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Elucidation of environmentally influential substance dynamics in the lake bottom sediment using stable isotope ratio

安定同位体を用いた湖底堆積物および植物を介した環境影響化学物質の循環の解明

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Introduction

The stable isotope techniques have been used to investigate the interactions of plants with their biotic and abiotic environments and their responses to resources that mediate or influence them. They are particularly useful to elucidate the nutrient dynamics between plants and lake-bottom sediments (Dawson et al., 2002). Here, the interaction between charophytes and sediment nutrients were investigated at Myall Lake, New South Wales, Australia (32°S 152°E).

Method

We collected plant biomass, bottom sediment, and water samples at Corrigans Bay, Russels Bay and Neranie Bay (Fig. 1). We determined interstitial water nutrient concentrations (i.e., NH_4-N, NO_3-N, NO_2-N, and PO_4-P) using a TrAAcs-800 Autoanalyzer after thawing the samples. Total nitrogen in the overlying water was determined using a spectrophotometer following Koroloff's method, and total phosphorus was determined by the molybdenum blue colorimetric method after digestion with $K_2S_2O_8$ in an autoclave (120°C) for 30 min (APHA, 1995). Carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) were determined for sediment samples and for samples of charophytes (*Chara fibrosa* var. *fibrosa* (A. Br.) and *Nitella hyalina* (DC.) Ag.) and *Najas. marina* L. Sediments and charophyte samples were acidified with 1 N HCl in a silver cup, dried, and covered with a tin cup. Other samples were directly covered with a tin cup and combusted at 1020°C in an EA1108 Fisons elemental analyzer (Fisons Instruments, Milan, Italy). The combustion products (CO_2 and N_2) were introduced into an isotope-ratio mass spectrometer (Delta Plus, Finigan, Bremen, Germany) in a continuous flow of helium carrier. The ratios of ^{13}C:^{12}C and ^{15}N:^{14}N were expressed relative to the Vienne-PeeDee Belemnite (V-PDB) standard for carbon (Coplen, 1996) and N_2 in air for nitrogen, respectively. The ratios ^{13}C:^{12}C and ^{15}N:^{14}N were calculated as:

$$\delta^{13}C \text{ or } \delta^{15}N = \left[\left(\frac{R_{sample}}{R_{reference}}\right) - 1\right] \text{ x } 1000\ (‰),$$

where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

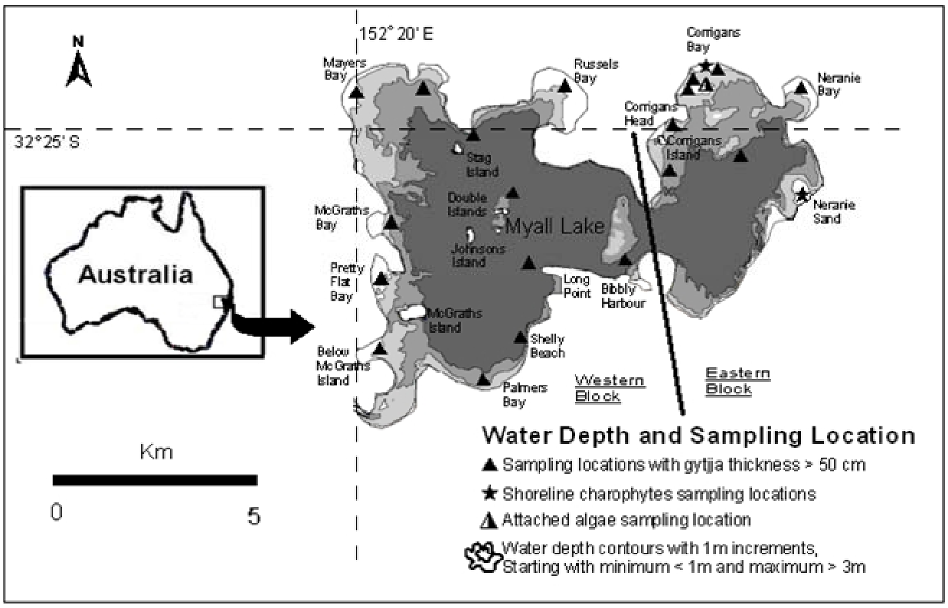

Figure 1. Map of Myall Lake showing sampling locations and water depth in 1 m increment contours.

Results

Water quality

During the two-year sampling period, water column temperature, pH, and dissolved oxygen varied between 12.7–29.5°C, 6.5–9.5, and 6.3–12.1 mg/L, respectively. However, the difference between the water temperatures at the surface and the bottom or between the central parts and bays was less than 1°C, indicating no thermocline existed at any time of the year. The formation of a thermocline was inhibited by the shallowness of Myall Lake and the strongly windy climate. Water transparency was always high, Secchi depth could not be determined because the disc was still visible even at depth 4.5 meter on very windy days.

General trend of biomass distribution

The distribution of *Chara* and *Nitella* biomass when compared against depth indicated that although the dry mass oscillates somewhat seasonally, high in summer and low in winter, the dry mass of charophytes was nearly zero in zones less than 40 cm deep, then it reached a peak value in areas around 1 m to 2.5 m deep, and it again gradually declined towards the deeper area (Asaeda et al., 2007).

Among the bays in the northern and western shorelines, Corrigans Bay almost always had a higher biomass of charophytes than the other bays, while Mayers Bay occasionally possessed a high biomass. However, the other bays, particularly Russels Bay and Neranie Bay, had small amounts of charophytes, except for short peak periods. Compared with the northern and western bays, where the charophyte mat started from about 50 cm deep downwards to the deeper side, the southern bays had charophytes starting from 1.5 deep downwards except during the calm season in autumn, when some amount of biomass was observed even at 1 m deep.

Accompanying formation of the charophyte mat, the *gyttja* layer always developed beneath the growing charophyte bed, which was otherwise sandy. The deep central area was covered with patchy charophyte mats, with total coverage declining from the eastern side to the western side, and with the smallest area being around the deepest point (Shilla et al., 2006a; Asaeda et al., 2007).

Other than charophytes, the only other major species widely distributed was *Najas marina*, which was, however, dominant only in autumn, from March to July. *Najas marina* frequently formed patches about 100 m wide in the central part, at the entrance of Corrigans Bay and along the eastern to western sites at 3 m deep, outside McGraths Bay, and inside Neranie Bay. However, the tops of the *N. marina* canopies were submerged at about 30 cm below the surface, although the entire water column other than the surface to 30 cm depth was fully occupied by *N. marina* biomass.

Ammonium and Phosphate phosphorus concentration in the interstitial water and sediment

Ammonium, nitrate and nitrite nitrogen, ort-phosphate phosphorus in the interstitial water and total nitrogen, total phosphorus and total carbon in the sediment varied spatially. At the center of the bays, ammonium concentration was relatively higher with 170 to 360 μM at 2.5 cm deep in the sediment, enhanced significantly downwards, being lowest at Corrigans Bay, 300 μM at 17.5 cm deep, and highest at Neranie Bay, 2500 μM at 17.5 cm deep. Nitrate and nitrate concentrations were at most 2 μM, negligibly smaller than that of ammonium. Total nitrogen concentrations in the sediment, slightly decreasing depth-wise, were generally similar between these bays, such as 130 to 180 μM for Corrigans Bay, 140 to 210 μM for Neranie Bay and 90 to 200 μM for Russels Bay (Siong and Asaeda, 2006; Siong et al, 2006; Shilla et al, 2006b).

Phosphate phosphorus concentrations of the interstitial water in the sediment were relatively similar between these bays; it enhances with depth below the bottom, ranging in 0.3 μM to 1.2 μM. Total phosphorus contents in the sediment distributed relatively uniformly in the sediment, slightly decreasing below 20 cm deep, however, remaining at a similar magnitude to the concentration of phosphate phosphorus in the interstitial water.

Total carbon concentrations in the sediment, slightly declining depth-wise, were relatively similar between these bays, such as 1200 μM to 2400 μM.

Stoichiomerical ratios in the interstitial water and sediments

Stoichiometrical ratios between nitrogen and phosphorus, N/P, were as follows. N/P values are generally extremely high in these bays because of high ammonium concentration, such as 100 to 200 in the sediment, and even much higher in the interstitial water; 170 to 250 in Corrigans Bay, 1000 to 2000 in Neranie Bay, and 900 to 1400 in Russels Bay.

Total nitrogen and total phosphorus in plant tissues

There was no particular trend between TN and TP, while, the ratios of TN and TP are relatively higher, particularly with charophytes and epiphytic algae, such as 80 to 200, while angiosperms were slightly lower, such as 30 to 130. For both groups, the ratios were at the same magnitude of that of sediment and lower than that of the interstitial water.

Nitrogen stable isotope ratios

The nitrogen stable isotope ratio, $\delta^{15}N$, of ammonium in the interstitial water and nitrogen in the sediments are relatively lower in Corrigans Bay than other bays, and higher in Russels Bay. All values are between -1 to 5‰ then enhanced with depth at all bays within upper 20 cm deep layer (Table 1).

Table 1. Mean and standard deviation of total carbon (TC), total nitrogen (TN), total phosphorus (TP), calcium (Ca), water content (H_2O), $\delta^{13}C$ and $\delta^{15}N$ in Myall Lake sediment and plant

Sediment Cores						
	n	**TC**	**TN**	**TP**	**Ca**	**H_2O**
Dec 2003†		mg/g				%
0-5 cm	15	251.1±25.9	17.8±1.0	0.63±0.20	5.8±3.8	95±3
5-10 cm	15	207.7±55.5	15.1±1.8	0.47±0.23	4.3±2.1	94±3
10-25 cm	15	192.2±42.2	9.9±3.8	0.43±0.21	6.1±4.7	92±3
		TC	**TN**	**TP**	**$\delta^{13}C$‡**	**$\delta^{15}N$‡**
March 2006		mg/g			‰	
0–5 cm	6	251.3±29.4	27.2±2.6	0.57±0.21	−13.8±1.3	−0.7±0.6
5–10 cm	6	248.4±25.4	26.0±2.7	0.62±0.23	−14.2±1.2	−0.4±0.6
10–15 cm	6	244.3±26.0	24.1±3.5	0.59±0.19	−14.2±1.2	−0.2±0.7
15–20 cm	6	239.0±19.8	23.9±2.3	0.57±0.21	−14.4±0.9	−0.3±0.6
20–25 cm	6	230.7±33.6	21.9±4.3	0.49±0.21	−14.7±0.8	−0.1±0.9
25–30 cm	6	219.32±37.6	21.7±4.5	0.34±0.24	−14.7±0.8	0±0.8
30–35 cm	6	212.1±34.6	19.3±3.9	0.41±0.28	−15.1±0.6	−0.1±0.8
35–40 cm	6	192.3±37.6	16.9±3.5	0.36±0.21	−15.3±0.5	−0.2±0.6
Plant Samples						
		TC	**TN**	**TP**		
Sep 2003-May 2005		mg/g				
C.fibrosa	68	348.1±18.6	34.9±5.9	0.45±0.15		
N.hyalina	68	333.2±15.4	27.3±2.6	0.42±0.08		
N.marina	52	306.0±8.0	20.2±3.2	1.25±0.40		
Attached algae	4	285.9±54.5	25.7±7.3	0.52±0.02		
Chara (shoreline)	6	302.4±45.7	27.8±6.3	0.60±0.10		

† Total carbon in Dec 2003 samples was estimated using LOI data where TC (mg/g) = 0.465*LOI (mg/g) (Westlake, 1965)

‡ Six sets of core samples collected in March 2006 were analyzed at least in triplicate until the results of $\delta^{13}C$ and $\delta^{15}N$ for each sample had a standard deviation less than 0.5‰.

Discussion

As the interstitial water was extremely richer in ammonium-nitrogen than the overlying water, the nitrogen source of plants is likely the interstitial water, rather than the overlying water. The agreement of N/P ratio of the sediment with that of plant indicates the gyttja layer is originated from decomposed plants (Shilla et al., 2006a) while N/P ratio in the interstitial water was much larger than these values with its high ammonium concentration.

Ammonium concentration in the interstitial water was highest at Neranie Bay, followed by Russels Bay, and lowest at Corrigans Bay, which corresponds to the inverse order of the period covered by plants. N/P ratio of the plant tissues indicates the consumption of nitrogen was about 100 times larger than phosphorus stoichiometrically. Phosphorus concentration in the interstitial water was less than 2 μM, introducing only 200 μM of nitrogen consumption, which causes one order smaller difference than that in ammonium concentration of the interstitial water between bays.

The effects of fractionation in nitrogen uptake

Values of nitrogen stable isotope ratio were extremely low for plants in the lake particularly with tissues sampled around the center of bays. The $\delta^{15}N$ values of the ammonium in the interstitial water were 3 to 5‰, therefore low

$\delta^{15}N$ in the plant tissues were attributed to not the original nitrogen in the interstitial water but the fractionation of low ammonium by plants.

As the stoichiometrical ratio of N and P in the interstitial water was 200 in Corrigans Bay to 2000 in Neranie Bay, while the ratio in the plant tissues were only 50 to 100. Therefore, the plants were likely capable of intensive fractionation of light ammonium in the uptake.

It is evident from the analysis that $\delta^{15}N$ lowers substantially with increasing N/P ratio in the interstitial water, particularly when N/P in the interstitial water is low. However, with higher N/P ratio, the intensity of fractionation lowers. Compared with other data, $\delta^{15}N$ value of *N. marina* at the shoreline was relatively higher. However, as there were no *N. marina* communities at the shoreline before, the tissue, although growing there at the time, seems to have been drifted from the center of the bay.

Although the N/P ratio of the overlying water was relatively high in natural water, such as about 120, it is nearly the same as the ratio of tissues in epiphytic algae. Therefore, the fractionation of light nitrogen in the uptake is likely limited. Therefore, $\delta^{15}N$ of the epiphytic algae was about 1‰, and much higher than that of charophytes, *N. marina* and *Ruppia maritima* at the same spot.

There is no correlation between these N/P ratios between the plant tissues and the interstitial water, where the values of the interstitial water at the shoreline was assumed to be same as that of the overlying water, however, N/P in the plant tissues are relatively high in charophytes and epiphytic algae, such as from 80 to 370, while between 20 and 80 for angiosperms, under the high ratio in the interstitial water. The reason for the difference is not clear yet, however, likely similar to the reason for the uptake preference of nitrate by angiosperms and ammonium for algae as nitrogen sources. Therefore, although the N/P in the interstitial water was same, there supposed to be a difference in the fractionation intensity between algae and angiosperms, such as relatively high with charophytes and epiphytic algae and low with angiosperms.

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