzinger, 1988; Jensen *et al.*, 1992; Hessen, Hindar & Holtan, 1997; Wetzel, 2001; Harrison *et al.*, 2009). Most combined N is lost to the atmosphere through denitrification (Keeney, Chen & Graetz, 1971; Berge *et al.*, 1997; Seitzinger *et al.*, 2006; McCarthy *et al.*, 2007), and freshwater ecosystems have high denitrification capacity, with rates per unit area that are up to 10 times higher than in terrestrial ecosystems (Seitzinger *et al.*, 2006).

Lakes are also sinks for N owing to burial of organic N in the sediments, and assimilation of N by macrophytes and algae. This may represent only temporary storage because larger plants and algae, and organic sediment resuspended by wind and animal activity, eventually decompose releasing inorganic nitrogen that can be denitrified. An increase in areal N loading should result in higher water column N loss, but may reduce the proportion of N assimilated and denitrified in lakes if the water column becomes NO_3^- saturated, although only a few studies have found evidence of this (Kemp & Dodds, 2002; Bernot & Dodds, 2005). Nitrogen loss is defined here as the total N processed in, and lost from, the water column, in absolute units per unit area and time, or expressed as percentage change per unit time, and (perhaps misleadingly) referred to as 'retention' by some previous authors (Vollenweider, 1975; Fleischer, Stibe & Leonardson, 1991; Molot & Dillon, 1993; Fleischer *et al.*, 1994; Windolf *et al.*, 1996). The original use of 'retention' by Vollenweider (1975) was in the context of what was delivered to a lake (the loading) and what was exported from it (the washout), the difference being what was retained by the lake. Vollenweider (1975) concentrated on phosphorus rather than nitrogen, and phosphorus truly was retained in the lake sediment, whereas he underestimated the role of denitrification and loss to the atmosphere. Nonetheless, the term 'retention' persists, even for nitrogen.

Nutrient enrichment is clearly a problem, but climate change may further burden lake ecosystems by altering temperature, chemistry and hydrology (Coops, Beklioğlu & Crisman, 2003; Fragoso Jr *et al.*, 2011; Baron *et al.*, 2013). Higher temperature increases nitrification and denitrification (Christensen & Sørensen, 1986; Pinay *et al.*, 2007; Baron *et al.*, 2013), but also N uptake by macrophytes and algae (Madsen & Brix, 1997; Agawin, Duarte & Agusti, 2000; Feuchtmayr *et al.*, 2009). Increasing temperature can also alter the ratio of carbon (C) to N in freshwater biota, with implications for mineralisation rates (Woods *et al.*, 2003; Gudasz *et al.*, 2010). Warming is also expected to increase tidal intrusion to coastal fresh waters, and more frequent storms transport more sea spray inland; there will also be raised surface evaporation. All these effects will lead to salinisation and variation in water level in lakes. Lower water level has been shown to increase (Özkan *et al.*, 2010; Bucak *et al.*, 2012) or decrease (Loverde-Oliveira *et al.*, 2009) submerged macrophyte resilience to shading by periphyton and phytoplankton, and also to influence the water–sediment contact, with variable effects on sedimentation and positive effects on denitrification. Raised salinity may decrease water column N loss by decreasing growth and reproduction of freshwater organisms (Jeppesen *et al.*, 2007; Tilley *et al.*, 2007; Brucet *et al.*, 2009; Jensen *et al.*, 2010), or increase it by aggregating suspended matter for subsequent sedimentation (Lick, Lick & Ziegler, 1992; Håkanson, Gyllenhammar & Brolin, 2004). The overall consequences of climate-induced changes in N processing are thus complex and variable.

Estimates of lake N assimilation, denitrification and sedimentation usually depend on monitoring, or models using mass balances, relying often on site-specific studies or synthetic analyses across lakes that lack controlled nutrient manipulation (Jensen *et al.*, 1992; Molot & Dillon, 1993; Havens *et al.*, 2001; Cook *et al.*, 2010), and most research on N processing has been restricted to temperate lakes. Keeping track of the overall processing of nitrogen in ecosystems is not easy because of the involvement of nitrogen gas, which is not easily monitored, and to some extent this may have contributed to a neglect of the role of nitrogen in eutrophication. Phosphorus, having a gaseous phase at Earth temperatures only in extreme reducing conditions (e.g. phosphine production in marshes), is much more easily budgeted. Nitrogen fixation is rarely measured directly but assessed by surrogates such as the concentration of cyanobacterial heterocysts, and it is difficult to separate assimilation and mineralisation in complex systems where autotrophic and heterotrophic organisms are freely intermixed. Analysis of existing mesocosm experiments, despite similar limitations in the range of processes measured as in whole-lake studies, at least offers more control in assessing the net effects of the processes on water column nitrogen loss and is a first step in better design of future experiments.

Here we have analysed data from six outdoor mesocosm experiments, performed under contrasting hydrological and climatic conditions, simulating shallow lakes to estimate bulk water column N loss. We used a uniform statistical approach to seek common features as well as to examine responses in individual experiments. Our measure of nitrogen processing was the change (almost always a decline) in nitrogen content in the water column, which we refer to as 'water column loss'. We hypothesised that N processing would increase with temperature, owing to stimulation of denitrification, and would also increase with increased N and P loading, through higher primary production by macrophytes and associated periphyton on the plants and other surfaces, such as the mesocosm walls. Moreover, we hypothesised decreased water column loss at high salinities owing to higher metabolic stress on organisms, and decreased water column loss at higher water levels, because of a greater water volume to sediment area (and its stock of denitrifying bacteria) and because less light reaches plants on the bottom.

Methods

Experimental design

We used total nitrogen (TN) and NO_3^--N concentration data from six (five previously published) mesocosm experiments that included Özkan *et al.* (2010), conducted in Turkey (TR), Li, Zhang & Jeppesen (2008) in China (CH), Feuchtmayr *et al.* (2009, 2010) and Moran *et al.* (2010) in England (U.K.), and González Sagrario *et al.* (2005), Jeppesen *et al.* (2007) and M. Søndergaard (unpubl. data) in Denmark (DK-1, DK-2 and DK-3) (Table 1). Each experiment was factorial in design and had different N loads as a treatment and reference controls (CN = no N addition), which enabled us to examine systematic variation across these experiments under different conditions.

Nitrogen was added as $Ca(NO_3)_2$ (TR, DK-1, DK-2 and DK-3) or $NaNO_3$ (CH and U.K.) and, in experiment CH, also as NH_4Cl. Additions of NO_3^- -N were sorted into the following groups: very high nitrogen (VHN) >190 mg m^{-2} day^{-1}, high nitrogen (HN) 150–190 mg m^{-2} day^{-1}, medium nitrogen (MN) 110–150 mg m^{-2} day^{-1}, low nitrogen (LN) <110 mg m^{-2} day^{-1} and control (CN) 0 mg m^{-2} day^{-1}. All mesocosms were cylinder-shaped and contained from 0.2 m^3 to 3 m^3 of water and were embedded into the sediment, when installed in lakes, or contained sediment when in containers, and all were open to the atmosphere; they had no outlets. The mesocosms were made of polyethylene (TR, DK-1, DK-2 and DK-3), concrete (CH) or fibreglass (U.K.). Sampling and analytical methods are given in Özkan *et al.* (2010), Feuchtmayr *et al.* (2009), Li *et al.* (2008), González Sagrario *et al.* (2005) and Jeppesen *et al.* (2007).

Turkish (TR) experiment

This experiment used 24 mesocosms set in the littoral zone of Lake Pedina in north-west Turkey, from 27 June 2007 to 27 September 2007. Mean water depth in the mesocosms was 0.7 m at the start of the experiment and 0.4 m at the end. Three NO_3^- loadings (CN, MN and VHN) were crossed with two phosphate loadings (MP = medium phosphorus, HP = high phosphorus). The NO_3^- additions were designed to maintain concentrations of 4 and 10 mg N L^{-1} and phosphate additions to maintain concentrations of 100 (MP) and 250 μg P L^{-1} (HP). Each treatment had four replicates. The mesocosms were planted with *Myriophyllum spicatum* at similar initial densities [7% as percentage volume of mesocosm inhabited by macrophytes (PVI)]. Fishes (*Carassius carassius*, *Cyprinus carpio* and *Scardinius erythrophthalmus* in each mesocosm), size <10 cm, from the lake were also stocked into each mesocosm at a density of 40 g (fresh weight) m^{-2}. All mesocosms were covered with nets to prevent bird predation.

Nitrate and PO_4^{3-} were added weekly. The first two additions were higher, to obtain the desired nutrient levels. Remaining additions were based on nutrient measurements preceding each addition. Sampling was weekly at the start and later every 2 weeks. Variables measured were TN, total phosphorus (TP), NO_3^-, NH_4^+, PO_4^{3-}, PVI, phyto- and zooplankton biomass, periphyton chlorophyll a (Chl-*a*), and phytoplankton Chl-*a*. Owing to evaporation, the water level decreased on average by 30 cm during the experiment in all mesocosms and also in the lake. The main objective of the original experiment was to examine the combined effect of increasing N and P concentrations on submerged macrophyte growth.

United Kingdom (U.K.) experiment

Forty-eight, 1-m-deep mesocosms were established in the University of Liverpool Botanic Gardens in north-west England, between 17 January 2006 and 28 August 2007. The mesocosms received three nutrient loadings (CN, LN and HN) crossed with two temperature treatments: unheated and heated by +4 °C above ambient temperature, and two fish treatments: presence or absence of four sticklebacks (*Gasterosteus aculeatus*). Mesocosms containing fish were intermittently restocked to achieve mean biomass of 4.3 g (fresh weight) m^{-2}. In

Table 1 Duration, location, design and mean (±SD) pre-experiment concentration of chlorophyll *a* (Chl-*a*), total nitrogen (TN) and total phosphorus (TP) of the six mesocosm experiments

Experiment abbreviation	Duration	Location	Mesocosm diameter/ height (m)	Number of replicates	Additional treatments to N-dosing	Type and no. of nitrogen addition levels*	Chl-*a* (μg L^{-1})	TN (mg L^{-1})	TP (mg L^{-1})
TR	27 June 2007– 26 September 2007	Lake Pedina, Turkey	1.2/1	4	2 phosphorus levels: medium (MP) and high (HP)	3, Aiming for target concentration	18.1 ± 14.1	0.5 ± 0.2	0.04 ± 0.01
U.K.	17 January 2006 – 28 August 2007	Ness Botanic Gardens, north-west England	2/1	4	Heating +4 °C (h), Fish (f)	3, Fixed addition	34.1 ± 28.8	1.8 ± 0.6	0.5 ± 0.3
CH	10 August 2005 – 29 December 2005	Wuhan Botanical Garden, China	1/1	5	No additional treatments	5, Aiming for target concentration	6.4 ± 2.8	0.4	0.05
DK-1	28 May 2002 – 12 August 2002	Lake Stigsholm, Denmark	1.2/1	4	Phosphorus addition: HP (high phosphorus)	3, Fixed addition	40	2	0.1
DK-2	1 May 1999 – 21 February 2000	Kogleaks brackish lagoon, Denmark	1.2/0.8	2	8 salinity levels (0.5, 1,2,4,6,8,12 and 16‰)	3, Fixed addition	21 ± 9	0.16 ± 0.12	0.11 ± 0.02
DK-3	13 June 2000 – 2 October 2000	Kogleaks brackish lagoon, Denmark	1.2/0.8	2	2 salinity levels (2, 12‰) and 6 water levels (30, 45, 60, 75, 90 and 105 cm)	2, Fixed addition	18 ± 9.7	1.8 ± 0.3	0.3 ± 0.2

TR, Özkan *et al.* (2010); U.K., Feuchtmayr *et al.* (2009, 2010); Moran *et al.* (2010); CH, Li *et al.* (2008); DK-1, González Sagrario *et al.* (2005); DK-2, Jeppesen *et al.* (2007); DK-3, M. Søndergaard *et al.* unpubl. data.

*Including control treatment, no nitrogen added.

the low N treatment, 17 mg N m^{-2} day^{-1} were added and in the high, 170 mg N m^{-2} day^{-1}. Mesocosms with N treatments also received 3 mg P m^{-2} day^{-1}. Nitrogen and P additions were made every 2 weeks, and the amount added was kept constant throughout the experiment. Every treatment had four replicates. During the experiments, the mesocosms developed an aquatic plant assemblage containing *Elodea nuttallii, Lemna trisulca, Ceratophyllum demersum, Lemna minor, Lemna minuta, Potamogeton berchtoldii, Potamogeton crispus, Potamogeton natans* and *Spirodela polyrhiza*.

The mesocosms were sampled every 2 weeks for TN, TP, NO_3^-, NH_4^+, PO_4^{3-}, PVI, phytoplankton biomass, zooplankton abundance, periphyton Chl-*a* and phytoplankton Chl-*a*, macroinvertebrate abundance, fish mass, and fresh and dry mass of macrophytes at the end of the experiment. The study aimed at elucidating the effects of warming and N loading on shallow lake communities in the presence and absence of fish.

Chinese (CH) experiment

An experiment with 45, 1-m-deep mesocosms was conducted in Wuhan Botanical Gardens, China, between 10 August 2007 and 29 December 2007. Five different N additions (CN, LN, MN, HN, VHN, with five replicates each) were applied to maintain the following NO_3–N concentrations: 2.5 (LN), 5 (MN), 7.5 (HN) and 10 mg N L^{-1} (VHN), and NH_4^+-N concentrations: 2.5 (LN), 5 (MN), 7.5 (HN), 10 mg N L^{-1} (VHN). Additions were made based on N data obtained from previous measurements. Nitrogen was added weekly to the mesocosms; however, no NO_3^- additions were made after 70 days of the experiment (after 20 October) because the concentration remained stable. The first addition was high compared with those following, to reach target N concentration. The mesocosms did not receive any PO_4^{3-} during the experiment. Five individuals of *Vallisneria spinulosa* were planted into each mesocosm at the beginning of the experiment.

TN, TP, NO_3^-, NH_4^+ and phytoplankton Chl-*a* were measured weekly, and N content, ramet number, fresh mass and dry mass of macrophytes were recorded at the end of the experiment. The main objective was to observe the effects of different NH_4^+ and NO_3^- concentrations on phytoplankton Chl-*a* and growth of *V. spinulosa*.

Danish (DK-1) experiment

Twenty-four mesocosms were placed in the littoral zone of Lake Stigsholm, Denmark, from 28 May to 12 August 2002. The mesocosms had a mean water depth of 1 m and a diameter of 1.2 m. The experiment had six nutrient treatments with four replicates each: CN, LN, MN, HP, LNHP and MNHP. At the beginning of the experiment, nutrients were added to the mesocosms to attain starting conditions of 0.2 mg P L^{-1} in HP: 4 mg N L^{-1} in LN and 10 mg N L^{-1} in MN. Thereafter, nutrients were added weekly at rates of 8.7 mg P m^{-2} day^{-1} in the HP treatment, 25 mg N m^{-2} day^{-1} in the LN treatment and 127 mg N m^{-2} day^{-1} in the MN treatment. All macrophytes were removed from the mesocosms before the experiment to achieve similar starting conditions, and they reappeared in the majority of the mesocosms. Four (1+) perch (*Perca fluviatilis*) of sizes between 6 and 8 cm fork length were introduced to each mesocosm prior to the start of the experiment at a density of 26 g (fresh weight) m^{-2} to minimise the zooplankton grazing on phytoplankton.

Total Nitrogen, TP, NO_3^-, NH_4^+, PO_4^{3-}, total suspended solids (TSS), phytoplankton Chl-*a*, and phytoplankton and zooplankton biovolumes were measured every second week. Transparency (Secchi depth) and fish abundance were monitored weekly, and macrophyte dry mass was measured at the end of the experiment. The purpose of the study was to investigate the effect of HN loading on the trophic structure of shallow lakes at moderately HP concentrations.

Danish (DK-2) experiment

An experiment with 48 mesocosms was conducted in the shallow Kogleaks brackish lagoon in Denmark, from 1 May 1999 to 21 February 2000. The mesocosms were on average 0.8 m deep and treated with three N loadings (CN, LN and HN) crossed with eight salinities: 0.5, 1, 2, 4, 6, 8, 12 and 16‰. All treatments had two replicates. One male three-spined stickleback 4–7 cm fork length, on average 5.9 g (fresh weight) m^{-2}, was added to each mesocosm to maintain modest zooplankton predation. Each mesocosm also received a mixture of plankton pooled from three nearby fjords: Lund Fjord (1‰), Østerild Fjord (4‰) and Limfjorden (22.4‰). The mesocosms did not contain macrophytes, and plants appearing during the experiment were removed.

A salt solution containing NaCl, $MgSO_4$ and $NaHCO_3$ that approximated the composition of seawater was added to the mesocosms every 2 weeks to maintain target salinities. Phosphorus and N were added weekly to at rates of 0, 1.5 and 7.5 mg P m^{-2} day^{-1} and 0, 23 and 115 mg N m^{-2} day^{-1}. The additions were kept constant during the experiment. TN, TP, NO_3^-, NH_4^+, PO_4^{3-} and phytoplankton Chl-*a* were initially measured weekly and then, from September 1999 to February 2000, monthly. The purpose was to determine the effects of contrasting salinities and nutrient loadings on regime shifts in brackish lagoons with low fish densities.

Danish (DK-3) experiment

Another experiment with 48, 1-m-deep mesocosms in the Kogleaks brackish lagoon, Denmark, was conducted from 26 June 2000 to 2 October 2000. Mesocosms were with (VHN) or without (CN) nutrient addition, crossed with high (12‰) and low (2‰) salinities and six different water levels. Mesocosms with nutrient addition had a fixed dose of NO_3^- based on concentration, corresponding to 60–280 mg N m^{-2} day^{-1} and – 6–30 mg P m^{-2} day^{-1} (at different water levels). Six different water levels, 30, 45, 60, 75, 90 and 105 cm, were used. All combinations of N addition, salinity and water level were doubly replicated. Nitrogen and P were added every 2 weeks throughout the experiment, and TN, TP, NO_3^-, NH_4^+, PO_4^{3-}, salinity and phytoplankton Chl-*a* were measured every 2 weeks. The mesocosms contained no macrophytes.

Data analysis and statistics

We defined N processes in the water column as sink to sediment, mineralisation, combined uptake by macrophytes and attached algae, and as loss from the mesocosms through denitrification. Total N and NO_3^- loss from water column was determined using the mass balance equation of Vollenweider (1975) and Messer & Brezonik (1978), as change in N content of the water column. Changes between successive samplings of each mesocosm in each experiment, respectively, were converted to daily water column loss per unit area (mg m^{-2} day^{-1}) using the following equation:

$$N_{loss} = (N_t + N_{load} - N_{t+1})/(At)$$

where, during a time interval t to $t + 1$ (days), N_{loss} is the amount of nitrogen (mg) lost from the water, N_t is the initial amount of nitrogen (mg) at time t, N_{load} is the amount of nitrogen added to the system (mg), N_{t+1} is the amount of nitrogen measured at $t + 1$ (mg) and A is surface area of the mesocosm (m^2). If additions were made more than once before the subsequent recording of concentration, all these additions were pooled. Daily water column N loss was also determined on a volumetric basis (mg N L^{-1} day^{-1}) for all treatments and experiments, since water column loss expressed per unit area will suppress the effect of loading at different water levels.

Relative water column N loss ($N_{pool\%}$ of TN, NO_3^- and NH_4^+) was defined as percentage of N lost from the water column N pool per unit time [N_{pool} = combined N stock (N_t) (mg) at time t plus N addition (N_{load}) (mg)] during the period. It was calculated by dividing the amount of nitrogen lost from the water at time $t + 1$ (mg) by the N pool in the mesocosms, to evaluate the proportion of N that was removed from the water.

$$N_{pool\%} = ((N_t + N_{load}) - N_{t+1})/(N_t + N_{load}) * 100$$

The time component is inherent in the measures at times t and $t + 1$. In addition, relative water column N loss ($N_{load\%}$) was also estimated as a function of external loading alone.

$$N_{load\%} = (((N_t + N_{load}) - N_{t+1})/N_{load}) * 100$$

The relative water column loss ($N_{pool\%}$) and loss as function of loading ($N_{load\%}$) were normalised to a period of 2 weeks for all experiments. Time-weighted means for each treatment of each experiment were calculated as the sum of time period (days) multiplied by the water column loss during that time period divided by the sum of each time period. For experiments TR, CH and DK-1, the first addition was excluded from the mean since the first N addition was considerably higher than those following to achieve the initial concentrations. From the time-weighted means of $N_{pool\%}$, we also calculated the theoretical water column N loss time (T) as the theoretical number of days to reach 100% removal (by the combined effects of denitrification, sedimentation and assimilation by periphyton and macrophytes) of the N pool in the water of each mesocosm. We divided the average number of days (d) between measurements by the mean $N_{pool\%}$ of the corresponding treatment and then multiplied it by 100.

$$T = [(days)/N_{pool\%}] * 100$$

Data expressed per unit area from the six experiments frequently did not meet the assumptions of parametric tests. Therefore, an autoregressive covariance model for SAS [$AR_{(p)}$] was used to evaluate differences in water column loss per unit area and unit volume among the different treatments. $AR_{(p)}$ fits a high-order model with many autoregressive lags and then gradually removes autoregressive variables until all those remaining have significant t tests. The covariance structure for the repeated measurements for water column N loss of each measurement time is assumed to be an autoregressive process of order 1. This format gave the best description of the covariance structure. Results of the [AR($_p$)] are given both as the main effect and as the interaction effect. Differences in the relative water column loss of loading ($N_{load\%}$), relative water column loss of the pool ($N_{pool\%}$) and the theoretical replacement time *T*, among treatments, were analysed with one-, two- or three-way ANOVA, depending on the number of fixed factors in the corresponding experiment. The results of the ANOVA analyses are also presented for main effects and interaction effects.

Results

Turkish (TR) experiments

Both water column TN and NO_3^- loss rates per unit area increased significantly with increasing N load (Tables 2 & 3), but did not change with time. As hypothesised, water column loss (as $N_{pool\%}$) increased consistently with load compared with controls (Table 3a,b; Figs 1 & 2), with mean NO_3^- concentration 1.2 mg L^{-1} and 7 mg L^{-1} in the MN and VHN treatments (Fig. 2). Phosphate additions increased the water column loss of TN and NO_3^- $N_{pool\%}$ on average by 10% in all mesocosms, and differences between treatments were significant for TN, but not for NO_3^- (Table 3a,b; Figs 1 & 2). Controls consistently accumulated N (data not shown).

United Kingdom (U.K.) experiment

Means of water column TN and NO_3^- loss increased greatly with loading (Table 2a,b). Warming in the U.K. experiment did not affect water column N loss per unit area in mesocosms with different N treatments (Table 3a,b), and there was no significant change in

Table 2 Mean (±SD) daily water column total nitrogen (TN) (a) and NO_3^- (b) loss and average NO_3^- addition (c) per unit of area in different N treatments in the six mesocosm experiments

(a) TN mg m^{-2} day^{-1}	N treatment				
	CN	LN	MN	HN	VHN
TR	−1.9 ± 0.2		119 ± 7. 4		251 ± 12.0
U.K.	0.3 ± 1.4	17.5 ± 0.3		169 ± 2.1	
CH	−6.6 ± 2.8	139 ± 25.1	171 ± 10.3	206 ± 17.0	230 ± 28.7
DK-1	−0.7 ± 9.8	55.3 ± 13.3	218 ± 16.6		
DK-2	−7 ± 2.6	19.2 ± 2.3	111 ± 2.9		
DK-3	4.7 ± 0.5				181 ± 6.1

(b) NO_3^- mg m^{-2} day^{-1}	N treatment				
	CN	LN	MN	HN	VHN
TR	−1.9 ± 0.5		119 ± 8.9		251 ± 28.1
U.K.	0.1 ± 0.03	16.7 ± 0.1		168 ± 1.00	
CH	4.6 ± 1.9	17.5 ± 11.5	20.2 ± 13.2	7.3 ± 18.2	−8.2 ± 27.7
DK-1	<0.01	111.2 ± 39.2.2	272 ± 32.6		
DK-2	−0.1 ± 0.08	21.8 ± 0.4	107 ± 4.6		
DK-3	−0.02 ± 0.9				184 ± 0.8

(c) Addition mg N m^{-2} day^{-1}	N treatment				
	CN	LN	MN	HN	VHN
TR	0		140		330
U.K.	0	17		170	
CH	0	109	135	165	195
DK-1	0	25	127		
DK-2	0	23	115		
DK-3	0				200

CN, control treatment; LN, low N addition; MN, medium N addition; HN, high N addition; and VHN, very high N addition.

Table 3 Effects of nutrient loading (N, P), heating, salinity and water level on (a) water column total nitrogen (TN) and NH_4^+ and (b) NO_3^- loss per unit of volume and relative loss ($N_{pool\%}$) in the six mesocosm experiments. Left columns present the main effects of a SAS autoregressive model, and the right columns the main effects of ANOVA analysis

Autoregressive model (loss per unit volume)					ANOVA (relative loss)	
Experiment	Variable	Treatment	*F*-value	*P*-value	*F*-value	*P*-value
(a)						
TR	TN	N	109	<0.01	331	<0.01
		P	5.1	0.04	8.8	<0.01
U.K.	TN	N	6750	<0.01	1050	<0.01
		Heating	1	0.32	3	0.09
CH	TN	N	93.5	<0.01	462	<0.01
	NH_4^+	N	75	<0.01	433	<0.01
DK-1	TN	N	795	<0.01	160	<0.01
		P	8.8	0.01	0.4	0.52
DK-2	TN	N	1270	<0.01	53.2	<0.01
		Salinity	0.2	0.9	0.6	0.77
DK-3	TN	N	830	<0.01	744	<0.01
		Salinity	4	0.05	34	<0.01
		Water level	8.2	<0.01	13	<0.01
(b)						
TR	NO_3^-	N	133	<0.01	83	<0.01
		P	3.9	<0.01	0.3	0.11
U.K.	NO_3^-	N	21 120	<0.01	203	<0.01
		Heating	0.5	0.5	0.02	0.9
CH	NO_3^-	N	40.9	<0.01	7.6	<0.01
DK-1	NO_3^-	N	Missing data points			
		P	Missing data points			
DK-2	NO_3^-	N	3300	<0.01	16	<0.01
		Salinity	0.8	0.5	1.8	0.13
DK-3	NO_3^-	N	115 000	<0.01	380	<0.01
		Salinity	0.3	0.6	4.7	0.04
		Water level	468	<0.01	6.3	<0.01

water column N loss with time. Similarly, there was no significant difference in $N_{pool\%}$ between the ambient and heated mesocosms for time-weighted means (Table 3a,b: Figs 1 & 2), although non-time-weighted means were weakly significantly different (ANOVA, $P = 0.03$). Nitrogen loading significantly increased the $TN_{pool\%}$ by 40–43% in HN treatments and 11% in LN treatments, compared with controls, and $NO_3^-{}_{pool\%}$ by 60–70% in the HN treatments and 75% in the LN treatments, also compared with controls (Figs 1 & 2). The mean NO_3^- concentration was <1 mg L^{-1} during the experiment. Of the N added, almost all was removed from the water column in both N treatments (Fig. 3), whereas the controls consistently accumulated N (Fig. 1).

Chinese (CH) experiment

Higher N loading increased the mean water column loss per unit area of TN and NH_4^+, but not of NO_3^- (Tables 2a,b & 3a,b). In contrast to the other five experiments, the mesocosms of the VHN treatments accumulated NO_3^-, on average by 8 mg m^{-2} day^{-1}, whereas all controls were sinks for NO_3^- by 4.6 mg m^{-2} day^{-1}. Mean water column loss values of TN and NO_3^- in different N treatments are given in Table 2a,b. The mean NO_3^- concentration in the VHN treatment was >11 mg NO_3^- L^{-1}, the highest among the six experiments. No significant change in water column loss per unit area over time was observed for TN, NH_4^+ or NO_3^-. Water column TN loss calculated as $N_{pool\%}$ decreased with increasing loading (Fig. 1) and showed significant variation only among the CN, LN and VHN treatments (Table 3a). Mesocosms receiving NH_4^+ additions acted as sinks for NH_4^+, as opposed to the mesocosms receiving NO_3^-, in which NO_3^- accumulated (Figs 1 & 2).

Danish (DK-1) experiment

Means of water column TN and NO_3^- loss are given in Table 2a,b. $TN_{pool\%}$ was highest in the MNHP treatment (27.0 ± 0.47% per 2 week), but could not be calculated for NO_3^- because too many data are missing. As in the

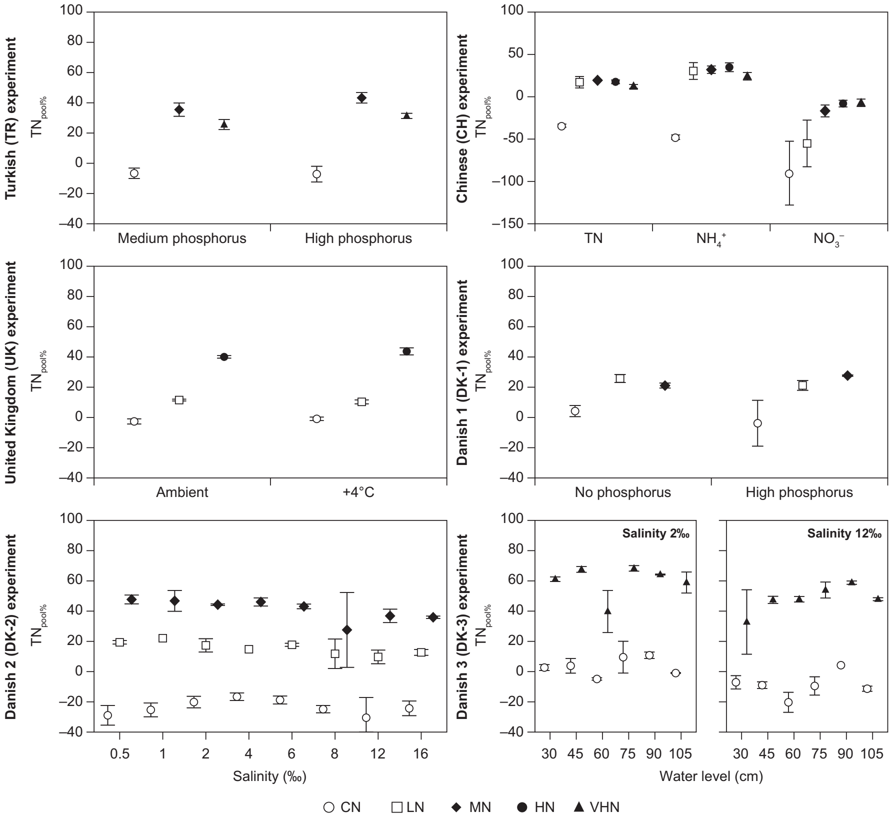

Fig. 1 Mean (±SD) relative total nitrogen (TN) loss (as per cent of N lost from the total N pool) for five different N treatments and additional treatments in the six mesocosm experiments. Negative values represent growth of the N pool.

Turkish experiment, HP addition resulted in increased water column TN loss, both per unit area and as $N_{pool\%}$ in the MN treatments, but not in LN treatments (Table 3a; Fig. 1).

Danish (DK-2) experiment

An increase in salinity did not significantly affect water column TN and NO_3^- loss (as $N_{pool\%}$) in the LN and MN treatments (Table 3a,b; Figs 1 & 2). Water column TN and NO_3^- loss per unit area and as $N_{pool\%}$ increased with increasing N load at all salinities (Table 3a,b). Mean NO_3^- concentration was <1.5 mg L^{-1} in all CN, LN and MN treatments (Fig. 2). On average, 100% of the nitrogen added was removed from the water column in 2 weeks.

Danish (DK-3) experiment

High salinity (12 ‰) significantly reduced the mean water column TN loss per unit area compared with low salinity (2‰), but not that of NO_3^- (Tables 2a,b & Table 3a,b). The mean NO_3^- concentration during the experiment was <0.2 mg L^{-1} in the CN and VHN treatments (Fig. 2). Both N addition and salinity had significant effects on the relative water column TN and NO_3^- loss (Table 3a,b), while no effect was observed among water levels. As in the DK-2 experiment, mean $TN_{pool\ \%}$ was 10–30% higher in the low-salinity treatments (2‰) than in the high-salinity treatments (12‰) (Table 3a; Fig. 1), and we also observed interaction between salinity and water level in $N_{pool\%}$ (salinity*water level, $P = 0.04$). Mesocosms with no addition gained TN and NO_3^-, and mesocosms with N addition were TN and NO_3^- sinks.

Cross-site analysis of theoretical water column loss time T and $N_{load\%}$

$N_{load\%}$ for TN showed no clear response to N treatments in the six experiments (Fig. 3). In the TR and DK-2 experiments, $N_{load\%}$ increased with increasing loading, while in CH and DK-1 experiments, $N_{load\%}$ decreased. The U.K. experiment showed no change in $N_{load\%}$ between LN and HN treatments. In the LN, MN, HN and VHN treatments of the six experiments, $N_{load\%}$ averaged 100% in 2 weeks, with more variation among LN treatments.

The relative water column loss time varied between 20 and 160 days in the six experiments and decreased

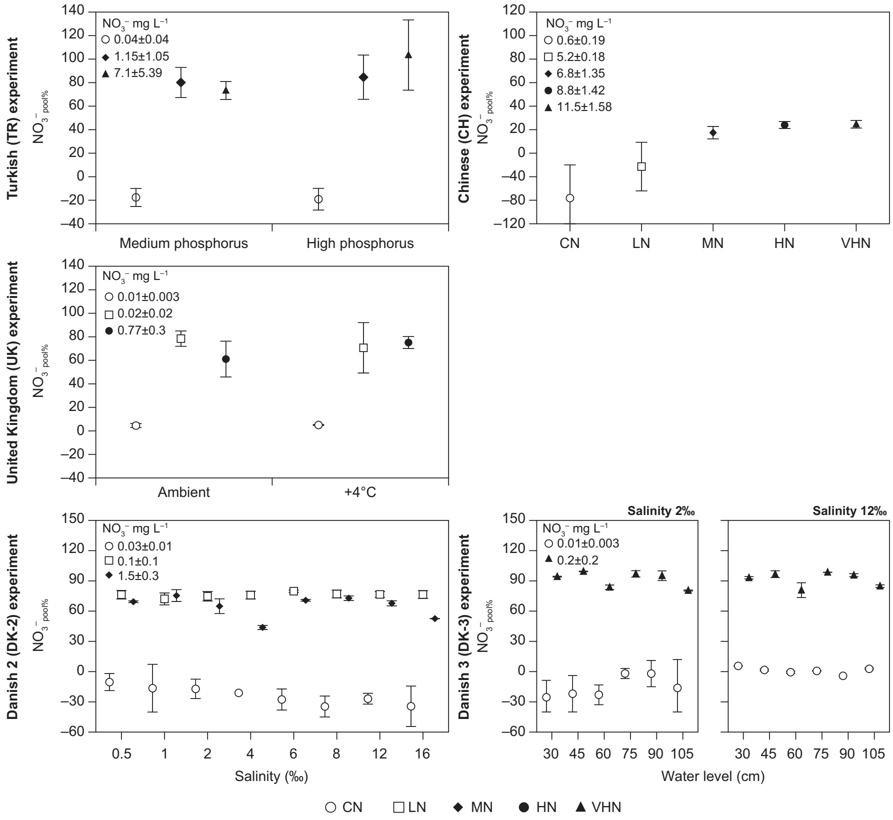

Fig. 2 Mean (±SD) NO_3^- loss (as percentage of N lost from the total NO_3^- pool) for five different N treatments and additional treatments in five mesocosm experiments where data were available. Legend in the left corner of each figure shows mean (±SD) NO_3^- concentration (mg N L^{-1}) during each experiment. Owing to too many missing data points, $NO^-_{3\,\mathrm{pool}\%}$ could not be calculated for DK-1. Negative values represent growth of the N pool.

consistently with increasing loading. Water column TN loss time was on average 50 days in the HN treatments of all experiments except CH, while turnover time of LN treatments in all experiments varied between 70 and 140 days (Fig. 4). In contrast to the other experiments, in the CH experiment, the TN turnover time increased with loading among MN, HN and VHN treatments, and NO_3^- accumulated. In all six experiments, controls were accumulated N, but to limited extents.

Discussion

The six experiments were conducted under different conditions, but some general patterns in water column loss emerged, as well as clearer specific patterns in individual experiments. Mean water column nitrogen loss per unit area increased with N load in all experiments and showed little change with time, whereas the theoretical time for all nitrogen to be lost from the water column decreased with increasing loading. Mean water column TN loss in the high N treatments varied from 111 to 250 mg m^{-2} day^{-1} (means for the whole experimental period), being similar to rates found in studies of natural shallow lakes in cultivated landscapes (Smith *et al.*, 1989; Jensen *et al.*, 1992; Tang & Xie, 2000; Dumont *et al.*, 2005).

As expected for mesocosms without outlets, in which there is longer contact time between water and possible processing surfaces such as the sediment and biota, we found a consistently higher $N_{\mathrm{load}\%}$ than usually recorded in natural lakes (Ekholm, Malve & Kirkkala, 1997; Havens *et al.*, 2001), with no observed differences in $N_{\mathrm{load}\%}$ between N treatments, except for the DK-1 and Chinese (CH) experiments, which showed a strong decrease with increasing N loading. The $TN_{\mathrm{load}\%}$ varied between 50 and 150% per 2 week in the high N treatments of the experiments. Removal efficiency does not seem to be sensitive to external N loading, as previously suggested by Jensen *et al.* (1992), but was affected by the N pool already present in the mesocosms.

The time calculated for complete loss at high N addition in all experiments was quite long, up to 50 days and, in CH, even longer, suggesting that N is remineralised several times before being permanently buried in the sediment, taken up by periphyton or lost through denitrification. Thus, the majority of the N in the water column of the HN and VHN mesocosms stayed as organic nitrogen. In these relatively long-term (up to more than 1 year) mesocosm experiments, periphyton assimilation of N may potentially be considerable, not least because the mesocosm walls provide a large surface area suitable for algal colonisation (Cattaneo &

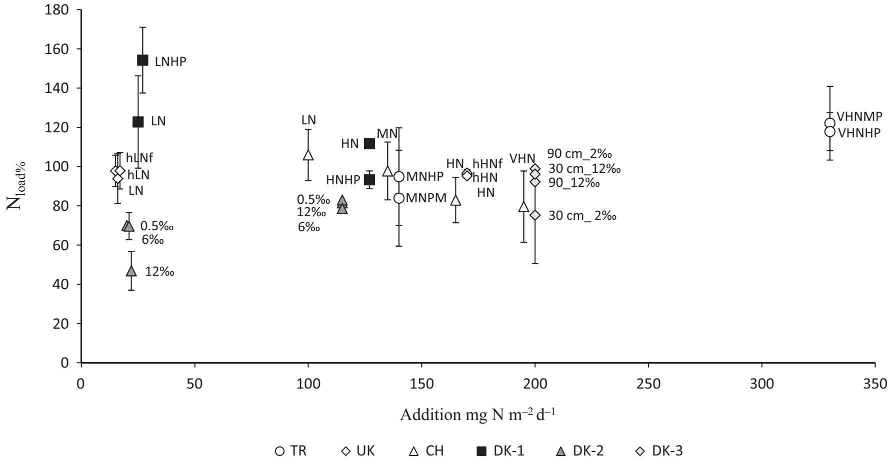

Fig. 3 Water column N loss as percentage of loading [$N_{load\%}$, mean (±SD)] versus N load with different temperatures, salinities and water levels in the six mesocosm experiments. Abbreviations for the four different N additions are as follows: LN, low nitrogen; MN, medium nitrogen; HN, high nitrogen; VHN, very high nitrogen. Abbreviations for additional treatments are as follows: MP, medium phosphorus; HP, high phosphorus; h, heating; f, fish; ‰, salinity in per mille; cm, water level in centimetres.

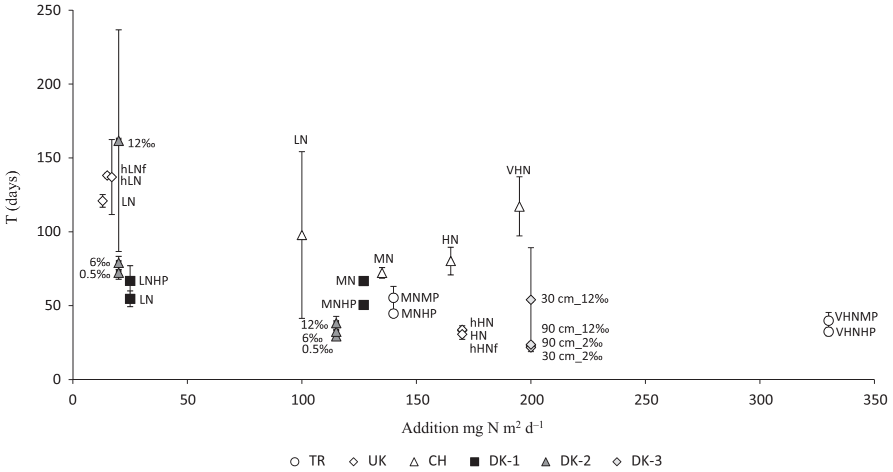

Fig. 4 Mean (±SD) water column N loss time (*T*), as the theoretical number of days to reach 100% removal, versus N load in different temperature, salinity and water-level treatments in the six mesocosm experiments. Abbreviations as in Fig. 3.

Amireault, 1992; Vadeboncoeur *et al.*, 2006). There are indications that increased mesocosm wall-area-to-volume ratio increases periphyton biomass and metabolism (Chen, Petersen & Kemp, 1997), and in oligotrophic lakes periphyton uptake can comprise up to 80% of primary production, whereas in mesotrophic and eutrophic lakes more primary production is allocated to phytoplankton than periphyton (Vadeboncoeur *et al.*, 2003). However, as all the six experiments had relatively nutrient-rich conditions, and all, except for DK-2 and DK-3, contained submerged macrophytes, the proportions of periphyton assimilation on mesocosm walls and epipelic growth on sediment surfaces were likely to be lower than reported in some previous studies, for example Wurtsbaugh *et al.* (2001), Vadeboncoeur *et al.* (2003, 2008), although notably high periphyton growth was recorded in the TR experiment [0.92 μg Chl-*a* (mg DW macrophyte^{-1}) in HNHP treatments].

While we found that N load alone did not determine water column N loss, high P additions in the TR and DK-1 experiments increased relative water column TN loss compared with treatments when no P or medium P was added. As observed by Persson & Broberg (1985), Ahlgren *et al.* (1994) and Berge *et al.* (1997), eutrophic lakes have a higher NO_3^- loss than oligotrophic lakes, and in eutrophic lakes loss is more related to biological processes, such as denitrification and phytoplankton assimilation, than to sedimentation (Jansson *et al.*, 1994). This concurs with a recent comparison among large lakes worldwide (Finlay, Small & Sterner, 2013). However, in our experiments, the percentage increase in water column loss owing to P addition was considerably smaller than observed in an experimental study of Kaste & Lyche-Solheim (2005) in a Swedish humic lake, who found an up to 70% higher N loss following a concentration increase of 10–12 μg P L^{-1}. By contrast, the approximately 100 and 500 μg P L^{-1} increase in the TR and DK-1 experiments resulted only in a 10% increase in N loss, perhaps reflecting N saturation because of the high N loading in these experiments. Uptake rates are limited by the capacity of macrophytes, algae and microbes, and these pools may have become saturated with N leading to decline in assimilation and hence the relative water column N loss over time (Dodds *et al*., 2002).

Contrary to our hypothesis, temperature had no effect on water column N loss, and no significant difference was found between ambient and +4 °C temperatures in the U.K. experiment. However, NO_3^- remained relatively constant (Fig. 2) and accumulated in the water column in the CH experiment, conducted in a subtropical climate, contrasting with the results from the five other experiments. In the Turkish (warm lake) experiment, no change in NO_3^- was observed. These results were somewhat unexpected, as the high temperatures in subtropical waters should potentially stimulate microbial processes, such as denitrification, and thus also NO_3^- consumption (Downing *et al.* 1999). Moreover, Dumont *et al.* (2005) and Baron *et al.* (2013) argued that pristine (sub)tropical lakes lose a larger portion of terrestrially derived N than their temperate counterparts, and the primary production is therefore usually N-limited. However, increased organic carbon and a reducing environment (e.g. low oxygen concentrations) also increase denitrification, especially if NO_3^- is abundant (Seitzinger, 1988; Mitchell & Baldwin, 1999; Šimek & Cooper, 2002; Taylor & Townsend, 2010). Organic matter mineralisation in the CH mesocosms may have been higher in the water column and at the sediment surface owing to the higher temperatures, leaving less organic matter available for the denitrifiers; we did not find higher oxygen concentration in the water column in this experiment than in the TR, DK and U.K. experiments. Our results are supported by Kosten *et al.* (2009), who found consistently high dissolved inorganic nitrogen (DIN) concentrations in some warm and shallow South American lakes; they also discovered that the contribution of NO_3^- and NO_2^- to DIN was higher in tropical lakes than in similar lakes at higher latitudes. Further indications of DIN accumulation in warmer climates have been observed in a mesocosm experiment conducted in subtropical Wuhan, China (S. Olsen et al. unpubl. data). There, the relative water column TN loss was on average 50% per 2 week and water column NO_3^- loss was 45% per 2 week in the high N treatment. Corresponding with the CH experiment, the relative water column loss of NO_3^- in this experiment decreased with increasing loading and NO_3^- accumulated in the water column, whereas NH_4^+ was rapidly consumed, perhaps due to nitrification.

However, the VHN loading in the CH experiment (195 mg m^{-2} day^{-1}) was among highest of the six experiments and thus may have contributed to the growth of the DIN pool. But, the loading in TR, DK-2 and U.K. experiments was also high, with 330 mg m^{-2} day^{-1}, 200 mg m^{-2} day^{-1} and 170 mg m^{-2} day^{-1}, respectively. Despite the high loading in the DK-2 and U.K. experiments, the relative water column NO_3^- loss in the VHN and HN treatments of these mesocosms was >60% per 2 wk and mean concentration <1 mg L^{-1}. Moreover, the mean NO_3^- concentration in the VHN treatment of the CH experiments was 11 mg L^{-1}. We propose that, although high loading could to some extent explain the low water column loss in the CH experiment, the effect of low carbon is also important. Temperature is one of the most important factors controlling the metabolism of aquatic organisms, but denitrifiers may be carbon-limited owing to fast mineralisation, thus counteracting the positive effect of temperature on metabolism.

As with the effect of temperature, the impact of salinity on water level N loss was variable, and significant only in the DK-3 experiment. The DK-2 experiment also showed indications of decreased relative water column loss with increasing salinity in all N treatments, but the results were not statistically significant. In support of our results, the study by Gophen & Paz (1992) of Lake Kinneret and the study by González *et al.* (1998) of the Salton Sea showed that a decline in salinity was followed by an increase in TN loss. They argued that an enhanced metabolism of nitrifiers at low salinity possibly produced more NO_3^- for denitrification, increasing the N loss. Moreover, Nowicki (1994) argued that, even though increased N availability also enhanced denitrification in saline estuaries, the mean N loss was <20% of the total N input $year^{-1}$. Thus, the denitrifiers could not cope with the N addition and quickly crossed the threshold for N saturation at higher salinities. However, as only a weak correlation was observed between salinity and N loss, this was perhaps a result of a positive relation between high salinity and sedimentation rates, particle aggregation and increasing Secchi depth (Forsgren, Jansson & Nilsson, 1996; Håkanson, 2006). Contrary to the results of Gophen & Paz (1992) and González *et al.* (1998), Heiskanen & Tallberg (1999) found a lower C : N ratio, and thus also raised N concentrations, with lower salinity in the partially enclosed Pojo Bay of the Baltic Sea. Further, they observed 30% higher sedimentation of N (in relation to C) in the open sea than in the bay. Thus, although an increase in salinity results in enhanced sedimentation which can account for up to 40% of the N loss in productive lakes, it does not necessarily compensate for the N accumulation resulting from decreased denitrification (Jensen *et al.*, 1992; Molot & Dillon, 1993; Benoy & Kalff, 1999; Saunders & Kalff, 2001).

Although water column N loss was not directly affected by changes in water level, we found an interaction between salinity, water level and relative water column loss. The effect of water level may be minor at a small scale, such as that of a mesocosm, but may have a larger effect in the littoral zone of a whole lake. Similarly, Chow-Fraser *et al.* (1998) and Coops *et al.* (2003) observed a lower N loss at high water levels in shallow lakes, mainly due to reduced macrophyte assimilation. For primary producers, water-level fluctuations mean significant variation in the light environment in the water column. A decreased water depth may increase macrophyte growth and assimilation of nutrients when plants become less restricted by light availability, thus potentially increasing water column N loss (Nõges & Nõges, 1999), but this may not be the case in warm lakes (Özen *et al.*, 2010). The phytoplankton biomass (expressed as Chl-*a*) in the DK-3 experiment (no macrophytes) increased gradually with decreasing water level, in both the high- and low-salinity treatments. However, no significant correlation was detected between Chl-*a* and water column NO_3^- loss in this experiment, suggesting that assimilation by phytoplankton had minor effect. Our results agree with previous findings that high salinity inhibits processes important for N processing and may be exacerbated by high water levels.

Because atmospheric deposition, nitrogen fixation and groundwater seepage were not considered, water column N loss was probably underestimated, but is likely to have been modest.. Atmospheric deposition may have accounted for a 2–4 mg m^{-2} day^{-1} increase in N in the DK and TR experiments (Hertel *et al.*, 2013; Im *et al.*, 2013); a 2–9 mg m^{-2} day^{-1} increase in the U.K. experiment (Stevens *et al.*, 2004; Fowler *et al.*, 2005); and a 6–15 mg m^{-2} day^{-1} increase in the CH experiment (Liu *et al.*, 2011; Shen *et al.*, 2013). These estimates suggest mean increases of 2%, 20% and 10% in the LN treatments and <6% in the HN treatments. Similarly, an unaccounted nitrate gain may have resulted from nitrification by sedimentary bacteria. Nitrogen fixation was not measured, and thus not accounted for, in the mass balance, but is likely to have been minor as most of the NO_3^- produced by nitrifiers is likely to have been denitrified locally in these organic sediments in coupled processes. Philips *et al.* (1997), Tõnno & Nõges (2003) and Nielsen, Enrich-Prast & Esteves (2004) estimated that N fixation can account for up to 2.5 mg m^{-2} day^{-1} of the N input to lakes experiencing blooms of N-fixing cyanobacteria, potentially contributing on average 2, 2.3, 10 and 14% in the LN treatments in the TR, CH, DK and U.K. experiments, respectively, and 1, 1.2, 2 and 1.5% in the HN treatments in the TR, CH, DK and U.K. experiments, respectively. However, cyanobacteria were scarce in the U.K. experiment. Mesocosms in Denmark and Turkey were placed in the littoral zone of a lake, thus allowing groundwater seepage and diffusion of water between lake and mesocosm through the sediment. Ground water is an important source of water into many lakes and, depending on the geology of the area, may comprise a significant input of the annual N loading (LaBaugh, Rosenberry & Winter, 1995; Hayashi & Rosenberry, 2002). However, waterlogged sediments are less permeable than soils. Belanger, Mikutel & Churchill (1985) estimated the mean nitrate flux from ground water to East Lake Tohopekaliga to be 0.09 mg m^{-2} day^{-1}, which would theoretically contribute on average <1% in the LN treatments and <0.1% in the HN treatments of the TR, CH, U.K. and DK experiments.

In conclusion, the mesocosms showed rapid water column loss of TN and NO_3^- in all the lakes, whether cool or warm. Although changes in salinity, water level and probably also temperature may to some extent influence the water column loss, the effects of N and P loads were of much greater importance. This implies that water column N loss in lakes is more sensitive to the nature of the catchment, reflected in changes in hydrology and loading, than to climate-induced effects related to temperature, salinity and water level. The results further indicate that lakes characterised by P limitation may become N-saturated faster than those with high P loading.

Although mesocosms are widely used to study eutrophication and nutrient dynamics in aquatic environments, there are limitations in extrapolating results from mesocosms to natural lakes. Particular problems include lack of water circulation and the presence of walls that create additional habitat for organisms. Therefore, some of the processes measured here may be overestimated; water column N loss may have been generally greater than that in a natural lake, but it is likely that the main conclusion that loading is the key consideration compared with other factors will pertain. In any case, mesocosms at least offer a means of assessing these processes in a much more controlled way than is possible in nature.

Acknowledgments

This project and Saara Olsen were supported by SDC (Sino-Danish Center for Education and Research), CLEAR (a Villum Kann Rasmussen Centre of Excellence project) and FP7/ENV-2009-1 under grant agreement 244121 (REFRESH project – http://www.refresh.ucl.ac.uk/), the MARS project (Managing Aquatic ecosystems and water Resources under multiple Stress) funded under the 7th EU Framework Programme, Theme 6 (Environment including Climate Change), Contract No.: 603378 (http://www.mars-project.eu). KÖ and the Turkish experiment were supported in part by The Scientific and Technological Research Council of Turkey (project no: 105Y332), Middle East Technical University (project no: BAP-2006-07-02-00-01, and MB was supported by Middle East Technical University. China's experiment was financed by NSF-China (30570291) and Wuhan Academic Leader Programme (201051730562). We would also like to thank the following scientists for valuable ideas and formulation of research questions: Henning Jensen and the staff at the Department of Bioscience – Lake Ecology Section, Silkeborg. We would like to thank an anonymous reviewer and Wayne Wurtsbaugh for insightful reviews of earlier versions of the manuscript and for helping to determine some of the difficult terminology. Anne-Mette Poulsen is acknowledged for linguistic assistance.

References

Agawin N.S., Duarte C.M. & Agusti S. (2000) Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. *Limnology and Oceanography*, **45**, 591–600.

Ahlgren I., Sorensson F., Waara T. & Vrede K. (1994) Nitrogen budgets in relation to microbial transformations in lakes. *Ambio*, **23**, 367–377.

Barker T., Hatton K., O'Connor M., Connor L. & Moss B. (2008) Effects of nitrate load on submerged plant biomass and species richness: results of a mesocosm experiment. *Fundamental and Applied Limnology/Archiv für Hydrobiologie*, **173**, 89–100.

Baron J.S., Hall E.K., Nolan B.T., Finlay J.C., Bernhardt E.S., Harrison J.A. *et al.* (2013) The interactive effects of excess reactive nitrogen and climate change on aquatic ecosystems and water resources of the United States. *Biogeochemistry*, **114**, 71–92.

Belanger T., Mikutel D. & Churchill P. (1985) Groundwater seepage nutrient loading in a Florida lake. *Water Research*, **19**, 773–781.

Benoy G.A. & Kalff J. (1999) Sediment accumulation and Pb burdens in submerged macrophyte beds. *Limnology and Oceanography*, **44**, 1081–1090.

Berge D., Fjeld E., Hindar A. & Kaste Ø. (1997) Nitrogen retention in two Norwegian watercourses of different trophic status. *Ambio*, **26**, 282–288.

Bernot M.J. & Dodds W.K. (2005) Nitrogen retention, removal, and saturation in lotic ecosystems. *Ecosystems*, **8**, 442–453.

Brucet S., Boix D., Gascón S., Sala J., Quintana X.D., Badosa A. *et al.* (2009) Species richness of crustacean zooplankton and trophic structure of brackish lagoons in contrasting climate zones: north temperate Denmark and Mediterranean Catalonia (Spain). *Ecography*, **32**, 692–702.

Bucak T., Saraoğlu E., Levi E.E., Nihan Tavşanoğlu Ü., İdil Çakiroğlu A., Jeppesen E. *et al.* (2012) The influence of water level on macrophyte growth and trophic interactions in eutrophic Mediterranean shallow lakes: a mesocosm experiment with and without fish. *Freshwater Biology*, **57**, 1631–1642.

Camargo J.A. & Alonso Á. (2006) Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. *Environment International*, **32**, 831–849.

Cattaneo A. & Amireault M.C. (1992) How artificial are artificial substrata for periphyton? *Journal of the North American Benthological Society*, **11**, 244–256.

Chen C.-C., Petersen J.E. & Kemp W.M. (1997) Spatial and temporal scaling of periphyton growth on walls of estuarine mesocosms. *Marine Ecology Progress Series*, **155**, 1–15.

Chow-Fraser P., Lougheed V., Le Thiec V., Crosbie B., Simser L. & Lord J. (1998) Long-term response of the biotic community to fluctuating water levels and changes in water quality in Cootes Paradise Marsh, a degraded coastal wetland of Lake Ontario. *Wetlands Ecology and Management*, **6**, 19–42.

Christensen P.B. & Sørensen J. (1986) Temporal variation of denitrification activity in plant-covered, littoral sediment from Lake Hampen, Denmark. *Applied and Environmental Microbiology*, **51**, 1174–1179.

Cook P.L., Aldridge K.T., Lamontagne S. & Brookes J.D. (2010) Retention of nitrogen, phosphorus and silicon in a large semi-arid riverine lake system. *Biogeochemistry*, **99**, 49–63.

Coops H., Beklioğlu M. & Crisman T.L. (2003) The role of water-level fluctuations in shallow lake ecosystems–workshop conclusions. *Hydrobiologia*, **506**, 23–27.

Dodds W.K., López A.J., Bowden W.B., Gregory S., Grimm N.B., Hamilton S.K. *et al.* (2002) N uptake as a function of concentration in streams. *Journal of the North American Benthological Society*, **21**, 206–220.

Downing J.A., McClain M., Twilley R., MElack J.M., Elser J., Rabalais N.N., Lewis W.M. JR., Turner R.E., Corredor J., Soto D., Yanez-Arancibia A., Kopaska J.A., & Howarth R.W. (1999) The impact of accelerating land-use change on the N-cycle of tropical aquatic ecosystems: current conditions and projected changes. *Biogeochemistry*, **46**, 109–148.

Dumont E., Harrison J., Kroeze C., Bakker E. & Seitzinger S. (2005) Global distribution and sources of dissolved inorganic nitrogen export to the coastal zone: results from a spatially explicit, global model. *Global Biogeochemical Cycles*, **19**, 1–13.

Ekholm P., Malve O. & Kirkkala T. (1997) Internal and external loading as regulators of nutrient concentrations in the agriculturally loaded Lake Pyhäjärvi (southwest Finland). *Hydrobiologia*, **345**, 3–14.

Feuchtmayr H., Moran R., Hatton K., Connor L., Heyes T., Moss B. *et al.* (2009) Global warming and eutrophication: effects on water chemistry and autotrophic communities in experimental hypertrophic shallow lake mesocosms. *Journal of Applied Ecology*, **46**, 713–723.

Feuchtmayr H., Moss B., Harvey I., Moran R., Hatton K., Connor L. *et al.* (2010) Differential effects of warming and nutrient loading on the timing and size of the spring zooplankton peak: an experimental approach with hypertrophic freshwater mesocosms. *Journal of Plankton Research*, **32**, 1715–1725.

Finlay J.C., Small G.E. & Sterner R.W. (2013) Human influences on nitrogen removal in lakes. *Science*, **342**, 247–250.

Fleischer S., Gustafson A., Joelsson A., Pansar J. & Stibe L. (1994) Nitrogen removal in created ponds. *Ambio*, **23**, 349–357.

Fleischer S., Stibe L. & Leonardson L. (1991) Restoration of wetlands as a means of reducing nitrogen transport to coastal waters. *Ambio*, **20**, 271–272.

Forsgren G., Jansson M. & Nilsson P. (1996) Aggregation and sedimentation of iron, phosphorus and organic carbon in experimental mixtures of freshwater and estuarine water. *Estuarine, Coastal and Shelf Science*, **43**, 259–268.

Fowler D., Smith R., Muller J., Hayman G. & Vincent K. (2005) Changes in the atmospheric deposition of acidifying compounds in the UK between 1986 and 2001. *Environmental Pollution*, **137**, 15–25.

Fragoso C.R. Jr, Motta Marques D.M., Ferreira T.F., Janse J.H. & Van Nes E.H. (2011) Potential effects of climate change and eutrophication on a large subtropical shallow lake. *Environmental Modelling & Software*, **26**, 1337–1348.

Galloway J.N. (1998) The global nitrogen cycle: changes and consequences. *Environmental Pollution*, **102**, 15–24.

González M.R., Hart C.M., Verfaillie J.R. & Hurlbert S.H. (1998) Salinity and fish effects on Salton Sea microecosystems: water chemistry and nutrient cycling. *Hydrobiologia*, **381**, 105–128.

González Sagrario M.A., Jeppesen E., Gomà J., Søndergaard M., Jensen J.P., Lauridsen T. *et al.* (2005) Does high nitrogen loading prevent clear-water conditions in shallow lakes at moderately high phosphorus concentrations? *Freshwater Biology*, **50**, 27–41.

Gophen M. & Paz J. (1992) Decreased salinity effects in Lake Kinneret (Israel). *Hydrobiologia*, **228**, 231–237.

Gudasz C., Bastviken D., Steger K., Premke K., Sobek S. & Tranvik L.J. (2010) Temperature-controlled organic carbon mineralization in lake sediments. *Nature*, **466**, 478–481.

Håkanson L. (2006) The relationship between salinity, suspended particulate matter and water clarity in aquatic systems. *Ecological Research*, **21**, 75–90.

Håkanson L., Gyllenhammar A. & Brolin A. (2004) A dynamic compartment model to predict sedimentation and suspended particulate matter in coastal areas. *Ecological Modelling*, **175**, 353–384.

Harrison J.A., Maranger R.J., Alexander R.B., Giblin A.E., Jacinthe P.-A., Mayorga E. *et al.* (2009) The regional and global significance of nitrogen removal in lakes and reservoirs. *Biogeochemistry*, **93**, 143–157.

Havens K., Fukushima T., Xie P., Iwakuma T., James R., Takamura N. *et al.* (2001) Nutrient dynamics and the eutrophication of shallow lakes Kasumigaura (Japan), Donghu (PR China), and Okeechobee (USA). *Environmental Pollution*, **111**, 263–272.

Hayashi M. & Rosenberry D.O. (2002) Effects of ground water exchange on the hydrology and ecology of surface water. *Ground Water*, **40**, 309–316.

Heiskanen A.-S. & Tallberg P. (1999) Sedimentation and particulate nutrient dynamics along a coastal gradient from a fjord-like bay to the open sea. *Hydrobiologia*, **393**, 127–140.

Hertel O., Geels C., Frohn L.M., Ellermann T., Skjøth C.A., Løfstrøm P. *et al.* (2013) Assessing atmospheric nitrogen deposition to natural and semi-natural ecosystems – experience from Danish studies using the DAMOS. *Atmospheric Environment*, **66**, 151–160.

Hessen D.O., Hindar A. & Holtan G. (1997) The significance of nitrogen runoff for eutrophication of freshwater and marine recipients. *Ambio*, **26**, 312–320.

Im U., Christodoulaki S., Violaki K., Zarmpas P., Kocak M., Daskalakis N. *et al.* (2013) Atmospheric deposition of nitrogen and sulfur over southern Europe with focus on the Mediterranean and the Black Sea. *Atmospheric Environment*, **81**, 660–670.

Jackson L.J. (2003) Macrophyte-dominated and turbid states of shallow lakes: evidence from Alberta lakes. *Ecosystems*, **6**, 213–223.

Jansson M., Andersson R., Berggren H. & Leonardson L. (1994) Wetlands and lakes as nitrogen traps. *Ambio*, **23**, 320–325.

Jensen E., Brucet S., Meerhoff M., Nathansen L. & Jeppesen E. (2010) Community structure and diel migration of zooplankton in shallow brackish lakes: role of salinity and predators. *Hydrobiologia*, **646**, 215–229.

Jensen J., Kristensen P. & Jeppesen E. (1990) Relationships between nitrogen loading and in-lake concentrations in shallow Danish lakes. *International Association of Theoretical and Applied Limnology. Proceedings*, **24**, 201–204.

Jensen J.P., Jeppesen E., Kristensen P., Christensen P.B. & Søndergaard M. (1992) Nitrogen loss and denitrification as studied in relation to reductions in nitrogen loading in a shallow, hypertrophic lake (Lake Søbygård, Denmark).

Internationale Revue der gesamten Hydrobiologie und Hydrographie, **77**, 29–42.

Jeppesen E., Søndergaard M., Pedersen A.R., Jürgens K., Strzelczak A., Lauridsen T.L. *et al.* (2007) Salinity induced regime shift in shallow brackish lagoons. *Ecosystems*, **10**, 48–58.

Karakoç G., Ünlü Erkoç F. & Katırcıoğlu H. (2003) Water quality and impacts of pollution sources for Eymir and Mogan Lakes (Turkey). *Environment International*, **29**, 21–27.

Kaste Ø. & Lyche-Solheim A. (2005) Influence of moderate phosphate addition on nitrogen retention in an acidic boreal lake. *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 312–321.

Keeney D. (1973) The nitrogen cycle in sediment-water systems. *Journal of Environmental Quality*, **2**, 15–29.

Keeney D., Chen R. & Graetz D. (1971) Importance of denitrification and nitrate reduction in sediments to the nitrogen budgets of lakes. *Nature*, **233**, 66–67.

Kemp M.J. & Dodds W.K. (2002) The influence of ammonium, nitrate, and dissolved oxygen concentrations on uptake, nitrification, and denitrification rates associated with prairie stream substrata. *Limnology and Oceanography*, **47**, 1380–1393.

Kosten S., Huszar V.L., Mazzeo N., Scheffer M., Sternberg L.D.S. & Jeppesen E. (2009) Lake and watershed characteristics rather than climate influence nutrient limitation in shallow lakes. *Ecological Applications*, **19**, 1791–1804.

LaBaugh J.W., Rosenberry D.O. & Winter T.C. (1995) Groundwater contribution to the water and chemical budgets of Williams Lake, Minnesota, 1980-1991. *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 754–767.

Lewis W.M. Jr, Wurtsbaugh W.A. & Paerl H.W. (2011) Rationale for control of anthropogenic nitrogen and phosphorus to reduce eutrophication of inland waters. *Environmental Science & Technology*, **45**, 10300–10305.

Li W., Zhang Z. & Jeppesen E. (2008) The response of *Vallisneria spinulosa* (*Hydrocharitaceae*) to different loadings of ammonia and nitrate at moderate phosphorus concentration: a mesocosm approach. *Freshwater Biology*, **53**, 2321–2330.

Lick W., Lick J. & Ziegler C.K. (1992) Flocculation and its effect on the vertical transport of fine-grained sediments. *Sediment/Water Interactions*, **75**, 1–16.

Likens G. (1972) Nutrients and eutrophication, American Society of Limnology Oceanography special symposium 1. *American Society of Limnology Oceanography, Lawrence*, **1**, 1–329.

Liu X., Duan L., Mo J., Du E., Shen J., Lu X. *et al.* (2011) Nitrogen deposition and its ecological impact in China: an overview. *Environmental Pollution*, **159**, 2251–2264.

Loverde-Oliveira S.M., Huszar V.L.M., Mazzeo N. & Scheffer M. (2009) Hydrology-driven regime shifts in a shallow tropical lake. *Ecosystems*, **12**, 807–819.

Madsen T.V. & Brix H. (1997) Growth, photosynthesis and acclimation by two submerged macrophytes in relation to temperature. *Oecologia*, **110**, 320–327.

McCarthy M., Lavrentyev P., Yang L., Zhang L., Chen Y., Qin B. *et al.* (2007) Nitrogen dynamics and microbial food web structure during a summer cyanobacterial bloom in a subtropical, shallow, well-mixed, eutrophic lake (Lake Taihu, China). *Hydrobiologia*, **281**, 195–207.

Messer J. & Brezonik P. (1978) Denitrification in the sediments of lake Okeechobee. *Verhandlungen des Internationalen Verein Limnologie*, **20**, 2207–2216.

Mitchell A. & Baldwin D.S. (1999) The effects of sediment desiccation on the potential for nitrification, denitrification, and methanogenesis in an Australian reservoir. *Hydrobiologia*, **392**, 3–11.

Molot L.A. & Dillon P.J. (1993) Nitrogen mass balances and denitrification rates in central Ontario lakes. *Biogeochemistry*, **20**, 195–212.

Moran R., Harvey I., Moss B., Feuchtmayr H., Hatton K., Heyes T. *et al.* (2010) Influence of simulated climate change and eutrophication on three-spined stickleback populations: a large scale mesocosm experiment. *Freshwater Biology*, **55**, 315–325.

Moss B. (2010) *Ecology of Fresh Waters: A View for the Twenty-First Century*, 480 pp. Wiley-Blackwell Publishing, London.

Moss B., Jeppesen E., Søndergaard M., Lauridsen T.L. & Liu Z. (2013) Nitrogen, macrophytes, shallow lakes and nutrient limitation: resolution of a current controversy? *Hydrobiologia*, **710**, 3–21.

Nielsen L., Enrich-Prast A. & Esteves F. (2004) Pathways of organic matter mineralization and nitrogen regeneration in the sediment of five tropical lakes. *Acta Limnologica Brasiliensia*, **16**, 193–202.

Nõges T. & Nõges P. (1999) The effect of extreme water level decrease on hydrochemistry and phytoplankton in a shallow eutrophic lake. *Hydrobiologia*, **408**, 277–283.

Nowicki B.L. (1994) The effect of temperature, oxygen, salinity, and nutrient enrichment on estuarine denitrification rates measured with a modified nitrogen gas flux technique. *Estuarine, Coastal and Shelf Science*, **38**, 137–156.

Özen A., Karapınar B., Kucuk İ., Jeppesen E. & Beklioğlu M. (2010) Drought-induced changes in nutrient concentrations and retention in two shallow Mediterranean lakes subjected to different degrees of management. *Hydrobiologia*, **646**, 61–72.

Özkan K., Jeppesen E., Johansson L.S. & Beklioğlu M. (2010) The response of periphyton and submerged macrophytes to nitrogen and phosphorus loading in shallow warm lakes: a mesocosm experiment. *Freshwater Biology*, **55**, 463–475.

Persson G. & Broberg O. (1985) Nutrient concentrations in the acidified Lake Gårdsjön: the role of transport and retention of phosphorus, nitrogen and DOC in watershed and lake. *Ecological Bulletins*, **37**, 158–175.

Philips E.J., Cichra M., Havens K., Hanton C., Badylak S., Rueter B. *et al.* (1997) Relationships between phytoplankton dynamics and the availability of light and nutrients in a shallow sub-tropical lake. *Journal of Plankton Research*, **19**, 319–342.

Pinay G., Gumiero B., Tabacchi E., Gimenez O., Tabacchi-Planty A.M., Hefting M.M. *et al.* (2007) Patterns of denitrification rates in European alluvial soils under various hydrological regimes. *Freshwater Biology*, **52**, 252–266.

Qiu D., Wu Z., Liu B., Deng J., Fu G. & He F. (2001) The restoration of aquatic macrophytes for improving water quality in a hypertrophic shallow lake in Hubei Province, China. *Ecological Engineering*, **18**, 147–156.

Saunders D. & Kalff J. (2001) Nitrogen retention in wetlands, lakes and rivers. *Hydrobiologia*, **443**, 205–212.

Schindler D.W. (1974) Eutrophication and recovery in experimental lakes: implications for lake management. *Science*, **184**, 897–899.

Schindler D.W., Hecky R., Findlay D., Stainton M., Parker B., Paterson M. *et al.* (2008) Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. *Proceedings of the National Academy of Sciences*, **105**, 11254–11258.

Seitzinger S., Harrison J.A., Böhlke J., Bouwman A., Lowrance R., Peterson B. *et al.* (2006) Denitrification across landscapes and waterscapes: a synthesis. *Ecological Applications*, **16**, 2064–2090.

Seitzinger S.P. (1988) Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. *Limnology and Oceanography*, **33**, 702–724.

Shen J., Li Y., Liu X., Luo X., Tang H., Zhang Y. *et al.* (2013) Atmospheric dry and wet nitrogen deposition on three contrasting land use types of an agricultural catchment in subtropical central China. *Atmospheric Environment*, **67**, 415–424.

Šimek M. & Cooper J. (2002) The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. *European Journal of Soil Science*, **53**, 345–354.

Smith S., Serruya S., Geifman Y. & Berman T. (1989) Internal sources and sinks of water, P, N, Ca, and Cl in Lake Kinneret, Israel. *Limnology and Oceanography*, **34**, 1202–1213.

Stevens C.J., Dise N.B., Mountford J.O. & Gowing D.J. (2004) Impact of nitrogen deposition on the species richness of grasslands. *Science*, **303**, 1876–1879.

Tang H. & Xie P. (2000) Budgets and dynamics of nitrogen and phosphorus in a shallow, hypereutrophic lake in China. *Journal of Freshwater Ecology*, **15**, 505–514.

Taylor P.G. & Townsend A.R. (2010) Stoichiometric control of organic carbon–nitrate relationships from soils to the sea. *Nature*, **464**, 1178–1181.

Tilley D.R., Ahmed M., Son J.H. & Badrinarayanan H. (2007) Hyperspectral reflectance response of freshwater macrophytes to salinity in a brackish subtropical marsh. *Journal of Environmental Quality*, **36**, 780–789.

Tõnno I. & Nõges T. (2003) Nitrogen fixation in a large shallow lake: rates and initiation conditions. *Hydrobiologia*, **490**, 23–30.

Vadeboncoeur Y., Jeppesen E., Vander Zanden M.J., Schierup H.-H., Christoffersen K. & Lodge D.M. (2003) From Greenland to green lakes: cultural eutrophication and the loss of benthic pathways in lakes. *Limnology and Oceanography*, **48**, 1408–1418.

Vadeboncoeur Y., Kalff J., Christoffersen K. & Jeppesen E. (2006) Substratum as a driver of variation in periphyton chlorophyll and productivity in lakes. *Journal of the North American Benthological Society*, **25**, 379–392.

Vadeboncoeur Y., Peterson G., Vander Zanden M.J. & Kalff J. (2008) Benthic algal production across lake size gradients: interactions among morphometry, nutrients, and light. *Ecology*, **89**, 2542–2552.

Vitousek P.M., Aber J.D., Howarth R.W., Likens G.E., Matson P.A., Schindler D.W. *et al.* (1997) Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications*, **7**, 737–750.

Vollenweider R.A. (1975) Input-output models. *Schweizerische Zeitschrift für Hydrologie*, **37**, 53–84.

Wetzel R. (2001) *Limnology: Lake and River Ecosystems*, 1006 pp. Academic Press, San Diego.

Windolf J., Jeppesen E., Jensen J.P. & Kristensen P. (1996) Modelling of seasonal variation in nitrogen retention and in-lake concentration: a four-year mass balance study in 16 shallow Danish lakes. *Biogeochemistry*, **33**, 25–44.

Woods H., Makino W., Cotner J., Hobbie S., Harrison J., Acharya K. *et al.* (2003) Temperature and the chemical composition of poikilothermic organisms. *Functional Ecology*, **17**, 237–245.

Wurtsbaugh W.A., Gross H.P., Budy P. & Luecke C. (2001) Effects of epilimnetic versus metalimnetic fertilization on the phytoplankton and periphyton of a mountain lake with a deep chlorophyll maxima. *Canadian Journal of Fisheries and Aquatic Sciences*, **58**, 2156–2166.