Suspended solids induced increasing microbial ammonium recycling along the river-estuary continuum of Yangtze River

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Abstract

Many large rivers worldwide are enriched with high levels of suspended solids (SS), which are known to be hotspots of many nitrogen (N) transformation processes (e.g., denitrification, nitrification). However, the influence of SS on microbial ammonium (NH4+) recycling remains unclear. Water column NH4+ regeneration rates (REGs) and potential uptake rates (Upots) as well as community biological NH4+ demand (CBAD) was measured in the river-estuary continuum of the third longest river in the world—Yangtze River, where shows dramatic SS gradients. We found that, REGs, Upots, and CBAD all showed increasing trends along the river flow, with higher REGs, Upots, and CBAD in the estuary than in the river sections. The regeneration and uptake of NH4+ were nearly balanced in the river sections, while the positive CBAD in the estuary indicated obvious NH4+ demand of microbes. Concentrations of SS, which also controls the content of chemical oxygen demand and particulate N, were the main factor influencing NH4+ recycling rates and CBAD. SS induced regenerated NH4+ in the river-estuary continuum of Yangtze River was estimated to be 21.81 × 108 kg N yr-1 and accounted for about 25% of total N inputs, suggesting that regenerated NH4+ is an important N source for microbes and may influence nutrient dynamics in lower coasts. To our knowledge, this is the first to report NH4+ recycling in Yangtze River with an emphasis on its influencing factors and contribution to N budgets.

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Abstract: Many large rivers worldwide are enriched with high levels of suspended solids (SS), which are known to be hotspots of many nitrogen (N) transformation processes (e.g., denitrification, nitrification). However, the influence of SS on microbial ammonium (NH_4^+) recycling remains unclear. Water column NH_4^+ regeneration rates (REGs) and potential uptake rates (U_{pot}s) as well as community biological NH_4^+ demand (CBAD) was measured in the river-estuary continuum of the third longest river in the world—Yangtze River, where shows dramatic SS gradients. We found that, REGs, U_{pot}s, and CBAD all showed increasing trends along the river flow, with higher REGs, U_{pot}s, and CBAD in the estuary than in the river sections. The regeneration and uptake of NH_4^+ were nearly balanced in the river sections, while the positive CBAD in the estuary indicated obvious NH_4^+ demand of microbes. Concentrations of SS, which also controls the content of chemical oxygen demand and particulate N, were the main factor influencing NH_4^+ recycling rates and CBAD. SS induced regenerated NH_4^+ in the river-estuary continuum of Yangtze River was estimated to be 21.81×10^8 kg N yr^{-1} and accounted for about 25% of total N inputs, suggesting

that regenerated $NH_4^+$is an important N source for microbes and may influence nutrient dynamics in lower coasts. To our knowledge, this is the first to report NH_4^+ recycling in Yangtze River with an emphasis on its influencing factors and contribution to N budgets.

Keywords: Suspended solids; Ammonium recycling; River-estuary continuum; Community biological ammonium demand

Introduction

Nitrogen (N) is an essential nutrient for organisms and the excessive inputs of N have led to serious eutrophication and frequent harmful algal blooms in aquatic systems (Diaz and Rosenberg, 2008; Di *et al.* , 2015). As an important reduced form of N, NH_4^+ needs the least energy for the uptake of phytoplankton and bacteria, and thus commonly shows low water column concentrations when the primary production is high (Gardner*et al.* , 2004; Paerl *et al.* , 2011). Despite within the conditions of low ambient NH_4^+concentrations and/or net fluxes (i.e., differences between the regeneration and uptake of NH_4^+), evidence have shown that raid NH_4^+recycling provides hidden N source for algal species (Bruesewitz*et al.* , 2015; Jiang *et al.* , 2019; Gardner *et al.* , 2017). Therefore, to comprehensively understand the sources and sinks of N and serve eutrophication control, it is crucial to evaluate NH_4^+ recycling and its contribution to the ecosystem.

Water column NH_4^+ recycling involves the intensively coupling of NH_4^+regeneration and uptake. The regeneration of NH_4^+ is primarily contributed by the bacterial ammonification of labile organic N and metabolic release of zooplankton (Gardner *et al.* , 2004), and the uptake of NH_4^+ mainly include planktonic (i.e., algae and heterotrophic bacteria) assimilation and bacterial nitrification (Hampel *et al.* , 2017). The balance between NH_4^+ regeneration and uptake can be reflected by calculating community biological NH_4^+ demand (CBAD), which indicates net microbial community demand for NH_4^+ (Gardner *et al.* , 2017).

Recycling rates of NH_4^+ and the CBAD varied among different water bodies under the influence of various environmental factors and ecosystem characteristics. Nutrient concentrations, organic matter, and chlorophyll *a* (Chl-*a*) are common factors influencing NH_4^+recycling rates in eutrophic lakes, estuaries, and coasts (Bruesewitz*et al.* , 2015; Gudasz *et al.* , 2015; Jiang *et al.* , 2019). In high turbid rivers, besides the factors mentioned above, suspended solid (SS) is also an important factor influencing NH_4^+ recycling processes (Xue *et al.* , 2019). SS supports the metabolism of attached heterotrophic bacteria (i.e., nitrification, denitrification, and nitrous oxide emission) by providing ideal oxic or anoxic interfaces with easily available substrates from SS or its surrounding water (Xia *et al.* , 2009; Liu *et al.* , 2013; Yao *et al.* , 2016; Zhou*et al.* , 2019; Zheng *et al.* , 2017). However, the influence of SS on microbial NH_4^+ recycling remains unclear.

Characterized by low retention time, drastic flushing effects, and dramatic SS changes, river-estuary continuums exhibit typical gradients of nutrient concentrations and dynamics (Mccarthy *et al.* , 2007b; Bruesewitz*et al.* , 2015; Liang and Xian, 2018). Previous studies in river-estuary continuums usually focused on N loads and its exports (Liang and Xian, 2018), long-term changes of nutrient concentrations (Liu *et al.* , 2018), and responses of phytoplankton community to increased nutrients input (Zhou *et al.* , 2008). Relatively fewer studies are focused on NH_4^+ recycling in river-estuary continuums and its effect factors. NH_4^+ recycling in river-estuary continuums may not support severe algal blooms as in eutrophic lakes, while it is still playing an important role in microbial growth and can impact nutrient dynamics in the systems and even lower coasts.

In recent decades, algal blooms increased dramatically in the estuary of Yangtze River in terms of frequency, area, and persistence time (Zhou*et al.* , 2008; Tang *et al.* , 2006; Dai *et al.* , 2011; Jiang *et al.* , 2014). It has been known that external N inputs play an important role in supporting algal blooms in the estuary of Yangtze River (Zhou *et al.* , 2008), while the contribution of water column N recycling is unclear. We hypothesized that NH_4^+ recycling would provide enormous N for microbes in the river-estuary continuum of Yangtze River, and the volume of regenerated NH_4^+ have the potential to support algal blooms in the estuary. The aims of this study are to: (1) quantify NH_4^+ uptake and regeneration rates as well as CBAD;

(2) examine the effects of SS and other environmental factors on NH_4^+recycling rates; (3) evaluate the contribution of regenerated NH_4^+ to N budgets and explore implications. This study is the first to report NH_4^+ recycling and CBAD in the river-estuary continuum of Yangtze River. The results of our study provide a basis for the comprehensive understanding of N sources in the Yangtze River and the formulation of N management strategies.

2. Methods

2.1 Study area and sampling sites

The Yangtze River (also called Changjiang River), originating from the Qinghai-Tibet Plateau, is the third longest river in the world, with a total mainstream length of about 6300 km, a basin area of 1.8×10^6 km^2 and an average annual discharge of 892 km^3 (Yan *et al.* , 2010)**(Fig. 1)** . In China, the river is divided based on watershed boundaries into three reaches, the upper reaches (upstream of Yichang), middle reaches (between Yichang and Hukou) and lower reaches (downstream of Hukou) (Wang *et al.* , 2008). The lower reaches areas of Yangtze River have dense river networks, numerous lakes, extensive plains and dense cities, forming a prosperous industrial belt, but also facing ecological environment problems such as the increasing concentrations of nutrient in the watershed, frequent occurrence of harmful algal blooms in lakes and severe red tide in estuaries (Yi *et al.* , 2011; Qin*et al.* , 2010; Tang *et al.* , 2006; Liu *et al.* , 2018). In addition, the lower reaches of Yangtze River can be subdivided into three parts, the Anhui section, Jiangsu section and estuary, according to the provinces and tidal **(Fig. 1)** .

In this study, 44 surface water samples (0.5 m below the water surface) were collected from the river-estuary continuum of the Yangtze River coastal zone in July, 2018, to investigate the spatial changes of REGs and U_{pot}s **(Fig. 1)** . There were 19 (CJ1 ˜ CJ19), 15 (CJ20 ˜ CJ34) and 10 (CJ35 ˜ CJ44) sites belonging to the Anhui section, Jiangsu section and estuary, respectively.

2.2 Sample collection and analysis

Surface water for NH_4^+ recycling experiments were collected in 1 L carboys. Water samples for nutrient concentrations (NO_3^-, NO_2^-, and NH_4^+) analyses were filtered through 0.7 µm fiberglass filters (Whatman GF/F) immediately following collection in the field. Water temperature, dissolved oxygen (DO), and pH were measured *in situ* using a multi-parameter water quality analyzer (YSI Professional Plus, 6600V2, USA). All samples were collected in triplicate and were immediately stored in a dark cooler. Total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), and phosphate (PO_4^{3-}) concentrations were analyzed in filtered samples (Jin and Tu, 1990). Concentrations of TDN and TDP were determined using the potassium persulfate digestion and spectrophotometric method (detection limits of 4 µmol N L^{-1} and 1 µmol P L^{-1} for TDN and TDP, respectively). NH_4^+ was determined using the nesslerization colorimetric method (detection limit 1 µmol N L^{-1}). $NO_3^-$and NO_2^- were determined using the phenol acid ultraviolet colorimetric method (detection limit 3 µmol N L^{-1}) and N-(1-naphthyl)-ethylenediamine colorimetric method (detection limit 0.2 µmol N L^{-1}), respectively. Total nitrogen (TN) and total phosphorus (TP) were determined on unfiltered water samples using the potassium persulfate digestion and spectrophotometric method (Jin and Tu, 1990). Particulate nitrogen (PN) was calculated as the difference between TN and TDN, and the standard deviation for PN was obtained using a propagation of error analysis. NO_x^- was the sum of NO_3^- and NO_2^-. Urea was measured using diacetylmonoxime reagent, and the detection limit was 0.04 µmol Urea-N L^{-1} (Mulvenna and Graham, 1992). Chl-*a*concentrations, chemical oxygen demand (COD), and suspended solid (SS) were determined using standard methods (Jin and Tu, 1990). Dissolved organic carbon (DOC) concentrations were determined using TOC-V CPN (Shimadzu, Tokyo, Japan) analyzer at high temperature (680 °C) after being acidified with 10 µL of 85% H_3PO_4.

2.3 NH_4^+regeneration and uptake experiment

Water column REGs and U_{pot}s were determined using isotope dilution methods. Isotope dilution experiments are usually conducted with low trace amendment level (about 10% of ambient) (Glibert *et al.* , 1982). However, low NH_4^+ concentrations and fast NH_4^+ recycling rates in summer flood season of Yangtze River may lead to depleted NH_4^+ pool before the end of incubation (Blackburn, 1979), excess$^{15}NH_4^+$(approximately 20 µmol N L^{-1}), as the reaction product rather than as a potentially limiting substrate, was added at the beginning of incubation (Mccarthy *et al.* , 2007a). Excessive$^{15}NH_4^+$ addition can promote NH_4^+ uptake rates, so the NH_4^+ uptakes obtained in this study were potential rates. On the other hand, because NH_4^+ is the end product rather than the substrate, excess additions will not affect regeneration rates (Blackburn, 1979).

Water from each site was enriched with 98%$^{15}NH_4Cl$ and decanted into duplicate clear polystyrene culture bottles (70 ml; Coring) after thoroughly mixed. Initial samples were filtered through a rinsed 0.2 µm syringe filter immediately after enrichment and mixing for total NH_4^+ concentrations and NH_4^+-^{15}N analysis. Bottles were incubated in a transparent bucket containing Yangtze River water to provide near-ambient light and temperature for 24 h. After incubation, final samples were collected in the same way as the initial samples. NH_4^+-^{15}N was measured using NH_4^+ oxidation membrane inlet mass spectrometry (OX/MIMS) (Yin *et al.* , 2014).

Water column REGs and U_{pot}s were calculated using a modified isotope dilution method (Glibert *et al.* , 1982; Blackburn, 1979). The relative abundance of NH_4^+-^{15}N (R) is required to calculate the REGs and U_{pot}s, which can be calculated as:

$$R =^{15} N/(^{15}N +^{14} N)$$

where ^{15}N and ^{14}N are the concentrations of NH_4^+-^{15}N and NH_4^+-^{14}N (µmol N L^{-1}), respectively.

The REGs can be calculated as follows (Bruesewitz *et al.* , 2015), which was derived from the logarithmic equations of Blackburn (1979):

$$\text{REG} = (R_0 - R_t)/t \ \times (C_0/R_t)$$

where R_0 and R_t are the relative abundances of NH_4^+-^{15}N at the initial and finial point, respectively, and t is the incubation time (h). C_0 is the initial concentrations of NH_4^+ (µmol N L^{-1}). REGs (µmol N L^{-1} h^{-1}) are absolutely positive values, indicating the actual regeneration rates of NH_4^+.

The U_{pot}s (µmol N $L^{-1}h^{-1}$) were calculated with the NH_4^+ concentrations change and regeneration rates (Bruesewitz *et al.* , 2015):

$$U_{\text{pot}} = (C_t - C_0 - REG \ \times t)/t$$

where C_0 and C_t are the initial and finial concentrations of NH_4^+ (µmol N L^{-1}).

CBAD characterizes internal NH_4^+recycling by representing the difference between measured potential NH_4^+ uptake rates and actual NH_4^+ regeneration rates in aquatic systems (Gardner *et al.* , 2017). Therefore, CBAD was calculated as:

$$\text{CBAD} = \ U_{\text{pot}} - REG$$

2.4 Statistical analysis

Statistical analyses were performed using SPSS 22.0. One-way analysis of variance (one-way ANOVA) combined with the Independent-Samples T-test were used to evaluate statistically significant differences between group average values. Pearson correlation analyses were applied to analyze the relationship between NH_4^+recycling rates and environmental factors. Differences and correlations were considered statistically significant at $p < 0.05$.

3. Results

3.1 Water column physicochemical characteristics

The physicochemical characteristics of water column in the Yangtze River are shown in **Table 1** . Water temperature in Anhui section, Jiangsu section and estuary ranged from 27.4 to 30.1, 27.6 to 30.2 and 28.6 to 30.0, respectively, and was significantly higher in estuary than in Anhui and Jiangsu section ($p< 0.05$). Chl-*a* concentrations were significantly higher in estuary than in river (Anhui and Jiangsu) sections ($p<$ 0.05). COD concentrations were highest in estuary (6.14 ± 1.74 mg/L) and lowest in Anhui section (4.05 ± 0.75 mg/L) ($p< 0.05$). Similar to the distribution of COD, DOC concentrations were highest in estuary (14.5 ± 5.3 mg/L) and lowest in Anhui section (6.6 ± 4.0 mg/L) ($p < 0.05$). SS concentrations ranged from 7 to 1315 mg/L, and the highest values were observed in estuary.

In all sites, N concentrations, including TN, PN, NH_4^+, NO_x^- and urea, ranged from 131 to 307, 1 to 158, < 1.0 to 13.0, 41 to 214, < 0.04 to 19.7 µmol N L^{-1} with average values of 196 ± 38, 56 ± 43, 4.5 ± 2.8, 112 ± 34, 5.5 ± 5.7 µmol N L^{-1}, respectively (**Table 1**). Along the river-estuary continuum, TN, PN, and NH_4^+ showed similar increasing trends with the direction flow, while no significant trends were observed in NO_x^- concentrations. TP concentrations ranged from 0.32 to 7.03 µmol P L^{-1}, with the highest values in Anhui section (3.35 ± 1.39 µmol P L^{-1}) and lowest in Jiangsu section (1.69 ± 1.23 µmol P L^{-1}) ($p < 0.05$).

3.2 NH_4^+regeneration and potential uptake rates

REGs in all water samples collected from Yangtze River ranged from 0.05 to 1.19 µmol N $L^{-1}h^{-1}$ with an average of 0.26 µmol N L^{-1} h^{-1}, showing an increased trend along the river-estuary continuum**(Fig. 2)** . The REGs were significantly lower in Anhui section (0.14 ± 0.09 µmol N L^{-1} h^{-1}) than in Jiangsu section (0.31 ± 0.18 µmol N L^{-1} h^{-1}) and estuary (0.44 ± 0.33 µmol N L^{-1} h^{-1}) ($p < 0.05$). U_{pot}s in the river-estuary continuum ranged from - 0.22 to 1.99 µmol N $L^{-1}h^{-1}$. Averaged highest U_{pot}s were observed in estuary (0.73 ± 0.56 µmol N $L^{-1}h^{-1}$), which were about 2 and 5 times higher than that in Jiangsu (0.32 ± 0.21 µmol N $L^{-1}h^{-1}$) and Anhui section (0.15 ± 0.14 µmol N L^{-1} h^{-1}) ($p < 0.05$), respectively. Similar to the spatial characteristics of REGs, U_{pot}s also increased along the river-estuary continuum **(Fig. 3)** , and there was a positive correlation between REGs and U_{pot}s ($r = 0.89, p < 0.01$) **(Fig. S1)** . In addition, the REGs accounted for 62% ± 18% of the U_{pot}s during the study period, this may suggest the processes of NH_4^+ recycling (NH_4^+ regeneration and uptake) are critical for regulating the supply of NH_4^+along the river-estuary continuum (Bruesewitz *et al.* , 2015).

3.3 Community biological NH_4^+ demand (CBAD)

Community biological NH_4^+ demand (CBAD) relates N dynamics to total microbial productivity and NH_4^+ deprivation in aquatic systems (Gardner *et al.* , 2017), and CBAD is approximated by observing differences between the potential NH_4^+uptake rates and actual NH_4^+regeneration rates (Gardner *et al.* , 2017). CBAD in the samples collected from Yangtze River ranged from - 0.43 to 0.80 µmol N L^{-1} h^{-1}, with the highest values found in estuary (0.30 ± 0.24 µmol N $L^{-1}h^{-1}$) and the relatively lower values in Anhui (0.02 ± 0.16 µmol N L^{-1} h^{-1}) and Jiangsu (0.01 ± 0.09 µmol N L^{-1} h^{-1}) section ($p < 0.05$) **(Fig. 4)** . The positive and negative values of CBAD indicate the relative magnitude of the NH_4^+potential uptake and regeneration rates. There were 6 and 8 sites in Anhui and Jiangsu sections showing negative values of CBAD, accounting for approximately 32% and 53% of the sampling sites, respectively. However, the CBAD rates of all sites in the estuary were positive values.

4. Discussion

4.1 NH_4^+recycling rates and contribution to the N budget in Yangtze River

REGs and U_{pot}s from Yangtze River were 0.26 (0.05˜1.19) µmol N $L^{-1}h^{-1}$ and 0.34 (- 0.22˜1.99) µmol N L^{-1} h^{-1}, respectively. Generally, the REGs and U_{pot}s in this study were within the range of values reported elsewhere (**Table 2**). In detail, they were comparable to several major polluted inflow rivers of Lake Taihu, China (Jiang *et al.* , 2019), but were higher than that from the low nutrients and low primary production Old Woman River (Mccarthy *et al.* , 2007b), Aransas River and Mission River (Bruesewitz *et al.* , 2015). Compared to other eutrophic lakes, such as Lake Taihu, Lake Petit saut and Lake Maracaibo (Gardner *et al.* , 1998; Collos *et al.* , 2001; Jiang *et al.* , 2019), insignificantly lower REGs and U_{pot}s were observed in the river-estuary continuum of Yangtze River. In addition, it is noteworthy that compared with lake ecosystems, river ecosystems tend to be found the greater REGs than U_{pot}s, suggesting a potential release risk of NH_4^+ in the river water column.

Sources of NH_4^+ to Yangtze River mainly include external nutrient inputs (river input, atmospheric deposition, and sediment release) and undocumented regenerated NH_4^+. Although it has been confirmed that the internal recycling processes play a vital important role in the NH_4^+ budget and ecological effect in eutrophic lakes (Wu *et al.* , 2017; Paerl *et al.* , 2011), the contribution of regenerated NH_4^+ to river-estuary continuum of Yangtze River is still unclear. Here we provide the first estimation of contribution from regenerated NH_4^+ to the N budget. Previous study has reported that river N input from the upper and middle reaches of Yangtze River was 57.14×10^8 kg N yr^{-1}, and N input in the lower reaches of Yangtze River (study area of this study) was estimated as 4.87 x 10^8 kg N yr^{-1} (Liu *et al.* , 2018). Atmospheric deposition in our study area was estimated as 2.76 x 10^8 kg N yr^{-1}, which was calculated as the deposition to emission ratio (Chen *et al.* , 2016). N input from sediments was estimated as 2.19 x 10^8 kg N yr^{-1}(estimated using the total mineralized N in the sediments minus the sum of denitrification, anammox and microbial N assimilation) (Lin *et al.* , 2016). Based on The Changjiang Bulletin issued by the Changjiang Water Resources Commission of the Ministry of Water Resources in 2018, the annual runoff of Datong station **(Fig.1)** in 2018 was 8028 x 10^8 m^3. Together with average REGs in the river-estuary continuum, we can estimate riverine regenerated NH_4^+ was approximately 21.8 x 10^8 kg N yr^{-1}. Compared with other N sources in the Yangtze River **(Fig. 5)** , the regenerated NH_4^+ is lower than riverine input from upper and middle reaches, but much higher than N input from lower reaches, atmospheric deposition, and sediments release. The regenerated NH_4^+ accounts for 25% of total N inputs in the study area, suggesting that regenerated NH_4^+ is an important internal N source for microbes and may influence nutrient dynamics in lower coasts. Although the summer rates of REGs can be significantly higher than in other seasons (Bruesewitz *et al.* , 2015; Jiang *et al.* , 2019), which made the annual regenerated NH_4^+ be overestimated, this ratio is high enough and could not be neglected.

4.2 Effects of SS on NH_4^+ recycling rates

No significant correlation between REGs and Chl-*a* was found in this study (r =0.220, $p > 0.05$) **(Table 3)** , indicating that algae was not the major source of regenerated NH_4^+. Unlike in eutrophic lakes (e.g., Lake Taihu, Jiang *et al.* , 2019), algae abundance in Yangtze River was low (2.8 +- 3.2 µg L^{-1}) due to high turbidity and washout from the high velocity of water flow in the Yangtze River, which leads to low concentrations of algae-derived organic N. However, significant correlations between REGs and SS instead of between REGs and DOC (r =0.197, $p > 0.05$) or DON (r =0.111, $p > 0.05$) suggested that REGs were mainly influenced by allochthonous particulate matters. SS is a complex mixture of organic detritus, microorganisms, and other organisms (Turner and Millward, 2002; Odman *et al.* , 1999), and SS in Yangtze River mainly come from the input of basin and soil erosion (Yang *et al.* , 2007). In this study, SS were positively correlated with COD (r =0.535, $p< 0.01$), and TN (r = 0.304, $p < 0.05$), and PN (r =0.478, $p < 0.01$) **(Fig. S2)** , suggesting that SS can act as a vector of nutrient to promote N cycling processes (Bilotta and Brazier, 2008; Zhang *et al.* , 2019). Moreover, significant correlations between REGs and COD (roughly represent the content of particulate and dissolved organic matter) (r =0.608, $p< 0.01$) and PN (r =0.455, $p < 0.01$) provide further evidence that heterotrophic bacterial (e,g., ammonifying bacteria) degradation of allochthonous particulate organic matters influences regeneration rates of NH_4^+. Ammonifying bacteria, a major participant for NH_4^+ regeneration activities, is abundant and widespread in river systems and tended to attach on SS (Xia*et al.* , 2013). Ammonifying bacteria population in culture with 5 g L^{-1} SS of river systems were shown two orders of magnitude higher than that without SS (Xia *et al.* , 2013). Thus, high regeneration rates of NH_4^+ in Yangtze River were deduced to be mainly contributed by heterotrophic bacteria attached on high levels of SS and PN in SS provides important substrate for bacterial metabolism.

NH_4^+ uptake is primarily composed of nitrification as well as assimilation by phytoplankton (Hampel *et al.* , 2017). However, the low biomass of phytoplankton in the turbidity river may contribute less to NH_4^+uptake. This is supported by the insignificant correlation between U_{pot}s and Chl-*a* (r =0.257, $p>$ 0.05). Study reported that nitrification rates increased with SS as a power function in the Yellow River, China, which was characterized by high SS concentrations (Xia *et al.* , 2009). Combined with high SS concentrations and low algae biomass in the Yangtze River, we can infer that NH_4^+uptake is mainly due to nitrification on SS. In this study, the SS concentrations were found to be significantly correlated with U_{pot}s (r =0.825, $p < 0.01$), which evidences the importance of SS in the NH_4^+ uptake process. Other factors such as the concentrations of COD, TN and PN can also affect NH_4^+ uptake rates. This may be due to the significant positive correlations between COD, TN, PN and the REGs, which provides abundant substrates for the uptake of NH_4^+.

4.3 Higher planktonic NH_4^+ demand in the estuary than river section of Yangtze River

Many studies have reported that Yangtze River estuary is characterized as P limitation due to sufficient N inputs (Wong *et al.* , 1998; Liang and Xian, 2018). However, results in this study suggest that planktonic NH_4^+ limitation may also occur in the river-estuary continuum of Yangtze River. CBAD as the index of NH_4^+ limitation in water, addresses the vital question whether or not the NH_4^+ demand of plankton could be met by NH_4^+ recycling in the water column (Jiang *et al.* , 2019; Gardner *et al.* , 2017). Our results showed that CBAD in the estuary of Yangtze River were significantly higher than in the Anhui and Jiangsu sections (near zero) **(Fig. 4)** , where NH_4^+ regeneration and uptake were nearly balanced.

Significant higher CBAD in the estuary could be due to several possible reasons. First, high concentrations of SS can not only promote microbial nitrification, but also accelerate NH_4^+ regeneration (Xia *et al.* , 2013; Xue *et al.* , 2019). Our results further indicated that SS concentrations promoted U_{pot}s faster than REGs (**Fig.6**), resulting in higher CBAD in the estuary of Yangtze River. Second, higher water temperatures (averaged 29.2 $\pm$ 0.5) in the estuary than other sections (averaged 28.4 +- 0.7 in Jiangsu section, averaged 28.1 +- 0.8 in Anhui section) of Yangtze River promote the higher CBAD values and NH_4^+limitation. Culture incubations in Lake Taihu showed that U_{pot}s increased faster than REGs in response to increasing water temperature between 5.0 and 32.9 (Jiang *et al.* , 2019). Another possible explanation for the higher CBAD in estuary is the higher plankton biomass. Previous studies reported that approximately 57% of bacterial cells were retained on the 0.7 µm filters and confirmed the presence of small phytoplankton and archaea through DNA analysis (Sipler *et al.* , 2017; Connelly *et al.* , 2014). Our results showed that PN was significantly correlated with Chl-*a* (r =0.314, $p < 0.05$) **(Fig. S3)** . Thus, PN concentrations can approximately represent biomass N of plankton intercepted by filters (GF/F). Comparing with the river sections, the estuary showed higher PN concentrations and CBAD increased with increasing PN concentrations (r =0.44, $p < 0.01$), implying that organisms may be increasing NH_4^+-deprived as plankton biomass increases, which was similar to eutrophic lakes (Gardner *et al.* , 2017).

4.4 Implications for management

Our results suggest that SS is a key factor controlling NH_4^+ recycling rates. High concentrations of SS in the water column of Yangtze River can promote both NH_4^+ regeneration and nitrification rates, resulting in the persistence of algal growth and potential high levels of NO_3^-concentrations. This is consistent with a previous study which showed that nitrification is one of the major sources of NO_3^- in the Yangtze River (Li *et al.* , 2010). Thus, effective measures to reduce SS concentrations in this turbid river may reduce the risk of N pollution. For example, restoration of shoreline vegetation, improvement of land use, and management of soil erosion are recommended to reduce SS concentrations in the lower reaches of Yangtze River.

Most previous studies in Yangtze River pay a special attention to NO_3^- exports into the East China Sea due to its high concentrations (Zhou *et al.* , 2008; Muller*et al.* , 2008; Liang and Xian, 2018). As a more preferred N nutrition for most phytoplankton species, NH_4^+ were overlooked in river systems because of low ambient concentrations. Our study and more evidences have shown that NH_4^+ recycling rates can be high even with low ambient concentrations (Jiang *et al.* , 2019; Gardner *et al.* , 2017), which can still influence estuaries and its coastal areas profoundly. High NH_4^+ recycling rates and demands reflect rapid microbial metabolism including both algal uptake and bacterial activities, which closely related to the growth and persistence of microbes (Hampel *et al.* , 2019). Compared to eutrophic lakes, NH_4^+ recycling in the estuary of Yangtze River was influenced in a larger part by bacterial activities. As large amounts of SS and organic matters flow downside into the coastal areas, rapid internal NH_4^+ recycling may also support algal growth and contribute to bloom persistence. Thus, diverse countermeasures should be focused on reducing estuarine inputs of SS or labile organic matters that potentially support high NH_4^+ recycling. Future studies are expected to understand how different properties of SS and organic matter influence water column NH_4^+ recycling. Results of this study is not only beneficial to the management of this third longest and most economically valuable river in the world, but also valuable to other large rivers and river-estuary continuum systems.

5. Conclusion

This study investigated NH_4^+ dynamics including its regeneration, potential uptake, and demand in the river-estuary continuum of Yangtze River. Higher REGs, U_{pot}s, and CBAD were found in the estuary, while relatively lower in the river sections. Moreover, compared to in the river sections, the higher regeneration and uptake rates of NH_4^+ as well as CBAD in the estuary are due to higher PN, COD, and SS concentrations. Faster microbial uptake of NH_4^+ than its regeneration result in obvious NH_4^+ demand in the estuary. In addition, NH_4^+regeneration is an important pathway of N supply in the Yangtze River, and regenerated NH_4^+ was estimated to be 21.81×10^8 kg N yr^{-1}, accounting for about 25% of total N inputs in the study area. This study indicates that NH_4^+ recycling is critical for regulating the supply and demand of NH_4^+ along the river-estuary continuum of Yangtze River. Effective managements to reduce SS inputs will alleviate N pollution and blooms (i.e., red tide) in the river-estuary continuum of the Yangtze River.

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CRediT authorship contribution statement

Jing-Ya Xue: Writing - original draft, Investigation, Conceptualization, Methodology, Data curation. Zhong-Hua Zhao: Formal analysis, Investigation, Data curation. Xiao-Long Yao: Conceptualization, Investigation. Wei-Ting Liu: Investigation. Lu Zhang: Investigation, Resources, Supervision, Project administration, Funding acquisition.

Conflicts of 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.

Data Availability Statement:

The data that support the findings of this study are available from the corresponding author (Professor Lu Zhang, luzhang@niglas.ac.cn).

Appendix A. Supplementary data

Supplementary data related to this article can be found in supporting information.

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