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Add a dash of salt? Effects of road de-icing salt (NaCl) on benthic respiration and nutrient fluxes in freshwater sediments

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ABSTRACT

Add a dash of salt? Effects of road salt (NaCl) on benthic respiration and nutrient fluxes in freshwater sediments

Winter road salt applications are increasing chloride concentrations in many freshwater ecosystems. This trend is alarming, given chloride's potential to impair aquatic ecosystems. Short- and long-term exposure to salt could affect ecosystem metabolism and nutrient cycles. Here, we examine connections between chloride concentrations, water quality conditions, benthic respiration, and sediment-water nutrient flux throughout a large (722 km^2) lake and its catchment. Aquatic locations experiencing high concentrations of chloride are indicators of anthropogenic activities and are often associated with additional pollutants. We used sediment core flow-through incubations under ambient and enriched chloride concentrations to determine the effects of road salt on benthic respiration and nutrient fluxes in stream, stormwater pond, and lake sites. Salt (as sodium chloride) additions caused a significant overall increase in benthic respiration. Acute exposure to road salt caused the strongest increase in benthic respiration when water was warm and at sites that had low (< 50 mg Cl$^-$/L) or high (> 400 mg Cl$^-$/L) ambient chloride concentrations or when water was cold and sites had intermediate (100-400 mg Cl$^-$/L) ambient chloride concentrations. Nitrate flux responded less uniformly to salt additions. Depending on waterbody type and season, ambient nitrate flux into the sediment was similar, increased, or decreased post-chloride addition. Dissolved phosphorus flux was not significantly impacted by salt additions. Across lake and stream sites, our results supported the hypothesis that chloride causes increased respiration while nutrient cycles were weakly and inconsistently altered under experimental pulse road salt additions.

Key words: aquatic biogeochemical cycles, road salt, nutrient flux, oxygen flux, sediment-water interface

RESUMEN

¿Agregar una pizca de sal? Efectos de la sal usada en carreteras (NaCl) sobre la respiración béntónica y los flujos de nutrientes en los sedimentos de agua dulce

El uso de sal de carreteras en invierno para el deshielo está aumentando las concentraciones de cloruro en muchos ecosistemas de agua dulce. Esta tendencia es alarmante, dado el potencial del cloruro para dañar los ecosistemas acuáticos. La exposición a corto y largo plazo a la sal podría afectar el metabolismo del ecosistema y los ciclos de nutrientes. En este estudio, examinamos las conexiones entre las concentraciones de cloruro, la calidad del agua, la respiración béntónica y el flujo de nutrientes entre el agua y el sedimento en un gran lago (722 km^2) y su cuenca. Los sistemas acuáticos que experimentan altas concentraciones de cloruro son indicadores de actividades antropogénicas y, a menudo, se asocian con contaminantes adicionales. Utilizamos incubaciones de sedimento con flujo continuo, en condiciones ambientales y con enriquecimiento de cloruro para determinar los efectos de la sal de carreteras en la respiración bentónica y los flujos de nutrientes, en un arroyo, una poza de aguas pluviales y en el lago. Las adiciones de sal (como cloruro de sodio) causaron un aumento general significativo en la

respiración bentónica. La exposición aguda a la sal causó el aumento más fuerte en la respiración bentónica cuando el agua estaba tibia y en los cuando tenían concentraciones de cloruro ambiental bajas (< 50 mg Cl⁻/L) o altas (> 400 mg Cl⁻/L); o cuando el agua estaba fría y tenían concentraciones de cloruro ambiental intermedias (100-400 mg Cl⁻/L). El flujo de nitrato respondió de manera menos uniforme a las adiciones de sal. Según el tipo de cuerpo de agua y el período del año, el flujo de nitrato ambiental hacia el sedimento fue similar, aumentó o disminuyó después de la adición de cloruro. El flujo de fósforo disuelto no se vio afectado significativamente por las adiciones de sal. En el lago y en el arroyo, nuestros resultados respaldaron la hipótesis de que el cloruro provoca una mayor respiración, mientras que los ciclos de nutrientes se alteraron de manera débil e inconsistente bajo las adiciones experimentales de sal de carreteras.

Palabras clave: *ciclos biogeoquímicos acuáticos, sal de carreteras, flujo de nutrientes, flujo de oxígeno, interfase sedimento-agua*

INTRODUCTION

Salinization is a global phenomenon occurring in many aquatic ecosystems (Cañedo-Argüelles, 2020; Dugan et al., 2017; Hintz et al., 2022; Kaushal et al., 2021; Marsalek, 2003). In cool temperate zones, winter de-icing agents (mostly in the form of sodium chloride; NaCl) used on roads, parking lots, and other paved surfaces to break ice-pavement bond and improve traction and safety are driving this trend of increasing chloride in freshwater (Kaushal et al., 2018). Chloride concentrations that exceed governmental guidelines for toxicity are becoming more common in streams, wetlands, and stormwater ponds (Herbert et al., 2015; Kaushal et al., 2018; Marsalek, 2003). Freshwater organisms tolerate specific ranges of salinity and chloride increases are not only threatening their survival (Elphick et al., 2011; Moffett et al., 2020) but also negatively impacting biodiversity and ecosystem services (Berger et al., 2019; Cañedo-Argüelles et al., 2013; Herbert et al., 2015). There is widespread concern that chloride increases could also disrupt terrestrial-aquatic linkages and aquatic biogeochemical cycles (Herbert et al., 2015; Hintz & Relyea, 2019). At present, however, there are only a small number of ecosystem process-based studies on road salt and the effects of salinization on ecosystem functions and biogeochemical cycles requires further targeted study.

Salinization influences the quality and quantity of organic matter and nutrients leaching from soils and sediments into aquatic ecosystems (Entrekin et al., 2019; Haq et al., 2018; Herbert et al., 2015). Chloride exposure can increase the release of cations (calcium, potassium, and magnesium) and nutrients (reactive phosphorus and dissolved nitrogen) from riparian soils, stream sediments, and biofilms (Duan & Kaushal, 2015; Haq et al., 2018; Martínez et al., 2020). Sodium exposure can disrupt cation exchange in soils, cause ammonium release from soil, interact with organic matter, and lead to increased alkalinity and pH of surface waters (Ramakrishna & Viraraghavan, 2005). Similar patterns have been observed in wetlands and streams where chloride increases are associated with decreased carbon storage and decreased nitrogen retention (Baldwin et al., 2006; Cañedo-Argüelles et al., 2013; Herbert et al., 2015). Chloride concentrations and toxicity often follow annual cycles, with the lowest concentrations occurring in fall and highest concentrations and toxicity in winter and early spring (Dugan & Rock, 2021; Marsalek, 2003). In waterbodies with limited prior exposure to chloride pollution, even relatively low chloride concentrations can negatively affect ecosystem processes (Arnott et al., 2020; Bartlett et al., 2012; Kjensmo, 1997). There is some evidence that nutrient cycles in waterbodies with a history of chloride pollution have acclimated and are less responsive to new chloride pulses (Hale & Groffman, 2006; Lancaster et al., 2016). Still, in stormwater ponds and urban aquatic ecosystems, seasonal chloride increases can cause chemocline-stratification, which fosters bottom water anoxia and abiotic phosphorus releases across the sediment-water interface (Kaushal et al., 2020; Marsalek, 2003; Novotny & Stefan, 2012; Song et al., 2013).

Salt pulses can also affect microbial respiration rates in wetland sediments, on aquatic biofilms, and in association with terrestrial organic matter decomposition (Almeida Júnior et al., 2020; Chambers et al., 2011; Martínez et al., 2020; Oliveira et al., 2021; Silva & Davies, 1999). At high chloride concentrations (> 1000 mg Cl^-/L), evidence of ionic stress causing a metabolic shutdown is often inferred through reductions in microbial respiration rates after chloride exposure (although see: Chambers et al., 2011). At low to intermediate chloride concentrations (10s to 500 mg Cl^-/L), the chloride pulses can increase microbial respiration, possibly indicating stimulated decomposition of organic matter or an early metabolic response to ionic stress (Cochero et al., 2017; Martínez et al., 2020; Oliveira et al., 2021; Silva et al., 2000). The magnitude of these respiration responses varies across sites and depends in part on site specific conditions, ambient chloride pollution levels, organic matter quality, and microbial community composition (Cochero et al., 2017; Martínez et al., 2020; Oliveira et al., 2021; Silva et al., 2000). Respiration responses have been found to be short in duration and reversable, but nonetheless signal that chloride pulses could have major episodic effects on aquatic carbon cycles and are a cause for concern.

In aquatic ecosystems, nitrate (NO_3) uptake into the sediment and benthic respiration tend to be coupled (Gold et al., 2021). As dissolved oxygen is drawn down in the sediment, redox conditions can shift towards phosphorus release into the water column (Novotny & Stefan 2012). It is unclear, however, if salt additions will reinforce nutrient cycling and respiration coupling or result in decoupling between nutrient cycles and benthic metabolism. Altogether, chloride may alter freshwater ecosystem metabolism and reduce the ecosystem's ability to retain/process nutrients, allowing more nutrients to escape downstream (Hintz & Relyea, 2019; Marsalek, 2003). Consequently, ecosystem responses could vary across chloride concentrations with biogeochemical systems showing signs of stress (increased respiration, low magnitude changes in nutrient cycling) at low to moderate chloride concentrations and full loss of metabolic function at high concentrations.

To determine how chloride affects nutrient cycling between the sediment-water interface and benthic respiration, we collected water samples and intact sediment cores from streams, stormwater ponds, and the littoral zone of a large lake. Sediment cores were spiked with low to moderate concentration of NaCl (40 to 500 mg Cl^-/L) to determine its short-term impact on dissolved O_2, NO_3, and total dissolved phosphorus (TDP) fluxes. These concentrations were chosen to approximate the concentration of salt pulses that this system received seasonally in runoff. We focus on chloride as the main toxicant because studies suggest chloride is more mobile than sodium (Haq et al., 2018, Kaushal et al., 2017; 2020) and the majority of winter road salt studies focus on chloride. We hypothesize that winter road salt additions increase benthic respiration, increase phosphorus flux out of the sediment, and decrease nitrogen flux into the sediment. Further, we hypothesize that salt additions have the largest effects on ecosystem processes in sites with low ambient chloride concentrations.

METHODS

Study sites and sampling methods

We studied Lake Simcoe (44° 25'N, 79° 25'W) and its watershed in Ontario, Canada where chloride concentrations have increased (~ 1 mg Cl^-/L/yr) since the 1980's (Fig. 1 a, b). We selected seven nearshore lake sites, five streams sampled just upstream of the lake, and four stormwater ponds located in Barrie, Ontario (Fig. 1a). Stream and stormwater ponds were sampled five times from October 2010 to August 2011 (about once every two months). Lake sites were sampled three times during the ice-free season (October 2010, May 2011, and August 2011). The sampling interval was chosen to cover the expected seasonal pattern of chloride, highest in early spring and lowest in late fall (Marsalek, 2003) and the annual range of bottom water temperatures.

Water was collected from the deepest part of the stream segment and intact sediment cores were collected from sediment depositional areas. Surface water samples and sediment cores were collected from stormwater pond sites just up-pond of the outflow structure in the permanent

pool. For lake sites, water samples were collected at the long-term monitoring locations and sediment cores were collected near the shore at < 2 m depth. In addition, two 23 L carboys were filled with water at each site for use in salt addition ex-

periments. Winter road salt and salt/sand mixtures were collected from ten municipal and provincial storage depots within the Lake Simcoe watershed during fall 2010. Salt/sand samples were then mixed together in equal proportions. This com-
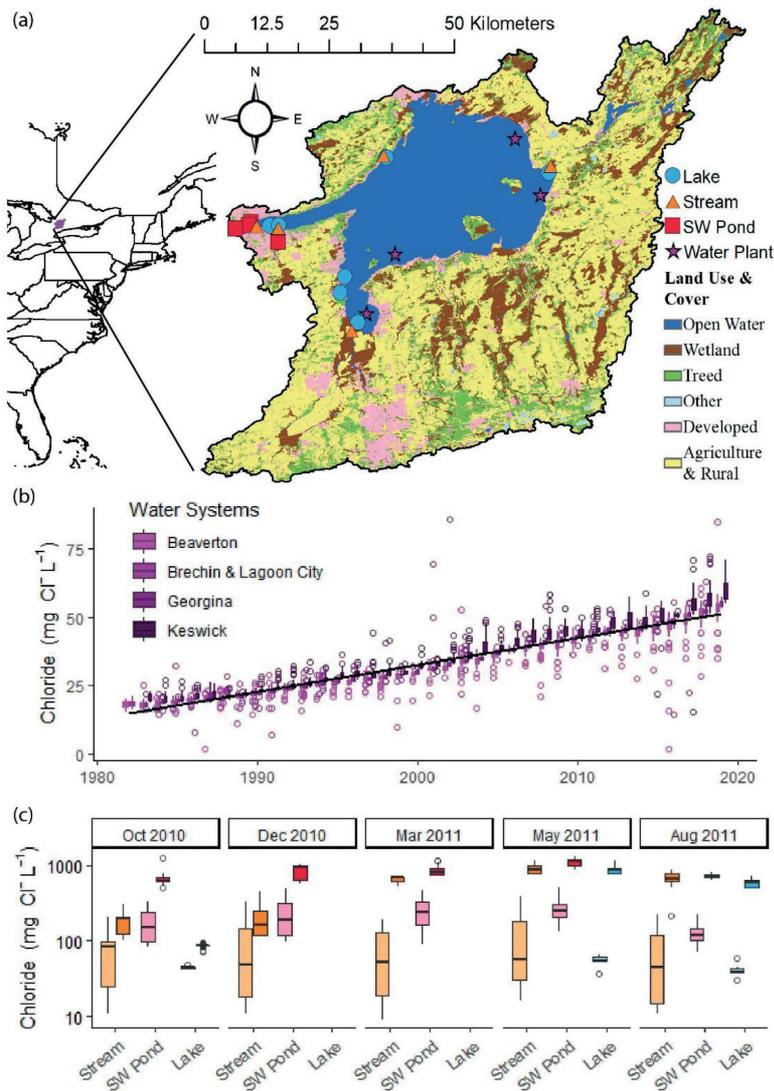
Figure 1. (a) Map of Lake Simcoe watershed land use and site locations (b). Long-term changes in lake chloride concentrations at four water treatment stations. Trend is the linear regression of the median annual chloride concentration for all combined water treatment station observations within each calendar year. (c) Ambient (light shades) and sediment-water flow through experimental salt addition (dark shades) chloride concentrations for the five sampling events of this study grouped by waterbody type. *(a) Mapa de los usos del suelo de la cuenca del lago Simcoe y la ubicación de los puntos de estudio (b). Cambios a largo plazo en las concentraciones de cloruro del lago en cuatro estaciones de tratamiento de agua. La tendencia es la regresión lineal de la concentración media anual de cloruro para todas las observaciones combinadas en las estaciones de tratamiento de agua dentro de cada año. (c) Ambiente (tonos claros) y en condiciones experimentales con la adición de cloruro en el flujo sedimento-agua (tonos oscuros), para los cinco muestreos de este estudio, agrupados por tipo de cuerpo de agua.*

posite sand/salt mixture was then used as the NaCl source for the experimental chloride additions.

At each site, two intact sediment cores were collected using an acrylic core barrel topped with a one-way plumbing valve affixed to a PVC pole (McCarthy et al., 2016). Core barrels were manually pushed into the sediment until hard substrates were reached, resulting in cores with a sediment depth ranging from 10 to 20 cm. At each site, in situ dissolved oxygen (DO; mg O$_2$/L), temperature (°C), and conductivity (µS/cm) measurements were taken at sampling depths using electronic handheld probes (YSI models 55 DO and 30/10 FT; YSI, Yellow Springs, Ohio, USA). Water samples were collected for total phosphorus (TP), total suspended solid (TSS), and chloride analysis and filtered in the lab within 24 hours using 0.2 µm polycarbonate membrane filter (Millipore) with a precombusted Whatman GF/F pre-filter for dissolved organic matter (DOM) and nutrients (NO$_3$ and TDP).

Flow-through sediment core experimental design

Sediment cores and water carboys were set up in a flow-through, environmental chamber system (Juckers et al., 2013; McCarthy & Gardner, 2003). Chamber light/dark cycles and temperature were set to best approximate water temperature and light conditions during the collection month. Chamber cycles were set to: (1) October 10.5 hr light at 7 °C and 13.5 hr dark at 10 °C, (2) December 9 hr light at 3 °C and 15 hr dark at 5 °C, (3) March 12 hr light at 3 °C and 12 hr dark at 5 °C, (4) May 15 hr light at 9 °C and 9 hr dark at 14 °C, and (5) August 14.75 hr light at 9 °C and 9.25 hr dark at 14 °C. The flow-through systems continuously circulated water from each site's carboy to the inflow of each sediment core at a flow rate of c.a. 1.5 mL/min over the sediment water interface of the core. The system pumped water directly over the sediment surface and then outflowing through a tube at the top of the core barrel to facilitate water exchange (Fig. S1, see Supplementary information, available at http://www.limnetica.net/en/limnetica). Water carboys were bubbled with an air stone to assure that the overlying water column of the core barrels was well oxygenated. The sediment surface and subsurface could still be anoxic as only the water column above the sediment was aerated and exchanged. Water was not pumped through the sediment. These steps prevented anaerobic metabolisms from taking place in the water column, helped the core system maintain the oxygenated water column observed during sampling, and provided horizontal water movement and exchange across the sediment surface.

Each sediment core was incubated for four days after an overnight stabilization period. Road salt additions to the inflow water carboy started at the end of the second light/dark cycle. During October and December 2010, NaCl additions doubled ambient chloride concentrations (an increase of 40, 100, and 500 mg Cl$^-$/L for lake, stream, and stormwater pond sites, respectively). After review of the preliminary data and sampling a mid-winter melt (data not included), we decided to make the same NaCl addition of 500 mg Cl$^-$/L to all sites during March, May, and August (Fig.1c). This adjustment caused NaCl additions to better match observed chloride concentrations in streams during melt periods and simplified the experimental design. Water samples were collected from the carboy and outflowing water of each sediment core just before dawn of the second light/dark cycle pre-NaCl addition and just before dawn of the second light/dark cycle post-NaCl addition. Water samples were used to determine ambient (pre-NaCl) and post-NaCl benthic respiration, NO$_3$ flux, and TDP flux. Samples for DO analysis were measured immediately or preserved with sodium azide for later analysis (Juckers et al., 2013).

DO, TDP, and NO$_3$ fluxes were determined by calculating the difference in concentration between the inflow and outflow, multiplying this difference by the measured flow rate over the sediment core, and dividing by the surface area of the core (52.8 cm^2). All measurements reflect the combined response of uptake, release and cycling of O$_2$, N and P across the sediment–water interface. For nutrients, negative values indicate uptake and positive values indicate release by the sediment. For benthic respiration, values are always positive, indicating that O$_2$ was taken up by the sediment as water flowed over the system. Primary production was not determined because

measurements were collected at the end of the dark period. Zero indicates that respiration was below our ability to detect change in input and output O_2 concentrations.

Laboratory chemical analyses

Chloride was measured using a Cl^- specific ion electrode (Thermo Fisher, model 9617BNWP). TP and TDP (µg P/L) were measured using the standard colorimetric method after persulfate (1.7 % f.c.) digestion in an autoclave for 1 hour at 121 °C (APHA, 1999). Following acidification (pH < 2), NO_3 was measured by second-derivative UV spectroscopy (Crumpton et al., 1992). TSS (mg/L) was measured as the mass of particles collected on GF/F filters after drying in an oven at 60 °C until constant mass (Williams et al., 2013).

DO concentration was determined using two methods for the flow-through sediment core experiment (membrane inlet mass spectrometry (MIMS) and O_2 probe). In October and December 2010, O_2 was measured using MIMS following McCarthy et al. (2016) within 2 months of sample collection (Juckers et al., 2013). In March, May, and August 2011, O_2 was measured immediately after sampling using a YSI 55-DO probe.

DOM composition was determined using a seven component parallel factor (PARAFAC) model of fully corrected absorbance and excitation-emission matrix data (Williams et al., 2013, 2016). The full PARAFAC model and site averaged data were reported previously (Williams et al., 2016). For this study, PARAFAC components were provided as percent of total fluorescence intensity and analyses limited to: C1 (humic-like), C2 (terrestrial, humic-like), and C6 (anthropogenic DOM).

Statistical analysis

Statistical analyses were carried out in R using Rstudio with packages: broom, psych, mgcv, scales, and tidyverse (Liaw & Wiener, 2002; Revelle, 2020; Robinson et al., 2020; Therneau & Atkinson, 2019; Wickham, 2016; Wickham et al., 2019; Wickham & Seidel, 2020). Pearson bivariate correlation was used to determine covariation between ambient chloride and water quali-

ty across all samples and within waterbody type. General additive models (GAMs) were used to conduct driver analysis of ambient nutrient flux and ambient benthic respiration across sites. Two-Way Mixed Effect Analysis of Variance (ANOVA) was used to determine the effect of waterbody type (between groups) and sampling event (within groups) on ambient benthic respiration and ambient nutrient flux for all sampling events.

For salt comparisons, Three-Way Mixed Effect ANOVA was used to determine the effect of waterbody type (between groups), NaCl treatment (within groups) and sampling event (within groups) on benthic respiration and nutrient flux for sampling events in 2011. When a significant effect was observed, pair-wise t-tests nested within or between groups were used to identify specific patterns. Salt additions in 2011 were consistent at 500 mg Cl^-/L. Sampling events in 2010 were not used in three-way ANOVA because stream and lake treatments received lower NaCl additions (40 and 100 mg Cl^-/L, respectively) than what occurred in 2011 and at stormwater ponds. Pair-wise t-tests within each waterbody type and event were used to determine if NaCl had an effect on benthic respiration and nutrient flux for October and December 2010 sampling events. For within site and event comparisons or two- or three-way interaction, we had reduced power to detect an effect because only two sediment core replicates were used at each site. Logistics prevent the incubation of three replicates. Instead of prioritizing number of within site and event replicates, our study design maximized the number of total sites we could visit per sampling event. When ANOVA indicated a significant NaCl effect, the post-NaCl addition flux was subtracted from the ambient flux, which enabled the magnitude of change in flux to be explored through GAM analysis. Negative change in flux and respiration indicated that the post-NaCl flux was farther away from zero than the ambient (i.e., salt addition increased the rate of respiration, nutrient uptake, or nutrient release). Positive change in flux indicated the post-NaCl flux was closer to zero than the ambient (i.e., salt addition lowered the rate of respiration, nutrient uptake, or nutrient release). The magnitude of change in flux indicated the effect size of NaCl additions on benthic respiration,

NO$_3$ flux, and TDP flux. For all GAMs, smoothing parameters were estimated using restricted maximum likelihood (reml). Akaike information criterion (AIC) and model fit via the gam.check function were used for model selection.

RESULTS

Ambient chloride, benthic respiration, and nutrient flux patterns

Ambient chloride ranged 9 to 501 mg Cl$^-$/L with waterbody mean ± standard error of 101 ± 23, 223 ± 31, and 47 ± 2 mg Cl$^-$/L for stream, stormwater pond, and lake sites, respectively (Table S1, see Supplementary information, available at http://www.limnetica.net/en/limnetica). Chloride concentrations were most variable across stream sites, due to the inclusion of urban and forested sites, and most stable across lake sites (Fig. 1). No seasonal patterns in chloride concentrations were evident during the sampling events of our study (Fig. 1). However, during a mid-winter melt that occurred in late February 2011, chloride concentrations measured at a subset of streams and stormwater ponds were two to five times higher than what was measured during our March sampling event (M.A. Xenopoulos Unpublished Data). Chloride concentration did not correlate to other water quality conditions within lake sites (Table S2, see Supplementary information, available at http://www.limnetica.net/en/limnetica). Within stream sites, chloride correlated positively with NO$_3$ (r = 0.79, p < 0.001) and DOM char-
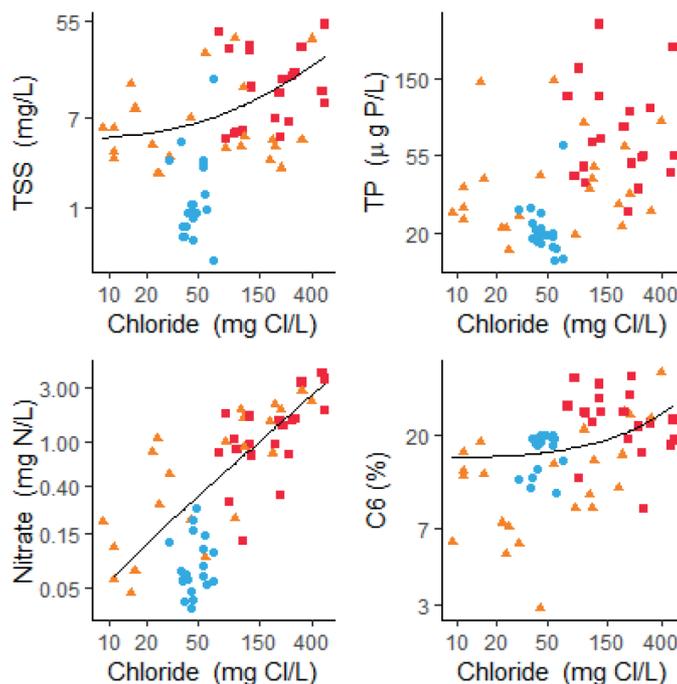
Figure 2. Bivariate relationships between ambient chloride concentrations and water quality variables (TSS = total suspended solids, TP = total phosphorus, C6 = anthropogenic, humic-like DOM) in lake (●), stream (▲), and stormwater (SW) Pond (■) sites across all sampling events. Note all axes are set to a natural log scale. Trend lines are provided for significant correlations across all water body types. Lines have a curved appearance because of the natural log scale. See text and Table S2 for correlation statistics. *Relaciones bivariantes entre las concentraciones de cloruro ambiental y las variables de calidad del agua (TSS = sólidos en suspensión totales, TP = fósforo total, C6 = DOM antropogénico, de tipo húmico) en el lago (●), en el arroyo (▲) y en la poza de aguas pluviales (SW) (■), en todos los muestreos. Todos los ejes están configurados en escala logarítmica. Se indican las líneas de tendencia para correlaciones significativas en todos los tipos de cuerpos de agua. Las líneas tienen una apariencia curva debido a la escala logarítmica. Consulte el texto y la Tabla S2 para ver la estadística de la correlación.*

acteristics C6 (r = 0.49, p = 0.017), an anthropogenic-like DOM component, and negatively with C2 (r = -0.62, p = 0.003), a terrestrial-like DOM component. Within stormwater pond sites, chloride correlated positively with NO_3 (r = 0.58, p = 0.008) and negatively with TDP (r = -0.52, p = 0.019). Chloride did not correlate with TP concentrations across or within waterbody sites. Across all waterbody types, chloride correlated positively to TSS (r = 0.45, p < 0.001), NO_3 (r = 0.77, p < 0.001), and C6 (r = 0.47, p < 0.001) and correlated negatively with C2 (r = -0.42, p < 0.00; Table S2, see Supplementary information, available at http://www.limnetica.net/en/limnetica; Fig. 2).

Ambient benthic respiration and ambient nutrient fluxes are presented in Table 1, Table S1 (see Supplementary information, available at http://www.limnetica.net/en/limnetica), and Figure 3. Ambient benthic respiration ranged 0.08 to 2.67 mmol $O_2/m^2/h$ with waterbody mean ± standard error of 0.68 ± 0.18, 0.92 ± 0.15, and 0.60 ± 0.07 mmol $O_2/m^2/h$ for stream, stormwater pond, and lake sites, respectively. Benthic respiration was significantly higher in August at all sites than the other sampling events (ANOVA $F_{1.3,15.7}$ = 26.2, p < 0.001; Table S3, see Supplementary infor-

mation, available at http://www.limnetica.net/en/limnetica). Benthic respiration was lowest during December and March. Ambient benthic respiration did not significantly differ by waterbody type. Temperature explained 53.8 % of the deviance in ambient benthic respiration (GAM edf = 6.5, p < 0.001; Table S4, see Supplementary information, available at http://www.limnetica.net/en/limnetica; Fig. 4a). Ambient NO_3 flux ranged -12.6 to 2.4 mg $N/m^2/h$ with waterbody mean ± standard error of -1.2 ± 0.6, -2.5 ± 0.8, and -0.03 ± 0.04 mg $N/m2/h$ for stream, stormwater pond, and lake sites, respectively. NO_3 flux significantly differed across sampling events with NO_3 uptake into the sediment being higher in October as compared to December (ANOVA $F_{4,44}$ = 2.95, p = 0.03). Ambient NO_3 flux into the sediment increased with ambient chloride concentration, with 36.3 % of deviance explained (GAM edf = 1, p < 0.001; Table S5, see Supplementary information, available at http://www.limnetica.net/en/limnetica; Fig. 4b). Ambient TDP flux ranged -0.23 to 0.82 mg $P/m^2/h$ with waterbody mean ± standard error of 0.12 ± 0.05, 0.01 ± 0.03, and 0.01 ± 0.01 mg $P/m2/h$ for stream, stormwater pond, and lake sites, respectively. Ambient TDP flux was statistically similar across season and

Table 1. Mean (standard error) ambient benthic respiration and nutrient flux rates organized by waterbody type for each sampling event and overall for the study period. Lake sites were not sampled in December and March (No Data; ND). *Respiración bentónica ambiental media (error estándar) y tasas de flujo de nutrientes por tipo de cuerpo de agua para cada muestreo y en general para el período de estudio. Los lagos no fueron muestreados ni en diciembre ni en marzo (sin datos; ND).*

Waterbody Type	Oct 2010	Dec 2010	Mar 2011	May 2011	Aug 2011	Overall
Stream						
Benthic Res. (mmol $O_2/m^2/h$)	0.48(0.05)	0.21(0.06)	0.16(0.04)	0.71(0.20)	1.56(0.62)	0.68(0.18)
NO_3 flux (mg N m^2/h)	-0.93(0.71)	-0.61(0.75)	0.19(0.18)	-1.17(0.86)	-2.91(2.44)	-1.24(0.63)
TDP flux (mg P m^2/h)	0.04(0.01)	0.02(0.00)	0.05(0.01)	0.07(0.05)	0.33(0.18)	0.12(0.05)
Stormwater Pond						
Benthic Res. (mmol $O_2/m^2/h$)	0.80(0.07)	0.44(0.06)	0.28(0.07)	1.20(0.23)	1.88(0.28)	0.92(0.15)
NO_3 flux (mg N/m^2/h)	-3.89(2.21)	0.06(0.84)	-3.27(2.87)	-3.15(1.40)	-2.03(0.95)	-2.46(0.79)
TDP flux (mg P/m^2/h)	-0.08(0.05)	0.01(0.03)	0.00(0.03)	0.01(0.02)	0.09(0.12)	0.01(0.03)
Lake						
Benthic Res. (mmol $O_2/m^2/h$)	0.43(0.06)	ND	ND	0.45(0.06)	0.94(0.09)	0.60(0.07)
NO_3 flux (mg N/m^2/h)	-0.14(0.11)	ND	ND	0.02(0.05)	0.00(006)	-0.03(0.04)
TDP flux (mg P/m^2/h)	0.01(0.02)	ND	ND	-0.02(0.01)	0.04(0.02)	0.01(0.01)
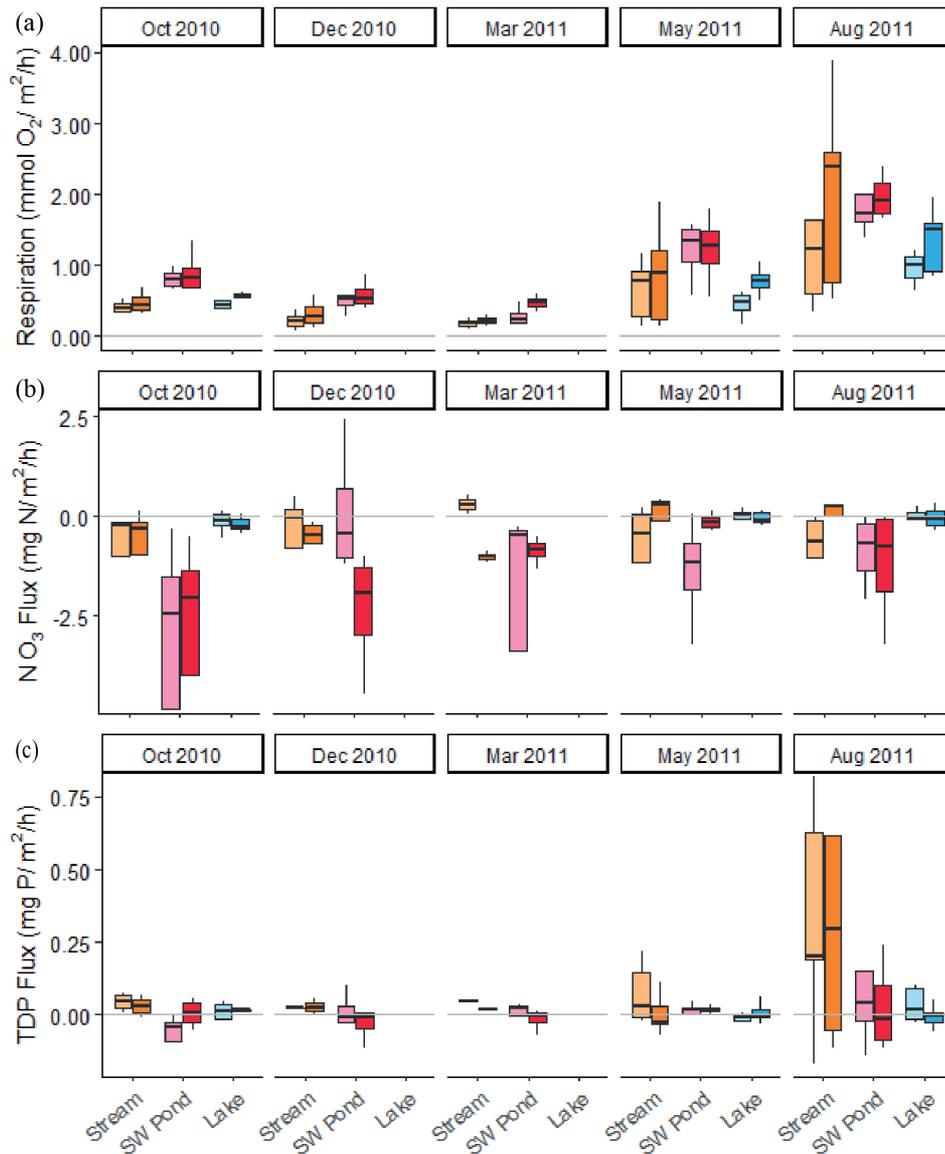
Figure 3. Oxygen (a), nitrogen (b), and phosphorus (c) flux during the five sampling events of this study grouped by waterbody type for ambient (light shades) and sediment-water flow through experimental salt addition (dark shades) conditions. For nutrients, negative values indicated uptake from water column into sediment and positive values indicate release from the sediment into the water column. Gray horizontal lines are set at zero for reference. Outliers (values > 1.5 times and < 3 times interquartile range) are not displayed. Please note that salt additions differed between 2010 and 2011 and salt addition responses are not fully comparable between 2010 and 2011 events. During October 2010, lake sites received 40 mg Cl-/L additions, during October and December 2010 stream sites received 100 mg Cl-/L additions, and stormwater management ponds in 2010 and all sites during 2011 received 500 mg Cl-/L additions. Ambient data are comparable across all events and sites. *Flujo de oxígeno (a), nitrógeno (b) y fósforo (c) durante los cinco eventos de muestreo agrupados por tipo de cuerpo de agua para las condiciones ambientales (tonos claros) y en condiciones experimentales con la adición de cloruro en el flujo sedimento-agua (tonos oscuros). Para los nutrientes, los valores negativos indican la absorción desde la columna de agua hacia el sedimento y los valores positivos indican la liberación del sedimento a la columna de agua. Las líneas horizontales grises se ajustan al cero como referencia. Los valores atípicos (valores > 1.5 veces y < 3 veces el rango intercuartílico) no se muestran. Las adiciones de sal difirieron entre 2010 y 2011 y por tanto, las respuestas a la adición de sal no son completamente comparables entre los eventos de 2010 y 2011. Durante octubre de 2010, los puntos del lago recibieron adiciones de 40 mg Cl-/L, durante octubre y diciembre de 2010 los puntos del arroyo recibieron adiciones de 100 mg Cl-/L, y las pozas en 2010 y todos los puntos durante 2011, recibieron adiciones de 500 mg Cl-/L. Los datos ambientales son comparables en todos los eventos y puntos.*

between waterbody types. Stormwater ponds and lake sites mostly had TDP fluxes around zero or indicative of P uptake by sediment. Drives of ambient TDP flux could not be modeled with GAM (Table S6, see Supplementary information, available at http://www.limnetica.net/en/limnetica).

Effects of salt additions on benthic respiration and nutrient flux

For 2011 sampling events when all sites received 500 mg Cl$^-$/L, three-way, mixed model ANOVA revealed that O$_2$ flux was significantly affected by sampling event ($F_{1.3,15.2}$ = 36.9, $p < 0.001$) and NaCl treatment ($F_{1,12}$ = 9.0, $p = 0.011$; Table S3, see Supplementary information, available at http://www.limnetica.net/en/limnetica). Benthic respiration increased from a mean of 0.9 ± 0.8 mmol O$_2$/m^2/h under ambient conditions to 1.1 ± 0.8 mmol O$_2$/m^2/h after NaCl additions (Fig. 3). During 2010, lake sites received 40 mg Cl$^-$/L, streams received 100 mg Cl$^-$/L, and stormwater ponds received 500 mg Cl$^-$/L. Paired t-test analysis within waterbody type and event indicated that NaCl addition caused an increase in benthic respiration within lake sites in October 2010 (t_{13} = -4.04, $p = 0.001$) and within stream

sites in December 2010 (t_7 = -2.56, $p = 0.038$), but NaCl additions did not alter benthic respiration within stormwater pond sites in 2010 and stream sites in October 2010. Thirty-six percent of the magnitude in change in respiration between ambient and post-NaCl conditions was explained by a GAM with the main effects of water temperature and ambient chloride concentration and their interaction (Fig. 5a; Table S4, see Supplementary information, available at http://www.limnetica.net/en/limnetica). At low temperatures and under intermediate chloride concentrations or under high temperatures and low chloride concentrations, NaCl additions caused benthic respiration to increase. Benthic respiration was less impacted by NaCl additions at high temperatures under intermediate to high chloride concentrations or at low temperatures under low or high chloride concentrations (Fig. 5a).

For 2011 sampling events, when all sites received 500 mg Cl$^-$/L, three-way mixed model ANOVA of NO$_3$ flux identified a significant three-way interaction (waterbody type * sampling event * NaCl treatment; $F_{2,22}$ = 6.7, $p = 0.005$; Table S3). Simple grouped two-way and one-way paired comparisons indicated that NaCl additions caused variable responses. For stormwater ponds,
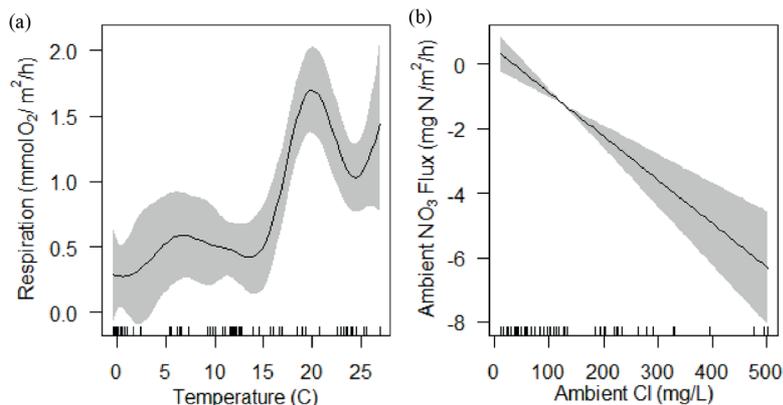
Figure 4. Best General Additive Models (GAMs) of ambient benthic respiration (a) and ambient nitrate flux (b). Ambient total dissolved phosphorus flux was not able to be modeled by parameters measured in this study. Temperature explained 53.8 % of the deviance in ambient benthic respiration (GAM edf = 6.5, $p < 0.001$). Chloride concentration explained 36.3 % of the deviance in ambient nitrate NO$_3$ (GAM edf = 1, $p < 0.001$). *Representación de los mejores modelos aditivos generales (GAM) de la respiración bentónica ambiental (a) y con flujo de nitrato ambiental (b). El flujo de fósforo disuelto total ambiental no pudo ser modelado por los parámetros medidos en este estudio. La temperatura explicó el 53.8 % de la desviación en la respiración bentónica ambiental (GAM edf = 6.5, $p < 0.001$). La concentración de cloruro explicó el 36,3 % de la desviación en el ambiente de nitrato NO3 (GAM edf = 1, $p < 0.001$).*
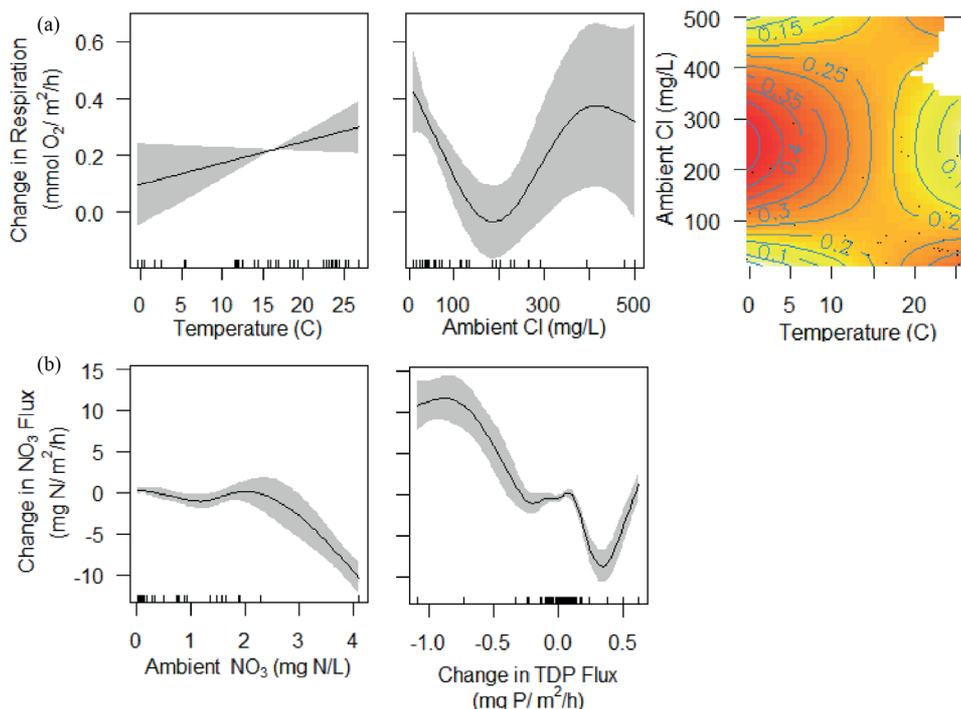
Figure 5. Best fit general additive models (GAMs) curves with 95 % confidence interval (gray) for predicting change in benthic respiration (a) and change in nitrate flux (b) post-NaCl addition. Hashed lines inside x-axis marked the observed conditions for independent variables. Change in respiration was best predicted by the main effects of temperature and ambient chloride concentration plus their interaction (displayed as a heatmap). Change in nitrate was best predicted by the main effects of ambient nitrate concentration and the change in TDP flux post-NaCl addition. Change is ambient minus post-NaCl respiration or flux. More positive change in respiration values indicate respiration increased post-NaCl addition. For change in nutrient flux, zero indicates no change, negative values indicate that post-NaCl flux was closer to zero (weaker/slower) than ambient, positive values indicate that post-NaCl flux was father away from zero (stronger/faster) than ambient, and the magnitude, independent of sign, indicates the change in rate post-NaCl addition. *Curvas de los modelos aditivos generales (GAM) con mejor ajuste con un intervalo de confianza del 95 % (gris) para predecir el cambio en la respiración bentónica (a) y el cambio en el flujo de nitrato (b) después de la adición de NaCl. Las líneas discontinuas dentro del eje x marcaron las condiciones observadas para las variables independientes. El cambio en la respiración se predijo mejor mediante los efectos de la temperatura y la concentración de cloruro ambiental más su interacción (mostrado como un mapa de calor). El cambio en el nitrato se predijo mejor por los efectos de la concentración ambiental de nitrato y el cambio en el flujo de TDP posterior a la adición de NaCl. El cambio se calcula como el valor ambiente menos el valor de la respiración o flujo posterior a la adición de NaCl. Un cambio positivo en los valores de respiración indican un aumento de la respiración después de la adición de NaCl. Para el cambio en el flujo de nutrientes, cero indica que no hubo cambios, los valores negativos indican que el flujo posterior al NaCl estuvo más cerca de cero (más débil/más lento) que el ambiente, los valores positivos indican que el flujo posterior al NaCl se alejó más de cero (más fuerte/ más rápido) que el ambiente, y la magnitud, independiente del signo, indica el cambio en la velocidad después de la adición de NaCl.*

NaCl additions caused an overall decrease in NO$_3$ flux into the sediment from a mean of -1.8 ± 3.3 mg N/m^2/h under ambient conditions to a mean of -0.7 ± 1.0 mg N/m^2/h post chloride treatment. Salt additions did not significantly impact benthic NO$_3$ flux at lake sites. In stream sites, NaCl additions generally did not significantly impact NO$_3$ flux, but with one exception. During March, NaCl additions caused increased NO$_3$ uptake from the water column into the sediment, shifting stream sites from releasing NO$_3$ into the water column (-0.9 ± 0.9 mg N/m^2/h) to taking up NO$_3$ from the water column (0.2 ± 0.3 mg N/m^2/h) post-NaCl addition. During 2010, paired t-test analysis within waterbody type and event indicated that NaCl addition generally did not significantly affect NO$_3$ flux within lake sites and within stream sites. Within stormwater pond sites, NaCl addi-

tions caused NO_3 flux uptake by the sediment to increase in December 2010 ($t_8 = 2.22$, $p = 0.062$), but did not significantly affect shift NO_3 flux in October 2010. Eighty-five percent of the deviance in the magnitude of change in NO_3 flux response to NaCl addition was explained by a GAM with the main effects of ambient nitrate concertation and the magnitude of change in TDP flux in response to chloride additions (Fig. 5b; Table S5, see Supplementary information, available at http://www.limnetica.net/en/limnetica). The rate of benthic NO_3 uptake and its relationship with NO_3 was non-linear but decreased at higher NO_3 concentrations at a single sampling site (Fig. 5b). The change in NO_3 flux after NaCl additions was also related to TDP flux in a non-linear way but overall, indicated that when NaCl additions caused benthic uptake of N to increase, uptake of P decreased and vice versa (Fig. 5).

For 2011 sampling events, three-way mixed model ANOVA of TDP flux showed that flux was similar across waterbody types and events and did not change in response to NaCl additions (Table S3; Fig 3). During 2010, paired t-test analysis within waterbody type and event indicated that NaCl additions generally did not significantly affect TDP flux within lake sites and within stream sites. Within stormwater pond sites, NaCl additions caused TDP flux to shift from net P uptake into the sediment to net P release into the water column ($t_7 = -3.47$, $p = 0.01$). In December 2010, NaCl additions did not significantly affect TDP flux. Change in TDP flux was not able to predicted using GAM. Change in NO_3 was the best predictor of TDP flux based on AIC (Table S6, see Supplementary information, available at http://www.limnetica.net/en/limnetica), but visual inspection of model fit revealed that observed and predicted data did not fit the GAMs (data not shown).

DISCUSSION

Freshwater salinization manifests as a distinct water chemistry signature, stemming from road salt applications, faster weathering, and altered soil cation properties in human dominated landscapes (Kaushal et al., 2018). Chloride concentrations (even those below toxicity guidelines for freshwater) effect food web and biotic structur-

al components of freshwater ecosystems (Greco et al., 2021; Hintz et al., 2022; Szklarek et al., 2022). The impacts of chloride on ecosystem processes and function, however, are more nuanced with variable responses being observed across ecosystems with similar levels of chloride exposure. Our results indicate that road salt additions at low (40 mg Cl^-/L) to moderate (500 mg Cl^-/L) concentrations increase benthic respiration and, depending on season and waterbody type, alter NO_3 cycles. Respiration and nutrient cycling responses to chloride are dynamic and might indicate resilience loss in ecosystems function, which is consistent with previous research (Arce et al., 2014; Baldwin et al., 2006; Berger et al., 2019; Cook & Francoeur, 2013; Hale & Groffman, 2006; McGuire & Judd, 2020). Our results, however, do not support the idea that low to moderate concentrations of chloride will directly mobilize nutrients in aquatic ecosystems and inhibit ecosystem function.

Urban land use and increases in human population density are synonymous with increased prevalence of anthropogenic-like DOM (McEnroe et al., 2013; Williams et al., 2016). This shift in DOM composition might be a symptom of the salinization syndrome. We found that the anthropogenic DOM (i.e., C6) to be correlated with ambient chloride, nitrogen, and phosphorus concentrations (Fig 2). In turfgrass soils, sodium additions increased the amount of humic-DOM leached from soils, which could also influence the amount and composition of DOM in freshwater ecosystems (Findlay & Kelly, 2011). Shifts in the DOM pool and nutrient availability could alter decomposition and nutrient cycling rates independent of NaCl effects. Hence, urbanization and winter road de-icing salting introduce a broad set of changes to surface water quality, which can yield complex biogeochemical responses.

The magnitude of ambient nutrient fluxes and benthic respiration are influenced by temperature and ecosystem conditions (Grüneberg et al., 2014; Juckers et al., 2013; Mulholland et al., 2009). In our study, ambient chloride concentration positively correlated with sediment NO_3 uptake from the water column (Fig. 4b). These trends seem to parallel trends observed in other ecosystem that linked human disturbance, nutrient pollution,

temperature, and organic matter availability to denitrification and benthic respiration rates (Fellows et al., 2006; Gold et al., 2021; Juckers et al., 2013; Mulholland et al., 2009). NO$_3$ flux rates observed within each waterbody type of the Lake Simcoe watershed were comparable to what has been observed in other lakes, but on the low side of the ranges reported for stormwater ponds and in streams (Gold et al., 2021; Juckers et al., 2013; Mulholland et al., 2009; O'Brien et al., 2012). Methodological differences, variation in benthic substrates, and ambient nutrient concentrations likely account for the low to average rates observed in here as compared to other ecosystems. Waterbody type differences in benthic respiration across the Lake Simcoe watershed could not be resolved because of the strong seasonal control of temperature on respiration (Fig. 4a). Benthic respiration rates were comparable to what has been observed in other streams, stormwater ponds, and lakes (Fellows et al., 2006; Gold et al., 2021; Mulholland et al., 2009). Altogether human activities have already altered aquatic ecosystems, leading to higher rates of ecosystem respiration and nitrogen cycling than what occurs in less impacted waterways.

During the open-water period in ecosystems experiencing salinization, low to moderate concentrations of chloride are frequently observed in lakes, streams, and stormwater ponds (Hintz et al., 2022; Szklarek et al., 2022). In our study, chloride levels at lakes sites were always well below the Canadian chronic exposure guideline of 120 mg Cl$^-$/L, but concentrations have been increasing steadily for three decades (Fig. 1). During our study, stream and stormwater pond sites occasionally exceeded the chronic exposure level but never exceeded the Canadian acute exposure guideline of 640 mg Cl$^-$/L but have at stream and stormwater pond sites elsewhere in the Lake Simcoe watershed. For the first two sampling events, NaCl additions did not cause lake sites to exceed chronic exposure levels, but did push stream sites above chronic exposure levels and stormwater ponds above acute exposure levels. During the last three sampling events, NaCl additions exceeded chronic exposure levels and stormwater pond sites exceeded acute exposure levels. Recent evidence suggests that chloride concentrations be-

low chronic exposure limits can cause zooplankton abundance to decrease and phytoplankton abundance to increase in lakes. Moreover, lower nutrient lakes were more impacted by low level, chronic exposure to chloride than higher nutrient systems (Arnott et al., 2020; Greco et al., 2021). Below chronic and acute level chloride exposure impacts on biogeochemical cycles and ecosystem metabolism are not frequently observed, but most studies focus on high chloride exposure levels (Haq et al., 2018; Hintz et al., 2022; Lancaster et al., 2016; Szklarek et al., 2022), which are more episodic in nature and rarely observed across freshwater ecosystems (Hintz et al., 2022; Szklarek et al., 2022). In our study, the impact on chloride was strongest under warmer temperatures at low ambient chloride concentrations and at low temperatures under intermediate ambient chloride concentrations. Hence, the timing of salt pulses into aquatic ecosystem might be as important as the prior exposure of the ecosystem to salt. At some sites, increased respiration corresponded to increases in benthic NO$_3$ uptake, indicating chloride pulses might have stimulated benthic metabolism (Silva & Davies, 1999).

A conceptual model of salinization impacts on aquatic biogeochemical cycles has emerged, which suggests, for most ecosystems, chloride increases will lead to increased respiration and nutrient mobilization (Herbert et al., 2015; Hintz & Relyea, 2019; Kaushal et al., 2021; Szklarek et al., 2022). Our results provide a good case study to explore how well the conceptual understanding matches observations from experiments across diverse aquatic ecosystems at low to moderate levels of chloride pollution. In our study, NaCl additions caused an increase in benthic respiration, no effect on TDP flux, and mixed effects on NO$_3$ flux. However, shifts in respiration were uncoupled from nutrient fluxes (Fig. S2, see Supplementary information, available at http://www.limnetica.net/en/limnetica). NO$_3$ uptake into the sediment and benthic respiration are often positively correlated (Gold et al., 2021). The lack of a coupled response here might indicate oxygen was not limiting in the experimental setup and NO$_3$ flux was associated with assimilatory nitrogen cycles. In our study, when salt additions caused a large alteration in nutrient flux, it impacted both

NO$_3$ and TDP but in different directions (Fig. 5b). When salt additions did not shift NO$_3$ flux, salt additions had little impact on TDP flux. Within each site, the magnitude of N and P response to salt addition seemed connected, but independent of benthic respiration effects. This could suggest salt additions disrupted both abiotic and biotic mechanisms the depending on specific site conditions induce a larger or null impact on benthic-pelagic nutrient and oxygen flux.

Increases in benthic respiration in response to salt additions should decreases bottom water DO, which is linked to phosphorus release (Novotny and Stefan 2012). In our study, the sediment-water interface was maintained under well-mixed oxygenated conditions using a flow-through system that preserves the sediment water interface. Perhaps under these conditions and lower doses of chloride, ecosystems respond differently to chloride (Arce et al., 2014) than what would occur in static chambers or sediment/soil-water slurry (Herbert et al., 2015; Haq et al., 2018; Hintz & Relyea, 2019; Kaushal et al., 2021). If our core system was allowed to stratify, as happens for short-periods (e.g., days) in stormwater ponds (Marsalek, 2003; Novotny & Stefan, 2012; Song et al., 2013), salt pulses could have indirectly led to phosphorus mobilization by increased decomposition and drawing down ambient DO as a result of increased benthic respiration.

Most ecosystems impacted by winter road salt pollution face cumulative stressors from eutrophication, land use and/or climate change. Stormwater ponds and, to a certain extent, streams and wetlands should have chloride-acclimated biogeochemical cycles already given their prior pollution exposure and close connection to the landscape. As such, lake ecosystems should show the strongest biogeochemical response to chloride pulses because those comparatively high chloride concentrations are rare. The results of our study conform to these expectations. Lake sediments increased respiration when exposed to 40 mg Cl/L NaCl additions in 2010 as well as when receiving a large dose (500 mg Cl/L) in 2011. Nitrogen and phosphorus cycles were not impacted by NaCl additions, which deviates from what salinization conceptual models predict. This distinction could stem from the water column remaining oxygenated. Given that chloride increased benthic respiration, chloride increases could have indirect effects that lead to physical conditions favoring phosphorus mineralization. These results might also suggest respiration rates is more sensitive to chloride pulses than benthic nutrient cycles and that overall shifts in benthic respiration rates were not sufficient to influence assimilatory and dissimilatory nutrient metabolic processes.

Ecosystem structural and functional responses to chloride additions seem dependent on environmental conditions and the context around exposure. Hence there is a need for researchers to repeat studies using similar conditions and expanding the environmental conditions under study. There is also a need to better understand the impacts of chloride on biogeochemical cycles at concentrations typically observed in ecosystems. Filling in gaps across environmental gradients and repeating experiments should enable a more holistic understanding of how winter road salting operations and chloride additions impact aquatic biogeochemical cycles. To our knowledge, this is the first report of chloride stimulated benthic nitrate uptake in an ecosystem not limited by cation availability, but the mechanism and drivers behind the chloride biogeochemical responses could not be fully explained. With variation being the norm, more spatiotemporally robust studies are needed to effectively predict ecosystem biogeochemical cycle response to salinization and incorporate multiple response under different conditions for the same resource. Across lake and stream sites, our results supported the hypothesis that low to moderate chloride additions cause increased benthic respiration while nutrient cycles across the sediment-water interface were weakly and inconsistently altered.

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