Article

Sterol and stanol compositions in sediments from the Bungaku-no-ike pond, Tokyo, Japan: Examination of stanol sources

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Abstract

The sterol compositions in sediments from an oxic pond of Bungaku-no-ike (Hachioji, Tokyo, Japan) using tetramethylammonium hydroxide (TMAH) thermochemolysis and gas chromatography-mass spectrometry (GC-MS) analysis were determined. In the sediments, four 5α(H)-stanols (cholestanol, campestanol, sitostanol, and brassicastanol) and their corresponding Δ^5-sterols were identified. We detected high concentrations of cholestanol ($1.08-1.07\,\mu g/g$), campestanol ($0.97-1.37\,\mu g/g$), and sitostanol ($2.78-3.88\,\mu g/g$) and high values of those 5α(H)-stanol/Δ^5-sterol ratios ($0.76-0.85$, $0.80-0.60$, and 0.29, respectively) in the sediments. In contrast, the concentrations of brassicastanol is low ($0.16-0.28\,\mu g/g$) with low brassicastanol/brassicasterol ratios ($0.11-0.16$). The 5α(H)-stanols are known to be typically formed by bacterial reduction of Δ^5-sterols under anoxic condition. However, the pond was confirmed to be in an oxidative condition (dissolved oxygen in bottom water, $5.5\pm0.5\,mg/L$). From these results, the high 5α(H)-stanol/Δ^5-sterol ratios, except the brassicastanol/brassicasterol, in the pond suggest that the source(s) of the 5α(H)-stanols may be terrigenous matter containing high 5α(H)-stanols resulting from preferential degradation of the Δ^5-sterols. In any cases, our study indicates that there is some possibility of 5α(H)-stanol inputs even though the sampling location is not anoxic conditions, and thus, the redox indicator using the 5α(H)-stanol/Δ^5-sterol ratio should pay attention to source effects.

1. Introduction

Sterols have been widely distributed from marine and lacustrine sediments (e.g., Ishiwatari et al., 2009; Bertrand et al., 2012; Yamamoto et al., 2015a, b; Huang et al., 2016). Sources of sterols in sediments are phytoplankton and terrestrial plants (Volkman, 1986). 24-Ethylcholest-5-en-3β-ol (β-sitosterol) and 24-methylcholest-5-en-3β-ol (campesterol) are major sterol components in higher plants (Yunker et al., 1995; Belicka et al., 2004; Killops and Killops, 2013). 24-Methylcholesta-5, 22E-dien-3β-ol (brassicasterol) is mainly derived from phytoplankton such as diatom and haptophyte algae (e.g., Volkman et al., 1981, 1998; Rampen et al., 2010). Utilization of their structural features, such as the number and position of the double bonds, types of functional groups, and the carbon content, sterols can be used as tracers for photo- and auto-oxidation (Christodoulou et al., 2009; Rontani et al., 2012, 2014). On the contrary, the 5α(H)-stanol/Δ^5-sterol ratio can be used as a tracer for

redox conditions because the Δ^5-sterols are reduced to 5α(H)-stanols by bacterial reactions under anoxic conditions (Rosenfeld and Hellman, 1971; Eyssen et al., 1973; Fig. 1C). Therefore, in anoxic conditions, high value of the 5α(H)-stanol/Δ^5-sterol ratio are expected (Nishimura and Koyama, 1977; Wakeham, 1989). In more recent study (Nakakuni et al., 2017), it was shown that this tracer was useful for reconstructing the redox events recorded in continuous sediment sequences (marine sediments off southern California, Ocean Drilling Program, Leg 167, Hole 1017E) over the last 45 kyr.

In contrast, inputs of 5α(H)-stanol were obtained by not only the bacterial reduction of Δ^5-sterol but also the other sources (e.g., Nishimura, 1977a; Gagosian et al., 1980; Volkman et al., 1990), which complicate the interpretation of 5α(H)-stanol/Δ^5-sterol ratio as redox tracer. In fact, high 5α(H)-stanol inputs were reported at various sites other than anoxic environments (e.g., Kondo et al., 1994; Rontani et al., 2014). Furthermore, it is suggested that living organisms such as higher plants and phytoplankton containing high 5α(H)-stanols might be important sources of 5α(H)-stanols in sediments

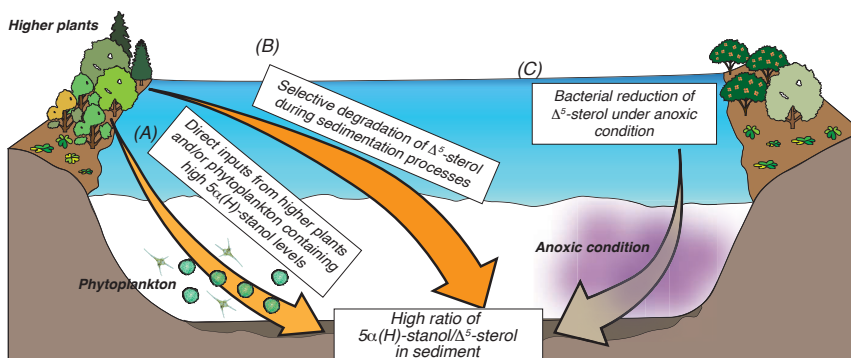Masatoshi Nakakuni, Keiko Takehara, Nobuyuki Nakatomi, Junpei Higuchi, Miyuki Yamane, Shuichi Yamamoto

Fig. 1 Scheme of the high 5α(H)-stanol/Δ^5-sterol ratio in sediment. (A) Direct inputs from higher plants and/or phytoplankton containing high 5α(H)-stanol levels. Contributions of 5α(H)-stanol derived from living organisms are few, although organisms with high 5α(H)-stanol levels have also been reported (Robinson et al., 1984; Volkman et al., 1990; Nishimura, 1977b). (B) Selective degradation of Δ^5-sterol during diagenetic processes. Since the Δ^5-sterols are more susceptible to decomposition than the 5α(H)-stanols under oxidative conditions, 5α(H)-stanols from living organisms can be concentrated during the sedimentation process (Nishimura, 1977a; Nishimura and Koyama, 1977). (C) Bacterial reduction of Δ^5-sterol under anoxic condition. The reduction occurs in the water column and surface sediments (e.g., Nishimura and Koyama, 1977; Wakeham, 1989). Therefore, the 5α(H)-stanol/Δ^5-sterol ratio can be used as a redox tracer (e.g., Bertrand et al., 2012; Zheng et al., 2015; Nakakuni et al., 2017).

(Nishimura and Koyama, 1977; Robinson et al., 1984; Volkman et al., 1990; Fig. 1A). Also, the sedimentary 5α(H)-stanol/Δ^5-sterol ratio can significantly change by selective degradation during diagenetic processes of Δ^5-sterols (Nishimura, 1977a; Nishimura and Koyama, 1977; Fig. 1B). Regarding the selective degradation of Δ^5-sterols, there have been reported in the sediments of Lake Shirakoma-ike (Nagano, Japan), suggesting 5α(H)-stanol compositions in the sediments were significantly affected by plant-derived degradation products because plant-derived 5α(H)-stanols (such as 24-ethyl-5α(H)-cholestan-3β-ol; sitostanol) could be abundantly found (Nishimura, 1977a). From these insights, it is suggested that such selective degradation of Δ^5-sterol can occur under oxidative environments, although our understanding of the origins and fate of 5α(H)-stanols still require further examination. The investigation of production and diagenetic processes for the 5α(H)-stanol in oxic environments should provide better understanding of its source and behavior.

In addition, tetramethylammonium hydroxide (TMAH) thermochemolysis was employed for the 5α(H)- stanol/Δ^5-sterol analysis of the sediments in our study. Organic compound analysis using the TMAH method was utilized since hydrolysis and methylation are simultaneously performed, and in a relatively short time. Using the TMAH method, various sterols including Δ^5-sterols, 5α(H)-stanols, and 4α-methyl-sterols were identified from marine sediments, and thus, this method makes it possible to analyze variety of sterols, as analyzed by trimethylsilyl derivatization (Asperger et al., 1999, 2001; Yamamoto et al., 2015a).

In the present study, we observed the Bungaku-no-ike pond (Pond of Literature) within Soka University as a new site where the 5α(H)-stanol/Δ^5-sterol ratios were high in the sediments of oxidative conditions, and report the sterol compositions to examine the applicability of redox indicator using the 5α(H)-stanol/Δ^5-sterol ratios.

2. Material and methods

2.1. Sampling location and pond samples

Bungaku-no-ike pond (Pond of Literature) is a small pond (ca. 3000 m^2) located in Soka University, Hachioji, Tokyo, Japan (35°41′24″N, 139°19′41″E) (Fig. 2). The water depth is $<$2 m, and the pond is not connected to a river system.

Sediment samples (St. 1 and St. 2) were obtained on August, 20, 2017 using an Ekman–Birge grab sampler. The sediment samples were frozen, lyophilized and finely powdered for the TMAH thermochemolysis. The vegetation around the pond includes: konara oak (*Quercus serrate*), several cherry trees (*Cerasus yedoensis*), and azalea (*Rhododendron*). The distribution of vegetation surrounding the pond is summarized in the Appendix.

At the sampling location, dissolved oxygen (DO) was measured in triplicate using a DO water test kit (Kyoritsu Chemical-Check Lab. Corp.). The DO at the water surface (ca. 10 cm in water depth) and bottom (ca. 1.8 m in water depth) were 7.3±0.6 mg/L and 5.5±0.5 mg/L, respectively, indicating that the pond is under oxidative conditions. The DO values were measured on the same

Table 1 Organic carbon and nitrogen data in the sediment sample from the Bungaku-no-ike pond.

	TOC (%)	TN	C/N	$\delta^{13}C$ (‰)
St. 1	13.7	1.3	10.5	-30.5
St. 2	12.8	1.2	10.7	-30.5

day of sampling of the sediments (August 20, 2017). Similar values of DO were also confirmed on July 25, 2017 during a preliminary experiment (the surface; ca. 6 mg/L, the bottom; ca. 5 mg/L). Although DO data are limited from July to August, a fountain water circulation system is operational throughout the year preventing stagnation of the aquatic conditions in the pond.

2.2. Plant samples around the pond

Leaf samples of *Quercus serrate, Cerasus yedoensis*, *Rhododendron* satsuki, and *Rhododendron* tsutsuji were taken on November, 5 and 6, 2017. The samples were lyophilized and powdered for sterol composition analysis.

2.3. Methods
2.3.1. Carbon content and stable isotope analysis

Total organic carbon (TOC), total nitrogen (TN), and the carbon isotope ratio in the sediment samples were analyzed using an elemental analyzer (EA1110, Thermo Fisher Co.) and an isotope ratio mass spectrometer (Delta V Advantage, Thermo Fisher Co.). Powdered sediment samples (ca. 10 mg) were wrapped in tin foil, and then analyzed with the instruments. The carbon isotope ratio was expressed in δ notation referenced to Vienna Pee Dee Belemnite limestone. The analysis error was $< \pm 0.24$‰.

2.3.2. Analysis of organic matter using the TMAH method

Sterol compositions in the sediments were analyzed using TMAH thermochemolysis gas chromatography-mass spectrometry (TMAH GC-MS). The samples (ca. 100 mg for the sediments and 5 mg for the leaf samples) were placed in a 10 mL glass ampoule and the TMAH reagent (25 wt.% in methanol; 150 μL) was added. Nonadecanoic-d_{37} acid (100 ng/μL in methanol; 50 μL) was added as an internal standard. After the methanol (MeOH) evaporated, the ampoule was sealed under vacuum conditions and placed in a 300ºC oven for 30 min. The combined extracts (with ethyl acetate) were dried in a vacuum desiccator and were re-dissolved in 100 μL of ethyl acetate. Lastly, 2 μL of the dissolved sample was injected (splitless injection at 290ºC) and analyzed with a GC-MS (6890N GC5973 MS; Agilent Technologies Co.) on a DB-5MS capillary column (0.25 mm internal diameter (i.d.), 0.25 μm film thickness (Agilent Technologies Co.), 30 m in length) using helium
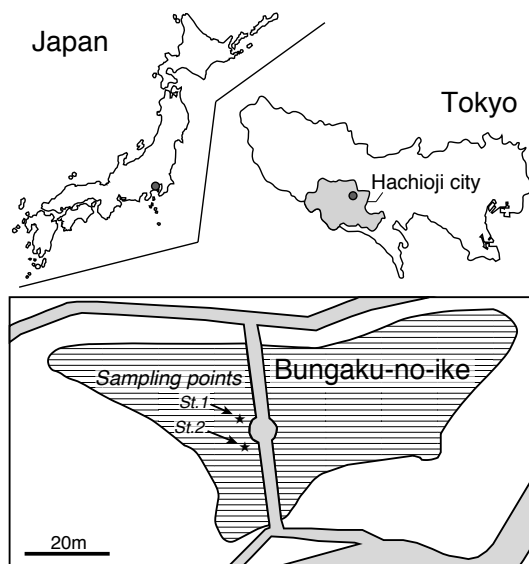

Fig. 2 Location of the Bungaku-no-ike pond. The stars (★) denote sampling sites. The upper and the lower sites are St. 1 and St. 2, respectively.

as the carrier gas at 1.0 mL/min. The oven temperature was set at 60ºC for 2 min, changed to 310ºC (6ºC/min) and was then maintained at 310ºC for 20 min. The mass spectrometer was set to a full scan ion monitoring mode (50–650 Dalton) with an MS scanning interval of 0.5 s. Identification of sterol compounds was performed by comparing the results with published spectral data (Yamamoto et al., 2015a). Sterol concentrations were calculated by comparing with internal standards. The analytical reproducibility of the 5α(H)-stanol/Δ^5-sterol ratios was within 10% error (Nakakuni et al., 2017).

3. Results

3.1. Bulk carbon, nitrogen content, and sterol compositions in the sediment samples

TOC and TN in the sediment samples are similar (Table 1); high TOC (12.8%–13.7%), low TN (1.2%–1.3%) with C/N ratio of 10.2–10.6. The carbon isotope ratios ($\delta^{13}C$) are -30.5‰ in both of the sediment samples.

The major sterols were identified in the sediment samples included cholest-5-en-3β-ol (cholesterol), brassicasterol,

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Table 2 List of major sterols detected in the sediment samples from the Bungaku-no-ike pond and sterols of higher plants collected the area around the pond.

| | Sediment samples | | | | Higher plants | | | |
| | St. 1 | | St. 2 | | Q.s. | C.y. | R.s. | R.t. |
	(μg/g)	(%)*	(μg/g)	(%)*		(μg/g)		
Δ^5-Sterols								
Cholesterol	2.00	7.5 (11.2)	1.42	7.7 (11.8)	-	-	-	-
Campesterol	2.28	8.5 (11.6)	1.21	6.5 (9.0)	-	9.29	-	-
Brassicasterol	1.77	6.6 (9.1)	1.48	8.0 (10.9)	-	-	-	-
β-Sitosterol	13.52	50.4 (69.1)	9.43	50.9 (69.6)	399.0	394.6	426.4	373.9
$5\alpha(H)$-Stanols								
Cholestanol	1.70	6.3 (23.5)	1.08	5.8 (21.6)	-	-	-	-
Campestanol	1.37	5.1 (19.0)	0.97	5.2 (19.5)	-	-	-	-
Brassicastanol	0.28	1.1 (3.9)	0.16	0.9 (3.3)	-	-	-	-
Sitostanol	3.88	14.5 (53.6)	2.78	15.0 (55.6)	trace	-	-	-
Ratios								
Cholestanol/cholesterol		0.85		0.76	-	-	-	-
Campestanol/campesterol		0.60		0.80	-	-	-	-
Brassicastanol/brassicasterol		0.16		0.11	-	-	-	-
Sitostanol/β-sitosterol		0.29		0.29	-	-	-	-

Q.s.= *Quercus serrata*, C.y.= *Cerasus yedoensis*, R.s.= *Rhododendron* satsuki, and R.t.= *Rhododendron* tsutsuji.
* The values are given as % of the eight sterols, and the values in parentheses are given as % of total Δ^5-sterols and total $5\alpha(H)$-stanols.
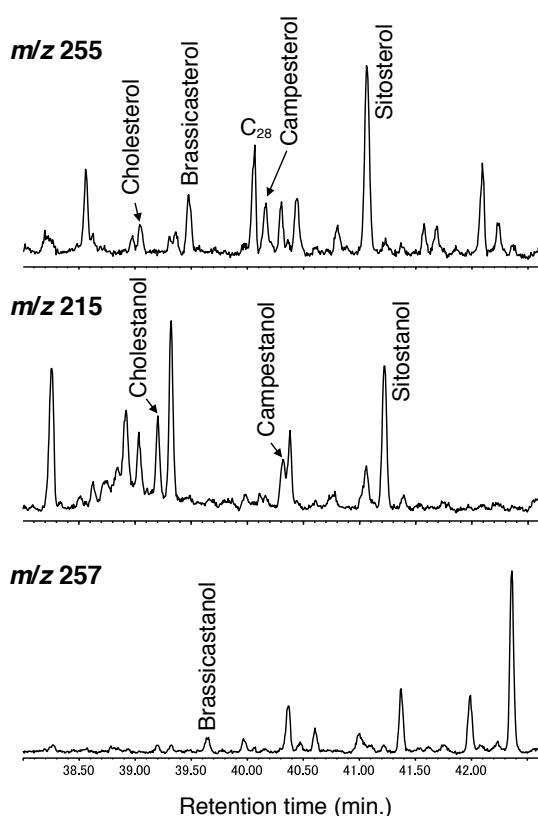

Fig. 3 Mass chromatograms (*m/z* 255, *m/z* 215, and *m/z* 257) of sterols from the sediment of St. 1.

campesterol, β-sitosterol, $5\alpha(H)$-cholestan-3β-ol (cholestanol), 24-methyl-$5\alpha(H)$-cholestan-3β-ol (campestanol), 24-methyl-$5\alpha(H)$-cholest-22E-en-3β-ol (brassicastanol), and sitostanol (Fig. 3). The sterol compositions are listed in Table 2. Among the Δ^5-sterols, concentration of β-sitosterol is the highest (9.43–13.52 μg/g), whereas that of brassicasterol is much lower (1.48–1.77 μg/g). Sitostanol is the major $5\alpha(H)$-stanol component in the sediments (2.78–3.88 μg/g). Although the brassicastanol was detected in the sediments, only trace amounts were found (<0.28 μg/g).

The ratios of cholestanol/cholesterol and campestanol/campesterol in the sediments show high values of 0.76–0.85 and 0.60–0.80, respectively. The sitostanol/β-sitosterol ratios are smaller (0.29) than the cholestanol/cholesterol and campestanol/campesterol ratios despite the high concentration of sitostanol. The brassicastanol/brassicasterol ratio has a significantly lower value (0.11–0.16) than the other ratios.

3.2. Sterols of higher plants from the area surrounding the pond.

Sterol compositions of the leaves obtained from the area surrounding the pond are listed in Table 2, and the representative mass chromatograms are shown in Fig. 4. The β-sitosterol was identified as the major sterol component from all leaf samples (373.9–426.4 μg/g). The concentrations of campesterol in leaf samples of *Cerasus yedoensis* are lower than that of the β-sitosterol (9.29 μg/g). As for the 5a(H)-stanols, only trace amounts of sitostanol were detected in *Quercus serrate*.
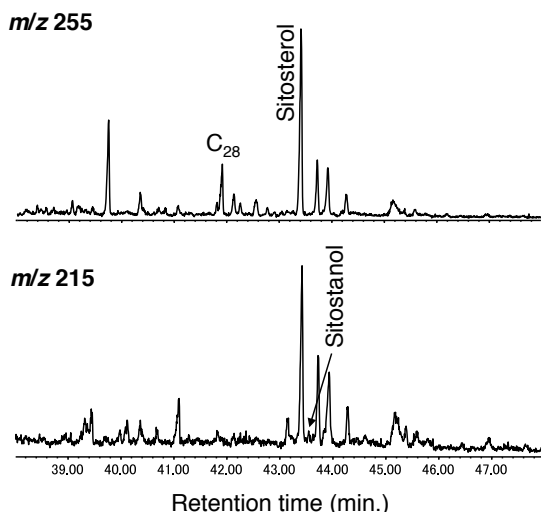
Fig. 4 Representative mass chromatograms (*m/z* 255 and *m/z* 215) of sterols from the plant (*Quercus serrate*).

Fig. 5 Distribution of C_{27}, C_{28}, and C_{29} sterols in aquatic plankton, higher plants, and sediment samples. Gray triangles (△)=plankton. White diamonds (◇)=higher plants. Red circles (●)=sediment samples from the Bungaku-no-ike pond. C_{27} sterols=22-dehydrocholesterol and cholesterol. C_{28} sterols=brassicasterol, campesterol, and 24-methylenecholesterol. C_{29}=stigmasterol and β-sitosterol. Plankton and higher plant distributions obtained from Huang and Meinschein (1979), including Attaway et al. (1971), Nishimura and Koyama (1976), Pryce (1971), and Meinschein and Kenney (1957).

4. Discussion

4.1. Major sterol sources in the sediment

β-Sitosterol is documented in higher plants as the major sterol components (Yunker et al., 1995; Belicka et al., 2004; Killops and Killops, 2013). The β-sitosterol has the highest concentration in the Δ^5-sterols in the sediment samples, and is the main sterol found in the leaf samples from the area around the pond (Table 2). Brassicasterol is found in phytoplankton including diatoms and haptophyte algae (Kanazawa et al., 1971; Orcutt and Patterson, 1975; Teshima et al., 1980; Volkman et al., 1981, 1998; Lin et al., 1982; Marlowe et al., 1984; Rampen et al., 2010). The concentration of brassicasterol is much lower in the sediments (Table 2). Huang and Meinschein (1979) indicated that the ternary plots of C_{27}, C_{28}, and C_{29} sterol compositions can be used as an ecological indicator. Phytoplankton contain high amounts of C_{27} sterols, while terrestrial plants contain high amounts of C_{29} sterols. Plots of C_{27}, C_{28}, and C_{29} sterol compositions in the sediments show closer distribution to terrestrial plants (Fig. 5), suggesting that the sterols in the sediments are mainly derived from higher terrestrial plants.

4.2. Environmental condition of the pond evaluated by 5α(H)-stanol compositions in the sediments

In general, high contribution of 5α(H)-stanol in natural environment is thought to be resulted from three routes; (1) bacterial reduction of Δ^5-sterol under anoxic condition (e.g., Gagosian et al., 1979; Wakeham, 1989, Fig. 1C), (2) direct inputs from unique living organisms containing high 5α(H)-stanols (Robinson et al., 1984; Volkman et al., 1990, Fig. 1A), and (3)

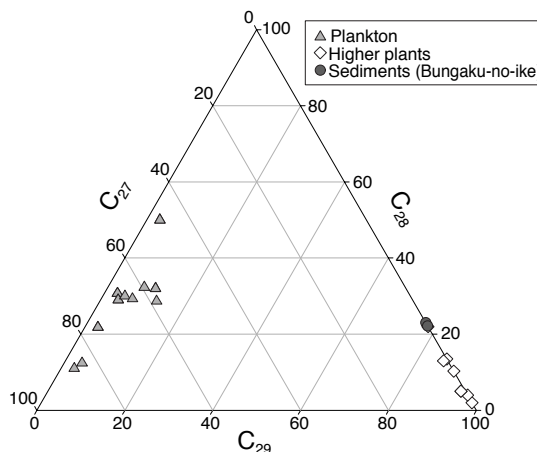selective degradation of Δ^5-sterol during sedimentation processes (e.g., Nishimura, 1977a, Fig. 1B). For the first route of bacterial reduction of Δ^5-sterols, the 5α(H)-stanol generates by the bacterial reduction of Δ^5-sterol under anoxic conditions (Rosenfeld and Hellman, 1971; Eyssen et al., 1973). Therefore, the 5α(H)-stanol/Δ^5-sterol ratio can be used as a tracer of bacterial anoxic activity, and also can apply to evaluation of depositional environments including paleo-studies (Canuel and Martens, 1993; Bertrand et al., 2012; Zheng et al., 2015; Nakakuni et al., 2017). Under anoxic condition, the microbial conversion of Δ^5-sterol to 5α(H)-stanol occurs in surface sediments and water columns (Nishimura and Koyama, 1977; Wakeham, 1989; Fig. 1C). Also, the 5α(H)-stanol/Δ^5-sterol records were reported in marsh environment and peat sequences in the Miocene, and increase of their ratios may be attributed to degradation and microbial hydrogenation of biosterols during the early diagenetic stage (Sawada et al., 2013; Stefanova et al., 2016). However, it should be noted that variations of the 5α(H)-stanol/Δ^5-sterol ratios can also be related to environmental conditions, and the 5α(H)-stanols are derived directly from organisms or that the Δ^5-sterols were converted to 5α(H)-stanols in the early diagenesis (Stefanova et al., 2016).

The higher 5α(H)-stanol/Δ^5-sterol ratios was reported in severe anoxic water and sediment of the Black Sea (0 mmol/kg in DO below ca. 100 m); remarkable high values at redox boundaries of the water column (>1; Wakeham, 1989) and the surface sediments (>1; Gagosian et al., 1979).

In sediments from offshore of the southern California, 24-nordehydrocholestanol/24-nordehydrocholesterol and brassicastanol/brassicaterol ratios indicated high values (~ 0.7) during suboxic (warming) intervals of the Marine Isotope Stage (MIS) 3 (Nakakuni et al., 2017).

Among the 5α(H)-stanol/Δ^5-sterol ratios, brassicastanol/brassicasterol ratio is suggested to be a good redox tracer, because inputs other than reduction of sterols in anoxic conditions is considered to be small in marine sediments (Nakakuni et al., 2017). However, the brassicastanol/brassicasterol ratios are low (<0.16) in the Bungaku-no-ike pond of the present study, which is strikingly lower than the 5α(H)-stanol/Δ^5-sterol records in the anoxic conditions reported previously. The result suggests that the pond in our work is an oxidizing environment based on the organic chemical trace, as well as the DO contents in the bottom water (5.5 ± 0.5 mg/L).

On the other hand, the ratios of cholestenol/cholesterol, campestanol/campesterol, and sitostanol/β-sitosterol shows markedly higher values compared to brassicastanol/brassicasterol ratio, similar to typical anoxic condition levels found in the Black Sea and southern California. The results indicate use of these ratios as a redox tracer is not suitable, and the higher contents of 5α(H)-stanol (cholestanol, campestanol, sitostanol) in the sediments are possibly caused by biological/geochemical effect(s) other than the bacterial reduction of the Δ^5-sterols.

4.3. Possibility of source of 5α(H)-stanol in the sediments

In previous studies, high 5α(H)-stanol contents (cholestanol, campestanol, and sitostanol) have been documented even in oxidative conditions. Kondo et al. (1994) found high choletanol (cholestenol/cholesterol ratio; 0.66 – 0.85), campestanol (campestanol/campesterol ratio: 0.63 – 0.67) and sitostanol (sitostanol/β-sitosterol ratio: 0.59 – 0.63) contents (Table 4) in the sediments from acidic lake such as Lake Fudo-ike (Kirishima, Japan). Nishimura (1977a) also reported high values of 5α(H)-stanol/Δ^5-sterol ratios (cholestenol/cholesterol; 0.56 – 0.82, campestanol/campesterol; 0.37 – 1.00, sitostanol/β-sitosterol; 0.67 – 1.22) from the sediments of Lake Shirakoma-ike (Nagano, Japan). Previous studies suggested that the contribution of 5α(H)-stanols except the bacterial reduction of Δ^5-sterols was significantly large to the sterol composition in freshwater sedimentary environments, which agrees with our results. The high 5α(H)-stanol inputs complicate interpretation of the 5α(H)-stanol/Δ^5-sterol ratio as an indicator of redox condition. Thus, it is important to explore the cause of high 5α(H)-stanol contents other than reducing environments, and as the results suggest, it is expected to provide better interpretation of the 5α(H)-stanol/Δ^5-sterol ratio as the indicator.

4.3.1. Terrestrial sources

The high 5α(H)-stanol contents in the sediments from the Bungaku-no-ike pond are likely to be related to terrigenous sources, because the ternary plots of C_{27}, C_{28}, and C_{29} sterols indicate significant contribution from higher plants (Fig. 5). In general, the content of 5α(H)-stanol in living higher plants are low (e.g., Nishimura, 1977b; Table 3), which is similar to the results in the present study (Table 2). However, according to Nishimura (1977b), only *Quercus serrata* is known to have relatively high sitostanol content (30.3% of all the sterols; sitostanol/β-sitosterol ratio: 0.48; Table 3). Since the Bungaku-no-ike pond is surrounded by *Quercus serrata*, there is a possibility that the plant-derived 5α(H)-stanols contributed to the sediments. However, content of sitostanol in the leaf samples of *Quercus serrata* around the pond were trace in the present study (Table 2). The difference of the sterol compositions in *Quercus serrate* between Nishimura (1977b) and our study may be attributed to chemical compositional variability as a result of seasonal and environmental effects.

Another possibility is a mechanism that the 5α(H)-stanols from living organisms are concentrated by selective degradation process of Δ^5-sterols under oxidative environments (Nishimura, 1977a; Nishimura and Koyama, 1977). Since the Δ^5-sterols are easily degraded in oxidative conditions compared to 5α(H)-stanols, high 5α(H)-stanol/Δ^5-sterol ratios in sediments are expected even if the direct contribution of 5α(H)-stanol from living organisms is low (Fig. 1B). Furthermore, Nishimura (1977a) suggests that 5α(H)-stanol, especially derived from terrestrial organism, could contribute to sediment through the degradation process. Since sitostanol was detected in the leaf samples, the high sitostanol/β-sitosterol ratio in the sediments can be interpreted as the results that the sitostanol was concentrated by the selective degradation process of β-sitosterol (Table 2). Although the sitostanol/β-sitosterol ratio in our work is lower than those in Lake Shirakoma-ike (0.67 – 1.22; Nishimura, 1977a), fresh plant inputs around the Bungaku-no-ike pond might be the cause. In fact, the sterol compositions in living plants around the pond showed high β-sitosterol concentrations (Table 2). Similarly, high 5α(H)-stanol contents in sediments from oxic conditions have been interpreted by inputs of degradation products from terrestrial sources associating with such the mechanism (Kondo et al., 1994; Arzayus and Canuel, 2005; Rontani et al., 2014). Since cholestanol and campestanol were not detected in the investigated plants (Table 2), it is difficult for the sources of campesterol and cholesterol to account for factors of the terrestrial plant sources and the degradation processes.

Table 3 Sterols of freshwater dinoflagellates and higher plants reported by previous studies (Robinson et al., 1987; Nishimura, 1977b; Nishimura and Koyama, 1977).

	Freshwater dinoflagellates			Higher plants			
	P.l.	P.c. (%)	C.f.	I.p.	P.d. (%)	Q.s.	Z.l.
Δ^5-sterols							
Cholesterol	trace	94.6	80.2	trace	trace	2.9	trace
Campesterol	-	1.8	-	3.0	7.8	3.6	5.3
Brassicasterol	-	-	-	-	-	-	-
β-Sitosterol	17.9	-	-	93.0	90.2	62.5	93.8
5α(H)-stanols							
Cholestanol	71.4	3.6	18.7	trace	trace	trace	trace
Campestanol	3.5	-	1.1	0.2	0.1	0.6	0.1
Brassicastanol	-	-	trace	-	-	-	-
Sitostanol	7.2	-	-	3.8	1.8	30.3	0.9
Ratios							
Cholestanol/cholesterol	-	0.04	0.23	-	-	-	-
Campestanol/campesterol	-	-	-	0.05	0.02	0.17	0.02
Brassicastanol/brassicasterol	-	-	-	-	-	-	-
Sitostanol/β-sitosterol	0.40	-	-	0.04	0.02	0.49	0.01

P.l. = *Peridinium lomnickii*, P.c. = *Peridinium cinctum*, C.f. = *Ceratium furcoides*. I.p. = *Ilex pedunculosa*, P.d. = *Pinus densiflora*, Q.s. = *Quercus serrata*, Z.l. = *Zizania latifolia*.
The sterol composition data in freshwater dinoflagellates and higher plants are taken from Robinson et al. (1987), Nishimura (1977b), and Nishimura and Koyama (1977), respectively.
*The values are given as % of the eight sterols.

Table 4 Sterols in sediment samples from other sites reported by previous studies (Nishimura, 1977a; Kondo et al., 1994).

	Sediment samples					
	S-1	S-2	S-3	S-4 (μg/g)	F-1	F-4
Δ^5-sterols						
Cholesterol	8.4	8	5.3	6.4	1.38	0.81
Campesterol	8.4	10.3	4.1	8.6	1.20	0.78
Brassicasterol	trace	6.6	trace	2.1	1.08	0.54
β-Sitosterol	45.8	39.7	27.6	47.3	2.38	1.76
5α(H)-stanols						
Cholestanol	6.9	4.5	3.2	4.2	1.12	0.69
Campestanol	5.4	3.8	4.1	4.6	0.80	0.49
Brassicastanol	-	-	-	-	0.35	0.15
Sitostanol	43.6	26.6	33.7	37.8	1.40	1.11
Ratios						
Cholestanol/cholesterol	0.82	0.56	0.60	0.66	0.81	0.85
Campestanol/campesterol	0.64	0.37	1.00	0.53	0.67	0.63
Brassicastanol/brassicasterol	-	-	-	-	0.32	0.28
Sitostanol/β-sitosterol	0.95	0.67	1.22	0.80	0.59	0.63

S-1 = Lake Shirakoma-ike (0-3 cm), S-2 = L. Shirakoma-ike (3-6 cm), S-3 = L. Shirakoma-ike (6-12 cm), S-4 = L. Shirakoma-ike (12-20 cm), F-1 = L. Fudo (0-2 cm), F-4 = L. Fudo (6-8 cm).
The sterol composition data in sediment samples of Lake Shirakoma-ike and Lake Fudo are taken from Nishimura (1977a) and Kondo et al. (1994), respectively.

4.3.2. Phytoplanktonic sources

In aquatic organisms, the 5α(H)-stanol contents are conclusively low, although the same 5α(H)-stanols have been identified in zooplankton and phytoplankton (Nishimura and Koyama, 1976, 1977; Chardon-Loriaux et al., 1976). However, high contents of 5α(H)-stanol are known in some organisms as unique cases (Volkman et al., 1990). In freshwater phytoplankton species, to the best of our knowledge, high 5α(H)-stanol contents were reported in some dinoflagellates only (Robinson

et al., 1984, 1987). Therefore, the 5α(H)-stanols from dinoflagellates could contribute to organic composition in sediments (Fig. 1A). In fact, the dinoflagellates have cholestanol and campestanol, which cannot be explained by the terrestrial sources. On the other hand, freshwater dinoflagellates are known to have high contents of 4α-methyl sterols, such as 4α-methyl-5α-cholestan-3β-ol and 4α, 23, 24-trimethyl-5α-cholest-22-en-3β-ol (Robinson et al., 1987). Although dinoflagellate was not investigated in the present study, the high contents

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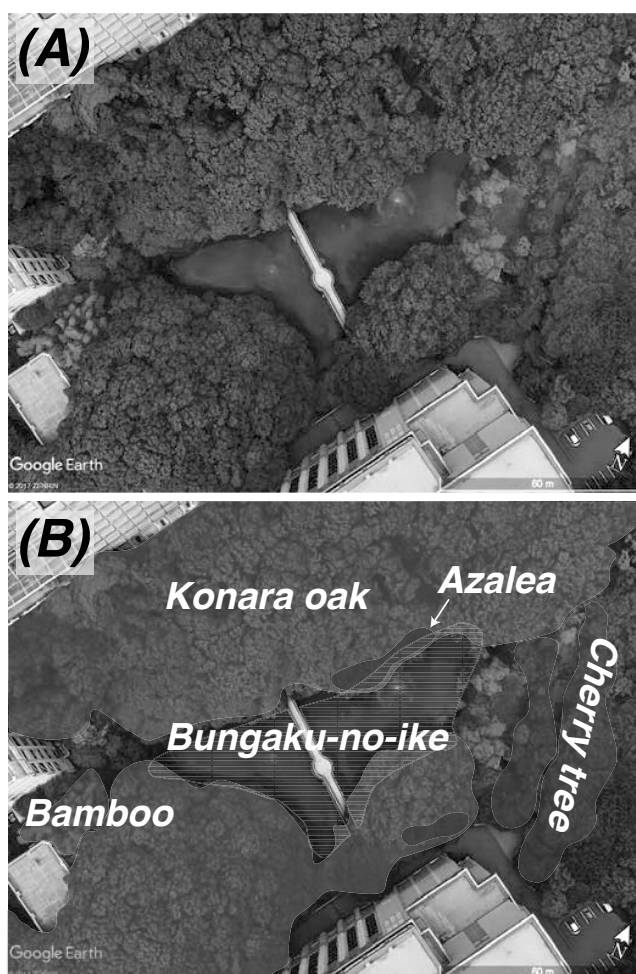
Appendix. Distribution of vegetation around the Bungaku-no-ike pond: (A) Original picture. (B) Distribution of vegetation. The original picture was obtained from Google Earth (https://www.google.com/earth/).