Ice duration drives winter nitrate accumulation in north temperate lakes

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Abstract

The duration of winter ice cover on lakes varies substantially with climate variability, and has decreased over the last several decades in many temperate lakes. However, little is known of how changes in seasonal ice cover may affect biogeochemical processes under ice. We examined winter nitrogen (N) dynamics under ice using a 30+ yr dataset from five oligotrophic/mesotrophic north temperate lakes to determine how changes in inorganic N species varied with ice duration. Nitrate accumulated during winter and was strongly related to the number of days since ice-on. Exogenous inputs accounted for less than 3% of nitrate accumulation in four of the five lakes, suggesting a paramount role of nitrification in regulating N transformation and the timing of chemical conditions under ice. Winter nitrate accumulation rates ranged from 0.15 µg N L⁻¹ d⁻¹ to 2.7 µg N L⁻¹ d⁻¹ (0.011–0.19 µM d⁻¹), and the mean for intermediate depths was 0.94 µg N L⁻¹ d⁻¹ (0.067 µM d⁻¹). Given that winters with shorter ice duration (< 120 d) have become more frequent in these lakes since the late 1990s, peak winter nitrate concentrations and cumulative nitrate production under ice may be declining. As ice extent and duration change, the physical and chemical conditions supporting life will shift. This research suggests we may expect changes in the form and amount of inorganic N, and altered dissolved nitrogen : phosphorus ratios, in lakes during winters with shorter ice duration.

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Data Availability Statement: Data are available in the North Temperate Lakes LTER repository at https://lter.limnology.wisc.edu/data. We used a merged winter data set of (1) chemical limnology, (2) physical limnology, and (3) ice duration https://lter.limnology.wisc.edu/node/56390 doi:10.6073/pasta/75d5bf82f0ac6d29ed2a0a8e8554c0c and the source data sets are available on the North Temperate Lakes LTER repository. Additional Supporting Information may be found in the online version of this article.

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Most of the world’s naturally formed freshwater lakes are located above 45° North and freeze during winter (Verpoorter et al. 2014). Effects of ice on physical properties of lakes include a convergence of bottom temperatures toward 4°C in freshwater systems, altered mixing dynamics, loss of gas exchange, and winter darkness (McKnight et al. 2000; Kirillin et al. 2012). Despite these fundamental changes, the influence of ice dynamics on ecology and biogeochemical cycling of seasonally frozen lakes are not well understood, often inferred indirectly by comparing lake conditions before and after ice is present (Weyhenmeyer 2009; Hampton et al. 2015). Broad scale declines in ice duration over the past century in the northern hemisphere (Magnuson et al. 2000; Benson et al. 2012) underscore the need to address this winter blind spot (Powers and Hampton 2016; Hampton et al. 2017) and better understand ecosystem responses to varying winter conditions (Ozkundakci et al. 2016) and ice duration (Denfeld et al. 2016).

Ice cover results in substantial changes to the physico-chemical environment and biogeochemical processes important to nitrogen (N) cycling (Bertilsson et al. 2012). N mineralization and nitrification—the microbial oxidation of ammonium to nitrite and nitrate—are central processes in N cycling that occur in diverse environments. Much understanding about these transformations comes from studies in soils, followed by coastal marine systems (Heiss and Fulweiler 2016), estuaries (Wankel et al. 2011; Beman 2014), permanently frozen Antarctic lakes (Voytek et al. 1999), and open water conditions in streams and freshwater lakes (Hall and Jeffries 1984; Bernhardt et al. 2002). As in marine environments (Konneke et al. 2005), Archaea are often the dominant ammonium oxidizers in lakes (Small et al. 2013; Hayden and Bemen 2014; Yang et al. 2017). The relative abundance of marine Archaea can increase during extreme cold under sea ice (Church et al. 2003), and nitrification is known to be a major influence on N speciation in permanently frozen lakes (Voytek et al. 1999). Consequently, nitrification may occur broadly in cold freshwater systems (<5°C) and at ecosystem-relevant rates, providing an ammonia sink and nitrate source that could affect other biogeochemical fluxes as well as many taxa that preferentially use ammonium over nitrate (Glibert et al. 2016). The expectation of significant nitrification during seasonal ice cover has been supported by isotopic evidence in subarctic lakes (Gu 2012), but to date there have been relatively few measurements of rates and controls on nitrification in freshwater lakes (Small et al. 2013), especially beneath ice.

Previous lake studies have reported nitrate accumulation during winter. Due to major exogenous N sources, it has often been difficult to elucidate the role of in-lake (endogenous) processes in generating this winter nitrate. For example, George et al. (2010) report winter nitrate concentrations > 500 μg N/L in eight lakes of the UK, Finland, Estonia, and Sweden, where drainage from surrounding agricultural land was identified as a primary source of the N. Similarly, Knowles and Lean (1987) reported rates of nitrate accumulation up to 13 μg L⁻¹ d⁻¹ (as N) using in situ water column measurements in Lake St. George, Ontario, a small (140 ha) kettle lake with contiguous shoreline development and likely exogenous N sources. Pettersson et al. (2003) report nitrate accumulation under ice in Lake Erken, Sweden, located in a moderately agricultural catchment. In contrast, ice-covered lakes in forested landscapes often have relatively low exogenous inputs of nitrate, potentially offering a simplified window on the N cycle during conditions not well represented in prior aquatic studies.

Nitrification has often been examined under warmer or more saline conditions than those typically observed during seasonal ice cover in freshwater lakes. When oxidized nitrogen species accumulate under lake ice, one simple constraint on the concentration is the number of days that ice has been present. Controls on nitrification in such lakes may also include: levels of connectivity between sediment microbes, N pools, and water column oxygen sources; exposure to excess light (French et al. 2012), ammonium (Chen et al. 2010), acidity (Jeschke et al. 2013), or anthropogenic pollutants such as metals or pharmaceuticals (Kraigher and Mandic-Mulec 2011; Rosi-Marshall and Royer 2012); competition for ammonium or nitrite by heterotrophic microbes or algae (Agbeti and Smol 1995), which may be elevated in lakes with high sediment-water contact or primary productivity. In unpolluted freshwaters, nitrite is thought to occur at trace concentrations and nitrite oxidation is not commonly viewed as a rate-limiting step of nitrification, although nitrite may sometimes accumulate to toxic concentrations in agricultural streams or groundwater (Stanley and Maxted 2008; Chen et al. 2010). While these factors can influence spatio-temporal variability in gross or net N transformation rates, understanding of total winter production of nitrate and ammonium could be enhanced through ecosystem-level studies that employ long-term winter data. Further, a simple predictor—the number of days since ice-on—may facilitate understanding of time-integrated process rates and the role of seasonal ice duration in N cycling.

The goal of this work was to examine dissolved inorganic N (DIN) dynamics and speciation during winter in a set of seasonally ice-covered north temperate lakes, focusing on the link between DIN and ice duration. We asked: (1) How rapidly and in what form (NH₄-N or NO₃-N) does DIN accumulate under ice? and (2) How are these rates related to ice duration? To address these questions and interpret potential roles of underlying mechanisms (e.g., nitrification, transfers between organic and inorganic N pools), we investigated temporal and vertical patterns of DIN, along with supporting measurements using a 30-yr dataset from five north temperate U.S.A. lakes.
Methods

We used 30+ yr of winter limnological data from five North Temperate Lakes Long-Term Ecological Research (NTL-LTER) study lakes (Allequash, Big Muskellunge (Big Musky), Crystal, Sparkling, and Trout Lakes; https://lter.limnology.wisc.edu) in northern Wisconsin, U.S.A. Maximum lake depth ranged from 8 m to 35 m. Lake depth, ice duration, winter data availability, and mean winter conditions are summarized in Table 1. Lakes were sampled 2–3 times each winter and N fractions were determined using consistent field and laboratory methodologies. Samples were collected at three depths at the deepest point of each lake (typically close to center of lake) through a 20-cm bore hole: surface (0–5 cm below the bottom of the ice), deep (1 m above lake bottom), and middle of the water column. Expanded methods are in the Supporting Information.

Relationships between concentration and days since ice-on

We examined winter NO\textsubscript{3}-N and NH\textsubscript{4}-N at three depths, and supporting physico-chemistry including total dissolved phosphorus (TDP, \(\mu\)g/L) and dissolved inorganic carbon (DIC, mg/L). With days since ice-on as our time variable, we used linear mixed effects models (Bates et al. 2015) to estimate lake-specific and depth-specific rates of change (Supporting Information). For Trout Lake only, a fitting window of 1–100 d since ice-on was examined for deep water, due to a hump-shaped curve for NO\textsubscript{3}-N. Analyses were conducted using R (R Core Team 2016). Loess fits were used for visualization.

Exogenous N inputs

Fitted rates of N change under ice were compared to rates of exogenous N input from streams and groundwater (Supporting Information). Exogenous N was quantified using three data sources: (1) groundwater NO\textsubscript{3}-N and NH\textsubscript{4}-N (n = 12 wells), typically sampled each year during autumn; (2) daily winter water fluxes to each lake from a data-driven hydrologic model based on daily precipitation and seasonal measurements of hydraulic head (Hunt et al. 2013; Hunt and Walker 2017); and (3) available stream chemistry data, sampled during 2007–2015. We assumed that N fixation and atmospheric N inputs were negligible during ice cover (Sampson and Brezonik 2003).

Results

Across lakes and depths, the ice-on period began with relatively low NO\textsubscript{3}-N concentrations, and higher NH\textsubscript{4}-N concentrations at the deep sampling depth. In deep water of Allequash, Big Musky, and Sparkling, NH\textsubscript{4}-N frequently reached concentrations > 500 \(\mu\)g/L. At middle and surface depths in all lakes, NO\textsubscript{3}-N was significantly higher in late winter compared to early winter (Fig. 1), attaining concentrations > 100 \(\mu\)g/L. These patterns at middle and surface

Table 1. Lake features and data availability.

| Lake name | Max depth (m) | Mean depth (m) | Lake area (km\textsuperscript{2}) | Catchment area (km\textsuperscript{2}) | Mean # obs. per year | NO\textsubscript{3}-N (\(\mu\)g/L) | NH\textsubscript{4}-N (\(\mu\)g/L) | O\textsubscript{2} (mg/L) | DIC (mg/L) | TDP (\(\mu\)g/L) | Water temp (deg C) | pH |
|-----------|---------------|----------------|-----------------------------------|----------------------------------------|-----------------------|-----------------------------|-----------------------------|-----------------|------------|----------------|-----------------|----|
| Allequash Lake, north basin | 2.9 | 1.2 | 11.2 | 182 | 31 | 1.44 | 0.83 | 138 | 0.36 | 105 | 1.36 | 84 |
| Big Musky Lake | 21.3 | 7.5 | 3.96 | 4.0 | 106 | 0 | 84 | 0 | 3.0 | 135 | 1.38 | 7.4 |
| Crystal Lake | 20.4 | 10.4 | 0.67 | 0.86 | 190 | 35 | 89 | 32 | 0.66 | 155 | 7.6 |
| Sparkling Lake | 35.7 | 14.6 | 10.9 | 47 | 53 | 133 | 170 | 77 | 0.76 | 155 | 7.6 |
| Trout Lake, south basin | 28 | 10.9 | 0.64 | 1.4 | 0 | 11 | 47 | 47 | 0 | 155 | 7.6 |
depths produced a consistent shift in DIN form over winter, from NH$_4$-N dominance to NO$_3$-N dominance. Model-fitted NO$_3$-N accumulation rates (Supporting Information Table S1) were highest at the middle sampling depth in Allequash, Big Musky, and Sparkling lakes, and at the deep sampling depth in Crystal and Trout (Fig. 2). Trout Lake deep water had the fastest NO$_3$-N accumulation rate among lakes and depths, at 2.7 µg L$^{-1}$ d$^{-1}$ (0.19 µM d$^{-1}$) prior to the NO$_3$-N decline around day 100. The selected NO$_3$-N model had a random intercept for year, and a heteroscedastic error structure (proportional to days since ice-on), producing a marginal r-squared of 0.79 (Supporting Information). NO$_3$-N accumulation rates at middle depth ranged from 0.35 µg L$^{-1}$ d$^{-1}$ (0.025 µM d$^{-1}$) in Crystal to 1.68 µg L$^{-1}$ d$^{-1}$ (0.12 µM d$^{-1}$) in Allequash, and had a mean of 0.94 µg N L$^{-1}$ d$^{-1}$ (0.067 µM d$^{-1}$). In deep water, NH$_4$-N also accumulated at ≥2.0 µg L$^{-1}$ d$^{-1}$ (0.14 µM d$^{-1}$), exceeding NO$_3$-N accumulation, but in these lakes most benthic surface area occurs above the deep sampling depth (Supporting Information). If middle depth is used to represent the whole lake, corresponding areal NO$_3$-N accumulation rates ranged from 260 µmol N m$^{-2}$ d$^{-1}$ in Crystal to 790 µmol N m$^{-2}$ d$^{-1}$ in Trout, with rates of 350 in Allequash, 580 in Big Musky, and 660 in Sparkling. The ratio of NO$_3$-N : DIN accumulation at middle depth was 0.41 in Crystal, and 0.64–0.83 in the other lakes, reflecting the substantial fraction of DIN as NO$_3$-N. In contrast to DIN, pronounced TDP accumulation was restricted to deep water (Supporting Information). At middle depth, between 30 and 120 d since ice-on, stable TDP combined with DIN accumulation gave predicted shifts in DIN : TDP ratios from 24 to 57 in Allequash, 25 to 42 in Big Musky, 32 to 53 in Crystal, and 39 to 67 in Sparkling; in Trout Lake between 30 and 100 d since ice-on, the predicted shift in DIN : TDP was from 33 to 50.

Exogenous inputs from streams and groundwater were insufficient to account for observed in-lake NO$_3$-N increases (Supporting Information). In three lakes that lacked perennial streams (Big Musky, Crystal, Sparkling), using the
median groundwater NO$_3$-N concentration of 4 µg/L and assuming middle depth is most representative, exogenous inputs accounted for <0.1% of the water column NO$_3$-N accumulation rate. Exogenous NO$_3$-N inputs were similarly negligible in comparison to aggregated (depth-ignorant) NO$_3$-N accumulation rates across the three depths. In Allequash, mean daily winter water input ranged from 17,000 m$^3$ d$^{-1}$ to 28,000 m$^3$ d$^{-1}$ depending on the year, equivalent to 49–113% of the lake volume (mean of 79%) when integrated over the duration of ice cover. However, using a stream and groundwater concentration of 15 µg/L NO$_3$-N, our calculations suggest exogenous NO$_3$-N inputs can only account for 3.8–6.0% of the in-lake NO$_3$-N increase in Allequash, and 0.62–1.1% in Trout. If a more liberal estimate of stream and groundwater NO$_3$-N concentration is used (35 µg/L, the 75th percentile of groundwater data), exogenous NO$_3$-N inputs still only account for 8.8–14% of the in-lake increase in Allequash, and 1.5–2.6% in Trout.

Positive correlations between DIN and DIC concentrations (Fig. 3) suggested organic matter decomposition and N mineralization supplied N available for nitrification, particularly in deep and intermediate waters. The highest DIN and DIC concentrations occurred in deep samples, pointing to likely benthic organic sources, with possible additional contributions from NH$_4$-N that was abiotically released from sediments. Breakdown of dissolved organic N (DON) represents a potential alternative source of water column NH$_4$-N, or NO$_3$-N (if DON breakdown is coupled with nitrification). However, there were no consistent, substantial declines in DON concentrations over winter (Supporting Information Fig. S5).

**Discussion**

While past work has demonstrated that nitrification rates can be inversely related to temperature (Randall and Buth 1984; Bruesewitz et al. 2009), this process may continue in cold winter waters when oxygen is present. There were strong positive relationships between NO$_3$-N concentration and days since ice-on in all five lakes, indicating NO$_3$-N accumulation in the water column under ice. External inputs could not account for these pronounced increases, leaving nitrification as a likely explanation for NO$_3$-N accumulation. In all five lakes, these integrated winter NO$_3$-N accumulation rates were ≥0.75 µg L$^{-1}$d$^{-1}$ at one or more sampling depths. Over shorter periods of winter, higher rates of NO$_3$-N accumulation may still be possible in these lakes. Higher NO$_3$-N accumulation rates have been reported under ice in more eutrophic lakes (Knowles and Lean 1987). Our reported rates reflect net processing, as some N may be consumed by processes such as denitrification or algal uptake, perhaps especially under thin or clear ice (Kerfoot et al. 2008, Katz et al. 2015). The regularity of inorganic N accumulation over these 30+ yr of data suggests the number of days since ice-on can be useful in predicting lake dynamics.

NO$_3$-N accumulation was more pronounced in middle and deep water compared to the surface, likely because nitrification occurs predominantly in the benthos, as has been
widely reported during ice-free conditions (e.g., Pauer and Auer 2000). Prior data on sediment nitrification rates below 5°C are scarce, even among water treatment studies (but see Abeysinghe et al. 2002). Benthic mineralization appears to be an important source of DIN in these lakes. This is suggested by elevated NH$_4$-N and DIC concentrations in deep and middle samples, positive NH$_4$-N~DIC relationships, and lack of predictable seasonal patterns for DON and suspended particle N (Supporting Information). The DIN accumulation rates at middle and surface depths were sufficient to regularly produce molar DIN : TDP ratios of 40–70 after 100 d of ice cover.

Water column DON turnover may have contributed a fraction of the DIN. However, there were no robust declines in DON concentrations over winter (Supporting Information Fig. S5) as might be expected if DON were the major DIN source. In addition, the hypothesis that water column DON supplies the majority of DIN runs counter to the paradigm of organic matter breakdown as a benthic-dominated process within inland lakes, perhaps especially during low light conditions that limit winter DOM photodegradation (Hampton et al. 2017).

One of the most conspicuous consequences of under-ice N transformation is the marked rise in NO$_3$-N : NH$_4$-N ratios over winter (Fig. 1), and its potential effects on autotrophic communities in late winter (Kerfoot et al. 2008) and spring (Gächter et al. 1974). For example, many taxa preferentially use NH$_4$-N as an inorganic N source, and will be at a disadvantage as NO$_3$-N becomes a larger fraction of the DIN pool at the expense of NH$_4$-N (Glibert et al. 2016). As additional examples, altered NO$_3$-N concentration could influence biogenic production of gases (N$_2$, N$_2$O) through coupled nitrification-denitrification, or spring/summer production of cyanobacterial secondary metabolites such as microcystin, which may be favored under lower NO$_3$-N : NH$_4$-N (Harris et al. 2016). Weyhenmeyer et al. (2007) indicate that nitrate-depleted summer conditions have become more common in shallow lakes of northern Europe, yet potential linkages between winter nitrification variability and summer NO$_3$-N remain an area of needed investigation. While P availability often limits productivity, N may co-limit autotrophs across a range of aquatic environments (Elser et al. 2007; Paerl et al. 2016), and can influence metabolic processes of phytoplankton and other organisms at non-limiting concentrations (Glibert et al. 2016).

Much benthic surface area in these lakes falls closer to middle sampling depths than surface or deep depths (Supporting Information), so the middle may be more representative of ecosystem-level process rates. Processes near the ice surface in these lakes are probably less influential on whole lake N transformation rates, as shallow samples represented a relatively thin layer of the water column according to temperature-DO profiles (Supporting Information Fig. S6), and shallow littoral sediments may become biogeochemically inactive if they freeze. Nonetheless, shallower lakes also often have higher surface area : volume ratios and

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**Fig. 3.** Positive relationships between DIN and DIC within each lake. Lines are the linear regression fits for the deep water samples (Supporting Information).
potentially higher sediment-water contact, which could influence N transformation rates. The two shallowest lakes, Allequash and Big Musky, had higher surface area : volume ratios (> 0.13 m²/m³) and in turn the highest NO₃-N accumulation rates at middle depth, yet estimated areal rates were intermediate (350 μmol m⁻² d⁻¹ and 580 μmol m⁻² d⁻¹) to the other lakes (260–790 μmol m⁻² d⁻¹). Understanding of the role of morphometry could be aided by research across a more diverse suite of lakes.

Given differences in DIN among depths, changes in NO₃-N concentrations may reflect depth-specific nitrification. However, in three lakes, surface and middle NO₃-N increases coincided with NH₄-N accumulation in deep waters (Allequash, Big Musky, Sparkling), raising the possibility of vertical transfer, perhaps especially during late winter. Below 4.0°C, water density increases as it warms (Wetzel 2001), and recent research has demonstrated the potential role of convective mixing under ice (Bruesewitz et al. 2014; Pernica et al. 2017). Such mixing may deliver oxygen to benthic nitrifiers, perhaps contributing to the high NO₃-N accumulation rate in deep water of Trout Lake (28 m). In larger, deeper lakes, nitrification may occur at depths >100 m when oxygen is present (Small et al. 2013). If NO₃-N accumulation in surface or deep water arrived through vertical transfer, our reported accumulation rates may still underestimate the contribution of intermediate depths to whole lake nitrification.

While nitrification is likely a spatially heterogeneous process, identifying habitats that contribute disproportionately to NO₃-N accumulation is often challenging (Clevinger et al. 2014).

Ice duration data from 1982 to 2017 indicate that winters with shorter ice duration (< 120 d) have become more frequent in these lakes since the late 1990s (Fig. 4), with much interannual variability. Since 1982, there were marginally significant simple linear declines in ice duration (p ≤ 0.08) of 4–5 d per decade in Allequash, Big Musky, and Crystal Lakes, but no significant declines in Sparkling or Trout Lakes (p > 0.1). A disproportionately long ice duration in 2013–2014 influenced significance of these regressions, as all lakes had significant declines (p ≤ 0.04) of ~6 d per decade when 2013–2014 was excluded. Other lakes in this region have shown similar declines of 4–9 d per decade over a longer period of record (Jensen et al. 2007). Given these patterns, it is possible that nitrate-rich winters are becoming less frequent in forested lakes experiencing shorter ice duration. The fitted accumulation rates can inform future expectations about peak winter NO₃-N concentration. For example, based on accumulation rates at middle depth, the expected NO₃-N concentration after 90 d of ice cover (corresponding to a short winter) is 21–40% lower compared to 140 d of ice cover, a decrease of 17–96 μg/L depending on the lake. Associated predictions at 90 d give peak DIN : TDP ratios of 30–60, a decrease of 10–20 units.
These predictions are approximate and can still vary across years and lake types. Nonetheless, truncated winter N dynamics are expected with shorter ice duration, and this could influence annual lake biogeochemical budgets, primary productivity, and biological communities.

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