

Biodegradability of algal-derived organic matter in a large artificial lake by using stable isotope tracers

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Abstract In order to understand the biodegradability of algal-derived organic matter, biodegradation experiments were conducted with ¹³C and ¹⁵N-labeled natural phytoplankton and periphytic algal populations in experimental conditions for 60 days. Qualitative changes in the dissolved organic matter were also determined using parallel factor analysis and the stable carbon isotopic composition of the hydrophobic dissolved organic matter through the experimental period. Although algal-derived organic matter is considered to be easily biodegradable, the initial amounts of total organic carbon newly produced by phytoplankton and periphytic algae remained approximately 16 and 44 % after 60 days, respectively, and about 22 and 43 % of newly produced particulate nitrogen remained. Further, the dissolved organic carbon derived from both algal populations increased significantly after 60 days. Although the dissolved organic matter gradually became refractory, the contributions of the algal-derived organic

matter to the dissolved organic matter and hydrophobic dissolved organic matter increased. Our laboratory experimental results suggest that algal-derived organic matter produced by phytoplankton and periphytic algae could contribute significantly to the non-biodegradable organic matter through microbial transformations.

Keywords Non-biodegradable organic matter · Stable isotope labeling · Phytoplankton · Periphytic algae · Biodegradability · PARAFAC

Introduction

The organic matter concentrations in water resources have been reported to increase in the UK, North America, Europe, and Japan (Driscoll et al. 2003; Evans et al. 2005; Hejzlar et al. 2003; Imai et al. 2001; Le et al. 2010; Worrall et al. 2003; Yamada et al. 2012). Apart from leading to increased water purification costs (Le et al. 2010), this also causes changes in the fate and transport of pollutants such as heavy metals in water bodies (Tessier and Turner 1995). This phenomenon has been observed in the Han River, which is one of the most important water sources in Korea (Lee and Choi 2009). In order to determine the reasons for this phenomenon, the Korean government is trying to understand the sources and long-term trends of organic matter.

In Lake Paldang, which is a large artificial lake located in the central region of the Han River, an annual rise of chemical oxygen demand (COD) has been observed since 1995. However, the biochemical oxygen demand (BOD) values are nearly constant (Fig. S1). On the other hand, the BOD/COD ratio has shown a decreasing trend since 1999, indicating increased contribution of refractory organic matter to the total organic matter (Badawy and Ali 2006; Vollertsen and

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Hvitved-Jacobsen 2002). These long-term trends support the accumulation of non-biodegradable organic matter (viz. recalcitrant organic matter) in natural water resources. However, limited information is available on the main source of non-biodegradable organic matter.

Although algal-derived organic matter is considered to be easily biodegradable, accumulating evidence suggests that a substantial fraction of algal-derived organic matter persists after several months of biodegradation (Kragh and Søndergaard 2009; Kristensen et al. 1995; Hanamachi et al. 2008). The labile organic matter can be converted to refractory matter, which remains for a long period in the water column, by microbial degradation. Ogawa et al. (2001) reported that glucose and glutamate, which are easily biodegradable, were rapidly consumed within 2 days by microbial transformation. They further stated that the refractory dissolved organic matter was released within a few days, after the addition of labile organic matter. In addition, Kragh and Søndergaard (2009) demonstrated the production of refractory organic components directly by marine algae or indirectly by food web processes. These results indicate that algal-derived organic matter in the water supply could be an important source of refractory organic matter, which increases the cost of water treatment.

Aquatic organic matter consists of autochthonous as well as allochthonous substances. Hence, it is not easy to separate the fate of organic matter produced by primary producers in a natural sample. In the case of periphytons, it is all the more difficult to distinguish newly produced organic matter from the complex mixture of algae, heterotrophic microbes, and detritus. However, this can be overcome by using isotopically enriched inorganic carbon and/or nitrogen. Stable isotope labeling has been used as a powerful tool to elucidate the fates and dynamics of algal-derived organic matter (Kritzberg et al. 2006; Pace et al. 2004; Veuger et al. 2004; Yoshimura et al. 2009).

During the biodegradation process, the organic matter preservation potential depends on the nature of the organic sources (Owen and Lee 2004). Combined excitation emission matrix fluorescence techniques with parallel factor analysis (EEM-PARAFAC) have been extensively used to infer changes in the qualitative composition of dissolved organic matter during biodegradation (Stedmon and Markager 2005; Yamashita et al. 2008). This new approach has a competitive advantage over conventional methods, i.e., it aids in understanding large EEM data sets (Kowalczyk et al. 2009).

To better understand the biodegradability of algal-derived organic matter, its properties must be determined in natural aquatic environments. Aside from phytoplankton, periphytic algal-derived organic matter is also an important source in aquatic systems. However, there is limited or no information available on the role of periphytic algae in the formation of refractory organic matter. Given that a considerable proportion of the organic matter newly produced by phytoplankton persists after biodegradation, periphytic algal-derived organic

matter could also participate in the formation of refractory organic matter. Therefore, in this investigation, an effort to understand the biodegradability of algal-derived organic matter originating from natural phytoplankton and periphytic algal populations was undertaken under laboratory conditions.

The present study was conducted to (i) determine the contribution of algal-derived organic matter during microbial degradation using stable carbon and nitrogen isotope tracers and (ii) understand the qualitative changes in dissolved organic matter during biodegradation.

Materials and methods

Sampling sites and sample preparation

Lake Paldang, constructed in 1973, is a major source of drinking water for the 20 million people residing in the Seoul metropolitan area of Korea (Fig. S2). Although the lake is a protected drinking water resource, its water quality is reported to have deteriorated because of increased algal production (Lee et al. 2015; Na and Park 2006). In order to determine the biodegradability of algal-derived organic matter, natural phytoplankton and periphyton populations were collected from Lake Paldang on May 24, 2011. Surface water containing natural phytoplankton communities was sampled at site P1 (Fig. S1), and the macro-zooplankton were removed by filtration through a 100- μm mesh (Hama et al. 2004; Yoshimura et al. 2009). The water samples were transferred to polycarbonate (Nalgene) bottles that were acid-washed. Several plastic bars (37.5 cm²) affixed to trays were placed underwater at site P2 to collect natural periphyton populations on April 28, 2011. After 1 month (viz. May 24, 2011), each plastic bar with the attached periphyton was placed directly into acid-washed polycarbonate bottles filled with filtrate (pre-combusted GF/F). These natural phytoplankton and periphyton populations were transferred to the laboratory within one-and-a-half hours of collection.

C and N labeling experiment

In order to separate the organic matter newly produced by algae from the bulk pool, natural algal populations were incubated with $\text{NaH}^{13}\text{CO}_3$ and K^{15}NO_3 under fluorescent light (viz. $\sim 140 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 14 h at 20 °C (which corresponds to the in situ temperature). A sub-sample was collected after 14 h (day 0), and the remaining samples were incubated in the dark at 20 °C for 60 days. Sub-samples for both algal populations were collected in triplicate at each sampling date (viz. days 5, 15, 28, and 60). After collecting the sub-sample, the periphyton was scraped off into the water and filtered. After 60 days, the concentrations of dissolved oxygen in the bottles containing natural phytoplankton and periphyton populations

were 5.6 and 5.9 mgO₂L⁻¹, respectively. These results indicate that the organic matter had been decomposed under oxic conditions. The particulate fractions in the samples were collected onto pre-combusted (4 h at 450 °C) Whatman GF/F filter, and the pigment composition, carbon, and nitrogen isotopic compositions and their concentrations were determined using HPLC (1200 series, Agilent Technologies, San Jose, CA, USA) and elemental analyzer–isotopic ratio mass spectrometer (EA–IRMS), respectively. The details of pigment analysis are described in Ha et al. (2014), and the detailed methodology for EA-IRMS analysis is provided below. The filtrate samples were analyzed to measure their nutrient concentration, concentrations of dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC), fluorescence property, and carbon and nitrogen isotopic compositions of hydrophobic dissolved organic matter. The concentrations of nutrients (nitrate + nitrite, phosphate, and silicate) were analyzed using a continuous flow nutrient analyzer (Bran + Luebbe, QuAAtro). The MOOS-2 was used as certified reference material. The concentrations of DIC and DOC were measured using a total organic carbon analyzer (TOC-V_{CPH}, Shimadzu, Japan). This method has been validated against a certified reference material (41–44 μmol L⁻¹; Hansell Laboratory, University of Miami).

EA–IRMS analysis

After removal of inorganic carbon (using 12N hydrochloric acid fumes), the isotopic composition and concentrations of particulate organic carbon (POC) were determined using an isotope ratio mass spectrometer (Isoprime, GV Instruments, Manchester, UK) with an elemental analyzer (Euro EA3028, EuroVector, Milan, Italy). The isotopic composition and concentrations of particulate nitrogen (PN) were also determined by a procedure similar to POC, barring the acidification step. The coefficients of variations of POC and PN concentrations using standard materials were 3.3 and 3.4 %, respectively. The analytical precisions for the carbon and nitrogen isotopic ratios, estimated by running standards (IAEA standards CH6 and N1), were 0.05 and 0.1 ‰, respectively. The standard reference materials for carbon and nitrogen were Vienna Pee Dee Belemnite (VPDB) and atmospheric N₂, respectively. The carbon isotope ratio of DOC was determined in the freeze-dried material after exposure to acid fumes to remove inorganic carbon (Urban et al. 2005). The removal of inorganic carbon with 12N hydrochloric acid fumes was verified using NaHCO₃.

Fluorescence analysis of DOM

The fluorescence EEM was measured with a luminescence spectrometer (LS-55, Perkin-Elmer, Liantrisant, UK) by scanning the emission spectra from 280 to 550 nm at 0.5 nm increments and stepping through excitation wavelengths from 250 to 500 nm at 5 nm increments. The methods of measuring the EEM and normalizing the fluorescence intensity were described in detail by Phong et al. 2014. After data acquisition, PARAFAC modeling was conducted using MATLAB 7.0 (Math Works, Natick, MA, USA) with the DOMFluor Toolbox (<http://www.models.life.ku.dk>), and the identified components and numbers were validated by split-half analysis. The details of the modeling have been provided elsewhere (Kowalczyk et al. 2009; Nguyen et al. 2013; Stedmon et al. 2003). The fluorescence emission spectra of the samples were recorded at 300 to 480 nm (under 254 nm excitation) to calculate the humification index (HIX). The HIX was estimated based on the equation proposed by Ohno (2002) and ranged from zero to one.

C and N isotopic compositions of hydrophobic DOM

The hydrophobic DOM was extracted using a solid phase C₁₈ extraction disk (3M Empore) following the method of Kim et al. (2003). The disks were conditioned using methanol before extracting the water samples. The water samples were passed through a GF/F filter, and the pH of the filtrates was adjusted to two using hydrochloric acid prior to extraction. Aliquots of 800–1000 ml of the filtrate water were loaded onto the SPE disk. The retentate containing hydrophobic DOM was eluted with methanol and dried using a centrifugal evaporator (EYELA, Tokyo, Japan). The carbon and nitrogen isotopic compositions of hydrophobic DOM absorbed onto the glass fiber filters (Whatman GF/F) were measured using an EA–IRMS as described above. However, the nitrogen isotopic composition of all hydrophobic DOM showed values less than the detection limit. The carbon isotopic composition of hydrophobic DOM is described in the “Results and discussion” section.

Data analysis

The concentrations of algal-derived organic matter in the subsamples were calculated according to the equation (Hama and Yanagi 2001; Hama et al. 2004) below:

$$\text{Concentration of algal-derived organic matter } (\mu\text{g C (or N) L}^{-1}) = [(a_{is}-a_{ns})/(a_{ic}-a_{ns})] \times [C \text{ (or N)}] \quad (1)$$

where a_{is} is the ^{13}C (or ^{15}N) atom percentage in an incubated sample, a_{ns} is the ^{13}C (or ^{15}N) atom percentage in a natural (non-incubated) sample, a_{ic} is the ^{13}C (or ^{15}N) atom percentage in DIC (or DIN), and C (or N) is the concentration of organic carbon (viz. POC and DOC) or PN in the incubated samples.

The kinetics of DOM degradation was expressed by a first-order exponential decay model taking the non-biodegradable pool into consideration, using the following equation:

$$OM(t) = BOM \times \exp(-kt) + NBOM \quad (2)$$

where $OM(t)$ is the amount of OM remaining at time t , BOM is the biodegradable pool, k is the degradation rate constant (day^{-1}), t is the time (days), and $NBOM$ is the non-biodegradable pool (Lønborg et al. 2009). Curve fitting was carried out using the software SIGMA PLOT 10.0 (Chabbi et al. 2006; Lønborg et al. 2009).

Statistical analysis

The statistical differences between day 0 and day t (viz. day 5, 15, 28, and 60) were tested using the Student's t test or Mann-Whitney U test. These statistical analyses were performed using the IBM SPSS Statistics 21 software (SPSS Inc., Chicago, IL).

Results and discussion

Biodegradability of algal-derived organic matter

The labeled DIC and DIN were successfully incorporated into the phytoplankton and periphytic algae over 14 h under light. Moreover, using enriched stable isotope tracers, it was possible to separate the newly produced organic matter from the bulk pool (Table 1). The initial (viz. day 0) concentrations of bulk POC, PN, DOC, and pigments were higher in the periphyton than the phytoplankton. Among the pigments, the highest concentrations recorded in both algal populations were fucoxanthin, which is a diatom marker pigment (Jeffrey et al. 1997), followed by chlorophyll a . These results suggest that the phytoplankton and periphytic algal communities were dominated by diatom taxa. The newly produced POC, PN, and DOC comprised 8.2, 5.0, and 0.43 % of the bulk pools, respectively, in the phytoplankton. On the other hand, in the periphytic algae, they comprised 2.6, 3.4, and 0.56 % of the bulk pools, respectively. The DOC fractions derived from both algal populations were 6.0 and 8.1 % of the newly produced total organic carbon for phytoplankton and periphytic algae, respectively. These values were comparable with those reported by Baines and Pace (1991) using ^{14}C as a tracer. The concentrations of nitrate + nitrite, phosphate, and silicate were 119, 0.27, and $77 \mu\text{mol L}^{-1}$, respectively, in bottles containing phytoplankton populations, and 105, 0.65,

and $77 \mu\text{mol L}^{-1}$, respectively, in bottles containing periphyton populations on day 0 (i.e., after 14 h of light incubation).

Time-dependent changes in concentrations of chlorophyll a and newly produced organic matter are shown in Fig. 1. The concentration of Chl a decreased rapidly during the initial 5 days in the phytoplankton-derived organic matter, while it decomposed from day 0 to day 28 in the periphytic algal-derived organic matter. The decay constant (k) of Chl a was three times greater in the phytoplankton than in the periphytic algae. Similar values of PO^{13}C were observed in both samples on day 0, but the degradation rate (k) was also faster in the phytoplankton-derived organic matter than in the periphytic algae. Louda et al. (1998) stated that during the senescence and death of algal cells, Chl a is converted to a degraded form. However, in contrast to phytoplankton, the Chl a of periphytic algae remained at 23 % of the initial value until day 15. Sekar et al. (2002) reported that the Chl a concentrations in biofilm were sustained for 15 days under dark conditions. They also observed a succession of algal communities in light-grown biofilms (Chlorophyceae–diatom–cyanobacteria), while the diatom mostly dominated the dark-grown biofilms for 15 days. Thus, as previous reports verify that periphytic diatom can survive until day 15, this could explain the slow degradation rates of periphytic algal-derived organic matter. The decay constant (k) of the POC in phytoplankton was comparable with those reported by a previous investigator (Pett 1989).

The concentrations of particulate organic matter were decreased in both populations, while the DOC either remained stable or increased. The initial DO^{13}C concentration was retained even after 60 days in the bottles containing phytoplankton populations. Further, its concentration was dramatically increased on day 5. This was probably caused by a significant decrease in Chl a and PO^{13}C (i.e., released as a labile fraction). In the case of periphytic algae, there was a 73 % decrease in PO^{13}C as compared to its initial value, but the DO^{13}C increased about three times compared to day 0. This increase coincided with 18 % of the biodegradable POC over 60 days.

The newly produced P^{15}N by both algal-populations declined rapidly within the first 5 days and continued to decline further until day 28. Although they showed different concentrations, the degradation rates were the same. Generally, organic nitrogen is preferentially degraded compared to organic carbon through the decomposition of organic matter during early diagenesis (Hecky et al. 1993; Talbot and Lærdal 2000). However, the disparate results were found in this study. The organic matter preservation has been known to be affected by the phytoplankton source (Hanamachi et al. 2008; Nguyen and Harvey 1997). In an oxic microbial degradation experiment using *Skeletonema costatum* (diatom), Kristensen et al. (1995) reported concentrations of POC and PON on day 38 to be 66 and 72 % of the initial concentration, respectively. In a decomposition experiment using *Thalassiosira weissflogii* (diatom), Harvey et al. (1995) also reported that the POC was

Table 1 Concentrations (mean \pm standard deviation, $n=3$) of particulate organic carbon, particulate nitrogen, dissolved organic carbon, and major pigments (Chl *a* and fucoxanthin)

Source of organic matter	Compounds				
	POC ($\mu\text{gC L}^{-1}$)	PN ($\mu\text{gN L}^{-1}$)	DOC ($\mu\text{gC L}^{-1}$)	Chl <i>a</i> ($\mu\text{g L}^{-1}$)	Fucoxanthin ($\mu\text{g L}^{-1}$)
Phytoplankton					
Bulk organic matter on day 0	1155 \pm 63	258 \pm 9.0	1431 \pm 11	19 \pm 0.89	14 \pm 0.97
Newly produced organic matter on day 0 ^a	95 \pm 3.9	13 \pm 1.5	6.1 \pm 0.26	–	–
Percentage of newly produced organic matter on day 0 ^b	8.2	5.0	0.43	–	–
Biodegradable organic matter ^c	82 \pm 3.5	10 \pm 1.2	–	19 \pm 0.29	14 \pm 0.84
Non-biodegradable organic matter ^c	13 \pm 1.8	2.8 \pm 0.80	–	0.25 \pm 0.15	0.021 \pm 0.63
Percentage of non-biodegradable organic matter ^d	14	22	–	–	–
Periphytic algae					
Bulk organic matter on day 0	3896 \pm 193	645 \pm 45	1612 \pm 27	77 \pm 7.5	72 \pm 0.37
Newly produced organic matter on day 0 ^a	102 \pm 38	22 \pm 2.5	9.0 \pm 0.93	–	–
Percentage of newly produced organic matter on day 0 ^b	2.6	3.4	0.56	–	–
Biodegradable organic matter ^c	71 \pm 4.6	12 \pm 0.92	–	76 \pm 5.1	60 \pm 6.4
Non-biodegradable organic matter ^c	31 \pm 2.9	9.5 \pm 0.60	–	0.17 \pm 3.5	10 \pm 4.3
Percentage of non-biodegradable organic matter ^d	30	43	–	–	–

^a ^{13}C or ^{15}N incorporated organic matter

^b (Newly produced organic matter on day 0/bulk organic matter on day 0) \times 100

^c Obtained by fitting the first-order exponential decay model using newly produced organic matter and pigment concentrations

^d (Non-biodegradable organic matter/newly produced organic matter on day 0) \times 100

preferentially degraded rather than protein over 20 days. Our results correspond to these previous findings involving degradation experiments using a single cell diatom.

Nguyen and Harvey (1997) also reported that almost half of the dinoflagellate-produced PN attributable to polypeptides and/or proteins was of a refractory nature. The algal-derived DOC remained at more than 70 % after biodegradation (Kragh and

Søndergaard 2009; Yoshimura et al. 2009). Although quite different proportions of biodegradable fraction of phytoplankton sources have been described in previous reports (Hanamachi et al. 2008; Nguyen and Harvey 1997), in this study as well, considerable photosynthetic organic matter could remain as non-biodegradable organic matter after the degradation process. On the other hand, the high proportions of newly produced

Fig. 1 Changes in **a** Chl *a*, **b** newly produced PO^{13}C , **c** DO^{13}C , and **d** P^{15}N of phytoplankton and periphytic algae. The plots represent observed mean values with standard deviations, and the *solid line* represents values predicted by the exponential decay model. The “*k*” is the degradation rate constant of each compound. Curve fitting was not attempted on DO^{13}C

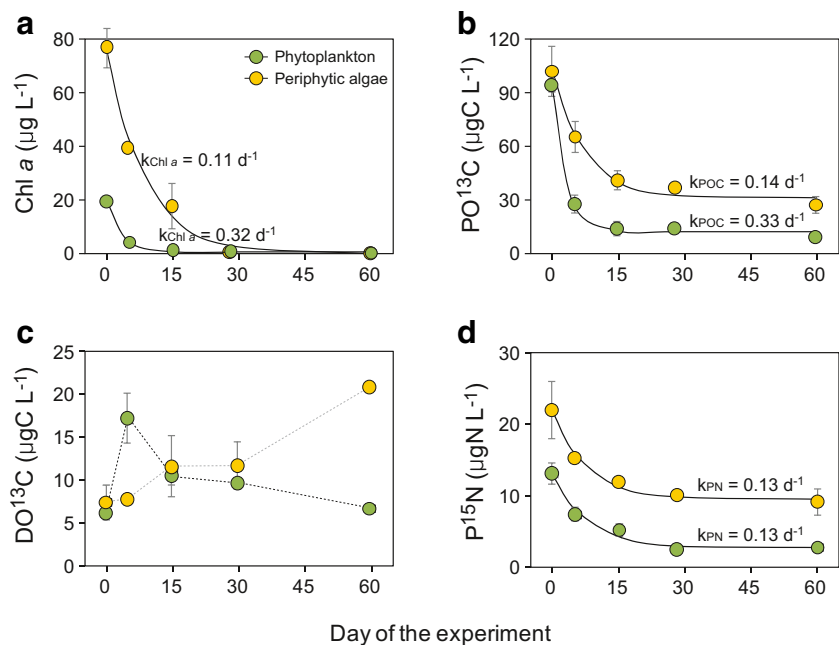


Table 2 Changes in concentrations of bulk dissolved organic carbon and newly produced dissolved organic carbon, and humification index in the dissolved water samples having natural phytoplankton and periphyton populations during the experimental period. Asterisks denote a significant difference compared to day 0 * $p < 0.05$; ** $p < 0.01$

Sources of organic matter	Bulk DOC (mgC L ⁻¹)	Newly produced DOC (μgC L ⁻¹)	Humification index
Phytoplankton			
Day 0	1.4 ± 0.011	6.1 ± 0.26	0.86 ± 0.0018
Day 5	1.4 ± 0.022	17 ± 2.9*	0.87 ± 0.0011**
Day 15	1.4 ± 0.072	10 ± 0.84	0.87 ± 0.0079
Day 28	1.5 ± 0.22	9.5 ± 0.23**	0.91 ± 0.0072**
Day 60	1.3 ± 0.029	6.7 ± 0.082*	0.88 ± 0.0038**
Periphytic algae			
Day 0	1.6 ± 0.027	7.4 ± 2.0	0.82 ± 0.036
Day 5	1.5 ± 0.051**	7.6 ± 0.24	0.84 ± 0.025
Day 15	1.5 ± 0.0029**	11 ± 3.6	0.86 ± 0.0067
Day 28	1.7 ± 0.080*	12 ± 2.7	0.89 ± 0.023*
Day 60	1.5 ± 0.028**	21 ± 0.26**	0.84 ± 0.025

periphytic algal organic matter persisted after 60 days. The periphyton not only consisted of algae but also comprised bacteria, fungi, and detritus (Carr et al. 2005). Kuehn et al. (2014) reported that photosynthetic products synthesized by periphytic algae were quickly transferred to and/or assimilated by heterotrophic microbial communities. In this context, the newly produced organic matter derived from periphytic algae could persist not only as detritus (which is of refractory nature) but also partially in incorporated form in the heterotrophic microbial biomass. In the present study, after 60 days, most of the Chl *a* was degraded in both algal populations, but some newly produced POC, DOC, and PN persisted.

Characterization of dissolved organic matter

The concentrations of bulk dissolved organic carbon were quite similar in the bottles containing phytoplankton

populations for 60 days. However, it was significantly altered in the bottles containing periphytic algae (Table 2). After 28 and 60 days, the concentrations of newly produced dissolved organic carbon by phytoplankton and periphytic algae increased compared to day 0. The HIX, which is an indicator of the degree of degradation (Ohno 2002), also increased in both bottles containing phytoplankton and periphytic algae communities during the degradation experiment. Generally, large amounts of organic matter produced by phytoplankton lead to an accumulation of dissolved organic carbon, which is labile in nature (Engel et al. 2012; Ittekkot et al. 1981). However, in the present study, it could be stated that the increase of newly produced DOC in both algal populations after 60 days was not related to the accumulation of labile DOC, considering the elevated HIX values. In addition, although the concentration of bulk DOC remained unchanged, the humification degree of DOC increased.

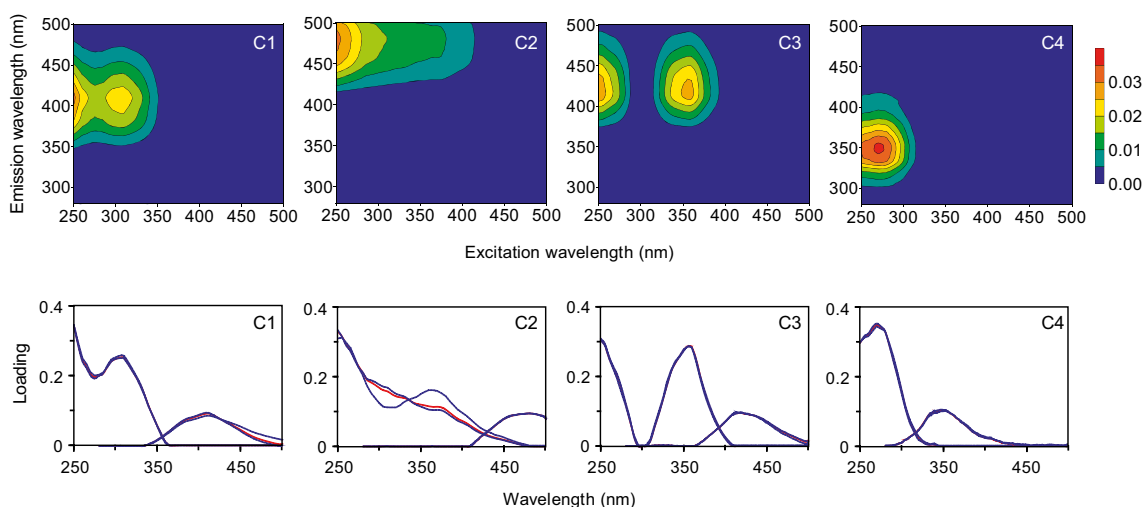


Fig. 2 PARAFAC model outputs representing the four fluorescent components (upper panel) and split-half validation results of four components (lower panel). The lines indicate excitation/emission loadings

(blue dotted lines indicate two independent halves of the dataset, while red line indicates the complete dataset)

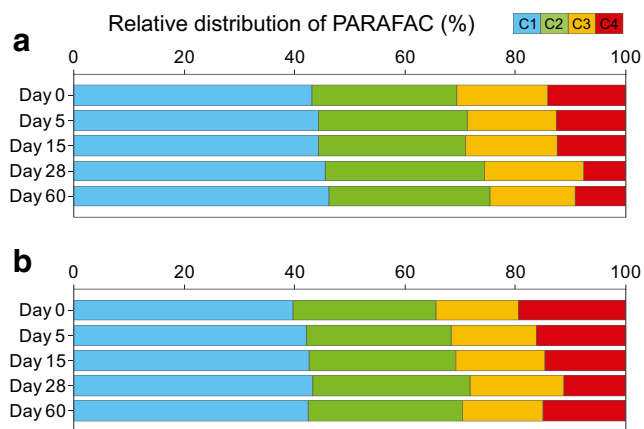


Fig. 3 Changes in relative distributions (%) of four components identified by PARAFAC in filtrate sample containing organic matter derived from **a** natural phytoplankton populations and **b** natural periphyton communities

Using the EEM-PARAFAC, the four fluorescence components (C1, C2, C3, and C4) were extracted from the EEM measurements for all 30 samples (Fig. 2 and Table S1). These components were similar to the peaks M, A, C, and T, respectively, described by Coble (1996). The filtrate samples containing organic matter derived from natural phytoplankton communities, wherein the relative abundances of C1 and C2 gradually increased until day 60, but the relative abundance of C4 showed a decreasing trend (Fig. 3). Although no statistically significant differences were observed, a similar pattern was recorded in the case of the filtrate samples comprising organic matter derived from natural periphyton populations. The F_{\max} values of four components also showed a pattern similar to relative abundance of each fluorescence component (Table S2). These results indicate qualitative changes in dissolved organic matter during the degradation experiments. Previous researchers have reported C1 to be assigned to the microbial humic-like component, and C2 was similarly assigned to the terrestrial fluorescent component (Cory and McKnight 2005; Yamashita et al. 2008). Stedmon and Markager (2005) demonstrated that C3 was ubiquitous in all environments, while Cory and McKnight (2005) explained it as an unknown terrestrial component. On the other hand, the

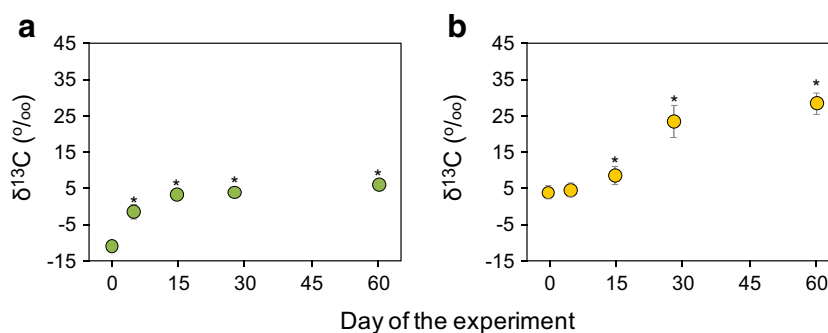
C4 is reported to be attributable to proteinaceous material related to recent biological activity (Determann et al. 1998). Parlanti et al. (2000) reported that a protein-like peak appeared first, followed by the β component (similar to C1 in this study, Table S1) during the first stages of algal degradation. Furthermore, the fluorescence intensity of the β peak increased, while the protein-like peak declined. Similar results were obtained in the present study. The dissolved organic matter in both samples seems to become increasingly refractory over the incubation time.

Contribution of algal-derived organic matter to hydrophobic DOM

The carbon isotope ratios of hydrophobic DOM were gradually enriched in both samples during the 60 days, even though they showed different trends (Fig. 4). The DOM composition changed as the degradation progressed; the protein-like component decreased significantly, while the humic-like components were increased. In addition, the humification index also increased. These results indicate that the dissolved organic matter changed into refractory organic matter as the decomposition advanced. According to Ogawa et al. (2001), labile organic matter can be transformed to a refractory form by heterotrophic bacteria. Lara and Thomas (1995) also found that a considerable fraction of algal-derived organic matter remained as hydrophobic DOM after 267 days. They also stated that 14 % of the newly produced POC was accumulated as hydrophobic DOC fractions. In general, hydrophobic organic matter is less prone to microbial degradation and is more recalcitrant (Jandl and Sletten 1999; Jandl and Sollins 1997). In addition, Aoki et al. (2008) reported that the hydrophobic acids consisted mainly of humic substances. Therefore, these results suggest that, after rearrangement, the algal-derived organic matter could probably be attributed to non-biodegradable organic matter in the lake.

The $\delta^{13}\text{C}$ values of hydrophobic DOM collected before adding tracers were -25.62 and -25.10‰ in the bottles containing phytoplankton and periphytic algal populations, respectively. On the other hand, its values were enriched on day 0 compared to natural samples. These results indicate that algal-derived

Fig. 4 Changes in the carbon isotope compositions of hydrophobic dissolved organic matter produced by **a** phytoplankton and **b** periphytic algae. The hydrophobic dissolved organic matter was extracted with a C_{18} solid phase disk. Asterisks indicate a significant difference compared to day 0 ($*p < 0.05$)



organic matter is rapidly transformed to hydrophobic DOM during photosynthesis. In the case of phytoplankton-derived organic matter, although the highest concentration of DO¹³C was observed on day 5, the δ¹³C values of hydrophobic DOM showed a different trend. These results suggest that the carbon isotopic composition in hydrophobic DOM can potentially represent non-biodegradable algal-derived organic matter in the present study. The higher δ¹³C values in the hydrophobic DOM derived from periphytic algae compared with phytoplankton are clearly related to the fast transformation of algal-derived organic matter by heterotrophic microbial communities in the periphyton.

Generally, the algal-derived organic matter is considered to be easily biodegradable, but the organic matter newly produced by algal populations can be transformed by microbial communities into organic matter that is refractory in nature. Although the present study, as designed, did not provide detailed information of microbial activity, it clearly indicates that algal-derived organic matter produced by phytoplankton and periphytic algae could contribute to the non-biodegradable organic matter in aquatic environments by microbial transformation. Therefore, primary production of phytoplankton and periphytic algae should be monitored and controlled to manage water quality in the water resources.

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