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Nitrate sources and watershed denitrification inferred from nitrate dual isotopes in the Beijiang River, south China

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Abstract The great spatial and temporal variability of nitrogen (N) processing introduces large uncertainties for quantifying N cycles in large scales, e.g. a watershed scale, and hence challenges the present techniques in measuring ecosystem N mass balance. The dual isotopes of nitrate (δ^{18}O and δ^{15}N) integrate signals for both nitrate sources and N processing, making them promising for studies on large scale N cycling. Here, the dual isotopes, as well as some ion tracers, from a subtropical river in south China were reported to identify the main nitrate sources and to assess the possible occurrence and degree of denitrification in the context of monsoon climate. Our results indicated that nitrification of reduced fertilizer N in soil zones was the main nitrate source, with sewage and manure as another important source in dry winter. Seasonal changes of denitrification was apparent by the ~1:2 enrichment of ^{18}O and ^{15}N from April to August, and suggested to occur over the watershed rather than in the river. The lowest denitrification (10%) occurred in April, when the fertilizer application was strongest and the monsoon rainfall abruptly increased, causing enhancement of leaching. The highest denitrification (48%) took place in August due to the high soil temperature and moisture. In December, denitrification was significant (26%) perhaps due to the high enough temperature for microbial activities, whereas the low soil moisture appeared to limit the degree of denitrification. This study suggests that the seasonal variations in denitrification should be taken into account when estimating regional N mass balance.

Keywords Nitrate · Dual isotope · Denitrification · River

Introduction

The concerns about the impact of excess nitrogen on ecosystems (Moffat 1998) and the contribution of gases, such as N_2O, to global warming (Pérez et al. 2000) have attracted numerous interests in nitrogen cycling. Flux and rate of Nitrogen (N) biogeochemical cycling have been recognized as spatially and temporally variable, with zones or periods of enhanced cycling popularly known as “hot spots” or “hot moment”, respectively (McClain et al. 2003). Both hot spots and moments change with the scale

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chosen for a study. Take denitrification, the conversion of NO_3^- to gaseous N (N_2O or N_2), as an example, it could occur around patches of labile organic matter in a meter of soil, in topographic depressions within a catena, in riparian and hyporheic zones from the upland to stream, and in wetlands of a large river basin (McClain et al. 2003). Temporal variation of denitrification is also noticed in many studies, which is mainly associated with moisture-related dry-wet cycles (e.g. Sextone et al. 1985; Lowrance 1992; Van Kessel et al. 1993; Koba et al. 1997; Priemé and Christensen 2001; Austin et al. 2004) or temperature-related seasonal cycles (e.g. Christensen and Sorensen 1988; Ostrom et al. 1998; Pattinson et al. 1998; Panno et al. 2006).

This heterogeneity of N cycling patterns thus makes precarious the extrapolation of small-scale measurements to larger scales, especially at ecosystem, landscape, and regional scales, where whole-system estimates of biogeochemical processes are hardly measured directly and often based on the extrapolation of plot-based measurements (McClain et al. 2003). The great deal of uncertainty in extrapolation indicates a clear need to develop better estimates of N cycling at scales relevant to N pollution issues. Among the continued improvement of new methods, the natural N isotope method, $\delta^{15}N$, which can be used as a “tracer” and/or “integrator” of N cycle processes (Robinson 2001), holds great promise for larger scale biogeochemical studies of N species origin and N processing. For example, NO_3^- originating from sewage and livestock effluent is typically more enriched in the heavier N isotope (^{15}N) compared with N and NO_3^- derived from atmosphere deposition, fertilizer N, and soil organic N (Kendall 1998). In addition to $\delta^{15}N$, researchers have included the measurement of oxygen isotope ratios ($\delta^{18}O$) of NO_3^-, decreasing uncertainties in source discrimination due to significant overlap of $\delta^{15}N$ values for NO_3^- derived from the latter three sources and producing more definitive results (Aravena et al. 1993; Burns and Kendall 2002; Chang et al. 2002). Moreover, this dual-isotope technique has been found powerful in determining whether or not denitrification has occurred by the $\sim$1:2 relationship between $\delta^{18}O$ and $\delta^{15}N$ (Böttcher et al. 1990; Aravena and Robertson 1998; Chen and MacQuarrie 2005), adding another means to discerning denitrification by the single-isotope studies that usually linked NO_3^- concentrations to its $\delta^{15}N$ values (Mengis et al. 1999; Lund et al. 2000).

In a watershed ecosystem, N processes occurring from upland to stream control the isotopic signature of N species in stream. Thus, the stream isotope measurement is more than just the study of water; it encompasses the biogeochemical interactions that control the biogeochemistry of the system, and provides information about nitrogen cycling that is integrated over the whole watershed. For example, using the $\delta^{15}N$ and $\delta^{18}O$ values for NO_3^- from the Mississippi River, Panno et al. (2006) found that the NO_3^- was primarily derived from agricultural fertilizers and soil organic N, and has undergone significant denitrification, occurring before discharge into the Mississippi River and varying by sample locations and seasons.

The Beijiang River is a subtropical monsoon-influenced tributary of the Pearl River in south China. The Pearl River estuary has been found very high in N concentrations, reaching 100 μM and comparable to the levels of the Mississippi River (Yin and Harrison 2008), and eutrophication is obviously accelerated in recent decades (Jia and Peng 2003; Hu et al. 2008). However, to date, there are no studies on N biogeochemical cycling occurring in the Pearl River system. And to our knowledge, few N isotope works have been done for tropical-subtropical rivers to investigate N processing in watershed ecosystems, especially under monsoon climate conditions. In this study, we report $\delta^{15}N$ and $\delta^{18}O$ values for NO_3^- from the Beijiang River. The objectives of our investigation are to identify the major sources contributing to the total dissolved NO_3^- in the river and to assess the possible occurrence and degree of denitrification in the context of monsoon climate.

Samples collection and analysis

Background

The Beijiang River is the second largest tributary of the Pearl River (Fig. 1); the latter is the second largest river in China next to the Yangtze River by discharge volume. The Beijiang River flows southward about 468 km from its major headwaters to converge with other main tributaries into the river net of Pearl River delta, and finally empties into the Pearl

River estuary and the South China Sea. Its catchments cover 46,710 km^2, accounting for about 22% of land area of Guangdong Province, one of the most rapid developing provinces in China.

The climate of the region is of typical monsoon nature, with hot and rainy summer from April to September, and cool and dry winter from December to February. In response to the climate, river runoff fluctuates obviously in an annual cycle, with 70–80% of the flux occurring from April to September (Li et al. 2005). Wet season is subdivided into early flood period (EFP, April–June) and late flood period (LFP, July–September). The precipitation of EFP is usually greater than that of LFP in Beijiang River basin, by a factor of ca. 1.8. The precipitation during the EFP is mostly attributed to frontal precipitation, while that during the LFP is mainly the result of tropical cyclones (Luo et al. 2008).

The Beijiang River Basin is a typical agricultural area, with 86% of land being used for agriculture, 4% for urban, and 10% as barren. Rice is the dominant crop in the river basin, which is planted in early March (early rice, harvested in July) and in early August (late rice, harvested in November). The heavy application of fertilizer takes place around April, when early rice and fruit trees grow rapidly. This fertilizer application can take effects for several months, so less fertilizer is used in later months. N fertilizer application in 2006 in the river basin is estimated to be approximate 1.3 × 10^5 t N (Chen and Jia 2009). In China, fertilizer N is almost in the reduced form, i.e., urea (71%) and ammonium (27%), e.g. NH_4HCO_3 and NH_4Cl, and the nitrate form (mainly NH_4NO_3) is negligible (Zhang 2006). The population of the region is 6.5 × 10^6, with great densities in the downtowns of county seats and two megalopolises, Shaoguan and Qingyuan (Fig. 1). Human activities make an important contribution to N input into the river basin from manure (human and animal wastes) and sewage (1.2 × 10^5 t N in 2006; Chen and Jia 2009), a great part of which is directly poured into the river system.

Samples and experiment

Seven sites in the mainstream of the Beijiang River located from the upper reach to the lower reach and five in tributaries close to the mainstream were chosen for water sample collection for this study (Fig. 1). These twelve sampling sites were close to the ongoing hydrologic stations of Guangdong Provincial Hydrologic Bureau. Water samples at

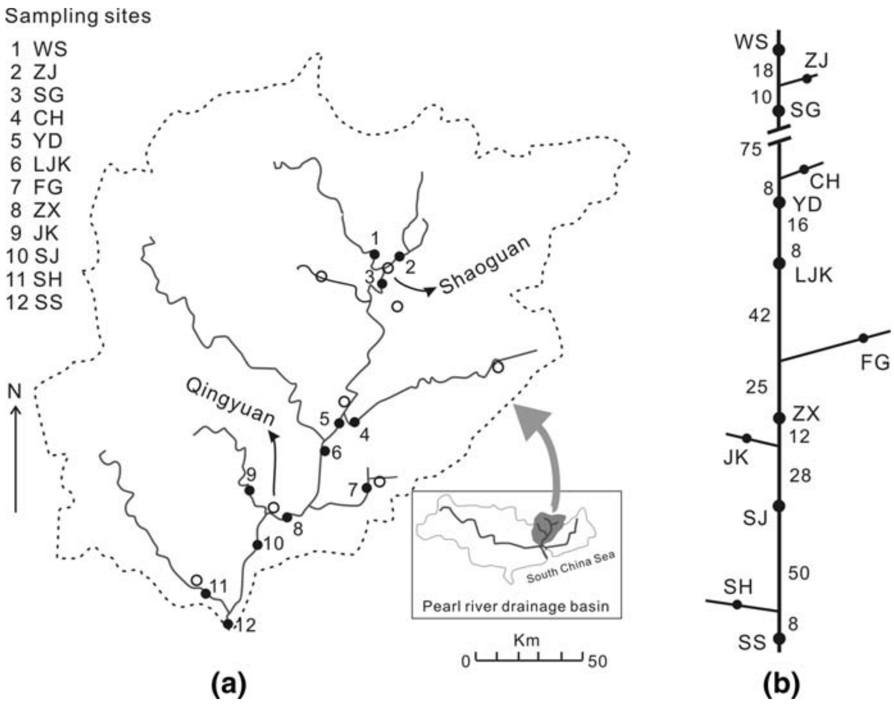

Fig. 1 **a** Map of the Beijiang River system. *Filled circles* are the twelve sampling sites, and *open circles* are towns and cities, including the two megalopolises, i.e., Shaoguan and Qingyuan. **b** Schematic showing the distance in river kilometers between sampling sites

~20 cm depth in the middle of the river were collected using Teflon containers three times from the twelve sites in a hydrological year: December 2006, April 2007, and August 2007. Every time our sampling was accomplished by sequentially sampling the mass of water as it moved downstream. All sampling equipment was pre-cleaned with non-phosphate detergent, rinsed thoroughly with tap water and deionized water. Collected water samples were filtered on site through glass fiber filters (Whatman, GF/F, 47 mm in diameter) into 2 l pre-cleaned polyethylene bottles, and then the nitrates in the filtered water samples were concentrated on the anion exchange column (DOWEX 1 × 8, 200–400 mesh, chloride form). Finally, about 100 ml filtered water samples were carried back to laboratory for anion concentration determination within at most 24 h.

In the laboratory, anion concentrations in water samples, including NO_3^-, Cl^-, and SO_4^{2-}, were determined using ion chromatography, with analytical precision of ±0.2 mg l^{-1}. Dissolved nitrate were converted to solid $AgNO_3$ according to the method introduced by Silva et al. (2000). $AgNO_3$ was then analyzed for $\delta^{15}N$ using a DELTA plus XL mass spectrometer connected with a CE EA1112 C/N/S analyzer, and for $\delta^{18}O$ using MAT 253 mass spectrometer connected with a high-temperature conversion elemental analyzer. Dissolved organic matter and oxygen-bearing ions other than NO_3^- were carefully removed during the preparation of $AgNO_3$ for $\delta^{18}O$ analysis. International standard IAEA-N3 was used for $\delta^{15}N$ and $\delta^{18}O$ calibration. Reproducibility of duplicate analyzes was <0.9‰ for $\delta^{18}O$ (averaging ± 0.3‰), and <0.3‰ for $\delta^{15}N$ (averaging ± 0.1‰).

Results

The nitrate concentrations ranged from 41.3 to 111.0 μM, with mean values for April, August, and December of 87.1 ± 10.7, 61.0 ± 10.9, and 75.7 ± 20.5 μM, respectively (Table 1). There appeared no large variations in nitrate concentration between seasons, in contrast with the great seasonal changes of Cl^- concentration. Because Cl^- is biologically and chemically inert and its concentration can change only by mixing within the river system, the ratio of NO_3^-/Cl^- has been considered more accurate for the study of N dynamics (Koba et al. 1997) and sources (Liu et al. 2006). We hence used the molar ratio of NO_3^-/Cl^- here and found that the differences in the ratio between seasons were quite apparent (Fig. 2): high values (2.46 average) in April, intermediate values (0.81 average) in August, and low values (0.34 average) in December.

The nitrate $\delta^{15}N$ ranged from 1.9 to 17.6‰, and averaged 7.9‰; $\delta^{18}O$ ranged from 5.6 to 17.3‰, and averaged 12.4‰. We did not find the intra-seasonal trends downstream for $\delta^{15}N$ and $\delta^{18}O$ values. There were no correlations between the two isotopes during April and December, respectively, but a positive correlation was evident ($\delta^{18}O = 0.52 \times \delta^{15}N + 9.2$, $r^2 = 0.32$, $n = 7$) during August. However, the nitrate for the 3 months can be clearly distinguished by their $\delta^{15}N$ values, showing an increasing trend from April (3.4‰ average) to December (8.3‰ average) to August (11.8‰ average). A similar increasing trend was also displayed for nitrate $\delta^{18}O$ (11.1‰ in April, 11.4‰ in December, and 15.0‰ in August), although there were some overlaps between seasons, especially between April and December. These inter-seasonal increasing trends for both isotopes are shown in Fig. 3 by the distributions of data points in the $\delta^{18}O$-$\delta^{15}N$ plot.

Discussion

Seasonal variations of nitrate sources and dynamics

Typical ranges of $\delta^{15}N$ and $\delta^{18}O$ for various natural and anthropogenic sources of NO_3^- are defined by boxes in Fig. 3 (Kendall 1998; Chang et al. 2002; Silva et al. 2002). NO_3^- originating from precipitation and synthetic NO_3^- fertilizer has much higher $\delta^{18}O$ values than that from nitrification of reduced N sources, i.e. fertilizer applied as NH_4^+ and urea, soil organic N, and NH_4^+ from sewage and manure. And NO_3^- from these kinds of reduced N could be distinguished by their different $\delta^{15}N$ values, although there are some overlaps in $\delta^{15}N$ between them. NO_3^- added to soils from precipitation and fertilizer would be quickly recycled, and usually no longer has the $\delta^{18}O$ value of the initial added NO_3^-, but instead would have a $\delta^{18}O$ value like that of reduced N sources that were nitrified in the soil zone (Mengis et al. 2001).

Table 1 The nitrate isotopic values and ion concentrations in the Beijiang River, and the calculated degree of denitrification over the watershed

Month	Sample site	Cl^- (μM)	NO_3^- (μM)	SO_4^{2-} (μM)	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)	Denitrification (%)
April	1	78.7	74.9	139.3	–	10.8	
	2	79.0	87.3	315.0	3.6	–	11.1
	3	93.5	85.4	231.6	2.8	12.8	6.1
	4	20.5	84.1	64.0	3.2	12.8	8.4
	5	19.6	81.0	66.3	4.4	11.2	15.6
	6	15.5	75.6	46.6	3.7	10.5	11.6
	7	84.1	111.0	195.6	3.0	12.1	7.5
	8	94.1	95.4	230.5	3.4	13.3	9.9
	9	17.9	82.2	29.6	3.5	12.3	10.7
	10	26.0	82.3	46.5	1.9	9.4	0.0
	11	–	–	–	3.8	11.0	12.1
	12	108.9	98.7	281.5	3.8	5.6	12.5
August	1	55.4	41.3	99.7	13.7	–	55.0
	2	69.2	59.5	303.1	7.8	–	33.1
	3	66.2	51.0	184.2	11.5	–	47.9
	4	55.7	82.9	190.0	13.4	14.5	54.3
	5	73.2	68.5	180.8	7.8	12.4	33.0
	6	65.8	57.8	180.6	10.3	12.8	43.4
	7	68.5	57.3	140.0	17.6	–	65.4
	8	68.2	62.4	162.7	10.2	16.9	43.0
	9	89.9	64.5	89.4	12.6	17.3	51.6
	10	196.2	69.8	211.6	12.1	14.9	49.9
	11	75.1	50.4	40.4	13.4	16.1	54.2
	12	116.7	67.4	188.8	11.0	15.2	46.1
December	1	125.7	42.2	233.1	8.7	–	
	2	313.0	100.7	356.2	8.3	–	
	3	352.2	77.3	484.5	7.6	11.8	27.9
	4	252.2	91.1	294.5	7.9	10.5	21.9
	5	399.9	83.2	314.9	7.8	–	
	6	199.1	75.6	265.8	11.0	12.3	42.0
	7	130.2	44.3	140.7	9.5	–	
	8	124.1	89.2	283.8	8.1	13.9	35.4
	9	212.5	80.9	158.4	7.5	–	
	10	307.2	85.6	285.5	9.5	7.0	0.0
	11	473.3	45.1	114.5	7.1	11.0	22.1
	12	201.6	93.7	292.5	7.1	13.5	31.5

–, Not determined due to sample destroyed during transportation, pretreatment or instrumental analysis

Denitrification and nitrification are the two important biologically mediated reactions causing the N and O isotopes to fractionate and leaving heavier isotopes (^{15}N and ^{18}O) in residuals. The fractionation factors for $\delta^{15}N$ and $\delta^{18}O$ during denitrification vary depending on the local conditions and rates of reaction; however, the ratio of the changes in $\delta^{18}O$ and $\delta^{15}N$ is typically close to 1:2 (Böttcher et al. 1990; Aravena and Robertson 1998; Kendall 1998; Mengis et al. 1999). During nitrification, in addition to preferential utilization of ^{14}N, NO_3^- generally utilizes one-third of the O from atmospheric O and

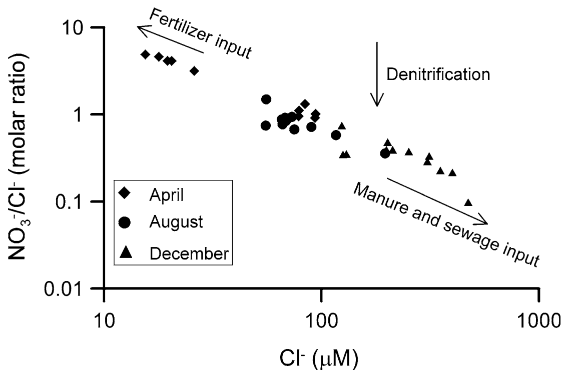

Fig. 2 Seasonal variations of NO_3^-/Cl^- ratios with Cl^- concentrations in the Beijiang River

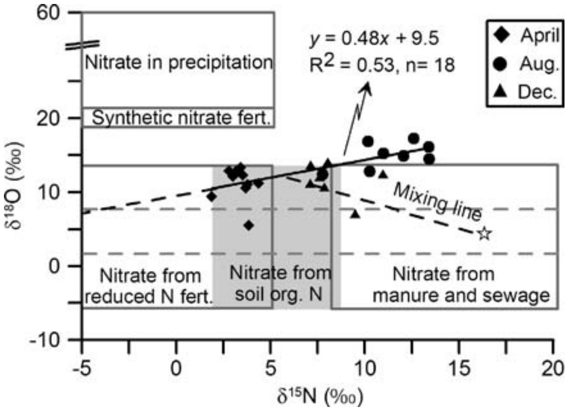

Fig. 3 Ranges of $\delta^{18}O$ and $\delta^{15}N$ values for potential nitrate sources and values measured in Beijiang River. The *boxes* bordered by *real lines* delineate the typical ranges of $\delta^{18}O$ and $\delta^{15}N$ values for various nitrate sources adopted from Silva et al. (2002). The *shaded area* defines the domain of nitrate from soil organic matter, which partially overlaps that of reduced N fertilizer and manure and sewage with respect to $\delta^{15}N$. The two *horizontal dashed lines* delineate the local $\delta^{18}O$ of NO_3^- from these reduced N sources as described in the discussion. The *regression line* is calculated from data for April and August. The *dashed* "*mixing line*" shows the compositions of nitrate in December from manure and sewage (marked by a *star*) and progressive denitrification of agricultural inputs (denoted by the *regression line*)

two-thirds from the surrounding water (Amberger and Schmidt 1987; Böttcher et al. 1990; Kendall 1998). It thus appears that the environmental settings and climatic conditions affect the initial $\delta^{18}O$ value of NO_3^- during nitrification of reduced N sources. Using this rule of NO_3^--O sources during nitrification and local monthly (amount-weighted average) range of precipitation $\delta^{18}O$ of −9.14 to +0.34‰ from nearby IAEA station of Guangzhou (IAEA 2003), an expected narrower range of nitrate $\delta^{18}O$ of +1.6 to +7.9‰ can be obtained for the reduced N sources of NO_3^-. This local theoretic range for nitrate $\delta^{18}O$ is shown in Fig. 3 as the domain delineated by the two horizontal dashed lines. We realize that this expected local nitrate $\delta^{18}O$ range is rather simplified, because (i) the relative contribution of ambient O from surrounding water and O_2 has been found not always keeping the rule of 2:1 (Mayer et al. 2001); (ii) there is also the possibility that the $\delta^{18}O$ of the ambient water may be enriched in ^{18}O due to respiratory isotope fractionation or evaporative effects (Kendall 1998; Burns and Kendall 2002). However, we believe that this theoretical estimation could be the most representative one for local $\delta^{18}O$ of nitrate oxidized from reduced N.

In our results, $\delta^{18}O$ values are mostly distributed close to the typical upper limit of $\delta^{18}O$ for nitrate from nitrified reduced N, and outside our estimated domain for local nitrate $\delta^{18}O$ (Fig. 3). These slightly greater $\delta^{18}O$ values might be due to the contribution of NO_3^- from atmospheric and synthetic NO_3^- fertilizer sources and denitrification. The contribution of synthetic NO_3^- can be readily eliminated, because it accounts for less than 2% of the synthetic N fertilizer applied in China. If contribution of unrecycled atmospheric NO_3^- was the dominant factor for the slightly higher $\delta^{18}O$ values, a strike contrast in river nitrate $\delta^{18}O$ between wet and dry seasons would be expected, with higher values in rainy April and August and lower values in dry December. However, our data are not consistent with this expected pattern; and hence suggest that atmospheric source reaching the river as precipitation, without passing through the soil zone, may be less important.

The increasing trend of our $\delta^{15}N$ values from April to December, and then to August could be explained by different degrees of denitrification, changes in mixing of different NO_3^- from nitrification of reduced N, or both. If mixing was the only process leading to the trend of $\delta^{15}N$, then samples with relatively elevated $\delta^{15}N$ values would require a large input of sewage and manure, which means that the most input would have to be in August. This scenario is obviously unrealistic, because precipitation and river water were high in rainy August, which would dilute the contribution of sewage and livestock effluent. In fact, the most contribution of sewage and livestock effluent should be in dry December, which is nicely suggested by the highest concentrations of Cl^- and SO_4^{2-}, and lowest NO_3^-/Cl^- ratios in December (Table 1; Fig. 2), because sewage and livestock effluent have been found high in Cl^- and SO_4^{2-} concentrations (Krapac et al. 2002; Karr et al. 2003; Yao et al. 2007), and low in NO_3^-/Cl^- ratios (Liu et al. 2006). The reason sewage and livestock waste have low NO_3^-/Cl^- ratios is partly because the N in them is ammonium and has not yet been converted to nitrate, and hence would discount the potential of the ratio as an indicator of sewage and manure in the case of intensive nitrification following, for example, application to fields. However, due to that most of sewage and livestock effluent is directly poured into rivers in the basin (Yang et al. 2005), we speculate that nitrification, even if occurring, is not so intensive in the flowing waters that could impair the signal of sewage and manure. Nevertheless, future investigations of the nitrification in rivers, as well as in soils, in the watershed are needed.

Since mixing of sources alone cannot account for the overall variation of isotopic values for the samples, it appeared that denitrification has played an important role. The close to ~1:2 linear relationships between $\delta^{18}O$ and $\delta^{15}N$ for April and August, as demonstrated by their regression line (Fig. 3), imply that denitrification was indeed the dominating process for these periods. The intersection of the linear relationship with the local nitrate source domain suggests that the NO_3^- in the samples comes primarily from reduced N fertilizer. However, this suggested NO_3^- is more or less negative in $\delta^{15}N$ and positive in $\delta^{18}O$, which might be caused by the contribution of precipitated NO_3^-, although it was less important as explained above. The impact of ^{18}O-enriched atmospheric NO_3^- on the nitrate $\delta^{18}O$ in river and soil water has also been suggested for the Mississippi River (Panno et al. 2006) and some forest areas (e.g. Burns and Kendall 2002). Nevertheless, the relative low values both for $\delta^{18}O$ and $\delta^{15}N$ in April, as well as the high NO_3^-/Cl^- ratios (Figs. 2 and 3), indicate that riverine NO_3^- in April was mainly from nitrification of reduced N fertilizer and less denitrified. This scenario corresponded to the heavy application of fertilizer in spring (typically April) for cropland in the study area and abrupt increase in monsoon rainfall, resulting in more rapid runoff and probably a better representation of initial isotopic composition of recently formed NO_3^- in soils. This would lead to relatively rapid movement of isotopic lighter NO_3^- from the soil zone into river systems. The ~1:2 increase in $\delta^{18}O$ and $\delta^{15}N$ from April to August demonstrates intensified denitrification in August, which may be a main cause for the lower NO_3^-/Cl^- ratios than in April. In the study area, monsoon and flood period continued from April to August, with soil temperature kept increasing and soil moisture maintained high, favoring the progress of denitrification.

Most isotopic values in December lie at the heaviest corner of typical soil derived nitrate domain, likewise suggesting the impact of denitrification (Fig. 3). However, as shown in Fig. 3, the ~1:2 linear line for isotopic values in April and August does not pass through the center of the isotopic data for December, but above most of them. This slight departure from the local denitrification line might be resulted from more contributions of nitrate from sewage and manure, as suggested earlier by high Cl^- and SO_4^{2-} concentrations and low NO_3^-/Cl^- ratios (Table 1; Fig. 2). This finding is quite similar to the results of Panno et al. (2008), in which nitrate from sewage and manure also make a more contribution in low flow period of the Illinois River. Therefore, the combination of mixing and denitrification could be applied for better accounting for the isotopes in December. Here, we simply assume that (1) the initial nitrate isotopic values from sewage and manure lied at the center of local domain for the source (+15‰ for $\delta^{15}N$ and +4.8‰ for $\delta^{18}O$; the star in Fig. 3) and did not undergo denitrification; (2) the denitrification line obtained in this study was constant through time. Then the relative contributions of the two sources, (1) sewage and manure; (2) fertilizer or soil underwent some degrees of denitrification, to the nitrate in any water samples collected in December can be estimated, as exemplified by the dashed line from the initial isotopic data point for sewage and manure-derived nitrate to the intersection with the denitrification line via a data point for a wintertime water sample (Fig. 3). Such estimations are not affected by the extent or timing of denitrification and mixing, or the spatial arrangement of point versus non-point sources (Kendall 1998). Using this method, the average contribution of sewage and manure in December was estimated to be 19%, ranging from 0 to 60%. This result is comparable to the approximately 14% contributions to the N load from sewage and manure discharge in the Upper Mississippi River basin (Panno et al. 2006).

Denitrification

In this study, denitrification signal was absent (April and December) or weak (August) within each sampling season. This suggests that denitrification did not occur, or less significant if any, in the river waters, because our samples within a sampling season are a set of Lagrangian samples. In a similar study, evaluation of a set of Lagrangian samples from Mississippi River at several different sites from Illinois to Louisiana also showed no evidence for denitrification in the river (Battaglin et al. 2001). These findings of the lack of in-stream denitrification in big rivers is consistent with the model of Alexander et al. (2000) and the ^{15}N tracer studies of Peterson et al. (2001), which concluded that there is more opportunity for contact and exchange of small, shallow stream water with the hyporheic zone, and hence N loss in streams declines rapidly with increasing channel size. In fact, denitrification fingerprinting may be more effective in small (5 km^2), heavily fertilized watersheds (Kellman and Marcel 1998). Therefore, the lack of isotopic evidence for denitrification in large rivers does not mean that denitrification has not occurred in river basins. Then a concerned issue is if nitrate isotopic analysis for a large river is applicable for basin-scale nitrogen cycling studies, which is an examination for the “integrator” role of the isotopes.

In our results, denitrification signal was apparent by inter-seasonal comparisons, with strongest in August and weakest in April. This finding is quite similar to the results of Panno et al. (2006), in which seasonally collected samples in the Mississippi River followed a well-defined denitrification pattern, although Battaglin et al. (2001) and Chang et al. (2002) were not able to distinguish denitrification in their studies. Panno et al. (2006) inferred from the seasonal pattern that denitrification has progressed mainly in soil zone or groundwater before discharging to surface water, which was controlled by seasonal changes in fertilization activity, soil moisture and temperature. This inference is consistent with the conclusion that terrestrial soils and groundwater are responsible for much denitrification at the watershed scale (Seitzinger et al. 2006). Consequently, the suggested denitrification from our results can be viewed as the overall effects of denitrifying processes occurred in the Beijiang River basin with seasonally varying degrees of denitrification. In the δ^{18}O-δ^{15}N plot (Fig. 3), inter-seasonal data are well separated, and intra-seasonal data cluster into each group of three with relatively narrow ranges of δ^{18}O and δ^{15}N, although there were some overlaps between December and August. This pattern indicates that the watershed-scale spatial variance in degree of denitrification in each season was less significant than the seasonal variance. It should be noted that this conclusion does not contradict with the idea of “hot spot” or spatial heterogeneity of denitrification within the watersheds in each season, because isotopic signal integrated watershed-scale nitrogen process that can only give a spatially mean state of the process as evidenced by the narrow δ^{18}O-δ^{15}N ranges for each season (Fig. 3).

As denitrification obviously changed its intensity between seasons in the study area, we are thus quite interested in approximating the temporal pattern of denitrification by our data. The linear correlations of δ^{18}O and δ^{15}N values with the natural logarithm of nitrate concentrations (Fig. 4), respectively, for April and August corroborate the applicability of the classical Rayleigh equation to denitrification process in this study. This equation describes that during denitrification, the isotopic values of the residual nitrate increases in proportion to the logarithm of the residual nitrate fraction, which can be expressed as (Kendall 1998):

$$\delta_R = \delta_{R0} + \varepsilon \ln(f)$$

where δ_R is the δ^{15}N or δ^{18}O value of the reactant nitrate at time t, δ_{R0} is the initial δ^{15}N or δ^{18}O value of the nitrate, f is the remaining fraction of the nitrate, and ε is the enrichment factor. As can been known from Fig. 4, ε values for ^{15}N and ^{18}O in this particular study should be −14.8 and −8.5‰, respectively. This estimation for ε values is quite similar to the widely cited results (−15.9 and −8.0‰ for ^{15}N and ^{18}O, respectively) observed by Böttcher et al. (1990) in an unconfined shallow ground water aquifer in an agricultural setting.

To calculate the degree of denitrification from our data, an initial isotopic composition (δ_{R0}) is needed. Here we simply chose the lowest δ^{15}N value in April, 1.9‰, as the initial value. By using this δ_{R0} value and −14.8‰ for ε, the amount of denitrification for the nitrate in the Beijiang River samples collected in April and August was calculated according to their δ^{15}N values (δ_R). Due to the significant contribution of sewage and manure nitrate in December as discussed above, δ^{15}N values of the intersection between mixing and denitrification lines (Fig. 3) were adopted for winter sample δ_R. The calculated results for degree of denitrification are presented in Table 1. Overall, the average percent of denitrification for nitrate are 9.6% in April, 48.1% in August, and 25.8% in December, respectively. Comparatively, using a similar method, Panno et al. (2006) estimated that 0–55% of the original nitrate has been denitrified in the Upper Mississippi River basin, with most of the samples falling between 10 and 40%. Our result is also similar to those from mass balance approaches used to estimate denitrification in terrestrial soils at regional scales: 40% for Europe (van Egmond et al. 2002), 30% for Asia (Zheng et al. 2002), and 33% for land areas draining to the North Atlantic (Howarth et al. 1996).

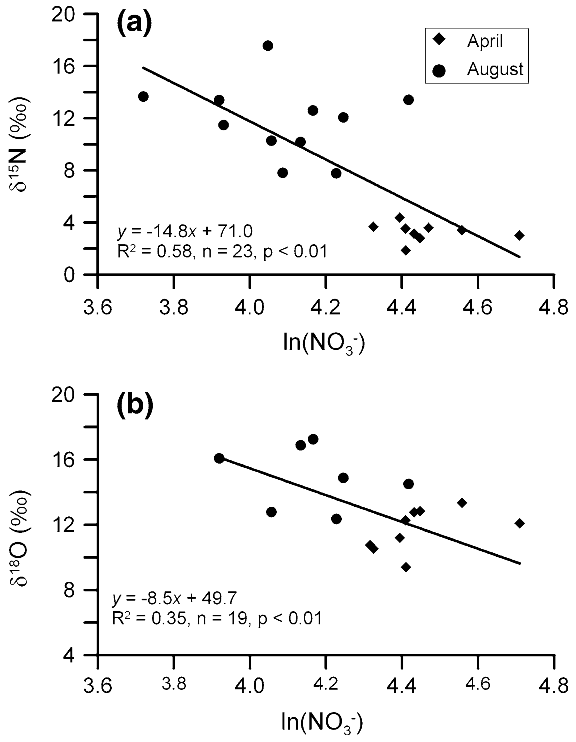

Fig. 4 The natural log of nitrate concentration verses δ^{15}N (**a**) and δ^{18}O (**b**)

Our results on the degree of denitrification indicate a legible seasonal variability of denitrification at the scale of watersheds. In April, low denitrification and high NO_3^-/Cl^- ratios correspond to enhanced fertilization activities and abrupt increase in monsoon precipitation, resulting in increased leaching of nitrate from the soil zone. This would lead to relatively rapid movement of NO_3^- from the soil zone into the river systems, causing less denitrification. The finding of the highest amount of denitrification in August confirms many field-scale investigations revealing that peak denitrification occurs generally in hot summertime (Cooke and White 1987; Christensen and Sorensen 1988; Groffman et al. 1996; Pattinson et al. 1998; Panno et al. 2006). However, the still quite high denitrification in December in this study has not been often observed, perhaps due to that most observations are located in temperate climate zone (Groffman et al. 1996; Ostrom et al. 1998; Panno et al. 2006), where winter temperature is too low to stimulate microbial mediated denitrification process. Whereas subtropical climate could maintain >10°C average in winter for our study watershed, higher than the limitation of 4–6°C for denitrification (Jordan 1989; Ruz-Jerez et al. 1994). Low relative winter temperature would decline the microbial activity, and hence the degree of denitrification, but we also speculate that the low soil moisture, as well as low groundwater table, during the dry wintertime might be more critical to weaken denitrification in December, because dry conditions have been found tending to be aerobic so as to favor the nitrate accumulation other than denitrification (e.g. Panek et al. 2000; Hefting et al. 2004).

Conclusions

The Beijiang River basin is a subtropical agricultural area influenced by monsoon climate. Seasonal changes in precipitation and hydrology play an important role in modulating the river nitrate sources and watershed N processing. In April during the early flood period when fertilizer application begins to be promoted, abrupt increase in monsoon precipitation and runoff caused enhancement of nitrate input leaching from soil and weak denitrification over the watershed. In August during the rainy hot summertime, strong denitrification occurred as indicated by the ∼1:2 increase in $\delta^{18}O$ and $\delta^{15}N$ relative to in April, appearing to denitrify nearly half of the nitrate at the watershed scale. In December during the dry wintertime, high Cl^- and SO_4^{2-} concentrations and low NO_3^-/Cl^- ratios, as well as the slight departure from April to August denitrification vector, suggest significant nitrate input from manure and sewage. Denitrification maintained still strong in December, although not stronger than in August due perhaps mainly to low soil moisture, as winter temperature was high enough for microbial activities. Consequently, our results demonstrate that the dominant controlling factors for watershed-scale N processing are highly variable over time, and that sampling of N species in river water at one point in time is clearly not sufficient to assess origins or the predominant processes occurring in the watershed.

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