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ORIGINAL ARTICLE

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Effect of a nitrogen pulse on ecosystem N processing at different temperatures: A mesocosm experiment with $^{15}NO_3^-$ addition

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Abstract

1. Shallow lakes may play an important role for the nitrogen (N) balance in drainage basins by processing, transferring and retaining N inputs. An increase in the frequency of storm-induced short-term N pulses and increased water temperatures are both likely outcomes of climate change, potentially affecting the N processing in lakes.
2. An experiment with a $K^{15}NO_3^-$ pulse addition (increase in NO_3^- concentration from *c.* 0.1 to 2 mg/L) was carried out in 12 mesocosms with relatively low (applies to Danish lakes) total N (TN) and total phosphorus (TP) concentrations (*c.* 0.3 mg N L^{-1} and 0.04 mg P L^{-1}) to assess the effects of an N pulse on N processing and storage in shallow lake ecosystems. The mesocosms have a hydraulic retention time of approximately two and a half months, and at the time of the experiment, they had been adapted to contrasting temperatures for a period of 10 years: ambient, T3 (heating according to the Intergovernmental Panel on Climate Change 2007 A2 scenario, +3.7–4.5°C, depending on season) and T5 (heating with A2 + 50%, +4.9–6.6°C).
3. Macrophytes and filamentous algae retained up to 40% and 30% of the added ^{15}N, respectively, reflecting their high biomass in the mesocosms. Macrophytes and filamentous algae constituted between 70% and 80% of the biomass of all primary producers during the experiment in the T3 and ambient treatments and between 20% and 40% in T5. By comparison, less than 1% of the added ^{15}N diffused to the sediment and less than 5% was lost to the atmosphere as N_2 gas. Snails represented the long-term storage of ^{15}N, retaining up to 6% of the tracer and with detectable enrichment 100 days after tracer addition.
4. We found no significant differences among the temperature treatments in the ^{15}N turnover after pulse dosing. However, a larger percentage of ^{15}N was stored in macrophytes in the ambient and T3 mesocosms, reflecting higher biomasses than in T5 where filamentous algae were more abundant. Macrophytes and filamentous algae rather than temperature were therefore key controllers of N processing during the summer N pulse in these shallow, relatively low TP lakes.

KEYWORDS
^{15}N addition, mesocosm, nitrogen pulses, primary production, temperature

1 | INTRODUCTION

Shallow lakes are of key importance in the global nitrogen (N) cycle as they process, transfer and store N inputs from surrounding terrestrial ecosystems, point sources and the atmosphere (Finlay, Small & Sterner, 2013; Moss, 2010). For decades, primary production was considered phosphorus (P)-limited (Schindler, 1974), but an increasing number of studies suggest that either N or N and P combined may limit production depending on the P concentration (González Sagrario et al., 2005; Jeppesen et al., 1997; Kosten et al., 2009). Phytoplankton and the algal–bacterial matrix on surfaces (periphyton) may be particularly sensitive to changes in N concentrations as they access nutrients mainly from the water column (Liboriussen & Jeppesen, 2003, 2006). Increased N in lakes may also decrease macrophyte abundance and reduce species richness due to algal shading (Barker, Hatton, O´Connor, Connor & Moss, 2008; James, Fisher, Russell, Collings & Moss, 2005; Olsen et al., 2015) or direct toxic effects of high ammonia (NH_4^+) (Cao, Ni & Xie, 2004).

Lakes experience short-term pulses of high N due to sewage outputs and agricultural run-off following heavy rain, potentially with a several-fold increase in N and P concentrations (Yang, Bastow, Spence & Wright, 2008). Climate warming may further increase the frequency of short-term nutrient pulses to lakes as storm events are expected to become more common (Beniston et al., 2007; Easterling et al., 2000). Furthermore, the Intergovernmental Panel on Climate Change (IPCC) predicts a temperature increase of 1.1–6.4°C by 2090–99 relative to pre-industrial levels (Solomon, 2007). Warmer lakes may also increase anoxic P release from sediments, and increasing P concentrations in the water column thereby lead to a higher phytoplankton biomass (Kosten et al., 2011) and a potentially higher risk of N limitation of primary producers.

Numerous studies have shown effects of temperature on aquatic organisms, such as phytoplankton (Adams & Sterner, 2000; Rolff, 2000), macrophytes (Madsen & Brix, 1997; Meerhoff, 2006) and microbes (Reay, Nedwell, Priddle & Ellis-Evans, 1999; Rysgaard, Risgaard-Petersen, Nielsen & Revsbech, 1993), including higher rates of nutrient uptake, increased denitrification, altered organism size distribution and modified food selection with warming (Barnes, Sweeting, Jennings, Barry & Polunin, 2007). A cross-system analysis including lakes, rivers and oceans by Piña-Ochoa and Álvarez-Cobelas (2006) found that denitrification rates increased with water temperature, concentrations of dissolved organic carbon as well as low oxygen concentrations of the overlaying water column. Some plants, animals and algae adapted to low temperatures also show changed stoichiometry with warming (Woods et al., 2003).

Information on how increasing temperatures affect the response of ecosystems to N pulses is scarce. Some studies have emphasised that the direct effects of warming are of low magnitude compared with the changes in hydrology or nutrient loading (Christoffersen, Andersen, Søndergaard, Liboriussen & Jeppesen, 2006; Kosten et al., 2009; Mckee et al., 2003; Moss et al., 2003; Olsen et al., 2014). However, it is often difficult to disentangle the effects of elevated temperature and excess nutrients from empirical data as the ecosystem responses to both stressors may be very similar (Jeppesen et al., 2014; Moss et al., 2011). Moreover, lack of comprehensive N budgets for lakes limits our understanding of N dynamics in fresh waters in future climate scenarios, and budgeting of N is challenging as it is difficult to estimate portions of nitrification and denitrification processes (Durand et al., 2011; Seitzinger, 1988). Stable N isotope techniques, a standard procedure in current ecological research (Post, 2002; Rundel, Ehleringer & Nagy, 2012), provide a more accurate estimate of the N flow-through ecosystems. It enables tracking the flux of N through all the different trophic pathways leading to and from an organism (Post, 2002) in addition to storage in the sediment and loss as N gas through denitrification. Thus, ^{15}N tracer experiments allow determination of the ambient rates of N uptake and transformation in lakes.

To investigate the effect of N pulses on N processing in ecosystems adapted to contrasting temperatures, we conducted an experiment involving ^{15}N pulse addition in flow-through mesocosms that have now run for over a decade and thus represent relatively mature ecosystems. At higher temperatures, we expected (1) a faster turnover of the inorganic N pulse at each trophic level with increasing metabolic rates and (2) a smaller portion of the N pulse stored in the mesocosms due to increased denitrification.

2 | METHODS

2.1 | Study site and experiment design

We used 12 mesocosms situated in Jutland, Denmark (56°14′N, 9°31′E). The mesocosms consist of cylindrical stainless steel tanks (1.9 m diameter, 1.5 m height [2.8 m^3]) that were established and have been maintained since August 2003 at three contrasting temperatures: ambient, T3 (heating according to the IPCC 2007 A2 scenario, +3.7–4.5°C, depending on season) and T5 (heating with A2 + 50%, +4.9–6.6°C) (Solomon, 2007), with four replicates of each (Liboriussen et al., 2005), hereafter referred to as ambient, T3 (A2) and T5 (A2 + 50%). The mesocosms received ground water approximately 10 cm above the sediment and had an outlet on the water surface. The water retention time during the experiment averaged 2.5 months and the water was continuously stirred with paddles to ensure circulation. At all three temperature treatments, the mesocosms also contained a macrophyte assemblage that was planted during the initial establishment of the mesocosm set-up. The assemblage comprised *Potamogeton crispus* (Potamogetonaceae), exotic *Elodea canadensis* (Hydrocharitaceae) or both, with *E. canadensis* being absent in the T5 mesocosms. The mesocosms also had naturally colonised filamentous algae among which *Cladophora* sp. and *Oedogonium* spp. were the dominant taxa. Temperature was continuously monitored with temperature sensors (sensor type: Pt 100; maximum error ± 0.15°C at 0°C, temperature transmitter type: TT-5333; PR electronics products, Rønde, Denmark) placed in the centre of each mesocosm.

2.2 | ^{15}N addition, sampling and analysis

To measure N uptake and transportation after an N pulse at three contrasting temperatures, we added 5.8 g ^{15}N as $K^{15}NO_3^-$ (98 atom % ^{15}N) to each mesocosm once to increase the NO_3^- in the mesocosms from *c.* 0.2 to 2 mg/L. The $K^{15}NO_3^-$ was applied after diluting it into a small amount of water from each mesocosm and pouring it evenly across the water surface.

Water chemistry, chlorophyll *a* (Chl *a*) and isotope samples were taken 4 days prior to the ^{15}N addition (day −4), subsequent to the ^{15}N addition (day 1) and 3, 8, 10, 15, 22, 31, 59, 79, 105 and 135 days after the addition. In addition, the nitrogen gas (N_2) content was measured hourly for 6 hr following ^{15}N addition. Samples for water chemistry included total nitrogen (TN), total phosphorus (TP), nitrate–nitrogen (NO_3^-) and ammonium–nitrogen (NH_4^+). Different N pools sampled for ^{15}N content included NO_3^- in the mesocosm water and inlet water, respectively (outlet water ^{15}N content was calculated using the inlet water volume (as they were presumed similar) and the ^{15}N content in the mesocosm at time *x*), N_2 in mesocosm water and particulate organic nitrogen (PON) including macrophytes, filamentous algae, epiphyton (attached algae on macrophyte hosts), periphyton (attached algae on metal plates), epipelon (algae + bacteria living on the surface of sediments, hereafter sediment), particulate organic matter (POM: phytoplankton + detritus + bacteria in the water), zooplankton, snails and other invertebrates, respectively.

Samples for TN, TP, NO_3^-, NH_4^+, $^{15}NO_3^-$ and POM were taken from 10 L pooled water samples collected with a core sampler, sampling from the surface to 10 cm above the sediment. Samples for $^{15}N_2$ were taken with a syringe approximately 10 cm below the water surface and transferred to 12-ml exetainers (Labco LTD, U.K.), leaving no headspace and preserved with 100 μl of 50% w/v $ZnCl_2$. Samples for NO_3^-, NH_4^+, $^{15}NO_3^-$ and POM analysis were pre-filtered with a 50-μm net to remove larger particles and then filtered with pre-combusted GF/C filters (Ø47 mm; Whatman, Maidstone, U.K.), except for TN and TP samples, which were analysed without filtration. Samples for TN, TP, NO_3^-, NH_4^+ and $^{15}NO_3^-$ were frozen prior to analysis. A helium headspace was added to $^{15}N_2$ samples, and the exetainers were shaken properly before storing the samples prior to analysis.

A single macrophyte individual was carefully collected with scissors (excluding roots) and epiphyton was separated from the macrophyte in the laboratory by placing it in a plastic bottle with distilled water, after which the bottle was vigorously shaken. The remaining epiphyton was gently brushed off from the leaves into the plastic bottle and filtered through pre-combusted GF/C filters (Whatman, U.K.), and the cleaned macrophyte was used for ^{15}N analysis. Filamentous algae were collected with a scraper from the walls of the mesocosm, approximately 20 cm below water. Periphyton was collected from metal plates (width 3.6 cm, length 60 cm) and placed on the sides of the mesocosms 1 year prior to the experiment. Samples were taken between 20 and 50 cm below the water surface with a scraper (sampling area 7.2 cm^2 per sample), preserved in ground water and filtered through pre-combusted GF/C filters (Whatman, U.K.). Sediment epipelon was collected with a sediment corer (diameter 5 cm), and the upper 2 cm of the core was separated for analysis. Snails were collected with a small net (mesh size 500 μm), and a subsample excluding the shell and stomach (to avoid measuring ^{15}N enrichment of their food source) was taken prior to storing. Zooplankton was collected with a 100-μm mesh by filtering 15 L of pooled mesocosm water and by picking up the animals individually from the filtered water samples.

Macrophyte biomass in the mesocosms was determined using an existing correlation between macrophyte PVI (percentage volume infested by macrophytes) and dry weight by Ventura, Liboriussen, Lauridsen, Søndergaard and Jeppesen (2008) for *E. canadensis* and *P. crispus*, respectively, based on macrophyte PVI measurements conducted on each sampling day. Although snails were present also on macrophytes and the sediment surface, only snails on the mesocosm walls were used as an indicator of snail density in the mesocosms as they were easy to detect and classify to different species and to count. We counted the number of snails on the whole wall area by visual inspection. If the number was high (>300), we counted a subarea of the wall until 300 was reached and extrapolated to the whole area.

TN, TP, NO_3^- and NH_4^+ samples were analysed with a Tecator 5012 flow-injection analyser supplied with a copper–cadmium redactor column (Grasshoff, Kremling & Ehrhardt, 2009; Solorzano & Sharp, 1980). Chl *a* samples were analysed spectrophotometrically by ethanol extraction (Jespersen & Christoffersen, 1987). The concentration of $^{15}N_2$ in water was analysed by mass spectrometry (20-22 isotope ratio mass spectrometer; Sercon Limited, Crewe, U.K.) according to Risgaard-Petersen and Rysgaard (1995). The ^{15}N-atom % of nitrate was determined according to Risgaard-Petersen, Rysgaard and Revsbech (1993), with the exception that a pure culture of *Pseudomonas aeroginosa* was used to convert the NO_3^- to N_2 gas. All PON samples were dried at 60°C for 48 hr and then homogenised and encapsulated in tin cups (6 × 4 mm; Sercon Ltd). The ^{15}N-atom% of the dried material was determined by mass spectrometry (Delta V plus isotope ratio mass spectrometer; Thermo Fisher Scientific, Bremen, Germany) after combustion in Flash EA (Thermo Fisher Scientific, Bremen, Germany), which measured the total organic N in these samples.

The ^{15}N content of all samples was expressed as ^{15}N-excess and defined as:

$$^{15}\text{N-excess} = (^{15}\text{N-atom\% of sample} - ^{15}\text{N-atom\% of control}) \times \text{concentration of N in pool X} \quad (1)$$

Here, the ^{15}N-atom% of control refers to the ^{15}N-atom% of a given pool (PON; NO_3; N_2) prior to tracer addition. The concentration is expressed in μmol N mg dw^{-1} for PON samples and in μmol N L^{-1} for gas and nitrate samples.

As $^{15}N_2$ in contrast to the other ^{15}N components may escape to the atmosphere, we estimated the flux of $^{15}N_2$-excess across the air–water interface using the following expression (Jähne et al., 1987):

$$f_g = k_g(C_{(wat,g)} - C_{(eq,g)}) \quad (2)$$

where f_g is the flux of N_2, k_g is the piston velocity of the gas, $C_{(wat,g)}$ is the actual concentration of gas in the water and $C_{(eq,g)}$ is the concentration of gas at equilibrium with the atmosphere. From our definition of $^{15}N_2$-excess (Equation 2) Equation 2 reduces to :

$$f_g = k_g(^{15}N_2\text{-excess water})$$

The estimated piston velocity for N_2 was 6.8×10^{-3} m/hr as calculated from the piston velocity of O_2 ($K_{O_2} = 8.8 \times 10^{-3}$ m/hr measured directly in the mesocosm; Liboriussen et al., 2011) and the diffusion coefficients for N_2 and O_2 (Soetaert, Petzoldt & Meysman, 2012) according to:

$$K_{N_2} = K_{O_2}\frac{D_{N_2}}{D_{O_2}} \quad (3)$$

2.3 | Data analysis

Similarity in nutrient concentrations and Chl *a* content among the twelve mesocosms before tracer addition was tested with one-way ANOVA. To investigate the effect of temperature on ^{15}N concentration in different N pools of the mesocosms, generalised additive mixed model (GAMM) was applied (model: gam (conc ~ s(time) + temp + s(tank, bs='re'), data = data, method = 'REML')), where time is a smooth term, tank is added as a random effect and temperature treatment is the fixed effect (Pinheiro, Bates, DebRoy & Sarkar, 2016). The GAMM analysis was carried out in the mgcv package (Wood, 2011) in R version 3.13 (R Development Core Team, 2015). The models with and without temperature as a fixed effect were compared using the likelihood ratio to see whether the addition of temperature significantly improved the model. Further, we compared the rate of $^{15}NO_3^-$ concentration reduction in the water among three contrasting temperatures with one-way ANOVA to detect potential differences in ^{15}N loss from the water column among the three temperatures.

3 | RESULTS

3.1 | Water chemistry

Nutrient concentrations did not differ significantly among the temperature treatments on the first sampling prior to ^{15}N addition (Table 1, one-way ANOVA, $p > .05$). Although concentrations of Chl *a* and TP were higher in the T5 mesocosms (by 6 μg/L for Chl *a* and 0.01 mg/L for TP). TN and NO_3^- reached peak concentrations in all three temperature treatments the day after ^{15}N addition (130–150 μmol/L), whereas no marked changes in NH_4^+ concentration were observed (Figure 1). The average weighted loss of ^{15}N as $^{15}NO_3^-$ from the water column between days 1 and 15 was 1.8 ± 1.4, 1.2 ± 0.4 and 1.0 ± 0.3 μmol L^{-1} day^{-1} in the ambient, T3 and T5 treatments, respectively. Loss of $^{15}NO_3^-$ through outflow was highest on day 8 in all treatments (Figure 2).

3.2 | ^{15}N-excess in N_2

Part of the added ^{15}N was denitrified. The $^{15}N_2$ excess concentration peaked 8–10 days after ^{15}N addition (Figure 3a). Correspondingly, the $^{15}N_2$ excess flux from the mesocosms peaked 8 days after ^{15}N addition, with peak rates of 85, 62 and 63 μmol m^{-2} hr^{-1} in the ambient, T3 and T5 treatments, respectively, but did not differ significantly among the temperature treatments (Figure 3b) (GAMM, $p = .3$ for temperature).

3.3 | ^{15}N-excess in macrophytes and filamentous algae

The most rapid and highest enrichment of the measured N pools was in macrophytes, followed by filamentous algae (peak levels were 1.5, 3 and 3 μmol mg dw^{-1} in macrophytes and 2, 2 and 1.3 μmol mg dw^{-1} in filamentous algae in the ambient, T3 and T5 treatments, respectively, Figure 4). Macrophytes also had the highest biomass (mean weighted biomass 45, 76 and 7 g dw^{-1} m^{-2}, Table 2) together with filamentous algae (mean weighted biomass 6, 7 and 7 g/m^2, Table 2) of all primary producers. The average weighted increase of ^{15}N-excess in macrophytes between days 1 and 15 was 0.1 ± 0.1, 0.8 ± 0.9 and 0.5 ± 0.6 μmol mg dw^{-1} day^{-1} and in filamentous algae 0.04 ± 0.02, 0.2 ± 0.2 and 0.2 ± 0.2 μmol mg dw^{-1} day^{-1} in the ambient, T3 and T5 treatments, respectively. The ^{15}N-excess did not increase with increasing temperature in either the macrophytes (GAMM, $p = .7$ for temperature) or in the filamentous algae (GAMM, $p = .2$ for temperature) but declined quickly after the peak had been reached at all temperatures.

3.4 | ^{15}N-excess in epiphyton and periphyton

The ^{15}N-excess in epiphyton peaked earlier than in the macrophytes (10 days after ^{15}N addition), peak values being 0.02, 0.03 and 0.03 μmol mg dw^{-1} in the ambient, T3 and T5 treatments, respectively. Total enrichment in epiphyton was modest compared with that of macrophytes and filamentous algae, the biomass of epiphyton being much smaller than that of macrophytes or filamentous algae (Table 2). Periphyton ^{15}N-excess was smaller than that of epiphyton (peak < 0.02 μmol mg dw^{-1}), but periphyton had a somewhat larger biomass (Table 2). We found no significant differences in ^{15}N-excess among the temperature treatments for either epiphyton (GAMM, $p = .6$ for temperature) or periphyton (GAMM, $p = .3$ for temperature).

3.5 | ^{15}N-excess in POM, zooplankton and sediment

The ^{15}N-excess of POM was also small compared with macrophytes and filamentous algae, peaks of 0.02 to 0.03 μmol mg dw^{-1} being observed for the first time 3 and then 15 days after tracer addition (Figure 4). Mean weighted POM biomass was also considerably

TABLE 1 Selected morphological and chemical (mean ± *SD*) data on the mesocosms before the experiment (6 June 2013). Temperature treatments are ambient (no heating), T3 (heating according to IPCC A2 scenario, average temperature increase +3°C above ambient) and T5 (heating according to A2 scenario + 50%, average temperature increase +5°C above ambient)

Temperature	Volume, m^3	Chl *a*, µg/L	TP, µmol/L	TN, µmol/L	NO_3, µmol/L	NH_4, µmol/L	Turbidity, NTU	Input $^{15}NO_3^-$, µmol/L
Ambient	2.8	2.9 ± 1.3	0.64 ± 0.18	6.3 ± 2.3	3.1 ± 3.4	0.2 ± 0.2	4.2 ± 0.9	0.01 ± 0.01
T3	2.8	3.9 ± 3.5	0.62 ± 0.14	4.2 ± 4.2	2.4 ± 3.9	0.7 ± 1.03	3.9 ± 0.7	0.01 ± 0.01
T5	2.8	10.3 ± 4.8	0.96 ± 0.31	2.1 ± 0.9	0.3 ± 0.1	0.1 ± 0.05	4.3 ± 1.1	0.01 ± 0.01

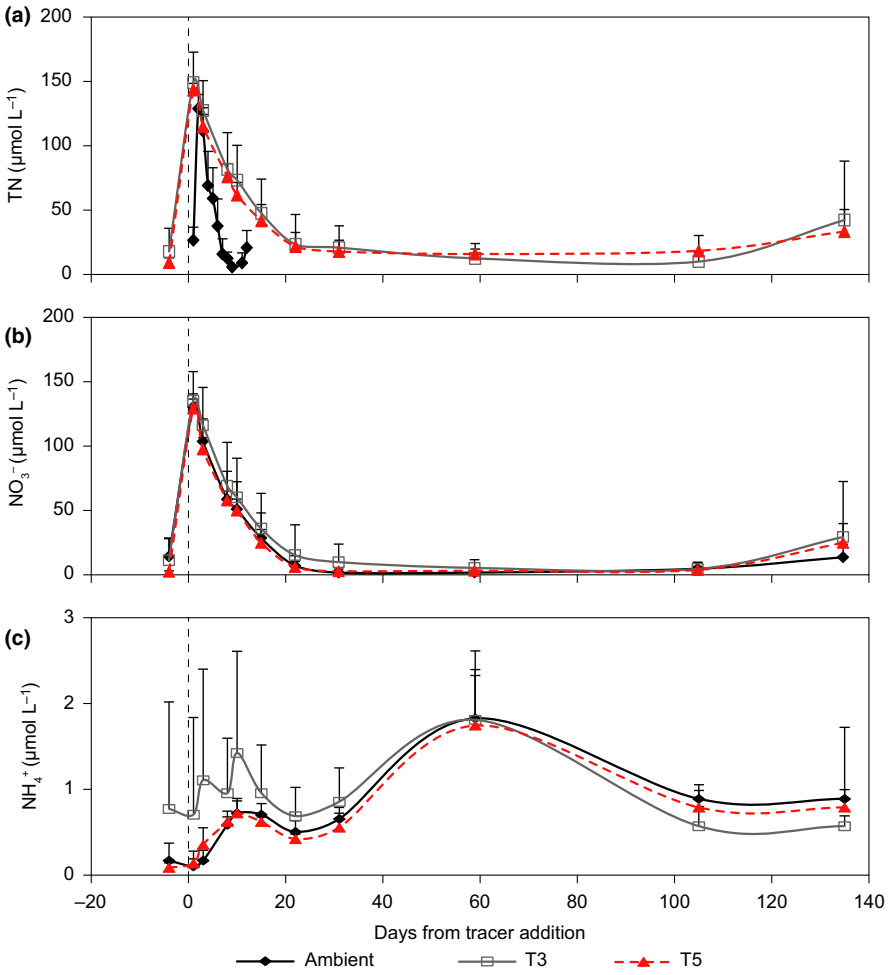

FIGURE 1 Mean (±*SD*) TN, NO_3^- and NH_4^+ concentrations (as µ mol L^{-1}) in three temperature treatments: ambient (no heating), T3 (heating according to IPCC A2 scenario, average temperature increase +3°C above ambient) and T5 (heating according to A2 scenario + 50%, average temperature increase +5°C above ambient) during the experiment. The dashed line represents the moment of ^{15}N addition

smaller than that of macrophytes and filamentous algae but larger than that of epiphyton and periphyton (Table 2). We found no significant differences in enrichment of POM among the three temperature treatments (GAMM, $p = .3$ for temperature). We observed no enrichment of sediment epipelon as the ^{15}N-excess did not increase beyond background values during the experiment (data not shown). The enrichment of zooplankton followed the two peaks of POM enrichment and reached similar values as that of POM in the T3 and T5 treatments (GAMM, $p = .4$ for temperature) but increased by a factor two in the ambient treatment (peak ~ 0.04 µmol mg dw^{-1}) compared with the POM ambient treatment (data not shown). However, the mean weighted zooplankton biomass was smaller than that of POM, being 0.02, 0.02 and 0.04 g/m^2 in the ambient, T3 and T5 treatments, respectively (Table 2).

3.6 | ^{15}N-excess in snails

Several snail species were observed in the mesocosms during the experiment, including *Lymnaea stagnalis* (dominant in most mesocosms), *Bithynia leachii*, *Potamopyrgus jenkinsi*, *Gyraulus albus* and *Radix balthica*. ^{15}N-excess in snails was low compared with macrophytes and filamentous algae (Figure 4), but their mean weighted biomass was high compared with other PON pools – 1.2, 9.8 and 0.9 g/m^2 in the ambient, T3 and T5 treatments (Table 2). The ^{15}N-excess and biomass of snails increased towards the end of the experiment, suggesting long-term storage and slow turnover of ^{15}N. We observed no significant differences in snail enrichment among the three temperature treatments (GAMM, $p = .3$ for temperature).

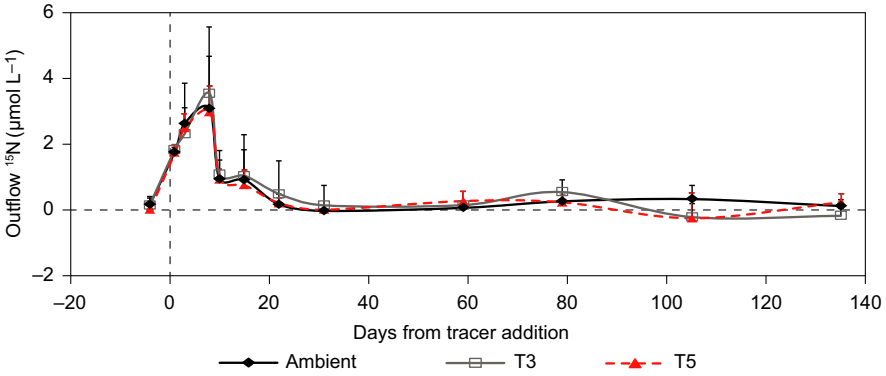

FIGURE 2 Mean (±*SD*) ^{15}N outflow (as combined NO_3^- and POM) from the mesocosms during the experiment in three contrasting temperature treatments (ambient, T3 and T5, as in Figure 1). The vertical dashed line represents the moment of ^{15}N addition and the horizontal line 0 µmol/L

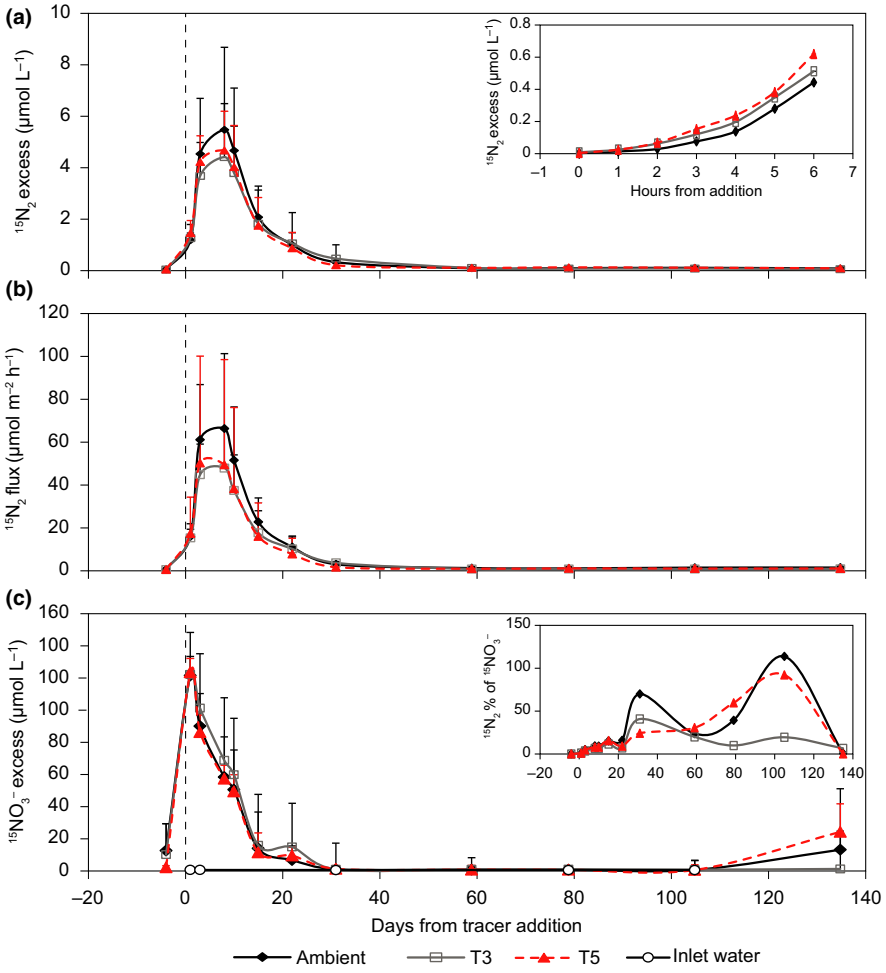

FIGURE 3 (a) The mean (±*SD*) concentration of $^{15}N_2$ (µmol/L) during the experiment, inset showing the first 6 hr; (b) mean (±*SD*) $^{15}N_2$ flux from the mesocosms to the atmosphere as µmol m^{-2} hr^{-1}; and (c) the mean (±*SD*) concentration of $^{15}NO_3^-$ (µmol/L) in the mesocosms, inset displaying % of $^{15}N_2$ concentration of the $^{15}NO_3^-$ concentration in three contrasting temperature treatments (ambient, T3 and T5, abbreviations as in Figure 1). The dashed line represents the moment of ^{15}N addition

3.7 | Tracer mass balance

For the first 10 days after the tracer addition, the ^{15}N stayed as $^{15}NO_3^-$ in the water column (day 1 *c.* 90% and day 3 *c.* 60%) or was released as $^{15}N_2$ (day 3 *c.* 3%) in all the three temperature treatments (Figure 5a, b, c). Fifteen days after the tracer addition, the two N pools with the largest biomasses, macrophytes and filamentous algae, retained a large portion of the remaining ^{15}N (*c.* 30%, 30% and 32% for macrophytes and 15%, 30% and 20% for filamentous algae in the ambient, T3 and T5 treatments, respectively, Figures 5a, b, c and 6a, b, c). Only *c.* 0.5%–1.5% of the tracer was found in the epiphyton, periphyton and POM pools (Figures 5a, b, c and 6a, b, c). Snails retained up to 3%, 6% and 1% of the tracer in the ambient, T3 and T5 treatments, respectively, with an increasing content towards the end of the experiment when sediment and zooplankton contained <1% of the tracer in total. Less than 1% of the added $^{15}NO_3^-$ was present in the water column after 22 days in the T5 treatment and after 31 days in the ambient and T3 treatments,

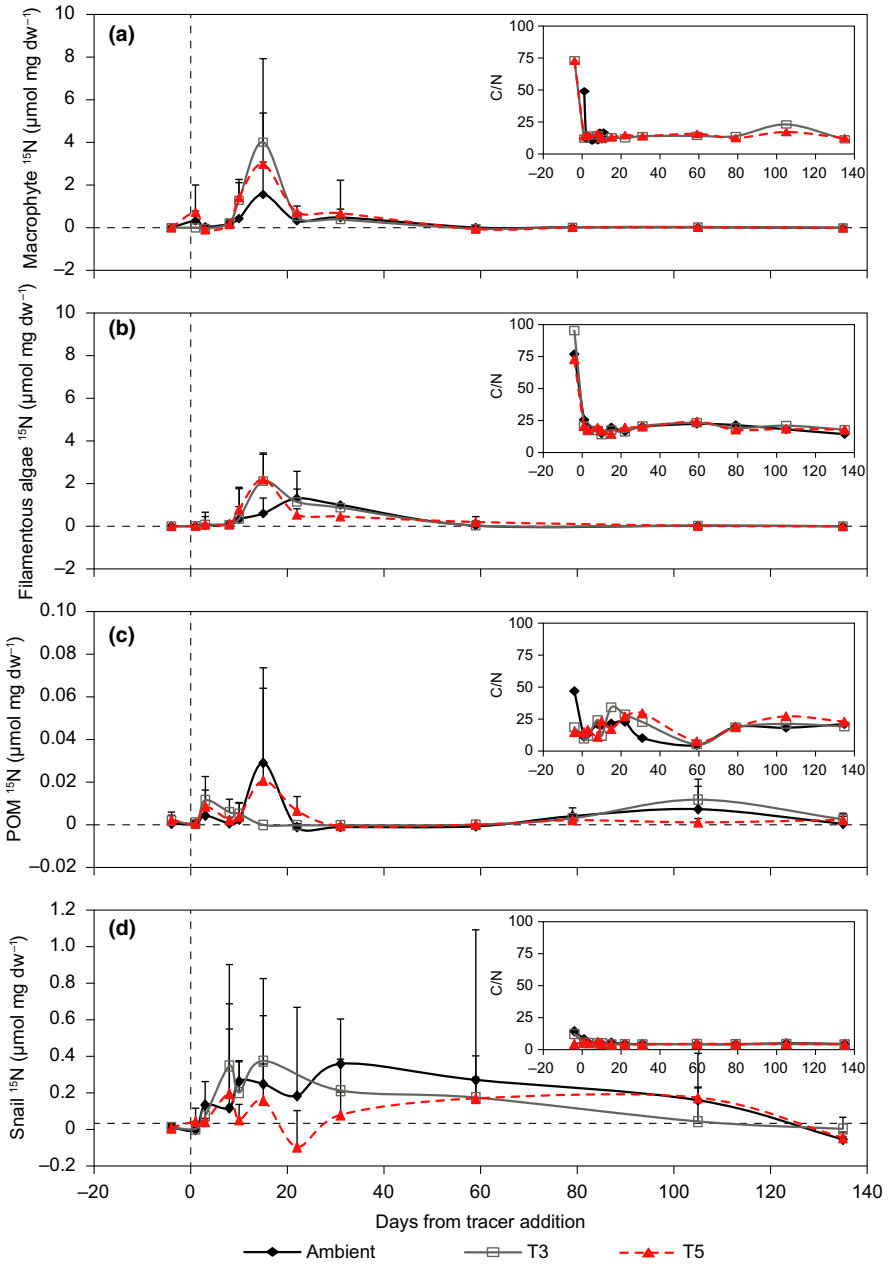

FIGURE 4 Mean (±*SD*) ^{15}N concentration (µmol mg dw^{-1}) and mean C/N ratio of macrophytes, filamentous algae, POM and snails during the experiment in three contrasting temperature treatments (ambient, T3 and T5, as in Figure 1). The dashed vertical line represents the moment of ^{15}N addition and the horizontal line 0 µmol mg dw^{-1}. Notice the different scale on the *y*-axes

and total loss of ^{15}N through $^{15}NO_3$ outflow, POM outflow and N_2 was 27%, 25% and 23% in the ambient, T3 and T5 treatments (one-way ANOVA, $p = .8$).

3.8 | Carbon

The C:N ratio in macrophyte stem and leaves, respectively, and filamentous algae decreased from 100 to <30 in all three temperature treatments immediately after tracer addition and remained <25 until the end of the experiment. We found no significant differences in C:N ratio among the three temperature treatments for macrophyte leaves, stems or filamentous algae: GAMM for leaves, $p = .3$; GAMM for stems, $p = .4$; and GAMM for filamentous algae, $p = .1$. In epiphyton, periphyton and POM, the C:N ratio fluctuated among sampling dates during the experiment; for epiphyton and POM, the C:N ratio ranged between 5 and 50 and did not differ among the temperature treatments (GAMM, $p > .1$ for temperature). The C:N ratio in snails and zooplankton was smaller than that of primary producers and was <20 during the experiment in all three temperature treatments (GAMM, $p > .1$ for temperature).

4 | DISCUSSION

Nutrient pulses increase the water column N concentration and potentially also the biomass of primary producers in lakes that are N-limited (Yang et al., 2010). Freshwater ecosystems may be particularly sensitive to resource pulses as they transmit more rapidly through aquatic systems compared with terrestrial systems,

TABLE 2 Mean (±*SD*) biomass (as g dw^{-1} m^{-2}) of different N pools during the experiment in three contrasting temperature treatments: ambient, T3 and T5 as in Table 1. POM = particulate organic matter

Days	−4	1	3	8	10	15	22	31	59	79	105	135
Ambient												
Macrophytes	24.4 ± 25.9	54.8 ± 3.6	60.8 ± 4	107.9 ± 55.3	131.4 ± 111.1	155 ± 117.4	149.7 ± 126.2	144.3 ± 212.4	245 ± 480.2	345.7 ± 480.2	311 ± 480.2	274 ± 380
Filamentous algae	16.9 ± 10	12.2 ± 6.1	10.4 ± 9.2	13.8 ± 6.5	12.3 ± 8.9	13.3 ± 17.7	13.4 ± 3.2	23.2 ± 10.9	22.1 ± 6.1	38 ± 12.9	46 ± 7.4	39 ± 34.2
Epiphyton	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	0.01 ± <0.01	0.01 ± <0.01	0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	0.02 ± <0.01	<0.01 ± <0.01
Periphyton	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.05 ± 0.02	0.06 ± 0.01	0.09 ± 0.03	0.08 ± 0.03	0.1 ± 0.06	0.06 ± 0.04	0.08 ± 0.03	0.06 ± 0.02	0.06 ± 0.02
POM	0.3 ± 0.3	0.4 ± 0.4	1.4 ± 0.8	0.7 ± 0.5	0.6 ± 0.8	0.6 ± 0.7	0.8 ± 0.8	0.7 ± 0.6	0.4 ± 0.2	0.2 ± 0.2	0.08 ± <0.01	<0.01 ± <0.01
Snails	3.1 ± 1.5	3.5 ± 0.9	3.4 ± 0.9	3.6 ± 1.1	3.6 ± 1.1	2.1 ± 1	3 ± 2	2.9 ± 3	5.2 ± 5.2	5.9 ± 5	10 ± 10.3	12.2 ± 13.4
Zooplankton	0.01 ± <0.01	0.03 ± <0.01	0.04 ± <0.01	0.06 ± <0.01	0.07 ± <0.01	0.07 ± <0.01	0.06 ± <0.01	0.07 ± <0.01	0.14 ± 0.02	0.04 ± <0.01	0.03 ± <0.01	0.02 ± <0.01
T3												
Macrophytes	61.9 ± 57.2	211.3 ± 303.1	223.1 ± 336.7	234.8 ± 489.2	306.0 ± 550.2	377.2 ± 515.2	340.3 ± 465.6	303.5 ± 509.3	339.9 ± 645.1	358.1 ± 645.1	376.3 ± 645.1	327.8 ± 525.5
Filamentous algae	9.5 ± 9.1	10.8 ± 4.8	9.4 ± 4	11.3 ± 4.6	25.4 ± 9	27.4 ± 19.4	26.8 ± 9.9	26.1 ± 18.3	43.4 ± 21.3	33.3 ± 15.3	66.3 ± 47.6	62.5 ± 41.1
Epiphyton	0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	0.02 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	0.01 ± <0.01	<0.01 ± <0.01
Periphyton	0.06 ± 0.01	0.07 ± 0.03	0.11 ± 0.04	0.06 ± 0.1	0.07 ± 0.08	0.08 ± 0.03	0.12 ± 0.04	0.15 ± 0.03	0.07 ± 0.03	0.15 ± 0.07	0.08 ± 0.03	0.08 ± 0.02
POM	0.5 ± 0.8	0.5 ± 0.8	2.5 ± 2.9	0.5 ± 1	0.4 ± 0.4	0.4 ± 0.3	0.3 ± 0.4	0.4 ± 0.5	0.5 ± 0.4	0.2 ± 0.2	0.2 ± <0.01	0.2 ± <0.01
Snails	21.4 ± 28.3	58.9 ± 10	58.9 ± 99.5	50.0 ± 70.3	50.0 ± 70.3	49.5 ± 73.8	42.4 ± 58.4	42.4 ± 58.4	21.5 ± 23.1	25.1 ± 27.8	24.0 ± 19.3	28.9 ± 37.7
Zooplankton	0.02 ± <0.01	0.03 ± <0.01	0.04 ± <0.01	0.05 ± <0.01	0.05 ± <0.01	0.06 ± <0.01	0.07 ± <0.01	0.09 ± <0.01	0.14 ± 0.01	0.23 ± 0.02	0.35 ± 0.02	0.50 ± 0.01
T5												
Macrophytes	32.6 ± 15.4	45.9 ± 7.9	50.9 ± 8.8	50.9 ± 19.6	52.5 ± 24	51.1 ± 18.8	37.8 ± 11.6	32.5 ± 9.9	4.2 ± 7.7	4.2 ± 4.9	4.2 ± 8	9.8 ± 11.7
Filamentous algae	17.7 ± 16.7	15.8 ± 2.2	9.5 ± 5.6	14.8 ± 18.5	15.7 ± 10	20.9 ± 15	22.3 ± 7.8	21.4 ± 7.2	52.9 ± 25.9	38.8 ± 12.3	64.7 ± 14.1	56.1 ± 38
Epiphyton	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	0.05 ± 0.01	0.04 ± 0.01	<0.01 ± <0.01	0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01
Periphyton	0.06 ± 0.01	0.06 ± 0.02	0.05 ± 0.02	0.08 ± 0.02	0.07 ± 0.05	0.12 ± 0.02	0.09 ± 0.02	0.19 ± 0.02	0.11 ± 0.02	0.15 ± 0.02	0.14 ± 0.02	0.12 ± 0.02
POM	1.72 ± 1.5	1.45 ± 1.3	2.00 ± 2.2	2.69 ± 2.3	2.44 ± 2.8	2.55 ± 3.9	2.45 ± 3.8	1.94 ± 2.6	5.85 ± 8.2	8.42 ± 12.5	9.61 ± —	5.64 ± —
Snails	0.8 ± 0.4	4.7 ± 5	4.7 ± 5	3.0 ± 1.4	3.0 ± 1.4	3.1 ± 2.5	3.1 ± 0.1	3.1 ± 0.2	2.4 ± 2.3	5.2 ± 6.8	4.8 ± 3	7.5 ± 6.2
Zooplankton	0.22 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.08 ± <0.01	0.06 ± <0.01	0.08 ± <0.01	0.10 ± <0.01	0.12 ± <0.01	0.21 ± 0.01	0.18 ± <0.01	0.14 ± <0.01	0.1 ± <0.01

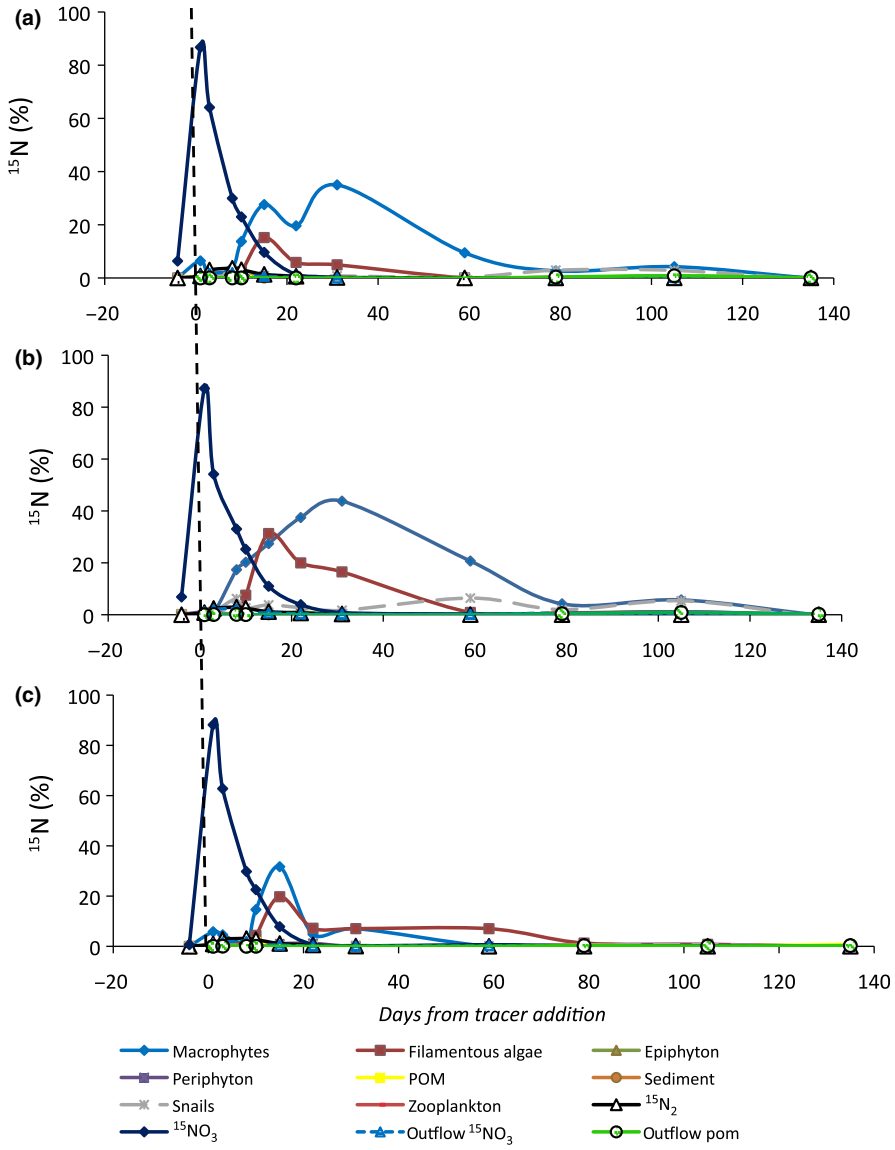

FIGURE 5 ^{15}N-excess in each N pool as percentage of the total $^{15}NO_3^-$ added on day 1 (5.8g is 100%) throughout the experiment in three contrasting temperature treatments (Ambient (A), T3 (B) and T5 (C)) (treatment abbreviations as in Fig. 1). Dotted line over the figures represents the moment of ^{15}N addition

strengthening both top-down and bottom-up effects (Scheffer, Van Nes, Holmgren & Hughes, 2008; Yang et al., 2008). Moreover, the turnover of N pulses may be faster at higher temperatures due to increased metabolism, decay of organisms and increased denitrification (Baulch et al., 2005; Hanisak, 1993). Yet, we found no significant differences among the three contrasting temperature treatments regarding ^{15}N-excess in the water column, denitrification or PON, suggesting that other factors rather than temperature determined the rate of N turnover in the mesocosms in summer. This concurs with some previous field studies, for instance Kosten et al. (2009), Nõges, Tuvikene and Nõges (2010) and Jeppesen et al. (2014), who concluded that changes in hydrology and loading were more important than changes in temperature for N processing in natural lakes. Moreover, we found that the N pulses were processed rapidly at all three temperatures as <1% of the added ^{15}N was present in the water column after 22 days in all temperature treatments. Accordingly, in a study in subtropical China, Zhang, Jeppesen, Li and Cao (2016) found that plant and algae communities in mesocosms with low initial nutrient levels were resilient to short-term extreme N pulses and that the sudden increase in N did not initiate a regime shift to a turbid state.

Submerged macrophytes are important components of clear shallow lakes and a key factor controlling ecological patterns and processes (Moss, 1990). They have strong impacts on the water column N concentration via direct uptake and by providing fresh organic matter for denitrification and epiphyton assimilation (Howard-Williams, 1981). Moreover, filamentous algae have shown fast uptake and particularly high N enrichment during N pulses (Dillon & Chanton, 2008; Worm & Sommer, 2000). As in the study of Kreiling, Richardson, Cavanaugh and Bartsch (2011) of a backwater lake of the Mississippi River, we found that most of the added ^{15}N was taken up by macrophytes and filamentous algae, both of which dominated the total biomass of biota in the mesocosms. The portion of ^{15}N in the macrophyte pool compared with other PON pools was greater in the ambient and T3 treatments than in the T5 treatment. This can be largely attributed to absence of *E. canadensis* in the T5

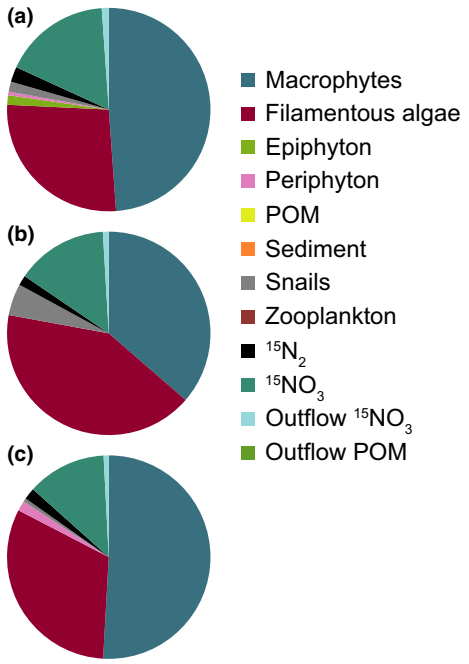

FIGURE 6 ^{15}N-excess in each N pool as proportion of 100% 15 days after the ^{15}N addition in three contrasting temperature treatments (ambient [a], T3 [b] and T5 [c]) (treatment abbreviations as in Figure 1

treatment and therefore a lower total biomass of macrophytes and primary producers in general. Moreover, *P. crispus* reached peak growth during the first 30 days of the experiment but then decayed rapidly in the T5 treatment. The portion of ^{15}N in filamentous algae, periphyton and POM was greater in the T3 and T5 treatments than in the ambient treatment. Filamentous algae thrive in warmer waters and benefit from excess nutrients released from macrophyte decay in summer (Liboriussen et al., 2011). The C:N ratio of both macrophytes and filamentous algae decreased significantly after the pulse addition and remained low until the end of the experiment, suggesting rapid uptake and storage of N during the pulse. Interestingly, the initial high ^{15}N in macrophytes was not maintained despite a continued lower C:N ratio after the dosing, indicating fast turnover of N in the plants.

Notwithstanding that macrophytes and filamentous algae constituted the largest N pool in the mesocosms, their storage of ^{15}N was of short-term duration compared with snails and zooplankton in which the tracer was still detectable 100 days after ^{15}N pulse addition. This concurs with previous ^{15}N studies that included consumer N turnover rates (Epstein, Wurtsbaugh & Baker, 2012; Woods et al., 2003). Snails, in particular, have been shown to significantly impact N processing by consuming a large portion of the gross primary production (75% in Hall, Tank & Dybdahl, 2003), not least when snail predators are scarce (Vanni, 2002) as the case in our mesocosms which contain a single stickleback per mesocosm. However, we found a maximum of 6% of the added ^{15}N in snail biomass towards the end of the experiment. Similarly, zooplankton may immobilise part of the N pulse by consuming phytoplankton. However, zooplankton represented a considerably smaller total N pool (as lower total biomass, Table 2) than snails, macrophytes and filamentous algae in the mesocosms, suggesting that the diet of zooplankton contained little of the added ^{15}N.

Peak $^{15}N_2$ flux in the ambient, T3 and T5 treatments varied between 60 and 80 μmol m^{-2} hr^{-1}, resembling previously reported rates for shallow freshwater and estuarine systems with similar loading (An & Gardner, 2002; Gardner et al., 2006; Scott, Mccarthy, Gardner & Doyle, 2008). However, compared with the ^{15}N retained in macrophytes, filamentous algae and snails, the ^{15}N loss through denitrification was low. Both the $^{15}N_2$ flux and the $^{15}N_2$ concentration in the water column peaked on day 8, a few days earlier than concentrations in other N pools but declined to levels close to those before addition by day 15. Similarly, the $^{15}NO_3^-$ concentration in the water column decreased to 10% of the added ^{15}N by day 15, suggesting that denitrification was by then already limited by the availability of the NO_3^- pool. Available NO_3^- from the pulses therefore seemed to generally bypass microbes and go directly to producers during the study period in these shallow plant-rich systems. The mesocosms may have shown differences in N turnover among temperature treatments if they had been dominated by phytoplankton instead of macrophytes and filamentous algae (as is the case for many lakes around the world, Abell, Özkundakci, Hamilton & Jones, 2012), with more favourable conditions for microbial activity due to a faster turnover of phytoplankton (Chróst & Siuda, 2006). Different results might be obtained in lakes with a long-term high N loading or with an initially higher N concentration or lower macrophyte cover.

In conclusion, the ^{15}N pulse in the mesocosms was quickly taken up by primary producers and channelled to secondary producers irrespective of the temperature treatment, emphasising the role played by shallow lakes in general in N processing within the landscape. Further, corresponding with the recent work of Davidson et al. (2015) studying greenhouse gases in the same mesocosms, our experiment underlines the importance of aquatic vegetation, rather than temperature, for modulating the effect of N pulses in lakes with low N concentrations.

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