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Nitrate sources and processes in the surface water of a tropical reservoir by stable isotopes and mixing model

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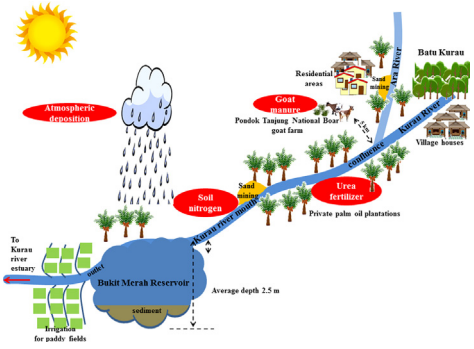
HIGHLIGHTS

- Nitrate dual isotope biplot showed mixing of nitrate sources.
- Nitrate processes in the surface water were derived from nitrification and mixing.
- Bayesian mixing model: highest contribution by manure and sewage.
- More atmospheric deposition contribution in the reservoir than in the river.

GRAPHICAL ABSTRACT



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ABSTRACT

Nitrate is one of the primary nutrients associated with sedimentation and fuels eutrophication in reservoir systems. In this study, water samples from Bukit Merah Reservoir (BMR) were analysed using a combination of water chemistry, water stable isotopes (δ^2H–H_2O and $\delta^{18}O$–H_2O) and nitrate stable isotopes ($\delta^{15}N$–NO_3^- and $\delta^{18}O$–NO_3^-). The objective was to evaluate nitrate sources and processes in BMR, the oldest man-made reservoir in Malaysia. The $\delta^{15}N$–NO_3^- values in the river and reservoir water samples were in the range +0.4 to +14.9‰ while the values of $\delta^{18}O$–NO_3^- were between −0.01 and +39.4‰, respectively. The dual plots of $\delta^{15}N$–NO_3^- and $\delta^{18}O$–NO_3^- reflected mixing sources from atmospheric deposition (AD) input, ammonium in fertilizer/rain, soil nitrogen, and manure and sewage (MS) as the sources of nitrate in the surface water of BMR. Nitrate stable isotopes suggested that BMR undergoes processes such as nitrification and mixing. Denitrification and assimilation were not prevalent in the system. The Bayesian mixing model highlighted the dominance of MS sources in the system while AD contributed more proportion in the reservoir during both seasons than in the river. The use of $\delta^{13}C$, $\delta^{15}N$, and C:N ratios enabled the identification of terrestrial sources of the organic matter in the sediment, enhancing the understanding of sedimentation associated with nutrients previously reported in BMR. Overall, the nitrate sources and processes should be considered in decision-making in the management of the reservoir for irrigation, Arowana fish culture and domestic water supply.

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1. Introduction

Reservoirs are highly dynamic systems, closely linked to the terrestrial ecosystem and flowing water (Ling et al., 2017). An increasing number of rivers have been gradually transformed into reservoirs, driven by expanding human demands for additional water sources for domestic and drinking water supplies, irrigation and agricultural requirements (Thornton et al., 1996; Pinto-Coelho et al., 2010; Padedda et al., 2017; Winton et al., 2019). Any human alterations in the upstream ecosystem and changes in water level may proactively affect the condition of a reservoir (Zorzal-Almeida et al., 2018), resulting in a shorter retention time, which in turn may affect nutrient cycling and phytoplankton production (Hou et al., 2013). Moreover, reservoirs are also vulnerable to accelerated eutrophication and implicated with issues such as habitat fragmentation, alteration of the physical and chemical quality of the released water (Winton et al., 2019) and siltation issues (Pinto-Coelho et al., 2010).

Typically, small or medium-sized reservoirs in Asia are shallow in depth, eutrophic and the major sources of nutrients are allochthonous (Hwang et al., 2002; Hwang et al., 2003; Mamun and An, 2017). Tropical reservoirs are in areas with high temperatures and humidity throughout the year, with cyanobacteria, algae, phytoplankton or floating macrophytes thriving due to the high sunlight exposure in equatorial regions (Dobson and Frid, 2009; Winton et al., 2019). Littoral communities typically dominate shallow reservoirs as the water depth is generally shallow enough to support sufficient underwater light for the growth of submerged macrophytes (Dobson and Frid, 2009) and substantial nutrient exchange may occur between the sediment and water, induced by wind mixing (Ansari et al., 2011; Sharip et al., 2018).

In eutrophic reservoirs, cyanobacterial blooms frequently occur, and the growth of phytoplankton in the reservoir waterbodies is triggered by the presence of nitrogen and phosphorus (Mamun and An, 2017; Moal et al., 2019). Excessive levels of nutrients (N and P) cause eutrophication in reservoirs across the globe, including America (Jacobson et al., 2017), Europe (Padedda et al., 2017) and Asia (Chen et al., 2019). Out of all nitrogen species, nitrate is the dominant form causing eutrophication in aquatic ecosystems, and methemoglobinemia in infants (Kendall et al., 2007). Thereby, it is crucial to identify the sources and transformations of nitrate for effective water quality management.

Quantitative contributions of the nitrate sources can be done using the basic mass balance approach based on two isotopes and three sources using the three-equation model (Moore and Semmens, 2008; Jackson et al., 2009; Parnell et al., 2010). The Bayesian mixing model implemented in the software package SIAR (Stable Isotope Analysis in R) is used to estimate the possible proportional source contribution to a mixture using the Markov chain, Monte Carlo with Metropolis–Hastings and prior distribution of source contribution using the Dirichlet distribution (Jackson et al., 2009). SIAR accounts for uncertainties in tempo-spatial variabilities as well as isotopic fractionations, concentration dependence and large number of sources (Moore and Semmens, 2008; Parnell et al., 2010). Initial applications of this model stemmed from quantifying the food sources in the diet of organisms and has been successfully applied in other environmental applications in aquatic systems (Yue et al., 2015; Matiatos, 2016; Wang et al., 2016; Zhang et al., 2018; Li et al., 2019).

Being the oldest man-made reservoir in Malaysia, beginning operation in 1906, Bukit Merah Reservoir (BMR) has experienced eutrophication as part of the ageing process associated with increasing productivity (Ismail and Najib, 2011; Hasan et al., 2011; Sharip et al., 2014; Zakeyuddin et al., 2016; Najib et al., 2017). Over 60% of nitrate and 30% of nitrite that entered the reservoir are retained in the sediment and most of the nutrients came from the Kurau River, the major inlet to the reservoir (Ismail and Najib, 2011; Talib et al., 2016). Apart from eutrophication, BMR is also experiencing severe sedimentation problems as a result of sediments, silts and organic matter accumulations that are filling up the reservoir (Hidzrami, 2010; Ismail et al., 2010; Hasan et al., 2011; Sharip et al., 2014; Najib et al., 2017). Accelerated productivity in the reservoir is also triggered by existing sand mining activities at the upstream end of the Kurau River, the expansion of agriculture and small-scale farming, and land use alterations from unplanned development activities surrounding the BMR water catchment (Hasan et al., 2011).

In this study, our sampling design involves combining the use of water quality, nutrient concentrations, stable isotope ratios of nitrate, hydrogen and oxygen in water samples and the Bayesian mixing model to identify and quantify the sources of nitrate and N transformation processes in the study area. Furthermore, this information can also provide information about the sources of water and the biogeochemical and ecosystem processes that control productivity and nutrient enrichment. In addition, a combination of $\delta^{13}C$, $\delta^{15}N$ and C:N ratios of the sediments were also concurrently used to differentiate between aquatic and terrestrial derived sources from sediments (Rogers, 2013). Knowing this could enhance our understanding of the sedimentation conditions previously reported that bring the nutrients into BMR (Ismail and Najib, 2011; Talib et al., 2016). It is crucial to assess the current condition of the reservoir to determine its longevity and sustainability, considering its important ecosystem services to both humans and nature. This effort is in line with the United Nations Environment Program sustainable development goals (UNEP, 2016) covering clean water and sanitation and life below water.

BMR is an interesting case because its N is derived primarily from diffuse sources such as atmospheric deposition, soil runoff, sedimentation and land use changes from unplanned development, conversion of forest into oil palm plantations, and mixed small-scale farming (Ismail and Najib, 2011; Zakeyuddin et al., 2016; Najib et al., 2017). Additionally, it is located in a tropical monsoon climate where aquatic biogeochemistry has not been as well studied as in temperate regions and its flow regime has been altered by humans for both irrigation and drinking water supply (Hidzrami, 2010; Hasan et al., 2011; Ismail and Najib, 2011; Sharip et al., 2014; Talib et al., 2016).

To our knowledge, the application of nitrate dual-stable isotopes in a tropical shallow man-made reservoir has not been documented. Thus, the objective of this study was to determine the variation of nitrate stable isotopes, to evaluate the sources of N in BMR and to quantify the contribution of the local sources from the study area using the Bayesian mixing model. This study is a first attempt to incorporate the stable isotopes monitoring tool that has the potential to greatly enhance our ability to manage our reservoir ecosystem.

2. Material and methods

2.1. Study area and sampling stations

BMR was built in 1902 in Perak, the northern state of Peninsular Malaysia, with a capacity of 70 million m^3 (Hidzrami, 2010). The reservoir has been divided into two sections; the northern part and the southern part, by a railway-line with a distance of 4.7 km. The length of the reservoir is 13.8 km, the width 4.5 km and the residence time is 33 days. The average depth is 2.5 m, which classifies BMR as a shallow reservoir with well-mixed waters (Sharip et al., 2018). BMR was built for the Kerian Irrigation Scheme to provide the water supply for the irrigation for double

cropping of about 24,000 ha of paddy fields in the Kerian district and also to provide a domestic water supply to the population in the Kerian and Larut Matang districts (Ismail and Najib, 2011). BMR is multipurpose in that it also acts as a tool for flood and drought control, serves as a tourism spot through the water theme park (Bukit Merah Reservoir Laketown Resort) which was built in the 1990s and is the water source used for Arowana fish culture in the downstream area after the outlet from BMR (Zakeyuddin et al., 2014). Major land use in this catchment (53.5%) covering Kerian, Larut Matang and Selama district is forest (264.4 km^2). Oil palm plantations covered 23.77% of the area (117.28 km^2) while 7.7% is covered by rubber plantations (37.92 km^2). The other land uses are fruit orchards (1.63 km^2), paddy (15.55 km^2), mixed agriculture (1.55 km^2), and chicken and goat farming (10.21 km^2) (JPBD, 2017). Based on the land use data (Fig. 1), there are also small and medium scale industries, including oil palm mill and wood or rubber manufacturing industries (0.51 km^2). Residential areas comprising mainly of traditional Malay houses covered 45.49 km^2 of the area (9.2%). Groundwater contribution was not included in the study because the locals in the area mainly used surface water as the source of domestic water supply.

2.2. Experimental design of sampling sites

We have selected ten sampling sites as a boundary that we set focusing on N sources and processes from the upstream of the main river, Kurau River system which flows to the inlet and the outlet of the reservoir. The largest water sources into the reservoir, Kurau River system (83.31 km^2) feeds into the southern part of the reservoir while Jelutong River (7.1 km^2), which connects with Merah River (4.25 km^2) feeds to the northern part of the reservoir (Ismail and Najib, 2011). Our study did not cover Jelutong and Merah River and the northern part of the reservoir since we want to focus on the effect of the main river system, Kurau River into the reservoir. Our selection was also based upon previous studies which reported the unplanned development and sedimentation originated from Kurau River (Ismail and Najib, 2011; Najib et al., 2017). The upstream catchment of the reservoir is located at Batu Kurau (St.1), which drains to the Kurau River (St.2). The Kurau River then flows through the confluence (St. 3) with another river, Ara River and the Kurau River mouth (St. 4) before it flows into the reservoir. The main inlet into the southern part of BMR is through the Kurau River mouth (St. 4), while the outlet of the water occurs through the Selinsing Dam (St. 9) and the Watergate (St. 7). For every sampling expedition, a total of four sampling sites were selected for the river, labelled as Batu Kurau (St. 1), Kurau River (St. 2), Confluence (St. 3) and Kurau River mouth (St. 4) while another six sampling sites were selected within the reservoir (railway, midpoint 1, Watergate, midpoint 2, Selinsing Dam, and Orang Utan Island, labelled as St. 5–10, respectively). St. 11 is the location for goat manure collection. We have designed our experiments to cover horizontal water sampling using a grab sampling technique. Details of the sampling sites and other descriptions are provided in Table S1.

2.3. Water quality, nitrogen and chlorophyll a concentration

Physico-chemical parameters (temperature, pH, dissolved oxygen (DO), total dissolved solids (TDS), electrical conductivity (EC)) were obtained *in situ* with the use of a YSI 556 MPS multiprobe meter (USA). The water samples were collected within 10–30 cm of the water surface of the river and the reservoir in August

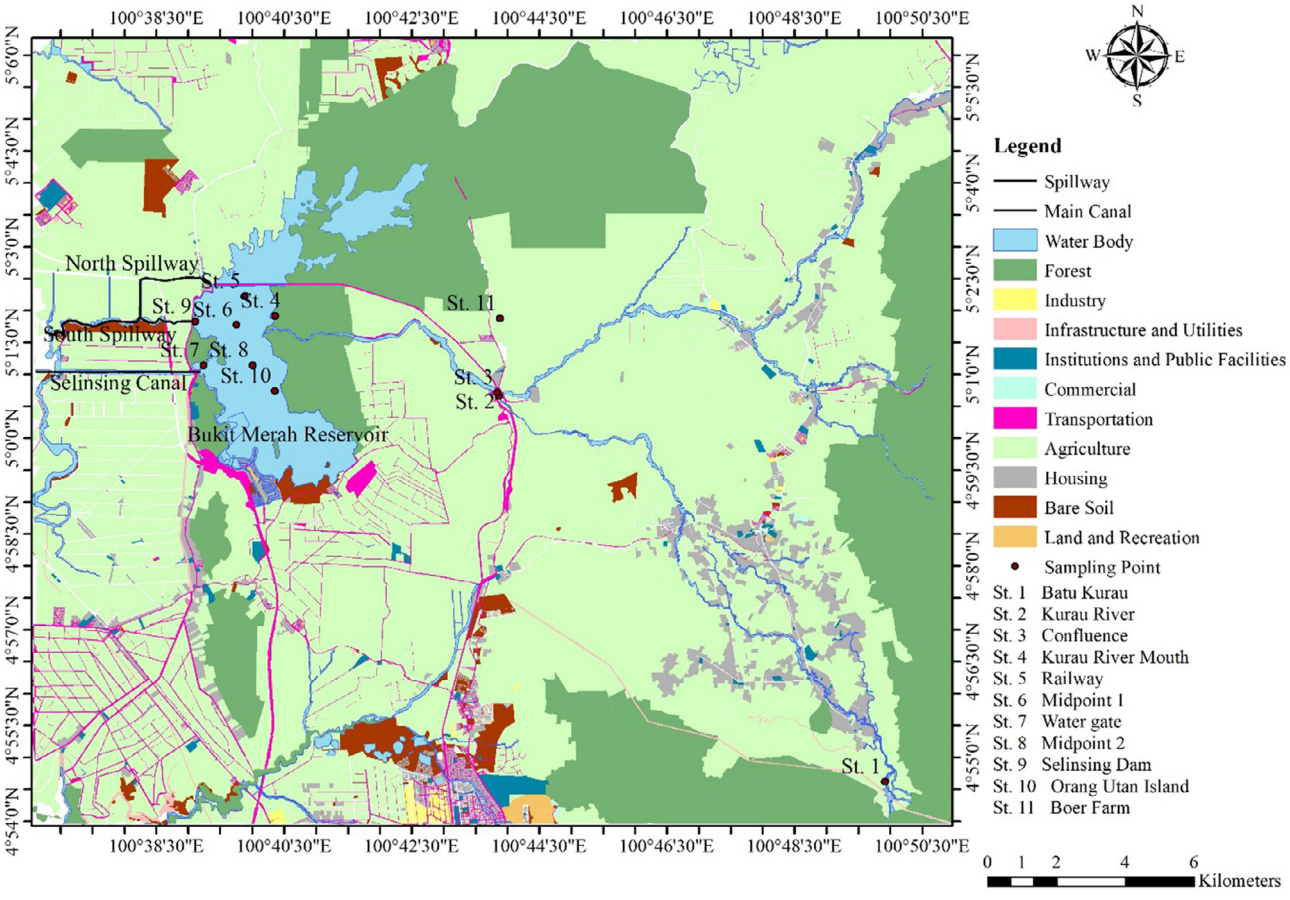

Fig. 1. Map of the sampling sites in the study area.

2016 and March 2017 (dry seasons) and Dec 2016 and October 2017 (wet seasons). After collection, the samples were kept below 4 °C to prevent significant degradation during storage. Only surface water was sampled because the reservoir is well-mixed, influenced by wind-driven motion. This wind-induced factor consistently mixed the water column between the surface and the bottom water (Sharip et al., 2018). The depth of the reservoir during the wet season was 2.8 ± 0.5 m and 2.5 ± 0.5 m in the dry season (N = 12). The shallowness of the reservoir water, and wind mixing result in no vertical stratification of the water during the study period.

All the analyses were conducted according to standard methods (ISONITRATE, 2009; HACH, 2015). Inorganic nitrogen concentrations (NO_3-N, NO_2-N and NH_3-N) and phosphate were analysed using a HACH DR 2800 spectrophotometer.

The chlorophyll *a* analysis was conducted following the method by Adams (1990). A volume of 250 mL of water samples was filtered with 0.45 μm cellulose nitrate membrane filters and added with 2.0 mL $MgCO_3$ during the final phase of the filtering process. The filter paper used was placed in a homogeniser glass and a volume of 2 to 3 mL of 90% acetone was added for the grinding process. It was ground for 1 min at 500 rpm to separate the chlorophyll pigment from the filter paper. The extraction was transferred into a centrifuge tube and allowed to stand in the dark for 10 min followed by centrifuging for 15 min under 4000 rpm. The supernatant was poured into a cuvette and the absorbance was read for the wavelength of 750 nm, 664 nm, 647 nm and 630 nm, respectively.

2.4. Total nitrogen (TN) and total phosphorus (TP) concentrations in the water samples

TN analysis (0–64 mg/L N) was determined using the Persulfate Digestion Method of Test N Tube™ vials (Method 10072) while TP (0.06–3.50 mg/L) was determined using PhosVer3 with the Acid Persulfate Digestion Method with Test 'N Tube™ Vials (Method 8190). Samples were analysed using a HACH DR2800 spectrophotometer and COD reactor. The difference between total nitrogen (TN) and dissolved inorganic nitrogen (DIN) (NO_3^--N, NO_2^--N, and NH_4^+-N) was used to determine dissolved organic nitrogen (DON). Similarly, the difference between total phosphorus (TP) and PO_4^{3-} was determined as dissolved organic phosphate (DOP).

2.5. Nitrate and water stable isotope analyses in water samples and isotopic calculation

Fieldwork collection and sample preparation adopted the standard guidelines by IAEA CRP F32007 and ISONITRATE manual. The water samples for $\delta^{15}N–NO_3^-$ and $\delta^{18}O–NO_3^-$ were collected and filtered *in situ* with a 0.22 μm PES Sartorius syringe filter, stored in acid-cleaned HDPE bottles and shipped to States Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences. The $\delta^{15}N–NO_3^-$ and $\delta^{18}O–NO_3^-$ of each water sample were analysed using the bacterial denitrification method (Sigman et al., 2001a,b; Casciotti et al. 2002; McIlvin and Casciotti 2011; Yue et al., 2014; Li et al., 2019). First, the bacterial strains, *Pseudomonas chlororaphis* subsp. *aureofaciens ATCC 13985* were cultured and grown on tryptic soy broth (TSB) for 7 days. The cells were concentrated by 5-fold, which was then split into 3 mL aliquots of 20 mL headspace vials. Once all the vials were subsequently sealed and capped, they were purged for at least 3 h with high-purity N_2 gas to ensure anaerobic conditions. After that, the water samples were injected into the sample vials with a volume equivalent to 20–50 nmol of NO_3^- to allow complete conversion of NO_3^- to nitrous oxide (N_2O). They were cultured overnight and the sample vials were injected with 0.1–0.2 mL of 10 mol/L NaOH the next day to remove any CO_2 gas and to stop any microbial activity (Casciotti et al., 2002).

The cultured denitrifier, *P. aureofaciens* was used for transformation of NO_3^- to N_2O because this denitrifier lacks the N_2O reductase enzyme (an enzyme that reduces N_2O to N_2). This characteristic allows conversion of NO_3^- and NO_2^- into N_2O but not to N_2. Hence, it can be used for simultaneous $\delta^{15}N$ and $\delta^{18}O$ analyses. The $\delta^{15}N$ and $\delta^{18}O$ of N_2O produced were analysed using a Trace Gas Pre-concentrator unit (Isoprime Ltd., Cheadle Hulme, Cheadle, UK) coupled to an isotope ratio mass spectrometer (IsoPrime, GV, UK). Four international standards were used; IAEA-N3 (4.7 ± 0.3‰, 25.6 ± 0.5‰), USGS-32 (180 ± 0.5‰, 25.7 ± 0.4‰), USGS-34 (−1.8 ± 0.2‰, −27.9 ± 0.4‰) and USGS-35 (2.7 ± 0.3‰, 57.5 ± 0.4‰) to calibrate the measured sample data after blank correction as proposed by Casciotti et al. (2002). All experimental reference materials were treated in an identical manner as the filtered water samples. Each sample was measured in duplicate and the standard error was 0.5‰ for $\delta^{15}N–NO_3^-$ and 1‰ for $\delta^{18}O–NO_3^-$. The standard error was high for $\delta^{18}O–NO_3^-$ due to larger volumes of water and lower concentration of bacteria were available than the normal water samples. Despite this issue, good standard calibration linearities were achieved ($R^2 = 0.9954$ for $\delta^{15}N$ and $R^2 = 0.9999$ for $\delta^{18}O$) at low nitrate-N amount (5 nmol), which deemed the data acceptable.

Water stable isotope analyses, $\delta^{18}O–H_2O$ and $\delta^{2}H–H_2O$, were measured using a Picarro L2140-I isotope water analyser at the Institute of the Surface-Earth System Science Research, Tianjin University, China. About 200 μL of surface water sample, rainwater sample or standard reference water was loaded into 300 μL vials and placed in a PAL autosampler tray. The δ^2H and $\delta^{18}O$ were measured six times for each vial from a 2 μL water injection, but the final value was the average of last four measurements to ensure between-sample memory effect was corrected. The recommended procedure to minimise between-sample memory is to ignore the first 3–4 results out of 8 injections per sample vial (Wassenaar et al., 2014). For calibration purposes and to overcome drift, three standards were measured for every seven samples (ranging from −96.4 to −9.5‰ for δ^2H and from −13.1 to −2.8‰ for $\delta^{18}O_{water}$). Placement of a maximum of 5–10 samples was recommended between groupings of water standards to minimise drift effects (Wassenaar et al., 2014). The $\delta^2H–H_2O$ and $\delta^{18}O–H_2O$ analyses had a precision of 1‰ and 0.2‰, respectively. All stable isotope ratios were expressed in per mil (‰) deviations as follows:

$$(‰) = \left(\left(R_{sample}/R_{standard}\right) - 1\right) \quad (1)$$

where, R_{sample} and $R_{standard}$ are $^{15}N/^{14}N$ or $^{18}O/^{16}O$ or $^{18}O/^{16}O$ or $^{2}H/^{1}H$ ratios of the samples and standards, respectively. The ratio of $^{15}N/^{14}N$ reference is atmospheric N (AIR) while values of $^{2}H/^{1}H$ and $^{18}O/^{16}O$ reference are Vienna Standard Mean Ocean Water (VSMOW).

2.6. Theoretical nitrification calculation

During nitrification process (NH_4^+ oxidised to NO_3^-) in surface water, the $\delta^{18}O\text{-}NO_3$ is a function of the amount of oxygen coming from the water molecule in nitrate (Andersson and Hooper, 1983; Xu et al., 2016). Hence, there is a relation between $\delta^{18}O\text{-}NO_3$ with the isotopic composition of the water ($\delta^{18}O\text{-}H_2O$). The variation range of ^{18}O offers useful information to identify nitrates derived from microbial nitrification as the mechanism is mediated by several different kinds of autotrophic bacteria (Kendall, 1998; Xu et al., 2016). The $\delta^{18}O$ value for nitrate formed through nitrification is expected to range between −5 and +15‰, including the soil nitrogen and ammonium from sewage and manure (Kendall

et al., 2007). In theory, the $\delta^{18}O$–NO_3 can be interpreted as a mixture of two oxygen atoms from H_2O and one oxygen atom from atmospheric O_2 (Andersson and Hooper, 1983; Böttcher et al., 1990; Kendall 1998). $\delta^{18}O$–O_2 value was assumed to be O atoms of ambient O_2; therefore the $\delta^{18}O$ of the atmosphere (+23.5‰) was used (Böttcher et al., 1990; Yue et al., 2014; Wang et al., 2016). Thus, the expected $\delta^{18}O$ value of NO_3^- can be calculated as follows:

$$\delta^{18}O - NO_3 = 2/3(\delta^{18}O - H_2O) + 1/3(\delta^{18}O - O_2) \quad (2)$$

where, $\delta^{18}O$–H_2O and $\delta^{18}O$–O_2 were assumed to be O atoms of ambient H_2O and O_2, respectively.

2.7. Bayesian mixing model

The Bayesian SIAR model was used to estimate the proportional contribution of the NO_3-N sources in aquatic systems following the method in Parnell et al. (2010, 2013). To estimate the contributions of different NO_3^- sources, nitrate is assumed to be sourced from atmospheric deposition (AD), soil nitrogen (SN), synthetic nitrogen fertilizer (SNF), and manure and sewage (MS). Nitrate fertilizer (NF) was not considered a source due to its unlikely use by locals. Isotopic values of NO_3^--N source end members were defined through field collection of these sources within the study area. After collection, all samples were put in an icebox for transportation to the laboratory. End member isotopic compositions of AD were collected from the surface of the reservoir during the sampling campaign in 2017 (March and Oct 2017; N = 5, $\delta^{15}N$-NO_3^- = −0.44 ± 1.69‰, $\delta^{18}O$-NO_3^- = +60.86 ± 2.89‰).

The samples of AD were collected using a tube dip-in water collector with pressure equilibration. This approach eliminates the need for paraffin oil (IAEA, 2014). This dip-in sampler consists of a tube leading from the funnel to the bottom of the sample accumulation bottle (>10 L high density polyethylene (HDPE) bottle). The funnel was closed with a ping pong ball to seal the collector bottle against evaporation and debris. When rainfall accumulates, the ball floats and opens the funnel. After the rain event, the ball returns to its original position (IAEA, 2014). During the collection period, the AD samples were stored in tightly capped HDPE bottles. AD samples from the accumulation bottle were transferred to 30 mL HDPE bottles with inlay caps for shipment. AD samples were shipped to Tianjin University, China for water stable isotope analyses using the same method as surface water samples as described in Section 2.5. AD samples for nitrate dual-stable isotopes, $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- were shipped to State Key Laboratory of Environmental Geochemistry, Chinese Academy of Sciences, Guiyang China and analysed using the same method as surface water samples described under Section 2.5.

Approximately 1 kg of composite soil samples (SN) pooled from three randomly selected points in the forested area of St. 1 in Batu Kurau was collected using a plastic scoop within 10 cm of the soil surface. The soil samples were prepared based on the procedure outlined by Rock et al. (2011). The soil samples were oven-dried for 24 h at 80 °C until the samples were considered fully dried and ground by using mortar and pestle. About 25 mL of 2 M potassium chloride (KCl) was added into 5 g of soil samples in a 100 mL beaker. This mixture was placed horizontally on a reciprocation shaker for 70 reciprocations per minute for 1 h (Rock et al., 2011). The soil-KCl suspension was then filtered using the Whatman filter paper.

SNF samples (urea fertilizer) were provided by local suppliers while goat manure (MS) samples were collected from the national Boer goat farm, which is located 5 km away from the confluence within the Pondok Tanjung area (St. 11 in Fig. 1). Both samples were prepared based on Heaton et al. (2012) method. SNFs were obtained from a freshly opened bag and both samples were ground into powder using a mortar and pestle. The samples were oven-dried at 80 °C for 24 h and slurred with deionised water on a shaker table and later filtered using Whatman filter paper.

SN, SNF and MS samples were sent to International Atomic Energy Agency, Vienna for $\delta^{15}N$-TN analysis. Mineralisation and subsequent nitrification of these end members usually produce NO_3^- which retains (within a few per mil) the N isotope of the δ15N-TN (Kendall et al., 2007). The $\delta^{18}O$ values of NO_3^- nitrified from SN, SNF and MS were calculated using the isotopic composition of water and O_2 (see Section 2.6) since the $\delta^{18}O$-NO_3^- reflects the $\delta^{18}O$ of the H_2O after the interaction of the source with the water molecules. Denitrification was not the prominent process in the surface water in the study area. This assumption was based on DO and isotopic plots, which we have explained in the Results and Discussion section (Section 3). To minimise uncertainty, the isotopic composition of each potential source used the local sources collected within the study area. However, since only one sample was obtained for SNF and MS sources, therefore we have also incorporated values from the literature as shown in Table S3 to consider the mean and standard deviation of data distribution for each source. The study area was also divided based on seasons (wet and dry) and the system (river and reservoir) to determine the sources. The model was run for 200 000 iterations, a burn in of 50 000 and a thinning of 15. The SIAR model was expressed as follows:

$$\begin{aligned} X_{ij} &= \Sigma P_k(S_{jk} + C_{jk}) + \varepsilon_{jk} \\ S_{jk} &\left(N \sim \mu_{jk}; \omega_{jk}^2\right) \\ c_{jk} &\left(N \sim \lambda_{jk}; \tau_{jk}^2\right) \\ \varepsilon_{ij} &\left(N\ 0; \sigma_j^2\right) \end{aligned} \quad (3)$$

where X_{ij} is the isotope value j of the mixture i, in which i = 1, 2, 3, N and j = 1, 2, 3, J; S_{jk} is the source value k on the isotope j (k = 1, 2, 3, K) and is normally distributed with mean μ_{jk} and standard deviation $\omega^2_{j,k;}$ P_k is the proportion of source k, which needs to be estimated by the SIAR model; C_{jk} is the fractionation factor for isotope j on source k and is normally distributed with mean λ_{jk} and standard deviation τ^2_{jk}; and ε_{jk} is the residual error representing the additional unquantified variation between individual mixtures and is normally distributed with mean 0 and standard deviation σ^2 (Jackson et al., 2009; Parnell et al., 2010; Wang et al., 2016).

2.8. Sediment $\delta^{13}C$, $\delta^{15}N$ and C:N ratio procedures

Surface sediment samples were collected using plastic scoops in the river and an Ekman grab sampler in the reservoir. Sediments were collected at the same sites as the water samples (Fig. 1), but only five out of ten data sets were presented in the Results and Discussion (Section 3) due to non-detected TN values in some of the samples. The sediment samples analysed for $\delta^{13}C$ were acid fumigated following the steps by Harris et al. (2001). Prior to stable isotope analysis, ~2.0 mg of a sample was weighed into small tin capsules (8 × 5 mm) in duplicates. These samples were then folded and compressed before being loaded into an auto-sampler for the analysis of stable carbon and nitrogen isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) using Flash 2000 elemental analyser (ThermoScientific, Waltham, MA) coupled to a Delta V Advantage isotope ratio mass spectrometer (Thermo, Milan, Italy) at the Analytical Biochemistry Research Centre (ABrC), University Sains Malaysia. Raw isotope ratios from the analysis were then normalised to the international scales using USGS-40 ($\delta^{13}C$ = −26.39‰ and $\delta^{15}N$ = −4.52‰), USGS-41 ($\delta^{13}C$ = 37.63‰ and $\delta^{15}N$ = 47.57‰) and urea with known iso-

tope values of $\delta^{13}C = -37.32‰$ and $\delta^{15}N = -0.45‰$ (IVA-Analysentechnik GmbH & Co., Germany) to make up a three-point calibration line for normalisation. All the certified reference materials were weighed at about 0.5 mg each and were assayed with the unknown samples in a batch run. Casein was used as a quality control material to correct for drift and was measured for every 12 samples with known values of $\delta^{13}C = -20.36‰$ and $\delta^{15}N = 5.83‰$.

The typical precision for the duplicated samples was ± 0.3‰ for $\delta^{13}C$ and ±0.2‰ for $\delta^{15}N$. Variations in stable isotope ratios were reported as parts per thousand (‰) deviations from internationally accepted standards which are Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric nitrogen (AIR) for nitrogen, in the delta (δ) notation.

The carbon/nitrogen (C:N) ratios were determined from the calculation of molar ratios of total organic C and total N. A Perkin Elmer 2400 Series II CHN Elemental Analyzer instrument was used to determine the abundance of carbon and nitrogen concentrations in the sediment samples.

2.9. Statistical analysis

Normality test was conducted using skewness, kurtosis, Kolmogorov-Smirnov, Shapiro Wilk test, histogram and Q-Q plots using SPSS v24.0 and GraphPad Prism 8. Majority of the data were normally distributed; therefore, we have used the parametric tests for statistical analysis. Comparisons of water quality and nitrogen concentrations between the seasons (dry and wet) and the systems (river and reservoir) were conducted using paired *T*-test because the experimental design included the use of the same sites for each period of sampling. Pearson's correlation analysis was performed to determine the degree of association between water quality variables and nutrient concentrations in the river and reservoir during each season.

3. Results and discussion

3.1. Water quality and nitrogen concentrations in the river and reservoir during dry and wet seasons

The water quality and nitrogen concentrations of the river and reservoir water samples in the study area are summarised in Table S2. The NO_3-N concentrations at all sampling sites ranged from 0.01 to 0.49 mg/L with an average of 0.13 ± 0.10 mg/L in the dry season (August 2016 and October 2017) and 0.17 ± 0.12 mg/L in the wet season (December 2016 and March 2017). No significant difference was detected between the two seasons (Paired *T*-test, N = 39, $p > 0.05$; Fig. S1). NO_3–N concentrations were found to be higher in the river (0.21 ± 0.04 mg/L) than in the reservoir (0.10 ± 0.08 mg/L) but the concentrations were well below the limit set for class IIA under the National Water Quality Standards for Malaysia (NWQSM), 7 mg/L suitable for use as water supply requiring conventional treatment. The NO_3-N concentrations were also well below class IV of the NWQSM, which is suitable for irrigation. Therefore, the NO_3-N concentrations indicated that the source of water flowing from the river to the reservoir was suitable to be used for domestic water supply and irrigation at the downstream part, which fits the main purpose of the creation of BMR. The other nitrogen contents, NO_2-N, NH_3-N and TN were not significantly different between seasons and between the river and reservoir (Paired *T*-test, N = 39, $p > 0.05$).

Meanwhile, Chlorophyll *a* (Chl *a*) was significantly different between seasons (Paired *T*-test; $t(9) = 3.332$, $p = 0.0088$). Chl *a* concentration in the river was the lowest during the dry season (N = 8, 1.07 ± 1.6 μg/L) while the highest chl *a* was detected in the wet season in the reservoir (N = 12, 20.30 ± 13.18 μg/L). This pattern suggests that the dry season in the river has the lowest phytoplankton biomass whilst the highest was detected during the wet season. Our finding is consistent with a previous study reporting that Chlorophyta was more abundant in the wet season compared to the dry season in BMR (Sharip and Yusoff, 2017). The water temperature was 26.90 ± 2.72 °C from the dry seasons in the river and 26.50 ± 1.99 °C during the wet seasons in the river. Meanwhile, the water temperature was 30.71 ± 0.91 °C in the reservoir during the dry season and 30.73 ± 1.22 °C in the wet season (Fig. S4c). The temperature was higher in the reservoir during both seasons compared to the lower temperatures in the river during both seasons, which potentially affect the growing regime of phytoplankton. The difference in temperature between river and reservoir could be related to the larger surface area of the reservoir (20.69 km^2) compared to the river (1.98 km^2) which permits direct sunlight to the reservoir.

The Chl *a* concentration in this study during the dry season in the river (N = 8, 1.07 ± 1.6 μg/L), wet season in the river (N = 8, 9.97 ± 9.96 μg/L), dry season in the reservoir (N = 11, 10.43 ± 2.76 μg/L) and wet season in the reservoir (N = 12, 20.30 ± 13.18 μg/L) were used as proxy indicators of algae (Mamun and An, 2017). Our previous publication has used the trophic state index (TSI) (Carlson, 1977) to determine the eutrophic condition of BMR (Yaccob et al., 2017). The TSI value for Chl *a* in BMR was within the range of 14.8–68.16 μg/L, which falls under eutrophic condition (Carlson, 1977). This condition is characterised by the presence of algal scums and macrophyte, and dominated by the blue-green algae in the waterbodies (Carlson, 1977). In BMR, the surface waterbodies were covered by macrophytes, *Hanguana malayana* (Bakong) near to St. 4 and St. 10 during our sampling expedition (Table S2) and was reported covering certain parts of BMR by previous studies (Hasan et al., 2011; Najib et al., 2017). Our findings were also in agreement with other previous studies by Najib et al. (2017) and Sharip and Yusoff (2017), which have reported the eutrophic status of BMR. Cyanobacterial bloom was also reported to possibly affecting the reservoir in the dry season (Sharip and Yusoff, 2017). However, based on observation, the appearance of the water was brownish instead of greenish, the green masked by the sediments (Sharip and Yusoff, 2017).

pH was significantly different between seasons (Paired *T*-test, N = 39, $t(9) = 9.548$, $p < 0.0001$). DO was also significantly different between seasons (Paired *T*-test, N = 39, $t(9) = 6.767$, $p < 0.0001$). pH and DO play an important role in determining the nitrogen species (Libes, 2009). DO was negatively correlated with NO_3-N in the river (N = 16, $r = -0.534$, $p < 0.05$). In the dry season, other nitrogen species, NO_2-N and NH_3-N, were also correlated with pH (N = 19, $r = 0.656$, $p < 0.05$ and $r = 0.601$, respectively). Meanwhile, in the wet season, NO_2-N was negatively correlated with DO, whereas NH_3-N was negatively correlated with DO (N = 19, $r = -0.588$, $p < 0.05$) and pH ($r = -0.509$, $p < 0.05$). In low oxygen conditions, the reduced forms of nitrogen, NO_2-N and NH_3-N, dominate (Galloway et al., 2004).

3.2. Total nitrogen (TN), dissolved inorganic nitrogen (DIN) and dissolved organic nitrogen (DON) ratios in the river and reservoir during wet and dry seasons

TN is the sum of DIN (NO_3-N, NO_2-N and NH_4-N) and organic nitrogen. The TN in surface water ranged between 1 and 64 mg/L, with an average of 19.40 ± 22.55 mg/L in the wet season (Fig. S1d and Table S2). NO_3-N occupied the largest proportion of TIN (total inorganic nitrogen) in both dry and wet seasons in the river and reservoir except for during the dry seasons in the reservoir (Table 1). NO_2-N is easily oxidised to NO_3-N due to the fast conversion by nitrobacteria, leaving low NO_2-N concentrations (Wang

Table 1
Ratios between NO_3-N, NO_2-N, NH_3-N, DIN, DON, TN, PO_4^{3-}, DOP and TP in dry and wet seasons in the river and reservoir.

	NO_3-N/TN (%)	NO_2-N/TN (%)	NH_3-N/TN (%)	DIN/TN (%)	DON/TN (%)	PO_4^{3-}/TP (%)	DOP/TP (%)
Dry Season- River	4.47	0.055	2.73	7.26	92.74	17.69	82.31
Dry season- Reservoir	0.35	0.009	0.9	1.26	98.74	40.48	59.52
Wet season-River	1.19	0.018	0.44	1.65	98.35	12.16	87.84
Wet season-Reservoir	0.69	0.009	0.59	1.29	98.71	29.6	70.4

et al., 2016). Therefore, the proportion of nitrite in TIN was the lowest, accounting for <0.055%. NH_3-N was found to be a high proportion of TIN in the reservoir during the dry seasons in the reservoir compared to NO_3-N. Lower concentrations of NH_3-N during the wet season could possibly be due to the dilution effect of rainfall. Our findings are in parallel with a eutrophic tropical reservoir in Brazil (Dellamano-Oliveira et al., 2008) showing lower nitrogen concentrations during wet seasons.

DIN only occupied up to 7.26% whereas DON largely occupied BMR to about 98.74% (Table 1). This finding is in parallel with the results of experimental studies showing that DON may constitute up to >85% TN in river and lake waters, with an average of 40–50% TN (Kroeger et al., 2006). This DON will flow out of the reservoir (BMR) through the water gate to the irrigation canal for paddy plantations and eventually to the Kurau estuary and the coastal zone. One plausible effect of the high TN dominated by DON in BMR is the probability of causing coastal eutrophication as DON is a bioavailable form of N and can be used by phytoplankton which may contribute to the growth of primary producers as shown from bioassay studies (Bronk et al., 2007). High concentrations of DON in surface waters of both rivers and reservoirs in dry and wet seasons could be related to the humus-rich black soil and the sandy soil which are carried by the Kurau River and are deposited into the reservoir (BMR). In rivers, the majority of DON is derived from terrestrial leaching and run-off and consists mainly of humic substances (Bronk et al., 2007).

3.3. TP and phosphate concentrations in the river and reservoir during wet and dry seasons

TP in this study ranged between 0.06 and 2.04 mg/L (N = 30, mean = 0.33 ± 0.46 mg/L; Table 1). No significant differences were found between TP in the dry season and the wet season, and between the river and reservoir water (Paired *T*-test, $p > 0.05$, Fig. S3). TP values were within the range reported from the previous study by Talib et al., (2016). A study by Oliveira et al. (2014) also reported high TP concentrations, exceeding 0.1 mg/L, in both wet and dry seasons in the tropical eutrophic Apipucos reservoir in Brazil. A total of 11.32 tonnes of TP (60.3% of the total TP load input) was recorded from the Kurau River in 2008–2009 (Najib et al., 2017) and phosphate were associated with sediment input from the Kurau River (Ismail and Najib, 2011). Major land use changes associated with uncontrolled development and land activities were reported by Hasan et al. (2011), from the year 1965 to 1998, and these changes affected BMR throughout the years. Out of the different types of phosphorus, DOP clearly dominates the system in both wet and dry seasons (Table 1) due to the cumulative effects of the sedimentation inputs which flowed from the river into the reservoir. In wet seasons, this input can be explained by the allochthonous nutrients carried by the rain.

3.4. Source analyses of sediment organic matter $\delta^{13}C$, $\delta^{15}N$ and C:N ratio in sediment samples

The C:N ratio was calculated from only five samples (due to non-detected TN values) from the river sediments (Batu Kurau) and another four from samples from the sediments within the reservoir (Table S4). Nevertheless, from these five representative samples, the values of $\delta^{13}C$ and the C:N ratios fell within the C3 terrestrial plants derived ($\delta^{13}C$ of −32 to −22‰) and C:N ratio of +8 to >+25‰ (Rogers, 2013). Algae is highly likely to be an important source to the sediment organic matter because of the shallow and eutrophic condition of BMR. But, the range of C:N ratio from the sediments in the reservoir (15.73–25.67), suggested that the sediments mainly consisted of C3 terrestrial plants. Algae sources are within the range of 5 to 8 (Rogers, 2013), which was lower than the C:N ratio of the sediments in BMR. The highest C:N ratio (43.17) was observed at Batu Kurau, a forested area, indicating that organic matter in the aquatic system derived mostly from land plants (Meyers, 2003). The C:N ratios of terrestrial plants are in the ranges 175–400 for wood, 20–50 for tree leaves, and 25–80 for grass and herbaceous plants (Hedges et al., 1986). Although only five representative samples were analysed, our results point towards grass and leaves of terrestrial plants as the sources imprinting the sediments within BMR. BMR is surrounded by riparian vegetation, Bakong (*Hanguana malayana*), forests and palm oil plantations, so these seem likely sources of organic matter to the sediments.

3.5. Isotopic composition of water

The $\delta^{18}O$–H_2O and $\delta^{2}H$–H_2O values of surface water samples from the river and the reservoir showed isotopic values ranging from −7.48 to −5.4‰ (N = 30, mean = −6.43‰) and from −44.67 to −30.23‰ (N = 30, mean = −37.69‰), respectively. The $\delta^{18}O$–H_2O in rainwater samples ranged from −10.72 to +1.14‰ (N = 6, average = −5.28‰) while the $\delta^{2}H$–H_2O ranged from −73.13 to +4.61‰ (N = 6, average = −28.79‰). The $\delta^{18}O$–H_2O and $\delta^{2}H$–H_2O values from river water, reservoir water, and rainwater are plotted in Fig. 2 along with the Local Meteoric Water Line (LMWL) obtained as a courtesy of IAEA's GNIP project. The LMWL of $\delta^{18}O$–H_2O and $\delta^{2}H$–H_2O in river water, $\delta^{2}H = 6.47x + 4.74$, $R^2 = 0.796$, in reservoir water ($\delta^{2}H = 6.61x + 4.30$, $R^2 = 0.812$) and in rainwater ($\delta^{2}H = 8.15x + 14.23$, $R^2 = 0.996$) were compared with the LMWL for Malaysia with $\delta^{2}H = 7.43x + 8.14$, $R^2 = 0.895$ (Fig. 2). As shown in Fig. 2, the majority of the isotopic values of water from the river, reservoir and rainwater agreed well with the LMWL, suggesting that the source of water in the area was primarily derived from rain and there was noticeable evaporation of water in the water catchment (Wang et al., 2015).

$\delta^{18}O$–H_2O appears to be more enriched in the reservoir during the wet seasons than in the river in both seasons and in the reservoir in the dry season. Significant variability in the water isotope values was found between seasons (Paired *T*-test; $t(9) = 7.112$, $p < 0.0001$; Fig. S4a). This factor corresponds to higher rainfall amounts during the wet season (672.5 mm), much higher than the dry season (195 mm). No significant differences in $\delta^{18}O$–H_2O were found between river water and reservoir water (N = 20, $p > 0.05$).

Similar patterns were observed for the $\delta^{2}H$-H_2O in the river and reservoir during both wet and dry seasons (Fig. S4b). Significant differences were found in $\delta^{2}H$-H_2O between seasons (Paired *T*-test; $t(9) = 7.112$, $p < 0.0001$). The water temperature was 26.90 ± 2.72 °C from the dry seasons in the river and 26.50 ± 1.99 °C during the wet seasons in the river (Fig. S4c). Meanwhile, the water temperature was 30.71 ± 0.91 °C in the reservoir

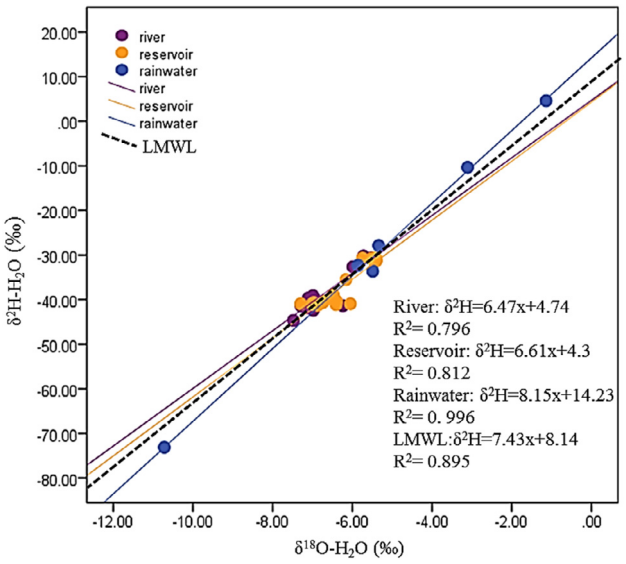

Fig. 2. A $\delta^{18}O$–$\delta^{2}H$ relationship of surface water in river, reservoir and rainwater of BMR and Local Meteoric Water Line (LMWL) of IAEA.

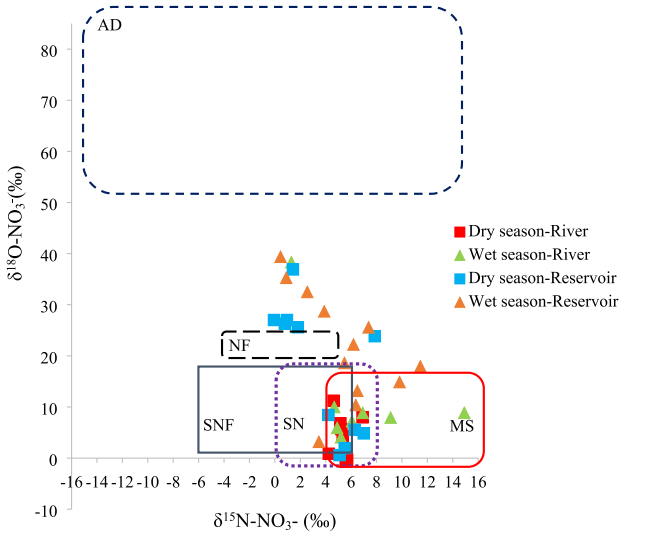

Fig. 3. $\delta^{18}O$-NO_3^- versus $\delta^{15}N$-NO_3^- values from river and reservoir water samples during dry and wet seasons embedded with the range of values reported in literature, as indicated by the coloured boxes representing atmospheric deposition (AD), synthetic nitrogen fertilizer (SNF), soil nitrogen (SN) and manure and sewage (MS) end members.

during the dry season and 30.73 ± 1.22 °C in the wet season (Fig. S4c). The difference between the seasons could be related to the temperature difference in the river and reservoir. At the same time, the rainfall amount also shows likely impact to the $\delta^{2}H$-H_2O patterns.

3.6. Identification of nitrate sources and processes in BMR using the dual isotope bi-plot and mixing model

A dual isotope bi-plot approach ($\delta^{15}N$–NO_3^- and $\delta^{18}O$–NO_3^-) was adopted to qualitatively identify predominant NO_3^- sources in BMR based on the dry and wet seasons in the river and reservoir water as shown in Fig. 3. The $\delta^{15}N$–NO_3^- in the surface water of the river and reservoir was found to range from −0.07 to +11.45‰ (N = 38, mean = 4.90‰). The average $\delta^{15}N$–NO_3^- in the dry season was 4.39 ± 2.31‰ (N = 19) whereas the average for the wet season was 5.40 ± 2.94‰ (N = 19). The $\delta^{18}O$–NO_3^- in the river and reservoir water samples varied between −0.29 and +39.40‰ (Fig. 3), which fall within the nitrification values in the literature (−5 to +15‰; Kendall et al., 2007) and mixing from fractionation processes (Kendall et al., 2007). The $\delta^{18}O$–NO_3^- values of more than the maximum nitrification values of +15‰ (Kendall, 1998; Kendall et al., 2007) may also suggest AD as an important nitrate source. Various fractionation processes may alter the initial composition of NO_3^- before or after mixing (Kendall, 1998). Other factors may include isotopic fractionation during NO_3^- formation caused by thunderstorms, the isotopic signature of the reactive oxygen in the atmosphere that combines with NO_x to form NO_3^- and any isotopic fractionation during reactions in the atmosphere (Kendall, 1998; Pardo et al., 2004; Xue et al., 2009). The $\delta^{15}N$–NO_3^- and $\delta^{18}O$–NO_3^- values of water samples in the dry season were not significantly different from those in the wet season and were also not significantly different between the river and reservoir (Paired *T*-test; $p > 0.05$).

By referring to the wet and dry season in the reservoir and one sample in the river during the wet season, samples with low $\delta^{15}N$–NO_3^- (near +0‰) and high $\delta^{18}O$–NO_3^- data points (>+25‰) may imply mixing with AD or fractionation processes affecting the nitrate sources. The $\delta^{18}O$–NO_3^- can be used to distinguish nitrate from nitrate fertilizers (NF) and atmospheric deposition (AD) sources due to the distinctive values compared to the overlapping N isotopic values (Kendall et al., 2007). The $\delta^{18}O$–NO_3^- from NF is close to that of molecular oxygen in the atmosphere with values around +23 ± 3‰ whereas in the atmospheric deposition, the $\delta^{18}O$–NO_3^- is generally enriched from >+50 to +94‰ (Michalski et al., 2015). Our samples did not fall within the range of NF as the $\delta^{18}O$–NO_3^- was more than +25‰. Furthermore, in Malaysia, the main fertilizers used are urea, ammonium sulphate, ammonium nitrate, ammonium phosphate and NPK compound fertilizers (FAO, 2004), therefore NF was eliminated as a potential nitrate source. The high $\delta^{18}O$–NO_3^- data points (>+25‰) in our samples were in fact, much lower than the typical values for AD but higher than the range of NF. We interpreted this as mixing with AD sources and fractionation processes affecting the system. Apart from that, a few of our samples with $\delta^{15}N$–NO_3^- of more than +5‰ and $\delta^{18}O$–NO_3^- data points between +18 to +26‰ also did not fell within the region of NF and AD. Therefore, fractionation process is responsible for the nitrate content in these samples, more so in the reservoir than the river (Fig. 3).

On the other hand, all samples during the dry season in the river suggested overlapping sources between synthetic nitrogen fertilizer (SNF), soil nitrogen (SN), and manure and sewage (MS). Some of the water samples during the dry season in the reservoir, and during the wet season in the river and reservoir also fell within these boxes (Fig. 3). The $\delta^{15}N$–NO_3^- range for manure and sewage in the literature is between +4 and +19‰ and +5 to +25, respectively and $\delta^{18}O$–NO_3^- of between −10 and +15‰ (Xue et al., 2009; Xu et al. 2016). The $\delta^{15}N$–NO_3^- range for SN is +0 to +8‰ and SNF ranges from −4 to +6.9‰, which clearly overlaps (Kendall et al., 2007). When referring to the literature for the local manure-N range, we could not find any other reported studies from Malaysia, therefore, we have opted from the literature compiled by Xue et al. (2009) and Xu et al. (2016).

Therefore, to ascertain how much nitrate was proportioned by these sources, we have calculated the quantitative contribution using the Bayesian mixing model in SIAR. In order to obtain the isotopic fractionation factor of each of the sources, Li et al. (2019) have proposed the use of the isotopic fractionation factor for denitrification from the linear regression of $\delta^{15}N$–NO_3^- and $\ln(NO_3\text{-}N)$. In our study, since denitrification can be interpreted as absent, the

isotopic fractionation factor is therefore considered 0 (Zhang et al., 2018). Overall, the SIAR model outputs reflected the contributions of different nitrate sources at a specific sampling period, but it is expected that this may change on a temporal basis. As shown in Table 2, the results showed that 37% was contributed by MS, followed by 31% from SNF and 28% from SN in the river during the dry season, which clarifies the overlapping range in the qualitative assessments in Fig. 3. During the wet season, 37% was contributed by MS in the river and 31% by SN with a lesser proportion by SNF (18%). Meanwhile, during the dry season in the reservoir, the difference between proportional contribution of nitrate sources was small, 26% by SN, 25% MS, 24% SNF and 23% AD. This proportion explains the overlapping pattern observed in Fig. 3 for SN, MS and SNF while the spread of the isotopic composition above these sources pointed out towards AD contribution. MS contributed the largest proportion in the reservoir during the wet season (33%), followed by AD (29%) and 25% of SN. The mixing model pointed out that MS was the dominant nitrate sources during the dry and wet season in the river and reservoir except for in the reservoir during dry seasons. MS sources might be due to the presence of residential areas (45.49 km^2), and the presence of the national Boer goat farm (9.94 km^2; St.11 in Fig. 1) and chicken farming in the area (2.61 km^2). Spatial factors such as land use type including residential areas and agricultural farmland affect the nitrate concentration in the water catchment (Xu et al., 2016).

Interestingly, AD contribution was observed in the reservoir during both seasons albeit the dominant source of MS (Table 2 and Fig. 6). This result highlighted that rainfall contributed as a significant factor affecting the nitrate sources in the reservoir but with a smaller contribution to the river in the dry (3.5%) and wet seasons (13%). AD contribution during the dry season in the reservoir implies that occasional rainy days (19 days) with lesser amount (195 mm) in the dry season compared to the wet season (38 rainy days and 672.5 mm of rainfall) also impacted the nitrate sources. However, the wet seasons in the river area seem to suggest increasing N sources in SN (31%) due to leaching from SNF inputs compared to the dry season (Table 2). Water discharges data from previous existing studies in the study area were reported within the range of 6.6–65.7 m^3/s (average discharge of 27.2 m^3/s) in the year 2008–2009 (Ismail et al., 2010; Najib et al., 2017) and 13 m^3/s in the year 2016 (Sharip et al., 2018). In the previous study by Najib et al., (2017), higher water discharge was recorded in BMR from September to December 2008 during the north-east monsoon compared to the other months. Hypothetically, the higher water discharge consistent with the higher rainfall amount from the previous study may also imply the possibility of higher water discharge due to the wet season in our study. Water discharge is influenced by rainfall and local topography of the area (Sani et al., 2012). Higher gradient at St. 1 (118 m) and St. 2, 3 and 4 in the river (23 m) relative to the reservoir (9 m) also supports the likely event of higher water discharge due to the elevation factor, which subsequently diluted the NO_3-N concentrations in the reservoir.

Table 2
Mean values of source contribution using SIAR.

System and season	Sources			
	AD	SN	MS	SNF
River				
Dry	3.5	28	37	31
Wet	13	31	37	18
Reservoir				
Dry	23	26	25	24
Wet	29	25	33	12

SN contributed approximately the same percentage in all groups (25–31% in Table 2 and Fig. 6), consistent with the findings by Ismail and Najib (2011), Talib et al., (2016) and Najib et al., (2017) which reported sedimentation associated with nutrient inputs in BMR. Sedimentation factor can be inferred as one of the processes responsible for nitrate based on sediment retained in the system with a total of 43053 t/year (Ismail and Najib 2011). Sediment retention would mean that the combination of nutrient additions coming from streams, rivers and recirculation of nutrients from the bottom can increase the productivity of the reservoir (Najib et al., 2017).

The total loading of the nitrate input in BMR was 47.14 t/year and the output was 17.31 t/year, resulting in net retention of 29.83 t/year of nitrate in the year 2008 (Ismail and Najib, 2011). Nitrogen retention in the system may occur due to three factors, assimilation by vegetation, denitrification, and sedimentation (Qiu et al., 2019). To find out whether the system (river and reservoir) during the dry and wet seasons in the study area was affected by assimilation processes by autotrophic organisms, we have plotted the Chl *a*, NO_3-N and $\delta^{15}N\text{-}NO_3^-$ concentrations (Fig. S2). Chl *a* concentration in the river during the dry season was not significantly correlated with NO_3-N ($N = 8$, $r = 0.290$, $p = 0.486$) and $\delta^{15}N\text{-}NO_3^-$ ($N = 8$, $r = 0.246$, $p = 0.556$) which suggests no assimilation taking place. Similarly, chl *a* during the wet season in the river was also not significantly correlated with NO_3-N ($r = 0.224$, $p = 0.629$) and $\delta^{15}N\text{-}NO_3^-$ ($r = -0.2664$, $p = 0.568$). Overall, the pattern between Chl *a*, NO_3-N and $\delta^{15}N\text{-}NO_3^-$ concentrations in the river during the dry season (Fig. S2a, b and c) suggests lower phytoplankton biomass compared to the wet season but both seasons could not point out towards assimilation process in the river. Meanwhile, the highest chl *a* was detected in the wet season in the reservoir (Fig. S2a), suggesting that rainfall inputs have increased the phytoplankton growth and the NO_3-N concentrations in the wet season compared to the dry season (Fig. S2a and b). During surface runoff, due to higher rainfall inputs in the wet season, soil nitrate (which is enriched in $\delta^{15}N\text{-}NO_3^-$) is likely to enter the reservoir, increasing NO_3^- concentration and favouring phytoplankton growth. On the other hand, $\delta^{15}N\text{-}NO_3^-$ in the reservoir was more enriched in the wet season than the dry season, which suggests assimilation process was also taking place as autotrophic organisms preferably uptake the ^{14}N, enriching the $\delta^{15}N\text{-}NO_3^-$. By referring to the ratio of $\delta^{15}N\text{-}NO_3^-$ to $\delta^{18}O\text{–}NO_3^-$, assimilation process is typically shown by the 1:1 relationship (Fry, 2006). This relationship is not shown in our samples (Fig. 4a). No significant negative relationship was also found between chl *a*, NO_3-N and $\delta^{15}N\text{-}NO_3^-$ in both dry and wet seasons in the reservoir ($p > 0.05$). Although the pattern did not support assimilation as the dominant process, the fact that there are certain indicators of assimilation may imply that we cannot completely rule out the assimilation process. Assimilation process can be co-occurring with other processes overlapping at the same time.

Although the $\delta^{15}N\text{–}NO_3^-$ and $\delta^{18}O\text{–}NO_3^-$ values depend mainly on their sources, biologically mediated processes such as denitrification are also important factors causing the nitrogen and oxygen isotopes to fractionate, leaving heavier isotopes behind (Kendall et al., 2007). To ascertain whether denitrification was responsible for the nitrate content in the study area, we have plotted the relationships between isotopic plots and other parameters such as DO and NO_3-N concentrations analysed in this study. Negative relationships were observed between $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO^{3-}$ in the river and reservoir during wet season and in the reservoir during dry season (Fig. 4a). This relationship suggested that denitrification did not occur as denitrification results in the enrichment of both $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO^{3-}$ (Yue et al., 2014; Wang et al., 2016; Li et al., 2019). Denitrifiers prefer the lighter isotopes (^{14}N and ^{16}O) leaving behind the heavier ^{15}N and ^{18}O isotopes, thus enriching the

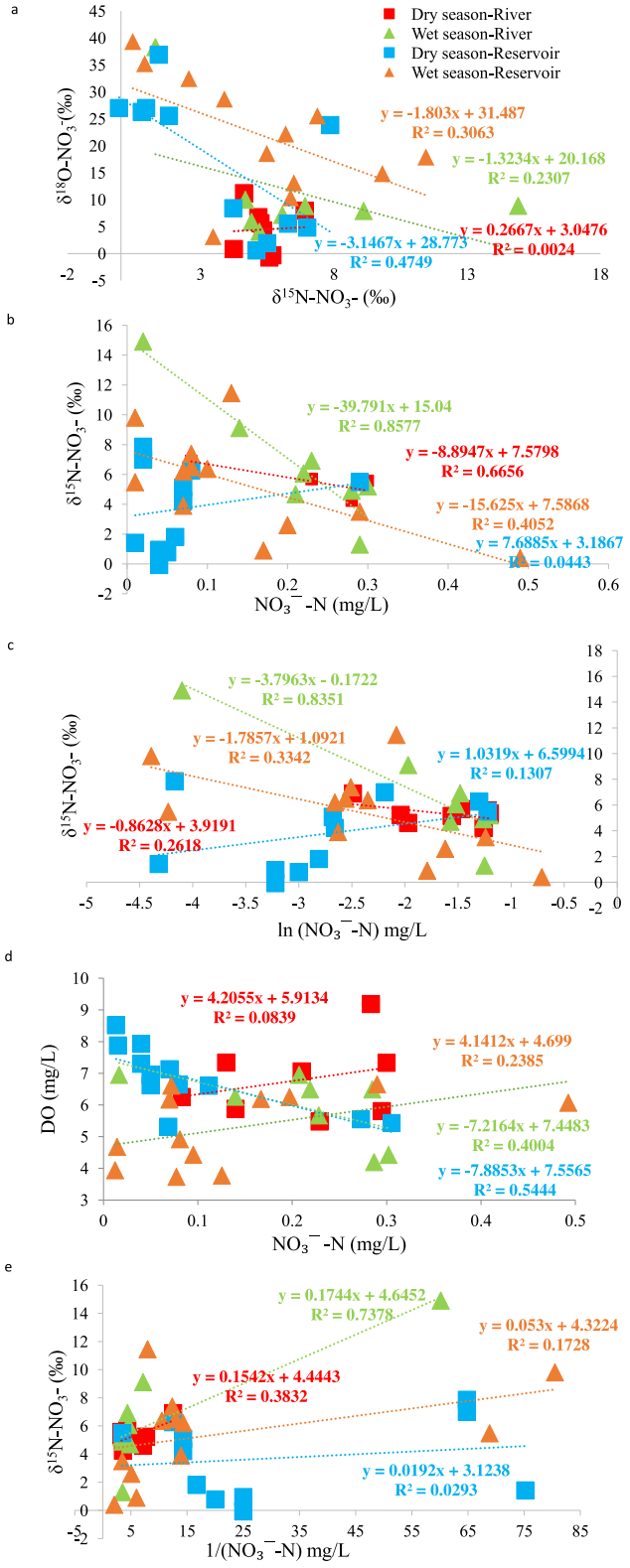

Fig. 4. a $\delta^{18}O\text{-}NO_3^-$ versus $\delta^{15}N\text{-}NO_3^-$ values from river and reservoir water samples during dry and wet seasons. b. $\delta^{15}N\text{-}NO_3^-$ versus NO_3-N from river and reservoir water samples during dry and wet seasons. c. $\delta^{15}N\text{-}NO_3^-$ versus ln NO_3-N from river and reservoir water samples during dry and wet seasons d. DO versus NO_3-N from river and reservoir water samples during dry and wet seasons. All plots showing denitrification was not the major process affecting NO_3^- in the system. e. $\delta^{15}N\text{-}NO_3^-$ versus $1/NO_3$-N shows mixing processes.

substrate. On the other hand, positive relationship in the river during the dry season could suggest potential denitrification ($y = 0.27 \times +3.05$, $R^2 = 0.002$) but this is not significant. The plot of $\delta^{15}N\text{-}NO_3^-$ and NO_3-N, and $\delta^{15}N\text{-}NO_3^-$ and ln NO_3-N was negatively correlated in the river during the dry season (Fig. 4b and c), which suggests denitrification as not the main N transformation process. This factor is also supported by the DO concentrations in Fig. 4d and Table S2. Denitrification in the surface water can be assumed as not the main process in the study area since the lowest DO concentration was 3.73 mg/L. The denitrification process requires anaerobic conditions (Yevenes et al., 2016) where DO concentrations could not be higher than 2 mg/L (Rivett et al., 2008); to be exact, <0.5 mg/L (ISONITRATE, 2009). In contrast, the linear trend between $\delta^{15}N\text{–}NO_3^-$ and inverse nitrate concentration (1/NO_3-N) indicate mixing of nitrate sources (Fig. 4e). Mixing of nitrate sources from 2 or more sources can result in parallel increase of both $\delta^{15}N\text{–}NO_3^-$ and NO_3^- concentration and results in a straight line on the Keeling plot (Kendall, 1998).

Apart from denitrification, the $\delta^{15}N\text{–}NO_3^-$ and $\delta^{18}O\text{–}NO_3^-$ also can be affected by nitrification process. Using the theoretical nitrification concept in Eq. (2), the $\delta^{18}O$ value in the atmosphere of +23.5, and the $\delta^{18}O$ values in the water of −7.48 to −5.40‰ in the river and reservoir; the range of $\delta^{18}O\text{–}NO_3^-$ derived from nitrification was within +2.85 to +4.23‰. The $\delta^{18}O\text{–}NO_3^-$ of water samples from the river and some from the reservoir, fell around the theoretical value (Kendall, 1998), which suggests nitrification was occurring in the river and reservoir during both the dry and wet seasons (Fig. 5). By referring to the $\delta^{18}O\text{–}NO_3^-$ found in other studies (−5 to +15‰ in Kendall et al., 2007), the $\delta^{18}O\text{–}NO_3^-$ values derived from nitrification in this study were within the literature values. In our results, many of the water samples from the reservoir had $\delta^{18}O\text{–}NO_3^-$ values on the upper limit of the theoretical nitrification values and values higher than the $\delta^{18}O$ in air (+23.5‰, Kendall, 1998), implying that the $\delta^{18}O\text{–}NO_3^-$ may come from atmospheric nitrate (Kendall, 1998; Kendall et al., 2007) or mixing processes among other sources and AD. The wet deposition contribution from atmospheric nitrate can display the oxygen isotope of mixing NO_3^- lower than the wet deposition contribution but higher than theoretical nitrification. In the meantime, we could only hypothesise that due to the higher temperature in the tropics, $\delta^{18}O\text{-}NO_3^-$ values of microbial nitrate are expected to be more

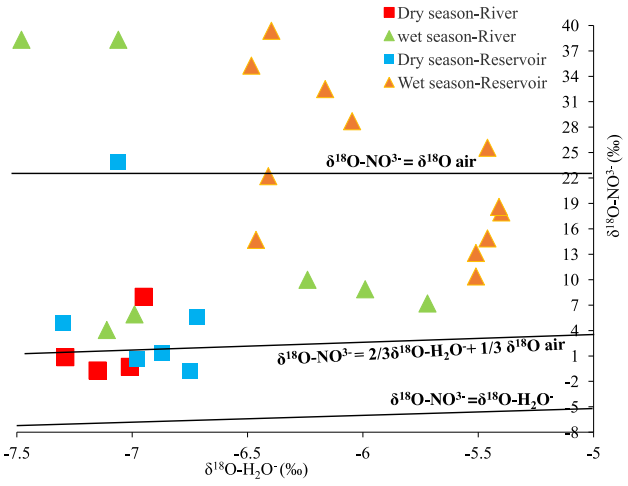

Fig. 5. $\delta^{18}O\text{-}H_2O$ versus $\delta^{18}O\text{-}NO_3^-$ in water samples from river and reservoir during the dry and wet season. Three lines represent the theory line in different condition.

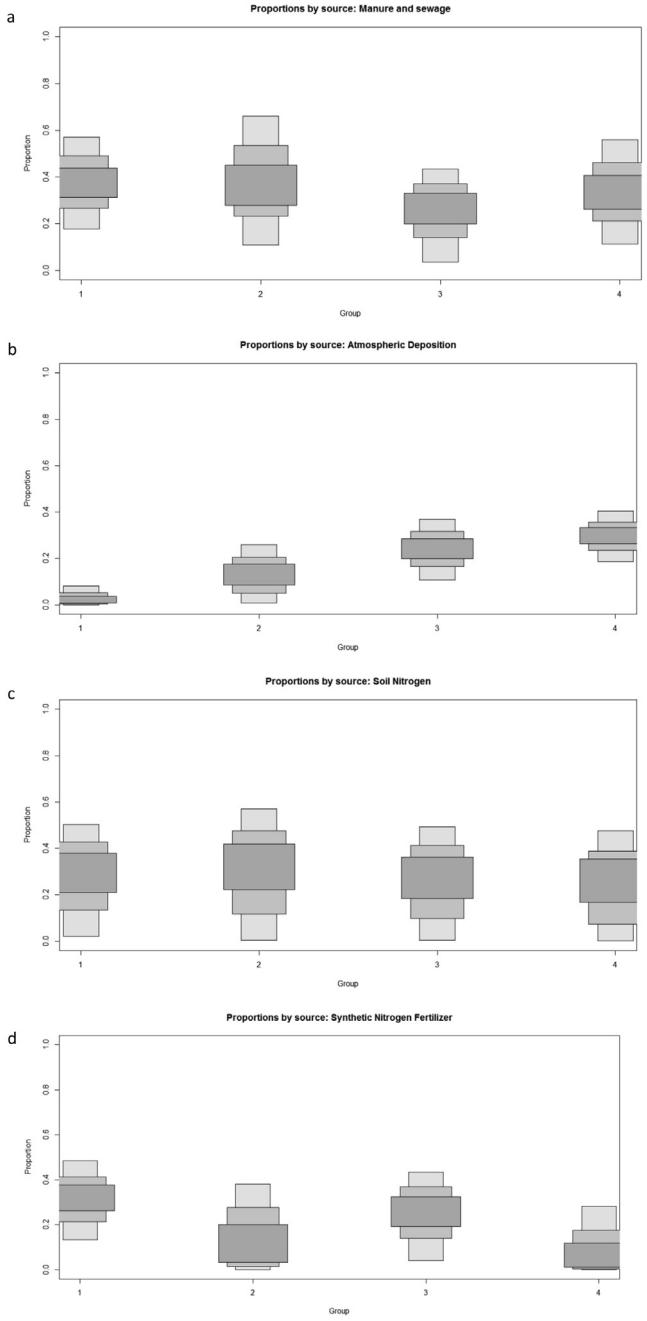

Fig. 6. Boxplots of mixing model proportions for each group, categorised by source. a. Manure and sewage, b. Atmospheric deposition, c. Soil nitrogen, d. Synthetic Nitrogen Fertilizer. Group 1 (dry season-river), group 2-wet season-river, group 3-dry season-reservoir and group 4-wet season-reservoir.

enriched due to several reasons. Firstly, higher temperature stimulates microbial respiration or respiratory oxygen consumption (Kendall 1998; Xu et al., 2016) which may consequently change $\delta^{18}O$–O_2 value from the atmospheric oxygen of (+23.5‰). Secondly, evaporation process may cause $\delta^{18}O$ enrichment of the soil-water available to nitrifiers (Snider et al., 2010), therefore the use of $\delta^{18}O$-H_2O of precipitation, surface water or groundwater may to a certain extent reflect the $\delta^{18}O$ of water available to nitrifiers (Spoelstra et al., 2007).

SNF contributed the least proportion in the reservoir during the wet season, potentially diluted by AD in the wet season. Nitrate from SNF used by private owners in oil palm plantations surrounding the Kurau River may leach into the river water through terrestrial run-off. The absence of canopy cover along the Kurau River (Mohammad et al., 2018) and the presence of sand mining activities in the Kurau River upstream of BMR further exacerbate river bank erosion, river bed degradation, river buffer zone encroachment and deterioration of river water quality (Teo and Noh, 2017). Therefore, to control nutrients carried by soil and run-off into BMR, the installation of filters, particularly in areas of sand mining activity, within the Kurau River is necessary to reduce soil-associated nutrient inputs. Additionally, the planting of riparian vegetation is required to act as a buffer to nutrients associated with run-off from terrestrial areas.

As a whole, the output of SIAR is acceptable and concurrent with the qualitative contribution presented in Fig. 3. Although our results provided important information for controlling nitrate concentrations in the reservoir, they have some limitations. Firstly, only the main river and the middle area of the reservoir were investigated in this study. Therefore, further research is necessary to understand the spatial variation of NO_3^- sources in the tropical reservoir. Secondly, the manure and SNFs sources incorporated values based on other relevant studies together with our local data, which may cause uncertainty of the proportions. Thirdly, the calculated results have some uncertainties due to ignoring fractionation during the calculations (Kendall et al., 2007; Yue et al., 2015; Matiatos 2016; Zhang et al., 2018).

In the absence of denitrification affecting the $\delta^{15}N$–NO_3^- and $\delta^{18}O$–NO_3^- in the study area, high NO_3^- concentrations were expected in the system since there was no loss of N. However, the system was dominated by low NO_3^- concentrations in the study area. Therefore, what might have caused the low NO_3^- in the study area? One factor was due to the dilution effect by rainfall during the dry and wet seasons in the reservoir as explained previously. The dynamic process of nitrogen transformation in the river was not explained by either denitrification, assimilation or rainfall effect. One plausible explanation would be that not one single biological process, but mixing processes were influencing the nitrate dynamics in the system. The low NO_3^- concentrations in our system could also be caused by low NH_4^+ concentrations from the low rate of organic nitrogen to NH_4^+ conversion. Ammonification or organic nitrogen decomposition hydrolyses organic nitrogen compounds into ammonium and is known as N mineralization (Xia et al., 2018). Thus, mineralization can be highlighted as one of the processes regulating the system.

4. Conclusions

In the present study, nitrate stable isotopes ($\delta^{15}N$–NO_3^- and $\delta^{18}O$–NO_3^-) and the water chemistry of river and reservoir water were evaluated to determine nitrate sources and processes within a shallow tropical reservoir system. A Bayesian mixing model in SIAR was used to estimate the proportional contribution of nitrate sources in samples collected from the study area. The qualitative plot of nitrate stable isotopes showed that nitrate sources in the river and reservoir were from mixing of AD, SNF, SN and MS sources. The trend of inverse NO_3-N concentration and $\delta^{15}N$–NO_3^- plot also supported that mixing was the dominant process affecting NO_3^- sources in the system. The water system in the study area was dominated by DON. In the presence of oxygen in the surface water, nitrification occurs, and it can be concluded that denitrification did not affect the nitrate isotopic composition. The relationship between chl *a*, NO_3-N concentration and $\delta^{15}N$–NO_3^- could not point out clear assimilation process in the system, potentially due to the mixing and overlapping nitrate sources. The results from the SIAR model showed that the dominant nitrate sources in the river and reservoir during the wet and dry season was contributed

mostly from MS. This information suggests that this N source should be reduced to minimise the nutrients impact to BMR. The emerging AD contribution in the reservoir during both seasons using SIAR, highlighted that AD impacted the reservoir more than the river. $\delta^{13}C$ and the C:N ratio of the sediments showed C3 terrestrial plants imprints, corresponding to run-off from the land to the water.

To complete the N cycle, diurnal sampling should be conducted to account for daytime and night-time effects on N concentrations and the denitrification process which depends on DO concentrations and is influenced by sunlight. Overall, the results obtained in this study provided important information for tropical reservoir water management and nitrate pollution control in a shallow tropical reservoir system.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.134517.

References

Adams, V.D., 1990. Water and Wastewater Examination Manual. Lewis Publishers Inc., Michigan.
Andersson, K.K., Hooper, A.B., 1983. O_2 and H_2O are each the source of one O in NO_2^- produced from NH_3 by Nitrosomonas: 15N-NMR evidence. FEBS Lett 164, 236–240.
Ansari, A.A., Gill, S.S., Khan, F.A., 2011. Eutrophication: threat to aquatic ecosystems. In: Ansari, A.A., Gill, S.S., Lanza, G.R., Rast, W. (Eds.), Eutrophication: Causes, Consequences and Control. Springer, USA.
Böttcher, J., Strebel, O., Voerkelius, S., Schmidt, H.L., 1990. Using isotope fractionation of nitrate-nitrogen and nitrate-oxygen for evaluation of microbial denitrification in a sandy aquifer. J. Hydrol. 114 (3–4), 413–424.
Bronk, D.A., See, J.H., Bradley, P., Killberg, L., 2007. DON as a source of bioavailable nitrogen for phytoplankton. Biogeosciences 4, 283–2007.
Carlson, R.E., 1977. A trophic state index for lakes. Limnol. Oceanogr. 22, 361–369. https://doi.org/10.4319/lo.1977.22.2.0361.
Casciotti, K.L., Sigman, D.M., Hastings, M.G., Bohlke, J.K., Hilkert, A., 2002. Measurement of the oxygen isotopic composition of nitrate seawater and freshwater using the denitrifier method. Anal. Chem. 74 (19), 4905–4912. https://doi.org/10.1021/ac020113w.
Chen, Z., Huang, P., Zhang, Z., 2019. Interaction between carbon dioxide emissions and eutrophication in a drinking water reservoir: a three-dimensional ecological modeling approach. Sci. Total Environ. 663, 369–379.
Dellamano-Oliveira, M.J., Vieira, A.H., Rocha, O., Colombo, V., Sant'Anna, C.L., 2008. Phytoplankton taxonomic composition and temporal changes in a tropical reservoir. Fundamental Appl. Limnol. 171 (1), 27–38.
Dobson, M., Frid, C., 2009. Ecology of Aquatic Systems. Oxford University Press.
FAO, 2004. Fertilizer use by crop in Malaysia. Land and Plant Nutrition Management Service Land and Water Development Division, first ed. Food and Agriculture Organization of the United Nations. Rome, Italy.
Fry, B., 2006. Stable Isotope Ecology. New York, Springer, USA.
Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S. P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A. F., Porter, J.H., Townsend, A.R., Vöosmarty, C.J., 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70 (2), 153–226.
Hach, 2015. Hach Water Analysis Handbook, Hach Company, USA.
Harris, D., Horwath, W.R., Van Kessel, C., 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Sci. Soc. Am. J. 65, 1853–1856.
Hasan, Z., Yusoff, M.S., Talib, S.H.A., 2011. Bukit Merah reservoir sedimentation assessment. Int. Conf. Environ. Sci, Eng. 8, 86–90.
Heaton, T.H.E., Stuart, M.E., Sapiano, M., Sultana, M.M., 2012. An isotope study of the sources of nitrate in Malta's groundwater. J. Hydrol. 414 (415), 244–254.
Hedges, J.I., Clark, W.A., Quay, P.D., Richey, J.E., Devol, A.H., de Santos, U.M., 1986. Compositions and fluxes of particulate organic material in the Amazon River. Limnol. Oceanogr. 31, 717–738.
Hidzrami, S.A., 2010. Bukit Merah Perak, Lake Brief. Managing Lakes and Their Basins for Sustainable Use in Malaysia (Lake Briefs Report Series 1). Kuala Lumpur: Akademi Sains Malaysia, 29–72.
Hou, W., Gu, B., Lin, Q., Gu, J., Han, B.P., 2013. Stable isotope composition of suspended particulate organic matter in twenty reservoirs from Guangdong, southern China: implications for pelagic carbon and nitrogen cycling. Water Res. 47 (11), 3610–3623.
Hwang, S.H., Jeon, J.H., Ham, J.H., Kim, H.S., 2002. Effects of physical characteristics on a nutrient-chlorophyll relationship in Korean reservoirs. J. Korean Soc. Agric. Eng. 44, 64–73.
Hwang, S.J., Kwun, S.K., Yoon, C.G., 2003. Water quality and limnology of Korean reservoirs. Paddy Water Environ. 1, 43–52.
IAEA, 2014. IAEA/GNIP precipitation sampling guide. http://www-naweb.iaea.org/napc/ih/documents/other/gnip_manual_v2.02_en_hq.pdf.
Ismail, W.R., Najib, S.A.M., 2011. Sediment and nutrient balance of Bukit Merah Reservoir, Perak (Malaysia). Lakes Reservoirs Res. Manage. 16 (3), 179–184. https://doi.org/10.1111/j.1440-1770.2011.00453.x.
Ismail, W.R., Rahaman, Z.A., Najib, S.A.M., Othman, Z., 2010. Sediment impacts on aquatic ecosystems of the Bukit Merah Reservoir, Perak, Malaysia. IAHS-AISH Publ. 337, 258–263.
ISONITRATE, 2009. Can Measurement of Nitrate, Oxygen, and Boron isotopes be useful for your nitrate problem? A guideline. http://isonitrate.brgm.fr/.
Jackson, A.L., Inger, R., Bearhop, S., Parnell, A., 2009. Erroneous behaviour of MixSIR, a recently published Bayesian isotope mixing model: a discussion of Moore & Semmens. Ecol. Lett. 12 (3), E1–E5. https://doi.org/10.1111/j.1461-0248.2008.01233. x.
Jacobson, P.C., Hansen, G.J.A., Bethke, B.J., Cross, T.K., 2017. Disentangling the effects of a century of eutrophication and climate warming on freshwater lake fish assemblages. PLoS ONE 12, (8). https://doi.org/10.1371/journal.pone.0182667 e0182667.
JPBD, 2017. I-PLAN Malaysia. https://iplan.townplan.gov.my/.
Kendall, C., 1998. Tracing nitrogen sources and cycling in catchments. In: Kendall, C., Mcdonnell, J.J. (Eds.), Isotope Tracers in Catchment Hydrology. Elsevier Science, pp. 519–576.
Kendall, C., Elliott, E.M., Wankel, S.D., 2007. Tracing anthropogenic inputs of nitrogen to ecosystems. In: Michener, R., Lajtha, K. (Eds.), Stable Isotopes in Ecology and Environmental Science. second ed.,. Blackwell Publishing, pp. 375–449.
Kroeger, K.D., Cole, M.L., Valiela, I., 2006. Groundwater-transported dissolved organic nitrogen exported from coastal watersheds. Limnol. Oceanogr. 51, 2248–2261.
Li, C., Li, S.L., Yue, F.J., Liu, J., Zhong, J., Yan, Z.F., Zhang, R.C., Wang, Z.J., Xu, S., 2019. Identification of sources and transformations of nitrate in the Xijiang River using nitrate isotopes and Bayesian model. Sci Total Environ. 2019 (646), 801–810. https://doi.org/10.1016/j.scitotenv.2018.07.345.
Libes, S.M., 2009. An Introduction to Marine Biogeochemistry. Academic Press, London UK.
Ling, T.-Y., Gerunsin, N., Soo, C.-L., Nyanti, L., Sim, S.-F., Grinang, J., 2017. Seasonal changes and spatial variation in water quality of a large young tropical reservoir and its downstream river Article ID 8153246 J. Chem. 2017, 16. https://doi.org/10.1155/2017/8153246.
Mamun, M., An, K.-G., 2017. Major nutrients and chlorophyll dynamics in Korean agricultural reservoirs along with an analysis of trophic state index deviation. J. Asia-Pac. Biodivers. 10, 183–191.
Matiatos, I., 2016. Nitrate source identification in groundwater of multiple land-use areas by combining isotopes and multivariate statistical analysis: a case study of Asoposbasin (Central Greece). Sci. Total Environ. 541, 802–814. https://doi.org/10.1016/j.scitotenv.2015.09.134.
McIlvin, M.R., Casciotti, K.L., 2011. Technical updates to the bacterial method for nitrate isotopic analyses. Anal. Chem. 83, 1850–1856.
Meyers, P.A., 2003. Applications of organic geochemistry to paleolimnological reconstructions: a summary of examples from the Laurentian Great Lakes. Org. Geochem. 34, 261–289. https://doi.org/10.1016/S0146-6380(02)00168-7.
Michalski, G., Kolanowski, M., Riha, K.M., 2015. Oxygen and nitrogen isotopic composition of nitrate in commercial fertilizers, nitric acid, and reagent salts. Isotopes Environ. Health Stud. 51 (3), 382–391.

Moal, M.L., Gascuel-Odoux, C., Ménesguen, A., Souchon, Y., 2019. Eutrophication: a new wine in an old bottle? Sci. Total Environ. 651 (1), 1–11.
Mohammad, M.S., Fadzil, N.F.M., Md. Sah, A.M.R., Zakeyuddin, M.S., Darwin, E.D., Hashim, Z.H., 2018. A freshwater fish biodiversity and distribution at Bukit Merah Reservoir river feeders, Perak, Peninsular Malaysia. Malay. Nat. J. 70 (4), 463–470.
Moore, J.W., Semmens, B.X., 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecol. Lett. 11, 470–480. https://doi.org/10.1111/j.1461-0248.2008.01163.x.
Najib, S.A.M., Ismail, W.R., Omar, M.A., 2017. Suspended sediment and phosphorus budget and trophic status of Bukit Merah Reservoir, Perak, Malaysia. J. Environ. Geography 10 (3–4), 1–8. https://doi.org/10.1515/jengeo-2017-0007.
Oliveira, Fábio H.P.C. De, Ara, André L.S. Capela E, Moreira, Cesar H.P., Lira, Osman O., Padilha, Maria Do Rosário F., Shinohara, Neide K.S., 2014. Seasonal changes of water quality in a tropical shallow and eutrophic reservoir in the metropolitan region of Recife (Pernambuco-Brazil). Anais da Academia Brasileira de Ciências, 86(4), 1863–1872. Epub October 14, 2014. https://dx.doi.org/10.1590/0001-3765201420140128.
Padedda, B.M., Sechi, N., Lai, G.G., Mariani, M.A., Pulina, S., Sarria, M., Satta, C.T., Virdis, T., Buscarinu, P., Lugliè, A., 2017. Consequences of eutrophication in the management of water resources in Mediterranean reservoirs: a case study of Lake Cedrino (Sardinia, Italy). Global Ecol. Conserv. 12, 21–35.
Pardo, L.H., Kendall, C., Pett-Ridge, J., Chang, C.C.Y., 2004. Evaluating the source of stream water nitrate using 15N and 18 O in nitrate in two watersheds in New Hampshire, USA. Hydrol. Process. 18, 2699–2712.
Parnell, A.C., Inger, R., Bearhop, S., Jackson, A.L., 2010. Source partitioning using stable isotopes: Coping with too much. variationPLoS ONE, 5.
Parnell, A.C., Phillips, D.L., Bearhop, S., Semmens, B.X., Ward, E.J., Moore, J.W., Jackson, A.L., Grey, J., Kelly, D.J., Inger, R., 2013. Bayesian stable isotope mixing models. Environmetrics 24, 387–399.
Pinto-Coelho, R.M., Brighenti, L.S., Bezerra-Neto, J.F., Morais Jr., C.A., Gonzaga, A.V., 2010. Effects of sampling effort on the estimation of spatial gradients in a tropical reservoir impacted by an oil refinery. Limnologica 40, 126–133.
Qiu, X., Huang, T., Zeng, M., Zhou, S., 2019. Three-year survey of nitrogen dynamics in a stratified reservoir of the North China. Plain. IOP Conf. Ser.: Earth Environ. Sci. 252, 052040. https://doi.org/10.1088/1755-1315/252/5/052040.
Rivett, M.O., Buss, S.R., Morgan, P., Smith, J.W.N., Bemment, C.D., 2008. Nitrate attenuation in groundwater: a review of biogeochemical controlling processes. Water Res. 42 (16), 4215–4232. https://doi.org/10.1016/j.watres.2008.07.020.
Rock, L., Ellert, B.H., Mayer, B., 2011. Tracing sources of soil nitrate using the dual isotopic composition of nitrate in 2 M KCl-extracts. Soil Biol. Biochem. 43 (12), 2397–2405. https://doi.org/10.1016/j.soilbio.2011.08.016.
Rogers, K.M., 2013. Using stable isotopes to detect land use change and nitrogen sources in aquatic systems. In Application of Isotope Techniques for Assessing Nutrient Dynamics in River Basins. IAEA, pp. 129–156.
Sani, S.F.M., Ismail, W.R., Najib, S.A.M., Omar, M.A., 2012. Evaluation of Sediment Budget of Bukit Merah Reservoir and its Catchment Area, Perak, Malaysia. Malay. J. Environ. Manage. 13 (1), 69–79.
Sharip, Z., Shah, S.A., Jamin, A., Jusoh, J., 2018. Assessing the hydrodynamic pattern in different lakes of Malaysia. In: Daniela Malcangio (Ed.), Applications in Water Systems Management and Modeling. https://doi.org/10.5772/intechopen.73274.
Sharip, Z., Yusoff, F.M., 2017. Plankton community characteristics of natural and man-made tropical lakes. J. Environ. Biol. 38, 1365–1374. https://doi.org/10.22438/jeb/38/6/MRN-356.
Sharip, Z., Zaki, A.T.A., Shapai, M.A.H.M., Suratman, S., Shaaban, A.J., 2014. Lakes of Malaysia: water quality, eutrophication and management. Lakes Reservoirs Res. Manage. 19 (2), 130–141. https://doi.org/10.1111/lre.12059.
Sigman, D.M., Casciotti, K.L., Andreani, M., Barford, C., Galanter, M., Böhlke, J.K., 2001a. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Anal. Chem. 73 (17), 4145–4153. https://doi.org/10.1021/ac010088e.
Sigman, D.M., Casciotti, K.L., Andreani, M., Barford, C., Galanter, M., Bolhlke, J.K., 2001b. A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. Anal. Chem. 73, 4145–4153.
Snider, D.M., Spoelstra, J., Schiff, S., Venkiteswaran, J.J., 2010. Stable oxygen isotope ratios of nitrate produced from nitrification: 18O-labeled water incubations of agricultural and temperate forest soils. Environ. Sci. Technol. 44, 5358–5364. https://doi.org/10.1021/es1002567.
Spoelstra, J., Schiff, S.L., Hazlett, P.W., Jeffries, D.S., Semkin, R.G., 2007. The isotopic composition of nitrate produced from nitrification in a hardwood forest floor. Geochim. Cosmochim. Acta 71, 3757–3771.
Talib, S.H.A., Yusoff, M.S., Hasan, Z.A., Ismail, W.R., Abustan, M.S., 2016. Nutrient concentration distribution in sediment and overlying water at Bukit Merah Reservoir Perak. MATEC Web Conf. 47, 05004. https://doi.org/10.1051/matecconf/20164705004.
Teo, F.Y., Md. Noh, M.N., Ab. Ghani, A., Zakaria, N.A., Chang, C.K., 2017. River sand mining capacity in Malaysia. Proceedings of the 37th IAHR World Congress August 13–18, 2017. Kuala Lumpur, Malaysia.
Thornton, J., Steel, A., Rast, W., 1996. Chapter 8 Reservoirs (Chapman, D.V. (ed.). in Water Quality Assessments: A guide to use Biota, Sediments and Water Environmental Monitoring. Second Edition. UNESCO, WHO, and UNEP. E & FN Spon, London, UK.
UNEP, 2016. UNEP: A Snapshot of the World's Water Quality: Towards a Global Assessment, available at http://www.unep.org/publications/ (accessed: 18 April 2019).
Wang, M., Lu, B., Wang, J., Zhang, H., Guo, L., Lin, H., 2016. Using dual isotopes and a bayesian isotope mixing model to evaluate nitrate sources of surface water in a drinking water source watershed, East China. Water 8 (355), 1–16. https://doi.org/10.3390/w8080355.
Wang, S., Zhang, M., Chen, F., Che, Y., Du, M., Liu, Y., 2015. Comparison of GCM-simulated isotopic compositions of precipitation in arid central Asia. J. Geogr. Sci. 25, 771783.
Wassenaar, L.I., Coplen, T.B., Aggarwal, P.K., 2014. Approaches for achieving long-term accuracy and precision of $\delta^{18}O$ and $\delta^{2}H$ for waters analyzed using laser absorption spectrometers. Environ. Sci. Technol. 48, 1123–1131.
Winton, R.S., Calamita, E., Wehrli, B., 2019. Reviews and syntheses: dams, water quality and tropical reservoir stratification. Biogeosciences 16, 1657–1671.
Xia, X., Zhang, S., Li, S., Zhang, L., Wang, G., Zhang, L., Wang, J., Li, Z., 2018. The cycle of nitrogen in river systems: sources, transformation, and flux. Environ. Sci. Process. Impacts 20 (6), 857–990.
Xu, S., Kang, P., Sun, Y., 2016. A stable isotope approach and its application for identifying nitrate source and transformation process in water. Environ. Sci. Pollut. Res. 23, 1133–1148. https://doi.org/10.1007/s11356-015-5309-6.
Xue, D.M., 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.
Yaccob, N.S., Syakir, M.I., Muhammad, S.A., Hashim, Z.H., Rahman, A.A., Fadhlullah, W., 2017. Water quality assessment of Bukit Merah Reservoir, Perak. In: Proceedings of the 5th ICERT 2017, pp. 91–96.
Yevenes, M.A., Soetaert, K., Mannaerts, C.M., 2016. Tracing nitrate-nitrogen sources and modifications in a stream impacted by various land uses, south Portugal. Water (Switzerland) 8 (9). https://doi.org/10.3390/w8090385.
Yue, F.J., Li, S.L., Jing, H., 2015. The contribution of nitrate sources in Liao Rivers, China, based on isotopic fractionation and Bayesian mixing model. Proc. Earth Planet Sci. 13, 16–20.
Yue, F., Liu, C., Li, S., Zhao, Z., Liu, X., Ding, H., Liu, B., 2014. Analysis of $\delta^{15}N$ and $\delta^{18}O$ to identify nitrate sources and transformations in Songhua River, Northeast China. J. Hydrol. 519, 329–339. https://doi.org/10.1016/j.jhydrol.2014.07.026.
Zakeyuddin, M.S., Md Sah, A.S.R., Hashim, Z.H., Puteh, K., Mohammad, S., Omar, W. M.W., 2014. The effect of seasonal changes on freshwater fish assemblages and environmental factors in Bukit Merah Reservoir (Malaysia). Transylv. Rev. Syst. Ecol. Res. 16 (1), 97–108.
Zakeyuddin, M.S., Md Sah, A.S.R., Mohammad, M.S., Fadzil, N.F.M., Hashim, Z.H., Omar, W.M.W., 2016. Spatial and temporal variations of water quality and trophic status in Bukit Merah Reservoir, Perak. Sains Malaysiana 45 (6), 853–863.
Zhang, M., Zhi, Y., Shi, J., Wu, L., 2018. Apportionment and uncertainty analysis of nitrate sources based on the dual isotope approach and a Bayesian isotope mixing model at the watershed scale. Sci. Total Environ. 639, 1175–1187.
Zorzal-Almeida, S., Salim, A., Andrade, M.R.M., Nascimento, M.A.N., Bini, L.M., Bicudo, D.C., 2018. Effects of land use and spatial processes in water and surface sediment of tropical reservoirs at local and regional scales. Sci. Total Environ. 644 (2018), 237–246. https://doi.org/10.1016/j.scitotenv.2018.06.361.