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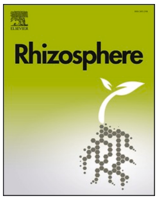

Short-term nitrogen enrichment induces sulfide intrusion in tropical oligotrophic seagrass meadows

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ABSTRACT

Sediment sulfide stress on seagrasses is related to anthropogenic nutrient enrichment and substrate type (e.g., sandy or muddy). This study investigated if anthropogenic nitrogen (N) enrichment resulted in sulfide stress in tropical seagrass *Thalassia hemprichii* inhabiting sandy habitats. Anthropogenic input resulted in 3.4-fold increase in sediment $\delta^{15}N$, and increase in the sediment organic matter and fine fraction content. N enrichment increased sediment sulfur (S) content and caused higher $\delta^{34}S$ depletion in sediment. Higher N availability contributed towards significant increase of *T. hemprichii* shoot density and leaf biomass. Conversely, it reduced the root biomass, and root branching index coinciding with depleted root $\delta^{34}S$ values. Additionally, in the sandy sediments the sediment Fe levels were lower than pristine conditions, suggesting Fe limitation and subsequent increased sediment sulfide intrusion into *T. hemprichii* roots. This highlights *T. hemprichii* growing in sandy sediments with low Fe levels are more prone to sulfide intrusion under N enrichment.

1. Introduction

Seagrass ecosystem provides various ecosystem services, and their decline threatens habitat and nursery for various marine fish, invertebrates and mammals, blue carbon sequestration and coastal nutrient cycles (Unsworth et al., 2022; zu Ermgassen et al., 2021). At present, the global seagrass ecosystems are under decline due to various anthropogenic activities related to coastal pollution and land-use changes derived from various human activities (Hu et al., 2021; Stockbridge et al., 2020). Coastal eutrophication includes nutrient run-offs from agricultural activities, industrial and domestic wastewater discharges etc., that alters the nutrient (e.g., nitrogen) and sediment organic matter (OM) content in the seagrass meadows(Mishra et al., 2025). These changes also lead to an increase in algal growth on the sediments which exacerbates formation of anoxic environment by blocking the direct interaction of water column oxygen and sediment (Jiang et al., 2022; Liu et al., 2022). Under the influence of this anoxic environment, sediment sulfide production increases from anaerobic decomposition of OM. This increased sulfide production combined with hypoxic sediment environment increases sulfide stress and associated toxicity on seagrass meadows leading to seagrass decline and die-offs. Globally several studies have demonstrated that sulfide toxicity due to anthropogenic nutrient influx and OM input is responsible for sudden seagrass die-off events, such as die-off of *Thalassia testudinum* meadows in Florida Bay, USA, *Zostera marina* meadows in Denmark, *Posidonia oceanica* in Balearic Islands, Spain, *Zostera mulleri* and *Amphibolis antarctica* in Australia (Erskine and Koch, 2000; Fraser and Kendrick, 2017; Frederiksen et al., 2006; Holmer and Kendrick, 2013; Marbà et al., 2007).

In general, seagrasses utilize their aerenchyma tissues for the diffusion of oxygen from leaves to roots (i.e., radial oxygen loss; ROL) and maintain an oxic-microsheild around roots (Frederiksen et al., 2006). This oxic environment readily re-oxidizes available sulfide to harmless sulfate, thus allowing seagrass to withstand low levels of sediment sulfide. However, during absence of photosynthetic activity at night the oxygen supply to the water column decreases. During this time the re-oxidation mechanism gets restricted and thus anoxic conditions prevail which increases the effects of sulfide toxicity on seagrasses. Other than surrounding water column factors, the sediment iron (Fe) content and substrate type (e.g., sandy, or muddy) also influences the sulfide dynamics in the sediment of seagrass meadows (Calleja et al., 2007; Holmer et al., 2005). Seagrass growing in carbonate rich sediments are

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particularly vulnerable to increased OM enrichment because the low Fe content in the sediment limits sulfide sequestration in solid-phase pools (e.g., pyrite), thus making the internal oxidation more important (Calleja et al., 2007; Hasler-Sheetal and Holmer, 2015). However, the negative effects of sulfide intrusion are expected when seagrass reoxidation mechanisms (e.g., internal oxygen detoxification or oxygen releasee from below ground tissues) are inhibited. Sulfide can penetrate bimolecular membranes and move rapidly to leaves by diffusion in the gas phase (Raven and Scrimgeour, 1997). Sulfide is considered as a phytotoxin, and negatively affects the photosynthesis by inhibiting photosystem-II, thus reducing growth and meristematic activity, resulting in reduced seagrass survival and increased die-offs (Papadimitriou et al., 2005; Vichkovitten and Holmer, 2005).

The Indian Ocean region hosts the highest diversity of seagrass species in shallow intertidal reef flats and tropical islands (e.g., Lakshadweep Islands of India and Maldives) that are dominated by coral reef derived carbonate sandy sediments (Mishra et al., 2023; Stankovic et al., 2023; McKenzie et al., 2020). In recent years, increase in anthropogenic nutrient (e.g., nitrogen) loading in these tropical islands are linked to the decline of various seagrasses such as *Cymodocea*, *Thalassia* and *Syringodium species* (Kaladharan and Anasukoya, 2019; Mishra et al., 2021a). These anthropogenic inputs include local untreated sewage disposal, aquaculture drainage, small scale industrial drainage and wastewater run-off (Kaladharan and Anasukoya, 2019; Mishra et al., 2024a,b). The effect of changing sediment and nutrient conditions on sulfide intrusion in the tropical seagrass *Thalassia hemprichii* is less understood. Literature archives indicate the demographic decline of *T. hemprichii* due to anthropogenic activities, despite this seagrass remains incredibly important for various endangered turtles (e.g., green sea turtle) and dugongs of the Indian Ocean region (Holmer et al., 2006; Mishra and Apte, 2020; Mishra et al., 2024b).

Therefore, this study aimed to determine if anthropogenic short-term (i.e., one season of < 6months) nitrogen enrichment leads to changes in sediment biogeochemistry (e.g., Fe levels and sulfide intrusion) resulting in decline of seagrass shoot density and root traits in tropical islands of the Indian Ocean region. We hypothesized that i) sulfide intrusion from sediment into seagrass roots and leaves (measured by $\delta^{34}S$) would be highest at sites with the maximum input of anthropogenic nitrogen (measured by $\delta^{15}N$), ii) seagrass $\delta^{34}S$ would be positively correlated with sediment OM concentrations, iii) sediment $\delta^{34}S$ would be negatively correlated with sediment iron (Fe) concentrations. This is a first study on sulfide intrusion in seagrass ecosystems of tropical islands of the Indian Ocean region and the results of this study can serve as a baseline in understanding seagrass response to sulfide intrusion under anthropogenic nitrogen enrichment in coastal waters of the Indian Ocean region, where similar species and environmental conditions exist for *T. hemprichii.* Furthermore, this study can aid in strengthening seagrass conservation, management and restoration practices by alleviating

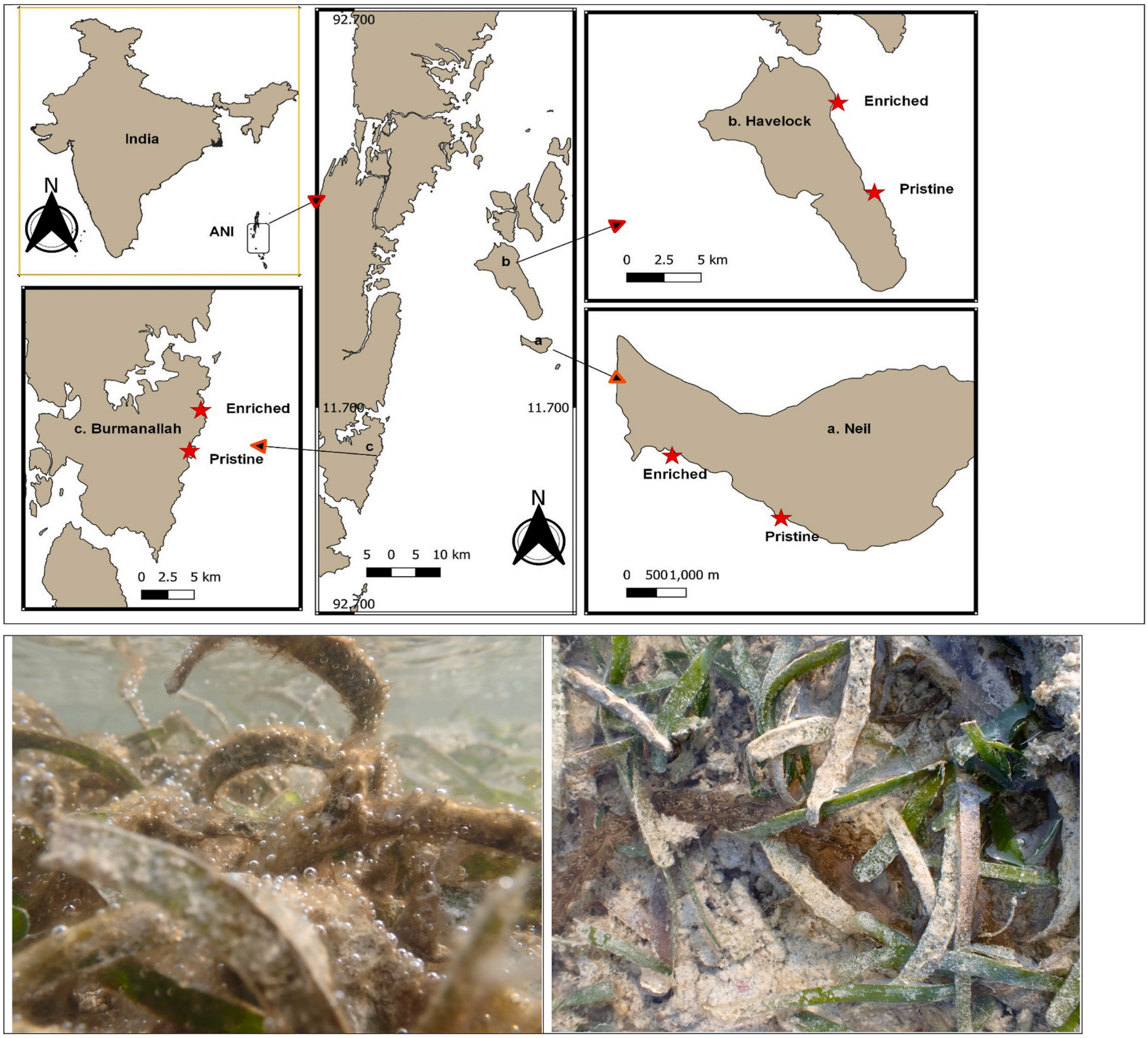

Fig. 1. a) Map of the Andaman and Nicobar Islands (ANI) of India showing the three study locations, and b) algal overgrowth over *T. hemprichii* leaves and sediment as observed during field sampling in ANI, India.

sulfide stress under different nutrient enrichment conditions this region.

2. Materials and methods

2.1. Study sites

Three locations of the Andaman and Nicobar Islands (ANI); Saheed Dweep (hereafter called as Neil), Swaraj Dweep (hereafter called as Havelock), and Burmanallah located in the southeast coast of India were sampled in April–May 2023 (Fig. 1). In each location, two sites were selected, i) pristine and ii) anthropogenically polluted due to nitrogen enrichment (hereafter enriched). The pristine sites were further away from the enriched sites (Fig. 1). These enriched sites were selected based on previous reports of seagrass decline at Neil, Havelock and Burmanallah due to anthropogenic activities (Gole et al., 2023; Mishra et al., 2021b; Sahu et al., 2023). In these enriched sites there is a seasonal chronic enrichment of nitrogen in dry season (when we have sampled) due to various anthropogenic activities. However, in wet season due to high rainfall (3000–3500 mm) and freshwater discharge the enrichment effects of nitrogen are reduced and these habitats change to oligotrophic conditions (Mishra et al., 2025; Sahu et al., 2023). *T. hemprichii* was the dominant intertidal species at all the three locations, inhabiting sandy-muddy substrates, under similar hydrodynamic conditions and was sampled within 0.5 m depth. At each site, five random 20 cm × 20 cm quadrats were placed 2m apart and the seagrass within the quadrat was collected using a hand shovel up to 10 cm depth. Additionally, approximately 50 g of sediment adjacent to each quadrat (surface sediment to 10 cm depth) was collected by inserting a plastic syringe with the tip cut-off. Both seagrass and sediment samples were stored in plastic zip-locked bags, stored at low temperature and transported to the laboratory for analysis.

2.2. Seagrass density, biomass, and morphometric traits

In the laboratory, the number of individual shoots within each quadrat were counted to determine the shoot density. Attached leaf epiphytes and sediment were removed from seagrass leaves by gently scrapping with a plastic blade and washed with deionized water. Seagrass was then sorted into above-ground (AG: leaves) and below-ground (BG: roots and rhizomes separately) tissues. From each quadrat, 10 random roots were measured for the root maximum length and the primary and secondary root ramifications were counted. The root branching index (RBI) was calculated as the total number of root ramifications divided by the root length (Mishra and Farooq, 2023; Sanmartí et al., 2018). After measurements, all AG and BG-tissues were dried at 60 °C for 48 h and weighed for AG and BG-biomass. Dried seagrass tissues were then finely grounded using a disc mill (Retsch, RS200, USA) for total sulfur (S), total nitrogen (N) and metal analysis.

2.3. Seagrass total S, N and isotope ($\delta^{34}S$ and $\delta^{15}N$) analysis

Ground leaves and root samples were weighed into tin capsules and analyzed for total S and N contents and stable isotope ratios (δ^{34}S and δ^{15}N) using a Flash Elemental Analyzer coupled to a Delta V Isotope Ratio Mass Spectrometer (IRMS, Euro Vector, EA3028 EA-Nu). Calibrations and precision (0.2 ‰) were verified using in-house acetanilide standards. Canyon Diablo Trolite (CDT) a meteorite of FeS and atmospheric air were used as standards for S and N, respectively. The δ^{34}S values can provide relative indication of sulfide intrusion into seagrass tissues (e.g., leaves or roots) from either sediment or sulfates from the water column. More negative values of δ^{34}S in seagrass tissues indicate a higher contribution of sulfides from sediment (i.e., −25 ‰ to −15 ‰), while a more positive value indicates the seagrass sulfate intake from the water column (average seawater: +21 ‰) (Frederiksen et al., 2006; Rees et al., 1978).

2.4. Sediment OM, total S, N, isotope ($\delta^{34}S$ and $\delta^{15}N$) and metal analysis

Field collected sediment samples were dried at 60°C for 48 h. Organic matter (OM) content in sediment was determined using the loss on ignition (LOI) method, by combusting at 550 °C for 5 h. For sediment total S and N content and isotope (δ^{34}S and δ^{15}N) analysis, the same method was followed as for the seagrass tissues (see methods section 2.3). For metal (Fe) analysis, the sediment and plant samples ($n = 5$) were digested in a microwave digestion system (Multiwave Pro, Anton Paar) using different temperature and time setting to achieve complete digestion (Mishra et al., 2022; Nazneen et al., 2022). For this purpose, 0.30 mg of sediment samples along with a certified reference material (HISS-1) were microwave digested with 4.5 ml ultrapure Hydrochloric acid (HCl) and 4.5 ml ultrapure Nitric acid (HNO_3). Seagrass tissues of 0.30 mg were microwave digested with 7 ml of ultrapure HNO_3 along with the reference material ERM-CD281. The samples were filtered (0.45-μm polycarbonate filter) and analyzed by a dual view ICP-OES (Mishra et al., 2024a).

2.5. Statistical analysis

All statistical analysis was performed using PRISM software version 10.3.2. Data were pre-checked for normality and homogeneity of variance. Differences in sediment and seagrass traits were investigated using two-way ANOVA. Where significant interactions were detected Holm-Šídák's multiple comparisons test was used. Principal Component Analysis (PCA) was used to determine relationships between sediment δ^{15}N with seagrass traits (root and leaf δ^{34}S ‰, root and leaf Fe levels) and sediment parameters (OM%, fine fraction (<65 μm) %, sand%, δ^{34}S ‰, Fe) between pristine and enriched conditions. C: N ratios were log (Y) transformed to derive linear regressions between sediment N% and leaf C:N ratios. Pearson correlation was used between sediment and seagrass traits for various relationships. Data is presented as mean ± standard deviation (SD).

3. Results

3.1. Influence of N enrichment on sediment traits

In *T. hemprichii* meadows, anthropogenic input resulted in a 3.4-fold increase in sediment δ^{15}N (6.54 ± 0.25 ‰) and 2.5-fold increase of the sediment N (0.72 ± 0.01 %) values compared to pristine conditions (Fig. 2, Supplementary Table S1). This enrichment also resulted in 3.3-fold increase in sediment S (1.51 ± 0.06 %) , resulting in more depletion of sediment δ^{34}S (−6.83 ± 1.15 ‰) than pristine conditions (Fig. 2). Anthropogenic input resulted in a 5.4-fold and 3-fold increase in the sediment OM (14.75 ± 0.49 %) and finer fraction (19.40 ± 1.11 %) respectively than pristine conditions. Under enrichment, increase in sediment fine fraction did not result in in an increase of the mean sediment Fe concentrations (enriched; 132.60 ± 51.28 mg kg^{-1}, pristine; 178.12 ± 38.43 mg kg^{-1}) (Fig. 2, Supplementary Table S2). However, positive relationship between sediment OM versus fine fraction ($R^2 = 0.72$, $p < 0.0001$), and sediment OM versus Fe concentrations ($R^2 = 0.77$, $p < 0.0001$) were observed (Fig. 3, Supplementary Table S3). Interestingly, the sediment Fe concentration showed negative relationship with sediment δ^{34}S ($R^2 = -0.69$, $p < 0.0001$) under enriched conditions (Fig. 3, Supplementary Table S3).

3.2. N enrichment on seagrass traits

N enrichment in sediment showed significant relation with leaf δ^{15}N ($R^2 = 0.33$, $p < 0.001$) and with leaf C:N ratios ($R^2 = 0.73$, $p < 0.0001$), indicating 3.3-fold higher utilization of N by *T. hemprichii* leaves (Fig. 4, Supplementary Fig. S4). Higher N availability resulted in a significant 1.2-fold and 1.6-fold increase of *T. hemprichii* shoot density (1424.06 ± 114.64 shoot m^{-2}) and leaf biomass (126.24 ± 18.74 g DW m^{-2})

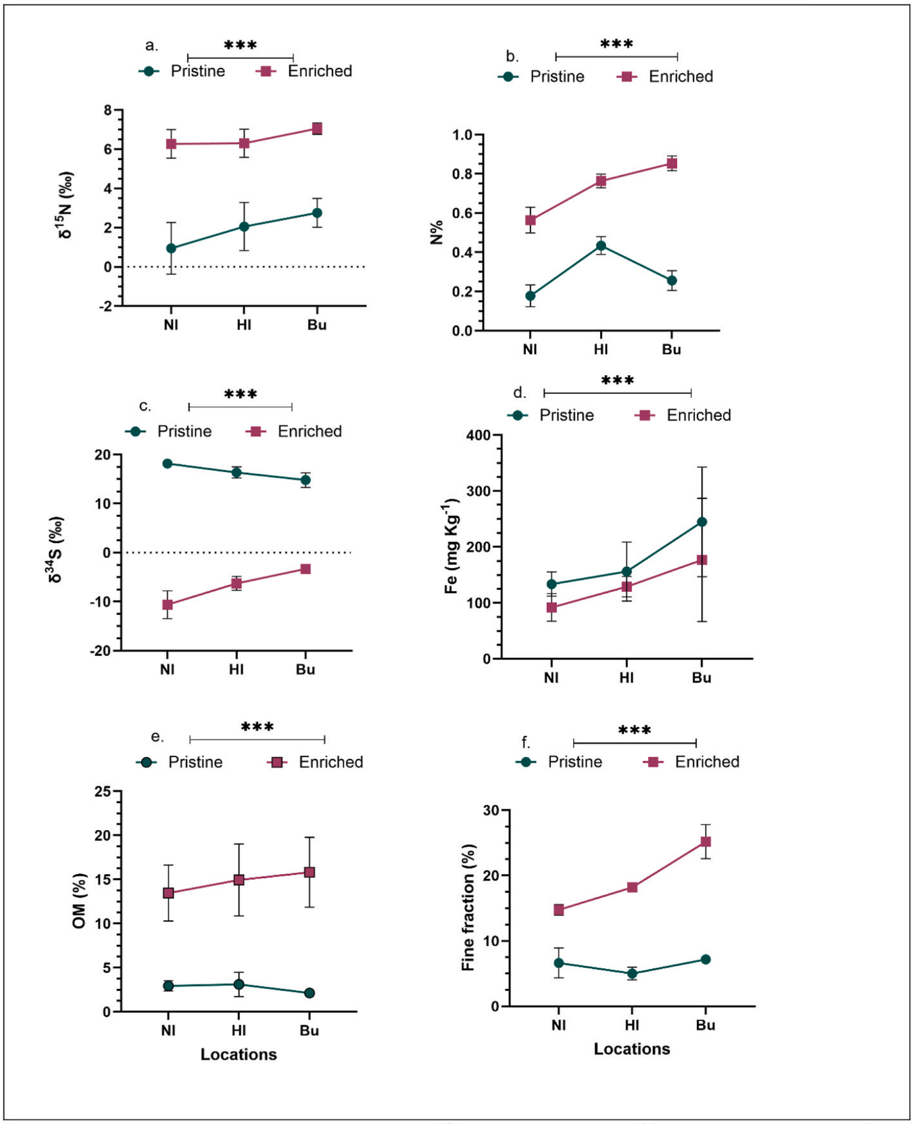

Fig. 2. Mean ± SD of sediment variables a) $\delta^{15}N$ (‰), b) N%, c) $\delta^{34}S$ (‰), d) Fe (mg Kg^{-1}), e) OM%, f) sediment fine fraction% of *T. hemprichii* meadows across pristine and enriched conditions across the three locations of ANI. Statistical significance ($p < 0.05$) values are derived from two-way ANOVA analysis (see Supplementary S1 for more details) using conditions (pristine vs enriched) and locations as fixed factors. ($p < 0.0001$***, $p < 0.001$**, $p < 0.01$*).

respectively, than pristine conditions (Fig. 5). Contrastingly, a significant 2-fold and 1.3-fold reduction in the root biomass (18.44 ± 2.32 g DW m^{-2}), and RBI (3.17 ± 0.17) of *T. hemprichii* was observed under enriched conditions (Fig. 5).

3.3. *Sulfide intrusion under the influence of N enrichment*

Overall, N enrichment related changes in sediment $\delta^{34}S$ were significantly correlated with *T. hemprichii* traits under enriched conditions compared to pristine conditions (Fig. 3, Supplementary Table S3). N enrichment resulted in significant 1.4-fold depletion in leaf $\delta^{34}S$ (13.15 ± 0.46 ‰) than pristine conditions (19.04 ± 0.54 ‰) resulting in a 2.5-fold increase in leaf S (0.87 ± 0.04 %) under enriched conditions (Fig. 4, Supplementary Fig. S5). Similarly, N enrichment showed inverse relationship (i.e. significant depletion) with root $\delta^{34}S$ (−2.17 ± 0.69 ‰) than pristine conditions (15.62 ± 0.70 ‰) resulting in 2.6-fold increase in root S (1.44 ± 0.04 %) under enriched conditions (Fig. 4, Supplementary Fig. S4). Root ^{34}S was positively correlated with sediment fine fraction ($R^2 = 0.59$, $p < 0.001$) and negatively related with sand ($R^2 = 0.60$, $p < 0.001$). Interestingly, at enriched locations despite lower sediment Fe levels than pristine conditions the sediment Fe was positively related with *T. hemprichii* root Fe ($R^2 = 0.98$, $p < 0.0001$) and root $\delta^{34}S$ ($R^2 = 0.70$, $p < 0.0001$) (Fig. 3, Supplementary Table S3).

4. Discussion

4.1. *N enrichment enhances sediment sulfide accumulation*

In this study, we observed anthropogenic N input led to significant site-specific enrichment in sediment $\delta^{15}N$ values compared to pristine conditions. The $\delta^{15}N$ values > 5, confirmed the source of N from anthropogenic input at our study locations (Archana et al., 2018; Thomsen et al., 2020). In this study, we expected an increase in anthropogenic OM input to be correlated with higher sediment sulfide levels and observed a positive correlation between sediment OM and sediment $\delta^{34}S$ (Fig. 3). The increase in sediment OM content also resulted in higher sediment total S, thus, resulted in more depleted sediment $\delta^{34}S$ values highlighting the effects of increased OM on increasing sulfide production through bacterial fractionated $\delta^{34}S$ (Fig. 3). Similar influence of increase in sediment OM content on

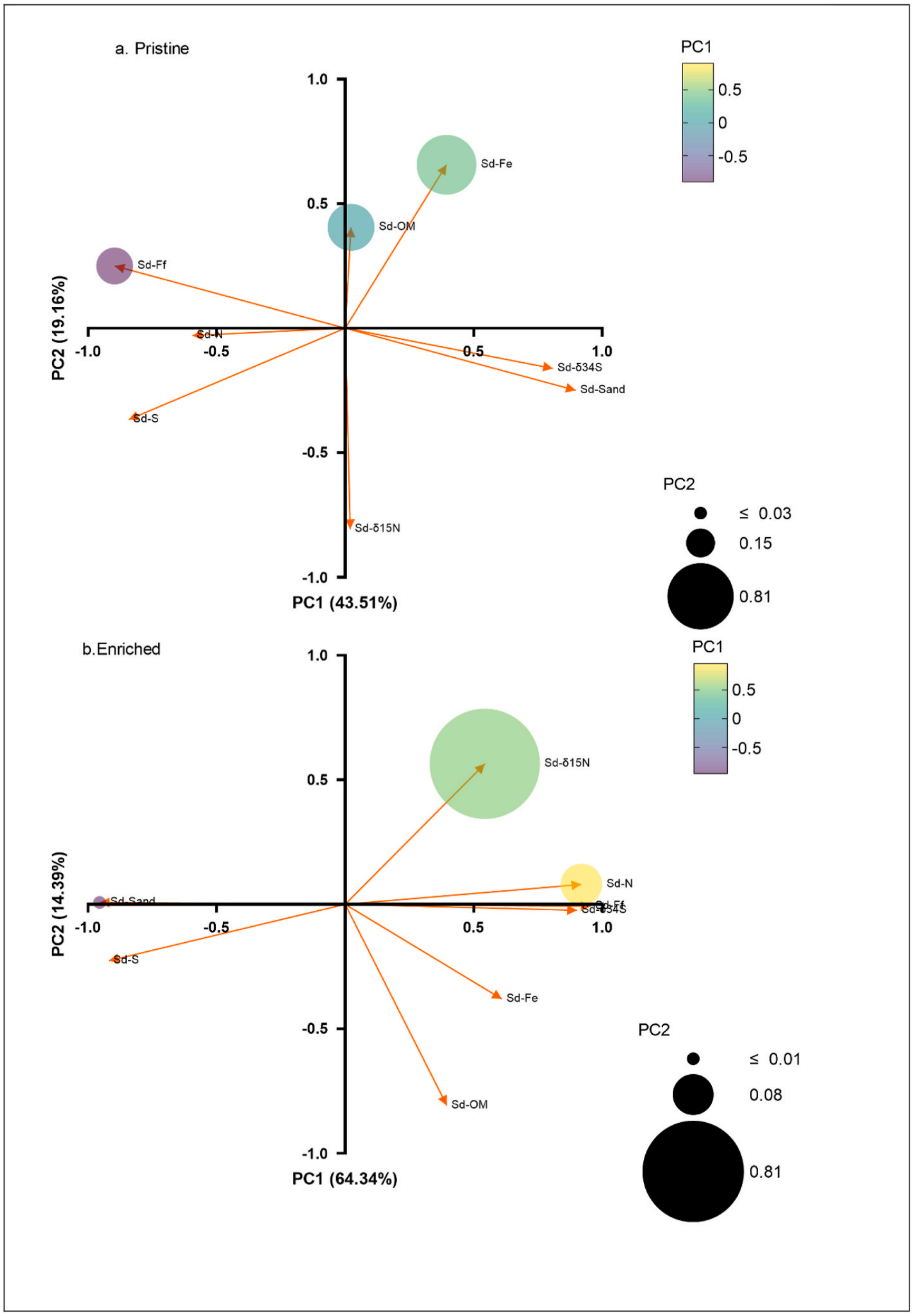

Fig. 3. Loadings of Principal Component Analysis (PCA) between *T. hemprichii* leaf and root ^{34}S with sediment OM, fine fraction, sand and Fe levels across pristine and enriched conditions of ANI, India.

increase in sediment S and depleted δ^{34}S has been observed for *T. hemprichii* meadows from Hainan Island of China under nutrient enrichment from aquaculture activities (Herbeck et al., 2014), and *Zostera muelleri* and *Zostera marina* under anthropogenically enriched conditions in the USA and Australia respectively (Haviland et al., 2022; Holmer et al., 2017). The range of sediment δ^{34}S across pristine (14.64–16.36 ‰) and enriched (−10.63 to −0.98 ‰) conditions of this study are within the global average sediment δ^{34}S values representing signals of seawater sulfate (+21 ‰) and reduced bacterial sedimentary sulfide signatures (−25 to −5 ‰) (Holmer and Hasler-Sheetal, 2014; Rees et al., 1978).

In this study, we expected sediment δ^{34}S to be negatively correlated with sediment Fe levels and observed negative correlation only under enriched conditions (Fig. 3). However, it is interesting to note here that anthropogenic input did not increase sediment Fe levels (Fig. 2). Therefore, the enriched sites with high total S content in sediment had more depleted δ^{34}S signatures, because the available Fe in these highly sandy sediments is bound with sediment S pools (as evidenced from low Fe:S ratios), causing Fe limitation, thus, resulting in low Fe levels in the sediment (Supplementary Table S2 and S6). This effect of Fe in reducing sediment sulfide in carbonated sediments has been observed for *T. testudinum* from the coast of the USA under experimental conditions (Ruiz-Halpern et al., 2008) and from mixed seagrass meadows from the coast of Thailand in the Andaman Sea (Holmer et al., 2006) and in other locations globally (Supplementary Table S7). However, it is for the first time sulfide intrusion in seagrass ecosystems of oligotrophic islands with no natural riverine inputs is studied making it difficult to compare our studies with similar environmental conditions globally (Supplementary Table S7).

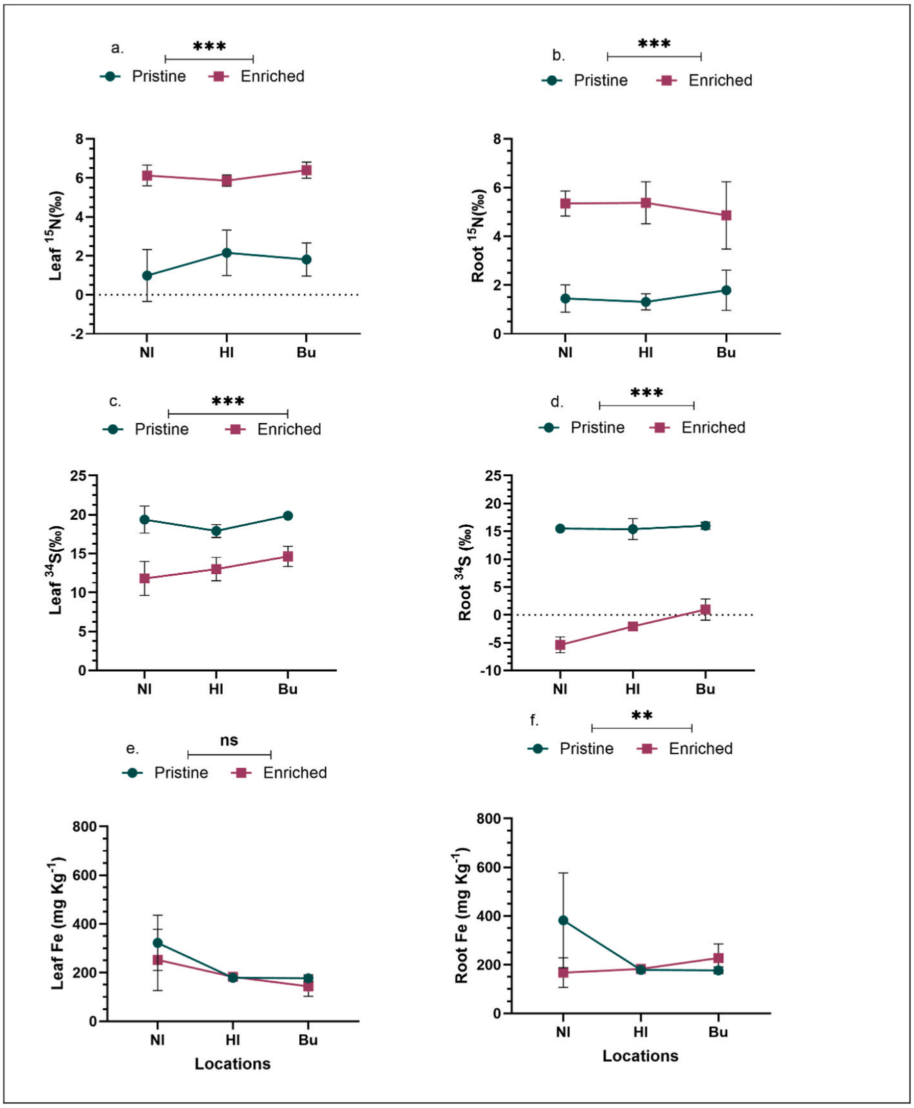

Fig. 4. Mean ± SD of *T. hemprichii* leaf and root variables across pristine and enriched conditions across the three locations of ANI, India. Statistical significance ($p < 0.05$) values are derived from two-way ANOVA analysis (see Supplementary S1 for more details) using conditions (pristine vs enriched) and locations as fixed factors. ($p < 0.0001$***, $p < 0.001$**, $p < 0.01$*, not significant ^{ns}).

4.2. N enrichment and sulfide intrusion from sediment into seagrass tissues

N enrichment resulted in growth and productivity of *T. hemprichii* in the enriched sites resulting in higher shoot density and leaf biomass compared to pristine conditions (Fig. 5). However, this N enrichment did not result in increase in root biomass and root branching index (RBI) indicating the intrusion of sediment sulfide into *T. hemprichii* roots through root tips as gaseous sulfide (MacLeod et al., 2023; Mascaró et al., 2009). This intrusion was further confirmed by the highly depleted δ^{34}S in the roots under enriched conditions (Fig. 4). This depletion of root δ^{34}S was also site-specific (i.e., enriched at Burmanallah (0.94 ± 1.89 ‰) and reduced at Neil (−2.07 ± 0.51 ‰) and Havelock (−5.39 ± 1.40 ‰)) across the three enriched conditions, and was correlated with the variation in anthropogenic N input, sediment OM, fine fraction and Fe levels (Fig. 3). Increased sulfide intrusion into roots reduces root energy (i.e., Adenosine triphosphate; ATP) production and inhibition of growth enzymes that may have affected root biomass formation and hence reduction in root branching (i.e., low RBI), which has been observed in *T. testudinum* under experimental sulfide exposures (Erskine and Koch, 2000). Furthermore, seagrass can mitigate sulfide toxicity by activating anaerobic metabolic pathways and specific biochemical routes to offset energy imbalance (e.g., increase ATP formation) and cytosolic acidosis. However, sulfide toxicity inhibits these mitigation pathways under anoxia or in dark conditions (e.g., at night) leading to severe toxic effects on seagrass root systems, as observed in *Z. marina (*Hasler-Sheetal, 2023*)*. Additionally, we also observed high algal growth on *T. hemprichii* leaf surface and sediments (Fig. 1b) confirming the role of N enrichment related algal growth, decomposition and sediment anoxia (Yang et al., 2018). This influence of N enrichment on increased contribution of algal organic matter to sediment pool of *T. hemprichii* meadows is well established from our study sites (Mishra et al., 2025). However, how decomposition of this algal matter and consequent sediment anoxia influences sulfide toxicity on seagrass roots and leaves is not well-established and needs further investigation.

In this study we expected, seagrass δ^{34}S would be positively correlated with sediment OM, fine fraction and Fe levels, but found no relationship between OM and leaf δ^{34}S, despite the positive relationship between increase in sediment OM and higher depletion of sediment δ^{34}S. However, we observed that δ^{34}S of *T. hemprichii* leaf across the enriched conditions (13.21 ‰) were 1.5-fold lower than the isotopic signature of seawater sulfate (+21 ‰), indicating sulfide intrusion from roots to the

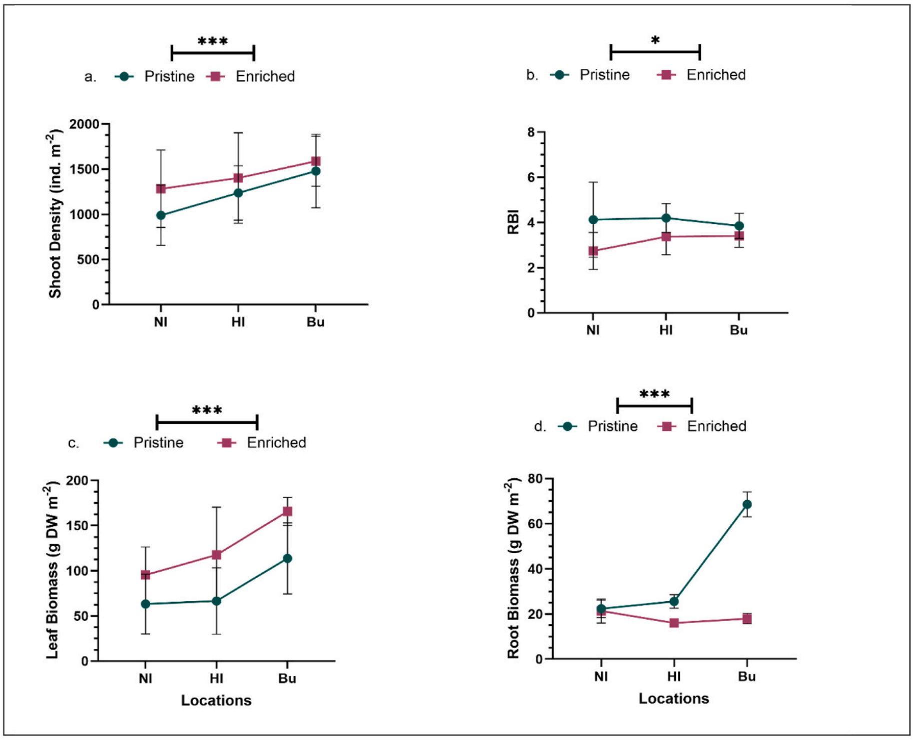

Fig. 5. Mean ± SD of *T. hemprichii* a) shoot density, b) RBI, c) leaf biomass and d) root biomass across pristine and enriched conditions across the three locations of ANI, India. Statistical significance ($p < 0.05$) values are derived from two-way ANOVA analysis (see Supplementary S1 for more details) using conditions (pristine vs enriched) and locations as fixed factors. ($p < 0.0001$***, $p < 0.001$**, $p < 0.01$*).

leaves. This further indicates that the leaves of *T. hemprichii* under enriched conditions were well oxygenated (due to absorption of oxygen directly from the water column) and thus were able to avoid sulfide toxicity by reoxidation of the available gaseous sulfide to non-toxic sulfate. These biological mechanisms helped in increased growth and productivity of *T. hemprichii* leaves and shoots by masking the effects of sulfide toxicity. This mechanism has also been observed in various other seagrasses like *Z. marina* and *T. testudinum* (Hasler-Sheetal, 2023; Oriol Mascaró et al., 2009). However, how these pathways can inhibit sulfide toxicity under increased N enrichment needs to be assessed for the seagrass meadows of ANI, India.

Sulfide is toxic to living cells due to its strong affinity towards iron-containing enzymes and thereby inhibit enzymatic activity in the cells (Raven and Scrimgeour, 1997). To avoid such toxicity, seagrasses have developed unique abilities to mobilize Fe within their rhizosphere via plant induced acidification causing reduction of insoluble Fe (III) oxy-hydroxides to dissolved Fe (II) (Brodersen et al., 2017; Garcias-Bonet et al., 2008; Pedersen and Kristensen, 2015). These may be one of the mechanisms of *T. hemprichii* under enriched conditions for reduction of sulfide within internal tissues, as the Fe levels were low at the enriched conditions (Fig.4). Furthermore, sulfide intrusion into seagrass tissues also causes severe metabolic stress such as decline in root ATP levels, which has been observed for *T. testudinum* (Erskine and Koch, 2000). This may be one of the reasons why the root biomass and RBI of *T. hemprichii* in this study was lower under enriched conditions (Fig. 5). Furthermore, this reduction in RBI can negatively affect the distribution of oxygen in seagrass roots/rhizosphere. This impacts negatively on the reoxidation of sediment sulfide and thus allow more entry of gaseous sulfide into the seagrass tissues (Elgetti Brodersen et al., 2016; Lamers et al., 2013). This study highlights that N enrichment in sandy seagrass sediments can cause severe negative effects on seagrass below ground productivity and growth through sulfide toxicity, when Fe levels are low. To avoid loss of seagrass ecosystems and their valuable ecosystem services reduction in anthropogenic N load and continuous monitoring of seagrass ecosystems should be prioritized to avoid decimation and die-offs.

5. Conclusions

In this study for the first time the impact of anthropogenic N enrichment and subsequent effects of sediment sulfide intrusion into seagrass (*T. hemprichii*) species growing in sandy sediments from the tropical ANI, India is presented. This study highlights that increase in anthropogenic activities results in increase of sediment N, OM, finer fraction of sediment and sediment S under enriched conditions. Consequently, increase of anthropogenic N input provided positive benefits to the seagrass resulting in increased density and biomass under enriched conditions compared to pristine. However, the increase in sediment sulfide production resulted in increased sediment sulfide intrusion (evidenced from root $\delta^{34}S$) into *T. hemprichii* roots and resulted in reduced root biomass and RBI under enriched conditions. Iron limitation in the sandy sediments under enriched conditions played an important role in increased sulfide intrusion into seagrass tissues and associated toxicity. Therefore, monitoring of seagrass ecosystems of ANI under the influence of anthropogenic nutrient (especially N) enrichment and its associated effects are necessary for better conservation and management of these vital ecosystems for climate change mitigation and other ecosystem services.

CRediT authorship contribution statement

Amrit Kumar Mishra: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Syed Hilal Farooq:** Writing – review & editing, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Amrit Kumar Mishra reports financial support was provided by The Rauf Ali Fellowship for Island Ecosystems. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rhisph.2025.101128.

Data availability

Data will be made available on request.

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