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Subsidy-stress responses of ecosystem functions along experimental freshwater salinity gradients

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Abstract Human activity is increasing salt concentrations in freshwaters worldwide, but effects of freshwater salinity gradients on biogeochemical cycling are less understood than in saline, brackish, or marine environments. Using controlled microcosm experiments, we characterized (1) short-term (one to five days) biogeochemical responses and (2) water column metabolism along a freshwater salinity gradient of multiple salt types. After one day, microcosms were oxic (4.48–7.40 mg O_2 L^{-1}) but became hypoxic (1.20–3.31 mg L^{-1}) by day five. After one day in oxic conditions, microbial respiration in magnesium-, sodium-, and sea salt-based salinity treatments showed a subsidy-stress response, with respiration increasing by over 100% as salinity increased from 30 to 350–800 µS cm^{-1}. Conversely, respiration consistently increased along a calcium-based salinity gradient, peaking at 1500 µS cm^{-1}. By day five, an inverse subsidy-stress response was observed with elevated respiration at upper or lower ends of the gradient except for the magnesium treatment, which had the lowest respiration at the highest salinity. Calcium- and magnesium-based salinity treatments also caused considerable changes in phosphorus concentrations and C:P and N:P. In a separate experiment, microbial respiration and water column primary production also displayed subsidy-stress responses, but imbalances in effect sizes caused consistently declining net community production with increasing salinity. Collectively, our results establish that short-term exposure to different salt ion concentrations can enhance freshwater biogeochemical cycling at relatively low concentrations and alter resource stoichiometry. Furthermore, the nature of effects of freshwater salinization may also change with oxygen availability.

Keywords Freshwater salinization · Respiration · Metabolism · Nutrient stoichiometry · Subsidy-stress

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Introduction

Microorganisms govern biogeochemical cycles that support aquatic ecosystem services and functions (Cotner and Biddanda 2002, Grossart et al., 2020). For example, diverse nutrient cycling pathways like nitrogen (N) fixation and denitrification control the bioavailability of N globally (Gruber and Galloway 2008). Assimilation and respiration of organic carbon (C) by microbes influences ecosystem metabolism, food-web dynamics, and dissolved oxygen concentrations, especially in hypolimnetic environments (Dodds and Cole 2007; Marcarelli et al. 2011; Rabalais et al. 2010; Sand-Jensen et al. 2007). Globally, inland freshwaters contribute significantly to carbon and nutrient cycling (Cole et al. 2007; Battin et al. 2023) and rates are influenced by environmental conditions like salinity, the concentration of dissolved salts. Salinity is increasing substantially in many freshwater environments (Campbell and Kirchman 2013; Kaushal et al. 2018) due to human activities like deicing, agriculture, urbanization, and resource extraction, as well as environmental changes leading to saltwater intrusion (Barlow and Reichard 2010; Corsi et al. 2010; Kaushal et al. 2018; Williams 2001). Importantly, different sources of freshwater salinization enrich receiving waterbodies with different ions, causing spatial heterogeneity of base cations like Ca^{2+}, Mg^{2+}, and Na^{+} in surface waters (Griffith 2014). However, the effects of ion-specific freshwater salinization on biogeochemical processes and ecosystem functions like photosynthesis, respiration, and nutrient cycling, are understudied in freshwater environments.

There is growing evidence that freshwater salinization affects many facets of aquatic ecosystems ranging from nutrient cycling to biodiversity, with effects differing by ionic composition (DeVilbiss et al. 2021, 2022, 2023; Timpano et al. 2018; Tyree et al. 2016). Anthropogenic salt inputs can mobilize both base cations and nutrients from the land to surface waters and affect the release and retention of bioactive elements within stream networks (Haq et al. 2018; Duan and Kaushal 2015; Kaushal et al. 2022; Galella et al. 2023). Once mobilized, prolonged exposure to elevated freshwater salt concentrations, *i.e.*, a press disturbance, can decrease leaf litter breakdown by detritivores and reduce microbial respiration rates (Tyree et al. 2016). Increasing freshwater salinity also decreases the abundance and diversity of many biological assemblages including macroinvertebrates, zooplankton, and crustaceans, potentially impacting higher trophic levels (Timpano et al. 2018; Lopatina et al. 2021; Hintz et al. 2022; Dettmers et al. 2003; Hebert et al. 2022). Conversely, freshwater salinization increases survival rates of *Escherichia coli*, an important indicator of bacterial water quality, as well as increases the diversity of freshwater bacterial communities, especially in freshwaters enriched with Mg^{2+} (DeVilbiss et al. 2021, 2022). Salinity effects on microbial function have been studied in brackish and marine environments (up to 60,000 μS cm^{-1}), where increased salinity alters processes like respiration and nitrogen cycling pathways (del Giorgio and Bouvier 2002; Chin-Leo and Benner 1992; Pakulski et al. 1995). Generally, however, the effects of small increases in salt concentrations (≤ 1500 μS cm^{-1}, APHA 2005, Cormier et al. 2013) on biogeochemical processes in freshwater environments are understudied. This is especially true for short exposure times, known as pulse disturbances, which are common in fluvial systems (Lake 2000). Collectively, salinity-induced shifts in nutrient and carbon processing can result in changes to stream metabolism.

Metabolism refers to the combined processes of gross primary production (GPP) and ecosystem respiration (ER) and is a fundamental property of stream ecosystems (Mulholland et al., 2008, Hall and Hotchkiss 2017). For example, ecosystems with GPP > ER are autotrophic and primary carbon sources supporting respiration are photosynthesized within the stream. Conversely, when GPP < ER, streams are heterotrophic, and respiration is subsidized by external carbon sources like leaf litter and detritus. Thus, environmental conditions that differentially impact either GPP or ER have the potential to fundamental alter energy flow and carbon fate in ecosystems. Additionally, hypoxia is far more common globally than once assumed and will also alter biogeochemical processes (Blaszczak et al. 2022). By utilizing fermentation or alternative electron acceptors like NO_3^{-}. SO_4^{2-}, Fe^{3+}, and CO_2, numerous microbial species can meet their energetic needs and alter dominant biogeochemical pathways and process rates (Falkowski et al. 2008). For example, in the absence of dissolved oxygen and presence of abundant nitrogen, rates of denitrification or dissimilatory reduction of nitrate to ammonia (DNRA) increase as bacteria switch to NO_3^{-} as their primary electron acceptor (McCarthy et al. 2015; Childs et al. 2002; Marzocchi et al 2022). Under anoxic conditions, both sediment organic carbon and phosphorus, depending on speciation, can diffuse from sediments to the overlying water column (Peter et al. 2017; Anderson et al. 2021; Hupfer and Lewandowski 2008). At the ecosystem scale, hypoxia increases C, N, and phosphorus (P) concentrations in the water column and alters nutrient stoichiometry as well as nutrient export downstream (Carey et al. 2022). Thus, understanding how freshwater salinization affects biogeochemical processes under variable oxygen conditions will establish mechanistic linkages between two prevalent, co-occurring anthropogenic stressors, freshwater salinization, and hypoxia.

The goal of this research was to experimentally document how biogeochemical processes including respiration and net changes in C, N, and P concentrations change along freshwater salinity gradients of different salt ions. Additionally, we quantified how net nutrient uptake might alter ambient water column stoichiometry. Lastly, we investigated how differences in salinity and salt type changed water column gross primary production, community respiration, and net community production during short-term incubations. Our hypothesis was based on the subsidy-stress concept (Odum et al. 1979), which describes how increases in certain environmental variables, freshwater salinity in this case, can enhance ecosystem processes like nutrient cycling at low levels but suppress the same processes as concentrations increase and surpass optimal conditions. Specifically, we hypothesized that slight increases in salinity would alleviate hypoosmotic stress, consequently inducing a subsidy-stress response of microbial respiration and changes in water column nutrient concentrations, with rates peaking at intermediate freshwater salinities. Because certain base cations like Ca^{2+} and Mg^{2+} are important micronutrients for microorganisms, we also hypothesized that responses to increasing salinity would vary by salt type, resulting in altered water column stoichiometry and thus, available resources.

Methods

Experimental approach

We designed two microcosm experiments to quantify the effects of freshwater salinization on biogeochemical process rates, including microbial metabolism, after relatively short exposures. The first experiment estimated respiration and changes in nutrient concentrations in controlled laboratory settings over a 5-day period (Experiment 1). The second experiment used a light–dark bottle approach to assess effects on water column metabolism by accounting for primary production in addition to respiration over a 24-h incubation in a local pond exposed to natural sunlight and temperature conditions (Experiment 2).

Experiment 1 – Microbial respiration and nutrient dynamics

To include representative microbial stream communities in microcosms, we submerged acid-washed sand in nylon bags in a forested headwater stream in Pembroke, VA (37° 20′ 27″ N 80° 37′ 21″ W) for two weeks. The site has naturally low nutrient concentrations and specific conductance (SC) ranging from ~5–50 µS cm^{-1} year-round. Acid-washed sand provided a realistic medium for microbial colonization but minimized the potential for sediment-bound salts or nutrients to be released into microcosm water as a result of experimentally altering salinity (Weston et al. 2010). After two weeks, we removed the sand from the stream and gently rinsed it with stream water to remove any natural organic matter or deposited silts. While we cannot be certain that rinsing did not alter biofilm communities, all incubations in the lab were treated identically, so any effect of rinsing the sand on bacterial communities would be identical across treatments. When we removed the sand-filled nylon bags, we also collected stream water in 20 L acid-washed carboys. Sand and water were immediately returned to the lab for microcosm setup on the same day. At the time of water collection, specific conductance = 39.0 µS cm^{-1}, pH = 7.1, temperature = 19.5 °C, and dissolved oxygen = 8.6 mg L^{-1}.

We used 300 mL biological oxygen demand (BOD) bottles as microcosms. Using a destructive sampling design, we tested two incubation exposure times: one day and five days. We used an additional set of microcosms to monitor dissolved oxygen concentrations during the study period using an Orion Star A223 dissolved oxygen portable meter (Thermo Fisher Scientific, Memphis, TN, USA). Each salt treatment including $CaCl_2$, $MgCl_2$, NaCl, and sea salt (sea) was tested in triplicate and separate sets of bottles were destructively sampled after one and five days (*i.e.,* each sample day had its own complete set of bottles). Each microcosm received 25 g of stream-inoculated sand to mimic a natural benthic environment. To prepare individual salt treatments, we spiked 2 L of stream water with either $CaCl_2$, $MgCl_2$, NaCl, or artificial sea salt in separate acid-washed bottles to target SCs of 125, 350, 800, and 1,500 µS cm^{-1} (four salt types, 4 conductivities, 16 total treatments) measured with an Orion Star A223 conductivity probe (ThermoFisher Scientific, Memphis, TN, USA). The primary goal of this experiment was to determine the potential for freshwater salinization to alter biogeochemical processes, so to eliminate any potential nutrient limitations and provide enough carbon to ensure measurable respiration rates, we primed each treatment with C (glucose-C), N (NH_3NO_3), and P (H_3PO_4) at the Redfield ratio (106:16:1) at 5 mg L^{-1} C. We chose 5 mg L^{-1} glucose-C because the respiratory quotient is one (*i.e.,* one mole of glucose respired consumes one mole of O_2), which would provide sufficient C to ensure observable oxygen consumption. After treatments were prepared, we aliquoted 300 mL of each treatment into six separate microcosms (triplicate sets destructively sampled for both one- and five-day measurements). In addition to salt treatments, we included triplicate control microcosms that received nutrients but no salt additions (conductivity = 30 µS cm^{-1}). Initial dissolved oxygen (DO) concentrations were measured using an Orion Star A223 dissolved oxygen portable meter (Thermo Fisher Scientific, Memphis, TN, USA), and we incubated bottles in the dark at room temperature to prevent photosynthetic activity.

After both one and five days, we destructively sampled each respective set of microcosms from each treatment, including reference microcosms with no salt additions. For each microcosm, we measured a final DO concentration using an Orion Star A223 dissolved oxygen portable meter (Thermo Fisher Scientific, Memphis, TN, USA) and syringe-filtered each sample through a 0.4 µm polycarbonate filter (Whatman®). Filtrate was stored in 60 mL, acid-washed HDPE bottles (Nalgene®) at -20℃ until analyses. For all samples, we measured concentrations of NO_3-N, NH_4-N, and SRP colorimetrically on a Lachat autoanalyzer (Lachat, Milwaukee, WI, USA) following manufacture protocol. Dissolved organic carbon (DOC) was measured as non-purgeable organic carbon (NPOC) on an Elementar varioTOC Analyzer (Elementar, Ronkonkoma, NY, USA) after acidifying samples. Respiration and the net rate of change in nutrient concentrations were calculated as:

$$R = \left[\left(C_{final} - C_{initial}\right) * V\right] / \Delta T$$

where R is the rate, $C_{initial}$ is the initial concentration of DO or nutrients in µM, C_{final} is the final concentration, V is volume of water in L (0.275 L), and ΔT is elapsed time (initial–final) in d. For all rates, negative values indicate net removal and positive values indicate net release into the microcosm water column. Effects of conductivity and salt type on respiration, nutrient fluxes, and stoichiometry were tested with second order polynomial regression. Differences in respiration or nutrient fluxes among salt types at the same conductivity were tested with ANOVA. All statistics and figures were generated in R Studio (version 2023.03.1; R Core Team).

Experiment 2—Effects of salinity and salt type on net community production

To test how freshwater salinization alters microbial metabolism (*i.e.*, microbial community respiration, primary production, and net production after accounting for respiration), we conducted a second microcosm experiment using a light–dark bottle technique, which allowed us to estimate rates of primary production as well as respiration. For this experiment, we also used 300 mL BOD bottles as microcosms. To best mimic realistic in situ conditions, we tested two salt profiles commonly observed in freshwater ecosystems experiencing salinization: a combination of Ca-, Mg-, and K- chloride salts at a ratio consistent with stream water in the region (Ca:Mg:K = 2.4:1.3:1, common in agricultural watersheds, see DeVilbiss et al. 2021) as well as NaCl, which is a common deicer. We chose not to prime mesocosms in this experiment with C, N, and P to focus on how freshwater salinity gradients impact ecosystem functions under ambient carbon and nutrient concentrations. To ensure there was a sufficient phytoplankton population in our incubation water to observe detectable changes in primary production, we collected water from Pandapas Pond in the Jefferson Natural Forest (37° 16′ 52″ N, 80° 28′7 W), which has an open canopy and significantly higher Chl-*a* concentrations than the forested stream sampled for Experiment 1. At the time of sampling, Pandapas Pond water specific conductance = 51.77 µS cm^{-1}, pH 7.07, and Chl-*a* = 86.39 µg L^{-1}. Background nutrient and ion concentrations were not measured at the time of collection but have been reported as (mean values) NO_3^- = 0.65 mg L^{-1}, PO_4^{3-} = 0.25 mg L^{-1},

Ca^{2+} = 2.5 mg L^{-1}, Mg^{2+} = 3.13 mg L^{-1}, Na^{+} = 7.33 mg L^{-1} (corresponding specific conductance = 74.25 µS cm^{-1}) (Potter 1978). We spiked pond water with either Ca:Mg:K or NaCl to achieve a salinity concentration range of 125, 350, 800, and 1500 µS cm^{-1}, as well as unaltered reference water at ~ 50 µS cm^{-1}. For each treatment, triplicate light and dark bottles wrapped with aluminum foil to prevent photosynthesis (6 total bottles per treatment) were deployed in floating chambers in a stormwater retention pond on Virginia Tech's campus (37° 12′ 59″ N, 80° 25′ 34″ W) for 24 h. Bottles were deployed and collected in the late afternoon from 16:00 to 17:00.

Dissolved oxygen concentrations were measured with an optical DO probe (YSI model #OBOD) prior to deployment and after 24 h. Community respiration (CR) was calculated as the difference between final and initial DO concentrations in dark bottles. We calculated net community production (NCP) as the difference between final and initial DO concentrations in the light bottle where both respiration and primary production occurred. Lastly, we calculated gross primary production (GPP) as the difference between NCP and CR, *i.e.*, GPP = NCP–CR.

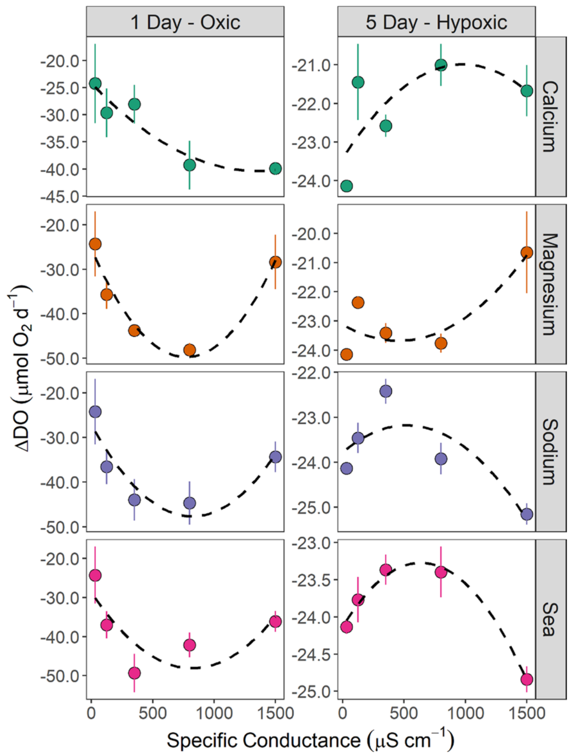

Fig. 1 Microbial respiration rates along experimental freshwater salinity gradients of $CaCl_2$ (top row), $MgCl_2$ (second row), NaCl (third row), and artificial sea salt (bottom row) under oxic (left column) and hypoxic (right column) conditions. More negative values indicate higher oxygen consumption

Results

Bacterial respiration and nutrient dynamics

Bacterial respiration rates responded to increasing salt concentration and salt type and varied between days 1 and 5 with changes in the oxygen availability. In the Mg^{2+}, Na^{+}, and sea treatments, a subsidy-stress response was observed, with the highest rates of oxygen consumption (*i.e.*, most negative ΔDO value) occurring at 350 or 800 µS cm^{-1} (Fig. 1, left column). In the Ca^{2+} treatment, however, oxygen consumption rates consistently increased with increasing salinity, peaking at 1,500 µS cm^{-1}. The largest change in rates of oxygen consumption occurred in the sea treatment at 350 µS cm^{-1}, an increase of 103% relative to unaltered reference water. At 350 µS cm^{-1}, oxygen consumption in the sea treatment was also significantly greater than in the Ca treatment (Fig. 2, left panel; $F_{3,8} = 5.67$, $p = 0.02$). No significant differences in oxygen consumption rates among salt treatments were observed at any other conductivity under oxic conditions on day 1 (all $p > 0.05$).

In addition to oxygen consumption, the rate of change in nutrient concentrations also varied considerably along the salinity gradient. Changes in concentrations of DOC and NO_3^- followed subsidy stress responses similar to oxygen consumption (Fig. 3, top panel). While there was net loss of DOC at all salinities, NO_3^- fluxes transitioned from net uptake at the upper and lower ends of the gradient to net release at intermediate salinities of 125–800 µS cm^{-1}, with the exception of the Ca treatment, where net fluxes were consistently positive (*i.e.,* NO_3^- release). Conversely, changes in NH_4^+ and PO_4^{3-} concentrations exhibited subsidy-stress responses, with higher loss occurring at intermediate salinities and lower loss at the upper and lower ends of the gradient. Similar to oxygen consumption and net NO_3^- release, rates of change in PO_4^{3-} in the Ca treatment differed from other salt treatments and increased monotonically with increasing salinity. The changes in nutrient concentrations

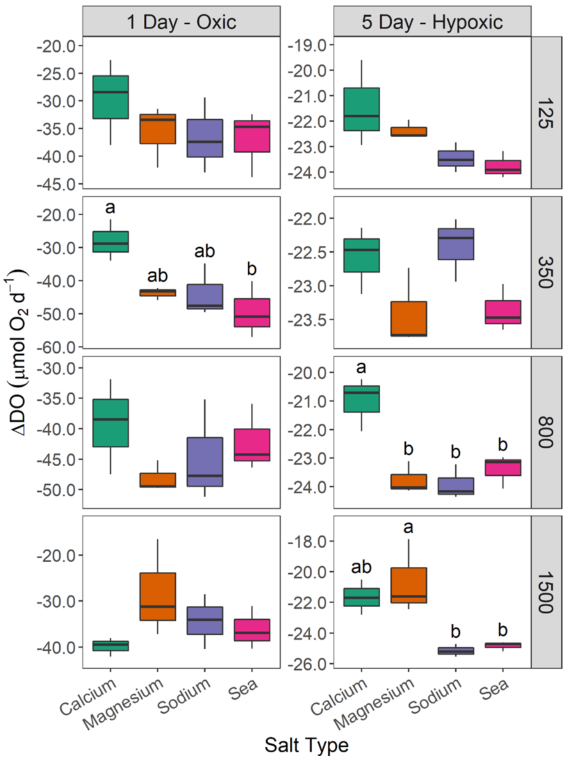

Fig. 2 Effects of different chloride salts on microbial respiration at four distinct salinities. The left column shows respiration after a 1-day incubation and the right column after a 5-day incubation that started in oxic conditions but became hypoxic during the 5 days. More negative values indicate greater oxygen consumption. Significant differences in respiration among salt types are shown with letters ($p < 0.05$)

caused shifts in ambient water resource stoichiometry along the salinity gradient. Imbalances between DOC and N resulted in lower C:N at intermediate salinities and higher C:N at the upper and lower end of the gradient (Fig. 4, top panel). Consistently increasing PO_4^{3-} removal from the water column in the Ca^{2+} treatment caused drastic increases in both C:P and N:P ratios at the upper end of the gradient that were not observed in the Mg^{2+}, Na^+, or sea treatments.

Respiration rates and changes in nutrient concentrations also varied significantly along the salinity gradient under hypoxic conditions on day 5. In both the Na^+ and sea treatment, oxygen consumption exhibited an inverse subsidy-stress response, with higher rates occurring at the upper and lower end of the gradient (Fig. 1). For both Na^+ and sea treatments, the highest oxygen consumption rates occurred at 1,500 µS cm^{-1} (Fig. 1, right panel). In contrast, increasing salinity generally decreased oxygen consumption rates with both divalent Ca^{2+} and Mg^{2+} treatments. For both Ca^{2+} and Mg^{2+} treatments, the highest respiration rates occurred at the lowest conductivity in the unaltered reference water. Respiration rates also differed significantly among salt treatments at both 800 µS cm^{-1} ($F_{3,8} = 11.53$, $p = 0.003$) and 1,500 µS cm^{-1} ($F_{3,8} = 8.17$, $p = 0.008$). At 800 µS cm^{-1}, oxygen consumption rates in the Mg^{2+}, Na^+, and sea treatments were all higher than in the Ca^{2+} treatment. At 1,500 µS cm^{-1}, oxygen consumption rates in the divalent Ca^{2+} and Mg^{2+} treatments were significantly less than the Na^+ and sea treatments (Fig. 2, right panel).

Salinity and salt type affected DOC and nutrient uptake rates after 5 days. Patterns of net DOC uptake were similar to oxygen consumption, showing an inverse subsidy-stress response with the exception of the Ca^{2+} treatment which had the lowest net DOC flux at the highest conductivity of 1500 µS cm^{-1} (Fig. 3. Bottom panel). Changes in NH_4^+ and NO_3^- concentrations exhibited opposite trends; NH_4^+ concentrations generally decreased with increasing salinity while NO_3^- concentrations tended to increase. In the Ca^{2+} treatment, NO_3^- concentrations indicated a shift from net loss to net release to the water column with increasing salinity. In the Mg^{2+}, Na^+, and sea treatments, rates remained negative indicating net N uptake at all salinity treatments. Changes in PO_4^{3-} concentrations along the freshwater salinity gradient also differed significantly among salt treatments ($r^2 = 0.37$, $p < 0.0001$). In both Ca^{2+} and Mg^{2+} treatments, PO_4^{3-} concentrations indicated a shift from net release (positive rates, increasing concentrations) to net uptake (negative rates, decreasing concentrations) as salinity increased. In contrast, net PO_4^{3-} release occurred at all salinities in the Na^+ and sea treatments, which also differed from the one-day oxic conditions where PO_4^{3-} uptake occurred in all salt and salinity treatments (Fig. 3, bottom panel).

Trends in water column stoichiometry also differed by salt type and salinity. In the Ca^{2+} treatment, C:N, C:P, and N:P all increased monotonically with increasing salinity. Both C:P and N:P also increased consistently with increasing salinity in the Mg^{2+} treatment as a result of PO_4^{3-} fluxes shifting from release to uptake. Relative to the Ca^{2+} and Mg^{2+} treatments, changes in nutrient stoichiometry in the Na^+ and sea treatments were minimal (Fig. 4, bottom panel).

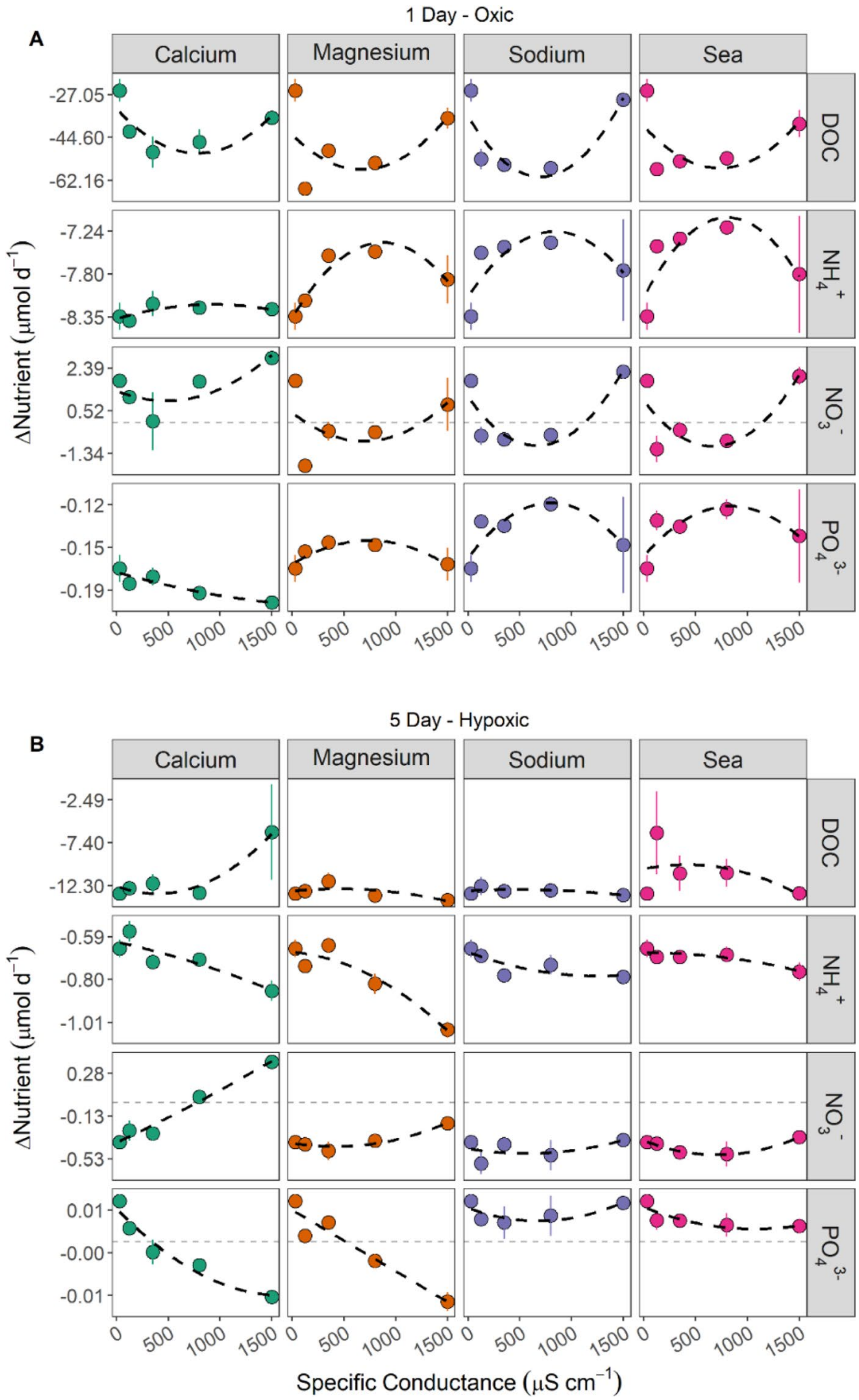

Fig. 3 Net fluxes of dissolved organic carbon (DOC, top row), NH_4^+ (second row), NO_3^- (third row), and PO_4^{3-} (bottom row) along experimental salinity gradients of $CaCl_2$ (first column), $MgCl_2$ (second column), NaCl (third column), and artificial sea salt (last column) in oxic (**A**) and hypoxic (**B**) conditions. Positive values indicate net release, negative values indicate net uptake. Dashed lines are at 0, indicating the transition from release to uptake

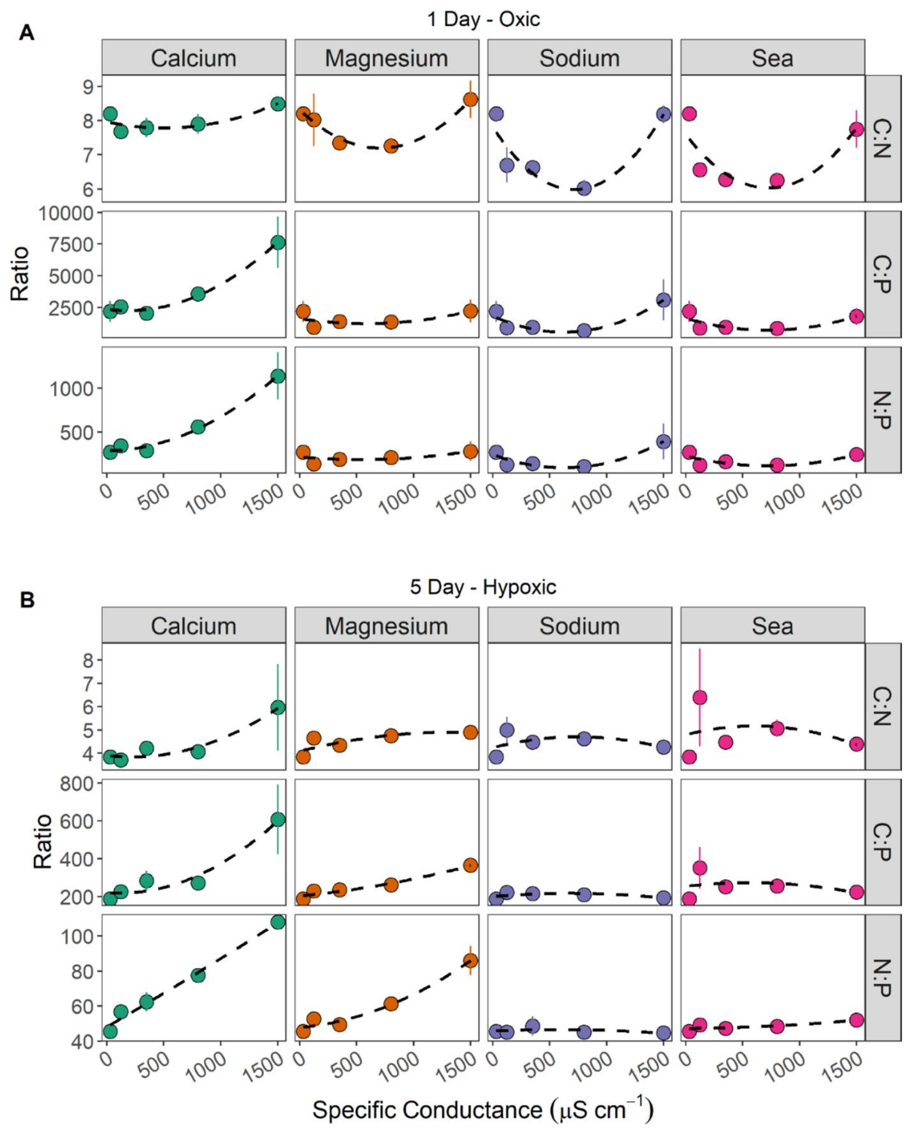

Fig. 4 Nutrient stoichiometric ratios (molar) including C:N (top row), C:P (middle row), and N:P (bottom row) along experimental freshwater salinity gradients of $CaCl_2$ (first column), $MgCl_2$ (second column), NaCl (third column), and artificial sea salt (last column) in oxic (**A**) and hypoxic (**B**) conditions

Effects of salinity and salt type on net community production

Community respiration (CR), gross primary production (GPP), and net community production (NCP) were all affected by increasing freshwater salinity. Similar to the oxic laboratory incubation, CR exhibited a subsidy-stress response with the highest oxygen consumption rates that were as much as 400% greater than reference water, occurring at intermediate freshwater salinities (Fig. 5). GPP also exhibited a subsidy stress response, but the effect size was less than CR, only increasing by ~ 50% relative to reference water. Imbalances between changes in CR and GPP in response to increasing salinity resulted in consistently decreasing NCP with increasing salinity. At the highest salinity treatment of 1,500 μS cm^{-1}, NCP decreased by ~ 75% relative to reference water for both salt treatments. Responses of CR, GPP, and NCP to increasing salinity were similar for both the Ca:Mg:K and NaCl treatments but NCP declined more drastically at lower salinities in the NaCl treatment while NCP decreased linearly in the Ca:Mg:K treatment.

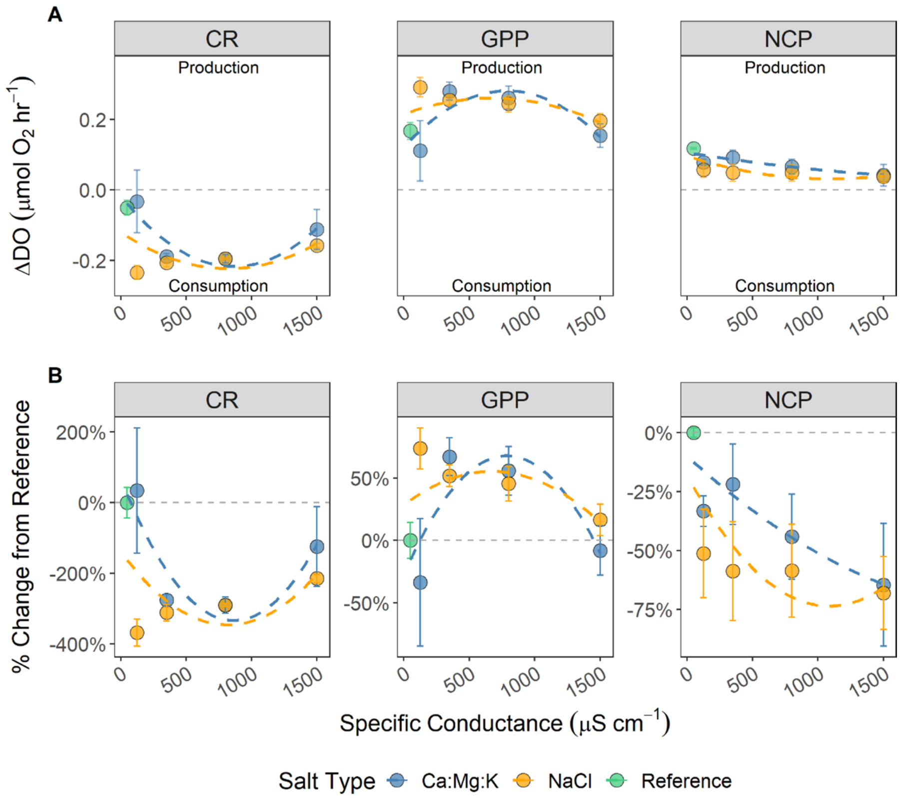

Fig. 5 Response of community respiration (CR), gross primary production (GPP), and net community production (NCP) along experimental salinity gradients of two salt profiles including a combination of calcium, magnesium, and potassium chloride salts (Ca:Mg:K) and sodium chloride (NaCl). Panel **A** shows measured oxygen flux values and panel **B** shows percent change from unaltered reference water. Positive values indicate oxygen production, negative values indicate oxygen consumption

Discussion

Impacts of freshwater salinization on biogeochemical cycling

A key finding of this work was that short term (one day) exposure to moderate freshwater salt concentrations (350–800 μS cm^{-1}) doubled microbial respiration in the presence of biologically reactive C. Maximum respiration rates also occurred at different salinities for different salt types. Dissolved salts, particularly at brackish and marine concentrations, can interact directly with natural dissolved organic matter (DOM) altering molecular weight, composition, lability, and downstream fluxes (Amon and Benner 1996, Sholkovitz 1976, Uher et al. 2001, Yamashita et al. 2008; Kaushal et al. 2022); however, the effects of freshwater salinization on microbially-mediated carbon dynamics were less understood. The increased respiration observed here suggests that ecosystems could become more heterotrophic with moderate levels of freshwater salinization, especially in ecosystems with low primary production like low-light headwater streams. Inland waters are already significant sources of CO_2 to the atmosphere, emitting an estimated 2.1 Pg C yr^{-1} (Raymond et al. 2013), some fraction of which is derived from respiration in excess of GPP (Hotchkiss et al. 2015). Freshwater salinization could further increase CO_2 production in and evasion from inland freshwaters to the atmosphere by stimulating microbial respiration. Furthermore, increased respiration due to freshwater salinization is also likely to impact the quantity, quality, and relative proportions of different C pools (i.e., dissolved, colloidal, particulate) exported downstream by enhanced degradation of more labile compounds (Benstead et al. 2009; Rosemond et al. 2015), especially for stream biotic communities adapted to use resources transported from upstream (Vannote et al. 1980). For example, bacterial community structure can change in response to altered dissolved organic matter (DOM) quality in aquatic systems (Osterholz et al. 2016). Thus, shifts in the quantity or quality of DOM exported downstream, resulting from freshwater salinization, could have cascading effects on downstream biotic communities and energy flow (Creed et al. 2018).

Freshwater salinization could also have important implications for carbon and DO dynamics in ecosystems prone to hypoxia. Hypoxia is a major concern impacting > 12% of rivers globally and causes altered nutrient cycling pathways (Testa and Kemp 2012), particularly under the stress of climate change (Hamidi et al. 2015; Justić et al. 1996; Blaszczak et al. 2022). Although we did not intend for 5-day incubations to go hypoxic, the longer exposure time and lower DO concentrations at the end of the incubation resulted in different trends in respiration and changes in nutrient concentrations relative to the 1-day oxic incubations. Thus, changing salt concentrations might further exacerbate hypoxic conditions by facilitating increased microbial respiration and DO consumption; however, our results also suggest that the salt concentrations at which such an effect might occur are dependent on salt type. For example, under hypoxic conditions, the highest respiration rates for NaCl and sea salt treatments (which is predominately Na^+) occurred at the highest salinity of 1500 µS cm^{-1} while respiration rates generally declined with increasing salinity from divalent $CaCl_2$ and $MgCl_2$. While increased concentrations of Na-based salts could exacerbate hypoxic conditions, increasing concentrations of Mg- and Ca-based salts could actually reduce DO consumption. Taken broadly, our results illustrate how considering both salinity and salt type, in addition to nutrient loading, may be a critical aspect in mitigating the effects of hypoxia in freshwater ecosystems.

Freshwater salinization alters resource stoichiometry

A second key finding of this work is that salinization differentially altered biogeochemical nutrient cycling, which resulted in large changes in resource stoichiometry under specific conditions. Resource stoichiometry considers how changes in pools of multiple chemical elements (primarily C, N, and P) influence and are influenced by biota (Welti et al. 2017). Collectively, respiration and net nutrient fluxes had only minor effects on N:P across a freshwater salinity gradient in oxic conditions. However, salinization from $CaCl_2$ caused increasing net SRP removal with increasing salinity. At high concentrations, Ca^{2+} can co-precipitate with phosphate ions forming hydroxyapatite (Simmons 2010) which could explain the increase in SRP removal at higher $CaCl_2$ concentrations. N:P also increased drastically with increasing $MgCl_2$ concentrations in hypoxic conditions, which could potentially be explained by struvite precipitation (Rivadeneyra et al. 1992). Regardless of the underlying mechanism, freshwater salinization has the potential to increase N:P by 300%. Changes in N:P as small as 70% impact biogeochemical processes (Rosemond et al. 2015), food web ecology (Glibert et al. 2011) and ecosystem metabolism (Williamson et al. 2016). For example, the relative abundance of species in algal communities shifts across an N:P gradient affecting food quality for algal consumers and other higher trophic levels (Stelzer and Lamberti 2001).

Hypoxic surface waters and benthic environments might be differentially affected by changes in N:P ratios. Hypolimnetic sediment–water interfaces can be significant areas of P regeneration, especially under low DO conditions, causing a positive feedback cycle that prolongs low-oxygen conditions in ecosystems (Conley et al. 2009; Lin et al. 2018). However, changes in biogeochemical process rates under elevated concentrations of Ca^{2+} or Mg^{2+} could reduce dissolved P concentrations via hydroxyapatite or struvite precipitation, potentially reducing the effects of sedimentary P regeneration on hypoxia (Koschel 1997). N:P also alters microbial assemblages. For example, internal P loading can decrease N:P to N-limiting conditions, which facilitates harmful blooms of N-fixing cyanobacteria or other diazotrophs in certain lakes (Watson et al. 2016; Nifong et al. 2022). However, freshwater salinization could potentially reduce blooms of harmful bacteria that thrive under low N:P conditions by scavenging and removing dissolved P from the water column. Generally, N:P of < 20:1 indicate N limitation while N:P > 50:1 indicate P limitation of biological processes (Guildford and Hecky 2000). Thus, a change in N:P of 150%, half of the observed % increase of N:P in this study, can alter nutrient limitations and microbial assemblages that are affected by stoichiometry.

Freshwater salinization alters net community production

Net community production (NCP) reflects the combined production and consumption of organic carbon by autotrophs and heterotrophs. Thus, NCP is an indication of energy sources and flow through an ecosystem and can reveal if an ecosystem's primary energy sources are external (allochthonous) or internal via primary production (autochthonous). GPP, respiration, and net production depend on and are sensitive to numerous controlling factors ranging from available resources like light and nutrients to disturbances like high flow events (Bernhardt et al. 2022; O'Donnell and Hotchkiss 2019, 2022). The effects of freshwater salinization on metabolism, however, are not well understood (Berger et al. 2019). Our first experiment documented how freshwater salinization alters respiration (organic C consumption) and biogeochemical cycling of C, N, and P in the absence of primary production. Our second experiment revealed that freshwater salinization also alters gross primary production (GPP) and overall NCP. Moderate freshwater salinization acts as a subsidy and stimulates GPP, but to a lesser extent than respiration, indicating that even in aquatic environments with ample light availability, freshwater salinization has the potential to make ecosystems more heterotrophic. However, the effects of freshwater salinization on GPP did substantially offset the salinization-induced increase in CR. Specifically, Both GPP and CR were enhanced and largely balanced each other resulting in less drastic effects on salinization on NCP). Therefore, light and nutrient availability are likely important factors in governing net impact of freshwater salinization on organic carbon, metabolism, and food web energy dynamics of aquatic ecosystems.

Integrating freshwater salinization and current biogeochemical frameworks

Current salinization frameworks (*i.e.,* Freshwater Salinization Syndrome) provide a robust understanding of sources, processes, and chemical changes of salinization; however, there is a recognized need to improve understanding of effects on ecosystem functions (Kaushal et al. 2018, 2023). Current conceptual frameworks for stream ecology emphasize the importance of stream flow as a master variable. For example, the Pulse-Shunt Concept and River Network Saturation Concept both posit that increasing flow and decreased water residence time reduce the capacity of headwater streams to process and remove nutrients, ultimately increasing downstream fluxes and biogeochemical opportunities (Knapp et al. 2020; Raymond et al. 2016; Wollheim et al. 2018). Salt loading and transport are also strongly influenced by flow (Lakoba et al., 2021). Depending on land use and season, high-flow events can either increase (i.e., flushing behavior) or decrease (i.e., diluting behavior) salt concentrations (Corsi et al. 2010; Wymore et al. 2019). Flushing events that increase salinity could have an antagonistic effect on downstream nutrient fluxes by stimulating microbial respiration and biogeochemical processes, creating hotspots or hot moments of biogeochemical cycling (Vidon et al. 2010). Effects of salinity pulses on stream carbon and nutrient dynamics could be further exacerbated by flow-induced reductions in GPP, which is less resilient and resistant to high flow events than respiration (O'Donnell and Hotchkiss 2022). These hotspots or hot moments could also depend on salt type, as exposure to different salt types produced significantly different respiration rates, even at the same salinity. Hypoxic and anoxic zones are also biogeochemical hotspots where hypoxia may increase the downstream flux of C, N, and P (Carey et al. 2022), which could be exacerbated by salinization (McClain et al. 2003). Our results indicate that nutrient fluxes from hypoxic systems could either be enhanced or suppressed by salinization depending on salt concentration and ionic composition. Incorporating salinity and major ion types could improve estimates of downstream nutrient fluxes and our understanding of hypoxia formation by accounting for salt-dependent changes in microbial process rates.

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Declarations

Competing interests All authors have no relevant financial or non-financial interests to disclose.

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