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Behavior of Stable Isotope Ratios of Attached Matter in a Drainage Canal

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Abstract

The behavior of stable isotope ratios of attached matter, which consisted of algae and detritus in a reconstructed drainage canal, was analyzed for one year. Stable carbon isotope ratios in most investigation stations increased during the summer. This was attributed to the growth and photosynthetic activity of attached algae. Only one investigation station showed stable carbon isotope ratios that did not peak in the summer. This station was located in an ecologically conserved section with abundant riparian woods remaining. There was no summer peak due to shading by woods that prevented algae from sufficient photosynthesis. We observed that gray water raised stable nitrogen isotope ratios in water and attached matter of the drainage canal. A topographical peak of nitric acid concentration lagged behind the stable nitrogen isotope ratio. Leaching of chemical fertilizers from paddy fields increased the nitric acid concentration, while decreasing the stable nitrogen isotope ratios at the same time.

Discipline: Agricultural environment
Additional key words: ecosystem, food web, paddy field, stable isotope ratio

Introduction

After revision of the Land Improvement Act in 2001, rural ecosystems in Japan became areas to be conserved. Mitigation of impacts that may have been caused by previous construction methods has been attempted since the revision. However, these energetic efforts seem to be insufficient at this stage, because most mitigation has focused on only promotion of fish runs and preparation of only space for organisms. Elimination of physical barriers or obstacles is obviously necessary, but, is not sufficient for mitigation.

If an injured food web in the environment is restored, an ecological pyramid will be able to feed organisms. Designers of land improvement projects must prepare better food resources for conserving organisms. Many organisms including rare species live in Japanese paddy fields[7]. It is urgently necessary to explicate food webs in paddy field ecosystems in order to prepare food resources for animals. This is because 60.1% of paddy fields and incidental canals which have important roles in the paddy ecosystem have already been improved by the Farm Land Consolidation Project[6] without sufficient consideration for the ecosystem. However there is still little knowledge about the ecosystem and food webs in the paddy area.

Stable isotopes provide an effective method to analyze food webs. This method has produced results with enclosed water areas and sea areas in Japan[5,11]. Application of a stable isotope method to a paddy field may generate many benefits. Stable isotope ratios of producers are the most important information for food web analysis, because stable isotope ratios of a producer affect all organisms within the same food web[9]. Therefore, examining the behavior of stable isotope ratios of producers at the periphery of a paddy field is a primary way to spread the use of the stable isotope method in a food web analysis of paddy field ecosystems.

In this study, the behavior of stable isotope ratios of attached matter in a drainage canal that feeds invertebrates and fishes was examined. Stable carbon isotope ratios in most investigation stations increased during the summer. This was attributed to the growth and activation of attached algae.

Analysis of a food web and stable isotope ratio

Some elements have isotopes that don't emit radiation and are called stable isotopes. Carbon-13 and Nitrogen-15 are two such isotopes often used to analyze a

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food web. These isotopes inevitably exist in nature, and also are present in the bodies of organisms. Stable isotope ratios for carbon and nitrogen are expressed as δ^{13}C and δ^{15}N, respectively.

Plants are classified into 3 groups by the photosynthetic mechanism, namely C_3, C_4 and CAM plants. Scarce plant species in the paddy fields of Japan belong to the CAM plant group. A considerable number of plants in the paddy area are C_3 plants, and all trees in forests belong to this group.

C_4 plants are considered to have developed from C_3 plants. Sugarcane (*Saccharum officinarum*), corn (*Zea mays*), eulalia (*Miscanthus sinensis*), crabgrass (*Digitaria ciliaris*), and giant foxtail (*Setaria faberi*) belong to C_4 plants. C_4 plants can often be found in the periphery of a paddy field. They are usually members of Gramineae that are natives of tropical regions. The δ^{13}C values for C_4 plants are approximately −13‰, with a range from −15‰ to −10‰[10]. The δ^{13}C values of organisms are considerably lower than C_4 plants, approximately –27‰, with a range from –35‰ to –25‰[9].

The values for algae in an environment that abounds in CO_2 are between C_3 plants and C_4 plants, at approximately –20‰ as shown by previous investigations[3]. The δ^{13}C of diatoms that were attached on an aerated aquarium were –19‰ (Mori, unpublished). The δ^{13}C values of periphytic diatoms cultured in an estuary of the Natori River were from –18‰ to –11‰[4]. The high values indicated by Itoh[4] may be caused by a low water velocity inhibiting CO_2 exchange from the atmosphere into the aquatic systems.

The type of plants present as producers in a food web can be predicted by analysis of δ^{13}C, because δ^{13}C changes little under prey-predator interactions. Therefore, when the δ^{13}C of an animal is approximately –30‰, the animal is considered to be in a food web derived from C_3 plants.

The δ^{15}N increases approximately 3‰ at every trophic level. This property can provide important information about prey-predator interactions in a food web. The origin of a food web can be predicted from δ^{13}C and a trophic level from δ^{15}N. Stable isotope ratios of producers are the most important data in a food web analysis.

The study site and methods

This study was conducted in *Harakawa* drainage canal (hereinafter called "*Harakawa*"), a main drainage canal in the *Isawa-nambu* Project (National Farm Land Consolidation Project, benefit area = 707 ha). The area is located in Iwate Prefecture, Northern Japan, at 39 degrees north latitude and 141 east longitude. *Harakawa* flows through the center of the area. Locations of the investigation stations and their profiles are shown in Fig. 1 and Table 1, respectively. A section of *Harakawa* including Station 5 was lined with L-type retaining walls and had a soil bottom classified as a silt fraction. Most flow of the canal was monotonous because the canal gradient and the cross-section of the canal were standardized. The biota in this section was poor. Other sections of the canal were lined with dry masonry and had various soil bottoms that contained boulders, gravel, silt, and clay with a steep bed slope and wide canal-width. These characters induced a canal environment with diverse biota.

The *Harakawa* basin was covered with forest in the upper stream of the benefit area with paddy fields in the area, as well as domestic wastewater from Station 2 to

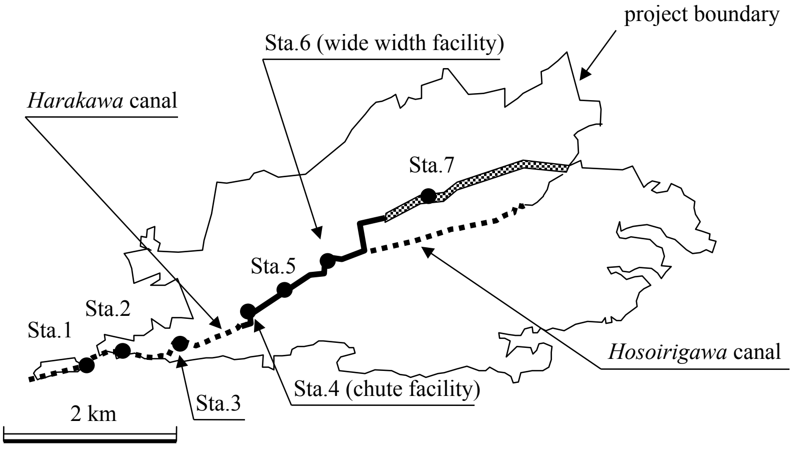

Fig. 1. Location of investigation stations

▪▪▪▪▪ : Concrete lining in the bottom and sides.
▬ : Concrete lining in the sides.
▨ : Conserved section.

Table 1. Profile of investigation stations

Investigation Station	Lining	Profile
Sta. 1	None	Not improved yet.
Sta. 2	Bottom and sides	Starting point of repaired *Harakawa* at the present time. A sample was collected from the concrete sides.
Sta. 3	Bottom and sides	A sample was collected from a pod made of porous concrete for a vegetative restoration test.
Sta. 4	Sides	A chute facility for ecosystem conservation. A steep angle made water velocity rapid. Small rapids and pools were shaped. Attached algae, invertebrates and fishes were abundant.
Sta. 5	Sides	A sample was collected from the sides because no attached matter was on the bottom.
Sta. 6	Sides	A wide section for a fish shelter. Attached algae, invertebrates and fishes were abundant.
Sta. 7	None	A conserved section with riparian woods.

Table 2. Annual average of $\delta^{13}C$ and $\delta^{15}N$

	Sta. 1	Sta. 2	Sta. 3	Sta. 4	Sta. 5	Sta. 6	Sta. 7	Average
$\delta^{13}C$	–22.2	–23.7	–23.2	–22.1	–23.1	–22.9	–25.4	–23.2
$\delta^{15}N$	1.0	2.7	3.8	5.4	5.4	4.9	3.5	3.8

Station 4. Station 4, Station 6 and Station 7 were distinctive stations compared to the other stations. Station 4 had a rapid steep slope instead of a drop facility to conserve fishes. Station 6 was a wide canal for fish shelter during a flood. Station 7 was located in a conserved section, in order to avoid repair and conserve the abundant biota. Investigations were made from April 2003 to February 2004 at two monthly intervals. Attached matter was gathered from boulders or from the surface of the concrete bottom when there were no boulders. Samples were fixed in formalin on site, then freeze-dried at –40ºC after examination under a microscope at a 400× magnification. Stable isotope ratios of the samples were analyzed with a mass spectrometer, Delta ^{plus} XP, manufactured by Thermo Finnigan.

Results and discussion

1. Structure and characteristics of attached matter

All samples of attached matter were composed of algae and detritus generated from plant material. Algae accounted for a small share at Station 7 as compared to the other stations. Blood worms (larvae of Chironomidae) and other invertebrates were captured in the attached matter at Station 3, even though both the walls and bottom of the canal were concrete lined, which has been considered to be unsuitable for organisms. Chain-forming algae had grown abundantly upon attached matter that seemed to contain a microbial community. These results suggest that some organisms may be able to live on the concrete lining of canals with remediation.

Diatom (*Melosira varians*) was observed to be dominant in June and replaced by *Spirogyra* sp. in August on the retaining walls. *Gyrosigma* sp. was observed preferentially at Station 4. Since little is known about the ecology of algae in the canal, more knowledge of algae, such as representative species, changes of algal dominant species and the properties of $\delta^{13}C$, is required.

2. Properties of $\delta^{13}C$

Annual averages of $\delta^{13}C$ for each investigation station are shown in Table 2. The $\delta^{13}C$ average of attached matter taken as a whole in *Harakawa* was –23.2‰. The $\delta^{13}C$ of detritus mostly from C_3 plants was measured to be approximately –26‰[8]. Since the $\delta^{13}C$ of algae was deduced to be approximately –20‰ as referred to above, the average $\delta^{13}C$ of attached matter indicated the mass of algae growing in *Harakawa* was approximately half of all attached matter.

Seasonal changes in the $\delta^{13}C$ values of each station are shown in Fig. 2. The highest $\delta^{13}C$ value was –20.2‰ at Station 4 in August. Annual averages for each station ranged from –25.4‰ to –22.1‰. In all stations except for Station 7, the highest values were observed in June or August. Regarding the monthly average of all seven stations, the highest value was –21.9‰ in June and second highest was –22.0‰ in August. The standard deviation and the annual disparity of $\delta^{13}C$ by station increased with

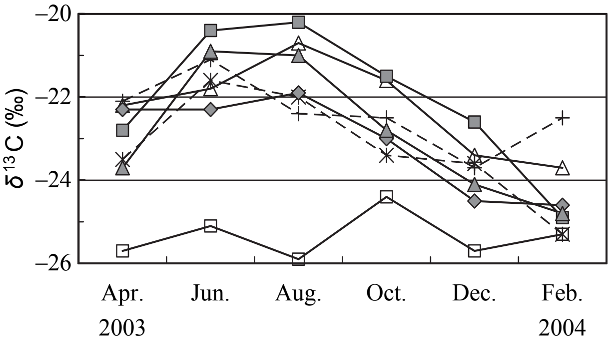

Fig. 2. Seasonal changes in δ^{13}C of attached matter

–△–: Sta. 1, –+–: Sta. 2, –✳–: Sta. 3, –■–: Sta. 4, –◆–: Sta. 5, –▲–: Sta. 6, –⊟–: Sta. 7.

the annual average of δ^{13}C. There was a significant correlation between the annual average and standard deviation ($R^2 = 0.67$, $p < 0.05$) and between the annual average and annual disparity ($R^2 = 0.67$, $p < 0.05$). These results suggest that growth of algae became higher during the summer because the δ^{13}C value of algae is higher than detritus.

Furthermore, δ^{13}C of algae increased if photosynthesis was activated, because isotope fractionations became smaller by the activation[1]. It is possible that the ascent of δ^{13}C in summer can be attributed not only to an increase of the algae on attached matter, but also to an increase in the δ^{13}C of algae itself. A positive correlation ($R^2 = 0.54$, $p < 0.05$) between water velocity and δ^{13}C of Station 6 was observed (Fig. 3). It is possible that the δ^{13}C of attached matter in a rapid stream increased because detritus in the attached matter was washed out and the proportion of algae increased relatively.

The lowest average of δ^{13}C was –25.2‰ at Station 7. The δ^{13}C value of Station 7 was compared with the other 6 stations; the range of seasonal change was narrow and the highest value in Station 7 was not recorded in the summer but in October. The reason for these results may be that the algal biomass on attached matter of Station 7 was lower than that of the other stations because of insufficient light that was essential for photosynthesis. The environment of Station 7 had similarities to a stream including much litter fall, high water velocity with growth of rapids, a gravel bottom, and a shady water surface. Organisms in a stream depend on organic matter derived from terrestrial plants, because algal production under insufficient light cannot sustain hungry invertebrates that act as a bridge between producers and secondary consumers in a food web. A gravel bottom catches litter and detritus that are very important for organisms as food in such an environment. A heterotrophic food web that is common in a stream may have been observed at Station 7.

From the aspect of material circulation, carbon for the food web is derived from two sources, 1) terrestrial organic matter and 2) dissolved inorganic carbon (DIC) in water absorbed from the atmosphere and fixed by algae. Both sources with their own δ^{13}C values would be transferred into the food web mainly via invertebrates. Therefore, the δ^{13}C values of attached matter and seasonal variation in the canal depend on the ratio of algae. It is possible that activation of algal production also influenced the ratios. We may say that material of a canal and solar irradiation affect the growth of the attached algae.

3. Properties of δ^{15}N

The annual averages of δ^{15}N by station are also shown in Table 2. Seasonal changes in the δ^{15}N values by station are shown in Fig. 4. Relationships between δ^{15}N and concentrations of nitric acid in June and December are shown in Fig. 5 and Fig. 6, respectively. The average of δ^{15}N for all attached matter was 3.8‰. As algae assimilate dissolved inorganic nitrogen (DIN) to grow in water, the δ^{15}N of algae indicates the DIN value. In general, δ^{15}N values in rain water are negative. The value becomes higher as water flows downstream because of biological accumulation. Therefore, the δ^{15}N of algae in the lower stream and in a section where bodily waste are discharged are almost always higher than in the upper stream.

As shown in Fig. 5 and Fig. 6, the δ^{15}N of attached matter closely followed that of the water, even though both of the values do not correspond exactly. We attribute the elevation of δ^{15}N of attached matter, including algae, from Station 2 to Station 4 to gray water that was discharged from houses. The δ^{15}N was higher than rain water. DIN absorbed by algae is considered to move inside the food web through invertebrates.

The maximum value of δ^{15}N of water was recorded at Station 4, but the peak of nitric acid concentration was at Station 6. The δ^{15}N values of chemical fertilizers are approximately zero, because they came from atmospheric nitrogen that is defined as the standard of the nitrogen stable isotope ratio. Therefore, discharged water from paddy fields showed lower δ^{15}N values than gray water. Nitric acid concentration continued to increase toward the lower reaches of the canal from household and paddy field discharges. However, the low δ^{15}N derived from chemical fertilizers in the gray water diluted the high δ^{15}N. The δ^{15}N values of attached matter and water in December were higher than in June. The differences were due to a low dilution with a decrease in canal water during the no-irrigation period because scarcely any water discharged from paddy fields to the canal. Denitrification generation increases the δ^{15}N of the remaining NO_3^- because the $^{14}NO_3^-$ consumption rate is higher than $^{15}NO_3^-$ in the denitrification process[2], namely fractionation. The elevation of δ^{15}N at Station 7 in December may include elevation by fractionation. Thus, more study is needed to understand definitely the dynamics of nitrogen in *Harakawa*.

Conclusions

It follows from what has been said that 1) the growth and activation of attached algae in the canal increased the δ^{13}C values of attached matter during summer; 2) insufficient photosynthesis in a shady ecologically conserved section with abundant riparian woods remaining prevented abundant production of algae; and 3) nitrogen from houses that had high δ^{15}N was absorbed by algae. It is likely that consumers in the food web are influenced materially by these characteristics.

This study showed that stable isotope ratios of producers indicate material flow and circulation in the canal. Stable isotope analysis helps us not only understand the food web and material transfer in the system but also the interactions between material circulation and the living world in the paddy ecosystem. We can survey the material flow in a paddy area by analysis of the biological community, adding isotopic investigations in paddy fields.

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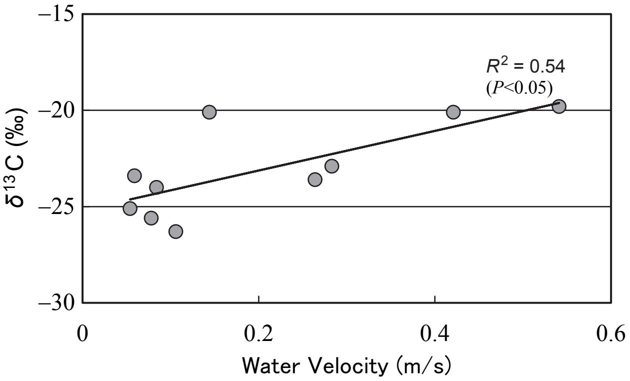

Fig. 3. Correlation between water velocity and δ^{13}C of attached matter

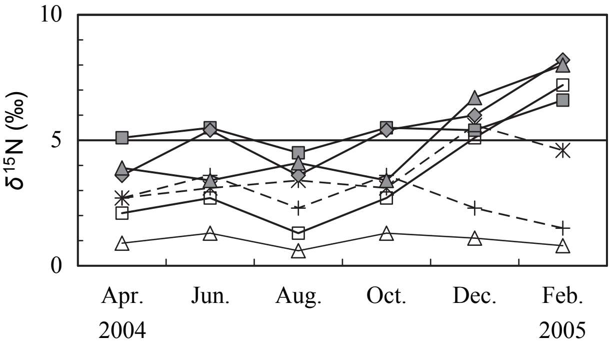

Fig. 4. Seasonal changes in δ^{15}N of attached matter

—△—: Sta. 1, -+-: Sta. 2, -✳-: Sta. 3, —■—: Sta. 4,
—◆—: Sta. 5, —▲—: Sta. 6, —□—: Sta. 7.

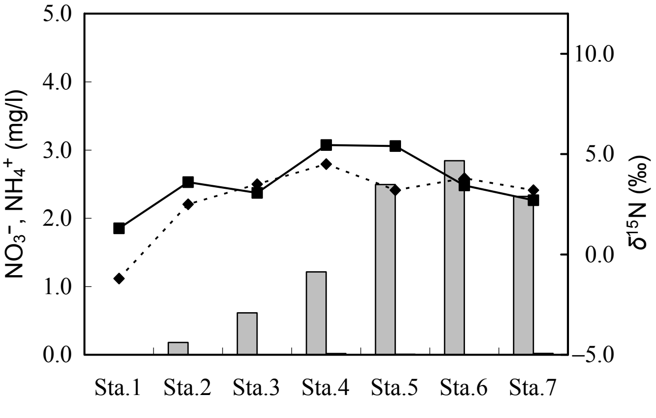

Fig. 5. Concentrations of nitric acid and δ^{15}N (Jun. in 2003)

▨: Concentration of NO_3^-, ■: Concentration of NH_4^+,
—■—: δ^{15}N of attached matter, -◆-: δ^{15}N of water.

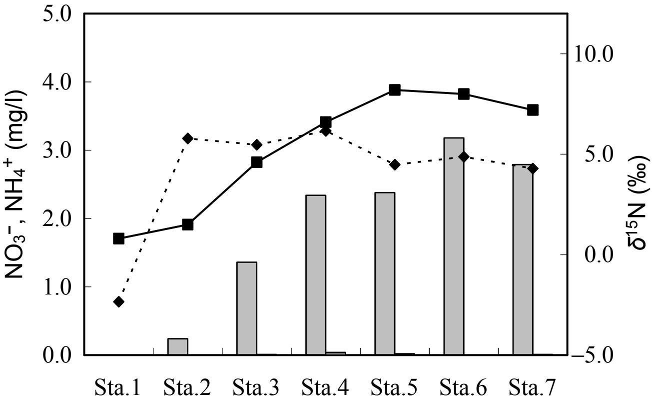

Fig. 6. Concentrations of nitric acid and δ^{15}N (Dec. in 2003)

▨: Concentration of NO_3^-, ■: Concentration of NH_4^+,
—■—: δ^{15}N of attached matter, -◆-: δ^{15}N of water.

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