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Nitrogen and oxygen isotope measurements of nitrate to survey the sources and transformation of nitrogen loads in rivers

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Abstract This paper reviews the studies on evaluation of river environments in terms of water pollution, ecosystem disturbances, excess nutrient (nitrogen) loads, and developments in the isotopic measurements of nitrate and present an update and future perspectives regarding the application of nitrate isotopes to river nutrient assessments. Then, we present the advantages of simultaneous measurement of the nitrogen and oxygen isotopes of nitrate in streamwaters. Dual isotope measurement has recently been used to identify the sources and paths of nitrogen loading in several stream systems. The most recently developed high-resolution denitrifier method is a promising tool with which to investigate the detailed spatial and temporal variation and mechanisms of nitrogen loading and transformation in rivers.

Key words nitrate, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, river environment, nutrient status, environmental assessment

INTRODUCTION

Although nitrogen is an essential nutrient in various biomes and ecosystems, excess nitrogen can act as a pollutant, seriously disturbing natural ecosystems. Atmospheric deposition is a major pathway for the loading of anthropogenic inorganic nitrogen into terrestrial ecosystems (Galloway et al. 1995; Galloway and Cowling 2002). In addition, excess nitrogen fertilizer and wastewater discharged from farms, livestock facilities, and residential areas are major direct inputs of nitrogen into aquatic ecosystems. These often constitute non-point sources of nitrogen loading into river systems. Excess nitrate (NO_3^-) and ammonium (NH_4^+) loads often cause the eutrophication of rivers, lakes, and reservoirs, accompanied by extreme algal blooms (Reynolds and Descy 1996).

A load factor method can be often used to estimate practical loads, although there are many uncertainties in the calculation of load factors for various land uses. In general, the nitrogen load in rivers is affected by both terrestrial factors (e.g., hydrology, geomorphology, and land use) as sources and in-stream biogeochemical processes. To estimate the load from each land use and elucidate the in-stream biogeochemical processes in river systems, mass balance investigations use the concentrations of various nitrogen compounds and discharge rate. In addition, models have been developed to describe these mass balances (Allan 1995; Kalff 2001; Ohte et al. 2007).

The isotope components of nitrogen compounds constitute a powerful resource that can provide more detailed insight into the nitrogen dynamics in a catchment; they can reduce uncertainty in a mass balance model performance because they can be used to

trace the nitrogen sources and sinks and to retrieve the transformation processes. The $\delta^{15}\text{N}$ of some nitrogen compounds reflects their source, isotopic fractionation resulting from physical and chemical reactions, and biological reactions and functions such as uptake, nitrification, denitrification, and assimilation in food webs. In addition to them, the stable isotopes of associated elements of nitrogen compounds, such as the ^{18}O of NO_3^- , can also be used as a powerful tracer. For instance, the $\delta^{18}\text{O}$ value of NO_3^- differs significantly between one generated biologically in soils and/or aquatic system and one formed atmospheric chemically in upper-air (Kendall 1998).

Therefore, the spatial and temporal variation in isotope signals, especially multi isotope signatures, can provide insightful information on nitrogen cycles, biological usage, and exports from ecosystems. This is also meaning that the isotope tracer techniques have been useful for an assessment on the anthropogenic nitrogen pollution in rivers and lakes. As we mentioned later, the applications of multi isotope technique to trace the nitrogen transport and transformation have been conducted for scientific researches since 1990's. The multi isotope tracer techniques, however, has not often applied on an operational assessments and survey of river environments.

The purposes of this paper are to briefly review the developments in the isotopic measurements of nitrate and present an update of this field. We then introduce the novel techniques for simultaneous measurements of nitrogen and oxygen isotopes of dissolved nitrate that has recently been developed and improved, and demonstrate a case study surveying the river nutrient status in a Japanese watershed using this method. Then, we address the future perspectives regarding the application of nitrate isotopes to river nutrient assessments.

METHODS FOR THE ISOTOPE MEASUREMENT OF NITRATE

The ammonium distillation method was generally used to measure the nitrogen isotopes of nitrate in natural waters before the mid-1990s. This method involves the reduction of nitrate to ammonium, which is distilled and concentrated as an ammonium sulphate salt and then combusted to produce nitrogen gas for isotope measurement using a mass spectrometer. Sigman *et al.* (1997) modified the ammonium diffusion method, which was formerly used for the tracer-level measurements of nitrogen isotope ratio. Dissolved nitrate is reduced to ammonium in a closed bottle, and the gaseous ammonia is trapped by a glass fiber filter impregnated with acid solution. The nitrogen isotope ratio of the ammonium ions on the filter is measured using an isotope ratio mass spectrometer connected to an elemental analyser (EA-IRMS) through a continuous flow system. This method is more accurate and facilitates sample preparation.

In the 2000s, Silva *et al.* (2000) proposed a new method using ion exchange resin. In this method, nitrate ions in a water sample are concentrated by adsorption on an anion exchange column. The adsorbed nitrate ions are removed from the resin by adding hydrochloric acid. This solution is neutralized using silver oxide, and the precipitated silver nitrate is extracted by freeze dehydration. Finally, the $\delta^{15}\text{N}$ of the silver nitrate is measured using an EA-IRMS. It is relatively easy to prepare a sample for isotope measurements using this method. Moreover, the concentration of the sample using an ion exchange column in the field after collection eliminates the need to bring a large volume of water to the laboratory. Chang *et al.* (1999) proposed a

method to determine the $\delta^{18}\text{O}$ of nitrate in fresh water using an ion exchange column.

Compared to these methods, the new “denitrifier method,” which can be used to measure the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate simultaneously, has many advantages (Sigman et al. 2001; Casciotti et al. 2002). The dissolved nitrate is reduced in a glass vial by denitrifying bacteria, which cannot reduce nitrous oxides gas (N_2O) to nitrogen gas (N_2). The nitrous oxide gas is input to a mass spectrometer for isotopic measurement through a continuous flow system. To produce N_2O gas from dissolved NO_3^- in a sample, the other reducing methods have also recently been developing using UV light or spongy cadmium for the reaction from NO_3^- to NO_2^- and using azide or hydroxylamine for NO_2^- - N_2O reduction (McIlvin and Altabet 2005).

The most critical advantage of the denitrifier method is that the required sample volume is less than 1/100 of that needed by the previous method. This feature is critically important for samples that contain a very small amount of nitrate. This method has made it possible to measure nitrate isotopes in samples such as ice cores, soil pore water, and even plant xylem water that could not be analysed using other methods. The denitrifier method can also be used for seawater because it is insensitive to the ionic strength of the solution. Moreover, whereas dissolved organic nitrogen can act as a contaminant in nitrogen isotope measurements using the previous method, it does not interfere with measurements performed using the denitrifier method because the bacteria only denitrify nitrate. The sample preparation is markedly faster than that for the previous method. However, this method has several weaknesses: the storage and maintenance of the denitrifying bacteria requires some special techniques, and the presence of ^{17}O in nitrate molecules, especially in rainwater, interferes with the $\delta^{15}\text{N}$ measurement of N_2O . The denitrifier method cannot be used to measure the $\delta^{15}\text{N}$ of nitrate and nitrite separately, but measures the total $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate and nitrite in the solution. Therefore, when the nitrite concentration is high relative to nitrate, it is necessary to check the measured $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values carefully. To measure $\delta^{18}\text{O}$, it is better to measure nitrate and nitrite separately because it is important to determine whether nitrate or nitrite is the dominant supplier of ^{18}O .

ADVANTAGES OF THE SIMULTANEOUS MEASUREMENT IN NITRATE $\delta^{15}\text{N}$ AND $\delta^{18}\text{O}$

Advantages of the Simultaneous Measurement of Nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$

In temperate climates, nitrate is a relatively mobile form of nitrogen in soil systems because of its solubility and inabsorbability, and it is the major form of dissolved nitrogen in streams and rivers, except in heavily reduced aquatic systems. Therefore, nitrate tracing is a useful approach by which to understand nutrient dynamics and transport in aquatic systems in temperate climate. However, there is no remarkable difference in the $\delta^{15}\text{N}$ of nitrate between precipitation and fertilizer (Kendall 1998). In addition, the $\delta^{15}\text{N}$ of soil water nitrate is often similar to that of precipitation.

As mentioned previously, the $\delta^{18}\text{O}$ measurement of nitrate was developed in the late 1980s and has been used to reveal new characteristics of nitrate in various environments (Amberger and Schmidt 1987; Voerkelius 1990; Aravena et al. 1993). Kendall compiled the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate in precipitation, soil water, river and lake water, and groundwater from these studies and showed that the range of $\delta^{18}\text{O}$ in precipitation is very large compared to that in other sources of water. Possible

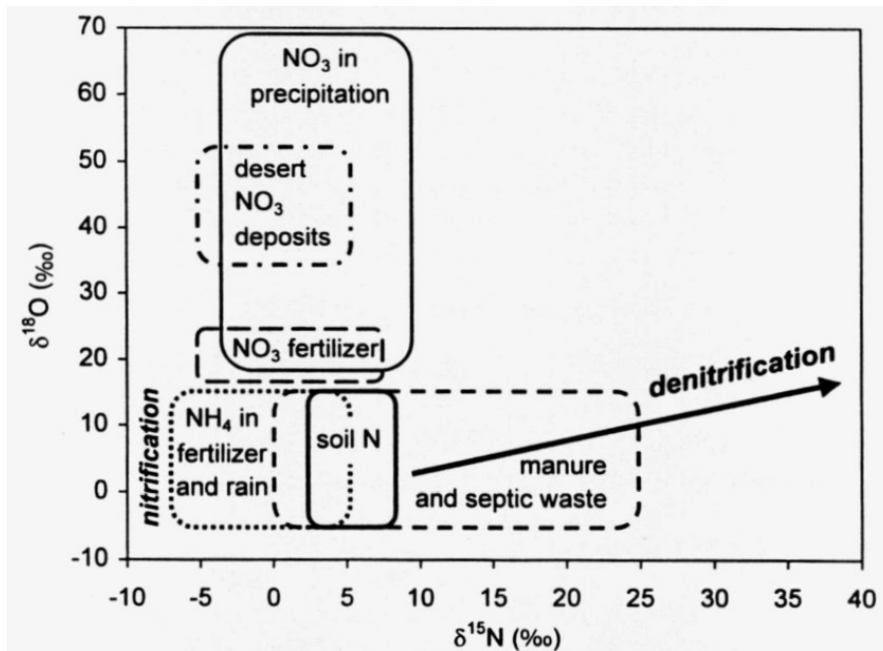


Fig. 1 Schematic diagrams of typical ranges of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of nitrate from various sources (presented originally in Figure 1) drawn by Kendall (1998). Nitrification of ammonium and/or organic-N in fertilizer, precipitation, and organic waste can produce a large range of δ values. Soil waters tend to have higher NO_3 - $\delta^{18}\text{O}$ values, and a larger range of NO_3 - $\delta^{18}\text{O}$ values, than groundwaters because of the higher $\delta^{18}\text{O}$ values of O_2 and/or H_2O in soils.

mechanisms for this large range in $\delta^{18}\text{O}$ involve various geochemical and atmospheric processes. Fractionation associated with nitrate formation occurs during thunderstorms, in the incomplete combustion of fossil fuels in power plants and vehicle exhaust, and in atmospheric photochemical reactions. A likely mechanism for the high $\delta^{18}\text{O}$ of nitrate is the reaction of ozone (O_3) with nitrogen gas (N_2), producing NO_x (Wahlen and Yoshinari 1985; Krankowsky et al. 1995).

Using this characteristic of $\delta^{18}\text{O}$, the nitrate supplied by precipitation can be distinguished from the nitrate produced by microbial activity in soils or added to soil as fertilizer, whereas this separation cannot be made using $\delta^{15}\text{N}$. Kendall (1998) summarized previous reports, and proposed a diagram of the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ ranges of major sources of nitrate (Fig. 1). Denitrification causes both the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of the residual nitrate to increase as the nitrate concentration decreases. Therefore, this diagram is useful to examine not only the origins of nitrate, but also the strengths of the processes involved in nitrogen transformation.

Studies have evaluated the contribution of atmospheric nitrate to stream nitrate in North America using the $\delta^{18}\text{O}$ of nitrate (Williard et al. 2001; Burns and Kendall 2002; Campbell et al. 2002). The samples are typically collected at intervals of several weeks or months, and the seasonal changes in the contribution of atmospheric nitrate to stream nitrate are discussed.

Ohte et al. (2004) measured both the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of stream nitrate at a very high frequency using the denitrifier method (Fig. 2). The greatest advantage of the

denitrifier method is its ability to measure the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate simultaneously and its high throughput. Fluctuations in the concentration and $\delta^{18}\text{O}$ of nitrate in streams indicated that atmospheric nitrate made a strong, direct contribution during the early snowmelt period in a forested watershed in the northeast United States as a result of highly concentrated nitrate stored in the snow pack during the winter. Another advantage of the denitrifier method is that it requires only a small amount of nitrate (several tens of nanomoles). Thus, Elliott et al. (2007) were able to measure the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of rainwater collected from across the United States and showed clear geographical patterns depending on the NO_x source such as stationary fuel combustion at electric power plants and vehicular emissions.

CASE STUDY ON TWO RIVERS IN THE LAKE BIWA BASIN

We have been evaluating the “environmental health” of a river basin in central Japan since 2003 using multiple isotope techniques. In this extensive investigation, we have

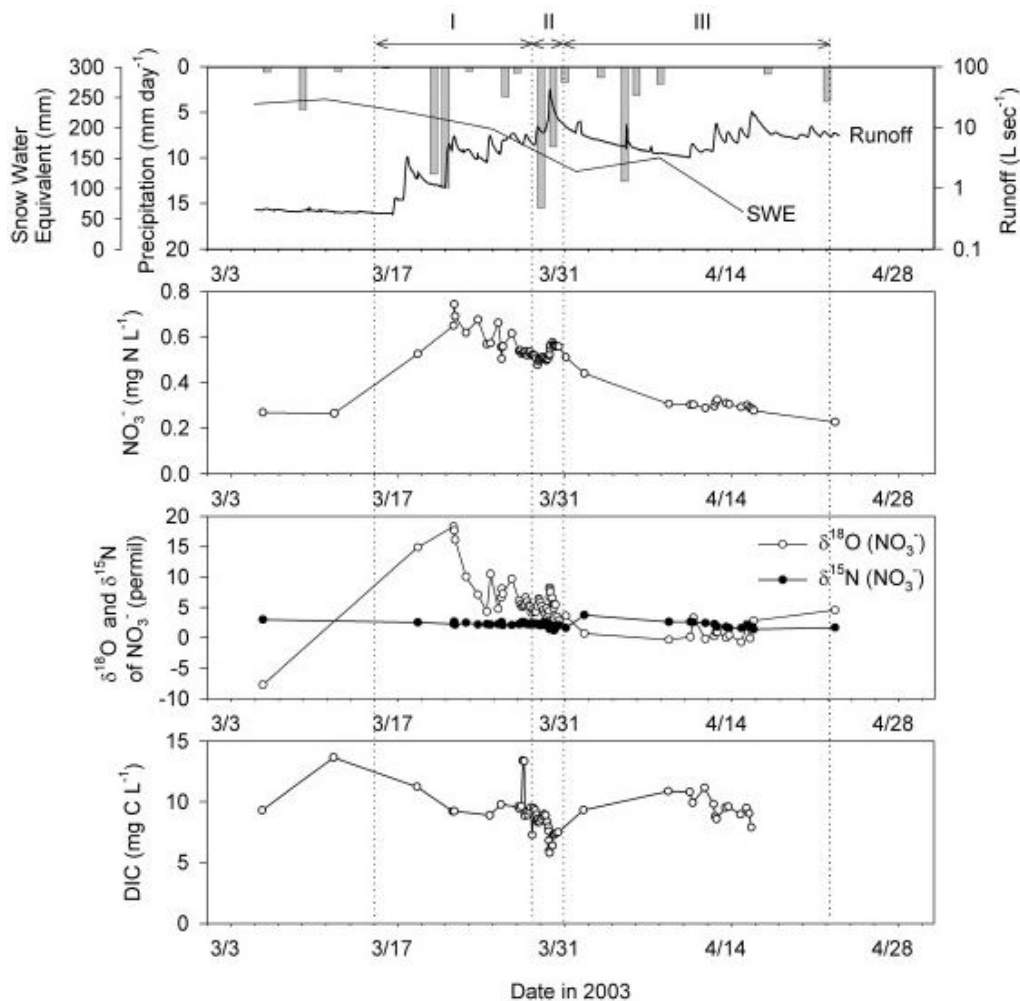


Fig. 2 Precipitation, runoff, snow water equivalent (SWE), nitrate concentration, $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of nitrate, and DIC concentration during snowmelt 2003. Phase I was the early snow melt, 16 to 29 March, Phase II was the peak flow period, 29 to 31 March, and Phase III was 1 to 22 April. Precipitation includes rain and snow that fell in this period (Modified from Ohte et al. (2004)).

examined 32 rivers in the Lake Biwa basin. For these rivers, we are attempting to find useful isotope information to describe the water quality (pollution status), nutrient conditions, and ecological status and to propose indexes for integrated “health” evaluation. Here, we introduce some new findings from this project and state our perspectives for the future.

Lake Biwa is the largest freshwater lake in Japan (670 km²). It has more than 32 inflow rivers, but only one outflow river. The Lake Biwa basin is located in the warm temperate climate region. Details of its climatic and hydrological features are described by Suzuki and Fukushima (1985). The basin has forests in the northern and western parts, agricultural land (mainly rice paddies) in the eastern part, and urban areas in the southern part.

We have investigated the changes in the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate between rivers with and without anthropogenic nitrogen inputs (Fig. 3). The Yasu River basin contains an agricultural region that consists mainly of paddies and residential areas in the middle to downstream parts, whereas forest dominates the Ado River basin. The nitrate

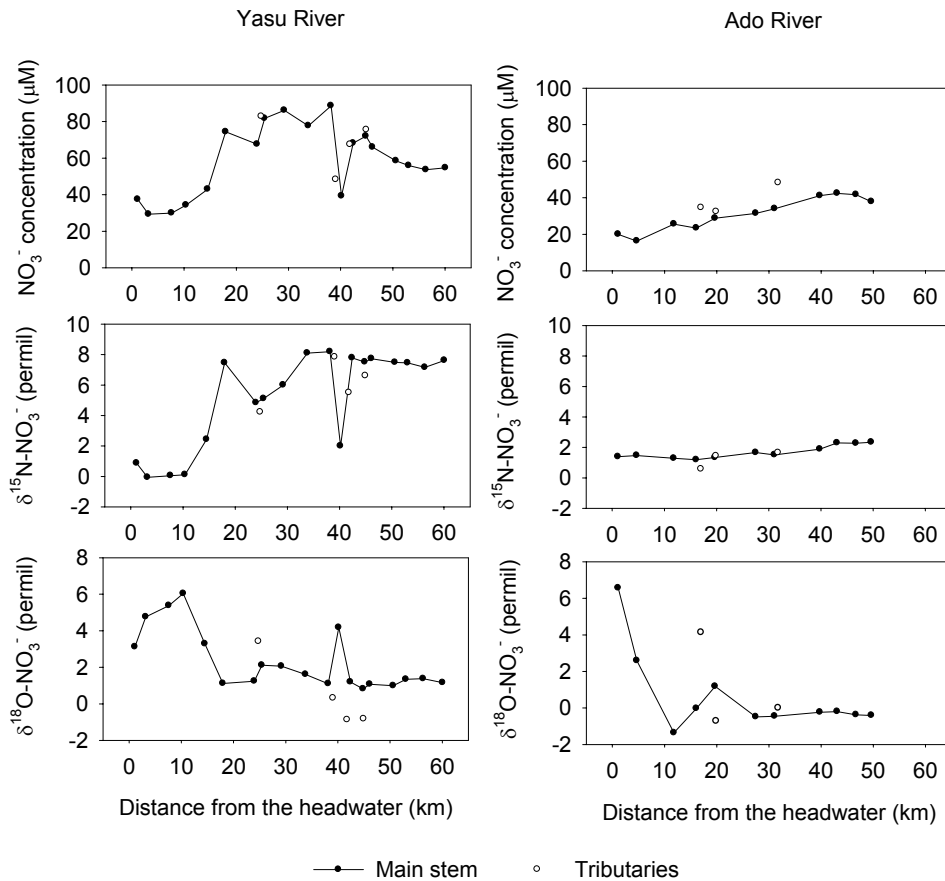


Fig. 3 Comparison of the changes in the concentration, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ of nitrate between rivers with (Yasu River) and without anthropogenic nitrogen inputs (Ado River). The Yasu River basin contains an agricultural region that consists mainly of paddies and residential areas in the middle to downstream parts, whereas forest dominates the Ado River basin (Nagata et al., in preparation).

concentration and $\delta^{15}\text{N}$ increased slightly downstream in the Ado River, whereas both increased dramatically in middle portion of the Yasu River. The $\delta^{15}\text{N}$ of organic matter also increased in the riverbed sediments. This clearly indicates the marked effects of the inflow of wastewater from agricultural fields and residential areas. In contrast, the $\delta^{18}\text{O}$ of nitrate in the Yasu River was higher (+4 to +7‰) upstream and decreased to +1 to +2‰ as the $\delta^{15}\text{N}$ increased downstream. This phenomenon may be explained in the same manner as the relatively high $\delta^{18}\text{O}$ in river water from forest-oriented watersheds. It may be reasonable for stream water in upstream forested catchments in relatively steep mountainous areas to contain more atmospheric nitrate than that drained from agricultural or urbanized regions because forested watersheds act as natural storage systems.

FUTURE PERSPECTIVES

Although conventional monitoring of discharge, water quality, and biotic communities of rivers has generated useful diagnostic information, this approach is costly.

Expensive instruments and intensive maintenance and labour are required to obtain high-quality data with sufficient spatiotemporal resolution. Despite the high cost, these data alone provide little information on the sources of water and materials, and even less on the geochemical and ecosystem processes that control material cycling and affect food webs. The use of multiple stable isotope ratios of water, nutrients, and organisms provides integrated diagnostic information in a timely, cost-effective fashion and overcomes the serious deficiencies of conventional approaches. One great advantage of the multiple stable isotope approach is that it combines the benefits of robust principles and a methodology that has already been developed in individual disciplines, including watershed hydrology, geochemistry, and community ecology. In addition, recent advances in mass spectrometry allow high-throughput measurements at a much lower cost than previously. The intensive monitoring of multiple environmental isotopes has become a practical option to be implemented in watershed diagnosis.

We expect that the carefully coordinated monitoring of multiple stable isotope ratios in a given watershed can provide comprehensive information on: the sources and flow paths of water and nutrients, especially nitrogen compounds; various metabolic parameters of ecosystems; and the energy base and food web structure of aquatic communities, depending on the watershed type and scale. These new data should supplement conventional data and aid in their interpretation to greatly enhance the ability to detect human-induced changes, as well as the incipient of changes of complex watershed systems.

Although in this paper we did not discuss the use of stable isotopes for food web analysis in river ecosystems, food web analysis is an indispensable part of any integrated assessment of ecosystem health, together with an investigation of nutrient dynamics using isotope tracing. For instance, Cabana and Rasmussen (1996) conducted a study based on this concept and developed a conceptual model to express the spatial distribution of the $\delta^{15}\text{N}$ of various aquatic organisms in rivers and lakes as a function of population density, which is a proxy of the strength of anthropogenic nitrogen loads. In addition, the preliminary results of our project suggest that the $^{15}\text{N}/^{14}\text{N}$ ratios of sediments and fish are an excellent indicator of the perturbation of the

nitrogen cycling and food webs of river communities. In future, efforts to establish protocols for the integrated evaluation of river ecosystem health are needed. These protocols will involve the use of multiple isotope signatures both to trace nutrient cycles and to examine the food web structure.

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