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Rising plant demand strengthens nitrogen limitation in tidal marsh

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Abstract

Nitrogen (N) is a limiting nutrient for primary productivity in most terrestrial ecosystems, but whether N limitation is strengthening or weakening remains controversial because both N sources and sinks are increasing in magnitude globally. Temperate marshes are exposed to greater amounts of external N inputs than most terrestrial ecosystems and more than in preindustrial times owing to their position downstream of major sources of human-derived N runoff along river mouths and estuaries. Simultaneously, ecosystem N demand may also be increasing owing to other global changes such as rising atmospheric [CO_2]. Here, we used interannual variability in external drivers and variables related to exogenous supply of N, along with detailed assessments of plant growth and porewater biogeochemistry, to assess the severity of N-limitation, and to determine its causes, in a 14-year N-addition × elevated CO_2 experiment. We found substantial interannual variability in porewater [N], plant growth, and experimental N effects on plant growth, but the magnitude of N pools through time varied independently of the strength of N limitation. Sea level, and secondarily salinity, related closely to interannual variability in growth of the dominant plant functional groups which drove patterns in N limitation and in porewater [N]. Experimental exposure of plants to elevated CO_2 and years with high flooding strengthened N limitation for the sedge. Abiotic variables controlled plant growth, which determined the strength of N limitation for each plant species and for ecosystem productivity as a whole. We conclude that in this ecosystem, which has an open N cycle and where N inputs are likely greater than in preindustrial times, plant N demand has increased more than supply.

KEYWORDS
elevated CO_2, eutrophication, oligotrophication, plant productivity, *Schoenoplectus americanus*, sea level rise

1 | INTRODUCTION

Nitrogen (N) is the most common limiting nutrient for plant growth in terrestrial ecosystems (LeBauer & Treseder, 2008) but can also be a harmful pollutant. N pollution that enters a terrestrial ecosystem has three primary fates: it can leach into waterways causing eutrophication; it may be lost as gas to the atmosphere partially as potent greenhouse gases; or it can be sequestered in plants and soils (Pastore et al., 2016). Coastal wetlands exert an outsized influence on the global N cycle because these ecosystems have an open N cycle, meaning that exchange with surrounding waters, gaseous loss and sequestration can each be greater in magnitude than in upland ecosystems on an area basis (Bowen et al., 2023; Valiela & Teal, 1974). The factors that control the balance of N inputs and losses in wetlands remain controversial (Childers et al., 2002; Tobias & Neubauer, 2019), limiting our understanding of how wetlands may mitigate, or respond to, future nutrient loads. Because N scarcity limits plant growth, and plant uptake is a major nutrient sink that ultimately allows long-term sequestration in soil, the severity of N limitation should relate to how much N wetlands can sequester.

The strength of N limitation of plant productivity is determined by two primary components, plant N demand and N availability. When demand exceeds supply, N limitation will be strong. Because both demand and availability are increasing globally (Gruber & Galloway, 2008; Mason et al., 2022), the end result for ecosystem N limitation remains unclear. While N inputs into ecosystems have increased owing to human influence, the demands of ecosystems may have also increased owing to lengthening of growing seasons, effects of elevated CO_2, altered precipitation and disturbance regimes (Craine et al., 2018; Mason et al., 2022) and because of centuries of increased erosion rates in upland ecosystems (Quinton et al., 2010). It remains controversial how the strength of present day N limitation compares to that of the past (Hiltbrunner et al., 2019). How the severity of N limitation will change in the future underlies enormous uncertainty in future ecosystem functions like global carbon uptake (Terrer et al., 2019; Zaehle & Dalmonech, 2011), so we need to understand how both components—plant N demand *and* N supply—will change in the future.

Ecosystem studies in uplands have yielded evidence for both source-driven (controlled by changes in N availability) and sink-driven (controlled by changes in N demand) variability in N limitation. In an arid woodland, the strength of N limitation was hypothesized to relate inversely to the strength of water limitation; the authors reasoned that if water is less limiting to plant growth, plants N demand will increase (Hooper & Johnson, 1999). However, it was concluded that the plant community reacts to changes in water availability such that total ecosystem productivity is commonly co-limited by two or more resources. In that study, how individual species responded to N addition could not be determined (Hooper & Johnson, 1999). In fire-prone prairies, the strength of N limitation related negatively to time since fire (Seastedt et al., 1991). As ecosystems recovered from fire, exogenous nutrient inputs increased such that experimentally added N had smaller effects on productivity. So, in uplands, the strength of N limitation relates to variation in the supply of N, but does this hold in tidal wetlands where external fluxes can be relatively large compared to plant demand?

In temperate tidal wetlands, N is nearly always at least partially limiting to primary productivity (Callaway et al., 1995; Levine et al., 1998; Morris & Bradley, 1999; Tyler et al., 2003; Valiela & Teal, 1974), but we do not know how the strength of N limitation may change in the future where other resources and abiotic factors are changing. In a brackish marsh, experimental N and CO_2 addition interacted positively to stimulate productivity, suggesting that elevated CO_2 can strengthen N limitation in the short term, but the effect wavered when the plant community shifted (Langley & Megonigal, 2010). At an adjacent experiment, low levels of experimental warming strengthened plant N limitation while further warming stimulated enough mineralization to meet plant N demand, though N limitation was not directly determined by experimental N addition (Noyce et al., 2019). One advantage of addressing N limitation in a wetland is that the dissolved nutrient pool can be assessed with porewater sampling. However, while some interpret porewater [N] as reflecting soil nutrient availability, it can also be influenced strongly by plant uptake (Drake, 2014; Negrin et al., 2011). Assessing the relationship of porewater N to other internal and external pools through time can illuminate its role in the N cycle.

We examined the controls on N limitation by taking advantage of the extended record of N effects from the 14-year $CO_2 \times N$ experiment at the Smithsonian Environmental Research Center (SERC) referenced above (Langley & Megonigal, 2010). Here, the degree of N limitation varies widely from year to year, but the cause of that variation remains unexplored. The long record of experimental N addition allows for direct determination of N limitation independent of common proxies (such as foliar [N] or C:N) and of the influence of important drivers, such as climate and sea level, that vary naturally in the background and represent the substantial influence of background anthropogenic climate change (Langley et al., 2018). The naturally simple plant community structure affords assessment of N limitation of individual species, which can help explain N effects on total ecosystem productivity (Langley & Hungate, 2014). We set out to answer this question: Is interannual variability in N limitation driven by interannual differences in plant demand or N availability? We hypothesized that if N limitation is driven by plant demand, then drivers that strongly influence plant demand, such as flooding, salinity and atmospheric CO_2, will control N limitation. Alternatively, if plant N limitation is driven by variability in supply, then we should observe larger proportional stimulation of plant growth in years with lower N availability as assessed by porewater N concentration. Identifying the controls on the strength of N limitation will afford generality in understanding past progression of N limitation and in forecasting future N limitation.

2 | METHODS

We used data from the Global Change Research Wetland at the Smithsonian Environmental Research Center near Edgewater, MD, a portion of the Kirkpatrick Marsh, located at the Smithsonian Environmental Research Center (SERC) in Edgewater, MD. The Global Change Research Wetland (GCReW) is adjacent to the Rhode River, a sub-estuary of Chesapeake Bay. The marsh has a 44 cm tidal range (Holmquist et al., 2021), and the plots used are positioned between 17 and 25 cm above NAVD88. The 95-year trend (1928–2023) in sea level rise is 3.8 mm year^{-1} and has accelerated such that the 20-year (2003–2023) trend is 7.4 mm year^{-1} (NOAA). Salinity of flooding waters ranges from 4 to 15 ppt typically peaking in the summer, but neither interannual variation nor long-term trends in salinity were related to sea level. Therefore, the effects of sea level rise on plants are driven by inundation and not associated salinization.

We focused on the $CO_2 \times N$ manipulation experiment that began in 2005. Twenty plots were located in the sedge-dominated zone of the marsh and fitted with octagonal open-top chambers. One of four treatments was randomly assigned to each chamber. Added N and elevated CO_2 treatments were imposed factorially beginning in May, 2006. For the N addition treatment, NH_4Cl was dissolved in 5 L of the tidal creek water that floods the marsh and sprayed onto 10 of the plots with backpack sprayers. Then, 5 L of unamended creek water was sprayed to rinse it to the soil surface. The 10 unfertilized plots received the same total amount of creek water without any added N. These treatments were applied at five points throughout each growing season achieving a total fertilization rate of $25\,g\,N\,m^{-2}\,year^{-1}$. All chambers receive ambient air from blowers delivered through octagonal manifolds that surround each plot. For the elevated CO_2 treatment, pure CO_2 was injected into a blower stream at a rate to increase atmospheric $[CO_2]$ by 340 ppm (Langley et al., 2009).

Only three plant species occur in these plots: the sedge, *Schoenoplectus americanus*, and two C_4 grasses, *Distichlis spicata* and *Spartina patens*. The sedge accounted for >90% of plant biomass in the control plots over the duration of the study. Each year, plant biomass and species composition was assessed at peak biomass in late July each year through a combination of clipping small subplots for grasses and counting and estimating ramet biomass allometrically for the sedge (Langley & Megonigal, 2010). Porewater chemistry was assessed from 2 to 5 times each year. At the beginning of the experiment in 2005, porewater wells were installed in triplicate in each plot to sample porewater at three target depths: 20, 40 and 80 cm. Samples were taken, stored, and analyzed following the methods of (Keller et al., 2009).

Climate data were recorded at the Smithsonian Environmental Research Center (Table S1). Sea level data were taken from the Annapolis NOAA gauge (NOAA tides and currents, station ID: 8575512). The gauge is 13 km from the site and exhibits close agreement with tidal maxima at the marsh (Langley et al., 2013). Susquehanna water quality data were taken from the Conowingo dam (Table S1), representing N concentrations in the main stem of Chesapeake Bay which can influences concentrations in the Rhode River. Kirkpatrick Marsh is located on the Rhode River, for which data were taken from a long-term monitoring dataset maintained at the Smithsonian Environmental Research Center. Samples were collected and analyzed according to Jordan et al. (1983).

2.1 | Analyses

Our goal was to determine the cause of interannual variability in N limitation by examining relationships between interannual variability in plant N limitation and other relevant datasets such as exogenous N inputs, climate, and sea level. We first examined the effect of N on plant growth across the full dataset with a linear mixed effects model using year, N and CO_2 treatments, and the interaction between treatments with a random intercept across plots. The response variables were total biomass, sedge biomass, and grass biomass. The interaction in the model allows us to investigate how elevated atmospheric CO_2 controls the effects of N. To further explore the temporal trend of N limitation, we employed a second model that included an additional interaction term of N treatment and year.

Having established in which cases N addition affected biomass, we explored the controls on N limitation directly using models with the strength of N limitation as the response variable. We define the strength of N limitation as the amount by which N addition increases plant biomass on an area basis ($g\,m^{-2}$). We estimated N limitation as mean aboveground biomass in N-fertilized treatments minus biomass in unfertilized treatments, separated by elevated CO_2 treatment ($n=5$) for each treatment year. We calculated this metric for total aboveground plant biomass (referred to herein as "total biomass"), and separately for both functional groups of plants present, sedge (including only *S. americanus*) and grass (including both *S. patens* and *D. spicata*). We excluded data from the pre-treatment year, 2005, resulting in 14 years of treatment data (2006–2019, inclusive).

We screened nine potential explanatory variables from an array of interannual datasets using correlation matrices between the responses and explanatory variables that we reasoned could influence the temporal variability of N effects. Five variables represented nutrient inputs or availability: (Susquehanna River [total N], Rhode River [total N], porewater $[NH_4]$ at three depths). Four variables represented physical drivers: air temperature, precipitation, porewater salinity, and sea level. Where clusters of similar explanatory variables were correlated, we chose one representative variable to screen. For instance, annual average porewater salinity at 40 cm was related to that at 20 and at 80 cm. Explanatory variables that had correlation coefficients >0.5 or <−0.5, and $p<.05$ with N effects, were explored further with multiple regressions for each response variable (Table S3).

Because sea level exhibited very tight relationships with plant growth, we further explored that driver. We reasoned that the effect of sea level on plants could lag such that effects from the previous year's growing season could explain current year's plant growth. We tested lag effects of 1, 2, 3, 6, 9, 12, 18, and 24 months by estimating running averages of mean sea level inclusive of July of the target growing season (Table S3). After the initial exploration, we conducted variable selection using simple linear regression models based on the Akaike Information Criterion to determine the strongest drivers of each category. We then ran a multiple linear regression model for N limitation on total, sedge, and grass biomass with the selected drivers, 24-month mean sea level, porewater ammonium at 40-cm deep, CO_2 treatment, and their interactions. Adding interactions, however, resulted in multicollinearity, meaning there was substantial linear dependency among predictors. Therefore, we centered predictors 24-month mean sea level and porewater ammonium at 40-cm depth around their respective means to reduce these linear correlations.

3 | RESULTS

The strength of N limitation of total plant biomass was positive across the duration of the study with and without elevated CO_2 (Figure 1). N addition resulted in an average stimulation of total plant biomass of 176 $g\,m^{-2}$ in ambient CO_2 ($t_{16}=2.78$, $p=.013$, Table 1) or 281 $g\,m^{-2}$ in elevated CO_2 ($t_{16}=1.18$, $p=.26$). The strength of N limitation differed sharply between plant functional groups. Sedge biomass production was only limited by N with elevated CO_2 ($t_{16}=2.60$, $p=.019$, Table 1). Grass production was N-limited under all conditions, but the strength of limitation was stronger at ambient CO_2 ($t_{16}=-3.75$, $p=.002$, Figure 1). The stimulation varied widely among years for sedge ($t_{258}=2.21$, $p=.028$, Table S4) and grass ($t_{258}=-4.93$, $p<.001$) but not for total biomass ($t_{258}=-0.31$, $p=.76$, Figure 1).

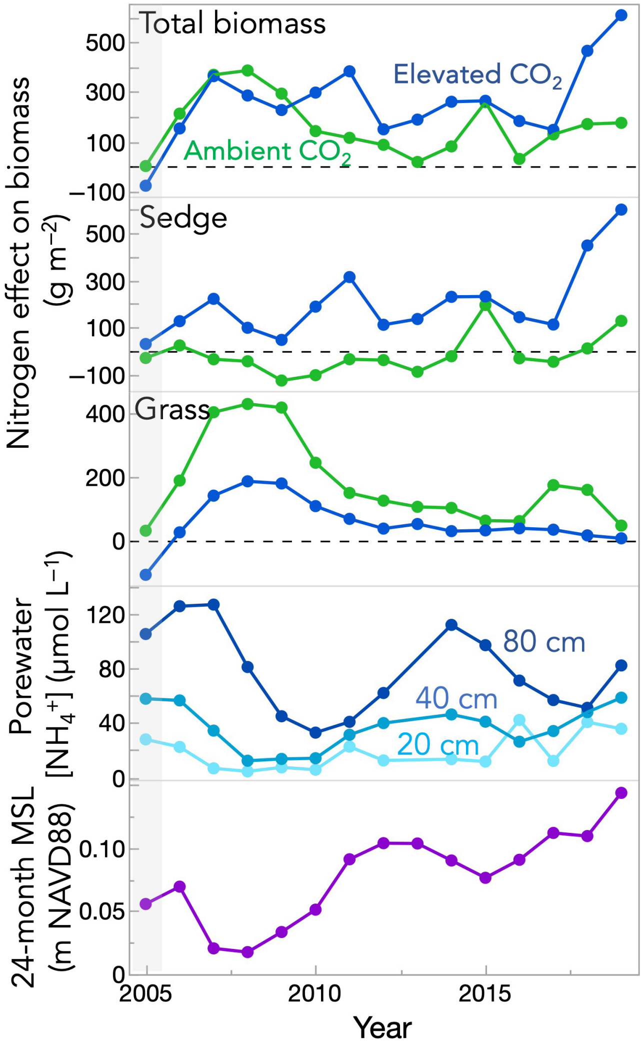

FIGURE 1 Annual strength of N limitation of total biomass, sedge biomass and grass biomass in ambient CO_2 and under elevated CO_2, porewater [NH_4^+] and 24-month running average mean sea level. Each N effect data point represents the N stimulation of biomass (fertilized plots − control plots, $n=5$) for each treatment year (2006–2019). Porewater [NH_4^+] is from 20, 40 and 80 cm deep in ambient plots. Dotted vertical line indicates when treatments were initiated in May 2006.

TABLE 1 Linear mixed effects model of N effects on plant biomass.

Biomass response	Predictor	*t*	df	*p* value
Total	Year	9.65	259	<.001
	CO_2	0.89	16	.388
	N	2.78	16	.013
	$N \times CO_2$	1.18	16	.257
Sedge	Year	13.85	259	<.001
	CO_2	1.18	16	.257
	N	−0.24	16	.810
	$N \times CO_2$	2.60	16	.019
Grass	Year	−6.42	259	<.001
	CO_2	−0.72	16	.480
	N	8.32	16	<.001
	$N \times CO_2$	−3.75	16	.002

Sea level and CO_2 treatment exerted strong control over N limitation for total biomass (Table 2). Over 70% (75% in ambient CO_2 and 78% in elevated CO_2) of the variability in N effect can be explained by sea level for grass, and 14% (ambient CO_2) and 30% (elevated CO_2) for sedge (Table 3). In years with high sea level, sedge biomass was high (Figure 2), and N limitation of sedge biomass was stronger, especially with elevated CO_2 ($t_{12}=2.29$, $p=.041$, Figure 3). In years of low sea level, grass biomass was high (Figure 2), and N limitation of grass was stronger for both ambient ($t_{12}=-6.04$, $p<.001$, Table 3) and elevated CO_2 ($t_{12}=-6.51$, $p<.001$, Figure 3). Different sea level metrics were consistent in their association with biomass of each species. Sedge biomass related most closely to July MSL of the current year, while grass biomass related more closely to integrated metrics of MSL over 24 months; however, the choice of metric did not affect the qualitative nature of the relationship (Table S3). Porewater [NH_4^+] related closely to N limitation of each plant functional type (Figure 3; Table 2). Multivariate analyses revealed few other strong correlations among any key external variables and N limitation (Figure S3), as assessed by the N treatment effect on plant growth.

4 | DISCUSSION

Using interannual variation in N pools and external drivers along with responses from a long-term N-addition experiment, we explored the controls on N limitation in this ecosystem. Interannual variability in plant N response related closely to variables reflecting both, plant N demand and N supply (Figure 3). Interpreting these findings in the context of previous literature and findings from this site, we conclude that sea level was the dominant driver of interannual variation in plant N demand which determined the strength of N limitation. Even though porewater N relates closely to N responses in some cases (Table 2), we feel the most parsimonious explanation is that variability in porewater [N] reflects patterns of plant uptake rather than drives plant growth responses.

TABLE 2 Multiple regression analysis of N effects on total biomass with 24-month mean sea level (MSL), porewater ammonium concentration (NH_4) at 40-cm depth and CO_2 treatment. The three-way interaction was not statistically significant and was excluded from the model.

Response	Predictor	t^a	p value
N effect on total biomass $R^2 = .638$ $p = .002$	MSL	−3.35	.003
	NH_4	1.82	.084
	CO_2	3.06	.006
	$MSL \times NH_4$	3.37	.003
	$MSL \times CO_2$	2.56	.019
	$NH_4 \times CO_2$	−0.36	.723
N effect on sedge biomass $R^2 = .752$ $p < .001$	MSL	−0.13	.898
	NH_4	1.84	.081
	CO_2	6.18	<.001
	$MSL \times NH_4$	1.84	.081
	$MSL \times CO_2$	1.05	.305
	$NH_4 \times CO_2$	−0.12	.904
N effect on grass biomass $R^2 = .876$ $p < .001$	MSL	−5.73	<.001
	NH_4	−0.27	.790
	CO_2	−6.36	<.001
	$MSL \times NH_4$	2.49	.022
	$MSL \times CO_2$	2.55	.020
	$NH_4 \times CO_2$	−0.41	.688

[a] The degrees of freedom are 19 due to two missing observations of porewater N.

TABLE 3 Simple linear regression models on N effects with porewater ammonium concentration (NH_4) at 40-cm depth or 24-month mean sea level (MSL) for different CO_2 treatments.

		Ambient CO_2			Elevated CO_2		
Plant	Predictor	t^a	p value	R^2	t^a	p value	R^2
Total	NH_4	−0.94	.366	.075	0.98	.349	.080
	MSL	−3.72	.003	.536	0.73	.481	.042
Sedge	NH_4	2.93	.014	.438	2.43	.034	.349
	MSL	1.38	.192	.138	2.29	.041	.304
Grass	NH_4	−2.73	.020	.404	−4.65	<.001	.662
	MSL	−6.04	<.001	.752	−6.51	<.001	.780

[a] The degrees of freedom are 12 for MSL models and 11 for NH_4 models due to one missing observation of porewater N in each CO_2 treatment.

Sea level, a factor that strongly controls plant composition and growth, related positively to the effects of N addition on total biomass and sedge biomass, and negatively to grass biomass, over 14 years. Porewater [NH_4^+], which can reflect interannual variability in N supply, also yielded tight relationships with N effects. The direction of causality underlying the relationships between annual mean sea level (driver) and plant growth (response) is clear. We know from extensive work here and elsewhere that total plant productivity, as well as growth of individual species, is extremely sensitive to the altered flooding regime driven by relative sea level changes (Langley et al., 2013; Morris et al., 2002). While plants can influence relative sea level by building elevation, these adjustments operate too slowly to explain the relationships across years observed herein.

The direction of causality in the relationships between porewater [NH_4^+] and plant growth is murkier (Negrin et al., 2011). In some cases, plant productivity and community composition are interpreted as driving variability in porewater [N] (Langley & Megonigal, 2010) and in other cases plants are interpreted as responding to gradients in porewater [N] (Bertness et al., 2002). Where plant growth is N limited, it should relate positively to N availability. However, plant growth also draws down porewater [N], so plant growth can relate inversely to N availability as assessed by porewater N concentration. With this bilateral influence in mind, we attempt to infer cause and effect in these biomass-porewater [N] relationships.

4.1 | Drivers of N limitation

Ultimately, the relationships between porewater [N] and N effects on plant growth were most likely driven by interannual variability in sea level. It is well established that sea level exerts a dominant control over plant growth and composition at this site (Langley et al., 2018) and tidal wetlands elsewhere (Janousek et al., 2016; Langley et al., 2013; Morris et al., 2002). High sea level years promote sedge

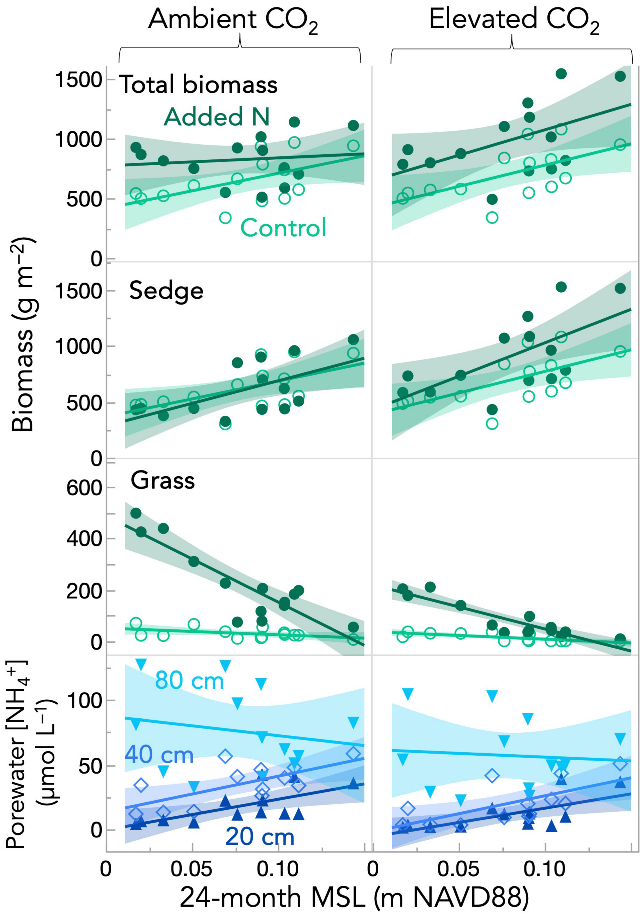

FIGURE 2 Relationship of mean annual total biomass, sedge biomass, grass biomass and porewater N with mean growing season sea level over 14 years. For biomass, dark green symbols represent plots with added N, while light green symbols represent no added N. Porewater depths are indicated in the bottom panels.

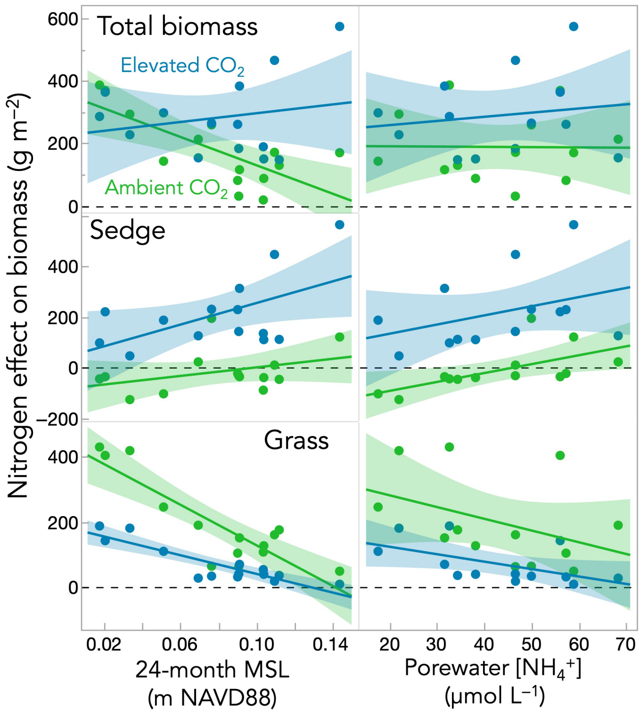

FIGURE 3 Relationships between N effect and MSL (left) and porewater [NH_4^+] (right) separated by CO_2 treatment (amb = green, eCO_2 = blue). N effects are estimated as mean biomass of N fertilized plots minus that of unfertilized plots for each year. Dashed horizontal lines show zero effect of N. Associated statistics are reported in Table 2.

growth at least in part by reducing competition with grasses, which are more sensitive to flooding than the sedge (Gabriel et al., 2022). Though the most flood-tolerant species here differs, the decline of high marsh grasses like *Spartina patens* and *Distichlis spicata* in response to increasing flooding is a widespread phenomenon across saltmarshes of this region (Valiela et al., 2024; Watson et al., 2016). This body of work suggests that the effects of sea level on plants is a direct response to flooding, suggesting that any relationships with porewater [N] we observed were indirect effects that were mediated by biomass production. In years when high sea level promotes sedge growth, near-surface porewater N availability is also high (Figure 2). This high porewater [N] may result from the distinctive rhizosphere activities of each plant functional group. First, the sedges root much more deeply than the grasses, which form dense mats of roots near the soil surface (White et al., 2012). When sea level is high, grass productivity is suppressed and can no longer draw down porewater [N] near the surface (higher porewater [NH_4^+] at 20 and 40 cm deep in high sea level years, Figure 2 bottom right). Second, the sedge delivers much more O_2 to the rhizosphere through aerenchyma, elevating soil redox and stimulating breakdown of organic matter (Noyce et al., 2023). In high sea level years, greater sedge biomass could stimulate N mineralization, thereby increasing porewater [N]. Also, we cannot rule out that sea level could have a *direct* influence on porewater biogeochemistry.

We have previously documented the strong effects of sea level on plant growth at this site (Langley et al., 2013), in accordance with findings from other wetlands (Morris et al., 2002), and effects of sea level on CO_2 stimulation of plant growth (Zhu et al., 2022). The relationship of sea level with nutrient limitation has not been well explored with in situ experiments. In field-deployed mesocosms, N addition interacted with manipulated sea level, having stronger positive effects on sedge plant growth as flooding stress increased (Langley et al., 2013). Here, we found indication of the same effect–the largest N effects on sedge growth occurred during high-sea-level years (Figure 3). We have seen indications of the same positive interaction between sea level and N stimulation of sedge biomass in two very different studies. The generality of this result should be tested in other flood-tolerant species.

4.2 | Elevated CO_2 strengthened ecosystem N limitation

The elevated CO_2 treatment had a strong influence on N limitation for each functional group and for the ecosystem. Elevated CO_2

strengthened N limitation of sedge and total biomass production while weakening N limitation of grass (Figure 2). The hypothesis of progressive N limitation predicts that elevated CO_2 will cause N to accumulate in recalcitrant organic pools, ultimately reducing plant N availability and exacerbating N limitation (Luo et al., 2004). We have observed evidence here that elevated CO_2 causing more N to accumulate in organic pools, but only where N was added (Pastore et al., 2016), and here, the strength of N limitation tended to increase through time in elevated CO_2 (Figure 1). However, we feel the temporal changes depend on other drivers such as sea level rise rather than slow-acting N-cycle feedbacks as predicted by progressive N limitation. That grass N limitation weakened under elevated CO_2 may reflect that sedges strongly outcompete the grasses. Elevated CO_2 stimulates sedge productivity especially with added N (Langley & Megonigal, 2010). Over multiple growing seasons, the sedges generate a thatch layer that excludes the grasses by light competition. Even though the grasses respond positively to added N at ambient CO_2, elevated CO_2 sways competition in favor of sedges so that grasses are unable to take advantage of added N.

4.3 | Salinity modified sedge N limitation

Salinity varies considerably among years and can have a large influence on plant growth that may also contribute to variability in N demand. Here, salinity had a secondary influence to sea level on sedge growth (Table S2). At this site, salinity varies independently of sea level (Table S3). High water-level years can result from high sea level in the region, which should also increase salt intrusion into this brackish estuary, but can also occur due to high regional rainfall and runoff, reducing salinity. Here, salinity relates negatively to the N effect on sedge growth but has no influence on N effects of grasses. *D. spicata* and *S. patens* are C_4 grasses known to exhibit salt-tolerance compared to the sedge (Erickson et al., 2007; Hansen et al., 1976). Salinity could also play a role by interacting with N supply and demand by inhibiting plant uptake or desorbing mineral N from soil particles. Without manipulation of salinity, we were unable to explicitly test these mechanisms.

4.4 | Common proxies of N limitation do not predict responses to added N

We consider response to N addition to be the standard by which to estimate N limitation. Previous studies have made inferences about N limitation based on proxies such as foliar N concentration (Craine & Jackson, 2010; Erickson et al., 2007), or plant morphology (Lu et al., 2019). We know from this N addition experiment that N addition increases foliar [N] by 15%, increases ramet width by 10% and reduces fine root production by 35% (Lu et al., 2019). So, we would expect that if N was most limiting to plant growth, years with the largest N effects might also have the lowest ambient foliar [N], narrowest ramets, and highest root productivity. Here, we found no relationship across years between foliar [N] in the control plots and the strength of N limitation as assessed by the fertilization responses (Figures S2 and S4). The relationship between stem width and N limitation was in the opposite direction of what would be expected if morphology were a useful indicator of N limitation. We found that years with larger ramets in the control plots, which should indicate less N limitation, actually had the *strongest* N limitation (Figure S4). There was some indication that high root growth in control plots corresponded to years of stronger N limitation, though the trend was driven strongly by 1 year with both high root growth and strong N limitation (Figure S4). The general lack of agreement between the strength of N limitation determined by direct manipulation of N and by other proxies, raises doubts about the validity of other proxies to infer N limitation. It is important to note that the fine root: shoot production ratio appeared to agree well with the patterns of nutrient limitation in each treatment early in the experiment (White et al., 2012), but eventually converged among treatments, possibly indicating an ecosystem-level adjustment in allocation (Figure S2).

4.5 | Future for N limitation

Atmospheric CO_2 is rising, driving climatic warming and accelerating sea level rise. Rising CO_2 and warming occur over nearly all land ecosystems, and sea level rise affects most tidal wetlands. Here we found that rising CO_2 increases the demand for N, corroborating observations of enhanced N demand in many other elevated CO_2 studies (Luo et al., 2004; Terrer et al., 2019) and recent syntheses indicating oligotrophication of terrestrial ecosystems (Craine et al., 2018). Elevating CO_2 concentration from ambient (390–410 ppm) to 720 ppm increased the strength of plant N limitation by 60% (177 vs. 284 $g\,m^{-2}$). Extrapolated globally, rising CO_2 could drive a great increase in the inordinately strong N sink activity in tidal wetlands, all else being equal. However, rising CO_2 ultimately drives warming and ice melt that accelerates sea level rise. We have previously concluded that CO_2 effects on plant growth are ultimately superseded by the flooding stress effects that accompanies sea level rise (Zhu et al., 2022). We anticipate a similar hierarchy of factors here– generally, strong abiotic drivers override resource effects on plant growth. Though exposure to elevated CO_2 can alter N cycling, increased flooding stress from accelerating sea level rise is overwhelming the ability of these plants to respond to elevated CO_2 in this ecosystem (Zhu et al., 2022) and will drive the N balance of this ecosystem in the long term.

In the future, atmospheric CO_2 will rise but for how long depends on sociopolitical factors such human energy regimes. Sea level, on the other hand, will continue to rise for centuries or millennia as ocean and ice temperatures equilibrate with warmer climates. In the short term, these drivers will rise together, so for the near term (25–50 year) projections, the most probable scenario is the high CO_2, high sea level condition. Though, CO_2 effects on N limitation were considerable over the duration of this experiment, we expect that relative sea level will primarily drive changes in N budgets of marshes and the estuaries they influence.

5 | CONCLUSIONS

Interannual variation in the strength of N limitation depends on plant species composition and abiotic factors such as sea level and CO_2. Ecosystem-scale plant productivity was always at least partially limited by N, and was more consistent over years than the strength of N limitation for sedges or grasses alone (Figure 3). The elevated CO_2 treatment and years with high sea level both tended to strengthen N limitation (Figure 3) and shifted it from grasses to sedges (Figure 4). Looking backward, these drivers may have already contributed to stronger N limitation (as in Mason et al., 2022) in coastal wetlands that have kept pace with sea level over the past century. Looking forward, we may expect N limitation to strengthen as atmospheric CO_2 and sea level continue to rise. However, when abiotic stressors become strong enough to inhibit growth of all plant species, in this case higher sea level or salinity for sedge, N limitation will be diminished due to declining plant N demand, and we should expect increasing export of N. Future work should test the generality of our finding that N sink strength inherently varies more than N sources such that sinks better explain variability in N limitation. These results from a tidal wetland have implications for any situation in which strong abiotic factors may interact with nutrient limitation. Where it is allowed to flourish, vegetation tends to naturally counteract chemical alteration of the biosphere through ecological response such as shifting species composition. But, where plant responses are inhibited by direct manipulation such as land-use change, or other strong physical drivers such as sea level rise or severe climatic changes, the capacity of vegetation to mitigate change will be diminished.

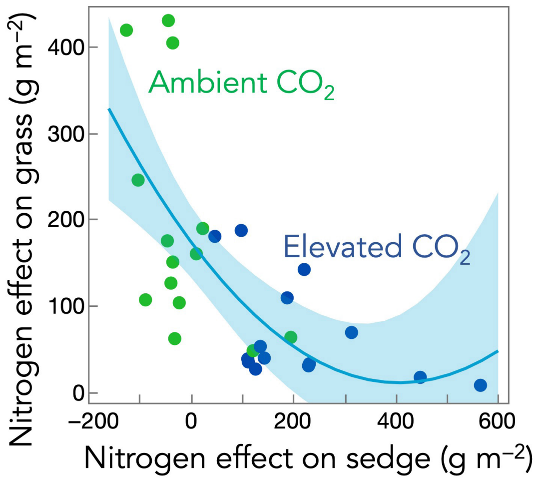

FIGURE 4 Strength of grass N limitation by strength of sedge N limitation. Each point represents a year. Green is annual average in ambient CO_2 and dark is elevated CO_2 ($n = 5$). Years of high sea level and plots with elevated CO_2 tend to have strong sedge N limitation but weaker grass limitation.

AUTHOR CONTRIBUTIONS

J. A Langley: conceptualization, formal analysis, writing, funding acquisition, review and editing. **L. Wang:** Formal analysis; writing – review and editing. **B. Yedman:** Conceptualization; formal analysis; writing – review and editing. **J. P. Megonigal:** Data curation; funding acquisition; project administration; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

The raw experimental data that support the findings of this study are openly available in the GCREW data archive on the Smithsonian website at https://serc.si.edu/gcrew/nitrogendata. Other publicly available data are available on USGS and NOAA sites as listed in Table S1. The data and code for statistical analyses are available in the Smithsonian Institution figshare repository (https://smithsonian.figshare.com) at https://doi.org/10.25573/serc.25777596.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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