I. Introduction

Lake water is clearly different from river water and groundwater in that the water body is stagnant and contains a high degree of biological activity because of its exposure to insolation. For this reason, lake nutrient levels are readily increased, and the composition of sediment cores, which reflects past environmental settings and climate modes, is generally well preserved under the relatively stable conditions and low levels of disturbance within lake settings.

Lakes possess a wide range of environmental characteristics, and are mainly classified according to their origin or the characteristics of biological productivity. Increasing numbers of studies have recently used stable isotopes of nitrogen ($^{15}$N) and carbon ($^{13}$C) to clarify the source of nutrients in lake water and sediments, the structure and energy flow of food webs, the bioaccumulation and biomagnification of hazardous chemicals in aquatic animals, and temporal trends in lake environments. In lake research in particular, the analysis of $^{15}$N and $^{13}$C is a powerful tool in analyzing the structure of the food web and energy flows, as well as the bioaccumulation and biomagnification of toxic chemicals in aquatic animals. Furthermore, the integration of data on food webs, stable isotope ratios, and environmental geochemistry can be useful in evaluating the risks of exposure to environmental contaminants. It is hoped that further research on lake environments using stable isotopic methods can be performed with the aim of conserving water quality and aquatic ecosystems.

II. Identifying the sources of dissolved nitrogen

Clarifying the source of nutrient pollution is...
the first step in the remediation of eutrophication in aquatic ecosystems. The stable isotope ratios of nitrogen ($^{15}$N/$^{14}$N; $\Delta^{15}$N) dissolved in lake water provide useful information regarding the nitrogen source and the biogeochemistry of the lake ecosystem. The isotopic ratios of nitrogen, expressed in delta notation, are calculated as follows:

$$\Delta^{15}N = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad (1)$$

where $R_{\text{sample}}$ is the raw ratio of heavy to light isotopes in the sample and $R_{\text{standard}}$ is the raw ratio of heavy to light isotopes of atmospheric nitrogen as an internationally accepted standard. (In the case of carbon isotope ratios ($^{13}$C/$^{12}$C; $\Delta^{13}$C), $R_{\text{standard}}$ is the raw value of $^{13}$C/$^{12}$C in Peedee belemnite.)

Organisms preferentially use the lighter isotopic species because of the lower energy "costs" associated with breaking the bonds in these molecules; this results in significant fractionation between the substrate (relatively heavy) and the biologically mediated product (relatively light) (Kendall, 1998). Water--dissolved N that originates from inorganic N fertilizers installed from atmospheric N2 records a $\Delta^{15}$N value in nitrate (NO$_3^-$) of between −7.4 and 6.8 $\delta$ (Ministry of the Environment, 2002); the biomass of N2--fixing plants and microorganisms also have $\Delta^{15}$N values of around zero (Wada and Hattori, 1976; Peterson and Fry, 1987). In contrast, NO$_3^-$ that originates from organic fertilizers such as manure has higher $\Delta^{15}$N values (Jin et al., 2004), and NO$_3^-$ derived from treated sewage has a $\Delta^{15}$N value of between 8 and 15 $\delta$ (Ministry of the Environment, 2002). Denitrification and ammonia volatilization result in isotope enrichment of the residual nitrate and ammonia, respectively.

The general hydrological budget for a lake system is written as

$$\Delta V = P + S_i + G_i - ET - S_o - G_o \quad (2)$$

where $\Delta V$ is the change in storage; $P$ is precipitation; $S_i$ is surface inflow, including any overland runoff; $G_i$ is groundwater inflow; ET is evapotranspiration; $S_o$ is surface outflow; and $G_o$ is groundwater outflow (Walker et al., 1998). Accordingly, lake water consists of groundwater, surface flow, precipitation, evapotranspiration, and biogeochemical processes within the lake basin.

The $\Delta^{15}$N value of NO$_3^-$ is not an accurate indicator of the source of N in lakes because NO$_3^-$ that originates from fertilizers and that exists naturally within the soil in particular may become isotopically enriched within groundwater and rivers before reaching the lake basin (Townsend–Small et al., 2007); biogeochemical factors might also affect $\Delta^{15}$N values during the residence period. Accordingly, few studies have employed $\Delta^{15}$N as a tool in identifying the origin of NO$_3^-$ in lake water.

Townsend–Small et al. (2007) investigated the origin of NO$_3^-$ in Lake Taihu, a hyper-eutrophic lake in eastern China, as well as the major inflow rivers. The authors collected lake and river water samples during the winter and spring of 2004 to analyze the concentration and $\Delta^{15}$N value of NO$_3^-$. Their results indicate that the major sources of summertime NO$_3^-$ in the lake watershed were the seasonal application of fertilizer and rainwater. In the winter months, sewage was the major source of NO$_3^-$ to urbanized areas of the lake. Isotopic techniques can therefore be used to identify the seasonal N contributions of different sources to aquatic ecosystems, especially when considered in the context of additional nutrient monitoring and flux analyses.

### III. Food web analysis

There is considerable interest in the use of
the stable isotope ratios of nitrogen and carbon in evaluating the structure and dynamics of ecological communities (e.g., Peterson and Fry, 1987; Kling et al., 1992; France, 1995; Vander Zanden et al., 1999a; Post et al., 2000; Beaudoin et al., 2001). The stable isotope ratios of organisms provide an averaged “picture” of feeding habits over significant periods of time corresponding to the turnover times of organic carbon or nitrogen; in contrast, traditional stomach content analysis provides just a “snap-shot” of the feeding habits of organisms (Fry and Arnold, 1982).

The use of stable isotope analysis in studies of animal ecology is founded on two central assumptions: (1) stable isotope ratios in consumers are proportional to those in their putative diet, and (2) differences in isotope ratios exist among the food sources available for consumers (Jardine et al., 2006). Carbon isotope shows considerable isotopic change during fixation by primary producers, while nitrogen isotope shows considerable change when processed by consumers; therefore, the combined analysis of these two isotopes enables the study of the different energy flow processes that shape the structure and function of food webs (Jardine et al., 2006). Unfortunately, it is difficult to capture the complexity of trophic interactions in ecological communities and details of omnivorous feeding behavior (Paine, 1988; Polis and Strong, 1996; Persson, 1999; Vander Zanden and Rasmussen, 1999) and to solve the problem of how to track energy or mass flow through ecological communities if all trophic links are to be of equal importance (Paine, 1988; Hairston and Hairston, 1993; Polis and Strong, 1996; Persson, 1999; Vander Zanden and Rasmussen, 1999).

The analysis of $^{15}\text{N}$ provides an alternative approach to conventional methods of determining the trophic level of consumers (e.g., Cabana and Rasmussen, 1994). The $^{15}\text{N}$ value of aquatic primary producers depends on the source of inorganic nitrogen, and the ratio tends to be enriched by about 3.0 $\delta$ 2.6 $\delta$ from diet to consumer (DeNiro et al., 1981) because of the preferential excretion of lighter isotope through metabolic processes.

$^{13}\text{C}$ can be used to distinguish between different food sources in aquatic ecosystems (Fry and Sherr, 1984). In lakes, $^{13}\text{C}$ is useful for discriminating between two major sources of available energy: littoral (near-shore) production from attached algae and detritus, and pelagic (open-water) production from phytoplankton. This distinction is possible because the $^{13}\text{C}$ of the base of the littoral food web tends to be enriched in $^{13}\text{C}$ (more positive $^{13}\text{C}$) relative to the base of the pelagic food web (France, 1995). The $^{13}\text{C}$ signatures of consumers are not statistically different from those of their food (mean difference, 0.8 $\delta$ 1.1 $\delta$) (DeNiro et al., 1978), or are slightly enriched in heavy isotope (mean difference within $\delta$ 2 $\delta$) (Orr et al., 2006).

Biota in aquatic food webs that rely on terrestrial organic carbon have a $^{13}\text{C}$ signature of approximately $-28\delta$ or are slightly enriched, because most terrestrial (C$_3$) plants have a $^{13}\text{C}$ signature of approximately $-28\delta$. In contrast, aquatic primary producers may exhibit a broad range of $^{13}\text{C}$ values (e.g., from approximately 1 $\delta$ to $-28\delta$) depending on the dissolved inorganic carbon source, whether it be the decomposition of terrestrial detritus or the weathering of limestone (Orr et al., 2006). Given that the obtained values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ might partly reflect the method of analysis and dietary differences, the utility of stable isotope methods can be improved if this information is incorporated into studies of trophic relationships (Vander Zanden and Rasmussen, 2001; Post, 2002; McCutchan et al., 2003).
The recent use of primary consumers over primary producers as a baseline of each food web has led to the modification of the following equation (Jardine et al., 2006):

$$\text{TL}_{\text{consumer}} = \frac{(\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{primary producer}})}{\delta^{15}N + 1}$$

(3)

where TL is “trophic level”, $\delta^{15}N_{\text{primary producer}}$ is assumed to occupy TL1, and $\delta^{15}N$ (the “enrichment factor”) represents the increase in $\delta^{15}N$ from one TL to the next. Following this modification, the trophic levels of organisms in the food web are now typically calculated according to

$$\text{TL}_{\text{organism}} = \frac{(\delta^{13}N_{\text{organism}} - \delta^{13}N_{\text{baseline}})}{\delta^{13}N + 2}$$

(4)

where $\delta^{13}N_{\text{baseline}}$ is the measured $\delta^{13}N$ of a long-lived primary consumer (TL = 2). A value between 2 and 5 is often assigned to $\delta^{13}N$, although this may vary depending on the types of organisms within the food web; this matter is the subject of some debate (Jardine et al., 2006).

For cases in which there are two potential food sources, each with a distinct isotope ratio, estimates of the proportional contribution of C from each food source to the growth of a consumer are based on the following two-source mixing model:

$$k_{\text{carbon}} = 1 - \frac{\delta^{13}C_A - \delta^{13}C_{\text{consumer}} + \delta^{13}C_{B}}{\delta^{13}C_A - \delta^{13}C_B}$$

(5)

where $\delta^{13}C_A$ and $\delta^{13}C_B$ are the isotope ratios of the potential food sources, $\delta^{13}C_{\text{consumer}}$ is the isotope ratio of the consumer, $\delta^{13}C_C$ is the trophic shift for C (typically assumed to be 0.0), and k is the proportional contribution of source A to the growth of the consumer (McCutchan et al., 2003). For three important food sources, the relative contributions are estimated following the assumptions of a three-source mixing model (Peterson and Howarth, 1987; Phillips, 2001).

In Lake Baikal, Russia, there exist two major primary producers–benthic plants and phytoplankton–with contrasting $\delta^{13}C$ values (Yoshii, 1999). In this case, the ratio of food sources of each benthic animal could be calculated by applying the two-source mixing model based on the assumption that the contribution of other sources such as terrestrial plants is negligible.

Applied research also makes use of stable isotope techniques; for example, Vander Zanden et al. (1999b) demonstrated that such techniques are useful in detecting changes in food web structure that occurred with the invasion of two non-native predators, smallmouth bass (Micropterus dolomieu) and rock bass (Ambloplites rupestris), into Canadian lakes. The authors found that the bass-induced shifts in the food web may have severe consequences for native species and ecosystems. Saito et al. (2001) investigated the effects on the reservoir food web of a new temperature control device within a dam reservoir at Shasta Lake, California, USA. The authors measured carbon and nitrogen stable isotope ratios and used a specialized reservoir water quality model to forecast operation-induced changes in phytoplankton production. They also applied a food web–energy transfer model to propagate predicted changes in phytoplankton up through the food web to the predators and sport fishes of interest. According to their results, some consumers were much more sensitive than others to perturbations in the supply of phytoplankton (e.g., bass and rainbow trout), the linked modeling predicted that fish biomass was generally unaffected by the new dam operations.

In general, to obtain the high level of resolution required to discern complex trophic interactions, stable isotopes must be used in conjunction with other data such as direct diet

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analyses.

IV. Indicators of the bioaccumulation and biomagnification of pollutants

Ecotoxicology studies that employ stable isotope analysis can be considered to fall into three general categories: (1) studies of the qualitative linkages between the dietary habits of animal populations and their contaminant concentrations, (2) studies of biomagnification in the food web, and (3) quantitative assessments of habitat-specific foraging as a means of explaining concentrations of biotic contaminants (Jardine et al., 2006). The most powerful studies combine categories 2 and 3, simultaneously assessing the importance of both the length of the food chain and the underlying dietary pathway in determining contaminant concentrations in an organism.

Jardine et al. (2006) reviewed the application, key considerations, and sources of uncertainty of stable isotope analyses in the field of ecotoxicology. Measurements of stable nitrogen and carbon isotopes in aquatic animals can provide valuable information regarding trophic relationships and the bioaccumulation and biomagnification of contaminants (Kidd et al., 1995a; Dominguez et al., 2003). The highest concentrations of contaminants such as heavy metals and/or hazardous organic compounds are commonly found in those fishes that occupy the highest trophic level (Rasmussen and Rowan, 1990; Power et al., 2002).

In 1991, it was reported that the lake trout (Salvelinus namaycush) and burbot (Lota lota) from Lake Laberge, one of the Yukon lakes in Canada, were contaminated with amounts of toxaphene and other lipophilic contaminants that were several times greater than those in the same species from other subarctic and arctic lakes and rivers (Muir et al., 1990; Kidd et al., 1993). Kidd et al. (1995a) analyzed the food-chain organisms, water, and sediments of Yukon lakes for toxaphene to distinguish between the “surreptitious dumping” and “food-chain length” hypotheses. The surface and maximum fluxes of toxaphene in the sediment cores obtained from Laberge, Fox and Kusawa lakes were comparable in these three lakes, similar to those found in other arctic cores and several orders of magnitude lower than the values found in temperate lakes previously treated with toxaphene. The contaminant–${\delta}^{15}$N relations obtained for biota and contaminant concentrations in the sampled water and sediments indicate that a longer than normal food chain is the sole explanation of the elevated toxaphene concentrations in fishes from Lake Laberge.

These results suggest that the highest contaminant concentrations are found in fishes from lakes with exceptionally long food chains and that ${\delta}^{15}$N might be useful as an initial screening mechanism in identifying such lakes. Moreover, some lakes in subarctic regions are contaminated by mercury (Hg) that originated from natural and/or anthropogenic sources via atmospheric transport (Lockhart et al., 1995; Downs et al., 1998), and elevated levels of Hg have been measured in fish from lakes in these regions (Björklund et al., 1984; Håkanson et al., 1990; Bodaly et al., 1993). The high biomagnification rates of subarctic lakes and the heavy reliance of aboriginal North Americans on such lakes makes it necessary to undertake additional studies of the dynamics of the accumulation of organic and inorganic contaminants in the food webs of subarctic lakes.

Among– and within–lake variability in the Hg levels of top consumers is affected by food chain length and individual growth rates and age (Kidd et al., 1995b). Kidd et al. (1995b) examined the relationship between trophic position (as determined by ${\delta}^{15}$N values) and Hg concentrations in fishes.
from six lakes in northwestern Ontario, Canada. The surface areas of these lakes range from 0.88 to 347 km². The dorsal muscle from lake trout (Salvelinus namaycush), burbot (Lota Lota), walleye (Stizostedion vitreum), northern pike (Esox lucius), white sucker (Catostomus commersonii), lake cisco (Coregonus artedii), lake whitefish (Coregonus clupeaformis), and yellow perch (Coregonus artedii) was analyzed for Hg and $^{15}$N. The authors also investigated the potential for $^{15}$N to be used in conjunction with a bioenergetics model (Norstrom et al., 1976) to predict Hg levels in fishes. The results revealed that log Hg was significantly related to $^{15}$N within each lake ($r^2$ ranged from 0.47 to 0.91, $p < 0.01$). For four species (yellow perch, northern pike, lake cisco, and lake trout), log Hg was positively related to $^{15}$N ($r^2$ ranged from 0.37 to 0.47, $p < 0.09$) across all lakes. These results demonstrate that $^{15}$N is a powerful tool in predicting Hg levels in fishes from various trophic positions within a given food web. In this case, the predicted results showed a strong correlation with measured Hg concentrations ($r = 0.91$, $p < 0.01$), indicating that $^{15}$N has the potential to be used in modeling and determining prey Hg levels, and therefore in predicting Hg levels in fishes.

In contrast, Blais et al. (2006) indicated that lake altitude is the best correlate of concentrations of Hg, polybrominated diphenyl ethers (PBDEs), and organochlorine (OC) pesticides in fish, accounting for as much as a 100-fold increase in 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene ($p,p'$-DDE) over the range in altitude from 450 to 2492 m in the French Pyrenees. The results of their study revealed that Hg in fish is enriched in high-elevation lakes to levels that can exceed fish consumption guidelines for humans, and that levels of dichlorodiphenyltrichloroethane (DDT) can exceed the thresholds identified for protecting fish-eating wildlife. The enhanced deposition of Hg into lake sediments at high altitudes has also been shown for lakes in Greenland (Bindler et al., 2001), possibly resulting from higher rates of orographic precipitation, suppressed evaporation due to cold temperatures, or increased photooxidation caused by high UV irradiance at high elevations. There also exists evidence that concentrations of several persistent organic pollutants (POPs) in air show an increase with altitude. Low temperatures and prolonged ice cover in high-altitude lakes may act to retain certain POPs (e.g., Blais et al., 2001).

It has been reported that hazardous elements such as selenium (Se) may be more easily assimilated into the food chains of lentic water bodies than those of lotic water bodies (Canton and VanDerveer, 1997; Lemly, 1999). Orr et al. (2006) sought to explain the occurrence of high Se concentrations in fish from lentic habitats compared with those from lotic habitats in the Elk River watershed, western Canada, including fish from ponds and wetlands. The authors found that the food chain lengths in lentic areas were similar to or shorter than those in lotic areas, thereby contradicting the hypothesis that elevated Se levels are associated with greater food chain length in lentic areas. The lentic environments were associated with elevated concentrations of Se in benthic invertebrates but not in primary producers, indicating that the key process of Se incorporation into the food web occurs via a pathway other than the direct consumption of primary producers. Indeed, this earlier study provides evidence of the importance of sediment–detrital processes that give rise to elevated concentrations in the benthos, probably via the uptake of microorganisms with elevated Se concentrations and/or direct uptake by the benthos of Se from the water (porewater or water...
near the water–sediment interface) or the incidental ingestion of sediment.

Stable isotope analysis is a powerful tool in evaluating the bioaccumulation and biomagnification of pollutants in populations of wild animals. To enhance our understanding of contaminant data, it is necessary that isotope analyses are carried out in tandem with more traditional measures of feeding behavior such as gut content analysis and visual observations. It is also desirable to perform laboratory experiments as required.

V. Paleolimnological studies

Nitrogen and carbon stable isotope ratios in sediment organic matter can be used as proxy indicators of historical changes in the trophic state of lakes, which in turn reflect environmental changes in the catchment and lake basin (e.g., Hollander and Haven, 1992; Wu et al., 2004, 2006). As trophic state increases, the $\delta^{13}C$ values of sediment organic matter also increase (Hodell and Schelske, 1998; Teranes and McKenzie, 1999). High levels of primary production caused by lake eutrophication may lead to depleted levels of dissolved CO$_2$. Under such conditions, phytoplankton show a reduced aversion to heavier $^{13}$CO$_2$, and sinking organic matter is progressively enriched in $^{13}$C.

The $\delta^{15}N$ value of organic matter in sediment can be used to reconstruct past productivity in surface waters because changes in $\delta^{15}N$ may reflect changes in environmental factors such as pH, temperature, species composition, and nitrogen limitation or fixation. Wu et al. (2006) compared the stable isotope signatures ($\delta^{15}N$, $\delta^{13}C$) of four sediment cores that span the entire 20th century with instrumental records of the trophic state of water within Lake Taihu, the third largest freshwater lake in China. Historical changes in the trophic state of lake water within each sub-basin were reconstructed from isotopic data. Despite the complexity of carbon and nitrogen cycles in lakes, the excellent agreement found between the stable isotope signatures and instrumental records for Lake Taihu suggests that $\delta^{13}C$ and $\delta^{15}N$ in sediment organic matter are capable of recording important shifts in the spatial and temporal evolution of the trophic state of lake water.

Herzschuh et al. (2005) inferred the history (45–0 ka BP) of the composition of aquatic vegetation within a shallow alpine lake, Lake Luanhaizi, in the NE Tibetan Plateau based on the frequencies of aquatic plant macrofossils and concentrations of aquatic pollen and algae in the sediments. The authors also measured values of $\delta^{13}C$, C/N, and n–alkane.

Analyses of pollen and plant macrofossils provide information on vegetation assemblages at high taxonomic resolution (Birks, 1993; Battarbee, 2000); however, the species characteristics of seed production and pollen representation mean that it is difficult to determine the quantitative composition of past aquatic vegetation. In contrast, approaches based on bulk organic matter (e.g., $\delta^{13}C$, C/N, biomarkers) enable quantitative estimates of whether planktonic algae, submerged macrophytes, or terrestrial plants made the primary contribution to sedimentary organic matter; however, these methods provide only low taxonomic resolution.

Melles et al. (2007) reported biogeochemical and isotope geochemical data (water content, dry bulk density, total organic carbon (TOC), total nitrogen, total sulfur, biogenic silica (opal) content, and $\delta^{13}C_{TOC}$) for core PG1351 recovered in 1998 from the central part of Lake El'gygytgyn, Siberia, providing a sensitive record of climatic variability in the East Siberian Arctic over the three most recent glacial–interglacial cycles. These data, combined with lithostratigraphic characteristics,
physical properties, and other proxies presented in Nowacyzk et al. (2002) and Melles et al. (2007), were used to infer repeating modes of major environmental change thought to have occurred in the catchment and in the water column of the lake over the past ca. 250 ka BP. The authors identified four depositional units of contrasting lithological and biogeochemical composition, reflecting changes in regional climate patterns.

As described above, $^{15}N$ and $^{13}C$ in sediment organic matter represent useful proxy indicators of historical changes in the trophic state of lake water, the composition of vegetation assemblages in lake basins, and the environmental factors of the catchment. Furthermore, multiple analyses that combine stable isotope ratios with lithostratigraphic data, physical properties, and other proxies provide a wide range of high-resolution data that is useful in paleolimnological studies.

VI. Conclusion

This review paper introduces studies of the water, sediment, and food webs of lake ecosystems that have employed stable isotopic methods. The stable isotope ratios of nitrogen ($^{15}N$) dissolved in water provide useful information regarding the N source and biogeochemistry; however, in the case of lakes, the $^{15}N$ value of NO$_3^-$ is not an accurate indicator of the N source because NO$_3^-$ may become isotopically enriched in groundwater and rivers before reaching the lake and because biogeochemical factors affect the $^{15}N$ values of retained lake water. Isotopic techniques can be used to identify seasonal N contributions from different sources to aquatic ecosystems, especially when considered in the context of analyses of other nutrients and fluxes.

$^{15}N$ and $^{13}C$ in sediment organic matter represent useful proxy indicators of historical changes in the trophic state of lake water, the composition of vegetation assemblages in the lake basin, and the environmental characteristics of the catchment. Furthermore, multiple analyses that combine stable isotope ratios with lithostratigraphic data, physical properties, and other proxies provide a wide range of high-resolution data that are useful in paleolimnological studies. These stable isotopes are powerful tools in analyzing the structure of the food web and energy dynamics. $^{15}N$ provides an alternative to conventional methods employed to determine the trophic level of consumers, while $^{13}C$ can distinguish between different food sources in aquatic ecosystems. In general, to obtain the high level of resolution required to discern complex trophic interactions, stable isotopes must be used in conjunction with other approaches such as direct diet analyses.

Stable isotope analysis can also be used to evaluate the bioaccumulation and biomagnification of toxic chemical compounds such as heavy metals and lipophilic organic chemicals in wild animal populations. Evidence also exists to suggest that pollutants are carried by long-range atmospheric transport to subarctic and/or high-elevation lakes. Hazardous contaminants are bioaccumulated in these regions, meaning that the concentrations of contaminants in fish of the upper trophic level can exceed fish consumption guidelines for humans and the thresholds proposed for protecting fish-eating wildlife. Combined data on food webs, stable isotopes, and environmental geochemistry can be used to evaluate the risks of exposure to environmental contaminants. It is hoped that further studies of lake environments, employing stable isotopic methods, can be carried out to identify effective measures in conserving lake water quality and aquatic ecosystems in the context of environmental conservation and the protection of human health.
Studies of lake environments using stable isotope ratios of nitrogen ($^{15}$N) and carbon ($^{13}$C)

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