ENVIRONMENTAL RESEARCH
LETTERS

PURPOSE-LED
PUBLISHING™

Part of the
ENVIRONMENTAL RESEARCH
SERIES

LETTER • OPEN ACCESS

Moderate nitrogen enrichment increases CO_2 sink strength in a coastal wetland

To cite this article: Wendi Qu *et al* 2024 *Environ. Res. Lett.* **19** 084044

IOP Publishing Environ. Res. Lett. 19 (2024) 084044 https://doi.org/10.1088/1748-9326/ad64e9

ENVIRONMENTAL RESEARCH
LETTERS

OPEN ACCESS

RECEIVED
15 March 2024

REVISED
29 May 2024

ACCEPTED FOR PUBLICATION
18 July 2024

PUBLISHED
26 July 2024

LETTER

Moderate nitrogen enrichment increases CO_2 sink strength in a coastal wetland

Wendi Qu[1,2], **Guangxuan Han**[2,3], **Josep Penuelas**[4,5], **Xiaoyue Wang**[1,2,*] and **Baohua Xie**[2,3,*]

[1] The Key Laboratory of Land Surface Pattern and Simulation, Institute of Geographical Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, People's Republic of China
[2] University of the Chinese Academy of Sciences, Beijing 100049, People's Republic of China
[3] Key Laboratory of Coastal Zone Environmental Processes and Ecological Remediation, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264000, People's Republic of China
[4] CREAF, Centre de Recerca Ecològica i Aplicacions Forestals, Cerdanyola del Vallès, Barcelona 08193, Catalonia, Spain
[5] CSIC, Global Ecology Unit CREAF-CSIC-UAB, Bellaterra, Barcelona 08193, Catalonia, Spain
[*] Authors to whom any correspondence should be addressed.

E-mail: wangxy@igsnrr.ac.cn and bhxie@yic.ac.cn

Keywords: nitrogen enrichment, coastal wetland, CO_2 sequestration, plant species composition, biomass distribution

Abstract

Coastal wetlands remarkably influence terrestrial carbon (C) stock by serving as natural reservoirs for 'blue carbon'. Anthropogenic nitrogen (N) enrichment shapes the dynamics of soil and plant communities, consequently affecting the C balance and ecosystem functions. The impacts of various levels of N enrichment on CO_2 sequestration in coastal wetlands, however, remain elusive. Here we conducted a long-term field study of N fertilization in a coastal wetland in the Yellow River Delta, China, to investigate N effects on soil properties, indicators of plant dynamics, and fluxes of ecosystem CO_2. The results indicated that moderate N enrichment (5 g N m^{-2} y^{-1}) stimulated C fluxes with increases in gross primary productivity (+26.4%), ecosystem respiration (+23.3%), and net ecosystem exchange (NEE, +31.5%) relative to the control. High (10 g N m^{-2} y^{-1}) and extreme (20 g N m^{-2} y^{-1}) amounts of N enrichment, however, had relatively minor impacts on these CO_2 fluxes. Overall, we observed a decrease in soil electrical conductivity (−24.6%) and increases in soil organic C (+25.2%) and microbial biomass C (+369.3%) for N enrichment. N enrichment also altered the composition of plant species, with a higher proportion of a local dominant species (*Phragmites australis*), and affected root biomass distribution, with more biomass near the soil surface. Structural equation modeling explained 65.2% of the variance of NEE and supported the assumption that N enrichment could alter the dynamics of soil properties and plant conditions and accelerate ecosystem CO_2 sequestration. These findings have important implications for forecasting the C cycle with increasing N deposition in coastal wetlands, contributing to the projections of the global C budget.

1. Introduction

Coastal wetlands are productive ecosystems where organic carbon (C) decomposes slowly in soil, so they function as reservoirs of 'blue carbon' and contribute as a natural climatic solution (Hopkinson *et al* 2012, Macreadie *et al* 2021). Recent studies on sinks of 'blue carbon' have emphasized the urgent need to restore and protect coastal wetlands due to their crucial role in global C sequestration (Han *et al* 2015). The influence of the increased deposition of nitrogen (N) on terrestrial C sinks, particularly in forests, has received growing attention over the years (Reay *et al* 2008, Liu *et al* 2022). The effects and underlying mechanisms of N deposition on ecosystem C sequestration in coastal wetlands, however, remain subjects of ongoing debate. This uncertainty introduces noticeable uncertainties in global C budgets and model projections (Ward *et al* 2020).

Global annual N deposition rates have experienced a three- to fivefold increase over the past century, and projections suggest a further two- to threefold rise by the century's end (Lamarque *et al* 2005). The N and C cycles are intricately linked

(Gruber and Galloway 2008). Numerous studies have indicated that the effect of N deposition on the soil C sink relies on the interplay between two factors: (1) N-induced increases in soil C input of by stimulated plant growth and (2) N-induced losses of soil C from processes such as the decomposition of soil organic C (SOC), respiration, and mineralization (Reay *et al* 2008). For coastal wetlands that have been widely identified as N-limited ecosystems, long-term N enrichment could increase soil C respiration by altering the dynamics of soil, microbe, and plant communities (Macreadie *et al* 2021). Soil N availability could also regulate environmental responses to higher CO_2 levels, increased temperatures, and alterations in water availability, further influencing soil C dynamics (LeBauer and Treseder 2008, de Vries *et al* 2014, Du *et al* 2020). Plant growth and ecosystem function can be influenced by alterations in N cycling and soil quality caused by N deposition. Field studies support that N deposition could enhance plant growth and biomass accumulation in coastal wetlands (Guan *et al* 2019, Qu *et al* 2022), indicating the ability to take up more C with increasing N deposition. Increased inputs of soil C from aboveground litterfall and belowground root biomass could amplify soil C decomposition and release more C into the atmosphere. Increased N availability may also alter the soil environment (e.g. pH and electrical conductivity) and soil microbial activity and biomass, further affecting the levels and forms of N in coastal wetlands associated with soil heterotrophic respiration (Tao *et al* 2018). For example, moderate N addition could increase the rate of soil respiration, but extreme N input could lower soil pH, decrease microbial biomass, and decrease soil respiration (Wang *et al* 2019). Recent studies have also suggested that N addition could influence plant biomass distribution, regulating soil C cycling and balance (Li *et al* 2021, Keller *et al* 2023). The effects of N deposition on the sequestration and balance of ecosystem C are thus intricate and involve interactive plant and soil dynamics. Assessing the impact of N enrichment on C cycling and sequestration in coastal wetlands is challenging due to its combined direct and indirect effects on carbon cycle processes (Geoghegan *et al* 2018).

Our knowledge of the N effects on C cycling is growing, but we need an integrated understanding of its processes and mechanisms in coastal wetlands. We, therefore, conducted long-term field experiments in a coastal wetland of the Yellow River Delta (YRD), China. These experiments involved eight years (2012–2020) of N enrichment, subsequent with a campaign of measurements of soil properties, plant traits, and fluxes of ecosystem CO_2. The study had a dual purpose: firstly, to investigate the impacts of different levels of N enrichment on CO_2 sequestration in a coastal wetland, and secondly, to establish a connection between N-induced alterations in the soil environment, plant dynamics, and the strength of the CO_2 sink in the wetland.

2. Methodology

2.1. N-fertilization experiment

The study was initiated in 2012 at the Ecological Research Station of Coastal Wetlands of YRD, Chinese Academy of Sciences (37°36′N, 118°57′E) (figure 1(a)). The YRD coastal wetlands are a relatively young and well-preserved ecosystem, with a rate of atmospheric N deposition of approximately 2.1 $g\ N\ m^{-2}\ y^{-1}$, a mean annual temperature of 13 °C, and a total rainfall of 560 $mm\ y^{-1}$ (Guan *et al* 2019). The soil water salinity is relatively high due to the tide, ranging from 5 to 30 $g\ l^{-1}$ (Chu *et al* 2018). The dominant herbaceous species in the area are *Phragmites australis* and *Suaeda salsa*, with average heights of ~1.7 m (Han *et al* 2015).

We established 20 experimental plots (1 m × 1 m) in an expansive field (50 plots in total), which were sparsely distributed to prevent the potential movement and loss of N (figure 1(b)). We tested four rates of N fertilization (N0: 0 $g\ N\ m^{-2}\ y^{-1}$, N5: 5 $g\ N\ m^{-2}\ y^{-1}$, N10: 10 $g\ N\ m^{-2}\ y^{-1}$, and N20: 20 $g\ N\ m^{-2}\ y^{-1}$), with each rate having five replicates, for a total of 20 plots. The selected fertilization rates were aimed at replicating levels of anthropogenic atmospheric nitrogen deposition ranging from normal to severe. Specifically, these rates were used to simulate normal (N0), moderate (N5), high (N10), and extremely high (N20) rates of atmospheric N deposition (Guan *et al* 2019, Wilcots *et al* 2022). We evenly sprayed ammonium nitrate (NH_4NO_3), a common fertilizer (Ahlgren *et al* 2008) dissolved in 1.2 l of deionized water, once a month to the fertilized plots since 2012. For N0 control plots, we applied deionized water without fertilizer to ensure a consistent soil-water content. To prevent the plants from intercepting the fertilizer, we applied the fertilizer directly onto the soil or the roots of the plants through spraying.

2.2. Measurement of fluxes of ecosystem CO_2

To measure fluxes of ecosystem CO_2, we used an Ultraportable Greenhouse Gas Analyzer (U-GGA 915, GLA132 Series, Los Gatos Research Inc., San Jose, USA) attached to a transparent chamber (diameter: 0.4 m; height: 1 m) (figure 1(c)). We inserted one circular frame (diameter: 0.4 m; height: 0.1 m) into the soil in each plot at a depth of 7 cm in July 2018. We kept two small electric fans operating continuously during the measurements to ensure proper air mixing inside the chamber. Additionally, when the plants exceeded a height of 1 m, we introduced an additional chamber measuring (diameter: 0.4 m; height: 0.7 m) for the measurements. After the gas concentration in the chamber began to increase linearly, nine

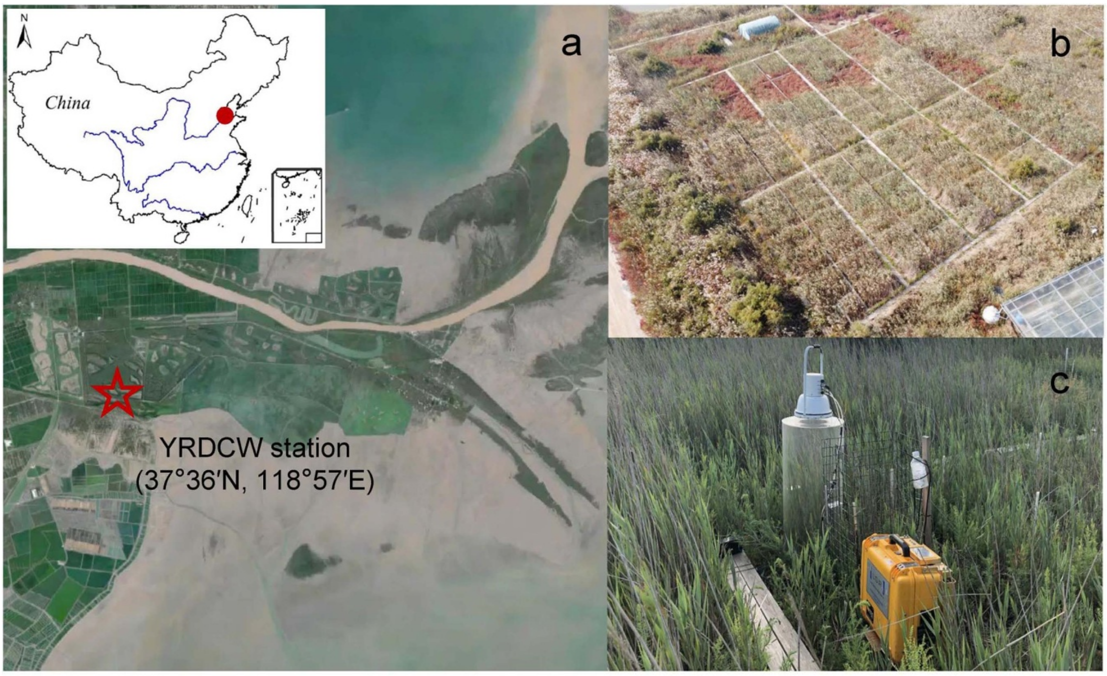


Figure 1. Descriptions of field experiment. (a) The location of the Yellow River Delta Coastal Wetlands (YRDCW) station with the corresponding latitude and longitude. The background is a high-resolution satellite image of YRD. (b) Bird view of the experimental field taken by a drone. (c) The equipment used to measure the fluxes of ecosystem CO_2.

CO_2 concentrations were measured in succession at 10 s intervals (Sun *et al* 2022). NEE was determined by calculating the rate of change in CO_2 concentration over time. Subsequently, the chamber was ventilated, repositioned on the frame, and covered with an opaque cloth to measure ER. In our study, GPP was determined as the difference of ER and NEE. It should be noted that positive values of GPP and ER represent opposite directions of C fluxes and negative (positive) NEE suggests an ecosystem C sink (source).

To examine the effects of eight-year N enrichment on C fluxes, we conducted two-year continuous measurements of CO_2 fluxes. Ecosystem CO_2 fluxes were measured twice a month on clear and sunny days at noon (around 12:00) from 1 January 2019 to 31 December 2020. The mid-day GPP and NEE represent the daily peak of corresponding CO_2 fluxes, to some degree, amplifying the N signal of CO_2 sink strength (Xia *et al* 2009, Sun *et al* 2022). It should be noted that the scaling of CO_2 fluxes that converts once-a-day values to daily total or averaged values is only possible with hourly continuous measurements due to varying diurnal patterns of CO_2 fluxes throughout different seasons (Reichstein *et al* 2005). In our study, we only focused on the directly measured dynamics of mid-day CO_2 fluxes under N enrichment, excluding the uncertainties caused by the scaling processes.

2.3. Measurement of plant indicators

We measured multiple plant traits including aboveground and root biomass, total number of plants, and the leaf area index (LAI). We applied an ACCUPAR LP-80 ceptometer (METER Group, Pullman, USA) to measure LAI in August 2020. We evenly divided each plot ($1\ \mathrm{m} \times 1\ \mathrm{m}$) into four quadrants using a cross at the end of the growing season. Then we randomly selected a quadrant, which represents 1/4 of the total area ($0.25\ \mathrm{m} \times 0.25\ \mathrm{m}$), to determine total number of plants and percentage of each plant species, and to collect plant aboveground biomass. For the sampling, we trimmed the aboveground vegetation to ground level, removed standing dead material, and classified the vegetation by type. The species composition was determined as the percentage of plant number for each species we sampled. For root sampling, we used a root auger (diameter of 10 cm) to take root samples, and then calculated the root biomass of each sample plot based on the area ratio. We collected the roots at multiple depths to measure their biomasses: 0–10, 10–20, 20–30, and 30–40 cm. The roots were divided into coarse and fine roots, defined by diameters $\geqslant 2$ and <2 mm, respectively. We placed the plant aboveground and root samples into sturdy envelopes and subjected them to a 48 hour drying process in an oven set at 80 °C to measure aboveground biomass and root biomass ($\mathrm{g\ m^{-2}}$).

2.4. Measurement of soil properties and microbes

We collected soil samples (20 cm depth) and measured data for soil properties in October 2020 in five randomly selected experimental plots (1 m × 1 m) in July 2020. The soil samples were air-dried for measuring soil properties. We measured extractable N (μg g^{-1} dry soil) in the soil samples, i.e. the total amount of NH_4^+-N and NO_3^--N, by mixing 3 g of soil (dry mass) with 15 ml of 2 M potassium chloride (KCl). Soil pH was potentiometrically determined in a 1:5 (w/v) soil: water suspension using a portable pH meter. We employed three parameter sensors for soil electrical conductivity measurement (EC, ds cm^{-1}) in the top 10 cm of soil. The soil samples for SOC analysis were pretreated with hydrochloric acid (1 mol l^{-1}) to remove inorganic C. The samples were subsequently analyzed using the elemental analyzer (Vario MACRO cube, Elementar Analysensysteme, Germany).

We used soil samples (fresh soil) to determine microbial biomass C (MBC) and microbial community structure under different N treatments. In our study, the measurement of MBC was carried out using a Shimadzu TOC analyzer (TOC-VCPH) along with the chloroform-fumigation extraction method (Brookes *et al* 1985). To examine the microbial community structure, we utilized the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc., USA) for the extraction of total DNA from the samples, which were subsequently stored at −20 °C. In order to identify functional microorganisms, the bacterial 16 S rRNA gene in V3-V4 fragments was targeted using the primers 341F and 806R (Mori *et al* 2014). The reaction setup consisted of a total volume of 50 μl, including 25 μl of 2 × Primer Taq (TaKaRa, RR902A), 1 μl of forward primer (10 mM), 1 μl of reverse primer (10 mM), 3 μl of g-DNA, and 20 μl of nuclease-free water. The thermal cycling protocol involved the following steps: (1) an initial denaturation at 94 °C for 5 min, (2) 31 cycles of denaturation at 94 °C for 30 s, (3) annealing at 52 °C for 30 s, (4) extension at 72 °C for 45 s, (5) a final extension at 72 °C for 10 min, and (6) a hold at 72 °C before purification (Xu *et al* 2016). Gel electrophoresis was employed to analyze the PCR products, while pyrosequencing was performed on the MiSeq platform. To ensure data quality, Trimmomatic-0.33 was utilized for data filtering. Additionally, the MOTHUR project (Li *et al* 2022) was used to identify and remove any reads suspected to be chimaeras. Operational taxonomic units (OTUs) at the species level were generated by setting a threshold of $\geqslant$97% to quantify the similarity between sequences (Xu *et al* 2016, Gu *et al* 2019). Taxonomic assignments were completed using the UCLUST method with the UNOISE3 algorithm for error-correction, adopting a similarity threshold of 0.5. Finally, reference sequences were obtained from the Greengenes OTU database (gg_13_8_otus, http://qiime.org/home_static/dataFiles.html). We measured the soil bacterial compositions for topsoil (0–20 cm) and subsoil (20–40 cm).

2.5. Statistical analyses

The N level was the only controlling factor in our experiment, so we performed a one-way analysis of variance (ANOVA) using IBM SPSS Statistics (www.ibm.com/spss). Statistical significance was determined at $p < 0.05$. We used Pearson correlation analyses to assess the associations among the soil properties, plant growth, species composition, biomass distribution, and CO_2 fluxes. We used structural equation modeling (SEM) to examine the underlying processes and mechanisms to quantitatively elucidate the direct and indirect effects of N enrichment on ecosystem C sequestration (i.e. NEE). We assumed that N enrichment regulated NEE by affecting the soil properties, plant growth, species composition, and distribution of root biomass. We excluded the extreme N treatment (N20) from the SEM to better examine the responses of NEE to N enrichment. To assess the goodness-of-fit of the SEM, we calculated several metrics outlined by Hair *et al* (2017): the Goodness-of-Fit Index (GFI), the standardized root mean square residual (SRMR), the ratio of χ^2 to the degrees of freedom, and the root mean square residual (RMR). Due to the limited sample size (<100), we utilized partial least squares in the SEM analyses using the 'Lavaan' package in R (v.4.1.1) (Rosseel 2012). In our study, we measured two-year C fluxes (from January 2019 to December 2020) and one-year plant-soil indicators (2020). Our main analyses were based on measurements in 2020 to avoid potential uncertainty caused by unmatched time frame.

3. Results

3.1. Impacts of long-term N enrichment on the fluxes of ecosystem CO_2

The temporal patterns of the CO_2 fluxes (i.e. NEE, ER, and GPP) in the N treatments were similar in 2019 and 2020 (figures 2(a), (c), and (e)), with an upward (downward) trajectory followed by a peak and subsequent decrease (increase). We detected considerable discrepancies, especially in the rainy season (from 15th July to 15th September). Moderate N enrichment (N5) stimulated CO_2 fluxes with increases in NEE (+31.5%, $p < 0.05$), ER (+23.3%, non-significant) and GPP (+26.4%, $p < 0.05$) throughout the full year, growing season, and rainy season

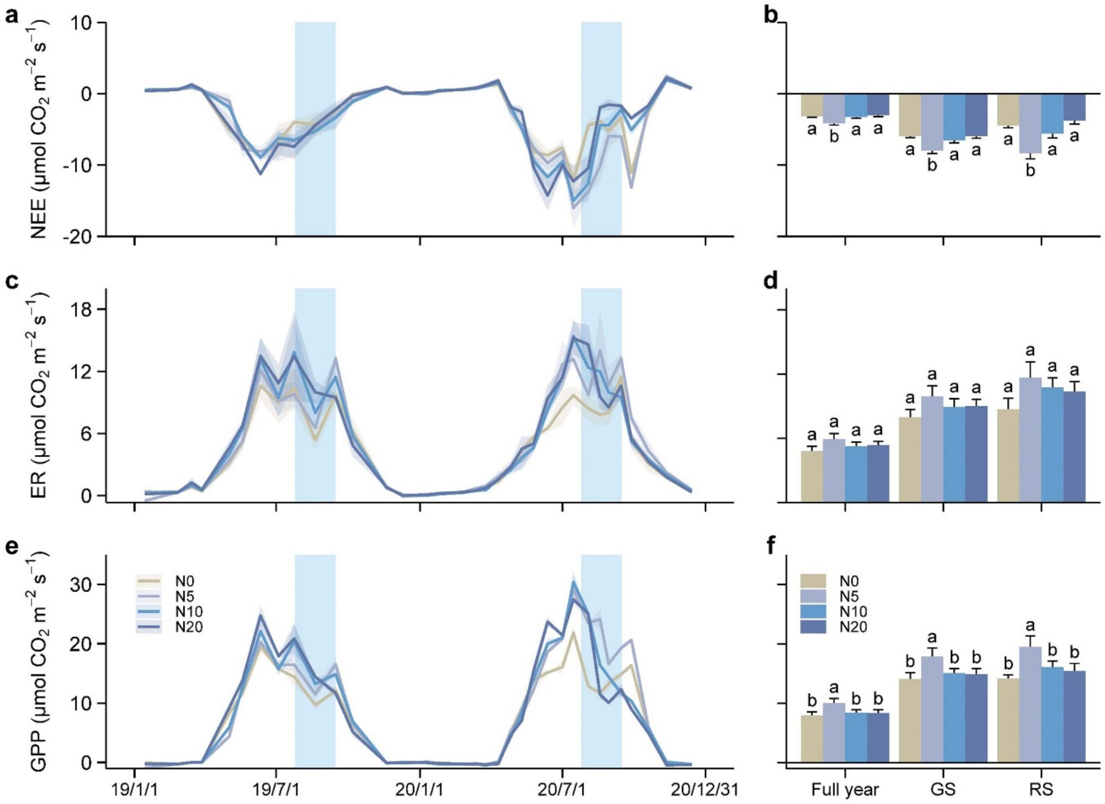


Figure 2. Effects of long-term N enrichment on the fluxes of ecosystem CO_2. (a), (c), and (e). Variations of net ecosystem exchange (NEE), ecosystem respiration (ER), and gross primary productivity (GPP) (means ± standard deviations) for 2019 and 2020, respectively. The light-blue bars represent the rainy season (RS, from 15th July to 15th September). (b), (d), and (f). Measurements for the entire year, the growing season (GS, NEE < 0), and the RS. The N-enrichment treatments, denoted as N0, N5, N10, and N20, correspond to the application of 0, 5, 10, and 20 g N m^{-2} y^{-1}, respectively. Different letters represent significant differences ($p < 0.05$).

(figures 2(b), (d), and (f)). High (N10) and extremely high (N20) N enrichment had relatively minor effects on ecosystem CO_2 fluxes.

3.2. Responses of plant dynamics to long-term N enrichment

Aboveground biomass was generally higher with N enrichment, 45.4% higher in N10 than N0 (figure 3(a)). The number of plants was slightly higher in N5 (+21.4%) and N10 (+21.3%) and slightly lower (−8.1%) in N20 relative to N0 (figure 3(c)). The impact of N enrichment on LAI was not obvious, but LAI was highest in N5 (figure 3(d)). The *P. australis* and *S. salsa* were dominant species over the fields (figure 3(b)). The ratio of these two dominant species showed a significant difference ($p < 0.05$) in species composition under N5 (+14.5%) and N10 (+12.8%) relative to N0 (figure 3(e)). The biomasses of coarse and fine roots were significantly higher ($p < 0.05$) by 126.7% and 50.8%, respectively, in N10 than in N0 (figure 4(a)). The ratio of coarse-root to fine-root biomass was slightly higher under N enrichment (figure 4(b)). N enrichment relocated more of the coarse-root biomass into near-surface soil (figure 4(c)) but had a minor impact on the distribution of fine-root biomass (figure 4(d)).

3.3. Effects of long-term N enrichment on soil properties and microbes

The concentration of extractable N was significantly higher ($p < 0.05$) in N5, N10, and N20 than in N0 by 71.8, 197.7, and 371.4%, respectively (figure 5(a)). Soil pH did not differ significantly among the N treatments (figure 5(b)). EC was significantly lower ($p < 0.05$) in N5, N10, and N20 than in N0 by 13.8, 29.4, and 30.5%, respectively (figure 5(c)). SOC concentration was significantly higher ($p < 0.05$) in N5 (22.5%), N10 (33.9%), and N20 (19.9%) compared to N0 (figure 5(d)). We found apparent increases in MBC under N enrichment, with 378.5%, 301.9%, and 427.5% increases in N5, N10, and N20, respectively, compared to N0 ($p < 0.05$) (figure 6(a)). Soil bacterial compositions and corresponding percentages were overall similar under different N treatments in topsoil (0–20 cm) and subsoil (20–40 cm) (figures 6(b) and (c)), with relatively higher proportions for *Proteobacteria* (topsoil: 29.7 ± 1.7%, subsoil: 34.2 ± 6.8%) and *Chloroflexi* (topsoil: 20.7 ± 0.7%, subsoil: 15.5 ± 3.3%).

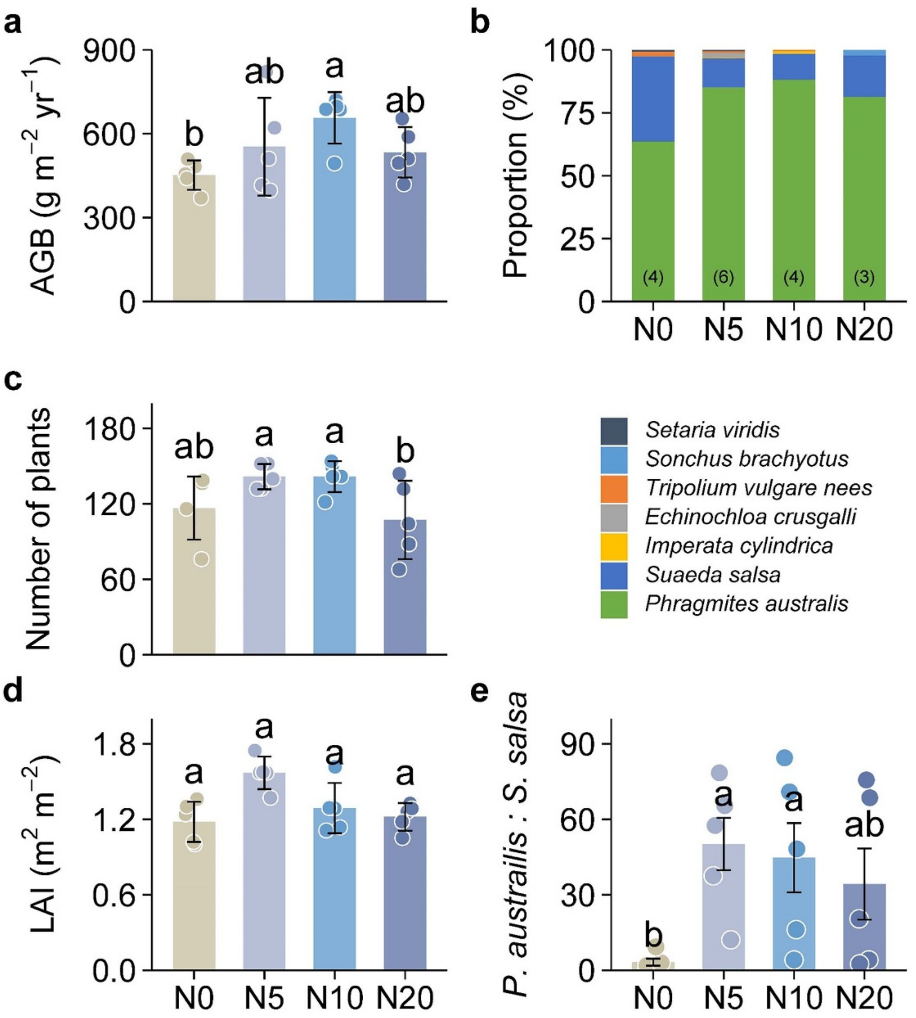


Figure 3. Effects of long-term N enrichment on plant growth and species composition. (a), (c), and (d). Distributions of aboveground biomass (AGB), number of plants, and the leaf area index (LAI) (means ± standard deviations), respectively. (b) Species composition. The numbers in the columns represent the total number of species (i.e. species richness) in the treatments. (e) Ratio of the two dominant plant species (*P. australis* and *S. salsa*). The N-enrichment treatments, denoted as N0, N5, N10, and N20, correspond to the application of 0, 5, 10, and 20 g N m^{-2} y^{-1}, respectively. The dots represent the value of replicated observations (n = 5). Different letters represent significant differences ($p < 0.05$).

3.4. Mechanisms of N effects on ecosystem C sink in the coastal wetland

Extractable-N concentration was correlated negatively with EC ($r = -0.51$; $p < 0.01$) and positively with SOC concentration ($r = 0.84$, $p < 0.001$), MBC concentration ($r = 0.63$, $p < 0.01$), aboveground biomass ($r = 0.62$; $p < 0.01$), number of plants ($r = 0.55$; $p < 0.05$), root biomass ($r = 0.77$; $p < 0.01$), and the biomass ratio between topsoil (0–20 cm) and subsoil (20–40 cm) ($r = 0.56$; $p < 0.01$) (figure 7). Extractable-N concentration was not significantly correlated with the CO_2 fluxes. The indicators of plant growth (aboveground biomass, number of plants, and LAI), however, were negatively correlated with NEE ($p < 0.05$). The SEM analysis generally confirmed the negative effects of N enrichment on NEE (figure 8). The criteria of the significance of the fitting of the SEM model were satisfied, with $\chi^2 = 8.433$, $p = 0.49$ (>0.05), GFI = 0.94 (⩾0.9), SRMR = 0.07 (⩽0.08), and RMR = 0.06 (⩽0.08). The fitted model explained 65.2% of the variance of NEE. Specifically, higher N availability could directly increase plant growth (i.e. plant number and aboveground biomass) and then decrease NEE. N enrichment could also reduce EC and further increase the ratio of coarse to fine roots and root biomass, leading to negative and positive effects on NEE, respectively.

4. Discussion

4.1. Changes in soil properties and plant dynamics induced by N enrichment

The coastal wetlands in YRD experience periodic tides that cause them to alternately submerge and become exposed, leading to the accumulation of salt and leaching with high EC (Han *et al* 2015). This environmental stressor could influence soil microbial activity and the cycling of soil C (Chambers *et al* 2011). We found that eight-year N enrichment substantially reduced EC (figure 5(c)), consistent with

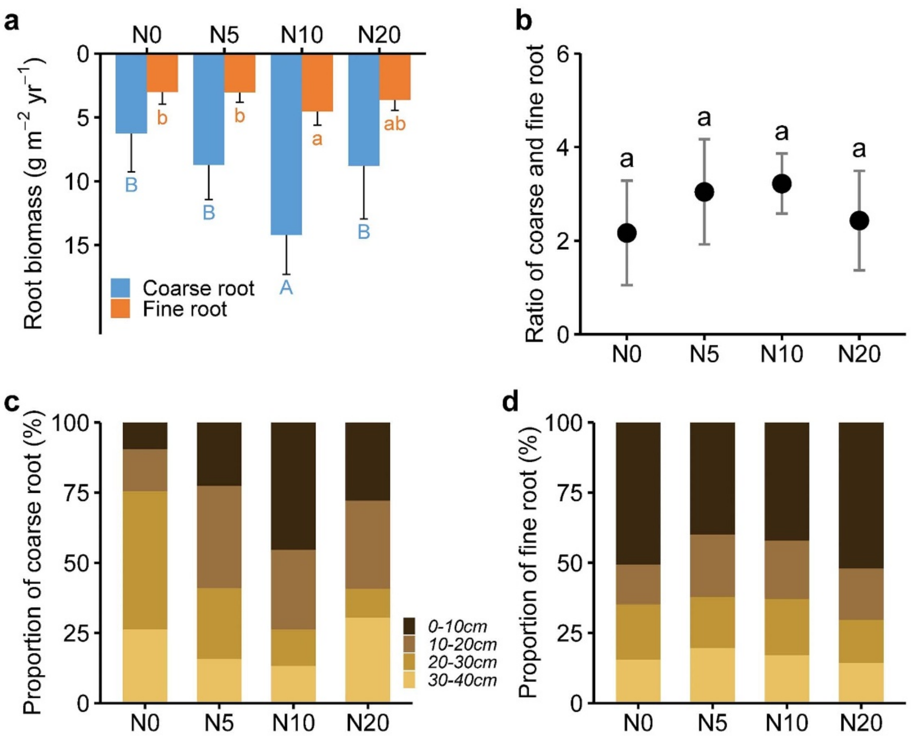


Figure 4. Effects of long-term N enrichment on the distribution of root biomass. (a) and (b) Distribution of root biomass and the ratio of coarse to fine roots (means ± standard deviations), respectively. (c) and (d) Distribution of biomass in gradients of soil depth (0–40 cm) for coarse and fine roots, respectively. The N-enrichment treatments, denoted as N0, N5, N10, and N20, correspond to the application of 0, 5, 10, and 20 g N m^{-2} y^{-1}, respectively. Different lower- and uppercase letters represent significant differences ($p < 0.05$).

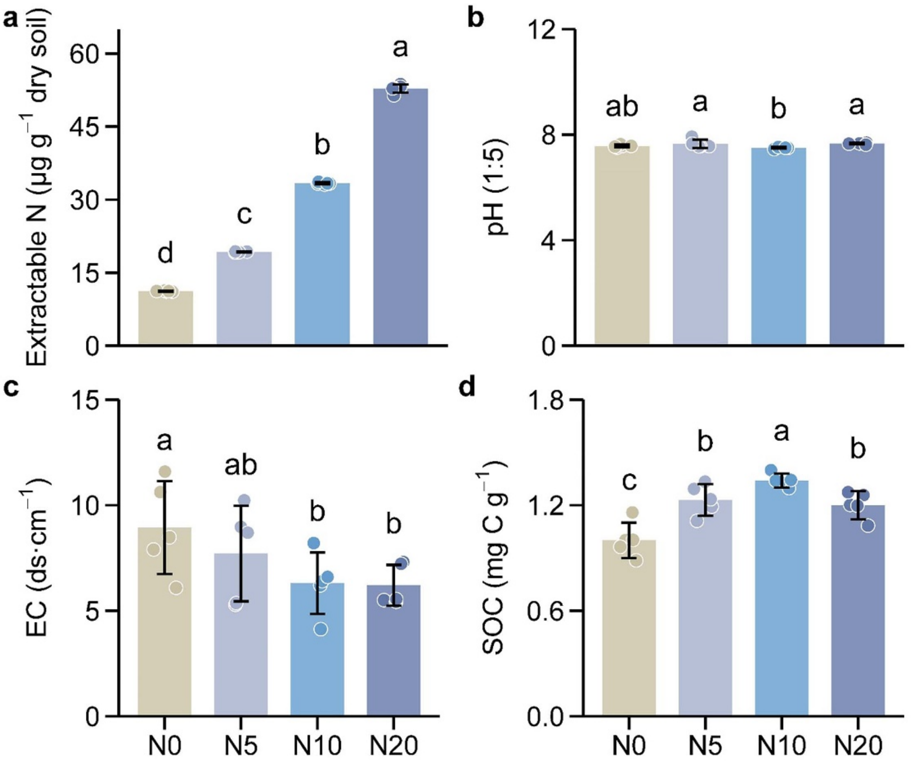


Figure 5. Effects of long-term N enrichment on soil physiochemical properties. (a)–(d). Distributions of soil extractable N (μg g^{-1} dry soil), pH, EC (ds cm^{-1}), and soil organic C (SOC) with N addition (means ± standard deviations), respectively. The N-enrichment treatments, denoted as N0, N5, N10, and N20, correspond to the application of 0, 5, 10, and 20 g N m^{-2} y^{-1}, respectively. The dots represent the value of replicated observations ($n = 5$). Different letters represent significant differences ($p < 0.05$).

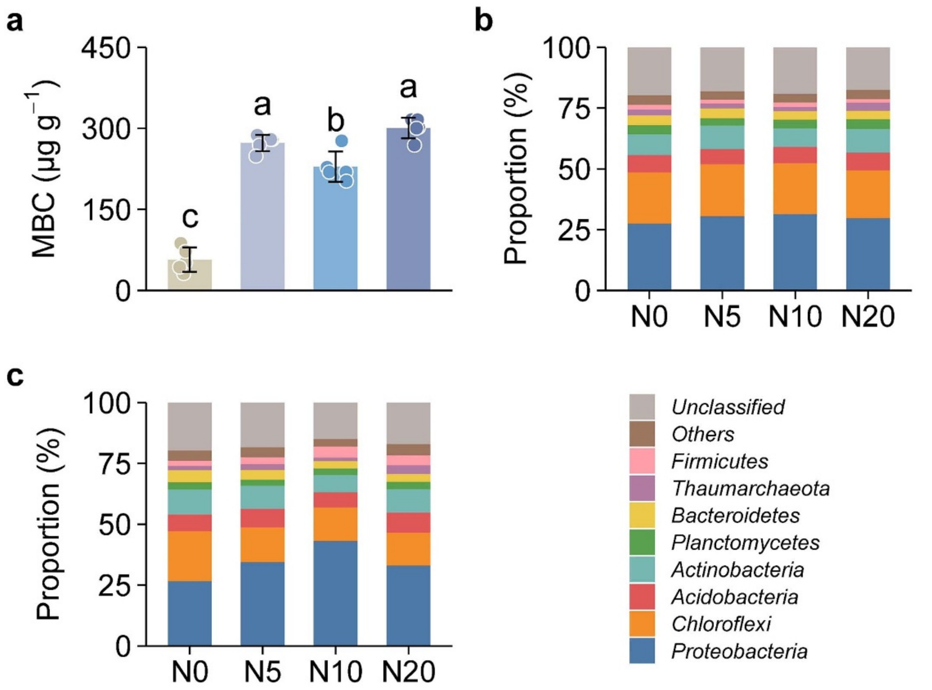


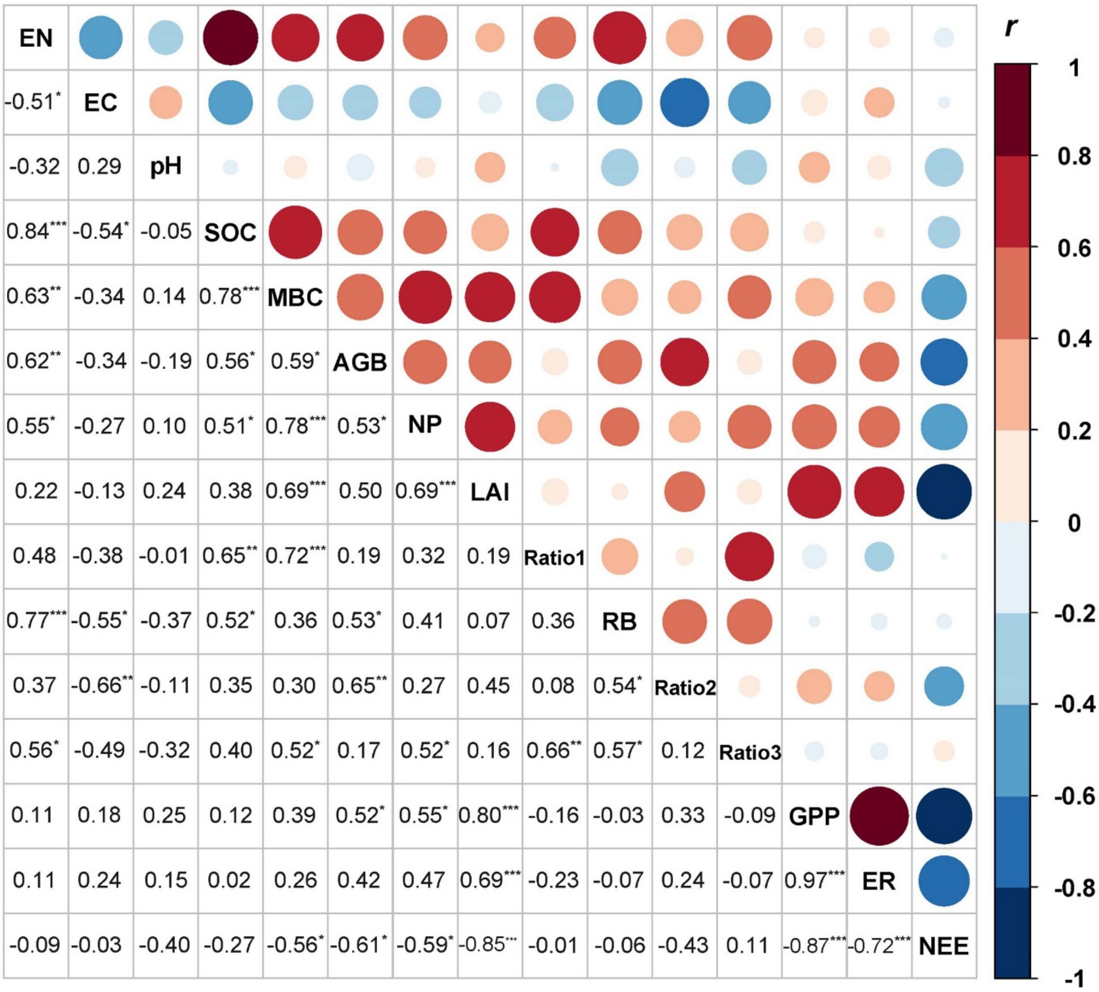
Figure 6. Effects of long-term N enrichment on soil microbes. (a) Distributions of soil microbial biomass C (MBC) with N addition (means ± standard deviations). The dots represent the value of replicated observations ($n = 5$). Different letters represent significant differences ($p < 0.05$). (b) and (c). Soil bacterial compositions with N addition in topsoil (0–20 cm) and subsoil (20–40 cm). The N-enrichment treatments, denoted as N0, N5, N10, and N20, correspond to the application of 0, 5, 10, and 20 g N m^{-2} y^{-1}, respectively.

previous studies (Guan *et al* 2019, Qu *et al* 2019). Increases in the availability of soil N potentially alleviate salinity stress in coastal wetlands, which is beneficial to soil microbes and plant communities (Qu *et al* 2020, Huang *et al* 2022). SOC concentration can be a proxy of the strength of sinks of soil C, and the SOC concentration in our study was significantly higher under N enrichment (figure 5(d)), supporting a stronger C sink controlled by the availability of N. The N-induced increase in MBC helps explain the higher ER under N enrichment (figure 6(a)), to some extent, offsetting the C sink strength in coastal wetlands. Due to the tidal erosion, only salt-tolerant microbes can survive in the coastal wetlands of YRD (Kearns *et al* 2016, Li *et al* 2022), leading to a stable diversity of microbes even with N addition (figures 6(b) and (c)).

So far, we have relatively limited knowledge of the effects of N on plant biophysical dynamics in coastal wetlands. WLong-term N enrichment strongly regulated the species composition of the plants in the YRD coastal wetland, with an increase in *P. australis* and a decrease in *S. salsa* (figure 3(e)). This alteration indicated a species-dependent adaption and suitability under anthropogenic nutrient enrichment (Porter *et al* 2013). Changes in species composition caused by N deposition have the potential to modify microclimates, sources of organic matter, and biogeochemical processes (Hu *et al* 2020). Root traits represent the primary source of input of SOC and C stocks in local ecosystems (Mokany *et al* 2006). Increased N availability in our study stimulated root biomass (figure 5(a)), confirming the nutrient stress of the plant community in this YRD coastal wetland (Guan *et al* 2019). Interestingly, the biomass of coarse roots was relocated from the subsoil to the near-surface soil (figure 4(c)), suggesting the influence of N stress on root production in coastal wetlands (McMurtrie *et al* 2012).

4.2. Effects of long-term N enrichment on the sequestration of ecosystem CO$_2$ in the coastal wetland

We found that moderate N enrichment increased the CO$_2$ fluxes in coastal wetlands. Excessive N input, however, can partially or fully offset these effects, consistent with N saturation previously reported (Wei *et al* 2012, de Vries *et al* 2014). Based on our correlations and SEM analyses, we found a potential link between N enrichment and C sink associated with dynamics of soil and plants (figure 8). Our study generally supported the N-induced increase in CO$_2$ release (Sun *et al* 2023), with a relatively high ER with large increase in MBC under N enrichment (figures 2(c) and 6(a)). More importantly, the increased uptake of CO$_2$ by plants by photosynthesis (GPP) was higher than the increased release of CO$_2$ by ER under N enrichment that led to a higher NEE, suggesting the potential of a stronger CO$_2$ sink under future scenarios with increasing N



EN														
-0.51*	**EC**													
-0.32	0.29	**pH**												
0.84***	-0.54*	-0.05	**SOC**											
0.63**	-0.34	0.14	0.78***	**MBC**										
0.62**	-0.34	-0.19	0.56*	0.59*	**AGB**									
0.55*	-0.27	0.10	0.51*	0.78***	0.53*	**NP**								
0.22	-0.13	0.24	0.38	0.69***	0.50	0.69***	**LAI**							
0.48	-0.38	-0.01	0.65**	0.72***	0.19	0.32	0.19	**Ratio1**						
0.77***	-0.55*	-0.37	0.52*	0.36	0.53*	0.41	0.07	0.36	**RB**					
0.37	-0.66**	-0.11	0.35	0.30	0.65**	0.27	0.45	0.08	0.54*	**Ratio2**				
0.56*	-0.49	-0.32	0.40	0.52*	0.17	0.52*	0.16	0.66**	0.57*	0.12	**Ratio3**			
0.11	0.18	0.25	0.12	0.39	0.52*	0.55*	0.80***	-0.16	-0.03	0.33	-0.09	**GPP**		
0.11	0.24	0.15	0.02	0.26	0.42	0.47	0.69***	-0.23	-0.07	0.24	-0.07	0.97***	**ER**	
-0.09	-0.03	-0.40	-0.27	-0.56*	-0.61*	-0.59*	-0.85***	-0.01	-0.06	-0.43	0.11	-0.87***	-0.72***	**NEE**

Figure 7. Pair-wise Pearson correlation coefficients among the indicators of the soil environment, plant dynamics, and CO_2 fluxes. Pearson correlation coefficients for extractable-N concentration (EN), soil electrical conductivity (EC), pH, concentrations of soil organic C (SOC) and microbial biomass C (MBC), aboveground biomass (AGB), number of plants (NP), leaf area index (LAI), and root biomass (RB). Ratio1, Ratio2, and Ratio3 represent the biomass ratios between *P. australis* and *S. salsa*, coarse and fine roots, and topsoil (0–20 cm) and subsoil (20–40 cm), respectively. GPP, ER, and NEE represent gross primary productivity, ecosystem respiration, and net ecosystem exchange, respectively. *, $p < 0.05$; **, $p < 0.01$; $n = 20$. The size and color of the circles in the upper right correspond to the correlation coefficients displayed in the bottom left.

deposition (Liu *et al* 2005). The positive impacts of N deposition on plant growth and ecosystem C sink in coastal wetlands could also be controlled by N availability, supported by previous studies (LeBauer and Treseder 2008, Johnson *et al* 2016, Li *et al* 2022). We found a lower response of NEE and GPP to extremely high N deposition than to moderate N deposition (figure 2), suggesting a potential tipping point of N deposition effect on ecosystem C sequestration in coastal wetlands. Divergent magnitudes and patterns of C fluxes across wetlands were widely reported, suggesting a high spatial heterogeneity of environmental and climatic responses of C fluxes (Lu *et al* 2017, Hu *et al* 2020). For example, CO_2 emissions (ecosystem respiration) in 21 coastal wetlands ($87.64\ \mathrm{mg \cdot m^{-2} \cdot h^{-1}}$) were much higher than that in inland wetlands ($39.17\ \mathrm{mg \cdot m^{-2} \cdot h^{-1}}$). Even within coastal wetlands, large differences in C fluxes in China and North America were reported (Hu *et al* 2020), highlighting the discrepancy of driving factors.

4.3. Limitations and implications

Limited field experiments inhibit our understanding of the effects of N deposition on CO_2 cycling and ecosystem functions in coastal wetlands, unlike intensively investigated forests. We conducted a field study using eight-year N fertilization to investigate how N enrichment affected CO_2 sequestration, considering relevant biotic and abiotic factors. Our study however, still contained limitations of study design, assumptions and constraints. First, we only measured CO_2 fluxes for two years to analyze the effects of N for only one site. This short study period and limited spatial scale may cause some uncertainty if other environmental factors, e.g. air temperature and precipitation, may jointly influence the effects (Greaver *et al* 2016). Second, limited observations especially in nighttime may influence the responses of C fluxes to N deposition. More frequent measurements (hourly) are needed to reduce the uncertainty and enhance the understanding of N enrichment on daily cycles of

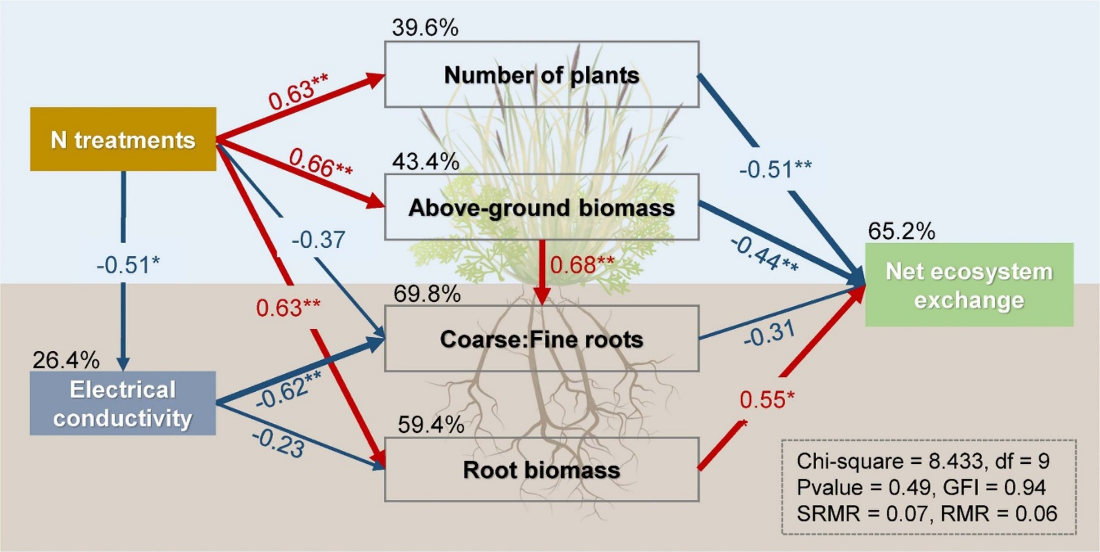


Figure 8. Structural equation modeling (SEM) describing the effects of N enrichment on net ecosystem exchange (NEE) by considering its impacts on soil electrical conductivity (EC), number of plants, aboveground biomass, the ratio of coarse to fine roots, and root biomass. The red and blue arrows represent positive and negative effects, respectively. The thickness of the arrows corresponds to the strength of the relationship. Standardized path coefficients are indicated next to the arrows. The percentages in gray next to the variables indicate the amount of variance explained by the model. *, $p < 0.05$; **, $p < 0.01$; n = 20. The box at the bottom right presents the criteria for assessing the significance of the SEM.

CO_2 fluxes. Third, N enrichment could modify CH_4 and N_2O fluxes, which are important components of the C cycle in wetlands. Related investigations on CH_4 and N_2O responses are still needed in the future to grasp the C-N interactions in coastal wetlands. Our study highlights the effects of N enrichment on the C cycling in a coastal wetland on the basis of long-term field experiments. We found level-dependent and positive impacts of N enrichment on the CO_2 sink in the coastal wetland, which is associated with N-induced shifts in soil and plants. Improving current C models by integrating the effects of N deposition on CO_2 sequestration is urgently and crucially needed. This improvement would contribute greatly to the global C budget and its accurate assessment.

5. Conclusions

Coastal wetlands have a crucial role in mitigating climate change as they serve as important C sinks. The impacts of N enrichment on ecosystem CO_2 cycling, however, still need to be identified, partly due to insufficient field experiments. We investigated the patterns and mechanisms of the effects of N on the sequestration of CO_2 in a YRD coastal wetland in China. We found that long-term N enrichment altered soil properties, plant growth, species composition, and patterns of root biomass. All these changes jointly and interactively stimulated the CO_2 fluxes in coastal wetlands. The N impacts depended on the level of N enrichment, indicating a nonlinear response of the functioning of the ecosystem CO_2 sink to nutrient stress in the wetland. Global N deposition is projected to increase, so our study highlights the potential of stronger CO_2 sinks in coastal wetlands under scenarios of future climate change.

Data availability statement

The data that support the findings of this study are openly available at the following URL/DOI: https://doi.org/10.5281/zenodo.11316363.

Acknowledgments

This research was supported by the National Key Research and Development Program in China (2022YFF0802104), the Natural Science Foundation of China (U2106209, U1906223, 42071126, 42101117), the International Science Partnership Program of the Chinese Academy of Sciences (121311KYSB20190029), the Youth Innovation Promotion Association of Chinese Academy of Sciences (2022051), the Grant TED2021-132627B-I00 funded by the Spanish MCIN, AEI/10.13039/501100011033, and the European Union Next Generation EU/PRTR, and the Fundación Ramón Areces Grant CIVP20A6621.

Author contributions

Baohua Xie and Xiaoyue Wang designed the study. Wendi Qu carried out the experiment, conducted all the analyses, constructed the figures and tables, and wrote the first draft. All authors contributed to the interpretation of the results and the text.

Conflict of interest

The authors declare no conflict of interest.

ORCID iDs

Wendi Qu https://orcid.org/0000-0002-2326-9243
Xiaoyue Wang https://orcid.org/0000-0002-9950-2259

References

Ahlgren S, Baky A, Bernesson S, Nordberg Å, Norén O and Hansson P A 2008 Ammonium nitrate fertiliser production based on biomass–environmental effects from a life cycle perspective *Bioresour. Technol.* **99** 8034–41

Brookes P C, Kragt J F, Powlson D S and Jenkinson D S 1985 Chloroform fumigation and the release of soil nitrogen: the effects of fumigation time and temperature *Soil Biol. Biochem.* **17** 831–5

Chambers L G, Reddy K R and Osborne T Z 2011 Short-term response of carbon cycling to salinity pulses in a freshwater wetland *Soil Sci. Soc. Am. J.* **75** 2000–7

Chu X, Han G, Xing Q, Xia J, Sun B, Yu J and Li D 2018 Dual effect of precipitation redistribution on net ecosystem CO_2 exchange of a coastal wetland in the Yellow River Delta *Agric. For. Meteorol.* **249** 286–96

de Vries W, Du E and Butterbach-Bahl K 2014 Short and long-term impacts of nitrogen deposition on carbon sequestration by forest ecosystems *Curr. Opin. Environ. Sustain.* **9** 90–104

Du E, Terrer C, Pellegrini A F, Ahlström A, van Lissa C J, Zhao X and Jackson R B 2020 Global patterns of terrestrial nitrogen and phosphorus limitation *Nat. Geosci.* **13** 221–6

Geoghegan E K, Caplan J S, Leech F N, Weber P E, Bauer C E and Mozdzer T J 2018 Nitrogen enrichment alters carbon fluxes in a New England salt marsh *Ecosyst. Health Sustain.* **4** 277–87

Greaver T L, Clark C M, Compton J E, Vallano D, Talhelm A F, Weaver C P and Haeuber R A 2016 Key ecological responses to nitrogen are altered by climate change *Nat. Clim. Change* **6** 836–43

Gruber N and Galloway J N 2008 An Earth-system perspective of the global nitrogen cycle *Nature* **451** 293–6

Gu S *et al* 2019 Application of organic fertilizer improves microbial community diversity and alters microbial network structure in tea (Camellia sinensis) plantation soils *Soil Tillage Res.* **195** 104356

Guan B, Xie B, Yang S, Hou A, Chen M and Han G 2019 Effects of five years' nitrogen deposition on soil properties and plant growth in a salinized reed wetland of the Yellow River Delta *Ecol. Eng.* **136** 160–6

Hair J, Hollingsworth C L, Randolph A B and Chong A Y L 2017 An updated and expanded assessment of PLS-SEM in information systems research *Ind. Manage. Data Syst.* **117** 442–58

Han G, Chu X, Xing Q, Li D, Yu J, Luo Y and Rafique R 2015 Effects of episodic flooding on the net ecosystem CO_2 exchange of a supratidal wetland in the Yellow River Delta *J. Geophys. Res.* **120** 1506–20

Hopkinson C S, Cai W J and Hu X 2012 Carbon sequestration in wetland dominated coastal systems—a global sink of rapidly diminishing magnitude *Curr. Opin. Environ. Sustain.* **4** 186–94

Hu M, Sardans J, Yang X, Peñuelas J and Tong C 2020 Patterns and environmental drivers of greenhouse gas fluxes in the coastal wetlands of China: a systematic review and synthesis *Environ. Res.* **186** 109576

Huang L, Bai J, Wang J, Zhang G, Wang W, Wang X and Cui B 2022 Different stochastic processes regulate bacterial and fungal community assembly in estuarine wetland soils *Soil Biol. Biochem.* **167** 108586

Johnson D S, Warren R S, Deegan L A and Mozdzer T J 2016 Saltmarsh plant responses to eutrophication *Ecol. Appl.* **26** 2649–61

Kearns P J, Angell J H, Howard E M, Deegan L A, Stanley R H and Bowen J L 2016 Nutrient enrichment induces dormancy and decreases diversity of active bacteria in salt marsh sediments *Nat. Commun.* **7** 12881

Keller A B, Walter C A, Blumenthal D M, Borer E T, Collins S L, DeLancey L C and Hobbie S E 2023 Stronger fertilization effects on aboveground versus belowground plant properties across nine US grasslands *Ecology* **104** e3891

Lamarque J F, Kiehl J T, Brasseur G P, Butler T, Cameron-Smith P, Collins W D and Thornton P 2005 Assessing future nitrogen deposition and carbon cycle feedback using a multimodel approach: analysis of nitrogen deposition *J. Geophys. Res. Atmos.* **110** D19303

LeBauer D S and Treseder K K 2008 Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed *Ecology* **89** 371–9

Li J, Han G, Wang G, Liu X, Zhang Q, Chen Y, Song W, Qu W, Chu X and Li P 2022 Imbalanced nitrogen–phosphorus input alters soil organic carbon storage and mineralisation in a salt marsh *Catena* **208** 105720

Li X, Zhang C, Zhang B, Wu D, Zhu D, Zhang W and Fu S 2021 Nitrogen deposition and increased precipitation interact to affect fine root production and biomass in a temperate forest: implications for carbon cycling *Sci. Total Environ.* **765** 144497

Liu J, Price D T and Chen J M 2005 Nitrogen controls on ecosystem carbon sequestration: a model implementation and application to Saskatchewan, Canada *Ecol. Modell.* **186** 178–95

Liu M, Shang F, Lu X, Huang X, Song Y, Liu B and Zhu T 2022 Unexpected response of nitrogen deposition to nitrogen oxide controls and implications for land carbon sink *Nat. Commun.* **13** 3126

Lu W, Xiao J, Liu F, Zhang Y, Liu C A and Lin G 2017 Contrasting ecosystem CO_2 fluxes of inland and coastal wetlands: a meta-analysis of eddy covariance data *Glob. Change Biol.* **23** 1180–98

Macreadie P I, Costa M D, Atwood T B, Friess D A, Kelleway J J, Kennedy H and Duarte C M 2021 Blue carbon as a natural climate solution *Nat. Rev. Earth Environ.* **2** 826–39

McMurtrie R E, Iversen C M, Dewar R C, Medlyn B E, Näsholm T, Pepper D A and Norby R J 2012 Plant root distributions and nitrogen uptake predicted by a hypothesis of optimal root foraging *Ecol. Evol.* **2** 1235–50

Mokany K, Raison R J and Prokushkin A S 2006 Critical analysis of root: shoot ratios in terrestrial biomes *Glob. Change Biol.* **12** 84–96

Mori H, Maruyama F, Kato H, Toyoda A, Dozono A, Ohtsubo Y, Nagata Y, Fujiyama A, Tsuda M and Kurokawa K 2014 Design and experimental application of a novel non-degenerate universal primer set that amplifies prokaryotic 16S rRNA genes with a low possibility to amplify eukaryotic rRNA genes *DNA Res.* **21** 217–27

Porter E M, Bowman W D, Clark C M, Compton J E, Pardo L H and Soong J L 2013 Interactive effects of anthropogenic nitrogen enrichment and climate change on terrestrial and aquatic biodiversity *Biogeochemistry* **114** 93–120

Qu W, Han G, Eller F, Xie B, Wang J, Wu H and Zhao M 2020 Nitrogen input in different chemical forms and levels stimulates soil organic carbon decomposition in a coastal wetland *Catena* **194** 104672

Qu W, Li J, Han G, Wu H, Song W and Zhang X 2019 Effect of salinity on the decomposition of soil organic carbon in a tidal wetland *J. Soils Sediments* **19** 609–17

Qu W, Xie B, Hua H, Bohrer G, Penuelas J, Wu C and Han G 2022 Long-term nitrogen enrichment accelerates soil respiration by boosting microbial biomass in coastal wetlands *Soil Biol. Biochem.* **175** 108864

Reay D S, Dentener F, Smith P, Grace J and Feely R A 2008 Global nitrogen deposition and carbon sinks *Nat. Geosci.* **1** 430–7

Reichstein M, Falge E, Baldocchi D, Papale D, Aubinet M, Berbigier P and Valentini R 2005 On the separation of net ecosystem exchange into assimilation and ecosystem respiration: review and improved algorithm *Glob. Change Biol.* **11** 1424–39

Rosseel Y 2012 lavaan: an R package for structural equation modeling *J. Stat. Softw.* **48** 1–36

Sun B, Jiang M, Han G, Zhang L, Zhou J, Bian C and Xia J 2022 Experimental warming reduces ecosystem resistance and resilience to severe flooding in a wetland *Sci. Adv.* **8** eabl9526

Sun H, Chen H, Li J, Zhang Y, Liu X, Li J and Nie M 2023 Nitrogen enrichment enhances thermal acclimation of soil microbial respiration *Biogeochemistry* **162** 343–57

Tao B, Wang Y, Yu Y, Li Q, Luo C and Zhang B 2018 Interactive effects of nitrogen forms and temperature on soil organic carbon decomposition in the coastal wetland of the Yellow River Delta, China *Catena* **165** 408–13

Wang J, Fu X, Zhang Z, Li M, Cao H, Zhou X and Ni H 2019 Responses of soil respiration to nitrogen addition in the Sanjiang Plain wetland, northeastern China *PLoS One* **14** e0211456

Ward N D, Megonigal J P, Bond-Lamberty B, Bailey V L, Butman D, Canuel E A and Windham-Myers L 2020 Representing the function and sensitivity of coastal interfaces in Earth system models *Nat. Commun.* **11** 2458

Wei X, Blanco J A, Jiang H and Kimmins J H 2012 Effects of nitrogen deposition on carbon sequestration in Chinese fir forest ecosystems *Sci. Total Environ.* **416** 351–61

Wilcots M E, Schroeder K M, DeLancey L C, Kjaer S J, Hobbie S E, Seabloom E W and Borer E T 2022 Realistic rates of nitrogen addition increase carbon flux rates but do not change soil carbon stocks in a temperate grassland *Glob. Change Biol.* **28** 4819–31

Xia J, Niu S and Wan S 2009 Response of ecosystem carbon exchange to warming and nitrogen addition during two hydrologically contrasting growing seasons in a temperate steppe *Glob. Change Biol.* **15** 1544–56

Xu N, Tan G, Wang H and Gai X 2016 Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure *Eur. J. Soil Biol.* **74** 1–8