Soil organic nitrogen content and composition in different wetland habitat types along the south-east coast of China

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A B S T R A C T

Soil organic nitrogen (SON) turnover regulates soil nitrogen (N) storage and availability. The coastal mudflats (MFs) in China have undergone drastic transformation due to invasive *Spartina alterniflora* (SAs) and subsequent reclamation of *Spartina* marshes to create aquaculture ponds (APs), but the impact on the amounts and compositions of soil nitrogen remains unclear. This study measured the topsoil total nitrogen (STN) and organic nitrogen (SON) compositions in 21 coastal wetlands in southeastern China. Results show that conversion of MFs to SAs increased STN by 38.5%, whereas subsequent conversion to APs decreased it by 16.4%, and the effect was consistent across the broad geographic and climate gradients. Most of the change occurred in the non-acid-hydrolysable fraction of SON, which accounted for 32–42% of STN. Within the acid-hydrolysable fraction, amino acid N, ammonia N and amino sugar N together accounted for about 57%, with the remaining 43% unidentified chemically. Our results suggest that invasion by *S. alterniflora* was the overwhelming driver to increase bioavailability of nitrogen and related biogeochemical processes in coastal soil, and the effects were partly reversed in subsequent reclamation of *Spartina* marshes to create aquaculture ponds.

Keywords: Coastal wetland; Invasive species; *Spartina alterniflora*; Land use change; Acid hydrolysis

1. Introduction

Nitrogen (N) is an essential mineral nutrient for primary production and microbial activity in terrestrial ecosystems (Abdul-Aziz et al., 2018; Vitousek and Howarth, 1991; Wang et al., 2022). As one of the largest active N reservoirs, soil plays a critical role in the global N cycle (Sollins et al., 2009; Yang et al., 2016). The soil N pool is composed of mainly organic N (> 90%) with a minor contribution from inorganic N (Schulten and Schnitzer, 1997). Despite its complexity and diversity, soil organic N can be separated by acid hydrolysis into the acid-hydrolysable nitrogen (AHN) and non-acid-hydrolysable nitrogen (non-AHN) fractions (Stevenson, 1982). The AHN fraction contains acid-hydrolysable ammonia, amino acids, amino sugars and unidentified nitrogen compounds, and is considered more labile and bioavailable than non-AHN (Johnsson et al., 1999; Silveira et al., 2008).

Notwithstanding their small areal coverage, coastal wetlands are disproportionately important as a terrestrial N inventory thanks to their high sedimentation rates and burial capacity for organic matter (Batjes, 1996; Yang et al., 2016). Coastal wetlands are facing multiple threats worldwide, including land-use change and invasion by exotic species (Murray et al., 2019; Sun et al., 2015; Walker and Smith, 1997), which can alter the soil properties and related N biogeochemistry (Yang et al., 2016, Peng et al., 2023; Tan et al., 2022).

There is an estimated 5.79×10^6 ha of coastal wetlands along the southern and eastern seaboards of mainland China (Sun et al., 2015). Large swaths of the native coastal mudflats were heavily impacted by the cordgrass *Spartina alterniflora* that was introduced originally to mitigate coastal erosion (Chung, 2006). As a mean to control the invasive vegetation and support food production, many of the *Spartina* marshes were subsequently cleared and converted to earthen aquaculture ponds (Duan et al., 2020; Ren et al., 2019). Such an extensive landscape alteration provides a rather unique opportunity to investigate how this sequential change of habitat type affects the soil biogeochemistry over a large geographical and climate gradient. This knowledge is particularly important for predicting future changes as the spread of *S. alterniflora* and expansion of coastal aquaculture continue in China (Duan et al., 2021; Mao et al., 2019). Some recent studies have looked at the effects of land use and land cover change, including reclamation and plant invasion, on wetland soil nitrogen pools (Sheng et al., 2022; Xu et al., 2019; Yang et al., 2019), but they were limited to a single location and did not examine the sequence of change, from native mudflat to *Spartina* marshes then to aquaculture ponds.

In the recent years, we have begun to investigate how the soil carbon pools, carbon remineralization, microbial functional compositions and greenhouse gas production potentials were affected by this landscape modification along China's coast (Yang et al., 2022a; Hong et al., 2023; Yang et al., 2023). One important finding was that soil N_2O production potential increased substantially when mudflats changed to *Spartina* marshes, but it then decreased when the marshes were converted to aquaculture ponds (Yang et al., 2023). It was hypothesized that these changes were partly attributed to the change in the availability in labile organic N that fueled N_2O production (Yang et al., 2023).

As a companion study to Yang et al. (2023) and to test the aforementioned hypothesis, here we analyzed and compared the soil's physicochemical variables in all three habitat types across 21 wetland sites, and derived common effect patterns of habitat modification on the soil organic N pool. Using the acid hydrolysis method, we quantified the ANH and non-ANH fractions of organic N, and assessed how habitat modification may affect soil organic N liability and turnover potential in coastal wetlands.

2. Materials and methods

2.1. Study area and sample collection

The study was conducted in coastal wetlands across five provinces in mainland China (20°42′ N to 31°51′ N; 109°11′ E to 122°11′ E) (Figure 1). Field samples were collected at twenty-one sites, with two sites in Shanghai (SH), six in Zhejiang (ZJ), nine in Fujian (FJ), three in Guangdong (GD) and one in Guangxi (GX). The sampling sites were influenced by tropical-subtropical monsoon climate, with a mean annual temperature of 11.0–23.0 °C and precipitation 100–220 cm. Coastal wetlands covered about 2.58×10^4 km^2 across these sites, or 44.5 % of the total area of coastal wetlands in China (Sun et al., 2015). Many of these coastal wetlands have undergone the same sequence of habitat modification, from native mudflat to *S. alterniflora* marshes (Xia et al., 2021), and subsequently from *S. alterniflora* marshes to earthen aquaculture ponds

(Mao et al., 2019; Meng et al., 2017). In our study area, the total areal coverage was approximately 334 km^2 of *Spartina* marshes (Liu et al., 2018) and 5,309 km^2 of aquaculture ponds (Duan et al., 2020), accounting for 61.2% and 36.9% of the total areas of *Spartina* marshes and aquaculture ponds (Yang et al., 2022a), respectively, in China.

During December 2019 and January 2020, three replicate plots were selected in each of the habitat types at each site: mudflat (MF), *S. alterniflora* marsh (SA) and aquaculture pond (AP). The upper 20 cm soils were collected using a steel corer, for a total of 189 soil samples (twenty-one sites × three habitats × three plots). All soil samples were transferred into sterile plastic bags and kept at 4°C in the dark prior to analysis (Hellman et al., 2019).

2.2. Measurement of soil physicochemical variables

In the laboratory, each soil sample was freeze-dried, homogenized and then ground to a fine powder for analyzing various physicochemical variables. Soil was mixed with deionized water in a 1:2.5 ratio (v/v) for measuring pH using an Orion 868 pH meter (Thermo Fisher Scientific, USA), and in 1:5 ratio for measuring salinity (‰) using a Eutech Instruments-Salt6 salinity meter (Thermo Fisher Scientific, USA) (Deng et al., 2015; Li et al., 2020; Tan et al., 2023). Soil particle size distribution (%) was measured with a Master Sizer 2000 Laser Particle Size Analyzer (Malvern Scientific Instruments, UK). Soil SO_4^{2-} (mg L^{-1}) and Cl^- (mg L^{-1}) contents were measured with an ion chromatograph (Dionex 2100, USA) (Chen and Sun, 2020), and soil organic carbon

(SOC, g kg^{-1}) content with a combustion analyzer (Elementar Vario MAX CN, Germany) (Liu et al., 2017). Soil microbial biomass nitrogen (MBN, mg kg^{-1}) content was measured by the fumigation-extraction method (Templer et al., 2003). Soil water content (SWC, %) and bulk density (SBD, g cm^{-3}) were determined based on weight loss before and after drying (Percival and Lindsay, 1997; Yin et al., 2019).

2.3. Determination of soil total nitrogen and nitrogen compositions

Soil total nitrogen (STN, mg kg^{-1}) was determined according to Xia et al. (2021), using an Elementar Vario MAX CN analyser (ELEMENTAR, Germany). Soil organic nitrogen (SON) fractions were analyzed by acid hydrolysis (Stevenson, 1996). Briefly, 6.5 g of soil sample was digested with 6 M HCl in an autoclave at 120 °C for 12 h. Afterward, the hydrolysate was filtered and subsequently neutralized to pH 6.5 by adding NaOH. The total acid-hydrolysable nitrogen (AHN, mg kg^{-1}) was determined by steam distillation using 10 M NaOH after Kjeldahl digestion of the acid hydrolysate. Acid-hydrolysable ammonia nitrogen (AMN, mg kg^{-1}) was measured by steam distillation with 3.5% (*w*/*v*) MgO (Tian et al., 2017). Amino acid nitrogen (AAN, mg kg^{-1}) was determined by steam distillation using phosphate-borate buffer after treatment with 5 M NaOH and ninhydrin powder to convert the amino N to ammonium N (Tian et al., 2017; Wang et al., 2017). Amino sugar nitrogen (ASN, mg kg^{-1}) was calculated by subtracting AMN from the sum of AMN and ASN obtained by steam distillation using phosphate-borate buffer at pH 11.2 (Tian et al., 2017; Wang et al., 2023). Hydrolysable unknown nitrogen (HUN, mg kg^{-1}) was calculated by subtracting AMN, AAN, and ASN from AHN. Non-acid-hydrolysable nitrogen (non-AHN, mg kg^{-1}) was calculated by subtracting AHN from STN.

2.4. Statistical analysis

All data were first checked for normality and homogeneity of variance. One-way analysis of variance (SPSS version 25.0; IBM, Armonk, NY, USA) was used to test for significant differences between habitat types in soil physicochemical variables, STN and individual SON components. Redundancy analysis (RDA) was conducted to examine the influence of different physicochemical factors on N variables, using the CANOCO 5.0 software package for Windows (Microcomputer Power, Ithaca, USA). All statistical significance was determined at $p < 0.05$.

3. Results

3.1. Soil total nitrogen across habitat types

The soil total nitrogen (STN) content varied considerably within each habitat type: 273.1–1,418.6 mg kg^{-1} in MFs, 623.7–2,723.9 mg kg^{-1} in SAs, and 354.4–2,516.3 mg kg^{-1} in APs (Figure 2). Overall, the mean STN content was significantly higher in SAs (1,101.1±54.5 mg kg^{-1}) than in both MFs (795.2±30.8 mg kg^{-1}) and APs (920.7±54.2 mg kg^{-1}) ($p<0.05$; Figure 2). Accordingly, the change from MFs to SAs and MFs to APs increased STN content by 38.5% and 15.7%, respectively; while the conversion of SAs to APs decreased STN content by 16.4%.

3.2. Acid-hydrolysable N and non-acid-hydrolysable N

Among all soil samples, the content of acid-hydrolysable N (AHN) averaged

524.29±21.25 mg kg^{-1} in MFs, 615.85±26.68 mg kg^{-1} in SAs and 585.83±33.90 mg kg^{-1} in APs (Figures 3a). Non-acid-hydrolysable N (non-AHN) averaged 256.36±17.64 mg kg^{-1} in MFs, 459.64±34.55 mg kg^{-1} in SAs and 316.61±25.55 mg kg^{-1} in APs (Figures 3b). Accordingly, the content of SON averaged 780.65±53.32 mg kg^{-1} in MFs, 1075.31±94.9 mg kg^{-1} in SAs and 902.43±94.46 mg kg^{-1} in APs (unpublished data).. AHN consistently made up a significantly higher portion of the SON than non-AHN in all three habitat types ($p<0.05$; Figure 3c). Between habitat types, the mean AHN (and non-AHN) was significantly higher in SAs than in MFs ($p<0.05$ or <0.01), which was in turn higher than that in APs (Figures 3a and 3b).

3.3. Compositions of acid-hydrolysable N

We measured the different components of AHN in the soil, including amino acid N (AAN), acid-hydrolysable ammonia N (AMN), amino sugar N (ASN) and hydrolysable unknown N (HUN). The mean AAN content varied among the three habitats in the order of APs (101.19±10.64 mg kg^{-1}) > SAs (83.68±6.42 mg kg^{-1}) > MFs (76.52±7.91 mg kg^{-1}) (Figure 4a). The mean AMN (Figure 4b), ASN (Figure 4c) and HUN (Figure 4d) were highest in SAs (respectively: 144.06±9.85, 121.31±8.16, 266.81±19.74 mg kg^{-1}), followed by APs (125.65±9.77, 107.04±6.52, 251.94±18.34 mg kg^{-1}) and MFs (113.87±6.38, 104.68±8.24, 229.22±14.52 mg kg^{-1}). Across all three habitat types, HUN accounted for the largest proportion of AHN (43.0–43.7%), followed by AMN (21.4–23.4%), ASN (18.3–19.9%) and AAN (13.6–17.3%) (Figure 5).

3.4. Relationships between N and physicochemical variables

Data on the individual soil physicochemical variables can be found in Yang et al. (2022a, 2023). Here we focused on examining their relationships with STN and the different SON components based on redundancy analysis (Figure 6). Within MFs, the soil N variables were negatively correlated to bulk density (SBD; 46%), whereas in both SAs and APs, soil N variables were positively correlated to soil organic carbon (SOC), which explained 40.7–58.4% of the variability of the former.

4. Discussion

4.1. Soil total nitrogen in different habitat types

The sampling sites in this study included three contrasting habitat types that are ubiquitous along the south-east coast of China: Non-vegetated mudflats (MFs), marshes colonized by *S. alterniflora* (SAs), and earthen aquaculture ponds (APs) with dense animal stocks and feed input. The soil N content in MFs was strongly and negatively correlated with SBD (Figure 6a), consistent with the negative relationship commonly observed between bulk density and organic content in different soil types (Avnimelech et al., 2001; Keller and Håkansson, 2010).

Compared to MFs and SAs, we expected that the deeper and stagnant water in APs would maintain an anoxic condition in the sediment and favor the accumulation of organics from unconsumed feed and biological residues (Hargreaves, 1998). Contrary to this expectation, the STN content in APs was only slightly higher than MFs, and both were significantly lower than SAs (Figure 2). These observations suggest that the *S. alterniflora* aboveground biomass prevented soil erosion and trapped allochthonous organic matter (Middelburg et al., 1997) while deposited autochthonous organics into the soil (Tong et al., 2011; Feng et al., 2017), leading to a higher STN content. The water-logged condition due to increased soil water content following the invasion by *S. alterniflora* would also create an anoxic condition that favored N accumulation (Feng et al., 2017; Yang et al., 2016), as shown by the positive influence of SWC on soil N content (Figure 6b). On the other hand, clearing of vegetation from aquaculture ponds and high nutrient utilization efficiency of the farmed animals was enough to lower soil N content in APs (Yang et al., 2021). Because most of the soil N would have been associated with organic matter, the added SOC from *S. alterniflora* and aquaculture operation would have increased STN and SON, as confirmed by the RDA results (Figure 6b,c).

4.2. Soil nitrogen compositions in different habitat types

Similar to other terrestrial ecosystems (e.g. forest, grassland and paddy) (Ren et al., 2023; Spargo et al., 2012; Tian et al., 2017; Wang et al., 2023), we observed that SON accounted for over 95% of STN and the majority of which was AHN (Figure 3c). Between habitat types, AHN differed by ~17% or less (Figure 3a). However, changing from MFs to SAs increased non-AHN by 79%, likely from refractory debris of *S. alterniflora* (Buchsbaum et al., 1991; Hopkinson and Schubauer, 1984). Subsequently, non-AHN decreased by 31% when the vegetation was removed to create aquaculture ponds (Figure 3b). Since AHN is considered the more labile and bioavailable fraction of SON, it is useful to further consider the sources of its different components e.g., AAN, AMN, ASN and HUN, as follows.

Unlike the wild habitats MFs and SAs, protein-rich feeds were added to APs regularly; unconsumed feeds and debris from stocked animals would then be decomposed into amino acids, as indicated by the rich acid-hydrolysable amino-acid nitrogen (AAN) content of its soil (Figure 4a).

Acid-hydrolysable ammonia nitrogen (AMN) has been described as organic-bound ammonia N that can be extracted chemically (Bremner, 1959) and is therefore included operationally in the SON pool. The significantly higher AMN content in SAs (Figure 4b) can be attributed to higher SOC derived from *S. alterniflora* (Hong et al., 2023) that bound and retained AMN in the soil.

Amino sugars are common components of bacterial and fungal cell walls and chitinous exoskeleton (Parsons, 2021). In our study, acid-hydrolysable amino sugar nitrogen (ASN) differed little among the habitat types, with a small but significant increase (16%) between MFs and SAs (Figure 4c). However, our previous study showed no significant difference in soil microbial biomass between habitat types (Yang et al., 2023) and ASN content was poorly correlated with MBN in this study (Figure 6b,c); therefore, the additional ASN in SAs may have been derived from arthropods living in the marshes.

A large fraction of soil nitrogen remains unidentified chemically, although it has been shown to be biodegradable (Ivarson and Schnitzer, 1979). In our study, some 43% of AHN was unidentified (HUN) across all three habitat types (Figure 5). Conversion of

MFs to SAs caused a small but significant increase in HUN (16%) (Figure 4d), some of which was likely derived from plant litter (Qiu et al., 2012; Wang et al., 2022).

4.3. Implications for soil biogeochemical processes

The coastal landscape in China has undergone drastic transformation in recent decades due to invasive species and land use change (Duan et al., 2020; Ren et al., 2019; Sun et al., 2015). In our earlier study, we determined that converting mudflats to *Spartina* marshes increased N_2O production potential of the soil by 128%, whereas subsequent reclamation of *Spartina* marshes to create aquaculture ponds decreased it by 30% (Yang et al., 2023). While N_2O production may involve multiple reactions by different microbes under different environmental conditions, the required inorganic N (e.g. NH_4^+-N and NO_3^--N) is often derived from microbial mineralization of organic matter in the soil (Feng et al., 2022; Noe et al., 2013). Therefore, our observations show that habitat modification could impact soil N_2O production by changing the soil organic N pools (Figure 7).

A meta-analysis study has shown that invasive vegetation often causes an increase in soil N availability by improving N retention, direct N exudation and stimulating microbial N_2 fixation, which facilitates further invasion leading to a positive feedback (Liao et al., 2008). Our data also showed that invasion of mudflats by *S. alterniflora* increased STN by 38.5% and non-AHN by 79.3%. This enrichment of soil N can contribute to the rapid spread of *S. alterniflora* along China's coast, increasing its coverage by 192-fold in just 35 years (Meng et al., 2020).

As a measure to control the spread of *S. alterniflora* and to boost food production, increasingly more *Spartina* marshes are being reclaimed for aquaculture (Duan et al., 2021). This in turn raises concerns about nutrient pollution and greenhouse gas emissions from the aquaculture ponds (Tong et al., 2021; Yang et al., 2021; Yang et al., 2022b). However, comparison of the different habitat types across 21 coastal wetlands has consistently shown that soil organic carbon (Hong et al., 2023) and nitrogen contents (this study, Figure 7), organic carbon mineralization rate (Yang et al., 2022a), CO_2, CH_4 and N_2O production potentials (Yang et al., 2022a; Yang et al., 2023) all decreased when *Spartina* marshes were converted to aquaculture ponds, regardless of geographical location, local climate condition or local aquaculture management. Therefore, the findings thus far all point to *S. alterniflora* invasion as the overwhelming driver of increasing soil organic matter contents and related greenhouse gas production, and reclamation of the marshes was able to partly reverse the effects (Figure 7).

5. Conclusions

This study evaluated the effects of coastal habitat modification on soil nitrogen content across a large latitudinal range in China. Our results show that the sequence of change from native mudflats to *S. alterniflora* marshes to aquaculture ponds has resulted in significant changes in STN and especially the operationally defined non-AHN fraction. The observed changes in the quantity and quality of soil nitrogen due to habitat modification were consistent across all 21 coastal wetlands, and aligned with earlier results on soil carbon and greenhouse gas production. Although converting *Spartina* marshes to aquaculture ponds may have unintended benefits of lowering soil organic content and greenhouse gas production, whether the practice itself has an overall positive environmental impact remains questionable because of potential pollution associated with fertilizer and feed production, transportation and pond discharge (Herbeck et al., 2013; Molnar et al., 2013). More importantly, the chemical identity of a large portion of the soil N, represented by non-AHN and HUN, remained unknown. Characterization of these N fractions will improve our understanding of how they influence soil health and microbial activities.

Declaration of competing interest

The authors 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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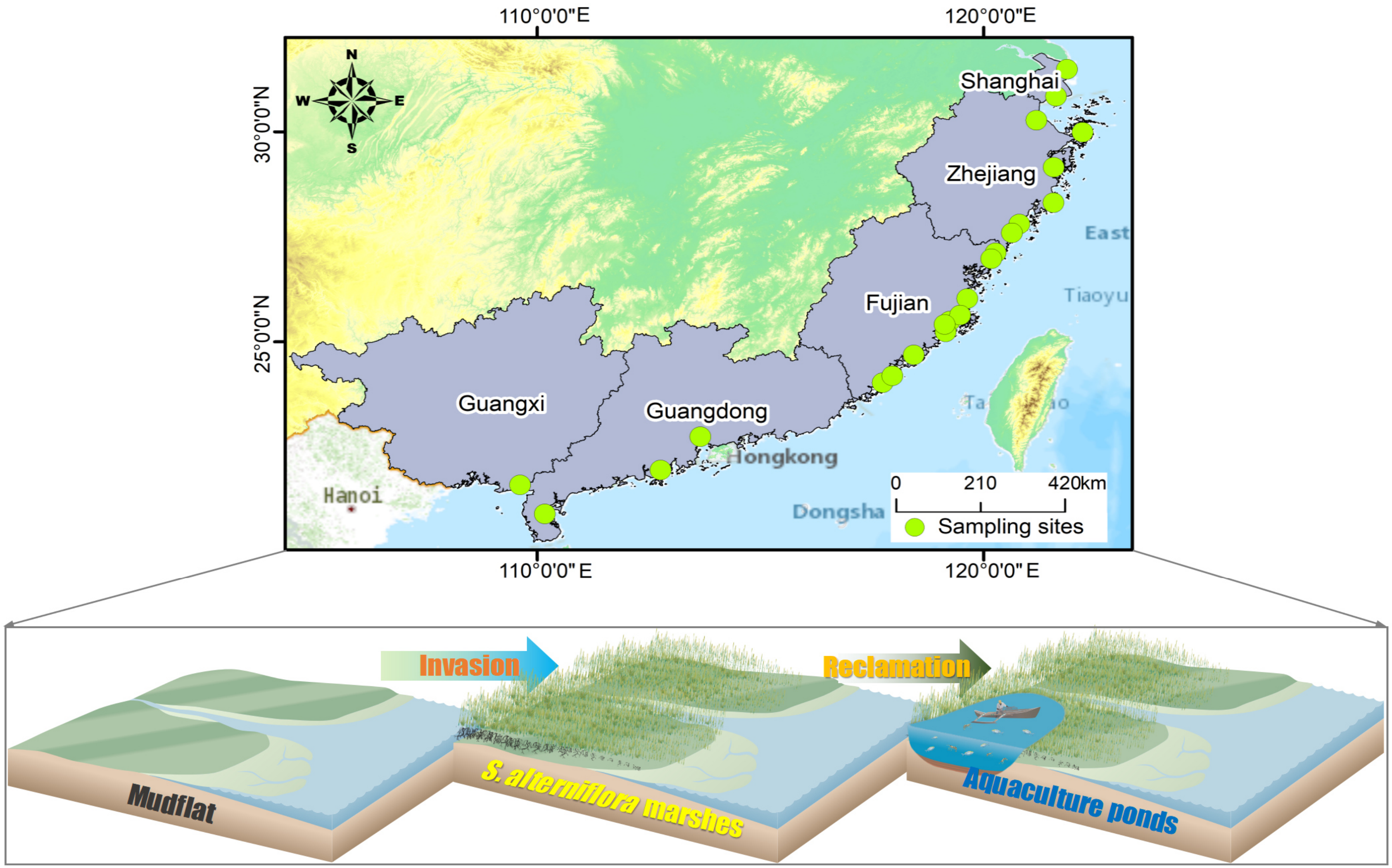


FIGURE 1 Locations of the 21 sampling sites across the coastal regions in southeastern China. Three wetland habitat types were investigated including mud flats (*MFs*), *S. alterniflora* marshes (*SAs*) and aquaculture ponds (*APs*).

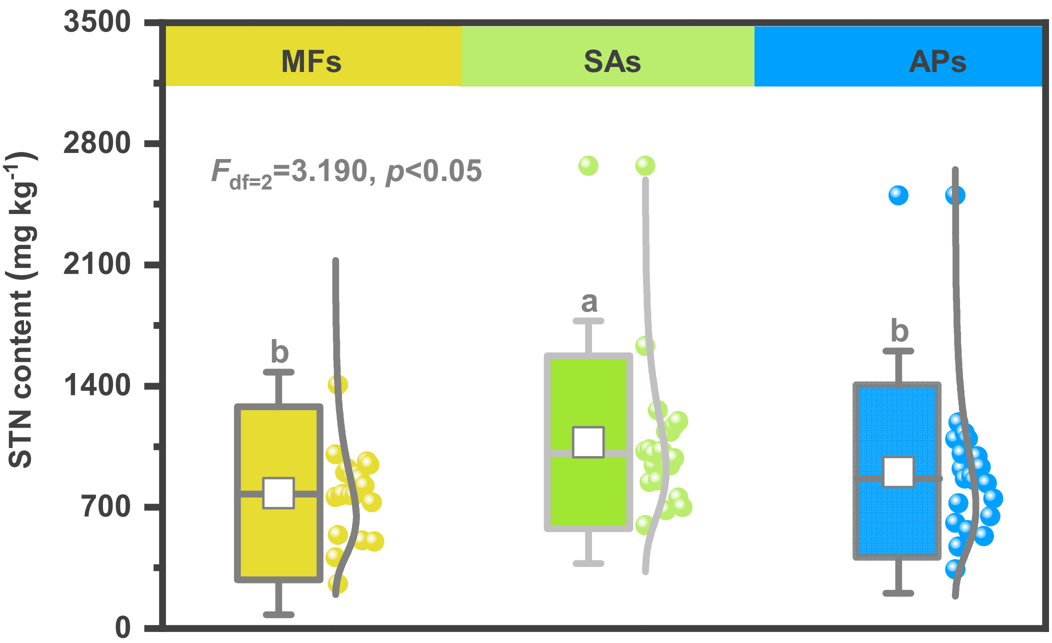


FIGURE 2 Box plots of total nitrogen (STN) in the top soil (0-20 cm) of the three wetland habitat types (MFs, mudflats; SAs, *S. alterniflora* marshes; APs, aquaculture ponds). Boxes with no shared letters are significantly different (p < 0.05).

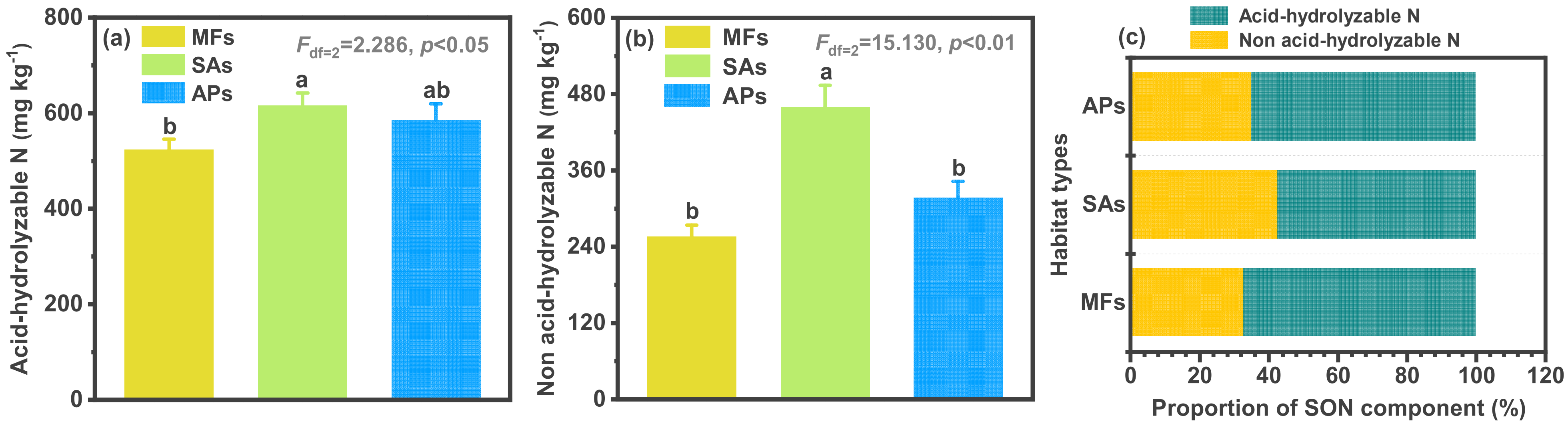
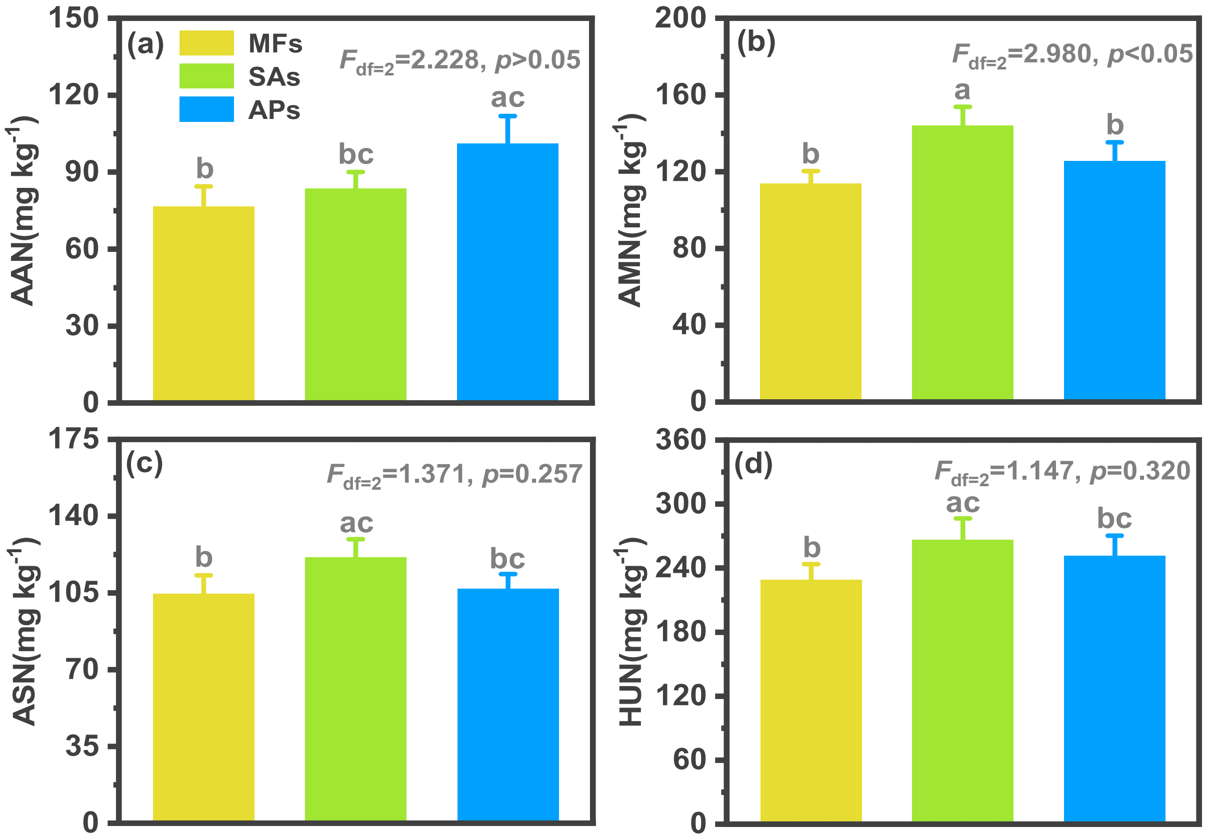


FIGURE 3 The amounts of acid-hydrolysable N (a) and non-acid-hydrolyzable N (b) (mean + SE) and proportion of SON components (c) in the top soil (0-20 cm) of the three wetland habitat types (MFs, mudflats; SAs, *S. alterniflora* marshes; APs, aquaculture ponds). Bars with no shared letters are significantly different ($p < 0.05$).



F I G U R E 4 Acid-hydrolysable N components in the top soil (0-20 cm) (mean + SE): AAN, amino acid N; AMN, ammonia N; ASN, amino sugar N; HUN, unknown N. MFs, SAs and APs represent mudflats, *S. alterniflora* marshes and aquaculture ponds, respectively. Bars with no shared letters are significantly different (p<0.05).

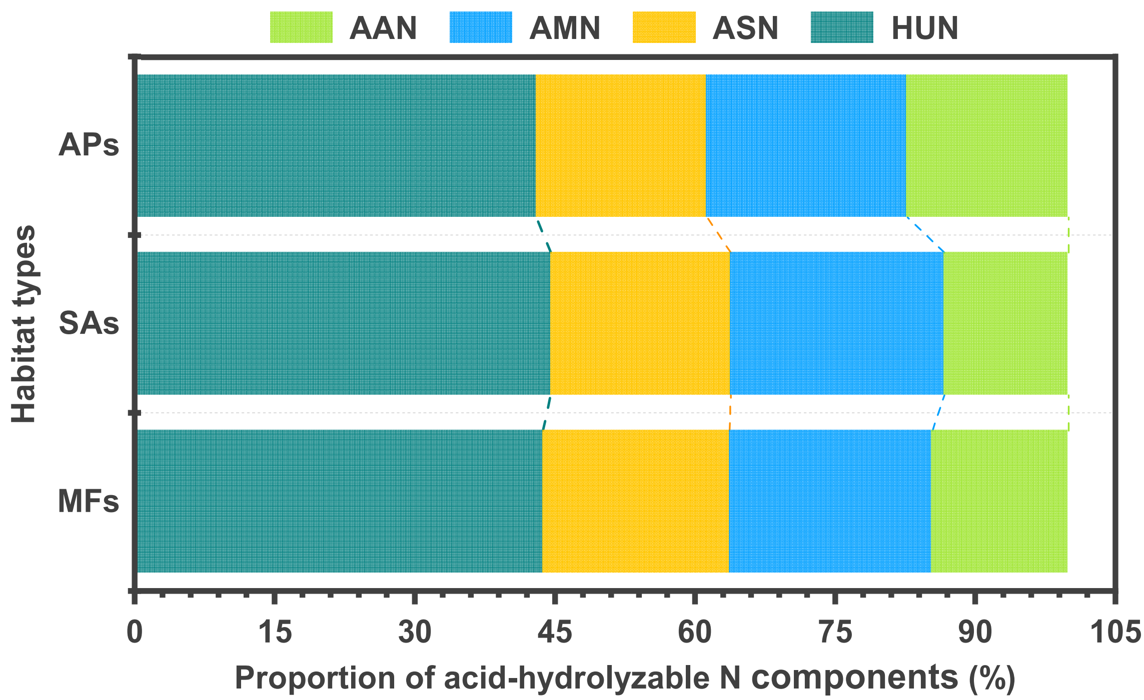


FIGURE 5 Proportions of different acid-hydrolysable N components in the top soil (0-20 cm) in the three habitat types. See Fig. 4 for explanation of abbreviations.

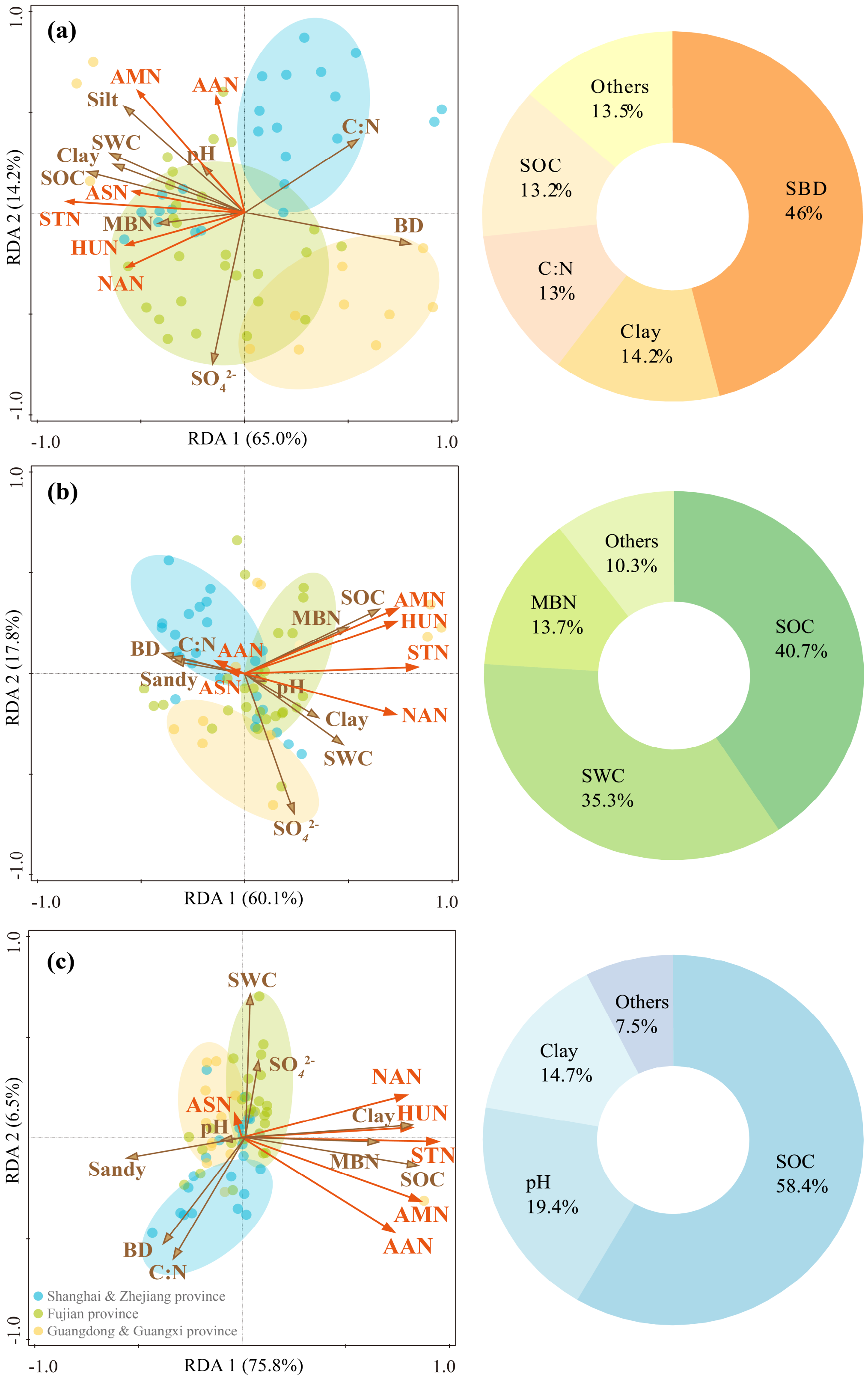


FIGURE 6 Redundancy analysis (RDA) biplots of the relationship between STN, individual SON components and soil physicochemical variables for the different habitats: (a) mudflats (MFs); (b) *S. alterniflora* marshes (SAs) and (c) aquaculture ponds (APs). The pie charts show the percent variations in STN and SON explained by the different variables. See main text for explanation of abbreviations.

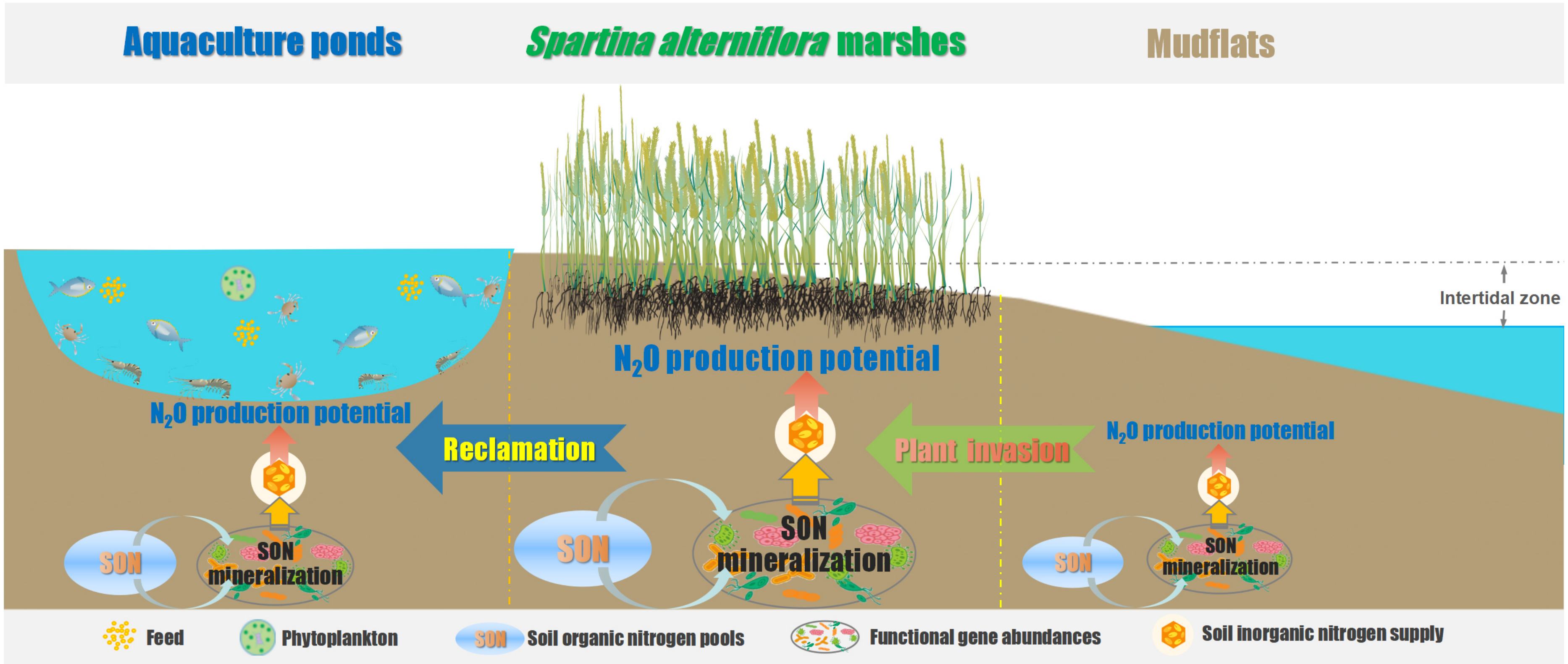


FIGURE 7 Schematic illustration of landscape change effects on soil nitrogen biogeochemical processes in impacted coastal wetlands in southeastern China.