Combining the Multivariate Statistics and Dual Stable Isotopes Methods for Nitrogen Source Identification in Coastal Rivers of Hangzhou Bay, China

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Abstract

Coastal rivers contributed the majority of anthropogenic nitrogen (N) loads to coastal waters, often resulting in eutrophication and hypoxia zones. Accurate N source identification is critical for optimizing coastal river N pollution control strategies. Based on a 2-year seasonal record of dual stable isotopes ($\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$) and water quality parameters, this study combined the dual nitrate (NO_3^-) stable isotope method and the absolute principal component score-multiple linear regression (APCS-MLR) model to elucidate N dynamics and sources in two coastal rivers of Hangzhou Bay. Water quality/trophic level indices indicated light-to-moderate eutrophication status for the studied rivers. Spatio-temporal variability of water quality was associated with seasonal agricultural, aquaculture and domestic activities, as well as the seasonal precipitation pattern. The APCS-MLR model identified soil + domestic wastewater (69.5%) and aquaculture tailwater (22.2%) as the major nitrogen pollution sources. Further, the dual stable isotope method identified soil N, aquaculture tailwater, domestic wastewater and atmospheric deposition N contributions of $35.3 \pm 21.1\%$, $29.7 \pm 17.2\%$, $27.9 \pm 14.5\%$, and $7.2 \pm 11.4\%$ to riverine NO_3^- in the Cao'e River (CER) and $34.4 \pm 21.3\%$, $29.5 \pm 17.2\%$, $27.4 \pm 14.7\%$, and $8.7 \pm 12.8\%$ in the Jiantang River (JTR), respectively. The APCS-MLR model and dual stable isotope method showed consistent results for riverine N source identification. Combining these two methods for riverine N source identifications effectively distinguished the mix-source components from the APCS-MLR method and alleviated the high cost of stable isotope analysis, thereby providing reliable N source apportionment results with low requirements for water quality sampling and isotope analysis costs. Moreover, this study highlights the importance of soil N management and aquaculture tailwater treatment in coastal river N pollution control.

1. Introduction

Increasing anthropogenic nitrogen (N) inputs have significantly elevated riverine N loading to coastal zones over the past decades, resulting in an expansion of eutrophication and hypoxia zones (dissolved oxygen $< 2 \text{ mg L}^{-1}$) and red tides worldwide (Breitburg et al. 2018). As the most important conduit for anthropogenic N delivery from land to coastal waters, coastal rivers contributed more than 43.2 Tg N to the ocean in 2000, which is expected to further increase in the future (Izett & Fennel 2018; Breitburg et al. 2018). Given the considerable N exported to coastal waters, reduction of coastal river N pollution is essential for mitigating coastal water eutrophication (Jiang et al. 2020). Therefore, it is warranted to quantitatively assess and identify the main N pollution sources in coastal rivers for optimizing pollution control strategies.

Watershed-scale mathematical models (including lumped models and distributed models) have been widely developed to identify riverine N sources (Borah & Bera 2004; Chen et al. 2014; Wellen et al. 2015; Imani et al., 2019). However, distributed models usually require large amounts of data (including hydrological, hydrochemical records, meteorological, land use, topography) for calibration and validation, resulting in many challenges for their applications in many regions (Chen et al. 2014). For lumped models, although they can provide a simplified understanding of N sources, requirements for watershed attributes and N discharge/loads from wastewater treatment facilities and industries limit their widespread application (Wellen et al. 2015; Hu et al., 2019). To overcome several of the limitations associated with these mathematical models, the absolute principal component score-multiple linear regression model (APCS-MLR) was adopted to quantify contributions from different pollution sources based on river water quality data. For example, the APCS-MLR method was used to identify the major contributors of riverine N in urban (Meng et al. 2018; Shen et al. 2021), suburban (Yang et al. 2013b), agricultural (Yuan et al. 2020) and mixed land-use catchments (Su et al. 2011). However, the APCS-MLR method cannot effectively distinguish contributions from sources with similar pollution characteristics (e.g., domestic wastewater and soil N are often identified as a mixed source) (Pasten-Zapata et al. 2014).

Alternatively, the dual nitrate (NO_3^-) stable isotope ($^{15}\text{N-NO}_3^-$ / $^{18}\text{O-NO}_3^-$) approach can be an efficient tool for assessing N transformations (e.g., nitrification and denitrification, Xia et al. 2017; Yi et al. 2017; Hu et al. 2019) and identifying/tracing N sources (Xue et al. 2009; Ji et al. 2017; Hu et al. 2019; Jiang et al. 2020). The dual stable isotopic information coupled with Bayesian statistics (i.e. MixSIAR, Liu & Han 2021) can effectively quantify N sources with unique isotopic signatures (e.g., atmospheric deposition, soil N pools, chemical fertilizers, manure/sewage, Xue et al. 2009). However, the high analytical cost has limited the widespread application of the dual stable isotope approach (irregular and sparse data sets), thereby hindering its rigorous adoption for N-source apportionment (Yang et al. 2013a; Kim et al. 2015). Therefore, combining the APCS-MLR method and dual nitrate (NO_3^-) stable isotope approach provides complementary information to enhance the accuracy of N source apportionment results while lowering the requirements/costs for water quality monitoring (Yuan et al. 2020).

In China, 16.6–37.5% of coastal waters experienced eutrophication and ~700 red tide events occurred due to excessive nutrients in 2011–2020, with 60% of these events occurring in the East China Sea (Ministry of Ecology and Environment, 2020). Large population density coupled with intensive agricultural, aquaculture and industrial activities in coastal areas of southeast China cause excessive N loading to coastal rivers, estuaries and ocean (Bu et al. 2011; Liu et al. 2018; Luo et al. 2018). The aggregation of multiple N sources in the coastal area of southeast China (Herbeck & Unger 2013) makes it difficult to quantitatively identify N sources in coastal rivers. Thus far, there have been only limited attempts to quantify the N sources in coastal rivers of China (Kang & Xu 2016; Wu et al. 2020; Herbeck et al. 2021).

This study collected a 2-year, seasonal record of dual isotopes ($\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$) and associated water quality data that was subsequently used for a synergistic analysis employing APCS-MLR and dual NO_3^- stable isotopes to quantify the primary N sources in two coastal rivers draining into Hangzhou Bay, southeast China. Based on the assessment of spatial and seasonal variations of eutrophication status for the coastal rivers, major N sources were identified by the APCS-MLR method. The dual nitrate (NO_3^-) stable isotope approach was then adopted to quantify riverine N sources. This study provides an integrated approach for quantifying N contributions from multiple sources in coastal rivers, thus providing theoretical support for developing effective N pollution control strategies.

2. Methods And Materials

2.1. Study area

As an important inlet to the East China Sea, Hangzhou Bay comprises 11 county-level administrative divisions with a total area of 4800 km² (Sun et al. 2016). Hangzhou Bay is one of the most severely polluted coastal waters in China and was the location of 60% of the extreme red tide events (max area >100 km²) in China during 2020 (Ministry of Ecology and Environment, 2020). Climate is subtropical monsoon with an average annual temperature and precipitation of 15–25 °C and 1300–1600 mm, respectively (Zhou et al. 2020). The water year was separated into four hydrologically contrasting seasons: dry season (spring: March–May; winter: December–February) and wet season (summer: June–August, autumn: September–November). Over the past 60 years, tideland reclamation projects have spurred the development of aquaculture ponds in the area by 138% (Zhou et al. 2020; Qiu et al. 2021). The southern coast of Hangzhou Bay (area with red boundary, Fig. 1) has an aquaculture zone comprised of 572.9 km² of ponds (accounting for ~12.0% of the total area). Major aquaculture species are fish, shrimp and crabs, with breeding areas of 9.1%, 26.2% and 42.2%, respectively (Ni et al. 2020).

The Cao'e River (CER) and Jiantang River (JTR) are two typical coastal rivers flowing into southern Hangzhou Bay. Aquaculture ponds averaged ~34% of the CER nearshore area (within 3 km of river channel, 72.77 km²), with developed lands and arable land contributing ~33% and ~24%, respectively. Primary land-use types in the JTR nearshore (accounting for 39.61 km²) were aquaculture ponds, arable land and developed lands, comprising 77%, 3% and 2%, respectively (Table 1). The major aquaculture species in CER and JTR were shrimp. Population density in the CER region was ~560 person km^{–2} and the JTR region has ~1300 person km^{–2}. In the CER and JTR regions, multiple-year average precipitation is ~1600 mm and ~1800 mm, respectively, with 40%–45% of rainfall occurring in the summer season due to typhoon events (Fig. 2).

Table 1 Land-use distributions for Cao'E River (CER) and Jiantang River (JTR)

	Total area (km ²)	A (%)	D (%)	G (%)	P (%)	Others (%)
CER	72.8	24.1	32.9	9.4	33.6	<0.1
JTR	39.6	3.2	1.6	<0.1	77.4	17.9

A-arable lands; D-developed lands; G-grasslands; P-aquaculture ponds

2.2. Field sampling and water quality and stable isotope analysis

We collected water samples from four sites (XS, CXF, CXW and PH, Fig. 1) around Hangzhou Bay for a total of 4 seasonal sampling events in 2019. Basic water quality indicators were measured, including total nitrogen (TN), total phosphorus (TP), ammonia (NH⁺ 4), nitrate (NO[–] 3), nitrite (NO[–] 2), dissolved organic carbon (DOC), chemical oxygen demand (COD), chlorophyll-a (Chl-a), pH, dissolved oxygen (DO) and transparency (Trans). Water samples for δ¹⁸O-NO[–] 3 and δ¹⁵N-NO[–] 3 analyses were collected seasonally in the CER and JTR from 2019 to 2020. Notably, water samples were only collected in summer (June) and autumn (September) in 2020 due to COVID-19 restrictions (i.e., water samples were collected in 6 seasons). Samples were collected uniformly at 3–5 points across the river channel and mixed to obtain a single depth-width integrated sample. All water samples were immediately sealed and stored in the dark at 4 °C.

The NO[–] 3-N, NO[–] 2-N and NH⁺ 4-N concentrations were measured after filtering through a sterile 0.45-μm Millipore polycarbonate membrane, whereas DOC was determined after filtering through a sterile 0.45-μm Millipore organic phase membrane. NH⁺ 4-N and NO[–] 3-N/ NO[–] 2-N were determined using the spectrophotometric salicylic acid and phenol disulfonic acid methods, respectively. DOC was measured with a Jena multi-N/C 3100 analyzer. TN concentrations were analyzed as NO[–] 3-N following alkaline potassium persulfate digestion. Transparency and chlorophyll-a (Chl-a) were measured by Secchi disk and chlorophyll-a fluorescence detector (FuloroQuik, AMI, USA), respectively. Temperature, pH and DO were measured in the field using YSI sensors (Xylem, New York, USA). All water samples were analyzed within 24 h of collection.

Water samples for δ¹⁵N-NO[–] 3 and δ¹⁸O-NO[–] 3 analyses were stored at -20 °C and subsequently transported to the Environmental Stable Isotope Lab (Chinese Academy of Agricultural Sciences, Beijing, China) for analysis. NO[–] 3 was converted to N₂O by the denitrifier method (Christensen & Tiedje 1988), and then detected by a continuous-flow isotope ratio mass spectrometer (IRAM, Isoprime 100). Isotope ratio values are reported in parts per thousand (‰) relative to atmospheric N₂ and Vienna Standard Mean Ocean Water (VSMOW) for δ¹⁵N-NO[–] 3 and δ¹⁸O-NO[–] 3, respectively (Xue et al., 2009). Sample analysis had an average precision of 0.31‰ for δ¹⁵N-NO[–] 3 and 0.55‰ for δ¹⁸O-NO[–] 3.

All stable isotope results were expressed as delta values by representing the deviations in per mil (‰) from the respective reference standard as follow:

$$\delta(\text{‰}) = 1000 \times (R_{\text{sample}} / R_{\text{standard}}) - 1 \tag{1}$$

where R_{sample} and R_{standard} are the isotopic ratios (¹⁵N/¹⁴N, ¹⁸O/¹⁶O) for the sample and standard, respectively.

2.3. Trophic level index (TLI)

To assess the river eutrophication status, we calculated trophic level index (TLI) values based on measured water quality parameters (China Environmental Monitoring Station, 2001). TLI is a weighted sum based on correlations between Chl-a and various water quality parameters, and was calculated based on the concentrations of Chl-a, TP, TN, Trans and COD_{Mn} (Wang et al. 2019):

$$TLI = \sum_{j=1}^m w_j \times TLI(j) \quad (2)$$

$$W_j = r_{ij}^2 / \sum_{j=1}^m r_{ij}^2 \quad (3)$$

where W_j is the weight of each index parameter and $TLI(j)$ is the calculated index based on each pollutant concentration; r_{ij} is the correlation coefficients between the reference *Chl-a* and each parameter j (*Chl-a*: 1, TP: 0.84, TN: 0.82, Trans: -0.83 and COD_{Mn} : 0.83), which were obtained from water survey data sets from China (Wang et al. 2019). TLI values range from 0 to 100 and characterize the eutrophication status based on five categories: oligotrophic ($TLI < 30$), mesotrophic ($30 < TLI < 50$), low-eutrophic ($50 < TLI < 60$), moderately-eutrophic ($60 < TLI < 70$), and hyper-eutrophic ($TLI > 70$) (Wang et al. 2019).

2.4. Nitrogen source identification methods

2.4.1 Absolute principal component score-multiple linear regression method

The absolute principal component score-multiple linear regression (APCS-MLR) method is derived from principal component analysis (PCA), and can be applied to identify pollutant sources (Yang et al. 2013a; Yuan et al. 2020). The overall PCA can be expressed as follow:

$$Z_{ij} = \sum_{k=1}^p g_{ik} h_{kj} \quad (4)$$

where Z_{ij} is the normalized value of element i concentration, g_{ik} represents the concentration of i in pollution source k (i.e., factor load), and h_{kj} is the contribution of pollution source k to the sample j (i.e., factor score).

Based on the rotation factor loads and principal eigenvectors obtained by PCA, multiple linear regression (MLR) was performed using the absolute principal component score (APCS) and eigenvectors to calculate the contribution rates (Su et al., 2009). The PCA and APCS-MLR analyses were performed with SPSS software (Ver. 17.0, SPSS, IL, USA).

2.4.2 Dual nitrate (NO₃) stable isotope method

The Bayesian tracer mixing model (e.g., SIAR) has been applied to identify contributions of multiple N sources in individual water bodies (Stock et al. 2018; Hu et al., 2019). In recent years, an inclusive, rich and flexible Bayesian tracer mixing model (i.e., MixSIAR) was further developed to include fixed and random effects as covariates explaining variability in mixture proportions (Stock et al. 2018; Liu & Han 2021). The MixSIAR (Ver. 3.0.2) was applied to apportion the contribution rates using R software (Ver. 4.0.3).

As MixSIAR needs pre-determination information for pollution sources, we adopted the results from APCS-MLR as the preliminary sources. Combining the field survey, land-use distribution and results from APCS-MLR (Table 1), this study apportioned riverine N to atmospheric deposition, soil N, aquaculture tailwater and domestic wastewater. Aquaculture tailwater and atmospheric deposition N isotope information were directly measured, while isotopic values for soil and domestic wastewater N were obtained from published values for a nearby watershed (Ji et al. 2017).

The principle of MixSIAR can be expressed by the following formula:

$$X_{ij} = \sum_{k=1}^p g_{ik} f_{jk} + e_{ij} \quad (5)$$

where X_{ij} is the tracer of species j of sample i , p represents the number of assumed sources, f_{jk} is the tracer value for species j in source k , g_{ik} denotes the contribution of source k to sample i , and e_{ij} is an error term.

All regression and correlation analyses were performed using SPSS (version 17.0, SPSS, IL, USA). One-way ANOVA with an LSD multiple comparisons test was used to examine the spatio-temporal variability of water quality parameters.

3. Results And Discussion

3.1 Spatio-temporal variability of water quality

According to China's national water quality standards (GB 3838-2002, Ministry of Environmental Protection of China, 2002), most of the coastal rivers discharging to Hangzhou Bay are considered severely polluted. Several pollutants concentrations exceeded the standard of "Grade IV" (including TP and COD_{Mn} , Table 2), which means severely polluted and can only be used for industry. Although no national river water quality standard is established for TN, TN concentrations (3.12 ± 2.14) were much higher than the 2.0 mg L^{-1} value classified as "Grade V" for reservoirs/lakes (Table 2), which is unable to support ecological functions. Riverine DO concentrations ($7.13 \pm 2.34 \text{ mg L}^{-1}$) were high due to natural reaeration by running water, as well as the artificial aeration of aquaculture discharge waters before release to rivers. Nutrient pollutant (TP, TN, NH_4^+ and NO_3^-) concentrations in aquaculture ponds were all much higher (1-2 times) than those measured in river water (Table 2), implying that aquaculture discharge waters were a potential pollution source for coastal rivers.

Table 2
Mean and standard deviation of water quality data for all sampling sites in coastal rivers of Hangzhou Bay

	TP	TN	NH+ 4	NO-3	NO- 2	DOC	COD	Chl-a	pH	DO
	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(mg L ⁻¹)	(µg L ⁻¹)		(mg
PH n=4	0.38±0.31 ^c (0.10-0.89)	3.27±2.06 ^b (1.64-6.80)	0.53±0.64 ^b (0.11-1.63)	1.07±0.74 ^b (0.16-2.07)	0.06±0.06 ^b (0.03-0.17)	11.5±9.6 ^b (2.8-27.8)	6.72±1.39 ^c (4.44-7.92)	15.5±15.3 ^c (1.3-40.8)	7.76±0.41 ^a (7.44-8.45)	6.30 (1.6
CXW n=4	0.23±0.18 ^c (0.04-0.46)	2.32±1.19 ^b (0.81-3.92)	0.93±0.74 ^b (0.20-2.16)	0.80±0.41 ^b (0.17-1.26)	0.10±0.14 ^a (0.01-0.34)	10.4±6.8 ^b (3.9-21.1)	8.78±1.82 ^c (5.70-10.40)	57.7±42.6 ^a (25.0-131.0)	8.05±0.40 ^a (7.45-8.59)	6.67 (4.6
CXF n=4	1.21±0.27 ^a (0.90-1.64)	6.58±5.11 ^a (3.11-15.4)	1.09±1.42 ^a (0.22-3.57)	3.88±3.77 ^a (0.28-10.20)	0.18±0.22 ^a (0.01-0.56)	15.8±16.9 ^a (4.7-44.9)	8.33±1.89 ^c (6.34-11.10)	33.3±11.1 ^b (15.8-46.7)	8.46±0.62 ^a (7.49-9.21)	6.66 (3.4
XS n=4	0.11±0.07 ^d (0.02-0.20)	0.85±0.23 ^c (0.56-1.14)	0.37±0.20 ^c (0.06-0.60)	0.35±0.09 ^c (0.18-0.40)	0.01±0.01 ^b (0.00-0.03)	7.7±1.3 ^c (6.4-9.5)	9.31±2.24 ^b (6.97-11.70)	32.7±15.3 ^b (12.4-49.4)	8.50±0.19 ^a (8.36-8.83)	8.89 (7.4-10.5
CER n=16	0.49±0.23 ^c (0.15-1.13)	3.08±0.96 ^b (1.50-4.80)	0.72±0.24 ^b (0.30-1.20)	1.92±0.61 ^b (1.10-3.78)	0.02±0.02 ^b (0.00-0.04)	–	16.14±8.77 ^a (0.80-28.90)	–	–	–
JTR n=17	0.74±0.41 ^b (0.11-1.34)	3.04±0.84 ^b (2.10-5.30)	0.56±0.27 ^b (0.25-1.50)	1.82±0.39 ^b (1.49-3.30)	0.01±0.01 ^b (0.00-0.05)	–	15.07±3.24 ^a (8.00-19.80)	–	–	–
River water n=49	0.58±0.41 (0.02-1.34)	3.12±2.14 (0.56-15.40)	0.67±0.57 (0.06-3.57)	1.75±1.44 (0.16-10.20)	0.04±0.09 (0.00-0.56)	–	13.2±6.49 (0.80-39.30)	–	–	–
Aquaculture ponds n=6	1.32±0.30 ^{**} (0.97-1.82)	5.88±0.41 ^{**} (4.20-8.20)	1.36±0.22 ^{**} (1.10-1.80)	3.20±0.87 ^{**} (2.3-4.9)	0.11±0.03 [*] (0.08-0.17)	–	27.15±3.28 ^{**} (21.3-31.6)	–	–	–
The numbers in the brackets represent the range of each parameter.										
Superscript letters after numbers denote significant differences (threshold is p<0.05, among PH, CXW, CXF, XS, CER and JTR).										
* denotes significant differences between river waters and aquaculture pond waters (*: p<0.05, **: p<0.01)										

Generally, water quality across rivers showed considerable spatio-temporal variability (Table 2). Pollutants concentrations (including TP, TN, NH+ 4-N, NO-3-N, NO- 2-N, DOC, and chlorophyll-a) at site CXF were 2.0-15.3 fold higher than the other sites. Site XS showed the lowest concentrations for most pollutants (including TP, TN, NH+ 4-N, NO-3-N, NO- 2-N and DOC, Table 2). Concentrations of NH+ 4-N and NO-3-N exhibited similar spatial variations (i.e., highest in CXF and lowest in XS). The higher NH+ 4-N and NO-3-N concentrations observed at CXF are attributed to the higher population density (1300 person km⁻², ~150% higher than other sites) and higher precipitation (Fig. 2). This implies that soil N and domestic wastewater might be important N sources to CXF. Most of the sampling sites were characterized as moderately-eutrophic (60<TLI<70); however, there was considerable spatial variation across sites (Table 2). The highest TLI was observed at CXF (hyper-eutrophic, TLI>70), followed by CXW, PH and XS (Table 2).

In terms of temporal variability, TN (p<0.01), NO-3-N (p<0.01) and NH+ 4-N (p<0.05) concentrations were significantly higher in the winter/spring dry season than in the summer/autumn wet season (Table 2). Moreover, the higher N concentrations in winter/spring may be associated with higher aquaculture tailwater discharge following the aquaculture harvest (Ni et al., 2021), as well as lower denitrification due to lower temperatures (Jiang et al. 2020). Conversely, no significant seasonal variations were observed in TP and COD_{Mn} concentrations across all sites (Table 2), implying differential processes affecting the various pollution sources (i.e., aquaculture tailwater, domestic wastewater, and agricultural runoff) and their transformations. TLI values for XS, PH, CXW and CXF were higher in summer or autumn (Table 2), which was mainly associated with seasonal variations of Chl-a as regulated by temperature and light conditions (Herb & Stefan 2003; Paerl & Paul 2012; Jiang et al. 2018).

3.2 Riverine N source apportionments

3.2.1 Source apportionment by the APCS-MLR method

Estimated sphericity values based on Kaiser-Meyer-Olkin (KMO) and Bartlett's test for the PCA incorporating 9 hydrochemistry parameters (TP, TN, COD_{Mn}, pH, DOC, NH+ 4, DO, NO- 2, NO- 3) were 0.580 and 212 (p<0.05), respectively. These values support the efficacy of the PCA approach for pollution source identification (Mei et al. 2014). Three principal factors were identified by PCA explaining a total variance of 84.5% (Table 3). In detail, factor 1 explained 50.3% of total variance with strong positive loadings on TN (0.951), TP (0.284), and NH+ 4 (0.920) and a moderate negative loading on DO (-0.190). These

characteristics are indicative of nutrient pollution from untreated domestic wastewater and agricultural runoff (Yang et al. 2013a; Mei et al. 2014; Yuan et al. 2020), implying mixed impacts from multiple pollution sources.

Table 3
Loadings of the water quality parameters on the principal components in coastal rivers of Hangzhou Bay

	Factor 1	Factor 2	Factor 3
TP	0.282	-0.139	0.913
COD _{Mn}	0.316	0.697	0.078
DO	-0.164	0.786	-0.302
TN	0.943	-0.008	0.06
pH	-0.324	0.652	0.621
DOC	0.915	0.162	-0.168
NH ⁺ 4-N	0.937	0.209	-0.195
NO ⁻ 2-N	0.954	-0.071	-0.039
NO ⁻ 3-N	0.839	0.132	0.222
% of variance	50.3	18.2	16.0
Cumulative %	50.3	68.5	84.5
Contributions to NO ⁻ 3	0.695	0.222	0.083
The absolute values of bold values exceeding 0.50 are the dominant components of each factor			

Factor 2 explained 18.5% of the total variance with strong positive loadings on DO (0.786), pH (0.652) and COD_{Mn} (0.697) and moderate correlation with NH⁺ 4 (0.209). Factor 2 characteristics are consistent with a pollution source from aquaculture tailwaters. The strong positive loading on NH⁺ 4, pH and DO is attributed to the usage of fertilizers (e.g., organic fertilizer, ammonia fertilizer, compound fertilizer, etc.) (Herbeck et al. 2021) and artificial aeration activities in aquaculture management practices (Ni et al. 2020). The high loading on COD_{Mn} was associated with large organic food inputs and excreta of aquatic animals, as well as organic-rich sediments in the tailwater (Wang et al. 2020; Kim et al. 2021).

Factor 3 explained 16% of the total variance with strong positive loadings on TP (0.913) and pH (0.621), as well as negative loadings on DO (-0.302) and NH⁺ 4 (-0.195). We ascribe these loading characteristics to point source pollution (Mei et al. 2014). The low loading on NH⁺ 4 may result from efficient domestic sewage treatment and high nitrification potential during transport (Yang et al. 2013a; Yuan et al. 2020). In summary, the PCA results identified pollutant sources in the rivers originating from soil, domestic wastewater (including untreated and well treated sewage) and aquaculture tailwater.

The APCS-MLR method was further applied to quantify the contribution of each N source to riverine N loads. The reasonably strong coefficient of determination ($R^2 = 0.845$) supports the efficacy of the APCS-MLR model (Yang et al. 2013b; Yuan et al. 2020). We interpret the results to indicate that a mixed source (soil N and untreated domestic wastewater) was the primary N source to coastal rivers (69.5%). Additional sources were assigned to aquaculture tailwaters (22.2%) and residual sources (8.3%). This quantitative information highlights the importance of controlling pollution from wastewater and soil erosion/leaching in coastal watersheds. It is important to note that limitations associated with the APCS-MLR method prevents further characterization of the mixed source into higher resolution components, which limits the use of the results for the development of effective pollution control strategies.

3.2.2 Source apportionment by the dual stable isotope method ($\delta^{18}\text{O}/\delta^{15}\text{N}$ -NO⁻ 3)

The overall $\delta^{18}\text{O}$ -NO⁻ 3 and $\delta^{15}\text{N}$ -NO⁻ 3 values were similar ($p > 0.05$) between JTR ($4.22 \pm 3.52\text{‰}$ and $5.11 \pm 4.20\text{‰}$) and CER ($4.02 \pm 3.98\text{‰}$ and $5.34 \pm 3.35\text{‰}$), respectively (Fig. 3). The ranges of $\delta^{18}\text{O}$ -NO⁻ 3 and $\delta^{15}\text{N}$ -NO⁻ 3 signatures were overlapping between soil N, domestic wastewater, aquaculture tailwater and river water, suggesting that all of these sources might contribute to riverine N loads (Hu et al. 2019).

More than 70% of the $\delta^{18}\text{O}$ -NO⁻ 3 values fell within the range of NO⁻ 3 formed as a product of nitrification ($-1.92 - 7.44\text{‰}$; calculated based on the $\delta^{18}\text{O}$ values of atmospheric O₂ and precipitation; Ji et al. 2017). The slope between $\delta^{15}\text{N}$ -NO⁻ 3 and $\delta^{18}\text{O}$ -NO⁻ 3 for 2019 and 2020 samples were 1.11 ($n=19$, $R^2=0.54$, $p<0.01$) and 1.45 ($n=12$, $R^2=0.48$, $p<0.01$), respectively. These values exceeded the range of slopes expected for N experiencing considerable denitrification (i.e., slopes between 0.43 and 1.0, Fig. 3, Xue et al. 2009; Hu et al. 2019). The absence of a strong denitrification signal is consistent with the high DO concentrations in river waters ($7.12 \pm 2.34 \text{ mg L}^{-1}$, Table 2). These high DO concentrations would be expected to hinder the denitrification process ($<2 \text{ mg L}^{-1}$ favors denitrification, Rivett et al. 2008). Overall, nitrification rather than denitrification was the likely dominant N transformation process in CER and JTR.

Similar $\delta^{18}\text{O}/\delta^{15}\text{N}$ -NO⁻ 3 results were observed between aquaculture pond and river waters, with more than 60% of $\delta^{18}\text{O}$ -NO⁻ 3 values falling within the range indicative of NO⁻ 3 formation as a product of nitrification ($-1.92 - 7.44\text{‰}$). No significant relationship ($p=0.90$, $n=15$) was observed between $\delta^{15}\text{N}$ -NO⁻ 3 and $\delta^{18}\text{O}$ -NO⁻ 3 for aquaculture pond waters (Fig. 3). Collectively, these results infer that artificial aeration of aquaculture ponds maintains adequate DO to

promote nitrification and inhibit denitrification (Jiang et al. 2020; Ni et al. 2020). Thus, aquaculture tailwaters may be an important source of NO_3^- pollution in both CER and JTR.

Based on our field observations, land-use distribution and APCS-MLR analysis, four major N sources (i.e., soil N, domestic wastewater, aquaculture tailwater, and atmospheric deposition) were identified for MixSIAR analysis. The MixSIAR analysis for JTR indicated that soil N ($34.4 \pm 21.3\%$) was the dominant riverine N source, followed by aquaculture tailwaters ($29.5 \pm 17.2\%$) and domestic wastewater N ($27.4 \pm 14.7\%$). The contribution from atmospheric deposition was deemed relatively low ($8.7 \pm 12.8\%$, Fig. 4c). Similar results were determined for CER, with riverine N source contributions from soil N, aquaculture tailwater, domestic wastewater and atmospheric deposition contributing $35.3 \pm 21.1\%$, $29.7 \pm 17.2\%$, $27.9 \pm 14.5\%$, and $7.2 \pm 11.4\%$, respectively (Fig. 4b).

High N contributions from soil N pools are derived from long-term excess N applications (i.e., legacy N) that have accumulated in the soil. The average soil N contents were 2.53 g kg^{-1} in JTR and 2.40 g kg^{-1} in CER, values much higher than the China national average (1.45 g kg^{-1}) for cropland soils (Zhou et al. 2020; China Soil Database). A large contribution from aquaculture is consistent with the high density of aquaculture ponds and the high N concentrations found in pond waters. Whereas pond waters are artificially aerated to avoid low DO concentrations in tailwaters, there are no specific management practices to address the release of N in tailwaters (Ni et al. 2021). High N contribution from domestic wastewater may be derived from high population intensity in both CER ($\sim 560 \text{ person km}^{-2}$) and JTR ($\sim 1300 \text{ person km}^{-2}$). Atmospheric deposition also contributes a considerable proportion N due to excess N fertilizer application and fossil fuels burning (Zhou et al. 2020).

In terms of temporal variability, the contribution of N from aquaculture tailwaters was significantly higher in winter in both CER and JTR ($p < 0.01$, Fig. 4b & 4c), which was consistent with the shrimp breeding cycle. Large amounts of N accumulate in the ponds during the breeding period (summer to autumn) and are subsequently discharged into the rivers during the winter following harvest (Ni et al. 2020). Similarly, the N contribution from domestic wastewater was highest in winter ($p < 0.01$, Fig. 4), owing to point source pollution being most strongly expressed during the winter dry season due to the lack of dilution (Hu et al. 2018). Seasonal variation of soil N contributions in CER and JTR were similar, with the lowest contribution in winter and the highest contribution in summer/autumn (Fig. 4b & 4c). This seasonal pattern can be attributed to the seasonal distribution of precipitation (Fig. 2). Greater soil erosion/leaching occurs in the summer when $\sim 45\%$ of annual precipitation occurs (CER = 7.85 and JTR = 8.89 mm day^{-1}), compared to only $\sim 13\%$ of precipitation in winter (CER = 2.48 and JTR = 2.38 mm day^{-1}). Higher rainfall amounts and intensities were shown to result in greater soil N losses to rivers (Deng et al. 2019; Zhang et al. 2021). The contribution of atmospheric deposition (wetfall + dryfall) was relatively stable with no significant seasonal fluctuations ($p > 0.05$).

3.2.3 Comparison between the two methods

The combined utilization of the APCS-MLR and dual ($\delta^{18}\text{O}/\delta^{15}\text{N}$ - NO_3^-) stable isotope methods provided consistent results and a limited verification step for estimating riverine N sources to rivers (Table 3 and Fig. 4). The estimated N contribution from soil N and domestic wastewater from the dual stable isotope with MixSIAR method (63.8%) was remarkably similar to the mixed source contribution calculated from the APCS-MLR method (69.2%). Similarly, the estimated N contributed from aquaculture tailwaters was consistent between the two methods (27.5% vs. 22.2%). These results provide compelling evidence that soil N, aquaculture tailwaters and domestic wastewater were the major riverine N pollution sources.

Notably, the APCS-MLR method failed to distinguish between the contributions from soil N pool and domestic wastewater (Table 3). While the dual stable isotope method was able to identify soil N and domestic wastewater as distinct sources, the estimated results incorporate uncertainty due to lack of independent verification (Xue et al. 2009; Hu et al. 2019) and are often constrained by the high cost of isotope analysis. In this study, the two methods were complementary with the APCS-MLR results providing the necessary source discrimination information for subsequent MixSIAR analysis, and reducing the cost of source screening for the stable isotope method. Furthermore, the two methods provide a measure of verification, which reduces the uncertainty associated with major source identification. Integration of the two methods provides an opportunity to achieve reliable N pollution source identification for rivers having limited data records for dual isotopes and water quality. Future studies incorporating relevant data from higher monitoring frequencies should be adopted to further evaluate the advantages/limits of the combined APCS-MLR model and dual isotope method by testing performance as a function of temporal data resolution.

3.3 Implications for N pollution control in coastal rivers

Results of this study confirmed the high eutrophication status of coastal rivers in Hangzhou Bay (Table 2), which contributes to persistent red tide events in coastal waters of eastern China (Breitburg et al. 2018; Jiang et al. 2020). Quantitative N source identification/apportionment provides critical information for adopting specific strategies to mitigate riverine N pollution in receiving waterbodies. Considering the high pollution contribution from cropland soil N runoff to Hangzhou Bay (Fig. 4a), sustainable nutrient management strategies are necessary to optimize utilization of soil N by crops and to reduce excessive N fertilizer inputs and subsequent N losses to runoff/leaching (Hu et al. 2019). Moreover, soil and water conservation practices (e.g., conservative tillage, plant/residue soil cover, and terraces) are important approaches for mitigating N pollution from soil erosion (Chen et al. 2018). For aquaculture tailwaters (Table 3, Fig. 4), it is necessary to reduce N and other pollutant discharge loads by improving aquaculture management practices (Turcios & Papenbrock 2014). For example, an integrated multi-trophic aquaculture (IMTA) system can greatly reduce aquaculture tailwater pollutant discharge by recycling by-products of waste from one aquaculture species to fertilize/feed other aquatic species (Neori et al. 1998; Naylor et al. 2000; Rakocy et al. 2006; Martan 2008). Zhang & Kitazawa (2016) estimated that IMTA can achieve maximum reductions of 30% for phytoplankton and 35% for N in tailwaters. Additionally, flow-through wetlands consisting of oxidation and reduction ponds connected in series can effectively enhance nitrification and subsequent denitrification to remove inorganic N species. A large quantity of untreated domestic wastewater is often discharged directly into coastal rivers in the Hangzhou Bay region due to the lack of efficient collection and treatment facilities ($\sim 46\%$ in 2019, Environmental Monitoring Center of Zhejiang, 2020). Our analysis indicated that domestic wastewater contributed considerable N to river waters, comprising a larger proportion of the riverine N load during the winter/spring dry period (Table 3, Fig. 4). Therefore, it is necessary to improve domestic waste collection for treatment.

Given the changing seasonal dynamics of riverine N sources (Fig. 4), management strategies must consider how specific sources change across different seasons. As eutrophication occurs disproportionately in summer and autumn due to higher temperature and light conditions (Paerl & Paul 2012; Jiang et al. 2018), management strategies should target N control measures to reduce riverine N concentrations especially during these two seasons. For example, effective soil and water conservation practices, as well as cropland nutrient management strategies, are warranted to reduce soil N losses during this summer/fall critical period for eutrophication. Correspondingly, more attention should be paid to domestic wastewater and aquaculture tailwater N reduction strategies during low flow periods (e.g., winter and spring) to reduce eutrophication potential.

4. Conclusion

This study combined the APCS-MLR model and the dual stable isotope ($\delta^{15}\text{N}$ -NO $_{3}^{-}$ and $\delta^{18}\text{O}$ -NO $_{3}^{-}$) method to quantitatively assess N dynamics and source contributions in coastal rivers discharging into Hangzhou Bay. This complementary and integrated approach achieved robust N source information with limited sampling requirements. Our water quality measurements confirmed that the studied rivers were severely polluted, with more than half experiencing moderate eutrophication (TLI>60). Spatio-temporal variability in water quality was associated with seasonal agricultural, aquaculture and domestic activities, coupled with the seasonal rainfall pattern. The APCS-MLR model identified a mixed source (soil N + domestic wastewater) and aquaculture tailwater as the main N sources, with contributions of 69.5% and 22.2%, respectively. The dual stable isotopes method identified considerable nitrification and minimal denitrification as N transformation processes. Furthermore, the stable isotope method apportioned riverine N contributions from soil N, aquaculture tailwater, domestic wastewater and atmospheric deposition N, with contributions of $35.3 \pm 21.1\%$, $29.7 \pm 17.2\%$, $27.9 \pm 14.5\%$ and $7.2 \pm 11.4\%$ in the Cao'e River (CER) and $34.4 \pm 21.3\%$, $29.5 \pm 17.2\%$, $27.4 \pm 14.7\%$ and $8.7 \pm 12.8\%$ in the Jiantang River (JTR), respectively. Moreover, the APCS-MLR and dual stable isotopes methods provided complementary/synergist information and a measure of verification for the quantitative apportionment results. Overall, the results highlight that coastal river N pollution control must focus on soil N management, aquaculture tailwater, and domestic wastewater treatment in the watersheds surrounding Hangzhou Bay.

Declarations

Authors contributions

Jia Zhou: Data curation, Formal analysis, Methodology, Visualization, Writing – original draft, Validation

Minpeng Hu: Data curation, formal analysis, methodology, visualization, writing – original draft, validation

Mei Liu: Sampling, measurement, data curation

Julin Yuan: Data curation, investigation

Meng Ni: Sampling, measurement, data curation

Zhiming Zhou: Data curation, investigation

Dingjiang Chen: Conceptualization, methodology, validation, formal analysis, investigation, writing – review & editing, supervision, project administration

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Availability of data and materials

The datasets used and analyzed in this study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Bai SW, Ren NQ, You SJ, Zhao XY, Li YF, Wang XH (2019) Modeling the oxygen-depleting potential and spatially differentiated effect of sewage organics in life cycle assessment for wastewater management. *Sci Total Environ* 655:1071–1080
2. Borah DK, Bera M (2004) Watershed-scale hydrologic and nonpoint-source pollution models: Review of applications. *T ASAE* 47:789–803
3. Breitburg D et al (2018) Declining oxygen in the global ocean and coastal waters. *Science* 359:46
4. Bristow LA, Mohr W, Ahmerkamp S, Kuypers MMM (2017) Nutrients that limit growth in the ocean. *Curr Biol* 27:R474–R478
5. Bu HM, Meng W, Zhang Y (2011) Nitrogen pollution and source identification in the Haicheng River basin in Northeast China. *Sci Total Environ* 409:3394–3402
6. Chai C, Yu ZM, Song XX, Cao XH (2006) The status and characteristics of eutrophication in the Yangtze River (Changjiang) estuary and the adjacent East China Sea, China. *Hydrobiologia* 563:313–328
7. Chen DJ, Hu MP, Dahlgren RA (2014) A dynamic watershed model for determining the effects of transient storage on nitrogen export to rivers. *Water Resour Res* 50:7714–7730
8. Chen DJ, Shen H, Hu MP, Wang JH, Zhang YF, Dahlgren RA (2018) Legacy nutrient dynamics at the watershed scale: Principles, modeling, and implications. *Adv Agron* 149:237–313
9. China Environmental Monitoring Station (2001) : Eutrophication assessment methods and classification technology requirements. Beijing
10. China Soil Database: <http://vdb3.soil.csdb.cn/>
11. Christensen S, Tiedje JM (1988) Sub-part-per-billion nitrate method - use of an N_2O producing denitrifier to convert NO_3^- or $(\text{NO}_3^-)\text{-N}^{15}$ to N_2O . *Appl Environ Microb* 54:1409–1413
12. Deng LZ, Fei K, Sun TY, Zhang LP, Fan XJ, Ni L (2019) Characteristics of runoff processes and nitrogen loss via surface flow and interflow from weathered granite slopes of Southeast China. *J Mt Sci-Engl* 16:1048–1064
13. Environmental Monitoring Center of Zhejiang (2020) : Ecological and Environmental Status of Zhejiang Province. 2020
14. Herb WR, Stefan HG (2003) Integral growth of submersed macrophytes in varying light regimes. *Ecol Model* 168:77–100
15. Herbeck LS, Unger D (2013) Pond aquaculture effluents traced along back-reef waters by standard water quality parameters, delta N-15 in suspended matter and phytoplankton bioassays. *Mar Ecol Prog Ser* 478:71–86
16. Herbeck LS, Krumme U, Nordhaus I, Jennerjahn TC (2021) : Pond aquaculture effluents feed an anthropogenic nitrogen loop in a SE Asian estuary. *Sci Total Environ* 756
17. Hu MP, Liu YM, Zhang YF, Dahlgren RA, Chen DJ (2018) A modification of the Regional Nutrient Management model (ReNuMa) to identify long-term changes in riverine nitrogen sources. *J Hydrol* 561:31–42
18. Hu MP, Liu YM, Zhang YF, Dahlgren RA, Chen DJ (2019) Coupling stable isotopes and water chemistry to assess the role of hydrological and biogeochemical processes on riverine nitrogen sources. *Water Res* 150:418–430
19. Imani S, Delavar M, Niksokhan MH (2019) Identification of nutrients critical source areas with SWAT model under limited data condition. *Water Resour* 46:128–137
20. Ji XL, Xie RT, Hao Y, Lu J (2017) Quantitative identification of nitrate pollution sources and uncertainty analysis based on dual isotope approach in an agricultural watershed. *Environ Pollut* 229:586–594
21. Jiang H, Lan WL, Li TS, Xu ZF, Liu WJ, Pan K (2020) : Isotopic composition reveals the impact of oyster aquaculture on pelagic nitrogen cycling in a subtropical estuary. *Water Res* 187
22. Jiang L, Li YP, Zhao X, Tillotson MR, Wang WC, Zhang SS, Sarpong L, Asmaa Q, Pan BZ (2018) Parameter uncertainty and sensitivity analysis of water quality model in Lake Taihu, China. *Ecol Model* 375:1–12
23. Jin XC, Liu SK, Zhang ZS, Tu QY, Xu NN (1995) Lake environment in China. Ocean Press, Beijing
24. Kang PP, Xu SG (2016) The impact of mariculture on nutrient dynamics and identification of the nitrate sources in coastal waters. *Environ Sci Pollut R* 23:1300–1311
25. Kim J, Kim Y, Park SE, Kim TH, Kim BG, Kang DJ, Rho T (2021) Impact of aquaculture on distribution of dissolved organic matter in coastal Jeju Island, Korea, based on absorption and fluorescence spectroscopy. *Environ Sci Pollut R. Preprints*
26. Kim KH, Yun ST, Mayer B, Lee JH, Kim TS, Kim HK (2015) Quantification of nitrate sources in groundwater using hydrochemical and dual isotopic data combined with a Bayesian mixing model. *Agr Ecosyst Environ* 199:369–381
27. Ling ZH, Guo H, Cheng HR, Yu YF (2011) Sources of ambient volatile organic compounds and their contributions to photochemical ozone formation at a site in the Pearl River Delta, southern China. *Environ Pollut* 159:2310–2319
28. Liu J, Zhenyao SY, Yan TZ, Yang YC (2018) Source identification and impact of landscape pattern on riverine nitrogen pollution in a typical urbanized watershed, Beijing, China. *Sci Total Environ* 628–629:1296–1307
29. Liu JK, Han GL (2021) : Tracing riverine particulate black carbon sources in Xijiang River Basin: Insight from stable isotopic composition and Bayesian mixing model. *Water Res* 194
30. Luo ZB, Hu SY, Chen DJ (2018) : The trends of aquacultural nitrogen budget and its environmental implications in China. *Sci Rep-Uk* 8
31. Martan BE (2008) : Polyculture of fishes in aquaponics and recirculating aquaculture. *Aquaponics J* 2008, 48, 28-33
32. Matiatos I (2016) Nitrate source identification in groundwater of multiple land-use areas by combining isotopes and multivariate statistical analysis: A case study of Asopos basin (Central Greece). *Sci Total Environ* 541:802–814

33. Mei K, Liao LL, Zhu YL, Lu P, Wang ZF, Dahlgren RA, Zhang MH (2014) Evaluation of spatial-temporal variations and trends in surface water quality across a rural-suburban-urban interface. *Environ Sci Pollut R* 21:8036–8051
34. Meybeck M (1982) Carbon, nitrogen, and phosphorus transport by world rivers. *Am J Sci* 282:401–450
35. Ministry of Ecology and Environment (2020) : Bulletin of marine environmental survey. Beijing
36. Ministry of Environmental Protection of China (2002) Environmental quality standards for surface water. Ministry of Environmental Protection of China, Beijing
37. Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MCM, Clay J, Folke C, Lubchenco J, Mooney H, Troell M (2000) Effect of aquaculture on world fish supplies. *Nature* 405:1017–1024
38. Neori A, Ragg NLC, Shpigiel M (1998) The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: II. Performance and nitrogen partitioning within an abalone (*Haliotis tuberculata*) and macroalgae culture system. *Aquacult Eng* 17:215–239
39. Ni M, Yuan J, Hua J, Lian Q, Guo A, Liu M, Xin J, Wang H, Gu Z (2020) Shrimp-vegetable rotational farming system: An innovation of shrimp aquaculture in the tidal flat ponds of Hangzhou Bay, China. *Aquaculture*, p 518
40. Ni M, Yuan JL, Zhang LM, Hua HQ, Rong HN, Gu ZM (2021) In-situ and ex-situ purification effect of ecological ponds of *Euryale ferox* Salisb on shrimp aquaculture, p 540
41. Paerl HW, Paul VJ (2012) Climate change: Links to global expansion of harmful cyanobacteria. *Water Res* 46:1349–1363
42. Pasten-Zapata E, Ledesma-Ruiz R, Harter T, Ramirez AI, Mählknecht J (2014) Assessment of sources and fate of nitrate in shallow groundwater of an agricultural area by using a multi-tracer approach. *Sci Total Environ* 470:855–864
43. Qiu LF, Zhang M, Zhou BB, Cui YZ, Yu ZL, Liu T, Wu SH (2021) : Economic and ecological trade-offs of coastal reclamation in the Hangzhou Bay, China. *Ecol Indic* 125
44. Qu MK, Wang Y, Huang B, Zhao YC (2018) Source apportionment of soil heavy metals using robust absolute principal component scores-robust geographically weighted regression (RAPCS-RGWR) receptor model. *Sci Total Environ* 626:203–210
45. Rakocy JE, Masser MP, Losordo TM (2006) : Recirculating aquaculture tank production systems: Aquaponics-integrating fish and plant culture. SRAC Publication 2006, 454, 16
46. Rivett MO, Buss SR, Morgan P, Smith JWN, Bemment CD (2008) Nitrate attenuation in groundwater: A review of biogeochemical controlling processes. *Water Res* 42:4215–4232
47. State Environment Protection Bureau of China (2002) Standard method for the examination of water and wastewater. Environmental Science Press, Beijing
48. Stock BC, Jackson AL, Ward EJ, Parnell AC, Phillips DL, Semmens BX (2018) : Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *PeerJ* 6
49. Su S, Li D, Zhang Q, Xiao R, Huang F, Wu J (2011) Temporal trend and source apportionment of water pollution in different functional zones of Qiantang River, China. *Water Res* 45:1781–1795
50. Sun TT, Lin WP, Chen GS, Guo PP, Zeng Y (2016) Wetland ecosystem health assessment through integrating remote sensing and inventory data with an assessment model for the Hangzhou Bay, China. *Sci Total Environ* 566:627–640
51. Tang DL, Di BP, Wei GF, Ni IH, Oh IS, Wang SF (2006) Spatial, seasonal and species variations of harmful algal blooms in the South Yellow Sea and East China Sea. *Hydrobiologia* 568:245–253
52. Thomas Z, Abbott BW (2018) Hedgerows reduce nitrate flux at hillslope and catchment scales via root uptake and secondary effects. *J Contam Hydrol* 215:51–61
53. Turcios AE, Papenbrock J (2014) Sustainable treatment of aquaculture effluents - what can we learn from the past for the future? *Sustainability-Basel* 6:836–856
54. Wang JL, Fu ZS, Qiao HX, Liu FX (2019) Assessment of eutrophication and water quality in the estuarine area of Lake Wuli, Lake Taihu, China. *Sci Total Environ* 650:1392–1402
55. Wang ML, Xiong X, Liu JJ, He C, Zhao QY (2020) Carbon and nitrogen stable isotopes evidence for the environmental impact of the cage fish farm in Poyang Lake, China. *B Environ Contam Tox* 105:860–867
56. Wellen C, Kamran-Disfani AR, Arhonditsis GB (2015) Evaluation of the current state of distributed watershed nutrient water quality modeling. *Environ Sci Technol* 49:3278–3290
57. Wu B, Zhao D, Zhang Y, Zhang X, Cheng S (2009) Multivariate statistical study of organic pollutants in Nanjing reach of Yangtze River. *J Hazard Mater* 169:1093–1098
58. Wu B, Jin HY, Gao SQ, Xu J, Chen JF (2020) Nutrient budgets and recent decadal variations in a highly eutrophic estuary: Hangzhou Bay, China. *J Coastal Res* 36:63–71
59. Xia YQ, Li YF, Zhang XY, Yan XY (2017) Nitrate source apportionment using a combined dual isotope, chemical and bacterial property, and Bayesian model approach in river systems. *J Geophys Res-Bioge* 122:2–14
60. Xue DM, Botte J, De Baets B, Accoe F, Nestler A, Taylor P, Van Cleemput O, Berglund M, Boeckx P (2009) Present limitations and future prospects of stable isotope methods for nitrate source identification in surface- and groundwater. *Water Res* 43:1159–1170
61. Yang LP, Han JP, Xue JL, Zeng LZ, Shi JC, Wu LS, Jiang YH (2013a) Nitrate source apportionment in a subtropical watershed using Bayesian model. *Sci Total Environ* 463:340–347

62. Yang LP, Mei K, Liu XM, Wu LS, Zhang MH, Xu JM, Wang F (2013b) Spatial distribution and source apportionment of water pollution in different administrative zones of Wen-Rui-Tang (WRT) river watershed, China. *Environ Sci Pollut R* 20:5341–5352
63. Yi QT, Chen QW, Hu LM, Shi WQ (2017) Tracking nitrogen sources, transformation, and transport at a basin scale with complex plain river networks. *Environ Sci Technol* 51:5396–5403
64. Yuan RY, Zheng TY, Zheng XL, Liu DS, Xin J, Yu L, Liu GQ (2020) Identification of groundwater nitrate pollution sources in agricultural area using PCA and SIAR methods. *Episodes* 43:739–749
65. Zhang JB, Kitazawa D (2016) Assessing the bio-mitigation effect of integrated multi-trophic aquaculture on marine environment by a numerical approach. *Mar Pollut Bull* 110:484–492
66. Zhang M, Wang XP, Liu C, Lu JY, Qin YH, Mo YK, Xiao PJ, Liu Y (2020) Quantitative source identification and apportionment of heavy metals under two different land use types: Comparison of two receptor models APCS-MLR and PMF. *Environ Sci Pollut R* 27:42996–43010
67. Zhang YF, Wu H, Yao MY, Zhou J, Wu KB, Hu MP, Shen H, Chen DJ (2021) : Estimation of nitrogen runoff loss from croplands in the Yangtze River Basin: A meta-analysis. *Environ Pollut* 272
68. Zheng Y, Luo X, Zhang W, Wu X, Zhang J, Han F (2016) Transport mechanisms of soil-bound mercury in the erosion process during rainfall-runoff events. *Environ Pollut* 215:10–17
69. Zhou BY, Wang JQ, Zhang HB, Shi HH, Fei YF, Huang SY, Tong YZ, Wen DS, Luo YM, Barcelo D (2020) : Microplastics in agricultural soils on the coastal plain of Hangzhou Bay, east China: Multiple sources other than plastic mulching film. *J Hazard Mater* 388
70. Zhou HX, Li X, Xu GR, Yu HR (2018) Overview of strategies for enhanced treatment of municipal/domestic wastewater at low temperature. *Sci Total Environ* 643:225–237
71. Zhou J, Zhang YF, Wu KB, Hu MP, Wu H, Chen DJ (2021) : National estimates of environmental thresholds for upland soil phosphorus in China based on a meta-analysis. *Sci Total Environ* 780

Figures

Figure 1

Location of study area and the distribution of sampling sites

Figure 2

Daily precipitation in Cao'E River (CER) and Jiantang River (JTR). The dotted line indicated a daily precipitation of 10 mm (the threshold of moderate rain).

Figure 3

Scatter plot of $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ values in rivers (2019 and 2020) and aquaculture ponds. Area shows the typical range of $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ values from Xue et al. (2009) and Yi et al. (2017).

Figure 4

The contribution of atmospheric deposition N (AD), soil N (SN), aquaculture tailwater (AQ) and domestic wastewater N (DW) in (a) coastal rivers of Hangzhou Bay; (b) Cao'E River (CER) and (c) Jiantang River (JTR) across different seasons based on the dual nitrate stable isotope approach (The MixSIAR model ran 3,000 simulations, i.e., $n = 3,000$ for each box). Box plots in (a), (b) and (c) illustrate the 25th, 50th and 75th percentile; the whiskers indicate the 2.5th and 97.5th percentiles.