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ORIGINAL ARTICLE

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Factors controlling the stable isotope composition and C:N ratio of seston and periphyton in shallow lake mesocosms with contrasting nutrient loadings and temperatures

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Summary

1. Carbon (C) and nitrogen (N) stable isotope composition (^{15}N:^{14}N, $\delta^{15}N$ and ^{13}C:^{12}C, $\delta^{13}C$) have been widely used to elucidate changes in aquatic ecosystem dynamics created by eutrophication and climate warming, often, however, without accounting for seasonal variation.
2. Here, we aim to determine the factors controlling the stable isotope composition and C:N ratio of seston and periphyton in shallow lakes with contrasting nutrient loadings and climate; for this purpose, we followed the monthly stable isotope composition (*c*. 1 year) of seston (SES) and periphyton (PER) in 24 mesocosms mimicking shallow lakes with two nutrient treatments (enriched and unenriched) and three temperature scenarios (ambient, +3 and +5°C).
3. Nutrient enrichment and warming had a stronger impact on the $\delta^{15}N$ and $\delta^{13}C$ values of seston than on periphyton, and the temporal isotopic variability in both communities was large.
4. $\delta^{15}N_{PER}$ did not differ markedly between nutrient treatments, whereas $\delta^{15}N_{SES}$ was lower in the enriched mesocosms, possibly reflecting higher N_2-fixation by cyanobacteria. $\delta^{15}N_{SES}$ was higher in winter in the heated mesocosms and its dynamics was linked with that of NH_4-N, whereas $\delta^{15}N_{PER}$ showed a stronger association with NO_3-N. $\delta^{15}N_{SES}$ demonstrated a positive relationship with mean monthly temperature, indicating less isotope fractionation among autotrophs when production increased.
5. $\delta^{13}C_{SES}$ was lowest in the enriched mesocosms during winter, whereas $\delta^{13}C_{PER}$ did not differ between nutrient treatments. $\delta^{13}C_{SES}$ and $\delta^{13}C_{PER}$ were positively related to pH, likely reflecting a pH-induced differential access to dissolved carbon species in the primary producers. The positive $\delta^{13}C$-temperature relationship suggested less fractionation of CO_2 and HCO_3^- and/or larger use of HCO_3^- at higher temperatures.
6. The C:N ratios varied seasonally and the differences between the enriched and unenriched mesocosms were stronger for seston than for periphyton. Particularly, the $C:N_{SES}$ ratios did not indicate deficiencies in N as opposed to the $C:N_{PER}$ ratios, supporting the observed changes in $\delta^{15}N$ and suggesting that seston

and periphyton have access to different sources of nutrients. We did not observe any clear effect of temperature warming on the C:N ratios.

7. Our study provides evidence of strong seasonality in the isotopic composition and C:N ratios of seston and periphyton across nutrient and temperature levels; also, we identified several factors that are likely to modulate the strength and variability in stable isotopes values and stoichiometry of sestonic and periphytic communities under these scenarios.

KEYWORDS
carbon stable isotopes, climate warming, eutrophication, nitrogen stable isotopes, primary producers

1 | INTRODUCTION

The global climate change is altering aquatic ecosystems in profound ways (Polunin, 2008). Warming may impact community structure and trophic interactions and have major ecological implications for the ecosystem structure and function of shallow lakes (e.g. Jeppesen, Søndergaard, & Jensen, 2003; Mckee et al., 2003; Meerhoff et al., 2007, 2012; Moss et al., 2003). Increased nutrient loadings are also largely recognised as a significant factor affecting the ecological structure and functioning of aquatic ecosystems (Smith, Tilman & Nekola, 1999).

For a while now, the relative abundances of stable nitrogen and carbon isotopes have been a common tool used for studying the structure and energy flow within food webs. Changes in temperature directly or indirectly impact the $\delta^{15}N$ of primary producers as major temperature-dependent N cycling processes, such as nitrification, denitrification and N-fixation, have severe and differential impacts on N isotopic fractionation (Hadas, Altabet, & Agnihotri, 2009; Owens, 1987). For instance nitrification implies oxidation of NH_4^+ to NO_2^- and NO_3^-, with NH_4^+ being strongly fractionated during the process, thus potentially producing NO_3^- with isotopically light N. Contrarily, denitrification returns light N_2 to the atmosphere, leaving the aquatic NO_3^- pool relatively enriched in ^{15}N (Miyake & Wada, 1971). Finally, N_2-fixation produces organic N, which is isotopically similar to or slightly depleted in ^{15}N relative to atmospheric N_2, which is around 0‰. A study by Veraart et al. (2011) showed that a three-degree temperature rise may double denitrification rates in the sediments of aquatic environments due to a systematic decrease in oxygen concentrations with rising temperatures. However, global warming may result in a positive selection pressure for N_2-fixing autotrophs, i.e. cyanobacteria, as a result of their optimal growth at higher temperatures compared with other algal groups (Reynolds, 1984; Wagner & Adrian, 2009). Consequently, global warming can alter the N budget of aquatic ecosystems (Woodland & Cook, 2014) and the phytoplankton and/or periphyton community of the aquatic ecosystem dominated by cyanobacteria could show lower $\delta^{15}N$ values compared with communities dominated by other algal groups (Gu & Alexander, 1993; Vuorio, Meili, & Sarvala, 2006).

Productivity may also impact the N isotope ratios of primary producers; for instance, in productive lakes fast-growing phytoplankton shows less isotopic fractionation (Owens, 1987; Wada & Hattori, 1978) and may deplete the nutrient pool, leading to further ^{15}N enrichment (e.g. Peterson & Fry, 1987). However, a simple relationship between lake trophic state and $\delta^{15}N$ is not always found as N_2-fixing cyanobacteria may dominate in eutrophic lakes (Estep & Vigg, 1985; Gu, Chapman, & Schelske, 2006; Gu, Schelske, & Brenner, 1996).

Warming and increased nutrient loading may also impact the $\delta^{13}C$ values of primary producers, which are affected by the preferred carbon source, carbon availability and potential effects of boundary layers (Doi et al., 2003; France, 1995; Hecky & Hesselein, 1995). Currently, many lakes have CO_2 concentrations above air equilibrium and act as major conduits for the transfer of terrestrial carbon to the atmosphere (Cole et al., 2007). In future climate scenarios, eutrophic lakes will have lower concentrations of atmospheric CO_2 as a result of higher water temperatures and higher concentrations of carbon originating from microbial decomposition and respiration (often depleted in ^{13}C compared with CO_2 from atmospheric sources) (Cole et al., 2007). Moreover, primary producers prefer the lighter C (^{12}C) during photosynthesis; thus, they have lower $\delta^{13}C$ values than their inorganic C source (Farquhar, Ehleringer, & Hubick, 1989). In aquatic ecosystems, carbon isotope fractionation varies between 0‰ and 20‰ depending on CO_2 availability (Kerby & Raven, 1985; O'leary, 1988). For comparison: at pH 5.5, 80% of the inorganic carbon occurs as CO_2 (aq), whereas at pH 8.5, CO_2 (aq) accounts for 1%, and at pH 10 HCO_3^- accounts for 50%, whereas most of the inorganic carbon occurs in the form of CO_3^{2-}, which is not available for uptake by plants. In the case of periphyton, the thickness of the benthic boundary layer affects CO_2 diffusion rates and consequently the $\delta^{13}C$ values of the algae within the periphyton matrix (Hecky & Hesselein, 1995).

Another factor that needs to be considered when interpreting isotopic values in lakes is time of the year (e.g. season). Numerous studies of freshwater ecosystems have reported that the $\delta^{15}N$ and $\delta^{13}C$ of primary producers vary greatly over the seasons (e.g. France, 1995; Gu, Schell, & Alexander, 1994; Kumar, Finlay, & Sterner, 2011; Post, 2002; Zohary, Erez, & Stiller, 1994). Particularly, different investigations have highlighted the importance of intra-annual

variation in the concentrations and $\delta^{15}N$ values of major nutrient sources (NO_3^- and NH_4^+) on the $\delta^{15}N$ of phytoplankton (Gu, 2009; Kumar et al., 2011; Sugimoto, Sato, Yoshida, & Tominaga, 2014). Moreover, Gu (2009) stated that a single or a few samples of particulate organic matter are not likely to represent the isotope characteristics of surface waters for the entire growth season. Besides, the $\delta^{13}C$ values of primary producers can vary at different timescales as they are affected not only by temperature and nutrient availability but also by, among other factors, primary production, pH, changes in species composition and microbial processes (Moschen, Lücke, Parplies, & Schleser, 2009).

Lastly, in aquatic ecosystems single-celled primary producers can alter their C:N ratios widely in response to temperature changes (Thompson, Guo, & Harrison, 1992) and to nutrient limitation as well (Elser et al., 2000). The effect of increasing temperatures on phytoplankton C:N appears to be species specific; thus, some species do not change their ratio, whereas others show a positive linear relationship between C:N and temperature (Thompson et al., 1992). Increases in the N influx produce changes in N availability, which potentially alter the C:N stoichiometry of autotrophs. Altered C:N stoichiometry in primary producers could, in turn, influence N storage in living and non-living organic matter, the lability of detritus, the movement of N through ecosystems and the flow of energy through food webs (Dodds et al., 2004). Moreover, primary producers show environmentally induced variation in C:N ratios because of their ability to affect structural C. Lake seston (mainly algae and detritus derived from algae) usually has C:N (mass ratio) between 5.5 and 17 (Hecky, Campbell, & Hendzel, 1993; Ventura, Liboriussen, Lauridsen, Søndergaard, & Jeppesen, 2008), whereas periphyton C:N can reach higher values, i.e. >40 (Hillebrand & Sommer, 1999).

To elucidate factors controlling the stable isotope composition and C:N ratio of seston and periphyton in shallow lakes, we used a long-term outdoor experiment with 24 mesocosms impacted by different nutrient treatments (enriched and unenriched) and different temperatures (ambient, +3 and +5°C) (Liboriussen et al., 2005). This state-of-the-art system of mesocosms provides a unique opportunity to assess the extent of warming and nutrient enrichment in the temporal heterogeneity of C and N isotope ratios in pond-like systems, not least because the experiment has a short water retention time (2.5 months) and have been running for 6 years, making them less influenced by the transient conditions that typically characterise short-term experiments.

We had the following hypotheses: H_1: Nutrient enrichment (increasing trophic state) would enhance the seasonal variability in $\delta^{15}N_{SES}$ and $\delta^{15}N_{PER}$, being highest in the heated enriched mesocosms where more extreme environmental forcing (e.g. in pH and nutrient level) may exert the greatest seasonal impacts on lake primary productivity; H_2: Nutrient enrichment would increase $\delta^{15}N_{SES}$ and $\delta^{15}N_{PER}$ values provided that the mesocosms were not dominated by N_2-fixing cyanobacteria; H_3: $\delta^{13}C_{SES}$ and $\delta^{13}C_{PER}$ would increase due to more carbon limitation (higher pH values) and to less carbon fractionation by autotrophs in the enriched and heated mesocosms, mainly in summer; H_4: Periphyton would have higher $\delta^{13}C$ values than seston due to the effect of boundary layers, reducing the CO_2 diffusion; and, H_5: C:N ratios of the sestonic and periphytic communities would differ across treatments, for instance showing lower values at high nutrient levels and low temperatures.

2 | MATERIALS AND METHODS

2.1 | Study site

In 2003, a mesocosm experiment was established in a lowland valley in Central Jutland, Denmark, to mimic present and future shallow lake environments (Liboriussen et al., 2005). The experimental set-up consisted of outdoor mesocosms (cylindrical stainless steel tanks with a diameter of 1.9 m and a total depth of 1.5 m [2.8 m^3]). Ground water entered the mesocosms *c.* 10 cm above the sediment with an outlet at the water surface. The system ran at two nutrient levels: unenriched and enriched ground water (augmented weekly with 54 mg P and 2152 mg N, N:P of 88 and $\delta^{15}N_{Ca(NO_3)_2}$ of 4.5 ± 1‰) crossed with three temperature treatments: ambient and heated according to the A2 scenario (*c.* +3°C, predicted temperature during the period 2071–2100 [IPCC, 2007]), down-scaled to local 25 × 25 km grid cells, and the A2+50% scenario (*c.* +5°C). Each treatment combination had four replicates. The target N and P concentrations were <0.02 mg P/L and <1 mg N/L in the unenriched mesocosms and >0.1 mg P/L and >2 mg N/L in the enriched mesocosms. The sediment and the mixture of active plankton communities inoculated in the mesocosms came from nearby lakes and ponds. The mesocosms also contained three-spined sticklebacks (*Gasterosteus aculeatus* Gasterosteidae) near to natural densities in Danish lakes relative to the nutrient treatment: one male in the unenriched mesocosms and a mixture of males and females (breeding allowed) in the enriched mesocosms. The water retention time of the mesocosms averaged 2.5 months and paddles provided continuous stirring. A higher modelled temperature difference for the A2+50% scenario from the control occurred from August to January (max. 6.6°C in September) compared with the rest of the year (min. 3.7°C in June). For further details, see Liboriussen et al. (2005).

2.2 | Temperature and pH

Water temperature is recorded continuously by temperature sensors (temperature transmitter type: TT-5333; PR electronics products, Rønde, Denmark) placed centrally in all mesocosms. pH is recorded every 30 min (Manta pH measurement system; OxyGuard, Water Management Technologies, Los Angeles, CA) in 12 of the mesocosms at the same time and rotated among the mesocosms every fourth week. The probes were calibrated weekly.

2.3 | Sample and field data collection

We collected monthly seston and periphyton samples from August 2008 to August 2009 and September 2008 to August 2009,

respectively, to determine elemental N and C and the stable isotopes $\delta^{15}N$ and $\delta^{13}C$.

To obtain seston samples, we pooled eight depth-integrated (water surface to sediment surface) water samples taken with a core sampler in the open water (to avoid resuspension of epiphytes). We pre-filtered the pooled sample through a 50-μm net followed by filtering through pre-weighed and pre-combusted GF/F filters (Whatman, Maidstone, U.K.). We used the water to analyse for chlorophyll *a* (Chl-*a*) and nutrients (see methods below).

To obtain periphyton samples, we placed sets of three 20-cm-long artificial plants (artificial ivy) hanging 15 cm below the water surface inside the mesocosms. We left the plants in the water for 1 month to allow periphyton colonisation and then replaced them on the same day that seston, phytoplankton Chl-*a* and nutrient samples were taken. We pooled the periphyton obtained from the three artificial plants from each mesocosm into one sample by placing the three artificial plants in a tray with distilled water. Periphyton was scraped off with a scalpel after which the water was pre-filtered through a 200-μm net and then through pre-weighed and pre-combusted GF/F filters. Filters with seston and periphyton were dried at 60°C for 2 days and then we scraped off the material from the GF/F filters and loaded it into tin capsules. Following Fernandes and Krull (2008), we did not fume the samples with HCl to avoid potential changes in the $\delta^{15}N$ values of the samples.

The C and N stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and the C:N mass ratio of solid material were analysed at the Stable Isotope Facility, University of California, Davis. We expressed stable isotope data in part per thousand (‰) deviations from international standards (Vienna Pee Dee Belemnite and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$ respectively) using the following equation:

$$\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1{,}000$$

where $X = {}^{13}C$ or ^{15}N and R = ratio of heavy/light isotope content ($^{13}C/^{12}C$ or $^{15}N/^{14}N$), and the working standards were glutamic acid and peach leaves. Internal precision was <0.2‰.

We reported C:N as atomic ratios and analysed them following Wetzel (2001), values >14.6 indicating severe N deficiency, values between 8.3 and 14.6 moderate deficiency and values <8.3 no N deficiency.

We collected monthly groundwater/inlet water samples for 13 months during 2008–2009 and analysed NO_3-N concentrations and isotopes ($\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$). The samples were collected in three time-rinsed 250-ml polyethylene bottles. Samples were kept on ice before filtering on a 47 mm GF/C filter (Whatman, Maidstone, U.K.) and then stored at −20°C. After determining the NO_3-N concentration in the samples (see method below), we forwarded 100 ml aliquots of each sample on ice for $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$ analyses at the Stable Isotope Laboratory, University of East Anglia, U.K. Denitrifying bacteria, *Pseudomonas aureofaciens*, converted 20 nmoles of NO_3^- into gaseous N_2O before isotope analysis (Casciotti, Sigman, Hastings, Böhlke, & Hilkert, 2002; Sigman et al., 2001). $\delta^{15}N$ and $\delta^{18}O$ analyses were made in duplicate. Isotopic compositions were reported in parts per thousand (‰) relative to atmospheric N_2 and Vienna Standard Mean Ocean Water for $\delta^{15}N$ and $\delta^{18}O$, respectively, using the equation previously described. Samples were analysed applying three international standards: IAEA N3, USGS 34 and USGS 35, each run in five replicates.

2.4 | Phytoplankton Chl-*a*, macrophytes, filamentous green algae and nutrients

We determined phytoplankton Chl-*a* and nutrient concentrations monthly from a pooled integrated water sample collected with a core sampler in open water at three sites We estimated Chl-*a* spectrophotometrically using a Shimadzu UV-160 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan) by ethanol extraction of filter residues (GF/C, 47 mm) according to Jespersen and Christoffersen (1987). We also calculated coverage of macrophytes and filamentous green algae once every month in the upper (0–0.5 m depth) and lower (0.5–1.0 m depth) parts of each mesocosm during 11 months, from August 2008 to June 2009. We assigned macrophytes and filamentous green algae coverage to the following categories: 0, >0%–5%, >5%–25%, >25%–50%, >50%–75% and >75%–100%. In addition, we measured upper and lower heights/lengths of the macrophytes and filamentous green algae, and we calculated total plant volume inhabited (PVI, *sensu* Canfield et al., 1984) for each mesocosm as %PVI = % coverage × plant height/water depth.

We measured concentrations of phosphate (PO_4-P) (Murphy & Riley, 1962), total phosphorus P (TP), nitrate (NO_3-N) (Grasshoff, Ehrhardt, & Kremling, 1983), ammonium (NH_4-N) and total N (TN) (Rebsdorf, Søndergaard, & Thyssen, 1989). PO_4-P, TP and NH_4-N were analysed using the Shimadzu UV-160 Spectrophotometer, and NO_3-N and TN were analysed with a FIA Star-5000 flow-injection analyser (Foss UK Ltd, Didcot, U.K.).

2.5 | Statistical analyses

We performed repeated measures analysis of variance (RM ANOVA) to compare physical, chemical and biological parameters in the two nutrient level (unenriched and enriched) and the three temperature scenarios (Ambient, A2 and A2+50%).

Next, we analysed the results from the isotope data using a two-step approach. First, we applied RM ANOVAs with a first-order autoregressive covariance structure and heterogeneous variances to compare $\delta^{15}N_{SES}$, $\delta^{15}N_{PER}$, $\delta^{13}C_{SES}$ and $\delta^{13}C_{PER}$ in unenriched and enriched mesocosms at the three temperatures across time (months). This approach allowed us to analyse the effects of nutrients and temperatures as categorical variables to explain the changes in the stable isotopes of the sestonic and periphytic communities. To avoid mask effects in the interaction term, which had large numbers of degrees of freedom in the numerator, we analysed the interaction results when two main effects were significant (Little, Bovaird, & Widaman, 2006). We also applied this analysis to $C:N_{SES}$ and $C:N_{PER}$ data.

Second, we ran general linear models using only quantitative covariates for all the response variables ($\delta^{15}N_{SES}$, $\delta^{15}N_{PER}$, $\delta^{13}C_{SES}$ and $\delta^{13}C_{PER}$) and included the factor time as season in the models.

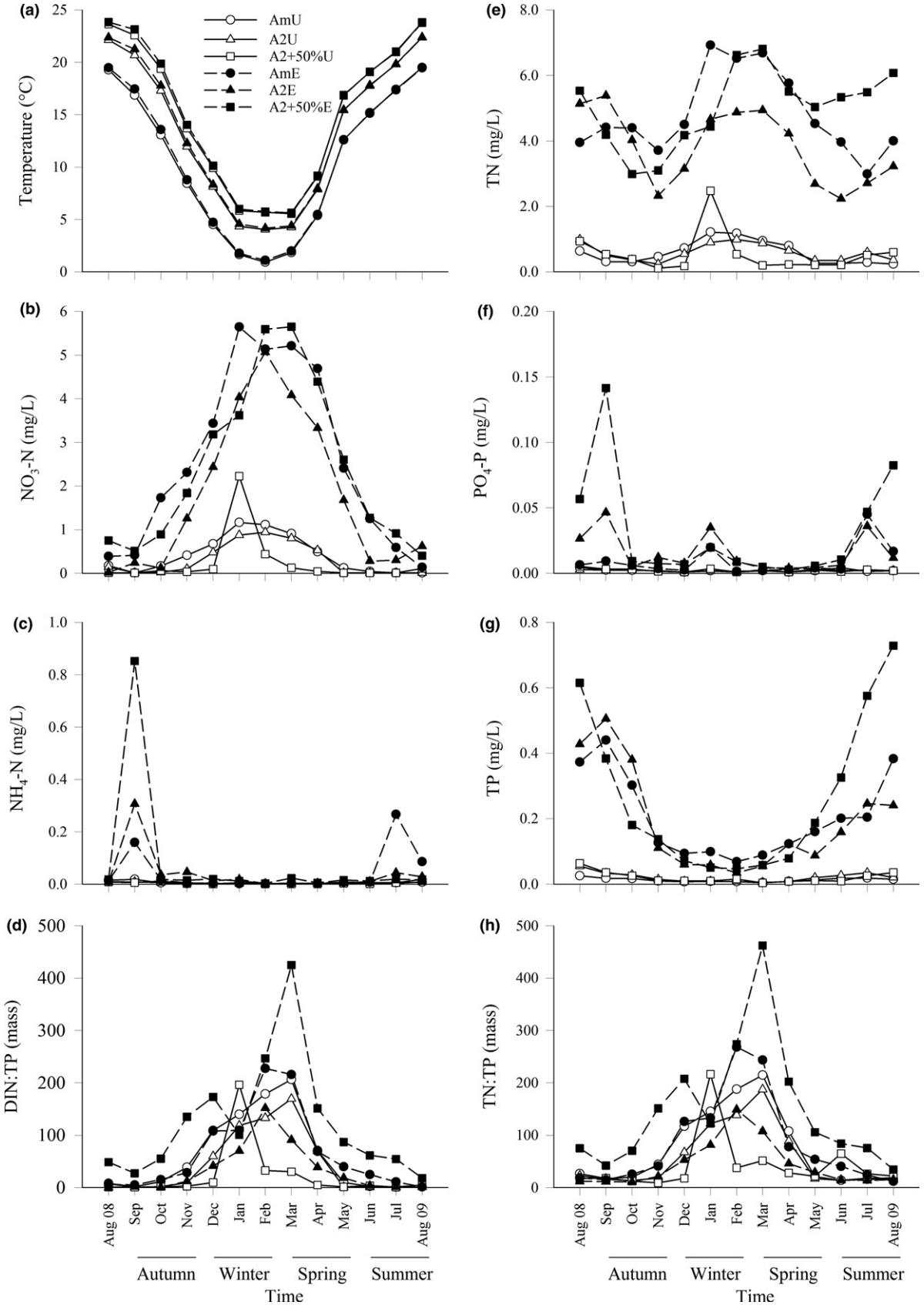

FIGURE 1 Mean values of temperature (a), nutrient concentrations (b, c, e, f and g) and nutrient ratios (d, h) during the sampling period for each treatment combination, ambient unenriched: AmU; A2 unenriched: A2U; A2+50% unenriched: A2+50%U (open symbols and solid lines) and ambient enriched: AmE; A2 enriched: A2E; A2+50% enriched: A2+50%E (filled symbols)

TABLE 1 Mean ± 1 *SE* and range (min–max) of stable isotope values, C:N ratios and physical, chemical and biological parameters for each treatment combination – ambient unenriched: AmU; A2 unenriched: A2U; A2+50% unenriched: A2+50%U and ambient enriched: AmE; A2 enriched: A2E; A2+50% enriched: A2+50%E. Results of repeated measures (RM) ANOVAs using the effects of nutrients (two levels, unenriched and enriched) and temperature (three levels, ambient, A2 and A2+50%) are also shown. FGA, filamentous green algae, PVI, plant volume inhabited; TN, total nitrogen; TP, total phosphorus, DIN, dissolved inorganic nitrogen, N.S., not significant

Variables/ Treatments	AmU	A2U	A2+50%U	AmE	A2E	A2+50%E	RM ANOVA and Bonferroni's Test $p < .05$
$\delta_{15}N_{SES}$ (‰)	5.2 ± 0.3 (−2.0 to 9.0)	5.4 ± 0.3 (0.4 to 8.7)	5.9 ± 0.3 (1.5 to 9.1)	3.2 ± 0.4 (−3.1 to 9.0)	4.2 ± 0.2 (−1.0 to 7.9)	4.3 ± 0.4 (−5.0 to 9.0)	
$\delta_{15}N_{PER}$ (‰)	3.6 ± 0.1 (1.1 to 5.3)	3.9 ± 0.1 (1.8 to 5.5)	4.2 ± 0.2 (1.8 to 6.8)	3.6 ± 0.2 (1.1 to 8.8)	4.5 ± 0.2 (2.1 to 9.8)	3.6 ± 0.2 (1.2 to 7.1)	
$\delta_{13}C_{SES}$ (‰)	−26.6 ± 0.4 (−31.9 to −22.3)	−26.9 ± 0.4 (−33.4 to −22.0)	−26.6 ± 0.4 (−35.4 to −22.2)	−28.2 ± 0.5 (−38.0 to −22.7)	−28.5 ± 0.3 (−34.5 to −24.5)	−26.8 ± 0.3 (−32.6 to −23.0)	
$\delta_{13}C_{PER}$(‰)	−27.5 ± 0.3 (−31.0 to −22.7)	−26.9 ± 0.4 (−32.0 to −21.0)	−26.7 ± 0.4 (−32.1 to −19.8)	−26.5 ± 0.3 (−30.5 to −20.6)	−26.8 ± 0.4 (−32.4 to −18.7)	−26.0 ± 0.4 (−30.0 to −18.6)	
$C:N_{SES}$	8.5 ± 0.2 (5.5 to 11.7)	9.0 ± 0.3 (5.7 to 14.0)	9.3 ± 0.3 (5.8 to 12.5)	7.7 ± 0.2 (5.4 to 11.6)	8.1 ± 0.2 (6.0 to 11.4)	8.3 ± 0.2 (5.6 to 11.8)	
$C:N_{PER}$	18.7 ± 1.3 (6.9 to 40.4)	18.2 ± 1.3 (8.0 to 44.0)	18.7 ± 1.1 (8.5 to 35.8)	16.2 ± 1.0 (7.7 to 45.9)	14.3 ± 0.7 (5.3 to 27.1)	16.3632 ± 1.1 (6.3 to 38.4)	
Temperature (°C)	10.5 ± 0.9 (0.9 to 19.9)	13.6 ± 1.0 (4.0 to 22.8)	15.1 ± 1.0 (5.4 to 24.2)	10.7 ± 0.9 (1.0 to 19.8)	13.7 ± 1.0 (3.9 to 22.7)	15.3 ± 1.0 (5.4 to 24.1)	Ambient < A2 < A2+50%
NH_4-N (mg/L)	0.005 ± 0.001 (0.001 to 0.053)	0.004 ± 0.001 (0.001 to 0.020)	0.005 ± 0.001 (0.001 to 0.046)	0.044 ± 0.019 (0.001 to 0.877)	0.040 ± 0.022 (0.001 to 1.142)	0.079 ± 0.046 (0.001 to 2.394)	Unenriched < enriched
NO_3-N (mg/L)	0.41 ± 0.07 (0.01 to 1.54)	0.30 ± 0.05 (0.01 to 1.10)	0.25 ± 0.12 (0.01 to 5.64)	2.57 ± 0.29 (0.01 to 6.44)	1.80 ± 0.26 (0.01 to 6.23)	2.39 ± 0.30 (0.01 to 7.23)	Unenriched < enriched
TN (mg/L)	0.59 ± 0.06 (0.50 to 1.55)	0.59 ± 0.05 (0.10 to 1.55)	0.55 ± 0.13 (0.05 to 6.40)	4.80 ± 0.24 (1.40 to 8.00)	3.81 ± 0.23 (0.60 to 7.45)	5.02 ± 0.38 (0.05 to 11.00)	Unenriched < enriched
TP (mg/L)	0.013 ± 0.001 (0.003 to 0.033)	0.021 ± 0.003 (0.004 to 0.110)	0.020 ± 0.004 (0.001 to 0.163)	0.205 ± 0.029 (0.009 to 0.852)	0.191 ± 0.025 (0.028 to 0.628)	0.265 ± 0.059 (0.004 to 1.629)	Unenriched < enriched
PO_4-P (mg/L)	0.002 ± 0.000 (0.001 to 0.008)	0.002 ± 0.00 (0.001 to 0.006)	0.002 ± 0.000 (0.001 to 0.015)	0.009 ± 0.003 (0.001 to 0.152)	0.016 ± 0.003 (0.001 to 0.125)	0.031 ± 0.010 (0.001 to 0.410)	Unenriched < enriched
DIN:TP (mass ratio)	60 ± 12 (0 to 316)	44 ± 9 (0 to 223)	22 ± 10 (0 to 435)	66 ± 20 (0 to 716)	33 ± 7 (0 to 188)	122 ± 31 (0 to 147)	N.S.
TN:TP (mass ratio)	73 ± 12 (3 to 325)	57 ± 9 (9 to 237)	42 ± 11 (4 to 492)	83 ± 22 (8 to 800)	43 ± 7 (8 to 225)	147 ± 33 (4.2 to 1487)	N.S.
pH	8.5 ± 0.1 (6.3 to 9.8)	8.5 ± 0.1 (7.2 to 10.2)	8.5 ± 0.1 (7.4 to 9.6)	8.7 ± 0.1 (7.4 to 10.3)	8.4 ± 0.1 (7.4 to 10.1)	8.6 ± 0.1 (7.4 to 10.2)	N.S.
Chl-*a* (μg/L)	4.6 ± 0.5 (0.5 to 22.4)	13.1 ± 2.7 (0.6 to 89.2)	14.2 ± 3.5 (1.1 to 142.2)	307.4 ± 46.6 (2.6 to 1306.0)	193.3 ± 34.9 (2.9 to 1228.9)	253.5 ± 58.5 (2.3 to 1988.0)	Unenriched < enriched
Macrophyte PVI (%)	18.3 ± 2.8 (0.7 to 78.3)	23.7 ± 2.6 (3.0 to 64.4)	28.1 ± 2.8 (0.0 to 69.6)	7.8 ± 2.5 (0.0 to 65.5)	0.0 ± 0.0 (0.0 to 0.0)	5.2 ± 2.0 (0.0 to 64.4)	Unenriched > enriched Ambient < A2 < A2+50%
FGA PVI (%)	0.6 ± 0.1 (0.0 to 4.0)	0.3 ± 0.2 (0.0 to 6.0)	7.4 ± 2.2 (0.0 to 56.0)	0.9 ± 0.4 (0.0 to 15.0)	3.0 ± 1.7 (0.0 to 50.0)	2.6 ± 1.1 (0.0 to 40.0)	N.S.

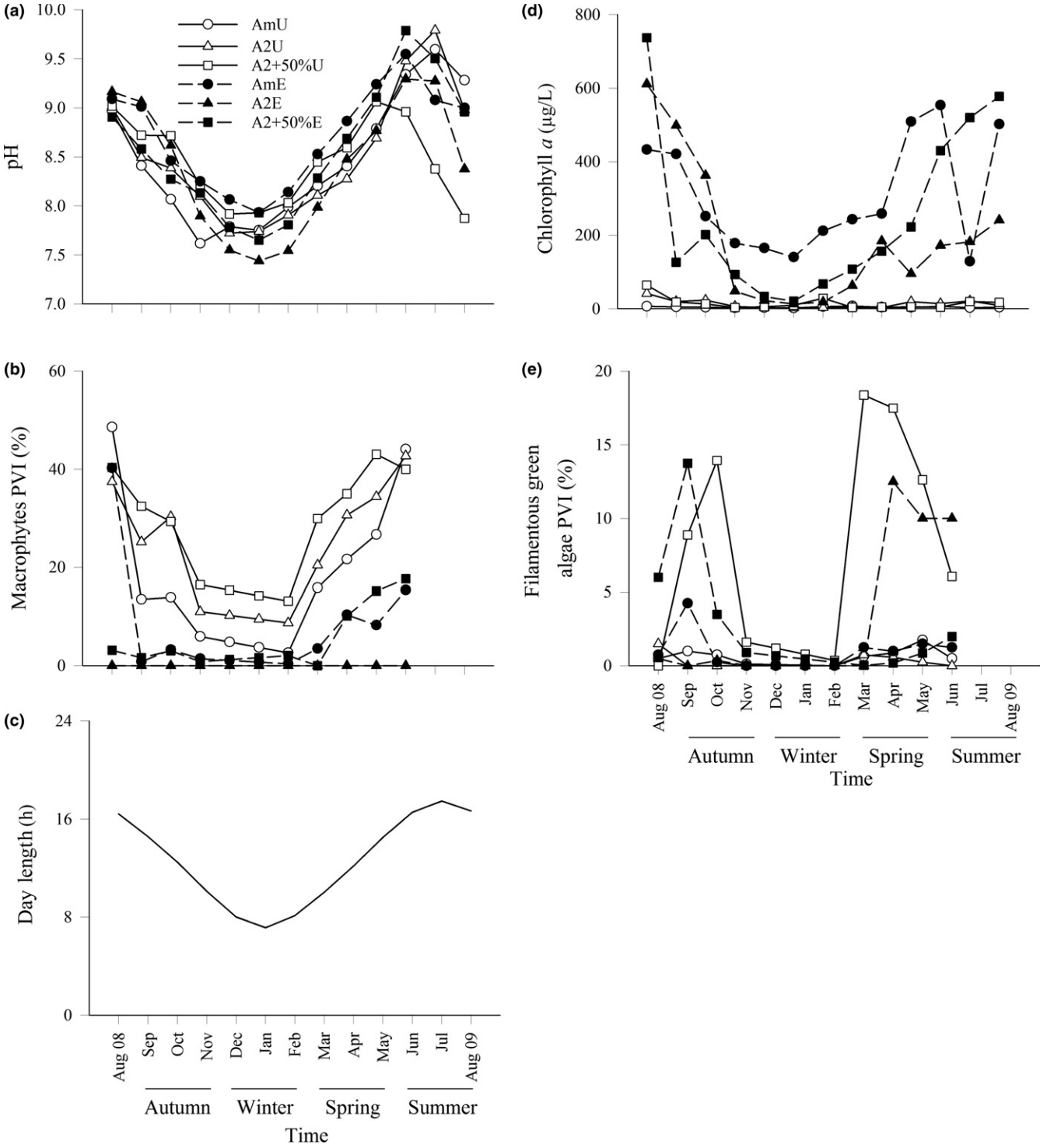

FIGURE 2 Mean values of pH (a), biological parameters (b, d, e) and day length (c) during the sampling period AmU; A2U; A2+50%U (open symbols and solid lines) and AmE; A2E; A2+50%E (filled symbols and dashed lines)

We generated several models replacing the nutrient treatment levels for the actual measurements of: (1) inorganic N (NO_3-N and NH_4-N) and inorganic P (PO_4-P); (2) Total N and Total P; (3) DIN (dissolved inorganic nitrogen):TP mass ratio; (4) TN:TP mass ratio in the mesocosms. The temperature treatments were also replaced with the mean monthly temperature of each mesocosm. We additionally included phytoplankton Chl-*a*, pH and day length as explanatory variables in the regression models. Besides, $\delta^{15}N_{NO_3}$ was included as a covariate in the model run for $\delta^{15}N$. We performed a correlation matrix with the explanatory variables to exclude multicollinearity. These models were also run using first-order autoregressive covariance and heterogeneous variances, and we reduced the initial models in a step-wise manner using the log-likelihood test to compare the models until only significant ($p < .05$) factors remained (Rawlings,

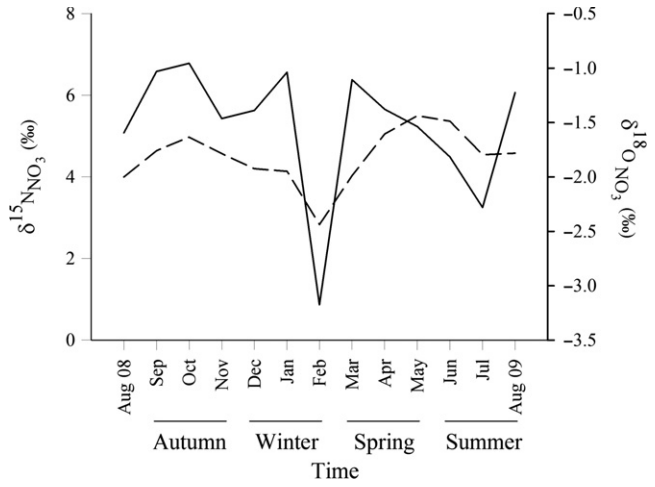

FIGURE 3 Monthly values of $\delta^{15}N_{NO_3}$ (solid line) and $\delta^{18}O_{NO_3}$ (dashed line) in the inlet water during the sampling period

1988). All variables were log10(*x*)-transformed or square-root arcsine-transformed except for pH.

It is worth mentioning that we performed a linear regression model using pH as response variable and phytoplankton Chl-*a*, macrophytes %PVI and filamentous green algae %PVI as explanatory variables to assess if the changes in pH were driven by primary productivity. The regression was run using data for 11 months (August 2008–June 2009). The regression model ($r^2 = .51$, $p < .001$) indicated that pH was significantly related to phytoplankton Chl-*a*, macrophyte %PVI and filamentous green algae %PVI in the enriched mesocosms ($p < 0.01$) and to macrophyte %PVI in the unenriched mesocosms ($p < .001$). Regrettably, as macrophytes and filamentous green algae were measured only for 11 (August 2008–June 2009) and not 13 (August 2008–August 2009) months, and periphyton Chl-*a* was not recorded, we decided to use pH in the models. Statistical analyses were performed using the software PASW statistics version 18.0.0 (IBM).

3 | RESULTS

3.1 | Physical, chemical and biological variables

The temperature in the mesocosms differed significantly in the three climate scenarios (Figure 1a; Table 1). The unenriched mesocosms had significantly lower concentrations of NO_3-N, NH_4-N, TN, PO_4-P and TP than the enriched mesocosms (Figure 1b–e, Table 1). pH

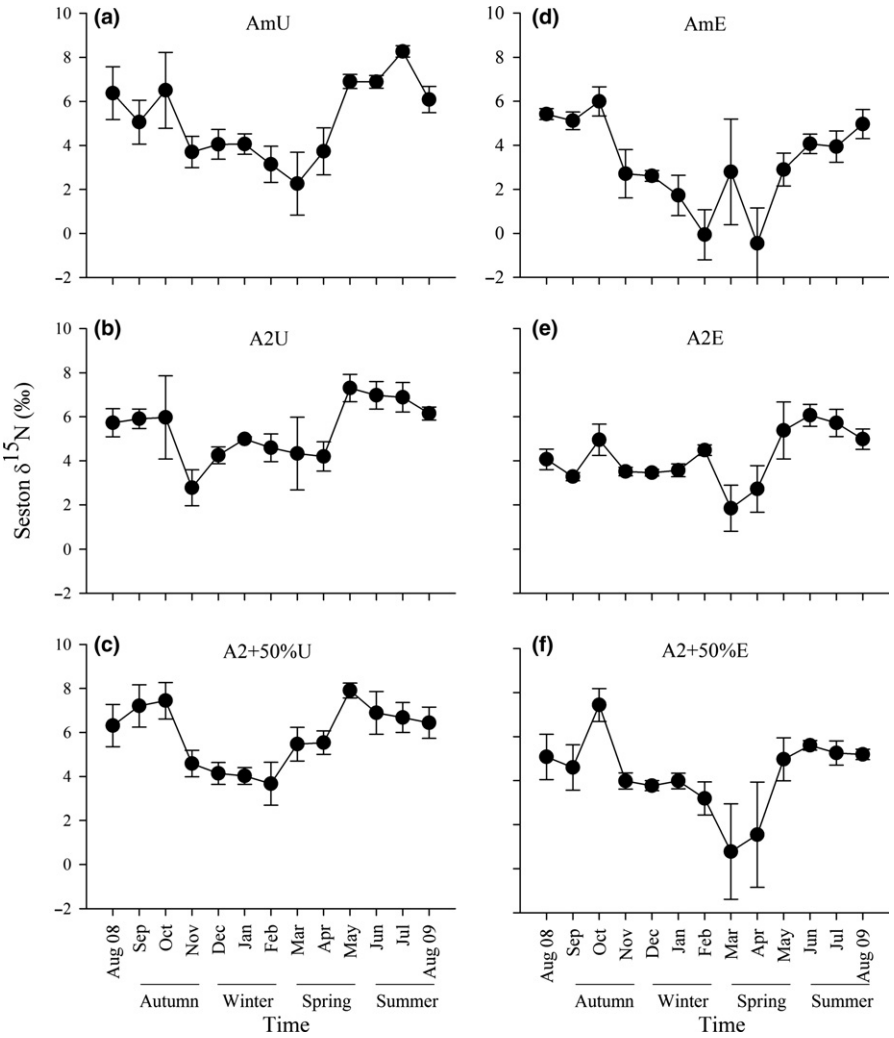

FIGURE 4 $\delta^{15}N_{SES}$ (mean ± 1 *SE*) (solid line and filled circles) for AmU (a); A2U (b); A2+50%U (c); AmE (d); A2E (e); A2+50%E (f)

TABLE 2 Results of RM ANOVA tests on $\delta^{15}N_{SES}$, $\delta^{15}N_{PER}$, $\delta^{13}C_{SES}$ and $\delta^{13}C_{PER}$ using the effects of month (time), temperature (three levels, ambient, A2 and A2+50%) and nutrients (two levels, unenriched and enriched). Significance levels: *$p < .05$, **$p < .01$, ***$p < .0001$, N.S., not significant

	$\delta^{15}N_{SES}$		$\delta^{15}N_{PER}$		$\delta^{13}C_{SES}$		$\delta^{13}C_{PER}$	
	df	*F*-values	*df*	*F*-values	*df*	*F*-values	*df*	*F*-values
Month	12	9.06***	11	11.89***	12	12.99***	11	13.96***
Temperature	2	4.43*	2	2.58 N.S	2	1.46 N.S	2	1.86 N.S.
Nutrients	1	39.57***	1	0.02 N.S	1	4.49*	1	2.80 N.S.
Month × temperature	24	0.97 N.S.	1	0.55 N.S	24	1.02 N.S	1	0.96 N.S.
Month × nutrients	12	1.25 N.S.	11	2.85**	12	1.38 N.S.	11	1.30 N.S.
Temperature × nutrients	2	0.61 N.S.	2	2.26 N.S	2	0.69 N.S.	2	0.31 N.S.
Month × temperature × nutrients	24	1.14 N.S.	22	0.84 N.S	24	0.87 N.S.	22	0.93 N.S.

values did not show significant differences among treatments (Figure 2a). Phytoplankton Chl-*a* concentrations were significantly higher in the enriched than in the unenriched mesocosms (Figure 2d; Table 1). Macrophyte %PVI obtained the highest values in the unenriched mesocosms and in the A2+50% temperature scenario (Figure 2b; Table 1). Finally, the treatments did not differ in DIN:TP mass ratio, TN:TP mass ratio (Figure 1d,h; Table 1) and filamentous green algae %PVI (Figure 2e; Table 1). Day length during the sampling period is also shown (Figure 2c).

3.2 | $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$

The NO_3-N concentrations in the inlet water ranged from 0.8 to 5.5 mg/L throughout the sampling period and $\delta^{15}N_{NO_3}$ varied between 4‰ and 5.5‰, except for February when it was lower (2.8‰) (Figure 3). Moreover, $\delta^{18}O_{NO_3}$ ranged between −2.3‰ and −1‰ (except −3.2‰ in February) (Figure 3), which is consistent with $\delta^{18}O_{NO_3}$ derived from ground water with no direct influence of atmospherically deposited NO_3^- (i.e. Burns, Boyer, Elliott, & Kendall, 2009).

3.3 | Seasonal variability in $\delta^{15}N$

The seasonal dynamics of $\delta^{15}N_{SES}$ in the unenriched mesocosms (Figure 4a–c) showed mean values >5‰ from August to October 2008 and a fast decline to minimum values during late autumn to early spring (November–April), after which the values increased rapidly in May and remained at >6‰ until August 2009 (Figure 1a–c). The patterns obtained for the different temperatures were similar (Figure 4a–c).

Seasonal $\delta^{15}N_{SES}$ data in the enriched mesocosms (Figure 4d–f) were comparable for the A2 and A2+50% temperature scenarios, the largest seasonal variation being found in the ambient temperature scenario (Figure 4d). The highest mean values were observed in October (between 5‰ and 7.4‰), whereas the lowest occurred in March and April in the A2 and A2+50% scenarios (between 0.8‰ and 2.7‰) and in February and April in the ambient temperature scenario (values near 0‰). At ambient temperature, March exhibited a higher mean but also the highest variation among the replicates (Figure 4d). For the rest of the annual cycle, $\delta^{15}N_{SES}$ varied between 3.2‰ and 6.1‰ in the heated mesocosms and between 1.7‰ and 5.4‰ at ambient temperature (Figure 4d–f).

The general RM ANOVA model run for $\delta^{15}N_{SES}$ showed significant variation in $\delta^{15}N_{SES}$ with time (month), temperature and nutrient level (Figure 4; Table 2). Particularly, $\delta^{15}N_{SES}$ was pronouncedly lower in the enriched mesocosms than in the unenriched mesocosms (except for October and November), and the lowest values occurred in the winter months in the ambient temperature mesocosms (Figure 4; Table 2 and Bonferroni's test $p < .05$).

The regression models for the isotope values of seston and periphyton obtained by replacing the nutrient treatment levels with TP and TN measurements or the ratios DIN:TP and TN:TP either excluded those covariates or had similar R^2 values than those using the inorganic forms of N and P (Tables S1–S6) and were therefore not included here.

The final regression model obtained for $\delta^{15}N_{SES}$ included pH, phytoplankton Chl-*a*, mean monthly temperature, NH_4-N and $\delta^{15}N_{NO_3}$ as significant covariates, all interacting with time (season) (Table 3). Phytoplankton Chl-*a* and $\delta^{15}N_{SES}$ showed a negative relationship during the winter, spring and summer months (Table 3). In contrast, mean monthly temperatures demonstrated a positive relationship with $\delta^{15}N_{SES}$ in autumn, winter and spring (Table 3). The covariates pH and NH_4-N exhibited an inverse relationship with $\delta^{15}N_{SES}$ in autumn/winter and autumn/spring respectively (Table 3). $\delta^{15}N_{NO_3}$ had a significant positive effect on $\delta^{15}N_{SES}$ only in autumn (Table 3).

The seasonal variation in $\delta^{15}N_{PER}$ was smaller than in $\delta^{15}N_{SES}$ (Figures 4 and 5). The mean values of $\delta^{15}N_{PER}$ were >2‰ in all the mesocosms and varied between 2.3‰ and 5.9‰ in the unenriched mesocosms (Figure 5a–c) and between 2.4‰ and 6.5‰ in the enriched mesocosms (Figure 5d–f). The lowest mean values appeared in February in all the unenriched mesocosms, whereas the enriched mesocosms had no period or month with markedly lower values, though an elevation occurred in the ambient and the A2 scenario in August 2008 (Figure 5).

The general RM ANOVA model run for $\delta^{15}N_{PER}$ showed that month and month-nutrient interaction explained its annual variability

TABLE 3 Results of backward regression models on the effects of pH, phytoplankton Chl-*a*, mean monthly temperature, day length, PO_4-P, NO_3-N, NH_4-N and $\delta^{15}N_{NO_3}$ on $\delta^{15}N_{SES}$ and $\delta^{15}N_{PER}$ respectively. The fixed factor season (time) was included in both models

	df	*F*-values	Partial coefficient	*p*-value
$\delta^{15}N_{SES}$ R^2 = .58				
Season	4	6.25	–	.0002
pH × season	4	5.24	–	.0005
Chl-*a* × season	4	25.74	–	<.0001
Mean monthly temperature × season	4	13.60	–	<.0001
NH_4-N × season	4	4.90	–	.0008
$\delta^{15}N_{NO_3}$ × season	4	4.75	–	.0010
pH: autumn	–	–	−1.27	<.0001
pH: winter	–	–	−1.13	.013
Chl-*a*: winter	–	–	−1.11	<.0001
Chl-*a*: spring	–	–	−1.33	<.0001
Chl-*a*: summer	–	–	−0.95	<.0001
Mean monthly temperature: autumn	–	–	0.33	<.0001
Mean monthly temperature: winter	–	–	0.12	.035
Mean monthly temperature: spring	–	–	0.38	<.0001
NH_4-N: autumn	–	–	−1.11	.0002
NH_4-N: spring	–	–	−1.22	.049
$\delta^{15}N_{NO_3}$: autumn	–	–	5.09	<.0001
$\delta^{15}N_{PER}$ R^2 = .385				
Season	4	12.61	–	<.0001
pH × season	4	4.56	–	.004
Chl-*a* × season	4	4.50	–	.003
Day length × season	4	6.45	–	.001
PO_4-P × season	4	2.79	–	<.0001
NO_3-N × season	4	4.81	–	.002
$\delta^{15}N_{NO_3}$ × season	4	13.23	–	<.03
pH: autumn	–	–	−0.55	.0019
pH: winter	–	–	−0.79	.0104
Chl-*a*: autumn	–	–	0.43	.0054
Chl-*a*: summer	–	–	0.32	.049
Day length: winter	–	–	0.89	<.0001
PO_4-P: spring	–	–	1.10	.0184
NO_3-N: winter	–	–	−0.27	.019
NO_3-N: spring	–	–	−0.43	.027
NO_3-N: summer	–	–	−0.45	.004
$\delta^{15}N_{NO_3}$: autumn	–	–	−1.39	.0096
$\delta^{15}N_{NO_3}$: winter	–	–	0.90	<.0001

(Table 2). The $\delta^{15}N_{PER}$ values from the unenriched and enriched mesocosms differed significantly only in February and April (Figure 5; Table 2 and Bonferroni's test $p < .05$).

The final regression model obtained for $\delta^{15}N_{PER}$ included pH, phytoplankton Chl-*a*, day length, PO_4-P, NO_3-N and $\delta^{15}N_{NO_3}$ as significant covariates, all interacting with time (season) (Table 3). pH exhibited an inverse relationship with $\delta^{15}N_{PER}$ during autumn/winter, whereas its relationship with NO_3-N was also negative in winter, spring and summer (Table 3). $\delta^{15}N_{PER}$ was positively related to phytoplankton Chl-*a* and day length in autumn/summer and winter respectively (Table 3). $\delta^{13}N_{PER}$ and PO_4-P showed a positive relationship in spring, whereas $\delta^{15}N_{PER}$ was negatively and positively related to $\delta^{15}N_{NO_3}$ in autumn and winter respectively (Table 3).

3.4 | Seasonal variability in $\delta^{13}C$

The seasonal dynamics of $\delta^{13}C_{SES}$ varied among treatments (Figure 6); the mean values of $\delta^{13}C_{SES}$ were relatively similar, though, ranging between −28.6‰ and −24.9‰ in the unenriched mesocosms (Figure 3a–c) and between −29.4‰ and −25.2‰ in the enriched mesocosms (Figure 6d–f). The seasonal patterns of $\delta^{13}C_{SES}$ in the unenriched mesocosms were rather similar for the three temperature scenarios until May when the values remained high in the ambient and A2 temperature scenarios, but exhibited a marked decline until August 2009 in the A2+50% scenario (Figure 6a–c). In contrast, in the enriched mesocosms the seasonal pattern of $\delta^{13}C_{SES}$ was similar in the ambient and A2 temperature scenarios with the highest values occurring during spring and summer (−24.0‰ to −27.4‰), followed by a marked decline in autumn to minimum values in winter (ranging between −29.6‰ and −32.1‰ from January to March). The A2+50% scenario exhibited less variability over the year (Figure 6d–f).

The variation in $\delta^{13}C_{SES}$ was explained by month and nutrient levels (Figure 6; Table 1). $\delta^{13}C_{SES}$ had lower values in the enriched than in the unenriched mesocosms in winter (December, January and February) (RM ANOVA and Bonferroni's test $p < .05$) (Figure 6; Table 2).

The regression model showed that the covariates: pH, phytoplankton Chl-*a*, mean monthly temperature, day length, PO_4-P and NH_4-N modelled $\delta^{13}C_{SES}$ and that their relationship with the dependent variable varied in intensity and significance with time (seasons) (Table 4). Particularly, we obtained a positive relationship between $\delta^{13}C_{SES}$ and pH all year round (Table 4), whereas phytoplankton Chl-*a* and $\delta^{13}C_{SES}$ demonstrated a negative relationship with variable slopes during the year, the most pronounced effect of this covariate occurring in winter (Table 4). $\delta^{13}C_{SES}$ showed a positive relationship with mean monthly temperature and a negative relationship with day length in winter (Table 4). The effect of the covariates PO_4-P and NH_4-N was significant in spring and summer, respectively, and its relationship with $\delta^{13}C_{SES}$ was negative (Table 4).

The seasonal variation in $\delta^{13}C_{PER}$ was larger than for $\delta^{13}C_{SES}$ (Figures 6 and 7). The patterns were similar for both nutrient treatments in the ambient temperature scenario, except for a sudden decrease in March values in the enriched mesocosms (−28.9‰) and later in April in the unenriched mesocosms (−28.2‰) (Figure 7a,d). $\delta^{13}C_{PER}$ dynamics in the two nutrient treatments was also similar for

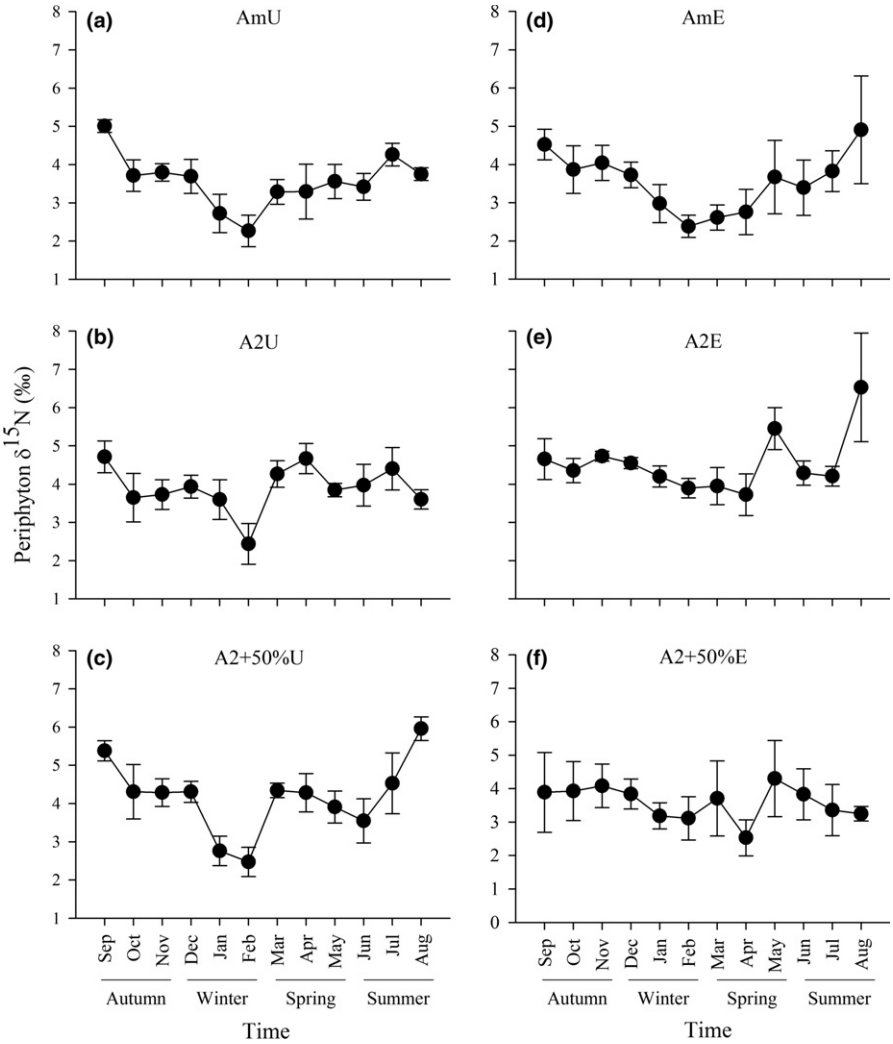

FIGURE 5 $\delta^{15}N_{PER}$ (mean ± 1 *SE*) (solid line and filled circles) for AmU (a); A2U (b); A2+50%U (c); AmE (d); A2E (e); A2+50%E (f)

climate scenarios A2 and A2+50% (Figure 7b,c,e,f). Particularly, the highest value of $\delta^{13}C_{PER}$ was recorded in May in the enriched A2+50% scenario (*c.* −22‰, Figure 7f).

The general RM ANOVA model run for $\delta^{13}C_{PER}$ showed that month was the key factor explaining annual variability (Table 2). Meanwhile, the regression model indicated that $\delta^{13}C_{PER}$ variations are related to mean monthly temperature, to pH-season interactions and to phytoplankton Chl-*a*-season and PO_4-P-season interactions (Table 4). $\delta^{13}C_{PER}$ showed a positive relationship with both mean monthly temperature and pH, the latter exhibiting different slopes along the seasons (Table 4). The effects of the covariates phytoplankton Chl-*a* and PO_4-P on $\delta^{13}C_{PER}$ were significant for autumn/spring and winter/summer, respectively, showing variable slopes (Table 4).

3.5 | Stoichiometry of seston and periphyton

The $C:N_{SES}$ ratios showed similar seasonal patterns in the unenriched mesocosms for all temperature treatments evidencing moderate N deficiency (values >8.3), the highest values occurring in winter (Figure 8a). The pattern of the $C:N_{SES}$ ratio in the enriched mesocosms was similar for the ambient and A2 temperature scenarios (values <8.3, Figure 8c), while A2+50% had higher values in November, December and January (pointing to a moderate N deficiency, Figure 8c) as in the unenriched mesocosms. The general RM ANOVA for the $C:N_{SES}$ ratio showed temperature by month and nutrient by month interactions (Table 5). The ambient temperature scenario differed from the other two scenarios in December and January, while the nutrient scenarios differed throughout the year except in May, June and July, evidencing a higher deficiency of N in the unenriched mesocosms (RM ANOVA and Bonferroni's tests $p < .05$) (Figure 8a, c; Table 5).

$C:N_{PER}$ values were usually high (>15, Figure 8b,d) from January until August, particularly in the unenriched mesocosms, indicating moderate or severe N deficiency. $C:N_{PER}$ ratios exhibited seasonality (month: $p < .0001$) and nutrient by month interaction (Table 5), demonstrating significantly higher N deficiency in warm months (from May to November) in the unenriched mesocosms (RM ANOVA and Bonferroni's tests $p < .05$). (Figure 8b; Table 5).

Finally, the differences in C:N between the enriched and unenriched mesocosms were greater for seston than for periphyton, and less clear effects of temperature were observed (Figure 8; Table 5).

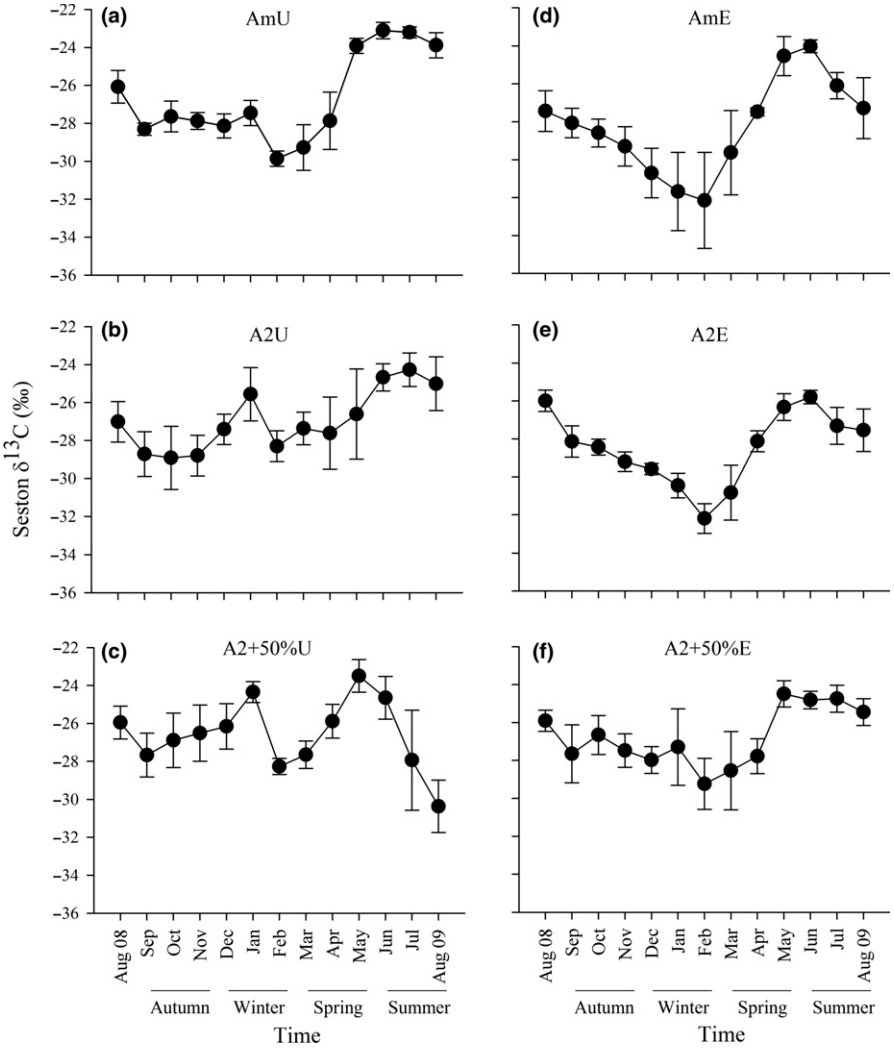

FIGURE 6 $\delta^{13}C_{SES}$ (mean ± 1 *SE*) (solid line and filled circles) for AmU (a); A2U (b); A2+50%U (c); AmE (d); A2E (e) and A2+50%E (f)

4 | DISCUSSION

Our investigation reveals changes in the $\delta^{15}N$ values of sestonic and periphytic communities under scenarios of contrasting nutrient and temperature levels and shows that those variations are governed by rather complex interactions among several environmental variables that are, in turn, time (season) dependent. Large variation in $\delta^{15}N$ among lakes reflects that multiple intertwining factors are at play such as variation in primary productivity, N-fixation, sources of nitrogen used and the isotope composition of dissolved inorganic nitrogen (Hadas et al., 2009). Our first hypothesis (H_1) was that the $\delta^{15}N$ values of seston and periphyton would increase with increasing nutrient level (e.g. Gu, 2009). We observed the opposite, as $\delta^{15}N_{SES}$ values were lower in the enriched than in the unenriched mesocosms during most seasons. Moreover, $\delta^{15}N_{SES}$ values were only higher in the heated scenarios than in the ambient temperature scenario in winter. The seasonal variation in $\delta^{15}N_{SES}$ was related to several variables, but mainly to phytoplankton Chl-*a* and mean monthly water temperature. As proposed in our second hypothesis (H_2), a negative relationship between $\delta^{15}N_{SES}$ and phytoplankton Chl-*a* would suggest a higher contribution of N_2-fixing cyanobacteria at increasing phytoplankton abundance. Indeed, our preliminary analysis of phytoplankton winter samples in a sub-set of the mesocosms (data not shown) indicated a high contribution of potential N_2-fixing cyanobacteria. The cyanobacteria *Chroococcus* spp. was the dominant algae group (>85%) in the unenriched mesocosms, whereas a mixture of cyanobacteria (*Chroococcus* spp.) and green algae (mainly *Monoraphidium* sp. and *Scenedesmus* sp.) dominated in the enriched mesocosms (averaging 57% and 19% respectively). Furthermore, the positive relationship between water temperature and $\delta^{15}N_{SES}$ indicated a primary productivity-driven isotope fractionation. During N uptake, phytoplankton preferentially uses ^{14}N, which results in an N pool enriched in ^{15}N. Isotope fractionation decreases as the phytoplankton growth rate increases and as the substrate level declines (Montoya & Mccarthy, 1995; Waser et al., 1998). Therefore, during the productive period of a seasonal cycle, i.e. the spring/summer months, phytoplankton was enriched in ^{15}N to a greater extent than during the unproductive period, i.e. the winter months.

Variations in the $\delta^{15}N$ of particulate organic matter may also reflect changes in inorganic and organic nutrient availability and utilisation (Altabet, 1996). Where NO_3-N is the primary N source, a negative relationship between the $\delta^{15}N$ of particulate organic matter

TABLE 4 Results of backward regression models on the effects of pH, phytoplankton Chl-*a*, mean monthly temperature, day length, PO_4-P and NH_4-N on $\delta^{13}C_{SES}$ and $\delta^{13}C_{PER}$ respectively. The fixed factor season (time) was included in both models

	df	*F*-values	Partial coefficient	*p*-value
$\delta^{13}C_{SES}$ R^2 = .67				
Season	4	90.35	–	<.0001
pH × season	4	22.79	–	<.0001
Chl-*a* × season	4	10.28	–	<.0001
Mean monthly temperature × season	4	6.59	–	<.0001
Day length × season	4	7.08	–	<.0001
PO_4-P × season	4	3.23	–	.013
NH_4-N × season	4	3.65	–	.0066
pH: autumn	–	–	1.36	<.0001
pH: winter	–	–	2.83	<.0001
pH: spring	–	–	3.61	<.0001
pH: summer	–	–	1.69	<.0001
Chl-*a*: autumn	–	–	−0.72	.0068
Chl-*a*: winter	–	–	−1.83	<.0001
Chl-*a*: spring	–	–	−0.66	.016
Chl-*a*: summer	–	–	−0.61	.0041
Mean monthly temperature: winter	–	–	0.34	<.0001
Day length: winter	–	–	−2.38	<.0001
PO_4-P: spring	–	–	−1.92	.0024
NH_4-N: summer			−1.21	.0013
$\delta^{13}C_{PER}$ R^2 = .52				
Season	4	144.55	–	<.0001
Mean monthly temperature	1	22.75	0.13	<.0001
pH × season	4	18.42	–	<.0001
Chl-*a* × season	4	3.91	–	.0043
PO_4-P × season	4	2.92	–	.022
pH: autumn	–	–	1.77	<.0001
pH: winter	–	–	1.35	<.0001
pH: spring	–	–	2.43	<.0001
Chl-*a*: autumn	–	–	−0.84	.0038
Chl-*a*: spring	–	–	0.59	.014
PO_4-P: winter	–	–	0.61	.027
PO_4-P: summer	–	–	−1.02	.027

and nutrient concentrations has been observed (Altabet & Mccarthy, 1986). However, $\delta^{15}N$ may not be a reliable indicator of nutrient utilisation when different nutrient sources with distinct isotopic signatures, such as NO_3-N, NH_4-N and dissolved organic N, are used by primary producers (Jones, King, Dent, Maberly, & Gibson, 2004; Kumar et al., 2011; Rau, Low, Pennington, Buck, & Chavez, 1998). We found no general relationship between the seasonal variations in $\delta^{15}N_{SES}$ and NO_3-N or $\delta^{15}N_{NO_3}$ availability in the inlet water. Instead, the variation in $\delta^{15}N_{SES}$ might, in part, be attributed to variations in the relative contributions of NO_3-N and NH_4-N to the N sources of the seston. In autumn/spring, higher NH_4-N correlated with lower $\delta^{15}N_{SES}$, indicating use of NH_4-N, while during spring/summer seston was enriched in ^{15}N compared with the inlet $\delta^{15}N_{NO_3}$, indicating that NO_3-N was not a key source of the N uptake (Kumar et al., 2011). A recent study by Olsen et al. (2017) shows that among the primary producers, macrophytes and filamentous algae are the main controllers of NO_3-N processing during the summer in the unenriched mesocosms. Most likely, the algae used NH_4^+ regenerated through zooplankton grazing or via microbial decomposition of organic N in the water or sediment (see Gu & Alexander, 1993).

$\delta^{15}N_{PER}$ negatively related to NO_3-N during all seasons and positively to PO_4-P also in all seasons except for summer. This pointed to a higher dependency of periphyton than of seston to NO_3-N, whereas the use of recycled N within the periphyton biofilm possibly resulted in the higher $\delta^{15}N$ observed. Few data are available in the literature on the relationships between $\delta^{15}N_{PER}$ and PO_4-P, but the study by Doi, Kikuchi, Shikano, and Takagi (2010) indicates as ours that high concentrations of PO_4-P lead to high $\delta^{15}N$ of benthic microalgae, likely because a higher primary production is driven by P (Gu, 2009).

The $\delta^{13}C$ values of seston and periphyton varied under scenarios of contrasting nutrients but showed a less significant effect of temperature scenarios; though, several environmental variables contribute to the variation in $\delta^{13}C$ across seasons. Our third hypothesis (H_3) was that an increase in primary productivity would result in a decrease in isotope fractionation and higher $\delta^{13}C$ of particulate organic matter (see Gu, Schelske, & Waters, 2011). We found the opposite as seston $\delta^{13}C$ differed among the nutrient treatments, being lower in the enriched mesocosms in winter. We also observed a negative relationship between phytoplankton Chl-*a* and $\delta^{13}C_{SES}$ during all seasons, most pronounced in winter. Variations in the species composition over the seasons may be the source of our result (Vuorio et al., 2006), but other factors may contribute as well. $\delta^{13}C_{SES}$ and $\delta^{13}C_{PER}$ were strongly and positively related to pH, indicating CO_2 limitation resulting from either reduced discrimination against $^{13}CO_2$ or transition to the use of HCO_3^- as carbon source at high pH (Liu, Chen, Li, & Gu, 2012; Vuorio et al., 2006). Particularly, within the pH range of the mesocosms (Figure 2a), most inorganic carbon is HCO_3^-, but the assimilated proportion of the two carbon forms (CO_2 and HCO_3^-) differs between species depending on their capacity of active transport of the HCO_3^- ion and on the proportion of CO_2 to HCO_3^- in the boundary layer (e.g. Keeley & Sandquist, 1992). $\delta^{13}C_{PER}$ followed more closely the variations in pH than did $\delta^{13}C_{SES}$. $\delta^{13}C_{PER}$ decoupled from pH during autumn/early winter, not least in the unenriched mesocosms, pointing to a higher carbon limitation with enrichment. This decoupling between pH and $\delta^{13}C_{SES}$ was also more pronounced in the heated than in the ambient mesocosms. Productive lakes typically have high pH because phytoplankton assimilation of dissolved CO_2 leads to elevated pH in the surface waters (Raven & Falkowski, 1999). Our results showed that the unenriched and enriched mesocosms had the same pH pattern (see

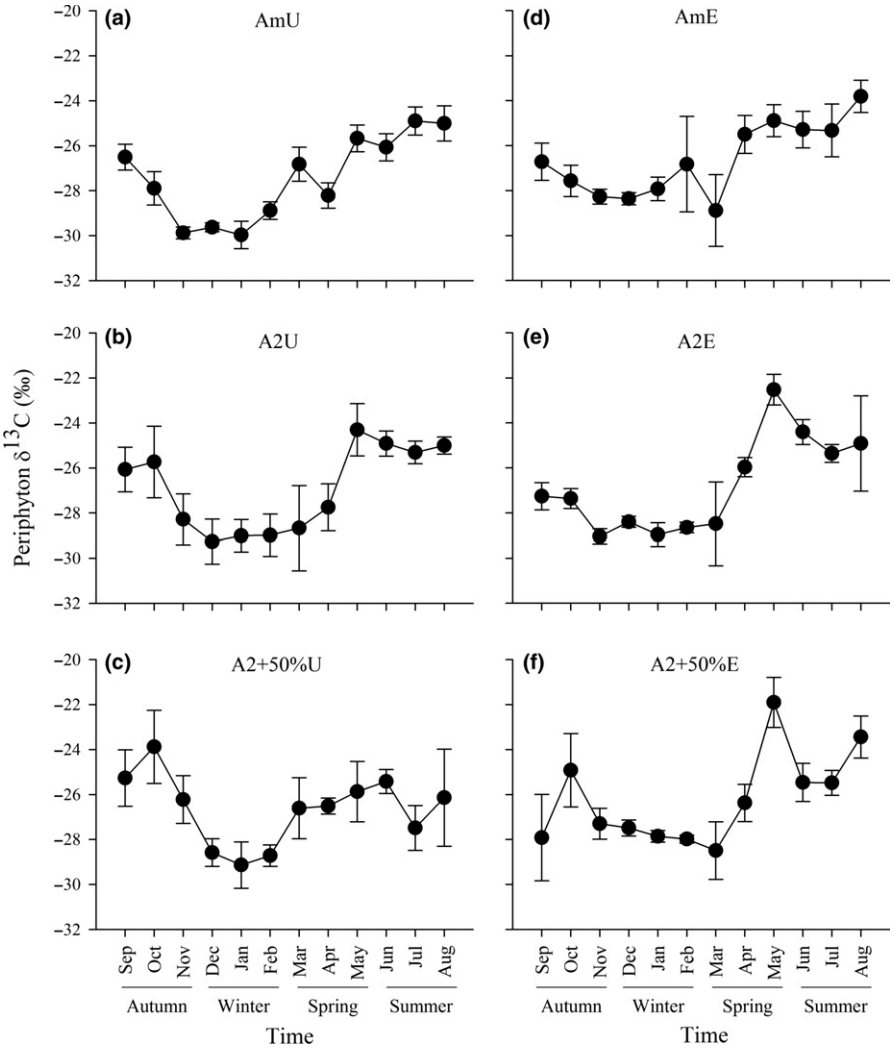

FIGURE 7 $\delta^{13}C_{PER}$ (mean ± 1 *SE*) (solid line and filled circles) for AmU (a); A2U (b); A2+50%U (c); AmE (d); A2E (e) and A2+50%E (f)

Figure 2a), suggesting that macrophytes assimilation of CO_2 or HCO_3^- affected pH in the former as phytoplankton does in productive environments. pH was not only significantly related to Chl-*a* but also to macrophyte %PVI and filamentous green algae %PVI in the enriched mesocosms and to macrophyte %PVI and filamentous green algae %PVI in the unenriched mesocosms. Moschen et al. (2009) found that the $\delta^{13}C$ dynamics of particulate organic matter in mesotrophic Lake Holzmaar (Germany) was highly responsive to phytoplankton primary production. Increased phytoplankton growth produced a decrease in the dissolved inorganic carbon pool followed by a reduction in phytoplankton discrimination against ^{13}C, resulting in isotopic enrichment of particulate organic matter. Moreover, Boll, Balayla, Andersen, and Jeppesen (2012), studying $\delta^{13}C$ of primary consumers and secondary consumers in a shallow lake in Denmark, found $\delta^{13}C$ to be markedly higher in years with high submerged macrophyte abundance, most likely reflecting elevated $\delta^{13}C$ of phytoplankton and periphyton mediated by a macrophyte-induced lowering of lake water CO_2 concentrations.

We also hypothesised (H_3) differences in the $\delta^{13}C$ values of seston and periphyton among the temperature scenarios. In contrast to our expectations, $\delta^{13}C$ of these communities did not differ among the temperature treatments; however, $\delta^{13}C_{PER}$ was positively related to the mean monthly water temperature in all seasons, while for $\delta^{13}C_{SES}$ a similar positive relationship was evident only in winter. This may also be attributed to changes in pCO_2 (Gu et al., 2011); besides, the fractionation of $\delta^{13}C$ between gaseous CO_2 and dissolved HCO_3^- is higher at low temperatures (up to 11‰ at 0°C) (Mook, Bommerson, & Staverman, 1974).

As hypothesised (H_4), our results showed that $\delta^{13}C_{PER}$ was generally higher than $\delta^{13}C_{SES}$ (especially in the enriched mesocosms), which may reflect that the carbon uptake was limited by diffusion in the boundary layer (Doi et al., 2010). In a previous investigation conducted by Ventura et al. (2008) in the same mesocosms, seston also had the lowest $\delta^{13}C$.

Our analyses of the elemental composition of seston and periphyton during the year also demonstrated a weaker effect of warming than of nutrient enrichment. Enrichment decreased (as expected, H_5) the C:N ratio of seston in late autumn/winter and of periphyton in late spring/summer (see also Ventura et al., 2008). The $C:N_{SES}$ ratios did not indicate deficiencies in N, whereas the $C:N_{PER}$ ratios did, matching the observed changes in $\delta^{15}N$ and suggesting that seston and periphyton had access to different sources

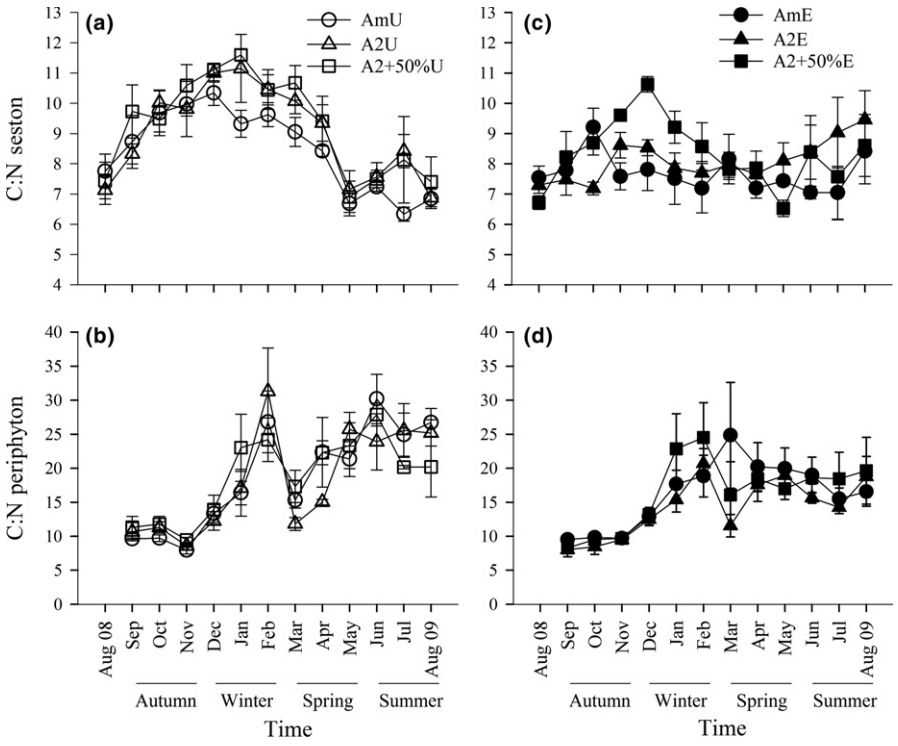

FIGURE 8 C:N ratios for seston and periphyton (mean ± 1 *SE*) for AmU, A2U and A2+50%U (a, b) (open symbols) and AmE; A2E; A2+50%E (c, d) (filled symbols)

TABLE 5 Results of RM ANOVA tests on $C:N_{SES}$ and $C:N_{PER}$ using the effects of month (time), temperature (three levels, ambient, A2 and A2+50%) and nutrients (two levels, unenriched and enriched). Significance levels: *$p < .05$, **$p < .01$, ***$p < .0001$, N.S., not significant

	$C:N_{SES}$		$C:N_{PER}$	
	df	*F*-values	*df*	*F*-values
Month	12	15.12***	11	44.71***
Temperature	2	4.36*	2	1.47 N.S.
Nutrients	1	18.25***	1	17.62***
Month × temperature	24	1.68*	1	0.76 N.S.
Month × nutrients	12	4.46***	11	5.03***
Temperature × nutrients	2	0.06 N.S.	2	0.50 N.S.
Month × temperature × nutrients	24	1.45 N.S.	22	0.66 N.S.

of nutrients. The access of periphyton to N may be restricted to recycled N within the periphyton matrix (Wetzel, 2001), whereas seston may access N in the water or N released from the sediments. The $C:N_{SES}$ ratios were lowest in the enriched treatment, which agrees with primary producers changing their elemental content as an adaptation to the differences in N availability (Sterner & Elser, 2002). Phytoplankton growing at high N concentrations can increase its protein content (and therefore have a higher N content) or decrease its lipid concentration (leading to a lower carbon content) (Beardall, Young, & Roberts, 2001), as well as show luxury consumption (Sterner & Elser, 2002). A complementary explanation could be a lower detritus content in the enriched mesocosms; indeed, lower C:Chl-*a* ratios were measured in these mesocosms by Ventura et al. (2008).

Increasing temperatures had only minor effects on the $C:N_{SES}$ ratio in our study. This was not in agreement with our expectations (H_5), but corroborates earlier results from the same mesocosms (see Ventura et al., 2008). A decrease in the C:N ratio would have been expected as aquatic plants increase their enzyme activity at low temperatures to compensate for the lower metabolic processes (Olesen & Madsen, 2000), which results in a higher N content. Nonetheless, phytoplankton changes in C:N with increasing temperatures are species specific, many species do not change their ratio as we observed for seston, whereas others increase their C:N with temperature (Thompson et al., 1992).

In summary, we found a stronger impact of enrichment and warming on the $\delta^{15}N$ and $\delta^{13}C$ of seston than of periphyton, and the temporal isotopic variability in both communities was large. Our results revealed a significant relationship between the isotopic values of phytoplankton and periphyton and physical and biogeochemical factors, which changes importantly with eutrophication and climate warming in temperate lakes. Nutrient enrichment did not increase the N isotope values, which may be linked to N-fixation by cyanobacteria. Periphyton showed higher $\delta^{13}C$ values than seston, which may be ascribed to differences in the size of the boundary layer. Also, seasonal variation was large in all treatment combinations, and even small changes in nutrients, as observed in the unenriched mesocosms, may have a profound impact on the isotope values of the sestonic and periphytic communities. There is little doubt that isotopic studies will remain at the cutting edge of aquatic ecology for the foreseeable future. The overall consequences of an alteration of aquatic environments with eutrophication and warming are difficult to oversee, but we believe that our investigation is important and may act as a basis for better

understanding the ecological and biogeochemical dynamics in aquatic ecosystems.

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