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C:N:P stoichiometry of particulate and dissolved organic matter in river waters and changes during decomposition

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Abstract

Background: Stoichiometry plays an important role in understanding nutrient composition and cycling processes in aquatic ecosystems. Previous studies have considered C:N:P ratios constant for both DOM (dissolved organic matter) and POM (particulate organic matter). In this study, water samples were collected in the six major rivers in Korea and were incubated for 20 days. C:N:P ratios were determined during the time course of the incubations. This allowed us to examine the changes in N and P contents of organic matter during decomposition.

Results: POM and DOM showed significant differences in N and P content and the elemental ratios changed during the course of decomposition; DOM showed higher C:N and C:P ratios than POM, and the C:N and C:P ratios increased during decomposition, indicating the preferential mineralization of P over N and N over C.

Conclusions: The N and P contents of organic matter in aquatic ecosystem are far from constant and vary significantly during decomposition. More detailed information on the changes in C:N:P ratios will provide improved understanding of decomposition processes and improved modeling of aquatic ecosystems.

Keywords: Organic matter C:N:P, POM, DOM, Labile and refractory organic matter, River water

Background

Nutrient cycling processes are important functions of freshwater ecosystems that affect water quality and the impacts of eutrophication. Changes in the stoichiometry of organic matter are associated with nutrient cycling and ecosystem biogeochemical processes. Transformations of nitrogen (N) and phosphorus (P) in organic forms reflect to the assimilation and dissimilation of these important nutrients. Organic matter is initially synthesized in aquatic ecosystems mostly through algal photosynthesis, and the C:N:P ratio is well known to be close to the Redfield ratio (106:16:1).

However, during the decomposition of organic matter, the behavior and the rates of mineralization of C, N, and P can vary. It is known that the mineralization rate of organic P is much faster than the mineralization rate of

organic C and N, resulting in the deviation of C:N:P ratio from the Redfield ratio (Islam et al. 2013; Jeanneau et al. 2018). Another factor that affects C:N:P ratio of organic matter is the input of allochthonous organic matter. Organic matter derived from vegetation in a watershed usually stays in the watershed for enough time to be degraded and transformed into recalcitrant humic substances which have low N and P contents before they are exported into streams. Sewage and manure can also undergo artificial humification in biological treatment plants or composting process.

Algae are known to have the ability of adjusting their C:N:P ratios according to food quality (Sterner and George 2000; Hall et al. 2005). Due to the large variation in P availability, freshwater organisms may alter their C:P and N:P ratios (Elser et al. 2005; Cross et al. 2007; Fitter and Hillebrand 2009). To maintain optimal body C:N:P ratios and growth, microorganisms can adjust their enzymatic activities to control the mineralization of organic N and P (Sinsabaugh et al. 2009). Cleveland and

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Liptzin (2007) reported that the average C:N:P ratio is 186:13:1 in soil and 60:7:1 in microbial communities. Other studies have found that the variations of C:N and C:P ratios of periphyton in stream ecosystems are remarkable (Stelzer and Lamberti 2001).

Because CNP stoichiometry can change significantly among different environments, this can cause uncertainty in the selection of correct coefficients when assessing nutrient cycling processes in modeling efforts. Usually one constant coefficient is selected for the stoichiometry in most models because we lack adequate knowledge to trace changes in stoichiometry, and it is practically difficult to estimate constituent coefficients as a function of environmental conditions. In this study, the changes in N and P contents of organic matter in river waters were measured and the changes in C:N:P ratios during decomposition process were examined. The constituent coefficients will help understand nutrient cycling processes in aquatic ecosystems and contribute to the selection of suitable parameters in ecosystem models.

Methods

Study area

The rivers sampled in this experiment included the major rivers in Korea. The Han River, the North Han River, and the South Han River are located between 37° 33' N and 126° 59' E. The Han River is the largest river based on basin area and river discharge with a basin area of 26,018 km². The Keum River is located between 35° 59' N and 126° 39' E. The Youngsan River and the Sumjin River are located in the central to southern area of Korea between 35° 20' and 35° 15' N and between 126° and 127° E. The watershed areas of the Youngsan River and the Sumjin River are 3522 km² and 4897 km², respectively. The river reaches are short, and the river basin areas in Korea are small compared with the other major continental rivers. The channel slopes are relatively steep upstream because of steep mountains and deep valleys in the uplands. Due to the natural topography and river regulation, the downstream regions have considerably lower slopes. The distribution of precipitation among the study watersheds is more varied than that of temperature. During the rainy season from June to September, Korea receives approximately 70% of its annual precipitation. River bank erosion in Korea is relatively low because of the dominance of rocky structures near the shoreline. However, the land uses within the river basins largely consist of residential, industrial, commercial, livestock, pasture, row crops, and forestry.

Collection of samples and measurement of decomposition rates

We measured the amount of particulate organic carbon (POC), particulate organic nitrogen (PON), particulate

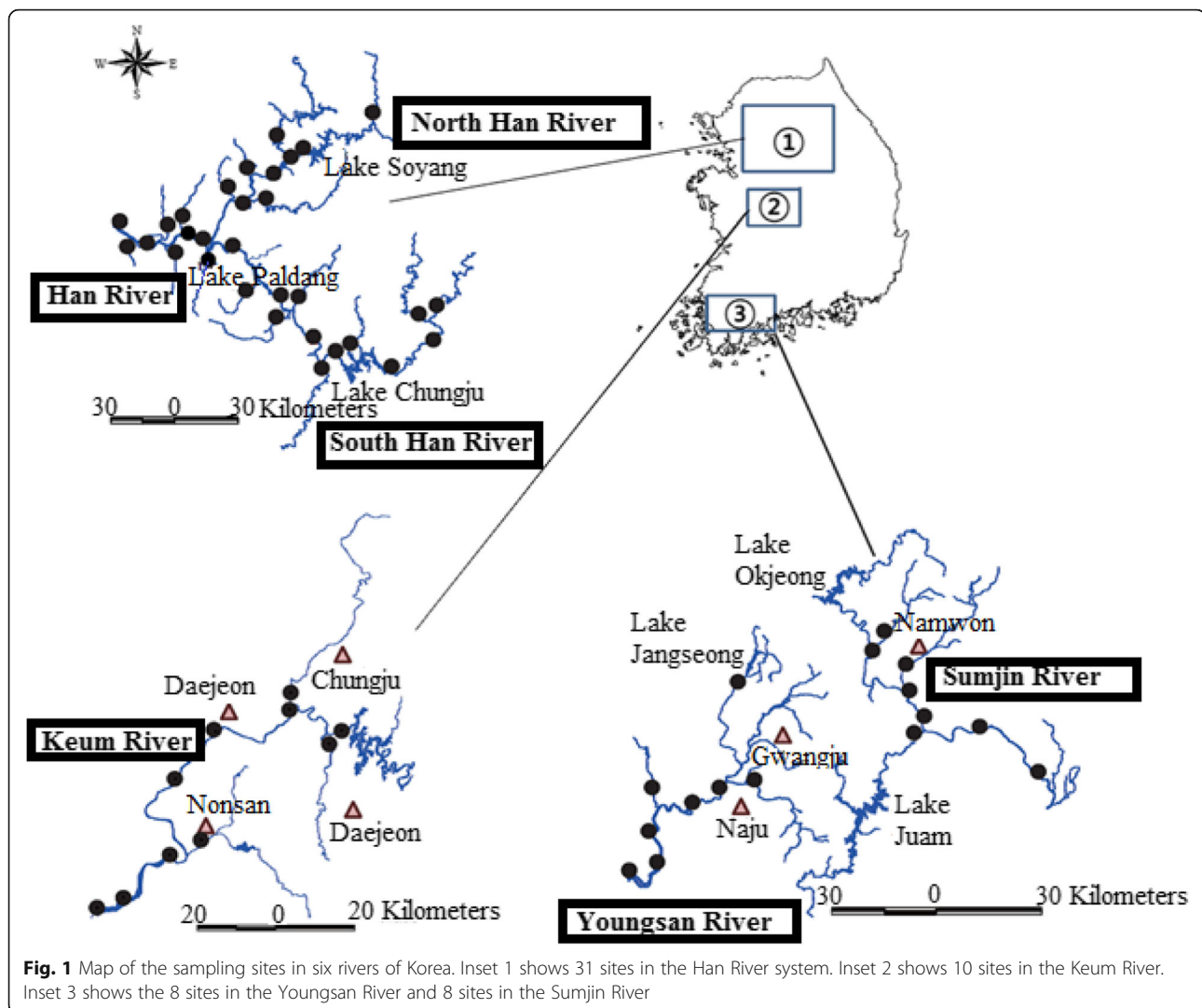
organic phosphorus (POP), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and dissolved organic phosphorus (DOP) in six rivers in Korea from October 2005 to October 2008 to assess changes in particulate and dissolved fractions of organic C, N, and P during decomposition. The sampling sites were the Keum River (10 sites), the Youngsan River (8 sites), the Sumjin River (8 sites), the North Han River (10 sites), the Han River (6 sites), and the South Han River (15 sites). The sampling sites are shown in Fig. 1. The number of replicates from each sampling site varied from 3 to 4 (Table 1). Water samples were collected in pre-cleaned polyethylene bottles thoroughly rinsed with surface water and stored in the dark at 4 °C to minimize deterioration prior to analysis. The water samples were filtered with 200- μ m mesh-sized net and were incubated in the dark at a constant temperature of 20 °C. Each incubation bottle was aerated by aquarium pumps to provide aerobic conditions and to reduce particle settling. When the water samples were collected, the entire bottle was shaken vigorously to resuspend any settled particles. Although the decomposition rates under aerobic and anaerobic conditions likely differ, this study focused on aerobic decomposition. In most cases, river water in Korea is aerobic.

In this study, organic matter was differentiated according to biodegradability as labile organic matter and recalcitrant organic matter. Organic matter was analyzed at the beginning of the incubation and analyzed again after the 20 days incubation. The organic matter remaining after 20 days was regarded as recalcitrant organic matter, and the amount of change during the incubation was regarded as labile organic matter. Therefore, total concentration means the initial labile N and P concentrations, and the final N and P concentrations of recalcitrant organic matter.

The glassware was cleaned with nutrient P-free detergent (Extrans), rinsed three times with ultra-pure water (Milli-Q), soaked in 10% (v/v) HCl for at least 24 h, and then rinsed three times with ultra-pure water. Using an incubation approach, measurements were done at 0 and 20 days from the start of the experiment.

DOC (dissolved organic carbon) concentrations were measured with a TOC analyzer (Shimadzu TOC-5000A) using the high-temperature catalytic oxidation method. After filtering the sample, the filters for the POC analysis were fumigated in an acidifying desiccator filled with 12 N HCl for approximately 4 h. After removing the DIC, the filters were wrapped in tin foil and analyzed by a CHN elemental analyzer (VARIO-EL).

The dissolved total N (DTN) and nitrate + nitrite ($\text{NO}_3^- + \text{NO}_2^-$) were sampled using the same procedure as DTP and DIP, respectively. The total N (TN) was determined by the cadmium-reduction method after



persulfate digestion, using a flow-injection autoanalyzer (SKALAR). Nitrate concentrations were measured with an autoanalyzer by the Cd-reduction method. DTN was oxidized to NO_3^- with potassium persulfate and analyzed in a similar manner to NO_3^- . Ammonia concentrations were measured by the spectrophotometry, and nitrite concentrations were measured by a flow injection autoanalyzer (APHA (American Public Health Association) 2005). DON was calculated as $\text{DTN} - (\text{NO}_2^- + \text{NO}_3^- + \text{NH}_4^+)$. PON was calculated as the difference between the TN and the DTN. The measurements of PON, DON, NH_4^+ , NO_2^- , and NO_3^- concentrations were performed at 0 and 20 days after the beginning of the incubation.

DIP (dissolved inorganic P), DTP (dissolved total P), and TP (total P) concentrations of the water samples were measured. DIP was analyzed after GF/F filtration

(0.45 μm) using the molybdenum blue method (APHA (American Public Health Association) 2005). DTP was estimated from the filtered sample as the DIP after persulfate digestion (APHA (American Public Health Association) 2005). DOP was obtained by subtracting DIP from DTP. TP was analyzed on the unfiltered sample as for DIP after persulfate digestion and determined using the molybdenum blue method (APHA (American Public Health Association) 2005). In both the particulate and dissolved organic forms of C, N, and P, the labile and refractory fractions were calculated as organic C, N, P = labile C, N, P + refractory C, N, P.

The temperature, pH, and EC were measured in situ with a YSI model 600 multi-parameter water analyzer. DO was measured using a DO meter. For COD_{Mn} , 30 mL samples were treated with 5 mN KMnO_4 in 1% NaOH for 1 h at 100 °C in an autoclave.

Table 1 Median concentrations of particulate organic carbon (POC), dissolved organic carbon (DOC), particulate organic phosphorus (POP), dissolved organic phosphorus (DOP), particulate organic nitrogen (PON), and dissolved organic nitrogen (DON) in the studied rivers and their molar ratios

Rivers	Date	Particulate organic matter						Dissolved organic matter					
		POC	PON	POP	C:N	C:P	N:P	DOC	DON	DOP	C:N	C:P	N:P
North Han	4/23/2007	700	640	8	1.28	226	185	1535	1108	4	1.62	991	656
	8/28/2007	810	350	27	2.70	78	29	1905	665	10	3.34	492	147
	10/1/2007	560	300	16	2.18	90	42	980	785	5	1.46	506	348
South Han	5/20/2008	1620	940	23	2.01	182	92	1770	206	4	10.03	1143	114
	7/15/2008	2170	440	20	5.76	280	49	1920	35	3	64.02	1653	26
	10/8/2008	930	340	11	3.19	218	72	1560	89	3	20.46	1343	79
Han	4/23/2007	2910	815	105	4.17	72	17	3340	1194	6	3.26	1438	459
	8/28/2007	1365	2540	118	0.63	30	48	2550	444	47	6.70	140	21
	10/1/2007	1110	2305	50	0.56	57	103	1900	690	8	3.21	614	191
Keum	10/15/2005	2175	746	49	3.40	115	34	4185	468	10	10.44	1081	104
	11/19/2005	2355	752	72	3.65	84	23	3750	499	8	8.77	1211	147
	3/10/2006	2050	368	94	6.50	56	9	3895	811	21	5.60	479	86
	6/9/2006	2025	654	80	3.61	65	18	3750	781	14	5.60	692	128
Youngsan	6/14/2006	2650	1555	44	1.99	156	78	4400	985	10	5.21	1137	218
	8/14/2006	2080	520	46	4.67	117	25	2820	945	13	3.48	560	161
	12/2/2006	1125	525	37	2.50	79	32	2250	655	8	4.01	727	193
	2/3/2007	1560	895	32	2.03	126	63	3570	725	14	5.75	659	119
Sumjin	6/14/2006	960	720	29	1.56	86	56	3300	915	8	4.21	1066	270
	8/14/2006	930	535	28	2.03	86	43	1830	1190	8	1.79	591	329
	12/2/2006	440	590	13	0.87	87	105	975	1140	4	1.00	630	631
	2/3/2007	605	530	12	1.33	130	102	1825	1165	3	1.83	1572	860
Mean		1482	812	43	3	115	58	2572	738	10	8	892	252
Median		1365	640	32	2	87	48	2250	781	8	4	727	161
SD		743	603	32	2	64	42	1073	351	10	14	410	225

(Concentration units are in mg m^{-3} , $n = 197$)

Results and discussion

Water quality characteristics and CNP content of organic matter

The median values of water quality parameters in the study rivers were pH 7.6, conductivity $220 \mu\text{S cm}^{-1}$, DO $9.8 \text{ mg O}_2 \text{ L}^{-1}$, COD $5.59 \text{ mg O}_2 \text{ L}^{-1}$, DIP 22 mgP m^{-3} , ammonia 120 mgN m^{-3} , and nitrate 1430 mgN m^{-3} . Of the six rivers in this study, two rivers were mesotrophic according to TP concentrations, but majority of sites were eutrophic due to the input of municipal sewage. The mean POC was 1482 mgC m^{-3} (Table 1) which is slightly lower than the world river median value of 2000 mgC m^{-3} (Wetzel 2001). However, DOC showed a mean of 2572 mgC m^{-3} , ranging $975\text{--}4400 \text{ mgC m}^{-3}$, which is much lower than the median value of world river (5000 mgC m^{-3}) as suggested by Wetzel (2001) (Table 1). DOC concentrations in this study was also much lower than the median value of world eutrophic lakes ($10,000 \text{ mgC m}^{-3}$), and it was rather closer to the average

concentration of oligotrophic lakes (2000 mgC m^{-3}). It is not known why DOC is low in Korean rivers, which is possibly associated with geological features and terrestrial ecosystems. Therefore, the DOC:POC ratio (1.6) in this study was lower than the median value of the world river of 3 (Dagg et al. 2004).

The median atomic C:N ratio of POM was 2.32, which is lower than the Redfield ratio (6.6). This implies that the median N concentration of POM is 23%, higher than the typical values for N concentration that are employed in water quality models (6–9%) (APHA (American Public Health Association) 2005) (Tables 2 and 3). The median C:P ratio of POM was 120, which is similar to the Redfield ratio of 106. This corresponds to a P concentration of 0.97%, which is close to the parameter values of water quality model; USEPA (Environmental Protection Agency) suggested 1.0% but the CE-QUAL-W2 model adopted 0.5%. Other studies showed that the C:P ratio of algae is similar to the Redfield ratio in

Table 2 CNP stoichiometry of particulate and dissolved organic matter

		TOC	TON	TOP	C:N	N(%)	C:P	P(%)	N:P
Total organic matter	Mean	4430	1667	63	3.10	17	181	0.64	59
	Median	3980	1560	39	2.98	18	263	0.44	89
	SD	2920	984	84	3.46	15	90	1.29	26
Total organic matter after 20 days decomposition	Mean	3080	894	38	4.02	13	209	0.56	52
	Median	2970	1030	26	3.37	16	295	0.39	88
	SD	1220	337	47	4.22	12	67	1.73	16
Particulate organic matter		POC	PON	POP	C:N	N(%)	C:P	P(%)	N:P
	Mean	1750	864	50	2.36	22	90	1.29	38
	Median	1350	680	29	2.32	23	120	0.97	52
Labile particulate organic matter		LPOC	LPON	LPOP	C:N	N(%)	C:P	P(%)	N:P
	Mean	710	447	17	1.85	28	108	1.08	58
	Median	460	310	9	1.73	30	132	0.88	76
Recalcitrant particulate organic matter		RPOC	RPON	RPOP	C:N	N(%)	C:P	P(%)	N:P
	Mean	930	407	33	2.67	20	73	1.60	27
	Median	690	240	17	3.36	16	105	1.11	31
Dissolved organic matter		DOC	DON	DOP	C:N	N(%)	C:P	P(%)	N:P
	Mean	2680	838	13	3.73	14	532	0.22	143
	Median	2280	780	8	3.41	15	735	0.16	216
Labile dissolved organic matter		LDOC	LDON	LDOP	C:N	N(%)	C:P	P(%)	N:P
	Mean	640	326	8	2.29	23	206	0.56	90
	Median	550	220	4	2.92	18	355	0.33	122
Recalcitrant dissolved organic matter		RDOC	RDON	RDOP	C:N	N(%)	C:P	P(%)	N:P
	Mean	1800	511	5	4.11	13	929	0.13	226
	Median	1540	410	4	4.38	12	993	0.12	227
	SD	970	703	4	1.61	33	626	0.19	389

Concentrations (mg m^{-3}) of total organic carbon (TOC), total organic nitrogen (TON), and total organic phosphorus (TOP); POC, DOC, POP, DOP, PON, and DON, and their labile and recalcitrant fractions in the studied rivers ($n = 197$)

optimum eutrophic conditions and high growth rates, but the C:P ratio can be as high as 1000 in P-limited conditions, deviating significantly from the Redfield ratio due to the stress of phosphorus deficiency (Goldman et al. 1979; Sterner and Elser 2002). The study sites in this study were mostly eutrophic river and reservoirs, and it can be inferred that the C:P ratio did not deviate much from the Redfield ratio because P deficiency stress was not likely.

The P concentration of algae has an important function in some water quality models, especially for estimating algae growth. Because P is the limiting nutrient in most rivers and reservoirs, maximum algal

densities are determined by P concentrations. In most of these models, algal growth is calculated on the basis of the limiting nutrient. The algal biomass is converted from P to corresponding dry weight or carbon by using the P composition coefficient of algae. Therefore, the uncertainty in P content is directly proportional to the uncertainty in algal density. For example, if the P concentration in algae is two times higher than at controlled condition, the maximum algal density can be the half of the controlled density. Therefore, it is very important for accurate model results to have the most appropriate coefficient for P concentration in algae.

Table 3 N and P contents in labile and recalcitrant forms of POM and DOM of river water measured after 20 days incubation, and the comparison with other model coefficients

	N (%)	P (%)	Reference
POM (0 day)	23	0.97	This study (median values, $n = 197$)
LPOM	30	0.88	
RPOM (POM after 20 days)	16	1.11	
DOM (0 day)	15	0.16	
LDOM	18	0.33	
RDOM (DOM after 20 days)	12	0.12	
Algae and organic matter in CE-QUAL-W2 model	8	0.5	(Cole and Wells 2017)
Phytoplankton constituent coefficient	6–9	1.0	(USEPA (United States Environmental Protection Agency) 1985)

When N:P ratios of POM and DOM were compared, DOM had higher N:P ratios than POM and greater deviation from the Redfield ratio. The median N concentration was 23% for POM, compared with 15% for DOM (Table 2). And the P concentration showed a much bigger difference than nitrogen; 0.97% for POM and 0.16% for DOM. The reasons why DOM had lower P concentrations than POM are not examined in this study. One plausible explanation is that P leach from particulate organic molecules faster than the loss of N and C.

In the comparison of labile and recalcitrant forms of organic matter, the median LPOC was 460 mg m^{-3} and RPOC was 690 mg m^{-3} which shows that 40% of POM was labile. For DOM, the proportion of labile form was 26%, LDOC was 550 mg m^{-3} , and RDOC was 1540 mg m^{-3} (Table 2).

Changes in stoichiometric ratios during decomposition

In accordance with the differences in N:P ratios in POM and DOM, N:P ratios showed remarkable change during the course of decomposition. The most remarkable change during the decomposition was the decrease in P concentration. The median value of the TOC:TOP was 263:1 at the start of the incubation, whereas the ratio was 295:1 after 20 days, showing that the P concentrations became lower during decomposition. When organic matter was differentiated into four categories of LPOM, RPOM, LDOM, and RDOM, it was found that LDOM portion showed a significant change of P concentration after decomposition. The DOC:DOP ratio of LDOM was 355 (P concentration 0.33%), but the ratio increased to 993 (P concentration 0.12%) after decomposition. The change of P concentration in LDOM was remarkable; however, the major reason for the decrease in P concentration during the incubation is thought to be the degradation of LPOM (higher P concentrations 0.88%) into DOM (lower P concentrations 0.16%) than POM (Table 2).

The changes in C:P indicate the preferential mineralization of organic P over organic C and organic N. This result coincides with reports about the

differential mineralization of organic P and organic C (Guo and Santschi 1997; Søndergaard et al. 2000; Islam et al. 2013). Because P is the limiting nutrient in freshwater, algae have developed efficient enzyme system for competitive utilization of organic P. Inorganic orthophosphate is preferably absorbed by algae, and then organic matter acts as a reservoir of potential P sources. Algae and bacteria are known to excrete an extracellular enzyme, alkaline phosphatase, in order to facilitate P absorption when inorganic phosphate is deficient. Alkaline phosphatase is attached to the cell surface or excreted out of cells as dissolved free enzyme, and cleave the ester bond between organic carbon and phosphate of dissolved organic matter (Wetzel 1991).

The result of this study confirms that the rate of mineralization can be quite different for organic C and organic P. Particulate organic matter in freshwater can be synthesized by algal growth initially and this “young” organic matter will have similar a C:N:P ratio with the Redfield ratio. Through enzymatic activities, POM is transformed into “old” dissolved organic matter with low P concentration and finally mineralized into inorganic matter. The large difference of P concentration in POM and DOM implies that the age and biochemical history of organic matter might be important factors in the consideration of stoichiometry in addition to the nutrient availability and species composition (Klausmeier et al. 2004).

Molar ratios of labile and recalcitrant P and N

Relationships between the C:N:P ratios of river organic matter offer an informative look at organic matter dynamics. The molar ratios of labile and recalcitrant P and N fractions in different seasons at different sampling sites varied widely. These variations were considered reasonable because the different sampling locations varied with respect to urban and industrial discharges and runoff, agricultural activities, and the bathymetric characteristics. The median ratio of LPON:LPOP in the studied rivers was 76 (Table 2). But in case of dissolved fractions, the median LDON:LDOP ratio was 122

(Table 2). In contrast, the median ratio of RPN:RPOP was 31 while the median ratio of RDON:RDOP was 227 (Table 2). The ratios of labile LPON: LPOP increased during decomposition while the ratios of LDON:LDOP decreased than the initial PON:POP and DON:DOP ratios reflecting faster decomposition of LPOP than LPON.

The spatial and temporal changes in the C:N:P ratios of POM could be due to the variations in proportions of the four major groups of biomolecules (carbohydrates, lipids, proteins, and P-compounds). The N:P ratio of DOM in unpolluted rivers is 30_170 (Meybeck 1982). In this study, there were also significant differences in the N:P ratios of the POM and DOM. In fact, the average N:P molar ratio of POM (Table 1) in the Keum River throughout the study period (N:P, 21), in the Youngsan River during August (N:P, 25), and in the Han River during April (N:P, 17) was close to the Redfield ratio of 16, typical of detrital organic matter derived from aquatic plants and phytoplankton (Anderson 1995). The N:P ratio of organic matter in the other rivers differed more widely from the Redfield ratio, perhaps due to the marked increase in the proportion of particulate carbohydrates at the expense of proteins and P-compounds. Depending on the type of vegetation, such as plants with soft tissues vs. woody tissues, terrestrial organic matter shows a wide range of N:P ratios. The terrestrial plants have low N and P contents with N:P ratios of 10_100 and 100_1000 for soft tissues and woody tissues, respectively (Ruttenberg and Goni 1997).

Conclusions

During and decomposition of organic C, P, and N, both labile and refractory fractions are essential to understand the C, N, and P dynamics in aquatic systems. There were major differences in the relative amount of labile and recalcitrant fractions of POC, DOC, PON, DON, POP, and DOP as a result of preferential mineralization of P over N and N over C. Our results provide some details on changes in the ratios of organic C, N, and P that will be useful for understanding organic matter decomposition and biogeochemical nutrient cycling and modeling applications in freshwater ecosystems.

Abbreviations

CNP: Carbon nitrogen phosphorus; COD: Chemical oxygen demand; DIP: Dissolved inorganic phosphorus; DO: Dissolved oxygen; DOC: Dissolved organic carbon; DOM: Dissolved organic matter; DON: Dissolved organic nitrogen; DOP: Dissolved organic phosphorus; DTN: Dissolved total nitrogen; DTP: Dissolved total phosphorus; LDOC: Labile dissolved organic carbon; LDOM: Labile dissolved organic matter; LDON: Labile dissolved organic nitrogen; LDOP: Labile dissolved organic phosphorus; LPOC: Labile particulate organic carbon; LPOM: Labile particulate organic matter; LPON: Labile particulate organic nitrogen; LPOP: Labile particulate organic phosphorus; POC: Particulate organic carbon; POM: Particulate organic matter; PON: Particulate organic nitrogen; POP: Particulate organic phosphorus; RDOC: Refractory dissolved organic carbon; RDOM: Refractory dissolved organic matter; RDON: Refractory dissolved organic nitrogen; RDOP: Refractory dissolved organic phosphorus; RPOC: Refractory particulate

organic carbon; RPOM: Refractory particulate organic matter; RPN: Refractory particulate organic nitrogen; RPOP: Refractory particulate organic phosphorus; TN: Total nitrogen; TOC: Total organic carbon; TON: Total organic nitrogen; TOP: Total organic phosphorus; TP: Total phosphorus

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

All authors contributed in the manuscript. BK designed the study. MJJ, CJ, JE, SJ, MSS, YL, and YC collected and analyzed the data. MJJ and BK wrote the initial draft of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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