	シュウ　シャワ　ガァン
氏　　　名	許　暁　光
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指 導 教 員	東北大学教授　西村 修
論 文 審 査 委 員	主査　東北大学教授　西村 修　　東北大学教授　原田 秀樹 東北大学教授　李　玉友　　東北大学准教授　坂巻　隆史 （災害科学国際研究所）

論 文 内 容 要 旨

1.Introduction

Lake sediments receive organic matters from both autochthonous and allochthonous sources. The former is produced by phytoplankton and the latter is derived from vascular plants. Knowledge of the source and composition of sedimentary organic matter (SOM) is very important as it closely related to current problems of shallow lakes such as occurrence of eutrophication, reduction of aquatic plants and disappearance of benthic animals (Schultz and Urban, 2008, Wijck et al., 1992, Omesová and Helešic, 2010). Recent studies about the source of SOM have mainly focused on the estuarine, coastal and marine ecosystems, however, information from freshwater lakes still remain poorly documented especially for large, shallow and eutrophic lakes. Therefore, one of the main objectives of this study is to reveal potential source contributions to SOM pool in eutrophic lakes and identify the dominant source using multiple molecular biomarkers. Furthermore, several controlling factors including primary production, animal consumption, microbial utilization and hydraulic influence related to SOM source and composition were conducted in order to get a better understanding on the mechanisms of these processes.

2.Material and methods

2.1. Site description

Lake Taihu (31º10′N, 120º09′E), located in southeastern part of Yangtze Delta, is the third largest freshwater lake in China. The lake has a surface area of 2,338km^2 and an average depth of 1.9m with more than 200 input rivers discharging about 7.6 billion m^3 water into the lake every year. Unfortunately, heavy cyanobacterial blooms occurred annually in the western and northern areas due to discharge of pollutants from surrounding basins. Aquatic macrophytes with more than 60% of total species composed of submerged macrophytes are mainly distributed in easterly portion of the lake. Lake Izunuma (38º43′N, 141º06′E) is a temperate and eutrophic shallow lake, located in a cropland of northern Miyagi, Japan. It has a maximum and average depth of 1.6m and 0.76m, respectively, with a total area of 3.69km^2. Owing to decades of nutrients discharge, the lake has been transformed into its present eutrophic state.

Approximate 80% of phytoplankton is green algae, however, emergent and floating-leaf plants are dominant among aquatic vegetation and cover almost the whole lake. Hence, the lake is also subjected to paludification as the average sedimentation rate is about 2 ~ 6mm/year. Lake Juni (40º34′N, 139º58′E) and Lake Towada (40º28′N, 140º53′E) are typically mountain lakes with the distance of about 80km, located in Aomori prefecture, Japan. Lake Juni consists of 33 pond waters with their total areas of 314km^2, however, the lake has transformed into current mesotrophic state since last century due to the influence of anthropogenic activities. Lake Towada, a double caldera lake, is the 12th largest lake in Japan with the maximum depth of 327m and a surface area of 61.1km^2. The lake is remote and oligotrophic with the extremely low concentration of nutrients.

2.2. Sampling strategy

To investigate the source and composition of SOM, sediment samples were collected from four studied lakes: (1) in Lake Taihu, thirty-three samples encompassing the whole lake were collected twice between August 22 ~ 30, 2012 and February 25 ~ March 5, 2013; (2) in Lake Izunuma, fourteen samples encompassing the whole lake were collected in June 24, 2013; (3) in Lake Juni, nine samples from different ponds were collected in June 25, 2013. (4) in Lake Towada, five samples from different coastal areas were collected in June 26, 2013. Triplicate surface sediment samples from each site were combined and homogenized before being stored in the dark at <4°C in the field, using the Ekman-Birge grab. After transported to laboratory rapidly, all samples were freeze-dried for 24h and stored at -40°C for fatty acid analysis.

To investigate the influence of primary production, animal consumption and microbial utilization on SOM source and composition, all field samplings were conducted in Lake Taihu. Surface water and corresponding sediment samples were collected from thirty-three locations of the whole lake, shrimps, fishes and benthic animals including snails and bivalves were collected in the northern region of the lake, and all these samples were collected during August 20 ~ 29, 2013. After transported to laboratory rapidly, all animals were dissected for removing shells or scales if there were, and then freeze-dried for 24h and stored at -40°C for fatty acid analysis. While for microbial utilization, sediment samples within benthic microbial were collected during November 3 ~ 13, 2011.

To investigate the hydraulic influence on SOM source and composition, all field samplings were conducted in Lake Izunuma, fourteen samples were conducted in June 24, 2013. After transported to laboratory rapidly, the sediment samples from each location were separated into two parts, one part kept intact and the other part was classified into four size groups (<32μm, 32-63μm, 63-125μm, >125μm) using the method of wet sieving. Sediment samples were gently shaken by hand in large breakers filled with ultrapure water. The weight of each fraction was carefully measured to estimate the relative contribution to mass of the sediment. At last, all the bulk and separated samples were freeze-dried for 24h and stored at -40°C until be analyzed for fatty acids and TOC analysis. Current velocity at 5cm above the sediment surface was measured from June to July 2013. Two locations were measured together and lasted for one week. These data were collected at 1Hz by repeated 30s bursts with 10 min intervals by electromagnetic horizontal two dimensional current velocity meters (Alec Electronics, compact-EM).

2.3. Sample analysis

Prior to analysis, sediment samples from each location were ground (<5um) and treated with the addition of 5ml 1M HCl to remove carbonates for determination of organic C and N contents. Thereafter, samples were dried to a constant weight (60 °C) and analyzed by elemental analyzer. Isotopic abundance analysis was carried out on an isotope analyzer. Samples were combusted and the resulting gases were separated by gas chromatography, and then analyzed by continuous flow-mass spectrometry. Stable isotope abundances were expressed in δ notation as the deviation from standards in parts per thousand (‰) according to the following equation:

$$\delta X = ((R_{sample}/R_{standard})-1) \times 1000 \quad (1)$$

Where, X is ^{13}C or ^{15}N and R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

One-step method (Abudulkadir and Tsuchiya, 2008) was used for extractions and esterification of all lipids to fatty acids methyl esters (FAMEs). Then, the FAMEs were separated and quantified using a gas chromatograph (GC-2014, Shimadzu) equipped with a split/splitless injector and a flame ionization detector (FID). The gas chromatograph was fitted with a Select FAME (100m, 0.25mm i.d., 0.20mm) capillary column. The GC oven was programmed as follows: 150°C (hold 5min) to 230°C (hold 10min) at 4°C/min and last hold at 250°C for 20min. The FAMEs assignments were made by comparison of retention times with those derivatives of authentic standards (Supelco, Aldrich, Sigma).

Stable carbon isotopic compositions of individual fatty acids were determined using a GC combustion system interfaced with an isotope ratio mass spectrometer (IRMS). The compounds were separated with a column with a stationary phase of 100% dimethylpolysiloxane. Peaks eluted from the GC were combusted to CO_2 over CuO/Pt wires at 850°C and on-line transported to the IRMS. The isotopic compositions of CO_2 peaks were measured with the IRMS operated at 10 kV acceleration potential and by magnetic sector mass separation. The $\delta^{13}C$ values of the compounds were calibrated with a reference CO_2 gas. The $\delta^{13}C$ values of FAMEs were corrected for the extra carbon atom added by methylation.

3. Results and discussion

3.1. Source and composition of SOM in shallow eutrophic lakes

For the purpose of assessing SOM source and composition in shallow eutrophic lakes, multiple molecular biomarkers including elemental ratios, stable isotope ratios and fatty acid biomarkers were used, however, fatty acid biomarkers were proved to be valid and the best compared with other two indicators. Results of fatty acids biomarkers illustrated that microalgae, aquatic plant, terrestrial plant and bacterial-derived organic matters were potential sources and composition of SOM in these freshwater lakes, however, among them, terrestrial plant and bacteria were predominant as evidenced by high correlations with TOC content. Additionally, multiple regression analysis was used for simulation the sediment TOC with all the potential sources, and results followed the most appropriate model: TOC total = 0.015 $FA_{terrestrial\ plant}$ + 0.024 $FA_{bacteria}$, further confirming the dominant sources of bacteria and terrestrial plants in shallow lakes.

3.2. Primary production, animal consumption and microbial utilization controlling SOM source and composition

In order to analyze the reason that terrestrial plant and bacteria were the dominant sources in shallow lakes, several controlling factors including primary production, animal consumption and microbial utilization were tested in shallow eutrophic Lake Taihu. Firstly, fatty acids in suspended particulate organic matters (SPOM) and their corresponding surface sediments were analyzed, results showed that terrestrial plants had a relatively strong correlation between SPOM and surface sediment while other sources were very weak, suggesting these potential sources may be subjected different influence during sinking processes. Secondly, fatty acid biomarkers and stable isotope ratios of shrimps, fishes and benthic animals in Lake Taihu were analyzed, results indicated that animals would prefer consuming more cyanobacteria than terrestrial plants, which is corresponding with the low contribution of cyanobacteria to SOM pools. Lastly, $\delta^{13}C$ of bacteria-specific fatty acids was used to assess the carbon sources utilized by bacteria in Lake Taihu, results showed that cyanobacteria contributing more than 90% for bacteria utilization, suggesting bacteria preferentially utilize labile cyanobacteria from the sediment carbon pool than higher plants.

3.3 Hydraulic influence on SOM source and composition in shallow eutrophic lakes

The hydraulic influence on SOM source and composition were conducted in shallow eutrophic Lake Izunuma. Surface sediments were fractionated into four size groups: <32μm, 32-63μm, 63-125μm and >125μm. Results showed that, compared with different size groups, bacteria largely accumulated in <32μm fraction, microalgae was mainly present in two fractions of <32μm and 63-125μm, aquatic plants appeared to be averagely distributed in all size fractions and >125μm fractionated sediments were mainly comprised of terrestrial plants. However, compared with different organic matter sources, terrestrial plant and bacteria were dominant in all fractions. Additionally, the strong relation between sediment TOC and percentage of <32μm fraction was demonstrated probably caused by a relatively large accumulation of bacteria in <32μm fraction. On the other hand, although it displayed very weak correlation between average current velocity above 5cm of surface sediment and sediment TOC content in Lake Izunuma, the relationship between frequency of current velocity and sediment TOC content exhibited a relatively strong and negative correlation occurred at the current velocity at 1cm/s without considering different current velocity characteristics of several sample locations.

Reference

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論文審査結果の要旨

浅い湖沼の富栄養化問題は世界的な環境問題であり，様々な富栄養化対策が進められているものの改善の兆しは見えない．また，富栄養化湖沼では水質のみならず底質の有機汚濁化，浅底化が進み，底生生物を中心とした生物・生態系に深刻な問題を発生させている．このような底質の問題に対して，これまで浚渫や覆砂などの土木工学的対策が行なわれてきたが効果に持続性がなく，また底質のシードバンク機能を損ねるなど様々な問題点も指摘されている．したがって，効果的な底質改善方法を開発する必要があるが，そのためには底質の有機汚濁化の原因を解明し，それに基づく合理的な対策手法の構築を行なう必要がある．しかし，浅い富栄養化湖沼の底質の有機汚濁機構に関する研究は非常に少なく，またこれまでの知見も水域によって傾向が異なり，あらためて底質の有機汚濁現象の把握，機構の解明が必要とされている．

そこで本研究では，浅い富栄養化湖沼の堆積物中有機物の起源，組成および制御因子を明らかにすることを目的として，安定同位体比および脂肪酸組成等を指標として堆積有機物の起源を解析する手法を開発し，それらの手法を応用して湖沼における一次生産，動物による消費，および微生物による利用等の有機物動態と堆積有機物の起源の関係を解析し，さらに堆積物中有機物の濃度に及ぼす水の流動の影響を検討したもので，全編6章よりなる．

第1章「緒論」では，本研究の背景，意義，目的について述べた．

第2章「既往の研究」では，本研究に関連する既往研究をレビューし，有機物の起源を解析するための指標，および有機物の起源の定量的評価方法等を研究する必要があることを明らかにした．

第3章「浅い湖沼の堆積有機物の起源および組成」では，従来指標である元素組成（C/N 比）や安定同位体比に比較して，本研究で着目した脂肪酸をバイオマーカーとした手法は堆積有機物の起源を明確に表す指標となること，および中国太湖のような超富栄養湖沼においても堆積有機物の起源は主に陸上植物由来有機物であり，量的には陸上植物由来有機物とそれを利用する細菌が堆積有機物のほとんどを占めることを明らかにした．これは極めて新規性の高い知見である．

第4章「堆積有機物の起源および組成を制御する一次生産，動物による消費および微生物による利用」では，太湖を対象として広域的に調査を実施し，懸濁有機物が沈降し表層堆積有機物に移行する過程で，藍藻類，珪藻類が減少し，陸上植物および細菌由来の有機物が増加する傾向が示され，一方で動物や底質中の細菌類は藍藻類を利用していることが明らかになった．これは新規性の高い知見である．

第5章「浅い湖沼の堆積有機物の起源および組成に及ぼす水の流動の影響」では，宮城県伊豆沼を対象として底質と水の流動を調査し，63μ以下の粒径を多く含む底質の有機物濃度は高く，起源は陸上植物と細菌類に由来すること，水の流動をある一定の流速を超える頻度（流速頻度）として指標化することで，堆積有機物濃度と流速頻度に負の相関があることを明らかにした．これは有用性の高い知見である．

第6章「総括および結論」では，本研究を通して得られた成果を総括した．

以上の通り，本研究は浅い湖沼の底質の有機汚濁化に起因する問題を改善するための基盤的知見として，脂肪酸組成から堆積有機物の起源を解析する方法を構築し，それを適用して富栄養湖沼の有機物動態を解析し，堆積有機物の起源としての陸上植物由来有機物の重要性を明らかにし，水の流動を制御因子として活用できる可能性を示したもので，環境工学の発展に寄与するところが少なくない．

よって，本論文は博士(工学)の学位論文として合格と認める．