Weak response of greenhouse gas emissions to whole lake N enrichment

Marcus Klaus [ID],* Ann-Kristin Bergström, Anders Jonsson, Anne Deininger, Erik Geibrink, Jan Karlsson
Department of Ecology and Environmental Science, Umeå University, Umeå, Sweden

Abstract

Global warming and land use scenarios suggest increased 21st century nitrogen (N) inputs to aquatic systems. Nitrogen affects in-lake processing and, potentially, atmospheric exchange of greenhouse gases, probably being most relevant in unproductive systems. Here, we test for the first time the effect of a whole-lake experimental increase (threefold) in external nitrate loads on the atmospheric exchange of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) from N-limited unproductive boreal lakes. Nitrate enrichment effects were assessed within a paired Before/After-Control/Impact framework based on 2-hourly to biweekly surface-water sampling of dissolved gas concentrations, and monthly whole-lake inventory surveys, carried out over 4 yrs in six lakes. Nitrate enrichment did not affect gas exchange during summer stratification and whole-lake gas inventories during summer and winter stratification. This finding specifically emphasizes the modest role of internal carbon fixation for the CO_2 dynamics of unproductive boreal lakes. A global synthesis of 52 published studies revealed a wide range of nutrient fertilization effects, both in systems similar to our experimental lakes, and other more productive systems. Effects depended mainly on the spatiotemporal scale of the study and became more pronounced when N enrichment was combined with phosphorous. Conclusively, although short-term and habitat-specific effects can occur, changes in N supply have only weak whole-ecosystem effects on greenhouse gas emissions from unproductive boreal lakes.

Land management and burning of fossil fuels have strongly perturbed the global cycles of carbon (C) and nitrogen (N). Anthropogenic reactive N applied to the earth surface heavily controls ecosystem functioning (Vitousek et al. 1997). In unproductive boreal lakes, atmospheric N deposition has enhanced phytoplankton growth due to the fact that phytoplankton is often N limited in these systems (Bergström and Jansson 2006; Elser et al. 2009; Lepori and Keck 2012). Additional N supply can also be expected to affect cycling and emissions of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (McCrackin and Elser 2011; Kortelainen et al. 2013; Yang et al. 2015). Such effects are poorly understood but urgently need to be revealed especially for boreal regions, given the ongoing and predicted regional future increases in N loads under climate warming (Hole and Engardt 2008), more intense local forestry activities (Laudon et al. 2011), and atmospheric deposition (Galloway et al. 2004).

Nitrogen alone, or combined with phosphorous (P), controls greenhouse gas dynamics in lakes by affecting primary production and respiration (Cole et al. 2000), methanogenesis (Bogard et al. 2014), methane oxidation (Deutzmann et al. 2014), denitrification, and nitrification (Mengis et al. 1997). In-lake greenhouse gas dynamics have been shown to respond to N or N + P enrichment by different types of studies with a wide range of spatiotemporal scales. Studies comprise of sediment incubations (Liikanen et al. 2003; McCrackin and Elser 2011), mesocosm- (Peixoto et al. 2013; Davidson et al. 2015) and whole-lake experiments (Kling et al. 1992; Findlay et al. 1999; Carpenter et al. 2001), and observations over time and space across gradients in nutrient inputs (Marotta et al. 2010; Gu et al. 2011; McCrackin and Elser 2011; Yang et al. 2015). These studies showed that N addition often increased N_2O accumulation in lakes but induced variable effects on CO_2 and CH_4 dynamics. Such mixed scale-dependent outcomes make it difficult to accurately predict nutrient enrichment effects on natural lake ecosystems.

To understand the complex effects of N on greenhouse gas emissions from lakes under natural conditions we need ecosystem-scale experiments (Schindler 1998; Solomon et al. 2015). Previous whole-lake experiments have mainly been carried out in relatively productive temperate systems heavily

influenced by land use and atmospheric N deposition, using combined additions of N and P (Kling et al. 1992; Findlay et al. 1999; Carpenter et al. 2001). However, it is unclear, whether insights from these experiments can be used to predict fertilization effects on greenhouse gas emissions from relatively unproductive boreal lakes, i.e., the dominant lake type in the boreal landscape. The N effects on lake ecosystems may differ from combined N and P effects, especially in regions with low N deposition (Bergström et al. 2008; Elser et al. 2009). This is highly relevant given that N and P inputs to lakes are often uncoupled when derived from forest clear cuts (Nieminen 2004; Tremblay et al. 2009) or atmospheric deposition (Mahowald et al. 2008; Camarero and Catalan 2012).

The whole-lake nutrient enrichment experiments by Kling et al. (1992), Findlay et al. (1999), and Carpenter et al. (2001) have provided insights into responses of CO_2 emissions during summer conditions. However, these experiments did not consider effects on CH_4 and N_2O and likely did not account for potential fertilization effects on benthic primary production (Vadeboncoeur et al. 2001) or decomposition of biomass sinking out of the epilimnion (Obrador et al. 2014). Such effects would not be detected until the gas accumulated during summer or winter stratification is being released during spring and autumn turnover which can lead to large biases in annual emission estimates (cf. Huotari et al. 2009; Karlsson et al. 2013; Ducharme-Riel et al. 2015). Therefore, sampling throughout the whole year across the whole water column is necessary to fully capture potential fertilization effects.

Here, we assess, for the first time, the effects of experimental nitrate addition on whole-lake inventories and lake-atmosphere fluxes of CO_2, CH_4, and N_2O. We included three pairs of unproductive boreal Swedish lakes covering a wide range of co-varying dissolved organic carbon (DOC) concentrations, light extinctions, and water residence times. Gas concentrations were sampled 2-hourly, biweekly (surface water) or monthly (depth profiles) during the open water season and once before ice-break-up 1 yr before and 3 yr after onset of nitrate addition to one lake of each lake-pair. Since primary production in northern Swedish lakes is N limited throughout the summer season (Bergström et al. 2008), we hypothesized a net decrease in CO_2 concentrations in response to nitrate addition during the growing season. Additionally produced algal biomass from fertilization is hypothesized to cause a net increase in CH_4 emissions facilitated by typically anoxic conditions in small boreal lakes (Bastviken et al. 2004; Chmiel et al. 2016). We also hypothesized a general increase in N_2O emissions from incomplete denitrification of the added nitrate. We finally placed our experimental results into a global context of all relevant published papers to test the generality of scale- and system-specific studies. Specifically, we assessed whether nutrient enrichment effects on greenhouse gas concentrations or emissions varied between unproductive systems similar to our experimental lakes and more productive systems,

depended on the spatiotemporal scale of the study, or whether lakes were enriched with N or P alone, or N and P combined.

Methods

Study sites and experimental design

Sampling was carried out in six unproductive headwater lakes located within 15 km from each other in northern Sweden (Table 1). During the experimental period of 2011–2014, mean annual air temperature was 1–3°C higher than normal (1°C, reference period 1960–1990) while annual precipitation was normal in all years (500–600 mm), except of in 2012 (800 mm, http://www.smhi.se/klimatdata/meteorologi). The catchments are mainly covered by mires (< 20%) and managed coniferous forest (> 80%) on podzol developed on glacial till on top of acidic bedrocks. Lake ice is present from late October to early May. The lakes are dimictic with seasonal mixed layer depths of 1–3 m during summer stratification (June–September) and a hypolimnion that turns anoxic toward the end of the stratification periods.

Whole-lake fertilization experiments were carried out utilizing a Before/After-Control/Impact (BACI) design with sampling periods of 1 yr "before" and 3 yr "after" onset of fertilization. The experiment included three lake pairs similar in DOC and nutrient concentrations, water residence time, light extinction (Table 1) and phytoplankton, zooplankton and fish communities (Deininger et al. 2017). For each lake pair, one control lake was left untreated and one impact lake was fertilized with dissolved potassium nitrate in 2012 and concentrated nitric acid in 2013 and 2014. Different sources of N were used due to practical reasons. Nitrate (NO_3^-) was used, because it is the most typical inorganic N form that leaches from headwater catchments, and elevated by atmospheric N deposition (Moldan et al. 2006) and forest clear-cutting (Schelker et al. 2016). We added an amount of 1–1.8 g N m^{-2} yr^{-1} (Table 1) equally across the lake surface to simulate conditions present today in southwestern Sweden where atmospheric DIN loads are 3–4 times as high as in our study area in 2011–2014 (0.8–1.7 g N m^{-2} yr^{-1} compared to 0.3–0.4 g N m^{-2} yr^{-1}, SMHI 2016). Nitrate was added biweekly (Lapptjärn, Fisklösan, Nedre Björntjärn 2012) or weekly (Nedre Björntjärn 2013–2014) throughout June to August each year and twice from the ice in late March 2012 and early April 2013 to mimic leaching events during storms and spring floods (Bergström et al. 2008).

Water sampling and analysis

Throughout the open-water period, surface water was sampled biweekly (05/2011–10/2014) and depth profiles (surface, mid-metalimnion, upper hypolimnion, 1 m from bottom) were sampled monthly (05/2011–04/2013) at the deepest points in each lake. Control- and impact- lake pairs were sampled the same day except for one lake pair in 2014 (5 d in between samplings). Six weeks before ice-breakup,

Table 1. Morphological and physicochemical features of the studied lakes given as arithmetic means during June–October 2011.

Lake pair	Lake	Catchment area (ha)	Lake area (ha)	Mean depth (m)	Water residence time (d)	a_{420} (m^{-1})	pH	DOC (mg L^{-1})	TP (µg L^{-1})	TN (µg L^{-1})	DIN (µg L^{-1})	Areal DIN loading (g m^{-2} yr^{-1})	
												Natural	Artificial
1	Övre Björntjärn	284.0	4.8	4.0	117	12.6	4.9	22.8	18.8	466	17.9	1.02	0.0
1	Nedre Björntjärn*	324.9	3.2	6.0	89	10.9	4.9	19.3	17.8	411	17.3	1.40	1.8
2	Mångstenstjärn	14.1	1.8	5.3	1034	4.6	5.9	10.1	12.2	317	11.1	0.15	0.0
2	Lapptjärn*	16.8	2.0	2.5	481	5.0	5.6	11.5	11.3	345	10.7	0.07	1.1
3	Nästjärn	3.4	1.0	4.2	2051	1.8	6.3	6.9	9.9	329	7.9	0.03	0.0
3	Fisklösan*	8.9	1.7	2.1	677	1.9	6.1	7.1	8.6	284	5.0	0.05	1.0

a_{420}, spectral absorbance at 420 nm; DOC, dissolved organic carbon concentration; TP, total phosphorous concentration; TN, total nitrogen concentration; DIN, dissolved inorganic nitrogen concentration.
* Fertilized in 2012–2014.

three depth profiles were sampled along a lateral transect (Supporting Information Fig. S1) to account for lateral variability of greenhouse gas accumulation under ice, assuming that whole-lake accumulation have reached its winter maximum at the time of sampling (Denfeld et al. 2015). Ice dynamics were inferred from direct field observations or temperature logger readings (see below). The timing of ice-in and ice-out was observed with an estimated error of 7 d and 4 d, respectively, as a mean over the study period, and behaved similarly between control- and treatment lakes ($\pm$ 1 d).

Partial pressure of CO_2 was measured in situ throughout 05/2011–10/2014 by a hand held non-dispersive infra-red CO_2 sensor (Vaisala GM70 Carbon dioxide meter) or an infrared gas analyzer (IRGA EMG-4, PP-Systems Inc,) coupled to a gas equilibrator (MINIMODULE 1.7 $\times$ 5.5 G542, Membrane Underling Performance) through which sample water was transferred by a peristaltic pump. Molar CO_2 concentrations were derived from Henry's law constants using water temperature-parameterizations in Wanninkhof (1992). For dissolved CH_4 sampling (05/2011–10/2013), 4 mL of water was injected into gas-tight 22 mL glass vials (PerkinElmer, U.S.) containing 50 μL 1.2M HCl, sealed with 20 mm natural pink rubber stoppers, flushed with N_2 gas prior to sampling. For dissolved N_2O sampling (05/2012–04/2013), we used the headspace equilibration technique where 50 mL of headspace gas (air taken in upwind direction 2 m aboveground) was equilibrated with 540 mL of lake water by vigorous shaking for 1 min and then transferred to a glass vial (as described above, but here without HCl) allowing any overpressure to be released during gas injection. CH_4 and N_2O concentrations in the vial headspaces were analyzed using a gas chromatograph (GC, Perkin Elmer Clarus 500, U.S.A.) equipped with a flame ionization detector (for CH_4 analysis) and an electron capture detector (for N_2O analysis). Median variation among 78 and 106 duplicates of CH_4 ($>$ 0.1 μM) and N_2O samples was 4% and 14%, respectively. Headspace concentrations were converted to molar concentrations by means of the ideal gas law using Bunsen solubility coefficients given in Wanninkhof (1992).

Time series of CO_2 were collected every 2nd hour throughout the whole open-water period of 2013 at 10 cm water depth using non-dispersive infra-red CO_2 probes (Vaisala CARBOCAP GMP 222, Helsinki, Finland) enclosed in a semi permeable PTFE membrane and coupled to Vaisala GMT 220 transmitters. These time series were validated against manual readings using a separate probe every second week. Times series were corrected for linear drifts based on probe-calibrations before and after the field season. We occasionally observed diel cycles in CO_2 concentrations which were greatly exaggerated given the observation that covariates (water temperature, lux or wind speed) did not show any conspicuous patterns. We used these covariates to identify and gap-fill erroneous patterns likely caused by biofouling following an approach described in Supporting Information Text S1. Wind speed was measured every 5 min using a cup anemometer (Hobo S-WSET-A) on an open mire 300 m

from one of the lakes (Övre Björntjärn). Anenometer height was 2.5 m until July 2011 and in 2014 and 10 m in the intermediate period. Lux was measured every 5 min using Hobo UA-002–64 light loggers installed within 30 m from each lake in an open environment. Water temperature was measured every 5 min at 50 cm depth intervals in each lake using Hobo TidbiT V2 temperature loggers.

To characterize lake color, spectral absorbance at a wavelengths of 420 nm (a_{420}) was measured on filtered water (acid washed Whatman GF/F 0.7 μm) using a Jasco V-560 UV-VIS Spectrophotometer (Easton, Maryland, U.S.A.). DOC and total N (TN) were analyzed using a HACH-IL 550 TOC-TN analyzer (*see* Deininger et al. 2017). For DIN analysis, water samples were filtered through 0.45 μm cellulose acetate filters prior to freezing and analyzed using an automated flow injection analyzer (FIA star 5000, FOSS, Denmark). Total P (TP) was analyzed by the same instrument following the molybdenum blue method (ISO 15681-1) after an acid persulfate (5%) digestion in an autoclave (120°C) for 1 h. All chemical analyses were performed at the Department of Ecology and Environmental Science (EMG), Umeå University.

Calculation of gas inventories and fluxes

Areal gas inventories were calculated for the whole water column by summing the product of gas concentration and volume of corresponding depth strata and normalizing this sum by lake surface area. Missing values (3 of 249) were replaced by the arithmetic mean value of neighboring strata. Depth strata were defined to be divided by horizontal planes located at depths corresponding to the mid-points of adjacent profile sampling points (Supporting Information Fig. S1). Depth-volume relationships were established using bathymetric data collected by a single beam echo sounder (Lowrance HDS-5 Gen2) and interpolated by ordinary kriging (RMSE = 0.3 m) using the geostatistical analysis package in ArcMap 10.1 (ESRI, U.S.). Lake volumes were calculated using the 3D analyst surface volume tool in ArcMap and corrected for ice volumes derived from ice-thickness measurements (0.5–0.7 m). Hereafter, the maximum gas inventory during the open-water period is referred to as "summer inventory" and the gas inventory accumulated under the ice as "winter inventory."

Gas fluxes across the air–water interface were calculated using Fick's law of diffusion, assuming a chemical enhancement factor of 1 and atmospheric CO_2 and N_2O concentrations of 429 ppm and 394 ppb (median of biweekly in situ measurements) and atmospheric CH_4 concentrations of 1.89 ppm (http://cdiac.ornl.gov/pns/current_ghg.html). Gas- and water-temperature specific atmospheric gas transfer velocities were calculated from k_{600}, the gas transfer velocity of CO_2 at 20°C following Jähne et al. (1987) using Schmidt-number parameterizations in Wanninkhof (1992), assuming a Schmidt number coefficient of -0.67. k_{600} was calculated using a model valid for small sheltered lakes (Cole and Caraco 1998) based on daily mean wind speed at a height of 10 m. Wind

speed measured at 2.5 m was extrapolated to 10 m assuming a logarithmetic wind profile following Crusius and Wanninkhof (2003). All fluxes from the lake to atmosphere reported here have a positive sign, if not declared otherwise.

Statistical analysis

We assessed fertilization effects on lake-atmosphere fluxes and summer and winter inventories of gases following the paired BACI approach of Stewart-Oaten et al. (1986). Treatment effects were analyzed in terms of effect sizes (ES), which is defined as the change (after-before) in the sampling specific differences between lake pairs (impact-control). The significance of the ES was tested using a linear mixed-effects model (LME) with "paired inter-lake difference" as the dependent variable, "Time" (Before/After) as a fixed effect and "lake pair" as a random effect on both slopes and intercepts. In cases where before-period data was missing (N_2O fluxes and summer inventories) we were forced to set the fixed effect to "1," assuming that there was no paired inter-lake difference in the before-period. All LMEs in this paper were analyzed by means of the "lme" function in the statistical program R (Pinheiro et al. 2015) using the restricted maximum likelihood approach after model assumptions were verified (Supporting Information Text S2). To account for within-lake spatial variability, we weighted inventory data in the LME by the volume-weighted standard errors of multiple observations per lake (Supporting Information Fig. S1, *see* also Supporting Information Figs. S2–S4) propagated for subtraction of inventories. Lake-atmosphere flux data was weighted equally in the LMEs, assuming that measurement errors did not vary seasonally. Analyses of the fluxes were restricted to the time of June to September, the period of water column summer stratification, for which data was available for all years.

To assess the statistical and biogeochemical significance of fertilization effects we used the *p*-value and slope (= ES) of the LMEs and Cohen's *D*, defined as $D = ES/2s$, where *s* is the standard deviation of paired inter-lake differences in the before period (Osenberg et al. 1994) or, in case of missing before-period data, in the after period. Cohen's *D* were "small" if $D < 0.2$, "medium" if $0.2 \leq D < 0.8$, and "large" if $0.8 \leq D$.

We further assessed if fertilization effects on lake-atmosphere gas fluxes or inventories varied with excess DIN loading. Excess DIN loading was defined as ΔDIN loading = artificial DIN loading – natural DIN loading (*see* Supporting Information Text S3 for details). We performed LMEs with the effect size as the dependent variable and ΔDIN loading as fixed effects and "lake pair" as a random effect on both slopes and intercepts. We weighted each observation by the root mean square of min-max-standardized standard errors of the effect size and ΔDIN loading, respectively. The standard error of the effect size was defined as the volume-weighted standard errors of multiple gas inventory observations within

each lake (Supporting Information Fig. S1) propagated for inter-lake and inter-year subtraction, or as the standard error of gas fluxes based on repeated surface water concentrations, propagated for inter-lake and inter-year subtraction, respectively.

In order to discuss if potential fertilization effects on CO_2 and CH_4 inventories would translate into lake-atmosphere gas fluxes during overturn periods (which were excluded from the BACI-analysis), we regressed the potential atmospheric release of stored gas during spring and autumn overturn against the gas flux calculated from surface water gas concentrations. Following Riera et al. (1999), we calculated the potential release of stored gases as the difference in total gas inventories between the seasonal (winter or summer) maximum and the following local minimum divided by the respective number of days with open water between the two dates. The lake-atmosphere gas flux was the arithmetic mean flux of all sampling occasions bound by the seasonal maximum and following local minimum inventory. In addition, we tested for a linear relationship between the two flux estimates using an LME with "lake pair" as random effects on both slopes and intercepts. In this LME, we weighted observations by the root mean square of propagated volume-weighted standard errors of multiple gas inventory observations per lake or standard errors of lake-atmosphere fluxes based on repeated surface water concentration measurements, respectively. Last, to test for potential systematic differences between control and impact lakes, we compared this LME with another LME where "lake pair" nested in "treatment" was included as a random effect using the likelihood ratio test.

Literature synthesis

To set our results in a global context, we compiled all peer-reviewed original studies listed on ISI Web of Knowledge addressing the question of how CO_2, CH_4, or N_2O concentrations or emissions in standing inland-waters (lakes, ponds, lagoons) respond to nutrient enrichment (with N or P alone or with N and P combined). Here, nutrients had to be explicitly shown or discussed to be a driving factor, e.g., by adding them to the system or by establishing gradients in space or time. Studies in reservoirs, temporal analysis of seasonal variability and spatial analyses of within-lake variability were excluded. We summarized study outcomes in contingency tables counting the number of studies which showed a significant increase, decrease or no significant change (alpha level = 0.05) in response to nutrients according to the (1) spatiotemporal scale of the system (sediment incubations, mesocosm experiments, whole-lake experiments, multi-annual trends, or spatial surveys), (2) whether systems were enriched with N or P alone or N and P combined, or (3) whether the systems were similar to our study lakes (unproductive boreal, (ant-)arctic or alpine soft-water lakes) or not. In the latter case, we

assigned systems to either group based on how the system was classified in the papers, or, if classification was missing, based on reported total P concentrations ("unproductive" if total P < 40 μg L^{-1}), geographic locations (following the Köppen climate classification) and pH values ("soft-water" if pH < 8). In case a study did not present *p*-values of statistical tests (10 cases), we also interpreted clear statements on the direction of the change as indicative of a significant change. For studies where nutrients where added at multiple levels we only considered the outcome of the lowest level of excess nutrient additions. For papers that presented outcomes from multiple experiments (with different types of treatments, study systems or spatiotemporal scales), or for multiple papers that refer to the same study system (but differed in treatment types or spatiotemporal scales) we counted each outcome separately. Hence, study outcomes may not be fully independent. Yet, our approach is common practice for literature records that are too sparse to allow systematic evaluation of non-independence (Downing et al. 1999).

We tested if study outcomes were differentially distributed over the aforementioned categories (1–3) using the Chi-square test $\chi^2 = \sum[(O\text{-}E)^2/E]$ where O is the observed frequency and E is the expected frequency given equal distribution. In case one row or column had only zero counts, we set associated grand totals of E to $3/n$ (n = total number of studies) to approximate the 95% confidence interval for the chance of this event occurring, following the *rule of three*. Associated *p*-values were generated using the "pchisq"-function in R 3.2.2 (R Development Core Team 2015).

Results

Quality assessment of gas flux observations

Gas fluxes and inventories showed strong seasonal and inter-annual variability (*see* Supporting Information Text S4 and Supporting Information Figs. S2–S4). Concentrations of CO_2 based on biweekly spot measurements and 2-hourly measurements followed each other well throughout the whole open-water period 2013 (Fig. 1). Even though diurnal cycles in CO_2 concentrations were not covered, this bias is similar in all lakes and biweekly sampling captured general seasonal trends well enough to accurately describe annual emissions. Total summer emissions (June–September) differed on average ($\pm$ SE) by 0.17 $\pm$ 0.05 mol m^{-2} or 7% $\pm$ 2% and total annual emissions by 0.40 $\pm$ 0.04 mol m^{-2} or 8% $\pm$ 1% between the two methods (Supporting Information Table S1). Summer emissions contributed to the majority of total annual emissions (51–67%).

Fluxes of CO_2 during spring and autumn turnover during the fertilization years increased linearly ($p < 0.001$) with increased changes in storage of CO_2 (Fig. 2A). The LME regression line was parallel to the 1 : 1 line but had a positive intercept (0.029 mol m^{-2} d^{-1}), which suggests that more gas was emitted than was stored before the onset of the turnover
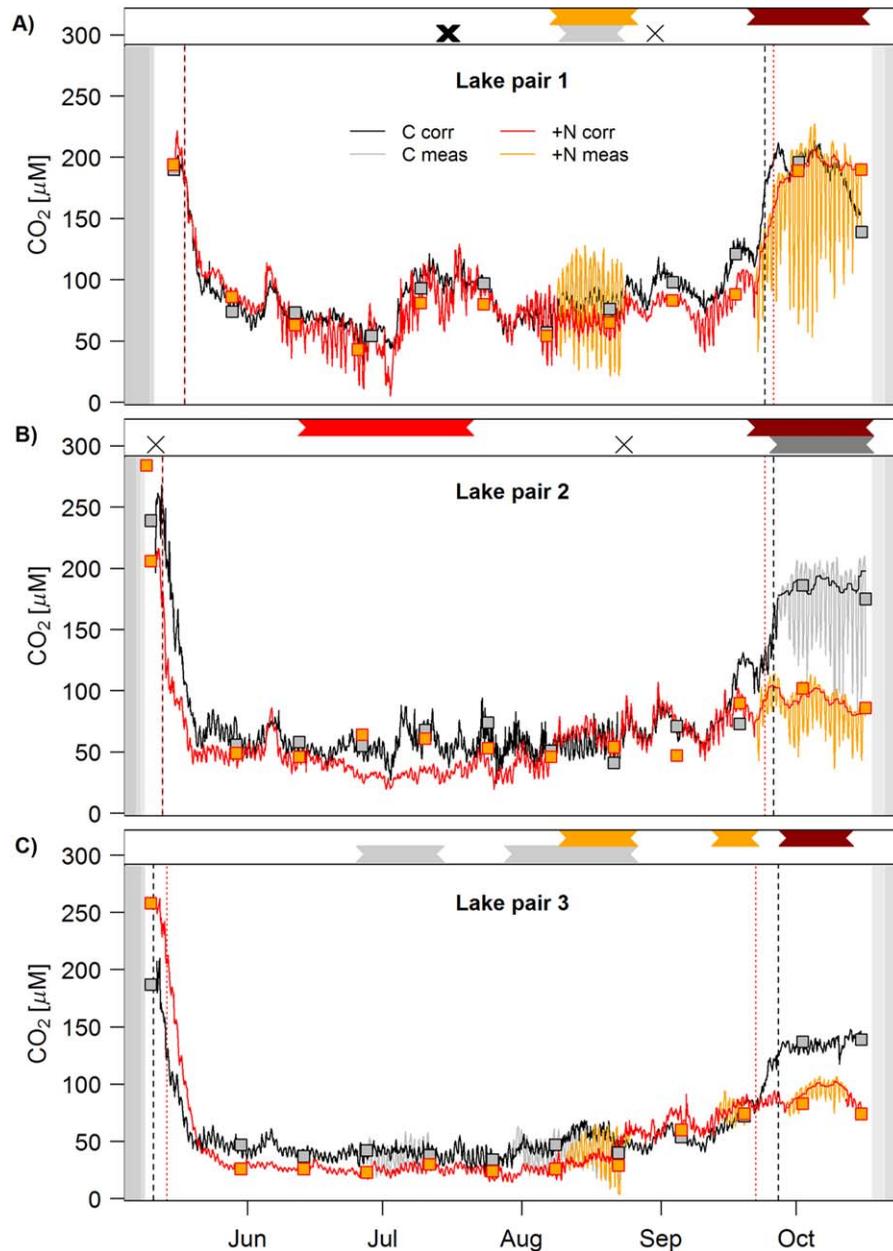
Fig. 1. Surface water CO_2 concentrations from May to October 2013 based on 2-hourly logger data (solid lines) and biweekly spot measurements (gray squares = control lake ("C"), orange squares = fertilized lake ("+N")) in three lake pairs. Shown are measured ("meas") and gap-filled values ("corr"). Erroneous or missing data points are marked by "x" above each figure. Red (orange) and black (light gray) crosses denote missing (erroneous) readings gap-filled by a multiple imputation algorithm while dark red and dark gray crosses denote erroneous readings replaced by moving median-filtered data (*see* Supporting Information Text S1). Dark and light gray bars show the minimum and maximum possible extent of the ice-cover period, respectively. The onset and end of the stratification period is marked by vertical dashed lines following the same color as the solid lines.

period. Including "treatment" into the random effects structure did not improve the LME (likelihood ratio = 3.36, $p = 0.34$), hence the relationship between gas storage and fluxes did not differ between control and impact lakes. The fact that summer and winter inventories strongly determined CO_2 fluxes during spring and autumn overturns, irrespective of the treatment, suggests that any potential nutrient

enrichment effect on CO_2 inventories should translate into atmospheric emissions during turnover periods. Hence, our inventory data is well suited to assess nutrient enrichment effects on annual CO_2 fluxes.

CH_4 fluxes during spring and autumn turnover were unrelated to the change in CH_4 inventories (Fig. 2B). Including "treatment" into the random effects structure improved the
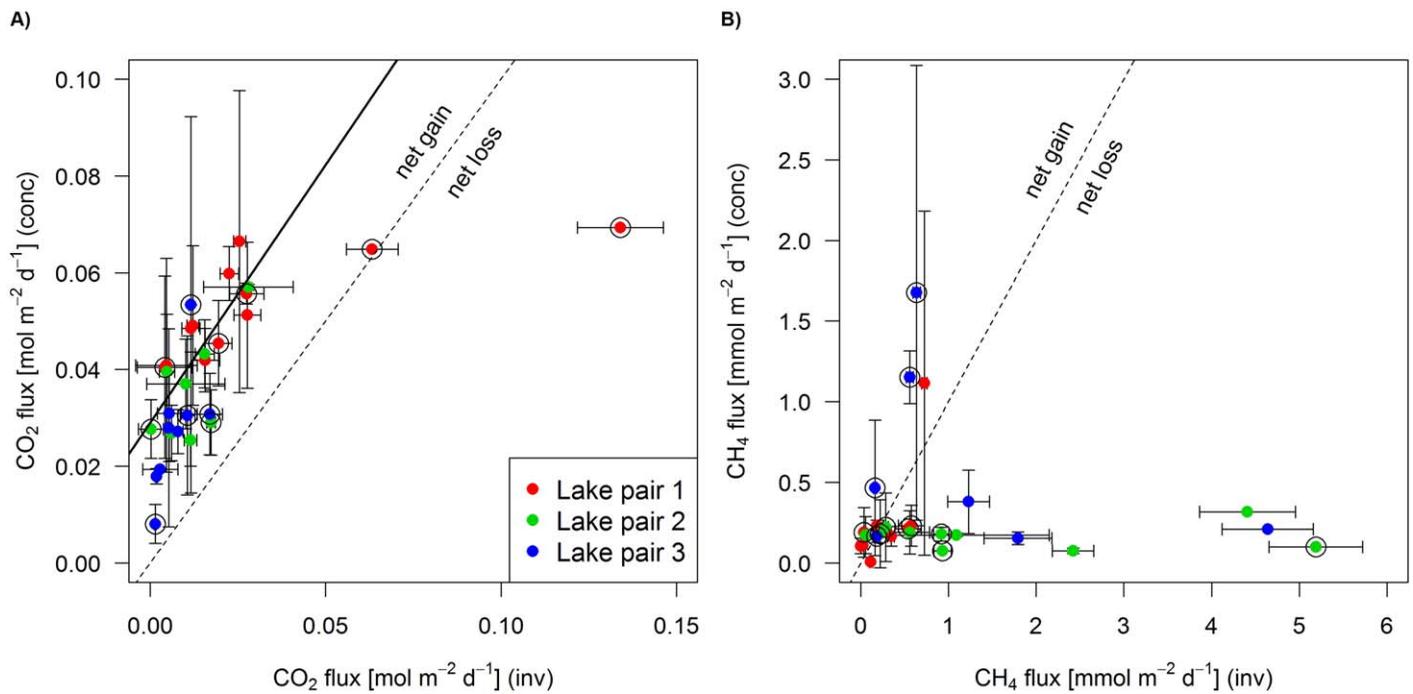
Fig. 2. Lake-atmosphere fluxes of (**A**) CO_2 and (**B**) CH_4 during spring and autumn overturn seasons 2011–2014 and 2011–2013, respectively, inferred from the rate of decline in total gas inventory ("inv") and from surface water concentrations ("conc"). Each circle represents an overturn period in a specific year and lake. The black hollow circles mark impact lakes. Error bars for inventories show propagated volume-weighted standard errors of multiple observations within each lake (*x*-axis). Error bars for lake-atmosphere fluxes show propagated standard errors based on surface water concentrations measured biweekly during the time when the inventory decreased (*y*-axis). The solid line marks the regression line of a linear mixed-effects model with "lake pair" as random effects on both slopes and intercepts weighted by the root mean square of measurement uncertainties expressed by error bars ($y = 1.067 \pm 0.219x + 0.029 \pm 0.004$, df = 23, $p < 0.01$ for both slope and intercept, marginal $R^2 = 0.84$, rse = 0.008, 2 outliers with $x > 0.05$ removed). The dashed 1 : 1 line divides regions of net-gain and net-loss of stored gas.

LME (likelihood ratio = 44.43, $p < 0.01$), but still did not reveal any significant relationship between the two CH_4 fluxes. In the majority of lakes, CH_4 fluxes based on surface water concentrations were < 0.4 mmol m^{-2} d^{-1}. These fluxes were 1–25 times lower than the fluxes based on changes in inventories, which suggests a general net loss of stored CH_4 during turnover periods. In some cases, CH_4 fluxes based on surface water concentrations were 1.5–5 times larger than fluxes based on changes in inventories, indicating a net gain in CH_4. This mismatch between fluxes based on surface water concentrations and changes in inventories suggests that maximum winter and summer CH_4 inventories cannot be used to infer CH_4 fluxes during spring and autumn turnover. Hence, our data was not suited to assess nutrient enrichment effects on annual CH_4 fluxes.

Responses to nitrate addition

Prior to fertilization, there was no statistically significant difference between control and impact lakes in physico-chemical parameters (Supporting Information Table S2) and gas fluxes and inventories (Table 2). As could be expected, the N fertilization resulted in a significant ($p < 0.01$) effect on the DIN and TN concentrations with an effect size of on

average 57 μg L^{-1} and 107 μg L^{-1}, respectively, yielding a *large* and a *medium* effect according to Cohen's *D*. Fertilization had, however, no effect on TP or DOC concentrations, pH and a_{420} (Supporting Information Table S2).

Lake-atmosphere CO_2 fluxes increased from an average of 27.4 mmol m^{-2} d^{-1} to 29.8 mmol m^{-2} d^{-1} in the control lakes but decreased from an average of 25.9 mmol m^{-2} d^{-1} to 24.6 mmol m^{-2} d^{-1} in the fertilized lakes between the before and after period. This decrease was, however, not significantly different from reference conditions ($p = 0.08$; -3.8 ± 2.2 mmol m^{-2} d^{-1}, Fig. 3A; Table 2). Further, summer and winter CO_2 inventories were unaffected by fertilization ranging from 0.9 mol m^{-2} to 1.5 mol m^{-2} (Fig. 3D,G; Table 2). Further support for the absence of any fertilization effect was given by the fact that effect sizes on CO_2 fluxes and inventories did not vary with DIN loadings (Supporting Information Fig. S5).

The CH_4 fluxes increased in both control- and impact lakes from 0.14 mmol m^{-2} d^{-1} to 0.31–0.37 mmol m^{-2} d^{-1} (Fig. 3B; Table 2). CH_4 summer inventories doubled and winter inventories increased 10-fold from 10.6–20.6 mmol m^{-2} to 17.1–44.2 mmol m^{-2} and from 2 to 23 mmol m^{-2}, respectively (Fig. 3E,H). Yet, these before-after changes occurred

Table 2. Effect of nitrate addition on lake-atmosphere fluxes and maximum inventories of greenhouse gases. Shown are the estimated intercept and slope of linear mixed-effects models with paired inter-lake differences (impact-control) as a dependent variable, time (before/after) as a fixed effect and "lake pair" as a random effect on both slopes and intercepts. The intercept represents the difference between the impact- and control lakes in the before period. The slope represents the effect size (the before-after change of the inter-lake difference).

Variable	Unit	Time period	Year	Before difference (Intercept)					Effect size (Slope)					Cohen's D	Cohen's Effect size
				est	SE	df	t	p	est	SE	df	t	p		
CO_2 flux	mmol m^{-2} d^{-1}	Summer	2011–2014	−1.49	1.93	89	−0.77	0.44	−3.81	2.18	89	−1.74	0.08	−0.27	MEDIUM
CO_2 inventory	mol m^{-2}	Summer	2011–2013	−0.43	0.67	5	−0.65	0.54	0.08	0.16	5	0.49	0.65	0.03	SMALL
CO_2 inventory	mol m^{-2}	Winter	2011–2013	−0.19	0.28	5	−0.70	0.52	0.00	0.15	5	0.03	0.98	0.01	SMALL
CH_4 flux	mmol m^{-2} d^{-1}	Summer	2011–2013	0	0.04	70	0.01	0.99	0.06	0.06	70	1.06	0.29	0.33	MEDIUM
CH_4 inventory	mmol m^{-2}	Summer	2011–2013	−9.32	10.27	5	−0.91	0.41	−16.71	10.11	5	−1.65	0.16	−0.46	MEDIUM
CH_4 inventory	mmol m^{-2}	Winter	2011–2013	0.23	1.55	5	0.15	0.89	−2.83	4.28	5	−0.66	0.54	−0.79	MEDIUM
N_2O flux	μmol m^{-2} d^{-1}	Summer	2012*	NA	NA	NA	NA	NA	1.89	1.24	28	1.53	0.14	0.14	SMALL
N_2O inventory	mmol m^{-2}	Summer	2012*	NA	NA	NA	NA	NA	−0.02	0.12	3	−0.15	0.89	−0.01	SMALL
N_2O inventory	mmol m^{-2}	Winter	2012–2013	−0.02	0.04	2	−0.47	0.68	0.02	0.04	2	0.61	0.60	0.17	SMALL

est, estimate; SE, standard error; df, degrees of freedom; t, t-value; p, p-value.
* Data from before-period missing.

parallel in control and impact lakes and did not reveal any significant fertilization effect (Table 2). Effect sizes on CH_4 fluxes and inventories were also independent of DIN loadings (Supporting Information Fig. S5).

In the after-period, N_2O fluxes and summer inventories tended to be slightly higher in fertilized lakes (2.4 μmol m^{-2} d^{-1} and 0.5 mmol m^{-2}, respectively) than in control lakes (0.5 μM m^{-2} d^{-1}, 0.1 mmol m^{-2}, respectively), yet this difference was not statistically significant (Fig. 3C,F; Table 2). N_2O winter inventories ranged from 0.08 mmol m^{-2} to 0.12 mmol m^{-2} and were not affected by fertilization (Fig. 3I; Table 2).

Literature synthesis

We synthesized results from 52 published papers covering 115 cases from 16 different countries, including the results of our study, the first whole-lake N-addition experiment (Supporting Information Fig. S6; Supporting Information Table S4). Responses of CO_2 concentrations or gas fluxes were reported in 57 cases, of CH_4 in 32 cases and of N_2O in 26 cases (Table 3). The majority of cases were spatial surveys (41) followed by sediment incubations (35). Equally represented were systems enriched with N (52) or P (10) alone and with N and P combined (53, Supporting Information Table S5), yet, of these studies, only one mesocosm experiment and one whole-lake experiment (this study) has investigated N enrichment effects alone (Supporting Information Table S4). Our literature synthesis revealed that nutrient effects varied widely across studies (Table 3). In the majority of cases, CO_2 did not change or decreased (47 of 57 cases), CH_4 did not change or increased (27 of 32 cases) and N_2O increased (17 of 26 cases). Responses differed significantly across spatiotemporal scales of the studies (Table 3). While spatial surveys tended to show increases in CO_2, multiannual trend observations and sediment incubations showed no clear response. In contrast, whole-lake (5 of 11) and especially mesocosm studies (7 of 11) often showed decreases. Similar patterns were found for CH_4, which increased in 9 out of 13 spatial surveys studies but showed no clear response according to any other study type. Across all types of studies, nutrient effects on CO_2 and CH_4 differed significantly depending on the type of nutrient enrichment (Supporting Information Table S5). CO_2 often decreased under combined N + P enrichment but showed no response under sole N or P enrichment. CH_4 typically increased under combined N + P or sole P enrichment but showed no response under N enrichment. Responses in N_2O were largely consistent across spatiotemporal scales and type of nutrient enrichment. Finally, responses varied widely in both unproductive boreal, (ant-)arctic or alpine soft-water lakes, and other, more productive temperate, tropical or hard-water, systems (Supporting Information Table S6). Hence, there was no statistical difference in the frequency distribution of responses between systems similar to our experimental lakes and other systems.
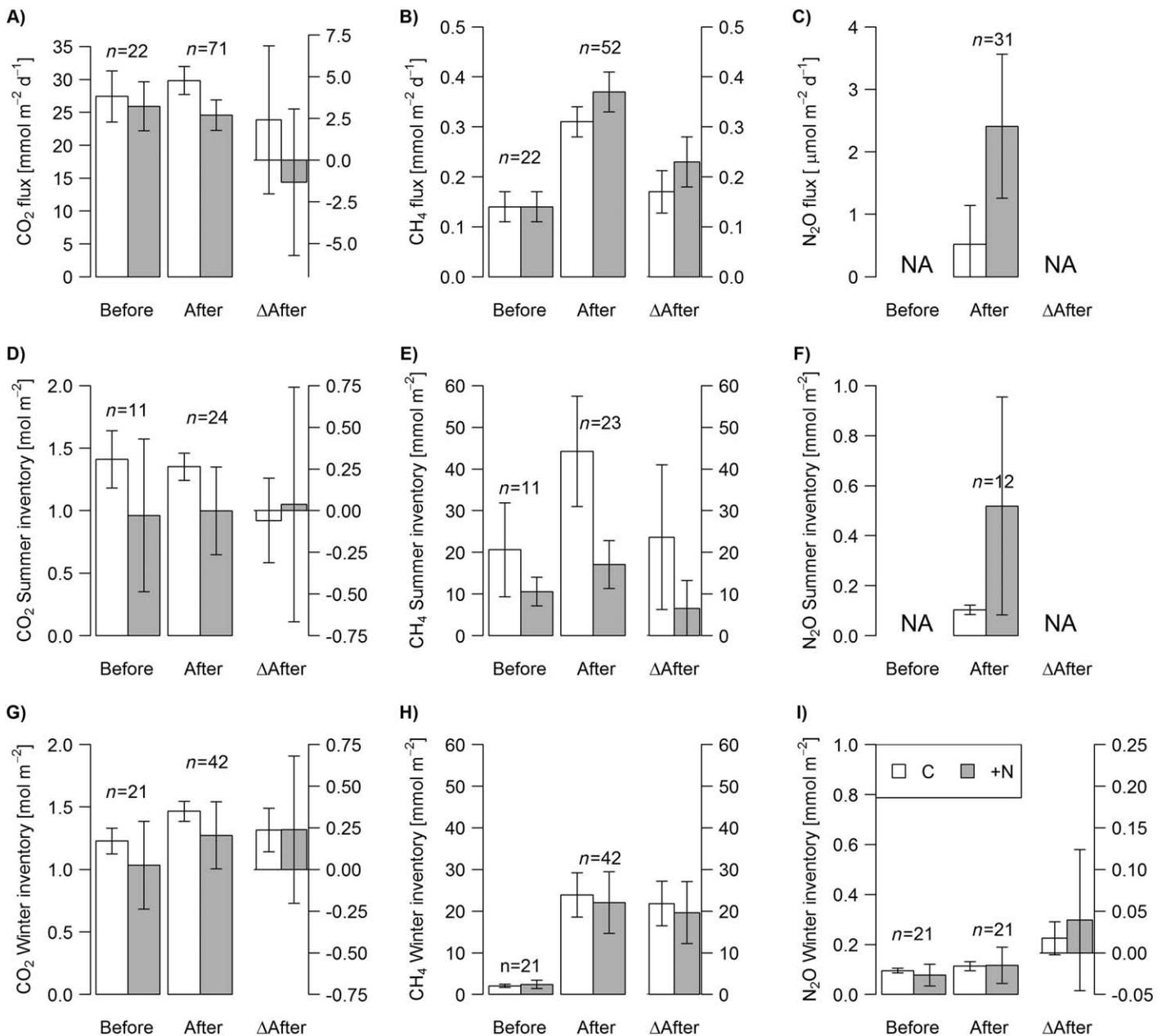
Fig. 3. Lake-atmosphere fluxes (**A–C**) and summer and winter inventories (**D–F** and **G–I**) of CO_2, CH_4 and N_2O in control ("C") and impact ("+N") lakes before and after onset of fertilization, and the respective differences between before and after ("ΔAfter"). Error bars show propagated standard errors of repeated observations over time and all lakes. Abbreviations: *n* = number of observations, NA = not available.

Discussion

We show, by conducting the first whole-lake N enrichment experiment on CO_2, CH_4 and N_2O emissions, that N per see has limited effects on greenhouse gas emissions from unproductive boreal lakes. Our study contrasts previous whole-lake experiments that have been limited to combined N and P effects on CO_2 emissions only, and many of the smaller habitat-specific studies and spatial surveys. Together, our experiment placed in the context of previous studies, demonstrates the scale and context dependence of greenhouse gas emissions to N enrichments. These findings are not necessarily limited to our study system, given that responses reported in the literature varied in lakes similar to ours as much as in other types of lakes (Supporting Information Table S6). The lack of a clear response in our experiment is either due to a limited increase in uptake or production of greenhouse gases in response to N addition or due to efficient buffering mechanisms that counteract N addition effects at the ecosystem scale. Our results emphasize

Table 3. Global literature synthesis on the effect of nutrient inputs (N or P) on CO_2, CH_4, and N_2O concentrations or emissions in standing inland-waters. Given is the number of study outcomes showing a significant increase, decrease or no significant effect according to the spatiotemporal scale of the system. Contingency table statistics: $Chi^2 = 16.8$, df = 8, $p = 0.03$ for CO_2, $Chi^2 = 13.2$, df = 6, $p = 0.04$ for CH_4, $Chi^2 = 7.0$, df = 6, $p = 0.32$ for N_2O.

Gas	Spatiotemporal scale	Increase	No change	Decrease	Grand total
		Response to nutrient addition (#cases)			
CO_2	Sediment incubation	2	6	1	9
	Mesocosm experiment	0	4	7	11
	Whole-lake experiment	0	6	5	11
	Multi-annual trend	0	2	3	5
	Spatial survey	8	6	7	21
	Grand total	10	24	23	57
CH_4	Sediment incubation	1	7	3	11
	Mesocosm experiment	2	2	1	5
	Whole-lake experiment	0	3	0	3
	Multi-annual trend	0	0	0	0
	Spatial survey	9	3	1	13
	Grand total	12	15	5	32
N_2O	Sediment incubation	11	4	0	15
	Mesocosm experiment	1	0	0	1
	Whole-lake experiment	0	3	0	3
	Multi-annual trend	0	0	0	0
	Spatial survey	5	2	0	7
	Grand total	17	9	0	26

that greenhouse gas emissions from lakes involve a complex suite of processes whose integrated response to individual drivers such as N can only be accurately assessed by whole-lake experiments.

Annual CO_2 emissions from our lakes were resilient to nutrient additions, despite a clear increase in gross primary production (GPP) following N fertilization (Deininger et al. 2017). Theoretically, two mechanisms could explain our observations. First, a large proportion of the additionally fixed C may have escaped bacterial respiration. This hypothesis is in line with our observation that the non-significant CO_2 decrease (-3.8 mmol m^{-2} d^{-1}) in response to nitrate addition during the summers 2012–2014 (Table 2) corresponded well to the net increase in pelagic GPP (3.2 mmol m^{-2} d^{-1}) during the summers 2012–2013 (Deininger et al. unpubl.). Bacteria may have avoided to respire the additionally fixed C because they sufficiently relied on abundant allochthonous C (Sadro et al. 2011; Solomon et al. 2013), or because they grew more efficiently in response to nutrient addition (del Giorgio and Cole 1998; Jansson et al. 2006). Data on ecosystem respiration would be valuable to fully reveal these mechanisms. Second, GPP was only a minor component of the whole-lake C budget. In our lakes, GPP (5 mmol m^{-2} d^{-1}, Deininger et al. unpubl.) was very low compared to baseline CO_2 emissions (26 mmol m^{-2} d^{-1}),

implying that changes in GPP following N enrichment could only have caused a maximum decrease in summer CO_2 emissions by 15%. Instead, primary production-driven CO_2 declines could be largely masked by high contributions of external CO_2 inputs to lake CO_2 emissions (Striegl and Michmerhuizen 1998; Wilkinson et al. 2016; Vachon et al. 2017) or mineralization of terrestrial DOC (Karlsson et al. 2007; Lapierre et al. 2013). Thus, even a clear GPP response following N enrichment does not necessarily translate into similar clear responses in lake-atmosphere CO_2 fluxes.

Our experimental results contrast with the significant CO_2 declines observed in five of eight previous whole-lake nutrient enrichment experiments (Supporting Information Table S4). Stronger CO_2 declines may be due to the larger importance of C uptake by primary producers for the whole-lake C budget, given that these experiments were carried out in more productive lakes than our systems (Carpenter et al. 2001; Wilkinson et al. 2016) or in seepage lakes with low terrestrial C inputs (Kling et al. 1992; Findlay et al. 1999). A further reason for stronger responses could be that N supply was similar or higher in these previous experiments (> 1.2 mg N m^{-2} d^{-1}) and combined with P addition. Our literature synthesis showed indeed that CO_2 decreased significantly more often in lakes enriched with both N and P compared to lakes enriched with either N or P

alone (Supporting Information Table S5), i.e., when phytoplankton are being substantially relieved from nutrient limitation. In our whole N enrichment experiments, phytoplankton likely switched from N- to P-limitation which constrained any further net uptake by CO_2 (Deininger et al. 2017). We should however be careful before drawing firm conclusions based on rather few experiments across a large diversity of lakes with potentially contrasting drivers of CO_2 exchange.

Effects of nutrient addition varied with the spatiotemporal scale of the system studied (Table 3). Specifically, CO_2 declines in mesocosm experiments were often more pronounced than in whole-lake experiments, likely because they exclude the masking effect of external C inputs. Hence, with increasing spatial scale (from pelagic- to whole-lake studies), nutrient effects on CO_2 emissions likely diminish, because other CO_2 controlling processes become more important, even though the absolute response of primary producers to nutrient addition may not change (Spivak et al. 2011). In contrast to experimental approaches, spatial surveys often reveal increases in CO_2 emissions with nutrient enrichment (Table 3). These net increases in CO_2 cannot be reconciled with nutrient stimulation of GPP. Instead, spatial patterns likely reflect region-specific covariation of nutrients with other factors such as DOC concentrations that fuel CO_2 emissions (Larsen et al. 2011; Lapierre and del Giorgio 2012). The same is true for studies of multi-annual trends which may not be purely due to changes in nutrient inputs, but instead to covariates such as climate (Rantakari and Kortelainen 2005; Finlay et al. 2009). Overall, strong scale-dependences make it difficult to generalize nutrient effects on lake CO_2 dynamics.

Scale-dependent responses to nutrient enrichment can also be expected for CH_4. The strong mismatch between experimental CH_4 responses and trends along spatial gradients may surprise. However, the CH_4 cycle includes many components of which only some may be involved in the system studied (Bastviken et al. 2004). For example, macrophytes can strongly modify CH_4 responses and can themselves be stimulated by nutrient additions (Davidson et al. 2015). Freshly produced biomass as a result of nutrient addition typically stimulates methanogenesis (Bogard et al. 2014; DelSontro et al. 2016) but does not necessarily cause measurable changes in diffusive CH_4 fluxes because it is efficiently oxidized on its way to the lake-water interface (West et al. 2016). Methane oxidization can even occur under anoxic conditions under the enhanced supply of nitrate that provides additional electron acceptors for CH_4 oxidizing bacteria (Deutzmann et al. 2014). This problem is well illustrated by the negligible coupling we found between CH_4 inventories and CH_4 fluxes during the following turnover periods (Fig. 2). We also cannot exclude that additional phytoplankton biomass in response to N addition has fueled CH_4 ebullition (West et al. 2016). We did not measure ebullition in our study. Given the observations that ebullition can

dominate over diffusive fluxes in boreal lakes (Chmiel et al. 2016; DelSontro et al. 2016) effects of N enrichment on total CH_4 emissions in our lakes are not known. Clearly, all relevant components of CH_4 cycling should be measured in future experiments to fully understand nutrient enrichment effects on annual whole-lake CH_4 emissions.

N_2O fluxes did not respond to nitrate enrichment in our experiment, despite DIN accumulation in the water column (Supporting Information Table S2) which suggests that nitrate was added in sufficient excess of the demand of denitrifiers. This contrasts with the typical increase observed in the large majority of previous studies. Yet, these studies assessed effects in mesocosms, sediment incubations or across spatial surveys and cannot be directly compared to our whole-lake experiment (Table 3). Our results on summer inventories and fluxes suffered from missing before-period data, and should therefore be treated with caution. However, we argue for minor differences between control- and impact lakes before onset of fertilization, because N_2O fluxes were consistently negligible in all control lakes, a pattern very typical for boreal lakes (Huttunen et al. 2003; Saarenheimo et al. 2015), especially with $pH < 6.2$ (Soued et al. 2016). The absence of any N_2O responses may be explained by the fact that nitrate was added to the oxic epilimnion where N_2O production during denitrification is inefficient (Mengis et al. 1997). Winter N_2O inventories did not increase with nitrate additions, despite elevated nitrate concentrations and anoxic conditions under the ice (data not shown), which could have been due to temperature limitation (Myrstener et al. 2016). Too few observations prevented us from evaluating N_2O fluxes during turnover periods. Since N_2O production during denitrification is most efficient at oxic-anoxic interfaces (Mengis et al. 1997), excess nitrate may have stimulated enhanced N_2O fluxes when oxic surface waters mix with anoxic bottom waters.

Our whole-lake experiment emphasizes that annual greenhouse gas emissions from unproductive boreal lakes generally do not respond to nitrate additions despite well-documented responses of primary production. We mainly attribute this lack of response to the fact that greenhouse gas cycling involves a large number of processes whose individual response to N additions may offset each other over time and space. Our results also emphasize the modest role of CO_2 fixation by primary producers after N addition for the CO_2 dynamics of unproductive boreal lakes. Hence, projected changes in N loading will likely not have any major impacts on the atmospheric CO_2 exchange of this type of lakes. Our global literature synthesis revealed that nutrient effects generally vary across spatiotemporal scales because of complex interactions of scale-dependent processes involved in greenhouse gas cycling. Therefore, nutrient effects should not be extrapolated to various spatiotemporal scales without caution for the system studied. Firm extrapolations and global predictions need to be informed by studies that reveal the underlying mechanisms behind the scale-dependence in nutrient responses.

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Acknowledgments

Conflict of Interest

None declared.