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RESEARCH PAPER

Seasonal and site-specific variability in terrigenous particulate organic carbon concentration in near-shore waters of Lake Biwa, Japan

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Abstract Identifying sources of particulate organic matter (POM) is important for clarifying fundamental mechanisms by which lake food webs are sustained. We determined carbon and nitrogen stable isotope ratios of POM in near-shore waters of Lake Biwa, a large, meso-eutrophic lake in Japan, to estimate relative contributions of terrigenous particulate organic carbon (T-POC), plankton-derived POC (P-POC) and epilithon-derived POC (E-POC) to POC in near-shore waters. Samples were collected during different months (November, February, May and July) at 29 sites located near the mouth of tributary rivers with different discharge and catchment land use. The data revealed that POC mainly consisted of P-POC and T-POC, with relative contributions varying widely over season and among locations. E-POC generally contributed little to the near-shore POC. Path analyses revealed that the concentration of riverine POC whose isotopic signatures were similar to those of rice straws increased with a larger %paddy field area in the catchment of tributary rivers, which subsequently enhanced T-POC inputs to near-shore waters through riverine transportation. Furthermore, our results suggested that T-POC contribution was influenced, with a time lag, by wave-driven turbulence and shore topography, which appear to affect sedimentation and resuspension of T-POC.

Keywords Allochthonous input · Isotope mixing model · Land use · Path analysis · Terrigenous POM

Introduction

Particulate organic matter (POM) is a fundamental energy source for food webs in lake ecosystems. POM in lake waters consists of diverse organic compounds with different origins, including phytoplankton-derived POM (P-POM), epilithon-derived POM (E-POM) and terrigenous POM (T-POM). Although P-POM is generally considered to be a major energy source for planktonic food webs in pelagic environments (Dodson 2005), E-POM and T-POM may also play an important role as food resources for suspension feeders. In fact, in small to medium-sized lakes in North America, some studies have revealed that terrigenous particulate organic carbon (T-POC) derived from tributary rivers can subsidize pelagic food webs (Cole et al. 2006, 2011; Pace et al. 2007). Other studies, however, have suggested that zooplankton growth and reproduction are slowed by the inputs of T-POM, which have high C/N ratios and area low-quality food (Karlsson 2007; Brett et al. 2009). In other systems, it has been reported that pelagic food webs were subsidized by benthic algal products (Rautio and Vincent 2007; Karlsson

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and Sawstrom 2009). For aquatic consumers, reliance on each POM originating from different sources appears to vary widely among lakes, depending on lake size, depth and trophic status (Chandra et al. 2005; Doi 2009; Vander Zanden et al. 2011). The relative contribution of POM with different origins may also vary among different locations of a single lake, especially in near-shore waters. For example, allochthonous input of T-POM might be large in near-shore environments adjacent to river mouths, although the extent and nature of this “river effect” may depend on river discharge, T-POM concentrations in river waters, and physical settings of the locations (shore topography and turbulence that affect sedimentation and resuspension of T-POM). Spatial and temporal variability in contributions of POM with different origins within a single lake has important implications for understanding mechanisms by which lake food webs are differentially organized in the same species pool. Indeed, a previous work has reported that allochthonous organic matter inputs to Lake Biwa from tributary catchments led to spatial heterogeneity in trophic pathways within coastal macro-invertebrate communities (Karube et al. 2010). However, data are highly limited to elucidate mechanisms underlying spatio-temporal variability in relative contributions of P-POM, T-POM and E-POM to total POM in near-shore environments.

Here, we measured carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of POM in near-shore water samples collected during four seasons at different locations set along the shore-line of Lake Biwa. In general, C_3 terrestrial plants (T-POM) have markedly different $\delta^{13}C$ (−27 ‰) than aquatic microalgae (P-POM and E-POM; Marshall et al. 2007). Moreover, among aquatic primary producers, phytoplankton and epilithic algae have different $\delta^{13}C$ because of the CO_2 diffusion limitation within the boundary layer surrounding algal cells (phytoplankton −32 ± 2 (SD) ‰, epilithic algae −17 ± 2 (SD) ‰; Fry and Sherr 1984; France 1995). In addition, $\delta^{15}N$ of aquatic primary producers may be variable among aquatic environments depending on the extent of anthropogenic nitrogen inputs (McClelland and Valiela 1998; Kohzu et al. 2008; Karube et al. 2010). The above differences in isotope signatures of POM with different origins are useful for estimating the relative contribution of P-POM, E-POM and T-POM by means of stable isotope mixing models (Fry 2006).

Lake Biwa, the largest lake in Japan, consists of two basins: the large (616 km^2), deep (average depth 41 m), mesotrophic northern basin and the small (58 km^2), shallow (average depth 4 m), eutrophic southern basin (Rossiter 2000; Somiya 2000). The lake receives water from 121 major tributary rivers and hundreds of other small creeks. The catchments of the major rivers show a wide range of land use patterns, including those with prevailing agricultural field, forest or residence areas (Somiya 2000). The 29 sampling sites of the present study covered a range of situations with variable influences of rivers that have different land use patterns in their catchments. We hypothesize that the quantity and composition of the near-shore POM can vary, on a within-lake scale, depending on land use patterns in tributary catchments as well as on coastal physical characteristics. Assuming that POM with different origins, i.e., P-POM, E-POM and T-POM, have different stable isotopic signatures, we aim to examine what factors determine within-lake variability in relative contributions of these potential organic sources to the near-shore POM, using an isotope mixing model and a path analysis.

Materials and methods

Sample collections

We collected POM samples at 29 sites along the shoreline of Lake Biwa in November 2005 and February 2006, May 2006, and July 2006 (Fig. 1). These sites were located near the mouth of tributary rivers, which have widely variable land use patterns (Appendix A). In most cases, the sampling sites were set within 100 m of the river mouth. For some sites, however, our research boat could not get access to the target location because these sites have a gentle coastal slope and/or are luxuriated by submerged plants. In such cases, the sampling site was set on the position of 5-m-depth contour where the distance between the site and river mouth was shortest. We measured pH and electric conductivity using a multiprofiler at each sampling site (U-22, Horiba).

At each sampling site, we used a water pump to collect water samples at the depth of 2 m. The water samples were first filtered through a 150-μm-mesh net to remove coarse particles and then filtered through precombusted (450 °C for 2 h) glass fiber filters (GF/F, 0.7 μm, Whatman). A particle size of 0.7–150 μm covers the usual size range of phytoplankton found in Lake Biwa (Tsuda et al. 1992). The POMs collected on the glass fiber filters were used for stable isotope analysis (see below). We extracted chlorophyll *a* from these POM samples in 90 % acetone solution and measured its concentration with a fluorometer (Turner Designs, 10-AU) according to Wetzel and Likens (2000). The chlorophyll *a* concentration was used as an indicator of local productivity in near-shore waters.

We assumed three end members (plankton, epilithon and terrigenous organic matter) as primary organic sources for the near-shore POM. First, to determine isotopic values of plankton-derived POM (P-POM), we collected water samples from the depth of 2 m at an offshore site (Station 30, see Fig. 1) in the same months as POM sampling in near-shore waters. The P-POM was filtered on precombusted GF/F filters after screening with a 150-μm-mesh net.

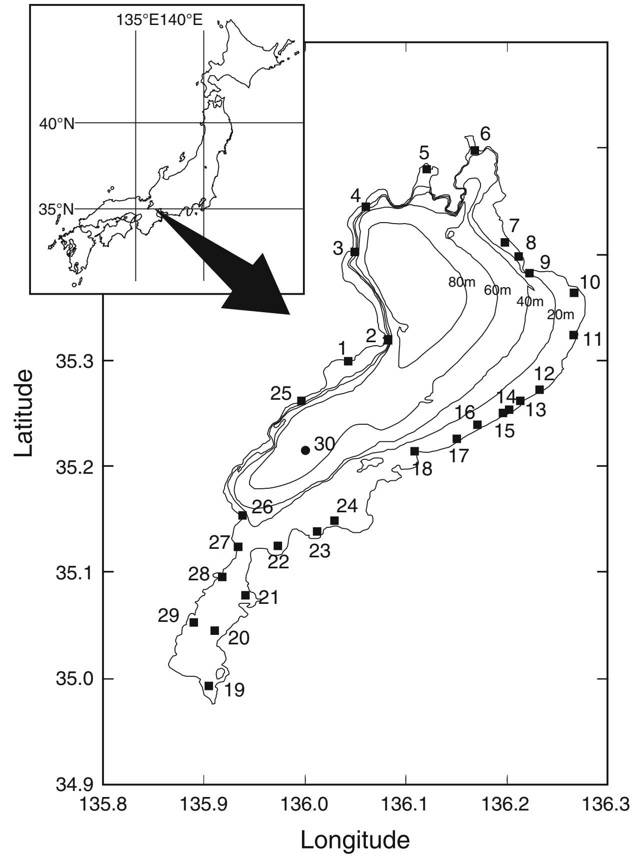

Fig. 1 A map of POC sampling sites (no. 1–30) in the near-shore waters of Lake Biwa. Number 30 is the reference site for P-POM. See Appendix A and B for details of site information

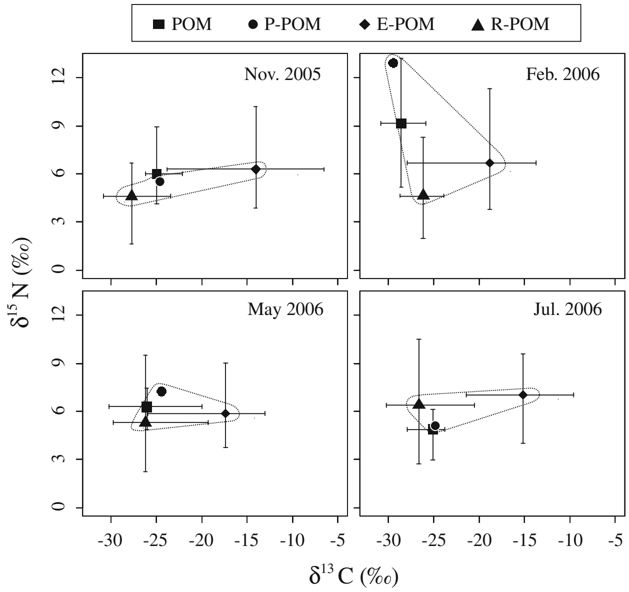

Fig. 2 Carbon and nitrogen isotopic signatures of near-shore POM and their potential organic sources (P-POM, E-POM and R-POM). Each plot represents the mean from all sampling sites, and *vertical* and *horizontal bars* indicate the range (also see Appendix C). Note that P-POM data are derived from only a pelagic site

The P-POM collected at this site was assumed to have isotope signatures typical for phytoplankton-derived organic matter in the lake because previous work has found that δ^{13}C values of POM collected there were identical to those of chlorophyll *a* extracted from offshore phytoplankton after the correction of isotope fractionation (Maki et al. 2010).

Second, to determine isotopic values of epilithon-derived POM (E-POM), epilithon samples were collected at all the sites except for some sites where boulders of appropriate size were not found (Fig. 1). At each site, epilithic organic matter was scraped off with a brush from five boulders of moderate size (30–40 cm) that had a surface area of more than 36 cm^2 on the upper side; it was mixed, filtered through precombusted GF/F filters and served for the stable isotope analyses.

Third, we collected riverine particulate organic matter (R-POM) as a proxy of terrigenous POM (T-POM) at sites 0.5–4 km upstream from the mouth, in the same months as the near-shore sampling but in the previous year (i.e., July 2004, November 2004, February 2005 and May 2005: see Kohzu et al. 2009 for details). In general, R-POM is composed of T-POM and fluvial E-POM (Kohzu et al. 2009). In our study rivers, however, δ^{13}C of R-POM was significantly different from that of fluvial E-POM in all 4 months (Tukey's post hoc test, R-POM vs. fluvial E-POM, November: $p < 0.001$, February: $p < 0.01$, May: $p < 0.01$, July: $p < 0.001$) but not from C_3 terrestrial plant detritus, i.e., rice straw collected from paddy fields in any month (Tukey's post hoc test, R-POM vs. T-POM, November: $p = 0.82$, February: $p = 0.65$, May: $p = 0.79$, July: $p = 0.91$), suggesting that R-POM is dominated by T-POM in tributary rivers of Lake Biwa.

One can expect that submerged macrophytes can be a dominant source for near-shore POM. However, we did not incorporate them as an end member of our mixing model for the following reasons. First, live macrophytes cannot contribute to the POM pool in near-shore waters during the summer growing season. It may be true that their detritus contributes to the near-shore POM during the decomposition process in winter, especially at sampling sites in the shallow south basin where macrophytes luxuriate vigorously (Haga et al. 2007). However, we have little knowledge on stable isotopic fractionation during their decomposition and have technical difficulty in separating their detritus from a mixture of POM with different origins in the near-shore waters for the stable isotope analysis. If the macrophyte contribution is not very large for the near-shore waters of the whole lake basin, we have the disadvantage of incorporating an additional end member into our mixing model, which will lead to low estimation accuracy for major organic sources. Therefore, we did not regard submerged macrophytes as primary organic sources in the near-shore water.

This is why we used the dominant three-end members, P-POM, E-POM and R-POM representative of T-POM, for our isotopic mixing model to estimate the composition of near-shore POM. All of these POM samples collected on precombusted GF/F filters were exposed to 0.1 M HCl to eliminate carbonates, rinsed with distilled water and then stored at −20 °C until the stable isotope analysis.

Stable isotope analysis

The GF/F filters were dried at 60 °C for 24 h, and then the residues were scraped off from the surface of the filters. Carbon and nitrogen stable isotope ratios of the residues were determined using continuous-flow isotope ratio mass spectrometers (CF/IRMS; Conflo II and Delta S, Finnigan MAT, Germany, and Conflo III, delta plus XP, Thermo Fisher, Germany), and their carbon content was measured using elemental analyzers (EA1108, Fisons, Italy and EA1112, Thermo Fisher, Germany). The isotope ratios were expressed as the per mil deviation from standards as follows:

$$\delta^{13}\mathrm{C} \text{ or } \delta^{15}\mathrm{N} = \left(R_{\mathrm{sample}}/R_{\mathrm{standard}} - 1\right) \times 1000\,(\text{‰}),$$

where $R = {}^{13}\mathrm{C}/{}^{12}\mathrm{C}$ or ${}^{15}\mathrm{N}/{}^{14}\mathrm{N}$. Vienna Pee Dee belemnite (VPDB) and atmospheric nitrogen were used as standards for carbon and nitrogen, respectively. The analytical precision based on working standards (Tayasu et al. 2011) was ±0.3 ‰ for both δ^{13}C and δ^{15}N.

Isotope mixing model

Relative contributions of three potential organic sources (i.e., P-POM, E-POM and T-POM) to POM collected at each near-shore site were estimated using an isotope mixing model with δ^{13}C and δ^{15}N (IsoSource Program; Phillips and Gregg 2003). This model enabled us to estimate all possible combinations of each source contribution (0–100 %) in 1 % increments to create a set of predicted sources. The initial tolerance was set at 0.1 ‰. If the estimated values were outliers (i.e., <0 or >100 %), the tolerance was increased in 0.1 ‰ steps to 2 ‰. The model outputs are expressed as the mean value and range (minimum-maximum) for each source. From the estimated mean contribution of each organic carbon source to POC concentration (mg L^{-1}) at each near-shore site, we calculated the concentrations of P-POC, E-POC and T-POC according to the following equation.

$$\begin{aligned}\text{P-POC}\left(\mathrm{mg\,L^{-1}}\right) &= \text{POC}\left(\mathrm{mg\,L^{-1}}\right) \times f_{\mathrm{p}}\\ \text{E-POC}\left(\mathrm{mg\,L^{-1}}\right) &= \text{POC}\left(\mathrm{mg\,L^{-1}}\right) \times f_{\mathrm{e}}\\ \text{T-POC}\left(\mathrm{mg\,L^{-1}}\right) &= \text{POC}\left(\mathrm{mg\,L^{-1}}\right) \times f_{\mathrm{t}}\end{aligned}$$

where f_{p}, f_{e} and f_{t} are the fraction of P-POC, E-POC and T-POC relative to the near-shore POC (i.e., $f_{\mathrm{p}} + f_{\mathrm{e}} + f_{\mathrm{t}} = 1$).

At some sites, we could not estimate each source contribution for the lack of E-POC data or because the estimated contribution remained an outlier for any of the three sources under the above criteria for our isotopic mixing model. In the present study, we present the results of the simulation model only for carbon source estimation, but analytical results on nitrogen sources were qualitatively the same as the carbon source estimation model.

Land use pattern and physical environments

Human population density (HPD in population km^{-2}) and proportions (%) of paddy field, forest and residential areas of the tributary catchment were calculated using a geographical information system (GIS: Appendix A). We assumed that these indices reflect the loading of terrigenous organic matter with different origins: e.g., the HPD or %residential area is an indicator of organic matter derived from sewage, the %paddy field area is an indicator of that from agricultural wastewater, and the %forest area is an indicator of that from leaf litter transported through upper streams.

Because there is a strong positive correlation between river discharge and catchment area, we estimated the river discharge, based on the following regression ($R^2 = 0.846$, $p < 0.01$) for tributary rivers for which discharge data are available (Shiga Prefecture 1992–2002):

$$\text{River discharge}\left(\mathrm{m^3\,s^{-1}}\right) = 0.0284 \times \text{catchment area}^{0.9502}$$

where the river discharge is the average for February, May, July and November during the recent 10 years (1992–2002). Although the river discharge shows daily variation depending on the weather condition, its monitoring date never accorded with our sampling date. Considering this variation, the inter-annual average can be a better indicator for river discharge compared to single monitoring data corresponding to our sampling months.

As physical characteristics of coastal environments, we used wave height (m), wave energy flux (kg m day^{-1} m^{-1}), shore energy flux (kg m day^{-1} m^{-1}, see Nakatsuji et al. 2006) and coastal slope (1 d^{-1}; d = horizontal distance from the shoreline to the point with a depth of 2 m). These data are available at http://www.biwakokasen.go.jp/others/kankyoujyouhou/index.html (Kinki Regional Development Bureau, Ministry of Land, Infrastructure, Transport and Tourism).

The GIS and coastal physical characteristics data are summarized in Appendix A and B.

Statistical analysis

We performed a nested analysis of variance (ANOVA) to test the temporal variation in POC concentrations, incorporating sampling sites as a random factor into the model (the nlme package of R Statistics; Pinheiro et al. 2010), which assumes that the error term distribution accords to Gaussian distribution. If the data distribution did not show normality and homoscedasticity, the dependent variable (POC concentration) was $\log(x + 0.5)$-transformed to satisfy the criteria for the ANOVA (Yamamura 1999). Then we performed a post hoc test for multiple comparisons using the Tukey method (the multcomp package of R Statistics; Hothorn et al. 2008) to compare POC concentrations among seasons. Furthermore, we performed ANOVA to compare the carbon concentrations among the three organic sources (P-POC, E-POC, and T-POC) in each season. All analyses were conducted using the statistical package R ver. 2.13.1 (R Development Core Team 2011).

We performed a path analysis to examine how the land use patterns in the tributary catchments and the physicochemical characteristics of the sampling sites may affect spatiotemporal variation in measured and estimated values of R-POC and T-POC concentrations, respectively, incorporating GIS and environmental variables into the model (see Appendix A and B). First, we screened the explanatory variables because models with too many variables generate redundant information. We checked the correlations among variables to remove multicollinearity and then selected the variables that best accounted for the variation in R-POC and T-POC concentrations. For instance, we selected wave height from among the three wave-associated variables and HPD but not %residential area. Because forest and paddy field are the most dominant land use types in the Lake Biwa Watershed, it is a matter of course that there is a strong negative correlation between their proportional data ($R^2 = 0.93$, $p < 0.001$). We performed two preliminary models to test effects on the R-POC for each of these two land use types. First, when we used %forest area solely as a GIS variable, instead of %paddy field area, we found that the former showed significantly negative but not positive effects (path coefficients, November: −0.44, February: −0.53, May: −0.83, July: −0.64, $p < 0.01$). Second, when we incorporated residuals of the %forest area regressed against the %paddy field area into the preliminary model, we did not find any significantly positive effect of %forest area on R-POC concentration, suggesting that forests cannot be a primary source of R-POC loadings in catchment areas. Based on results of these preliminary analyses, therefore, we excluded %forest area from a final version of our a priori model to reduce the redundancy and the multicollinearity between potentially correlated GIS variables.

After screening the variables, we constructed the basic framework of an a priori model consisting of two components (Fig. 3). The model was separated because the data distributions needed to be $\log(x + 0.5)$-transformed for some of explanatory variables in component 2 to ensure normality and homoscedasticity for statistical criteria. Component 1 was a sub-model that accounts for the variation in measured values of R-POC (upper panel in Fig. 3). Assuming that human sewage and agricultural wastewater have strong effects on R-POC, we incorporated HPD (path 1 in Fig. 2) and the %paddy field area (path 2) into the model. We also assumed that the HPD and %paddy field areas have a positive interactive effect because agricultural activities are usually high in areas with a high population density and vice versa (path 3).

Component 2 was constructed to examine which physical characteristics of coastal environments explain the site-specific variation in estimated values of T-POC concentration in near-shore waters, assuming that T-POC concentration can be affected by a dilution effect due to river discharge (path 5), resuspension due to wave-driven turbulence (path 6), and the potential for resuspension, which is intensified in sites with a gentle coastal slope (path 7). These paths were assumed to have an additive effect on T-POC concentration.

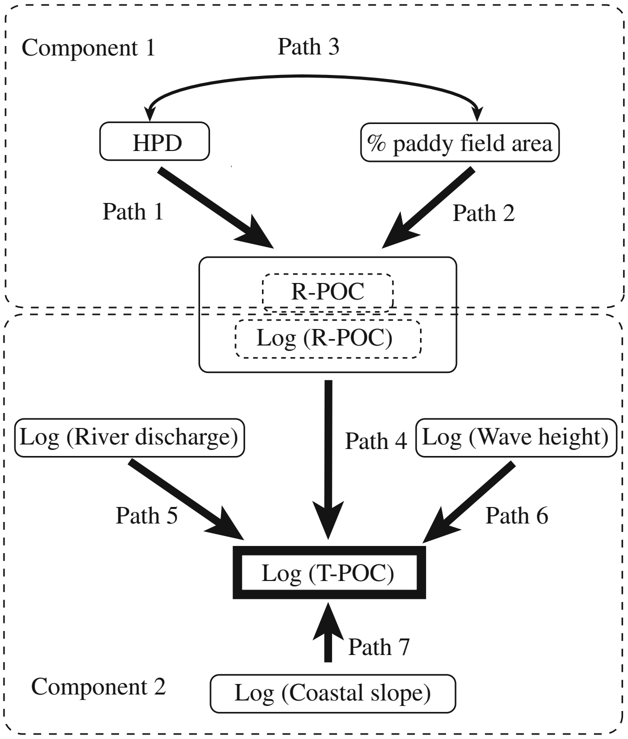

Fig. 3 An a priori path model to explain the variation in T-POC concentrations in the near-shore waters of Lake Biwa. The model consists of two components. *Unidirectional arrows* indicate a causal effect and *double headed arrows* an interactive effect

The model analysis consisted of two steps. First, we conducted a simultaneous analysis of several groups, considering the four seasons as different groups. This allowed us to test for significant differences in the causal mechanisms of the seasonal dynamics of R-POC and T-POC concentrations and provided more accurate model estimation than what would have been obtained from separate analyses of each of the four groups (Arbuckle 2007). Then we selected the best-fit model, setting equality constraints as a null hypothesis that the coefficient of each path is not significantly different among the four seasons (Tabei 2001; Arbuckle 2007). In the initial condition, the level of significance was set at 0.05 for the seasonal difference in each path coefficient and was then increased up to 0.6 in intervals of 0.05. In this way, we constructed a total of 12 models and compared them using the Akaike information criterion (AIC). We selected the best-fit model based on the minimum AIC. The best-fit model was tested using the chi-square goodness-of-fit. We also evaluated its goodness-of-fit based on the root mean square error of approximation (RMSEA; criterion RMSEA <0.05: good, RMSEA >0.08: mediocre, RMSER >0.1: not accepted, Browne and Cudeck 1993). The path analysis was performed using the statistical package AMOS (ver. 16, IBM-SPSS, Tokyo).

Results

Spatiotemporal variation in POC concentration and composition

Using the carbon and nitrogen isotope mixing model with three end members, P-POM, E-POM, and R-POM, we estimated the relative contributions of three organic sources (P-POC, E-POC, and T-POC) to POC at each near-shore site (Appendices C and D). As indicated by a narrow range of estimated values, our isotope mixing model showed high estimation accuracy (Appendix D), except for some sites (e.g., site no. 3, 10, 11, 13, 20 in November, 2005), in which P-POM and R-POM had similar isotopic signatures (Appendix C). Based on the relative contribution of these sources, we calculated the concentration of P-POC, E-POC, and T-POC (Table 1; Fig. 4). The near-shore POC concentrations differed among sites (Fig. 4) and seasons ($F_{3,83} = 13.56$, Jul. > May = Feb. > Nov., $p < 0.05$). P-POC and T-POC were the dominant organic sources of the near-shore POC, whereas the E-POC concentration was consistently low (Fig. 5). In July, the concentration of P-POC was generally higher than that of T-POC (Fig. 5). T-POC concentrations tended to be high at the sites located along southern and eastern shores, especially in February and May (Fig. 4).

Possible factors that influences the spatiotemporal dynamics of T-POC

T-POC concentrations in near-shore waters showed marked spatiotemporal variation at sampling sites (Fig. 4). In our path analysis, a sub-model of component 1 gave the best fit when the significance level for the equality constraints was set at 0.6 ($\chi^2 = 0.721$, $df = 7$, RMSEA = 0.000, AIC = 58.721). The total effect of human land use on R-POC concentration showed great seasonal variation (Fig. 6). Tributary rivers with a larger %paddy field area had a consistently high R-POC concentration year-round (path 2). In contrast, HPD did not have significantly positive effects on R-POC concentration (path 1).

Similarly, component 2 of our path model was the best fit when the significance level was set at 0.3 ($\chi^2 = 0.488$, $df = 42$, RMSEA = 0.057, AIC = 140.288). The total effect of proximate physical factors on T-POC showed great seasonal variation (Fig. 6). The allochthonous input of R-POC had a significantly positive effect on the near-shore T-POC concentration only in May (path 4), whereas river discharge had a negative effect on the near-shore T-POC concentration in July (path 5). Wave height always had a significantly positive effect on the near-shore T-POC concentration (path 6), and the near-shore T-POC concentration was higher for sites with a gentle coastal slope in February and May (path 7).

Discussion

Our data demonstrated that near-shore T-POC concentrations varied widely among sites and over season. The results of path analysis suggested that agricultural wastewater from the paddy fields was the primary driver affecting the near-shore T-POC concentration via increased inputs of R-POC. Especially in May, we detected a significant, robust path in that the %paddy field area had a positive effect on the near-shore T-POC concentration via R-POC inputs (paths 2 and 4). Large contribution of T-POC in May could be related to agricultural practices in this season. Previous work has reported that a large quantity of paddy-derived wastewater flows into the lake basin at the onset of the rice irrigation period between late April and early May (Fujii et al. 2002; Haga and Ohtsuka 2003; Ohkubo and Azuma 2005; Hama et al. 2010). The results of the present study support the notion that agricultural activities, especially paddy irrigation, can be a significant factor that affects T-POC concentrations in near-shore environments of Lake Biwa. It is also interesting to note that %paddy field area had a positive effect on R-POC concentration even in February when paddy field activities are considered to be minimal. This suggests the possibility

Table 1 Measured POC concentration and estimated P-POC, E-POC and T-POC concentrations (mg L^{-1}) at each coastal site in Lake Biwa

Site no.	Name of tributary rivers	Nov. 2005				Feb. 2006				May 2006				Jul. 2006			
		POC	P-POC	E-POC	T-POC	POC	P-POC	E-POC	T-POC	POC	P-POC	E-POC	T-POC	POC	P-POC	E-POC	T-POC
1	Kamo River	1.614	1.473	0.077	0.061	3.346	3.286	0.013	0.047	3.897	1.489	0.596	1.812	3.421	3.281	0.109	0.031
2	Ado R.	1.493				1.226	1.060	0.147	0.020	1.724	0.655	0.360	0.708	4.256	3.349	0.145	0.757
3	Ishida R.	1.446	0.450	0.139	0.857	0.981	0.801	0.061	0.120	1.796				6.145	5.408	0.277	0.461
4	Momose R.	2.300	1.851	0.007	0.442	1.242	1.226	0.009	0.009	2.531				5.720	3.318	0.686	1.722
5	Oura R.	1.222	1.213	0.004	0.004	2.274	1.569	0.000	0.705	0.921				7.029	5.286	0.056	1.687
6	Shiotsu-o R.	1.249	1.225	0.014	0.009	2.310	1.400	0.284	0.626	4.259				5.567	0.167	0.484	4.916
7	Chonoki R.	1.709	1.355	0.048	0.306	1.771	1.593	0.000	0.177	4.395	1.481	0.040	2.875	8.603	4.069	0.792	3.742
8	Ta R.	1.713	1.358	0.048	0.307	1.391				4.586	0.000	0.000	0.000	7.001			
9	Ane R.	1.963	1.580	0.075	0.308	1.862	1.178	0.013	0.670	2.595				6.974	6.939	0.035	0.000
10	Yone R.	1.607	0.754	0.212	0.640	2.953	1.603	0.009	1.343	3.291	0.000	0.000	0.000	10.145			
11	Amano R.	1.711	0.707	0.147	0.857	6.035	2.124	0.024	3.886	4.758				8.501	7.337	0.867	0.298
12	Seri R.	2.166	1.967	0.022	0.178	4.101	2.215	0.021	1.866	3.021	0.000	0.000	0.000	6.820	5.524	0.184	1.112
13	Inukami R.	1.866	0.507	0.140	1.216	8.151	3.831	0.000	4.320	4.348	1.991	0.009	2.348	6.617	6.432	0.119	0.066
14	Ajiki R.	2.116	0.224	0.421	1.473	9.595	2.178	0.384	7.024	3.378	0.000	0.000	0.000	6.102	5.504	0.531	0.073
15	Uso R.	4.483	4.339	0.031	0.112	6.672	3.303	0.000	3.369	4.984	3.908	0.030	1.052	6.047	5.654	0.181	0.212
16	Bunroku R.	2.601				2.533	1.824	0.000	0.709	4.699	0.000	0.000	0.000	5.470	5.054	0.284	0.137
17	Nomazu R.	2.794				3.336	2.605	0.400	0.327	6.483	3.468	0.000	3.015	9.929	9.055	0.109	0.764
18	Echi R.	3.920	3.175	0.008	0.737	2.327	1.682	0.016	0.628	3.701				12.012			
19	Nagaso R.	5.833	0.117	2.187	3.529	12.609				7.392	6.099	0.022	1.271	6.220	5.847	0.000	0.373
20	Hayama R.	6.262	1.828	0.645	3.782	21.669	1.517	1.408	18.744	8.838	0.168	2.121	6.549	9.259	9.027	0.000	0.231
21	Shin-moriyama R.	3.095	0.025	1.711	1.362	34.982	5.527	8.046	21.409	14.337	5.448	0.115	8.774	12.896	0.064	0.000	12.831
22	Yasu R.	6.179				3.684				6.571				11.995			
23	Yanomune R.	11.544				3.388	2.101	0.000	1.287	3.883	1.091	0.043	2.753	11.108	9.742	0.022	1.344
24	Hino R.	3.567	0.235	1.384	1.948	5.953	3.661	0.000	2.292	3.339	0.053	0.013	3.273	9.522	8.665	0.809	0.048
25	U R.	2.136	1.816	0.000	0.320	2.263	1.878	0.373	0.011	3.161	2.580	0.095	0.487	3.545	0.025	0.982	2.542
26	Wani R.	1.117	0.978	0.003	0.136	3.539	2.902	0.000	0.637	2.741	2.119	0.005	0.617	4.516	1.165	0.379	2.972
27	Mano R.	2.322	2.220	0.063	0.039	2.585	1.383	0.000	1.202	4.185	3.955	0.230	0.000	3.694	3.380	0.314	0.000
28	Tenjin R.	3.431	3.431	0.000	0.000	5.342	3.776	0.118	1.448	4.860	3.377	0.967	0.510	5.831	4.869	0.000	0.962
29	Fujinoki R.	2.081	0.010	2.071	0.000	8.922	3.953	0.625	4.345	6.095	2.206	3.870	0.018	6.197	4.709	0.000	1.487
Offshore reference site																	
30	Ie-1	2.38				2.22				3.01				4.73			

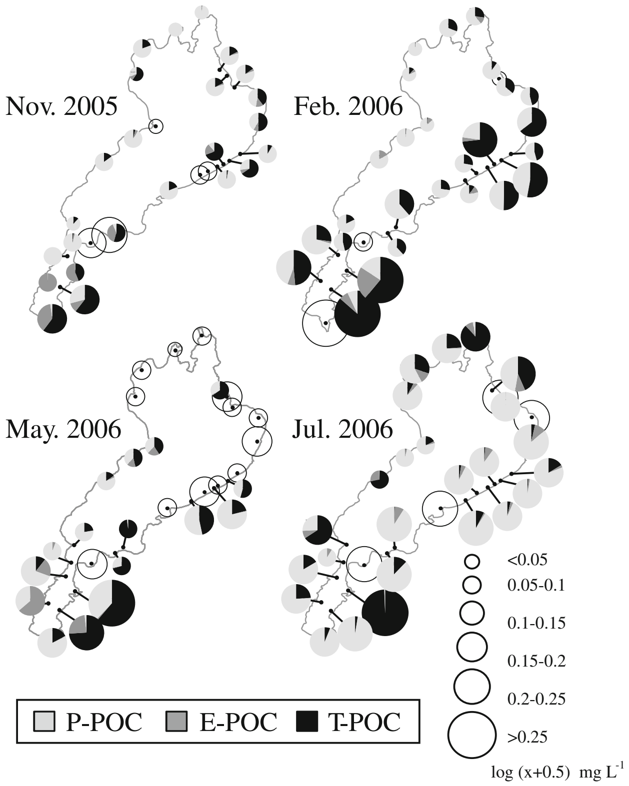

Fig. 4 Spatiotemporal variations in the concentrations of three components, P-POC, E-POC, and T-POC, of POC in the near-shore waters of Lake Biwa. The size of each *circle* is proportional to the POC concentration. *Transparent circles* represent sites where the relative contributions of three potential organic sources could not be estimated (see "Materials and methods")

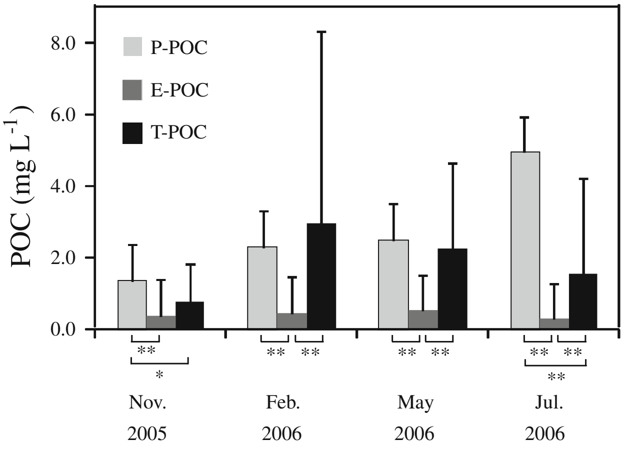

Fig. 5 Seasonal changes in the composition of three potential organic sources of POC in the near-shore waters of Lake Biwa. *Vertical bars* are the standard deviation

that organic-rich soils can be eroded from paddy fields even in winter because of snowing and melting. The underlying mechanisms are not yet fully understood, remaining to be investigated in the future.

The results of path analysis also suggested that physical characteristics of the coastal environment, including coastal slope and wave height, have significant effects on T-POC concentration in near-shore waters. We speculate that waves and gentle coastal slopes might synergistically enhance the resuspension of sedimentary T-POC, resulting in increased terrestrial contribution to the near-shore POC. This notion is partly supported by findings of Murase and Sakamoto (2000) who reported that the near-shore sediments of Lake Biwa contained a large quantity of terrigenous organic matter. Intriguingly, in the present study, although R-POC had a positive effect on the amount of near-shore T-POC only in May (path 4), the wave effects were strongest in November, after the rice field irrigation season (Fig. 6). These results suggest that agricultural wastewater loading had a time lag effect on the T-POC concentration via sedimentation and wave-driven resuspension in near-shore waters.

One might expect allochthonous inputs of R-POC to be magnified by river discharge as well as by its concentration. However, our analysis did not support this prediction and revealed that river discharge had strongly negative effects on the near-shore T-POC concentrations in July with high precipitation. In general, flooding has the potential to increase the concentration of suspended organic matter in river water and consequently organic matter loadings on near-shore waters. However, we avoided field samplings during flooding events because our interest was in land use effects on allochthonous inputs but not in physical transportation mechanisms under ordinary flow conditions. During the rainy season, discharge of larger rivers that receive larger quantity of rain waters in their larger catchment areas might have a greater dilution effect on near-shore POC.

In near-shore waters of Lake Biwa, POC was composed mainly of P-POC and T-POC, whereas E-POC contributed little to POC regardless of the season and site. In some shallow and oligotrophic lakes, it has been reported that epilithic-derived trophic energy flows dominate in coastal food webs (Rautio and Vincent 2007; Karlsson and Sawstrom 2009). There are two possible mechanisms causing the relative contribution of E-POC to be low in Lake Biwa basins. One is associated with lake morphological features: Lake Biwa has a deep structure with a steep slope, especially for the large north basin, in which the proportional area of shallow waters to the whole basin was considerably small (Sakamoto 2000), suggesting that habitats are limited for epilithic growth. Another mechanism is that an increase in P-POM and T-POM decreases light penetration to the lake bottom, which has negative effects on epilithic growth (Vadeboncoeur et al. 2001, 2003). Consistent with our observation, previous studies have reported that filter-feeding macrozoobenthos and zooplankton in near-shore

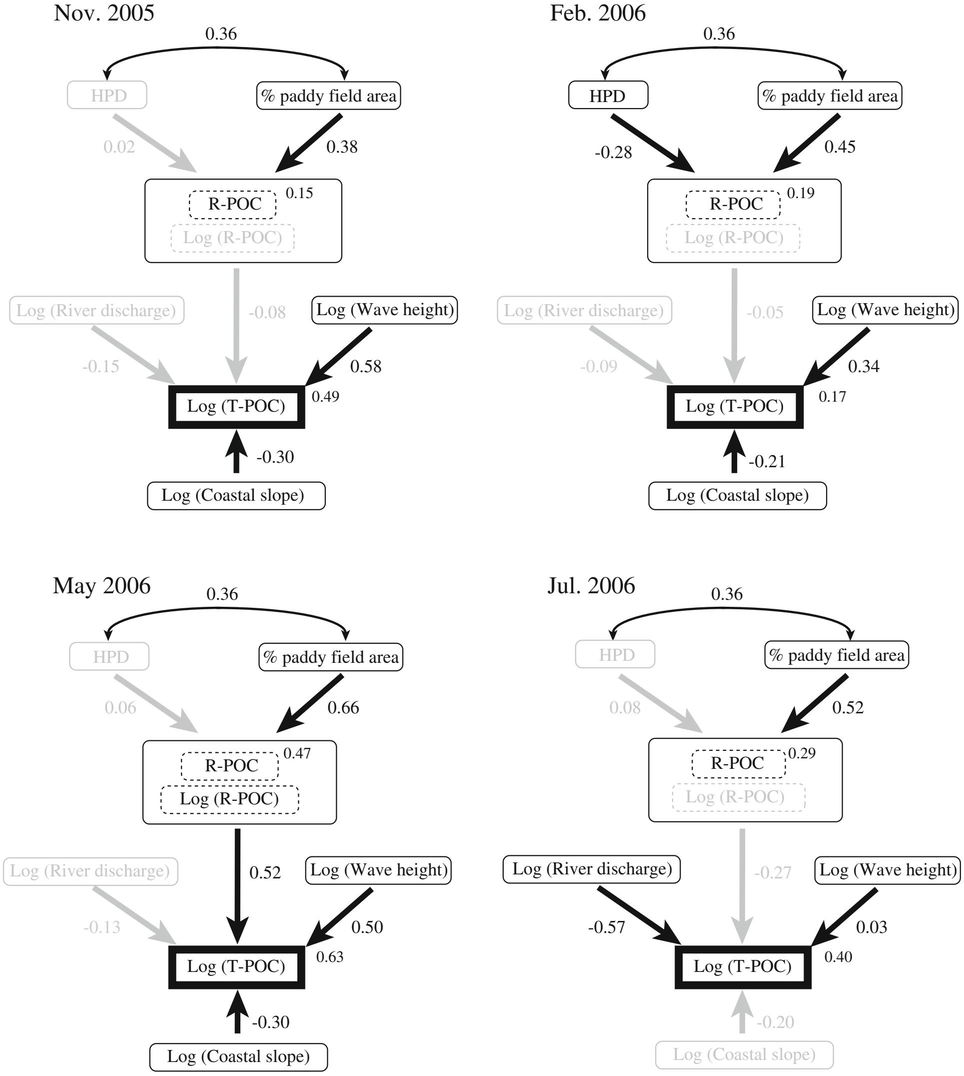

Fig. 6 The path model based on the simultaneous analysis of multiple populations with structured means. The basic framework is the same as that in Fig. 2. *Dark* and *light arrows* are significant ($p < 0.05$) and not significant ($p > 0.05$) paths, respectively. *Values with arrows* are path coefficients. The subscripts of R-POC and log (T-POC) indicate the total effect on these variables

environments of Lake Biwa relied mainly on P-POC and T-POC rather than on E-POC (Karube et al. 2010). Their production reliance is also trophically transferred to that of predatory fishes, characterizing the food web configuration of whole communities (Okuda et al. 2012).

In the present study, we did not consider the contribution of detrital macrophytes to the near-shore POM for the following two reasons. First, in Lake Biwa, especially in the deep north basin, the area of shallow habitats available to macrophytes is disproportionately small relative to that of the whole basin, so that their total primary production is much lower than that of phytoplankton and of even epilithon whose euphotic zone is deeper than that of the macrophytes (Sakamoto 2000). Second, we have technical difficulty the separating macrophyte detrital fraction from a mixture of POM with different origins in the near-shore water for the stable isotope analysis and thus in characterizing isotopic alteration during their decomposition in nature. To evaluate their contribution, one of the promising approaches is polyunsaturated fatty acid (PUFA) analysis, which can quantify each of the organic sources by characterizing the composition of primary producer-specific polyunsaturated fatty acids (Kelly and Scheibling2012). In floodplain lakes of the central Brazilian Amazon basin, for instance, the technique revealed that macrophyte detritus is a primary component of suspended POM during the low water season (Mortillaro et al. 2011). The application of PUFA analysis, even if it is beyond our scope in this study, will be a future challenge for better understanding of seasonal changes of inorganic matter flows in Lake Biwa, especially in the shallow south basin where submerged plants have recently luxuriated.

Using stable isotope approaches, we demonstrated that allochthonous inputs of T-POC to near-shore waters were affected not only by the land use pattern in tributary catchment areas, but also by the physical characteristics of local coastal environments. This suggests that the spatio-temporal heterogeneity of food quality and quantity for aquatic consumers will create a dynamic pattern of coastal food webs, altering the relative importance of the autochthonous grazing food chain and allochthonous microbial loop in lake ecosystems.

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