Anthropogenic point source and non-point source nitrogen inputs into Huai River Basin and their impacts on riverine ammonia-nitrogen flux

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Abstract

This study provides a new approach to estimate both anthropogenic non-point source and point source nitrogen (N) inputs to the landscape, and determines their impacts on riverine ammonia-nitrogen (AN) flux, providing a foundation for further exploration of anthropogenic effects on N pollution. Our study site is Huai River Basin of China, a watershed with one of the highest levels of N input in the world. Multi-year average (2003-2010) inputs of N to the watershed are 27,200±1,100 kg N km^{-2} yr^{-1}. Non-point sources comprised about 98% of total N input and only 2% of inputs are directly added to the aquatic ecosystem as point sources. Fertilizer application was the largest non-point source of new N to the Huai River Basin (69% of net anthropogenic N inputs), followed by atmospheric deposition (20%), N fixation in croplands (7%), and N content of imported food & feed (2%). High N inputs showed impacts on riverine AN flux: fertilizer application, point source N input and atmospheric N deposition were proved as more direct sources to riverine AN flux. Modes of N delivery and losses associated with biological denitrification in rivers, water consumption, interception by dams may influence the extent of export of riverine AN flux from N sources. Our findings highlight the importance of anthropogenic N inputs from both point sources and non-point sources in

heavily polluted watersheds, and provide some implications for AN prediction and management.

Keywords: watershed approach, net anthropogenic nitrogen input (NANI), nitrogen (N), ammonia-nitrogen (AN), point source pollution, non-point source pollution

1 Introduction

Nitrogen (N) enrichment in watershed ecosystems is an issue of global concern (Galloway et al., 2004). Human activities strongly influence the N loads to watersheds in a number of different ways, for example through fertilizer application driven by increased agricultural activities (Billen et al., 2001;Billen et al., 2013), or through point-source discharge as the result of increased industrial and domestic emissions (Van Drecht et al., 2009). Increased N input to watersheds is often accompanied by a high load of N into the river system and corresponding riverine N export (Han and Allan, 2012;Hong et al., 2012). These N impacts on N flux are very dependent on the modes of delivery to aquatic ecosystems. For example, the pollutants could be added into the river network by indirect routes such as rainfall-runoff and leaching, etc (Carpenter et al., 1998) or discharged directly into river systems. Therefore, to effectively guide N management for protecting aquatic ecosystem health, anthropogenic N accounting linked to riverine N export must be responsive to the modes of N delivery.

Net anthropogenic nitrogen input (NANI) is a watershed-budgeting approach that sums N contributions from atmospheric deposition, fertilizer application, agricultural biological fixation, and net import/export of N in food and feed to a watershed. This method was originally proposed by Howarth et al. (1996), and has been used as a simple yet powerful approach to estimate major anthropogenic sources of N to terrestrial and aquatic ecosystems. A large volume of published studies has since described the relationship between NANI and nitrogen fluxes in rivers (David and Gentry, 2000;Boyer et al., 2002;Howarth et al., 2006;Han and Allan, 2008;Swaney et al., 2012;Howarth et al., 2012;Billen et al., 2009). NANI has turned out to be a reliable predictor of riverine N exports, the magnitudes of which can also have strong relationships with hydro-climatic conditions such as precipitation, discharge and temperature (Schaefer and Alber, 2007;Schaefer et al., 2009;Howarth et al., 2012).

The accounting method of NANI has been refined since its first application in North Atlantic Ocean. Boyer et al. (2002) added a new input component to reflect the impact of natural fixation, and found this revised nitrogen accounting method (TNI, which is equivalent to NANI plus natural N fixation) would be a good predictor of riverine N export in watersheds dominated by "natural" systems. Han and Allan (2008) and Hong et al. (2013) refined the NANI methodology by comparing different calculation methods. Hong et al. (2011) then released an open source toolbox of NANI estimation, and greatly promoted the application of NANI methodology. However, all of the improvements in NANI methodology still did not address the modes of N delivery. Conventional NANI methodology emphasizes on the impact of non-point source N input (Howarth, 1998) and is inexplicit in specific N pathways, which can cause potentially large errors and poor predictions in some watersheds with heavy point source N pollution (Gao et al., 2014).

In addition, a number of studies incorporating NANI have been conducted extensively on total nitrogen (e.g., Hong et al. (2012)), nitrate flux (e.g., McIsaac et al. (2002)) or total dissolved N (e.g., Huang et al. (2014)); no single study has adequately addressed the ammonia-nitrogen (AN) component. This is problematic, because in many heavily impacted rivers, e.g. with high biological oxygen demand (BOD) due to untreated sewage and other sources of organic pollutants, the correspondingly low dissolved oxygen levels can provide an environment suitable for the persistence of AN as a component of riverine N fluxes. For example, in heavily polluted rivers in some regions, ammonia-nitrogen can account for more than 70% of total nitrogen across seasons and river sections (Pernet-Coudrier et al., 2012;Li et al., 2014). Furthermore, in many Chinese rivers, AN is the only component of riverine N that is regularly monitored (Ma et al., 2009;Xia et al., 2011;Shao et al., 2006), which indicates the prevalence of the problem and supports the need for better understanding of AN dynamics in these heavily impacted rivers. Extending the study of NANI dynamics to include the response of riverine AN will refine our understanding of nitrogen dynamics in river basins and will facilitate adaptive management of conservation policies and programs, especially in areas of poor riverine water quality.

Hence, the major purposes of this study are to: 1) differentiate the common NANI methodology into two parts: point sources and non-point sources; 2) investigate the impact of anthropogenic point source and non-point source N inputs on riverine AN flux; and 3) determine the potential influential factors of riverine AN export. We carried out our study in the Huai River Basin (HRB), which was previously reported as one of the watersheds with the heaviest pollution in China (Bai and Shi, 2006) and the highest N input in the world (Billen et al., 2013), giving it the worst water quality in the nation's top seven basins (Xia et al., 2011), and resulting in health consequences for its population (Bai and Shi, 2006). In addition, as a representative basin of rapid urbanization growth (a 15% increase during 2003-2010, according to MWR (2010)), results from the HRB would also have implications for other watersheds, since as claimed by Van Drecht et al. (2009), future continued population and economic growth in developing countries will almost certainly lead to further increasing N emissions in the coming decades. Below, we first estimate the amount of point source N and non-point source N inputs that occur for the whole basin. Then, we investigate the AN export in relation to NANI and analyze the factors most influential to AN export. Finally, parametric and sensitivity analyses of NANI methodology are also conducted since they could serve as guidance in applications to other watersheds and to adjust anthropogenic N inputs. Most of the data used to estimate NANI are presented in supplementary materials.

2 Material and methods

2.1 Watershed characteristics

The Huai River Basin (HRB) is located in eastern China (N30°55′～36°36′, E111°55′～121°25′; Fig. 1), lying between the Yangtze River Basin and Yellow River Basin. It has a drainage area of 270,000 km^2, ranking sixth by area of all river basins of China. The HRB can be divided into the upper, middle and lower and Yishusi sub-basins. The 1,000 km Huai River originates in the Tongbai Mountains of Henan province and flows eastward to the Yangtze River and Yellow Sea (Fig. 1). The Yishusi River stems from Yimeng Mountains of Shandong province and flows southward then eastward to Yellow Sea. The population dwelling in the basin is 165 million. Its average population density is 623 person km^{-2}, and approximately 5 times the nation's average.

Twenty-seven watersheds cover the total area of the four reaches, ranging in size from 1,095 to 16,460 km^2, and encompassing a wide variety of land uses, population density, and human activities. Land cover in HRB was 9.8% forest, 1.7% grass, 68.7% cropland, 6.1% wetland, and 13.5% residential area for the 1990s through 2000s, although land cover varies greatly across watersheds and time (see Part I in supplementary material). The area-weighted average of total annual precipitation is highly variable from year to year (ranging from about 637.0 mm yr^{-1} to 1,287.5 mm yr^{-1}), and 50-80% of annual precipitation is concentrated in the flood season (June–September) (Xia et al., 2011). Due to intensive agricultural production and rapid urbanization growth, industrial AN discharges reached 66,389 tons in 2010 (MWR, 2010), and the amount of N fertilizer application had increased to 0.50 million tons, raising the issues of impacts on water quantity and quality in this watershed.

2.2 Methodology

The N budget considered here was divided into non-point source and point source inputs because of the major differences in their modes of N delivery. This division facilitates the analyses performed below related to attribution of sources. The equation can be represented as:

$$NANI = NANI_n + NANI_p \qquad (1)$$

where *NANI* is the total net anthropogenic nitrogen input, $NANI_n$ is net anthropogenic nitrogen input that could potentially contribute to non-point source pollution, and $NANI_p$ is the anthropogenic nitrogen input that directly discharges into the river (i.e. sewage N discharge). The main differences between Eq. (1) and the previous version of NANI methodology are that: 1) human-induced N inputs were recalculated according to their modes of N delivery; 2) some new equations that can represent industrial and urban domestic loads were introduced to estimate point source N inputs.

The methodology of $NANI_n$ estimation was very similar to that reported in Han et al. (2014), which was in turn based on the methods developed by Howarth et al. (1996). Major input components included atmospheric deposition, fertilizer, net food and feed import, and biological N fixation. This non-point source component should exclude part of urban household N emissions that enter centralized sewage systems and then discharge into river systems as a form of point source (Van Drecht et al., 2009). Industrial and domestic centralized sewage nitrogen discharge are considered to be point source nitrogen inputs ($NANI_p$). Industrial byproducts (primarily structural forms, e.g., nylon, plastic, and synthetic fiber, etc.) are not considered as a new input since most of them tend to accumulate in human settlements due to their long service lives (Gu et al., 2013).

NANI are typically based on a watershed scale in order to estimate riverine N export from the watershed. We collected all the datasets covering 2003-2010 for each county. All the data were multi-year averaged (2003-2010) to avoid a storage effect in which N tends to be stored in the landscape in dry years and flushed into rivers in wet years (Howarth et al., 1996;Swaney et al., 2012;Chen et al., 2014). As suggested by Han and Allan (2008) and Hong et al. (2013), county-level datasets were aggregated to the catchment scale using a land-use weighting method: weighting by the fraction of the relevant land use type, such as crop or urban land, lying within each catchment. For agriculturally related indicators (such as fertilizer application, crop yields, etc.), we adopted the land use weighting by cropland area; for residentially-related indicators (such as population, industrial sewage discharge, etc.), we used the method of land use weighting by urban land area. Land cover data with a 30 m×30 m resolution was adopted to transform the scale (see Fig. 1A). To facilitate understanding our N cycle analysis, a diagram of the nitrogen accounting method, is presented in Fig. 2.

2.2.1 Non-point sources ($NANI_n$)

The total amount of non-point source nitrogen input ($NANI_n$) is estimated as:

$$NANI_n = N_{chem} + N_{fix} + N_{dep} + N_{im} - N_{urban} = N_{chem} + N_{fix} + N_{dep} + N_{r-im} \quad (2)$$

Where the individual N inputs are as follows: N_{chem} is N content of chemical N fertilizers applied; N_{fix} is crop N fixation; N_{dep} is atmospheric deposition of oxidized N; N_{im} is N content of the net import/export of food and feedstuffs; N_{urban} is the N content of food and feed consumed by urban populations.

To avoid double-accounting of point source inputs, N_{urban} was subtracted from $NANI_n$ for it is usually connected to municipal sewage systems and acts as point source pollution. Since human N emission usually is identical to N intake (100% excretion) (Han et al., 2011), N_{urban} here is considered a part of net food and feed import (N_{im}, including urban and rural N consumption). Thus, we can form a new input term defined as net food and feed import in rural region (N_{r-im}). All terms here are in kg N km^{-2} yr^{-1}; the definition and data sources for each term are presented below. All of the data used in calculating $NANI_n$ are provided in supplementary material (see Part II).

2.2.1.1 Fertilizer (N_{chem})

N_{chem} is defined as the amount of N in yearly N fertilizer application. N fertilizer application including forms of single N fertilizer (such as ammonium nitrate, anhydrous ammonia, ammonium bicarbonate, urea, and miscellaneous forms) and compound fertilizer (synthetic fertilizers also containing P, K or other nutrients) were adopted in our estimates. The data were obtained from the annual provincial census (e.g., Statistics (2010)). An average N content of 35% of compound fertilizer is commonly assumed in China (Li and Jin, 2011) and hence we used this value to calculate the elemental N input of compound fertilizer.

2.2.1.2 Biological nitrogen fixation (N_{fix})

N_{fix} refers to the sum of symbiotic N fixation by cultivation of legume crops and non-symbiotic N fixation by microorganisms in agricultural ecosystem. Biological nitrogen fixation in agriculture land was calculated by multiplying the area of crops in each subunit by published N fixation rates. When estimating symbiotic N fixation by leguminous crops, only soybean and peanut were taken into consideration since they were the most common leguminous crops in our study area. Fixation rates for each of these crop classes were estimated from reviews by Zhang et al. (1989), Lu et al. (1996) and Du et al. (2010). As suggested by Li and Jin (2011) and Du et al. (2010), the N fixation rate used for estimating rice and other non-symbiotic N-fixing crops was 30 kg ha^{-1} yr^{-1} and 15 kg ha^{-1} yr^{-1}, respectively (Table 1).

2.2.1.3 Atmospheric N deposition (N_{dep})

The deposition of ammonia and ammonium is not considered as a new input of nitrogen to a region, based on the idea that transport of these species through the atmosphere generally occurs only over fairly short distances (Prospero et al., 1996;Fangmeier et al., 1994;Schlesinger and Hartley, 1992). Thus, we viewed NH_x deposition as a recycling of nitrogen within a region rather than as an additional source of nitrogen to the region. Since NO_y comes largely from the combustion of fossil fuels, its deposition needs to be considered as a regional input of nitrogen (Howarth, 1998).

China is one of the areas of highest N deposition globally (Galloway et al., 2008), resulting from extensive use of fossil fuels in industry and transportation, chemical fertilizers in agriculture, and the expansion in intensive animal husbandry in the last three decades (Ti et al., 2011). However, there is no systematic nation-wide monitoring network to derive geographical and temporal distribution of the deposition rates. In this study, the data of wet and dry atmospheric N deposition from 2003 to 2010 referred to the simulated results of wet and dry deposition of NO_y by the Frontier Research Center for Global Change (FRCGC) (Ohara et al., 2007). The dataset of the inventory produces estimates of deposition at a 0.5°×0.5° latitude-longitude resolution.

2.2.1.4 Net food and feed import in rural area ($N_{r\text{-}im}$)

Net food and feed import (both in urban and rural regions) is usually based on the assumption that imports and exports are determined by the balance of local production and consumption, and thus defined as total N consumption (by livestock and humans) minus total N production (by crops and livestock) (Schaefer et al., 2009). This quantity will be negative (representing an export) when N production exceeds consumption. However, this study subtracted the N consumption by urban inhabitants from net food and feed to avoid double accounting. The amount of net food and feed import that potentially contributes to diffuse pollution in rural regions ($N_{r\text{-}im}$) is calculated as follows:

$$N_{r-im} = N_{selfo} + N_{selfe} - N_{harv} - N_{liv} \qquad (3)$$

where N_{selfo} and N_{selfe} stand for N consumption by rural inhabitants and livestock, respectively. N_{harv} stands for N in crops, and N_{liv} for N in animal products.

Human consumption of N in food (N_{selfo}) was estimated as the product of nitrogen consumption per capita and the number of rural inhabitants in each subunit. According to research carried out by Wei et al. (2008), nitrogen consumption per capita in rural China is 4.31 kg N yr^{-1}.

Animals are usually fed according to relatively straightforward dietary prescriptions designed for maintaining or gaining weight. Livestock consumption of N in feed (N_{selfe}) was calculated by N consumption per individual multiplied by the number of each animal type in each subunit. We chose the values of consumption reported by Han et al. (2014) and the values for the percentage N excreted reported by Van Horn (1998). The parameters along with their sources used to calculate N mass in animal products had been presented in detail in Han et al. (2014). The animal N production category includes meat, milk, eggs, etc. We estimated animal N production (N_{liv}) by the difference between animal feed consumption (intake) and animal excretion (waste production).

N in crop products (N_{harv}) was estimated from their N contents and total mass of products. Protein rather than N contents is ususally reported for products, and we assumed N content to be 16% of protein content (Ti et al., 2011;Jones, 1941). Protein contents for different crops were obtained from the book of China Food Ingredients Table (Yang et al., 2009). The parameters used to calculate N mass in crop products are given in Table 2.

2.2.2 Point sources ($NANI_p$)

Industrial and domestic centralized sewage nitrogen discharge is combined to estimate point-source nitrogen inputs. Environmental census data of 2003-2010, which include data from Chinese environmental protection agencies, were adopted in our estimation. This dataset (such as the AN generation load and sewage effluent from industrial and urban households) also can be found from the Anhui, Jiangsu, Henan, and Shandong provincial yearbooks. All of the data used in calculating $NANI_p$ were provided in supplementary material (see Part III). The total amount of urban domestic and industrial sewage can be calculated from:

$$NANI_p = (N_{urban} + N_{ind})(1 - I_{sew} I_{rem-tn}) \quad (4)$$

where $NANI_p$ represents the total nitrogen load from point sources; N_{ind} is N discharged by industrial production; N_{urban} is N discharged by urban inhabitants; $I_{rem\text{-}tn}$ refers the average removal rate by a sewage plant; I_{sew} is the percentage of sewage effluent that is treated by sewage plants.

While the atmospheric deposition of N onto impervious surfaces contributing drainage from urban and industrial areas is also a potential point source of N, preliminary calculations based on estimates of impervious surface area (Sutton et al., 2011) indicated an average contribution of only about 12-18% of the value of N load generated from Eq. (4), depending upon the extent of sewage treatment assumed. Given the added uncertainty associated with this term, and that it's overall effect would be partially cancelled by subtracting it from Eq. (2), its potential contribution was not included in this study.

Emitted N in wastewater by households and industries are connected to the same sewerage system (Van Drecht et al., 2009). Thus the calculation of I_{sew} can be obtained from the following equation:

$$I_{sew} = \frac{W_{sew}}{W_{ind} + W_{urban}} \quad (5)$$

where W_{ind} and W_{urban} refer the volume of wastewater generated by industrial production and urban household, respectively; W_{sew} refers the actual treatment volume by sewage plants. W_{sew} was obtained from the list of Nationwide Inventory of Urban Sewage Treatment Facilities (http://www.mep.gov.cn/gkml/hbb/bgg/201305/t20130508_251788.htm). W_{ind} and W_{urban} are provided in provincial yearbooks (see Part III of supplementary material).

N discharge from urban residents (N_{urban}) was estimated as the product of urban population and average nitrogen consumption per capita. We adopted the data of Wei et al. (2008), who reported that the average N emission per capita by urban inhabitant of China was 4.77 kg N yr^{-1}; Industrial N discharge (N_{ind}) was computed as the product of industrial sewage effluent flow (W_{ind}) and average nitrogen concentration. The average nitrogen concentration in industrial sewage had shown a wide range of values (e.g., 0.87～48.43 mg/L, Yang et al.

(2003)). In this study, we use the mean value of 25 mg/L that was reported in Changjiang River Basin of China to estimate industrial N discharge (Yan et al., 2010).

The average removal rate by a sewage plant (I_{rem-tn}) shows very large fluctuations depending upon influent load and season (Jin et al., 2014). According to Qiu et al. (2010), the total nitrogen removal rate by different sewage treatment systems in China ranged from 40% to 70%. We used an average reported value for N removal rate of 60% since N removal rate of the most common treatment systems (oxidation ditch (OD), anaerobic/anoxic-oxic (AO) process, sequencing batch reactor (SBR), and anaerobic-anoxic-oxic (AAO)) of the Huai River Basin was about 55～59%.

We also estimate the amount of ammonia-nitrogen discharge based on Equ. (4):

$$AN_p = (AN_{urban} + AN_{ind})(1 - I_{sew} I_{rem-an}) \qquad (6)$$

where AN_p represents the total AN load from point sources; AN_{ind} is AN load discharged by industrial production; AN_{urban} is AN load discharged by urban inhabitants; Here, I_{rem-an} refers the average removal rate of AN by a sewage plant and I_{sew} is the percentage of AN effluent that is treated by sewage plants. AN_{ind} and AN_{urban} can be directly found from the yearbook of Anhui, Shandong, Jiangsu, Henan provinces. I_{rem-an} here was set at 70%, since AN removal rate in most municipal sewage treatment systems was about 10% higher than total nitrogen removal (Qiu et al., 2010). The calculation of I_{sew} can be found in Equ. (5).

2.2.3 Riverine ammonia-nitrogen export

AN flux in the outlet of a watershed was calculated from stream discharge and water quality using the LOADEST regression model (Runkel et al., 2004). Stream discharge were collected automatically at the hydrometric stations (outlet of watersheds) from 2003-2010. Water quality data were obtained at the same hydrometric stations. AN was determined in the laboratory following the standard analytical method for water quality (Ministry of Environmental Protection of China, 2002). During 2003-2006, all water quality data were reported at a bimonthly time scale, while after 2007, these data were reported at a monthly time scale. Details on sample collection and laboratory analysis were described in the Huai River Commission (http://www.hrc.gov.cn/). There were very few missing water quality data during the study period (less than 1% of total). For this analysis, when a particular month's data was missing, the missing value was interpolated based on the previous and the following month's values of the monitoring station. The distribution of monitoring stations is presented in Fig. 1.

2.2.4 Sensitivity analysis

Relative sensitivity (*S*) of a variable *y* to a parameter *x* is evaluated by examining the effect of a change of *x* on the response of *y* relative to the baseline value. The sensitivity here is defined as the proportional change of variable *y*, relative to baseline y_b, divided by the proportional change in parameter *x*, relative to baseline value x_b (for example, if a 10% change in parameter *x* relative to its baseline results in a 10% change in *y* relative to its baseline, then *S* =1). Hence, the relative sensitivity of the input terms and parameters of NANI can be obtained from (Hong et al., 2013):

$$S(y|x, x_b, y_b) = \frac{(y - y_b)x_b}{(x - x_b)y_b} \quad (7)$$

Since the relationship of the parameters tested in this study to NANI is mostly linear, the choice of range of variation (i.e., 5%, 10% or 20%) has little effect on the result of sensitivity analysis. Therefore, we applied a ±10% change for each of the NANI components and estimated its sensitivity from the resulting proportional change in NANI.

3 Results and discussion

3.1 Nitrogen budgets and geographic differences

As a watershed with one of the highest levels of N inputs to its watersheds in the world (Billen et al., 2013), N input and its sources in the Huai River Basin should be carefully considered. Our study shows that NANI into the Huai River Basin was about 27,186±1,129 kg N km^{-2} yr^{-1} (mean±S.D.) from 2003 to 2010, about 98% of which could be potentially contributed to non-point sources and the remaining 2% is added to the watershed ecosystem as a form of point source (Table 3). This value was about five times the average intensity of NANI reported for mainland China (5,013 kg N km^{-2} yr^{-1} in 2009, Han et al. (2014)) and India (4,616 kg N km^{-2} yr^{-1} in 2000s, Swaney et al. (2015)), ten times that reported in US watersheds (Hong et al., 2013), and nearly twice that of the Beijing metropolitan region (15,236 kg N km^{-2} yr^{-1} averaged during 1991-2007, Han et al. (2011)). The results are comparable with previous studies. The N inputs to the administrative provinces of Anhui, Henan, Shandong, Jiangsu in Huai River Basin were 13,121, 21,090, 18,072 and 24,219 kg N km^{-2} yr^{-1} respectively, for the year 2009 (Han et al., 2014), very close to our results.

Fertilizer was the largest non-point source N to the Huai River Basin (68.7% of NANI), followed by atmospheric deposition (20.2%), N fixation in croplands (7.0%) and imported food and feed N (2.1%). Point source N inputs accounted for the least part of the input, with the value of 542±48 kg N km^{-2} yr^{-1}. The sub-basin with the highest input was Yishusi basin (Table 3), followed by the Lower basin.

Fig. 3 presents an overview of the geographic differences of point and non-point source N inputs into hydrologic units. NANI to watersheds showed a significant geographic difference across the whole basin. The headwater watersheds tended to exhibit lower N inputs (for example: No.1, 2, 3, 14, 15, 23, etc.), while in the "mountain-plain" transition watersheds or plain watersheds, higher N inputs appeared due to stronger effects of human disturbance. N inputs from point sources and non-point sources showed a positive correlation (r=0.82, $P<0.001$), indicating that many watersheds in Huai River have faced the dual-risk of contributions from both modes of N delivery. Geographic differences of NANI in individual watersheds were related to watershed characteristics, such as positive correlations with watershed population density (r=0.90, $P<0.001$), percentage of agricultural land area (r=0.84, $P<0.001$), and percentage of developed land area (r=0.88, $P<0.001$), while it was negatively correlated with percentage of forestland area (r=-0.83, $P<0.001$) and watershed average elevation (r=-0.61, P=0.004), consistent with previous findings (Howarth et al., 1996;Swaney et al., 2012).

AN flux in rivers can be observed from Fig. 3. Riverine AN loads ranged from 127 ton yr^{-1} to 31,611 ton yr^{-1}. Correspondingly, area-averaged flux of AN can vary from 116 to 655 kg N km^{-2} yr^{-1}. The watershed with lowest AN flux was located at the headwater of Huai River, while the highest (No. 9) was close to Luohe City, which was heavily polluted by domestic sources (population density ~ 635 ind km^{-2}) as well as direct discharges of industrial sewage (average rate of treated sewage was just 8% for 2003-2010).

3.2 Ammonia-nitrogen flux in relation with point source and non-point N inputs

AN flux in this region of high N inputs exhibits positive linear relationships to point source (R^2=0.61, $P<0.001$), non-point source (R^2=0.59, $P<0.001$) and total input (R^2=0.59, $P<0.001$)

(Fig. 4). Linear equations which describe the relationship between anthropogenic nitrogen input and nitrogen export were consistent with previous studies, such as in Schaefer and Alber (2007) and Swaney et al. (2012), but our result shows that exponential formulas show a better match between NANI and AN (R^2=0.73, P<0.001). This kind of equation also has been reported for other nitrogen forms such as in nitrate (McIsaac et al., 2001) and total nitrogen (Han et al., 2009). Howarth et al. (2012) evaluated the nonlinear effect as a possible threshold, below which a smaller fraction of NANI is exported as riverine N flux.

NANI is computed from atmospheric deposition, fertilizer, net food and feed import in rural, biological nitrogen fixation and point source N input (Fig. 2). Each NANI component contributes to riverine AN flux. Our results indicate that fertilizer application, point source N input and atmospheric N deposition have a more direct impact on riverine AN flux, while the biological nitrogen fixation and net food and feed import are not as strongly related across the subbasins (Fig. 4).

For all of 20 watersheds, fertilizer N is the single largest input. Perhaps it is not surprising, therefore, to observe that fertilizer input is significantly correlated with riverine AN flux (Fig. 4*e*). A more interesting finding is that atmospheric N deposition is also well correlated with riverine AN flux (R^2=0.77, Fig. 4*f*). This result coincides with the findings by Howarth (1998) for the North Atlantic watersheds, and also for 150 watersheds in Europe and North America (Howarth et al., 2012). The underlying reason may be the information conveyed by atmospheric N deposition, since atmospheric deposition originates largely from the combustion of fossil fuels which are associated with both agricultural and industrial production. AN flux is also strongly related to point source N input (R^2=0.61, P<0.001; Fig. 4*c*) and point source AN input (R^2=0.68, P<0.001; Fig. 4*d*), which is consistent with the conclusion of Xia et al. (2011) that industrial and municipal point source discharge were also major pollution sources in Huai River Basin.

In contrast, for biological N fixation, it has shown a positive but statistically insignificant relationship (P>0.05) with AN flux (Fig. 4*h*). Biological N fixation is a relatively small input (accounting for only 7% of the total NANI). Its role may be easily hidden by other inputs. The influence of net food and feed import was also unclear (Fig. 4*g*). Although Howarth et al. (2012) indicated that the flux of N in many rivers increases as the net import increases, there was not any clear relationship between food import and riverine AN flux in our study. In this case, the underlying reason may be due to poor linkages between AN and net food/feed; the organic nitrogen in human and livestock waste may not be consistently converted to AN, and thus may not contribute as a significant source that would be observed if we were considering the total nitrogen fluxes (TN) as has been done previously (Swaney et al., 2012;Hong et al., 2012).

The results also indicate it is possible to construct N source-based models to estimate riverine ammonia-nitrogen flux, because the major N sources have shown a more direct effect on AN export (Fig. 4). By this simple empirical model, further insight may be provided into how to adjust and balance point source and non-point source N inputs to effectively manage human-induced N. Riverine AN flux (*RAF*) can be predicted well by a linear function ($RAF = 0.27NANI_p + 0.0046NANI_n + 51.75, R^2 = 0.66, P < 0.001$). Since an exponential formula between non-point source N and AN showed a good fit (Fig. 4b), an exponential model ($RAF = 0.14NANI_p + 65.35\exp(0.000047NANI_n), R^2 = 0.73, P < 0.001$) was developed to test ammonia prediction and resulted in marginally better performance. However, we found that the accuracy of these empirical models is not very high. The underlying reason is probably due to the fact the simple regression equations cannot completely capture the variation of many influential factors of AN export. We discuss some of these factors below and their role in improving our understanding of nitrogen dynamics as a foundation for future exploration of some process-based models.

3.3 Factors influencing AN export

The influence of landscape and climate on riverine TN flux has been addressed in previous studies (Howarth et al., 2006;Schaefer et al., 2009;Hong et al., 2012;Howarth et al., 2012). Our results relating these influential factors to AN flux are also similar to those previously reported for total nitrogen. For example, AN flux showed a positive correlation with watershed average slope (Fig. 5*a*) and discharge (Fig. 5*b*), since gentle slopes and low discharge would increase nitrogen residence time in watersheds and ultimately prolong the time for biological N processing in the landscape (Swaney et al., 2012). The role of watershed average temperature in N export is less clear (Fig. 5*c*). For example, in southeastern US watersheds, temperature was interpreted as a strong explanatory variable in predicting percent N export (Schaefer and Alber, 2007), while in the western US (Schaefer et al., 2009), Baltic Sea basin (Hong et al., 2012) and European watersheds (Howarth et al., 2012), there was no direct evidence that temperature was an important factor controlling N export. Although some studies (Schaefer and Alber, 2007) suggested that the negative relationship between N export and temperature was due to the effect of increased denitrification rates, there may be an alternative explanation (Swaney et al., 2012) that it is due to correlation of temperature with other indicators such as evapotranspiration. In our study, watershed temperature showed a positive relationship with precipitation (R^2=0.62, P<0.001) and discharge (R^2=0.60, P<0.001), so it is not surprising to find that temperature also showed a positive relationship with percent AN export in our study.

The potential role of dams has also been addressed in previous studies (Dynesius and Nilsson, 1994;Nilsson et al., 2005;Schaefer and Alber, 2007). Our result suggests that the number of dams built to fully utilize regional water resources would greatly prolong the nitrogen residence time within aquatic ecosystems, and ultimately decrease the percent AN export (Fig. 5*d*). However, due to highly artificial control and the fact that dams have impacts on both water quantity and quality, other indicators (e.g., volume of dammed reservoirs; Fig. 5*e*) were not significantly correlated with percent AN export in our analysis.

The modes of N delivery would also affect the percent of NANI exported as AN flux (Fig. 5*f*). High proportions of anthropogenic N from point sources would significantly increase percent AN export because they are directly discharged into streams.

The most striking result found in our study was that the percentages of cropland and urban area are negatively correlated with the percent of NANI exported as riverine AN (Fig. 5*g* and Fig. 5*h*). One might interpret this to suggest that human-activities related to these land uses hinder AN export. This can be misleading, considering that human activities are responsible for introducing reactive N to the region. A possible cause for this relationship is permanent water loss due to consumption (containing N) in irrigation, drinking water and other uses. According to the Huai River Water Resources Bulletin of 2010, the amount of permanent water loss (e.g., *via* evaporation) had increased to 39.42 billion tons, which accounts for nearly 50% of total water resources (85.96 billion tons) in the region. Therefore, the water consumption by human activities may likely be a very important factor of nitrogen removal due to both physical extraction of N from rivers and increased residence time effects on N retention (Lassaletta et al., 2012).

In sum, from our results we can classify the major influential factors of AN export into: biological nitrification/denitrification (represented by slope and discharge), water consumption (represented by percentages of cropland and urban area), modes of N delivery (represented by $NANI_p$/NANI) and impact of dams (represented by numbers of major dams). However, we acknowledge that other undetermined factors could also partially explain the variation of riverine N export as a percent of NANI, including soil storage, infiltration into groundwater, or errors in accounting due to uncertainties in data and parameters. More comprehensive work should be carried out in heavily loaded watersheds such as this one to determine the roles of these processes in exporting NANI as riverine fluxes.

3.4 Implications for percent TN export

In addition to the AN export, estimation of percent TN export would provide useful information to compare with other watersheds, and address more interesting questions regarding the fate of anthropogenic N and the roles of climate and human activities (van Breemen et al., 2002). However, we lack long-term riverine monitoring data of total nitrogen for all the watersheds. In this section, percent TN export was approximated by determining the ratio between AN and TN in some monitoring stations where this information is available, then extrapolating the value of TN from AN. The resulting estimates could contain large uncertainties, since the percentage of AN to TN in rivers is highly dependent on season and pollution sources.

Studies from relatively undisturbed watersheds indicated that AN in rivers usually accounts for about 10% (or even less) of total nitrogen (Li et al., 2009;Singh et al., 2005), while it can be higher than 70% in urban or heavily polluted rivers in Asia (Li et al., 2014;Pernet-Coudrier et al., 2012). Evidence from the long-term monitoring studies in the mainstream of Huai River revealed that ammonia-nitrogen was the major form of dissolved nitrogen before 2000 (Mao et al., 2003). However, pollution management, especially in treatment of sewage and other sources of organic pollutants, has greatly reduced the possibility of riverine environments being suitable for the persistence of AN (MWR, 2010). In 2008, riverine nitrate was measured in a study conducted at several stations in the basin, with concentrations ranging from 0-15.7 mg/L NO_3-N, with a mean of 2.1 mg/L NO_3-N (Zhang et al., 2011), suggesting that nitrate is now an important constituent of riverine N flux. In addition, long-term monitoring data of TN, available for three inflow stations of Hongze, Nansi and Suya Lake in the Huai River Basin show that AN was correlated with TN ($P<0.001$), accounting for roughly 20% to 50% of TN (see Part V of supplementary material).

Our results found that an average of 0.91% of NANI was exported from the HRB as AN (Fig. 4a), with flow-weighted average riverine AN concentrations (supplemental materials, Table 7a) ranging from 0.2-3.3 mg/L N (average ~ 1 mg/L N, about half the average nitrate-N value reported by Zhang et al. (2011)). Assuming that this represents 20-50% of the total, much or all of the remainder is likely made up of nitrate, and TN export in the Huai River Basin is about 1.8%～4.5% of NANI. The value is comparable with a global study from Tysmans et al. (2013), which indicated that the percent TN export in this region is around 0～2%. While, our estimate is far below the average total nitrogen export ratio globally (25%) (Galloway et al., 2004) and for US watersheds (24%) (Swaney et al., 2012), N export ratios outside this 24-25% range have been reported in other parts of the world, possibly because of the various mechanisms dominating N export in different situations. TN export from the Huron River of Michigan (Bosch and Allan, 2008), Oldman River of Canada (Rock and Mayer, 2006) and Jurong Reservoir watershed of China (Kimura et al., 2012) accounted for 8%, 1.7% and 1% of the anthropogenic N input respectively, relatively close to our observed proportional export. The low values of percent TN export in these systems were explained by their great number of impoundments or water bodies, and relatively low runoff.

In the HRB, the riverine export is correlated with high consumption of water resources (containing N) and high impact of the dams and impoundments. Denitrification in river systems is often considered as an important pathway of N removal from watersheds (Seitzinger, 1990;Seitzinger et al., 2002;Billen et al., 2009) and the construction of dams and impoundments could significantly increase the nitrogen residence time within aquatic ecosystems, and thus increase the proportion of N removal through denitrification losses, assuming that nitrate is sufficiently available. The amount could be significant, given that more than 5,700 impoundments and 5,000 sluices have been constructed in most of the main streams and tributaries of the HRB (Xia et al., 2011). As in other Asian regions (Swaney et al., 2015), irrigation water consumption could be an important factor; the HRB is a very important food-producing region, which has produced nearly one-fourth of the country's marketed grain, cotton, and oilseeds on one-eighth of the nation's farmland (Bai and Shi, 2006). Under such intensive agricultural production, a high amount of riverine N is recycled through irrigation, and is subject to increase in residence times which favor denitrification (Lassaletta et al., 2012). In addition, other factors such as low slope and low runoff in some parts of the watershed (e.g., downstream) also limit NANI exported as riverine N flux (Rock and Mayer, 2006), and storage could be occurring in the soil and groundwater (van Breemen et al., 2002).

3.5 Parameters and sensitivity analysis

Accounting for the point source and non-point source components of anthropogenic N input would increase the complexity of its estimation. Since point sources and non-point sources both significantly impact riverine ammonia-nitrogen flux via different pathways, our anthropogenic N calculation that explicitly estimates the point source *vs* non-point source contributions could serve as a foundation for further exploration of anthropogenic effects on N pollution.

However, considering the fact that some data (such as the percentage of treated sewage (I_{sew})) used in $NANI_p$ calculation are not easily collected, some questions emerge: Are the parameters necessary to estimate point source N input? When we remove the parameters, how does the value of estimated point source N change? What kind of watersheds would easily be influenced by the removal of specific components? To answer these questions, the main components (I_{sew}, N_{ind} and N_{urban}) in Equ. (4) were each removed one at a time to determine the effect of being excluded from the NANI calculation (Table 4). By replacing the values of N_{urban}, N_{ind} and I_{sew} in Equ. (4) with 0 one at a time, we found that $NANI_P$ was changed by -81%, -19% and 40%, respectively. Obviously, domestic N discharges are important components of $NANI_p$ estimation, and show the largest impact on the headwater watersheds (No. 2, No. 4 and No. 1) when excluded from $NANI_p$ estimation. Followed by N_{urban}, estimated $NANI_p$ that ignored the role of sewage treatment systems (by setting I_{sew} to 0) would cause a larger error in the watersheds with high point source discharge and high rate of treated sewage (No. 21, No. 24 and No. 12). The least important component of $NANI_p$ estimation is N_{ind}. Removal of N_{ind} would affect the watersheds with high industrial discharge (No. 15, No. 9 and No. 3).

We also analyzed the sensitivity of NANI to input sources (Fig. 6), since as discussed by Hong et al. (2013) and Swaney et al. (2015), determination of the sensitivity of anthropogenic N inputs (both point source N and non-point source N) would help target N management appropriately (e.g., waste treatment *vs* fertilizer management) by providing first-order estimates of the relative importance of different sources of N loading to a watershed. For the non-point source N component of NANI, by far the most sensitive input terms of $NANI_n$ in the

Huai River Basin is fertilizer application, followed by feed N, crop N, atmospheric N deposition and finally biological N fixation. We found feed N is the second sensitive input source to $NANI_n$, indicating that N intake by livestock is a very important N source. Hence, the priority strategies of N management in non-point source system in the Huai River Basin should be focused on the reduction of fertilizer application rate and the control of livestock populations (e.g. reduction of the intensity of livestock breeding, manipulation of dietary N intake by animals and management of manure).

For the point-source N component of NANI, the sensitivity of urban domestic N discharge to $NANI_p$ is higher than that of industrial N discharge, indicating that decreased domestic N discharge is more important to point source N management. The result is consistent with a recent government report (Ministry of Environmental Protection of China, 2010) that states urban domestic point source N input accounts for about 75% of total load of point source. High sensitivities of N removal rate (I_{rem-tn}) and treated sewage effluent (W_{sew}) suggest that focusing on building more sewage treatment facilities to increase N recycling and improving technology of sewage plants to enhance N removal would be effective management strategies.

However, N management should not be only based on the overall anthropogenic N inputs, but also on local river water quality and the riverine and management processes affecting it. Including more spatially explicit biophysical details related to the response to N loading is needed to better support N management.

4 Conclusions

This work contributes to existing understanding of human-induced N pollution by differentiating the common NANI methodology into two parts (point sources and non-point sources of N inputs) and extending the analysis to AN, which has been largely neglected in previous studies. The results for the HRB show that multi-year average (2003-2010) NANI are 27,200±1100 kg N km^{-2} yr^{-1}. N inputs from point sources have been shown to be a much more important explanatory variable of riverine AN export than non-point source N, although they only account for about 2% of NANI. By examining the influence of N sources, we found that major N sources, such as fertilizer application, point source N input and atmospheric N deposition, directly impacted the AN flux in rivers. This result indicates that a source-based model can be used to predict AN fluxes in rivers.

The number of dams appears to be related to AN retention in the watershed, while volume of impoundments shows no significant relationship. AN retention could be the result of a combination of factors including biological denitrification and AN sorption onto settling sediment particles (both potentially increased by damming), losses associated with permanent water consumption (including irrigation), and storage in sediments, soils and groundwater. However, it is difficult to provide better assessments because N removal processes are dependent on the form of N. Monitoring of nitrogen in Chinese rivers has been largely focused on AN, neglecting nitrate and other N species. To better understand the processes of N retention, and to better inform N management strategies, we advocate changes in regional water quality monitoring policy to include more measurement of nitrate and total nitrogen in rivers, in addition to AN.

In sum, our results highlight the importance of attributing anthropogenic N inputs to point sources and non-point sources since this provides useful information relevant to N management. For the purpose of constructing a more accurate model of riverine N export, future work should address the study of mechanisms which promote or hinder N loss from these anthropogenic sources.

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Table 1. Biofixation rate and values used for calculating N fixation in HRB (kg ha^{-1} yr^{-1})

Type	Range of published fixation rate in China (Li and Jin, 2011)	Value used in this calculation
Symbiotic N fixation		
Soybeans	56.9-180	128.5 (Lu et al., 1996)
Peanuts	45-100	95.6 (Zhang et al., 1989)
Non-symbiotic N fixation		
Rice	30-62	30 (Du et al., 2010)
Other non-symbiotic crops	15	15 (Bao et al., 2006;Lu et al., 1996;Yan et al., 2003)

Table 2. N content of agricultural crop production (Yang et al., 2009)

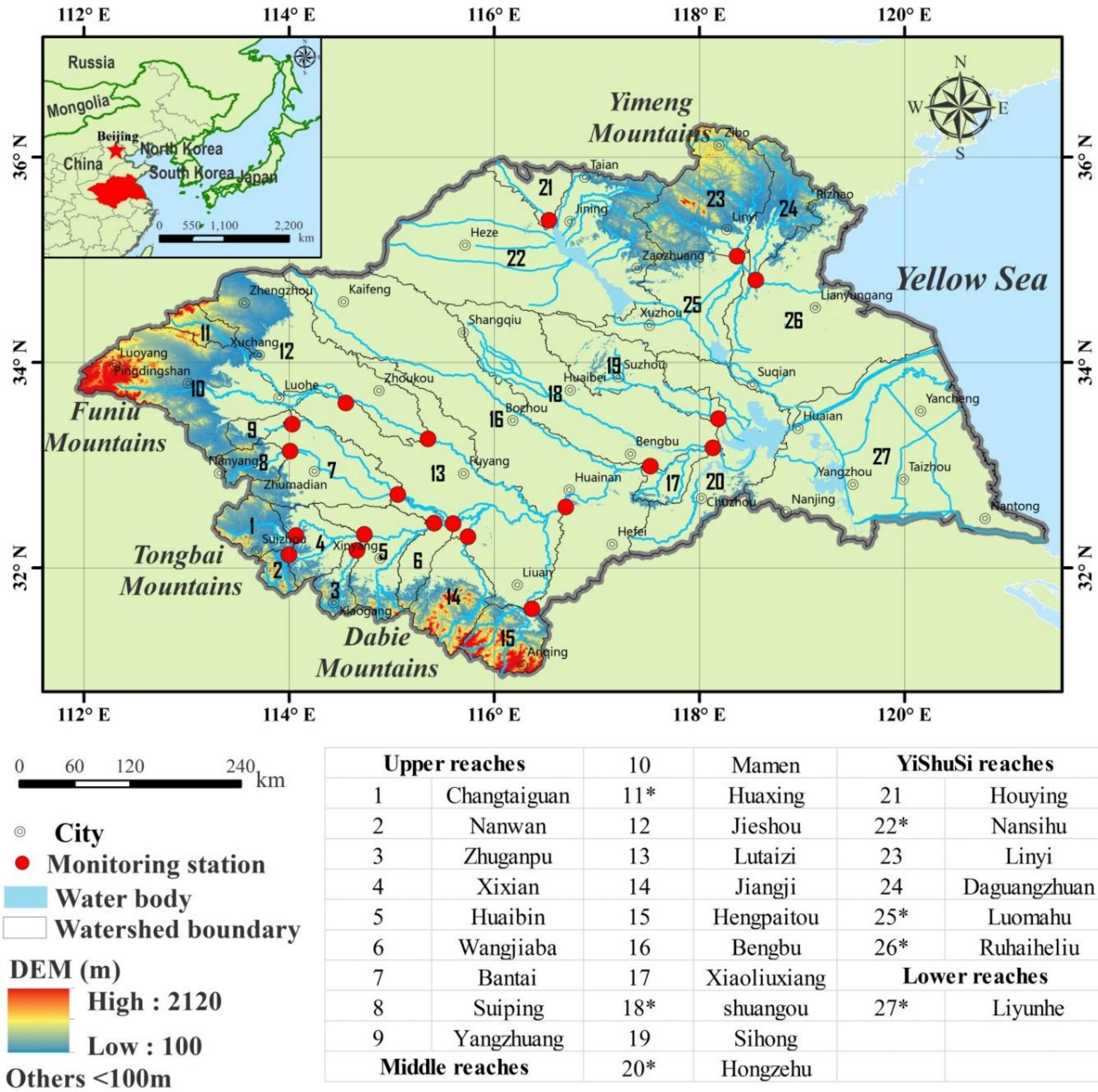
Parameter	Corn	Wheat	Paddy	Potatoes	Cabbage	Orange	Plum	Pear	Apple	Peach	Peanut	Soybean
Protein (%)	8.8	11.2	7.4	1.1	1.7	0.8	0.7	0.4	0.2	0.9	12.1	35.1
N (g kg^{-1})	14.08	17.92	11.84	1.76	2.72	1.28	1.12	0.64	0.32	1.44	19.36	59.16

Table 3. Average N inputs to Huai River basin during 2003-2010 (mean ± S.D., kg N km^{-2} yr^{-1})

Sub-basin	Non-point source N input ($NANI_n$)					Point source N input ($NANI_p$)		Total N input (NANI)
	$NANI_n$	Fertilizer N (N_{chem})	Atmospheric N deposition (N_{dep})	Biological N fixation (N_{fix})	Food and feed N in rural region (N_{r-im})	$NANI_p$	Point source AN input (AN_p)	
Upper	22,515±1,054	14,766±720	3,675±149	1,785±40	2,288±1825	307±12	182±16	22,822±1045
Middle	25,871±1,548	17,655±1,231	5,092±228	2,192±23	932±2,280	521±56	307±26	26,392±1053
Lower	26,030±440	20,591±1,393	5,535±257	1,511±84	1,596±1,193	743±97	391±31	26,773±471
Yishusi	29,769±1,156	21,190±688	6,805±515	1,610±153	164±1,320	591±61	297±18	30,560±1,100
HRB	26,644±1,172	18,687±1,002	5,480±273	1,900±61	576±1,825	542±48	300±20	27,186±1,129

Table 4. Percent change in point N input ($NANI_p$) resulting from component removal

Main components	Percent change in $NANI_p$ resulting from component removal (mean ± S.D.)	Three watersheds with the largest variation in $NANI_p$	Three watersheds with the smallest variation in $NANI_p$
Domestic N discharge (N_{urban})	-81% ± 0.11	No. 2 (97%)	No. 15 (-52%)
		No. 4 (97%)	No. 9 (-57%)
		No. 1 (94%)	No. 21 (-71%)
Industrial N discharge (N_{ind})	-19%±0.11	No. 15 (-48%)	No. 2 (-3%)
		No. 9 (-43%)	No. 4 (-3%)
		No. 21 (-29%)	No. 1 (-6%)
Percentage of sewage effluent (I_{sew})	40%±0.26	No. 21 (123%)	No. 9 (6%)
		No. 24 (79%)	No. 15 (12%)
		No. 12 (61%),	No. 3 (17%)



Upper reaches		10	Mamen	YiShuSi reaches	
1	Changtaiguan	11*	Huaxing	21	Houying
2	Nanwan	12	Jieshou	22*	Nansihu
3	Zhuganpu	13	Lutaizi	23	Linyi
4	Xixian	14	Jiangji	24	Daguangzhuan
5	Huaibin	15	Hengpaitou	25*	Luomahu
6	Wangjiaba	16	Bengbu	26*	Ruhaiheliu
7	Bantai	17	Xiaoliuxiang	Lower reaches	
8	Suiping	18*	shuangou	27*	Liyunhe
9	Yangzhuang	19	Sihong		
Middle reaches		20*	Hongzehu		

Figure 1. The boundaries of the 27 watersheds used in constructing N budgets. We did not have sufficient monitoring data for watersheds 11, 18, 20, 22, 25, 26 and 27 (labeled with a asterisk). The following AN flux analysis does not include these seven watersheds

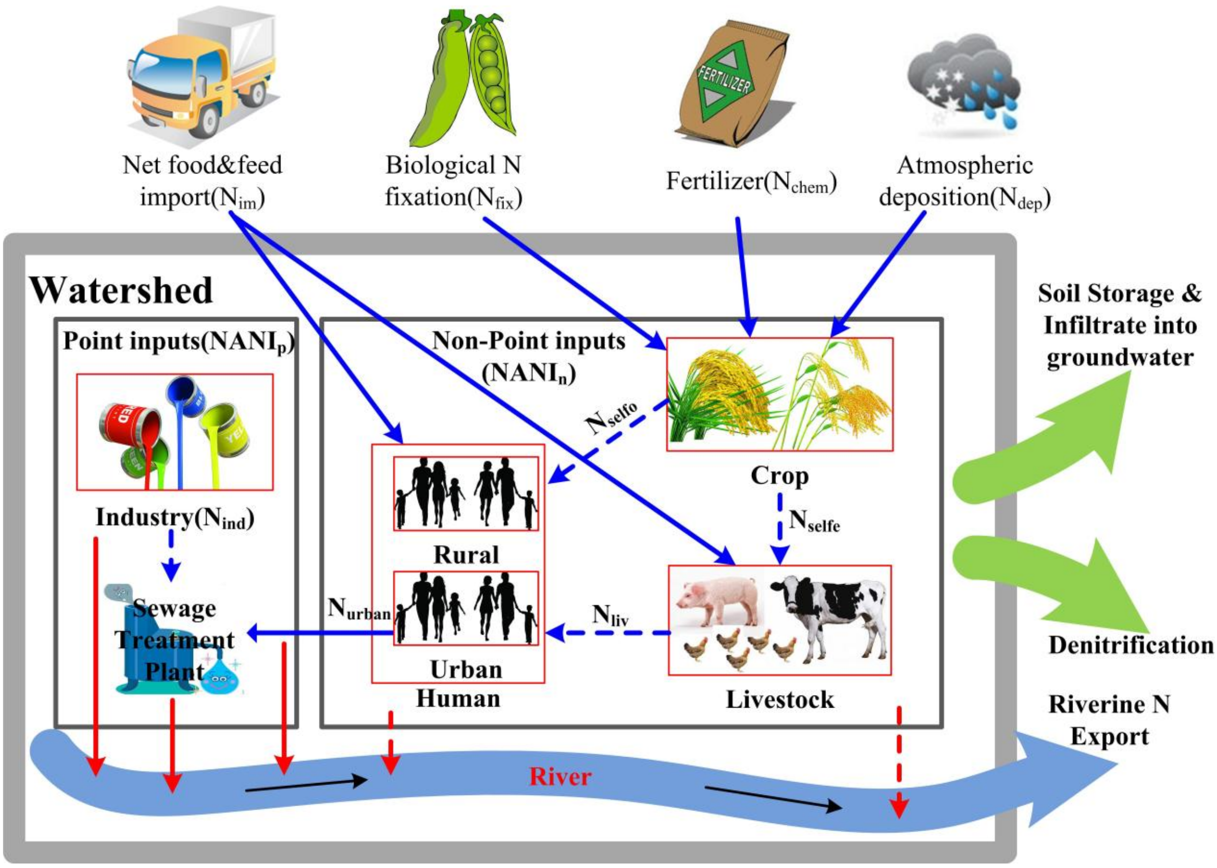


Figure 2. Diagram of major components of net anthropogenic nitrogen inputs (NANI) and exports from a watershed ecosystem (revised from **Swaney et al. (2012)**). Within a watershed ecosystem there are two kinds of input sub-systems: non-point and point systems, with large differences in the modes of N delivery. In the non-point source system, the pollutants can be added into the river network in indirect routes, such as rainfall-runoff, leaching, etc. In the point source input system, the pollutants usually are discharged directly into river systems. In this estimate, the N flows with abbreviations (e.g., N_{im}, N_{fix}, N_{urban}, etc.) were included as NANI estimate. The solid blue arrows in the figure represent anthropogenic nitrogen flows, and dotted blue arrows indicate an internal cycle of nitrogen within the watershed ecosystem. The solid red arrows represent the nitrogen flows directly into river systems. The dotted red arrows indicate indirect nitrogen flows.

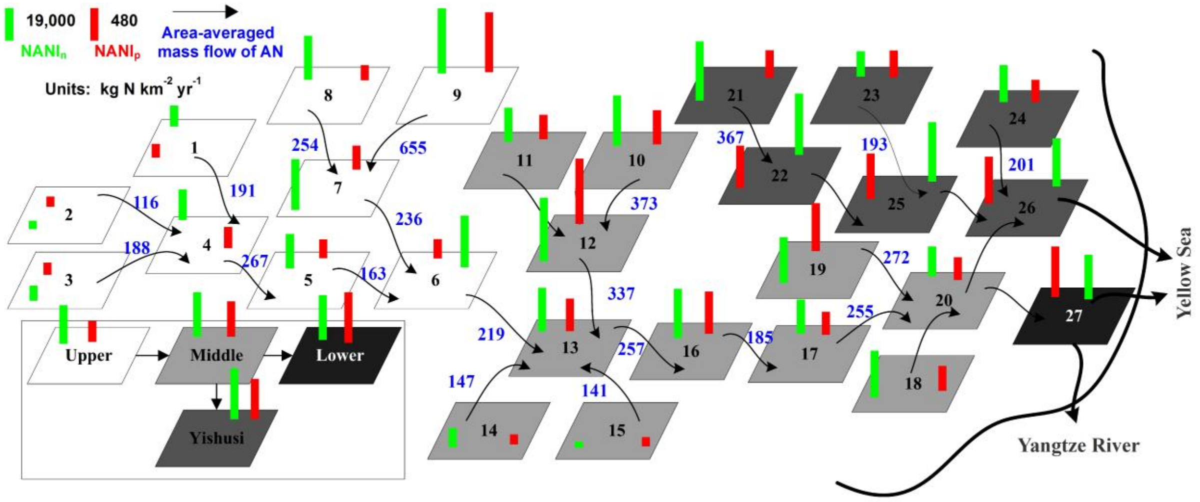


Figure 3. Average net anthropogenic nitrogen inputs and riverine ammonia-nitrogen flux in Huai River Basin of China

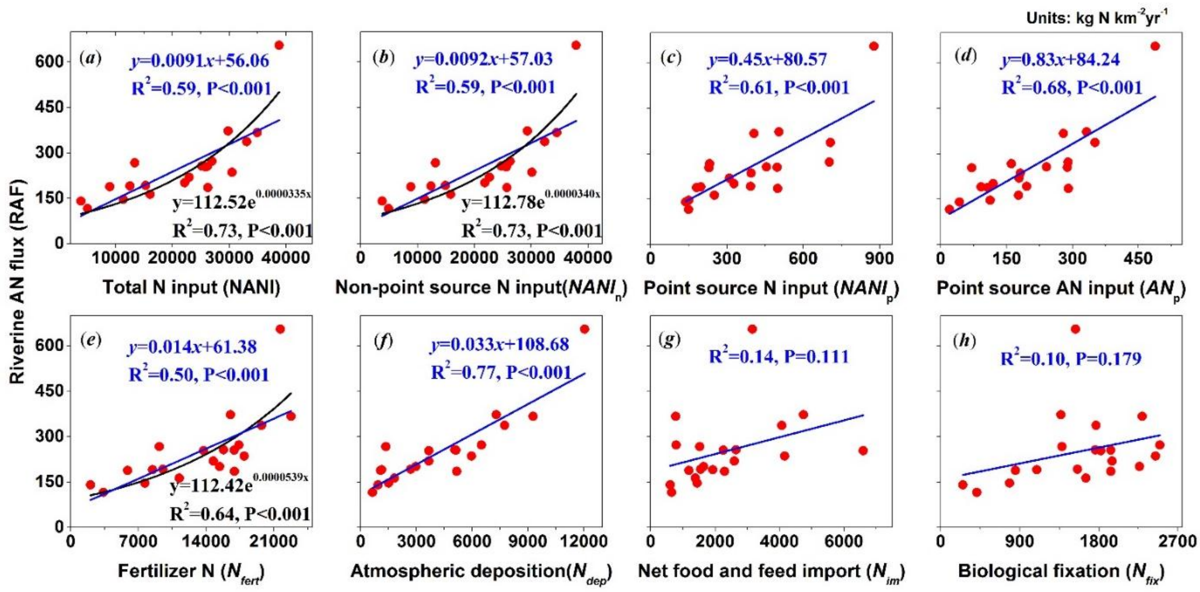


Figure 4. Linkage of AN flux with different N sources

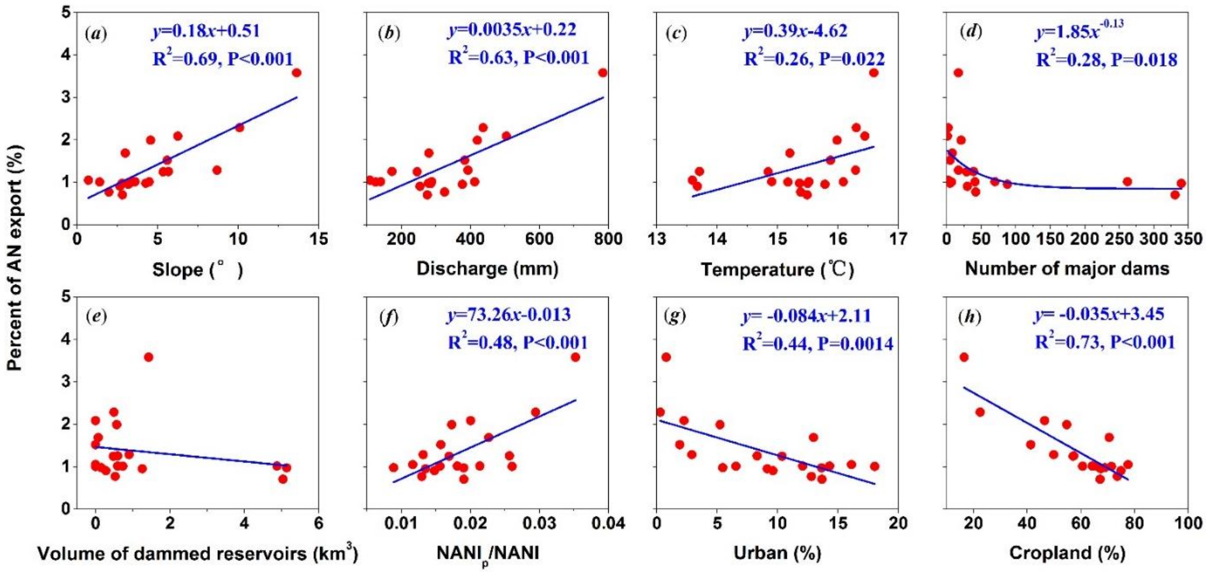


Figure 5. Regressions between AN export as a percent of NANI (%) and individual independent variables across the subbasins of the Huai River Basin (n=20)

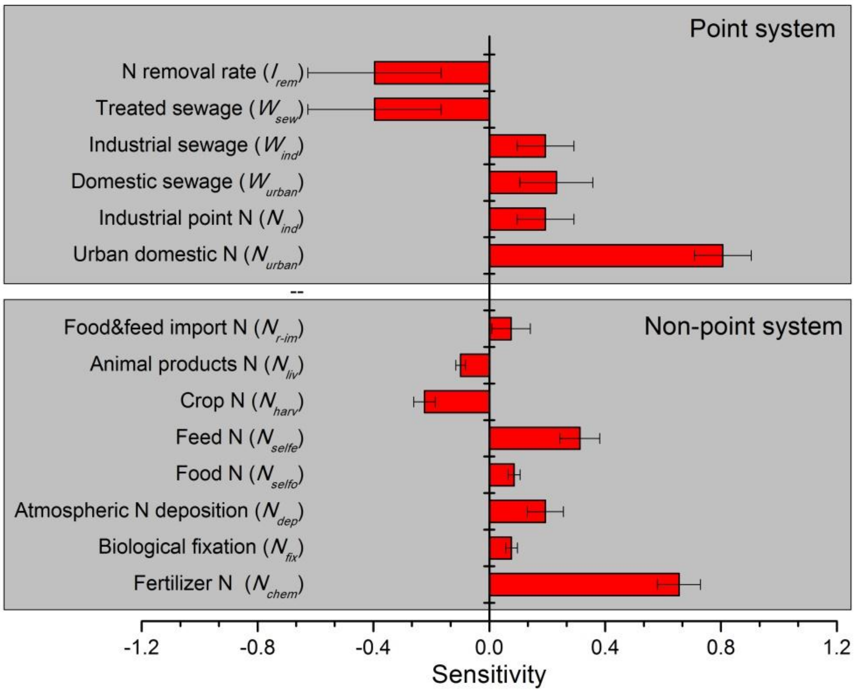


Figure 6. Sensitivity of major input terms calculated from 27 watersheds in Huai River Basin (mean ± S.D.). Sensitivities were calculated by applying ±10% change in input terms of NANI. We did not test the uncertainty and sensitivity of parameters used in $NANI_n$ estimation, since many other similar studies clearly discussed these for all of the parameters (e.g. Swaney et al. (2015), Hong et al. (2013), and Sobota et al. (2013))