自然生態系による水圏環境中の粗細粒・溶存・ガス物質の動態機構の解明

―安定同位体を用いた湖沼の物質動態の解析－

Behaviors of particulate, dissolved and gaseous materials in aquatic systems -Behavioral analyses of materials in a lentic system, indicated by carbon and nitrogen stable isotope ratios-

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1. Introduction

The use of stable isotope techniques in plant ecological research to investigate plant interactions with their biotic and abiotic environments and the responses to resources that mediate or influence them has grown steadily during the past two decades (Dawson et al., 2002). Plants preferably uptake and synthesize lighter elements rather than the heavier ones, thus fractionating stable isotopes of carbon and nitrogen from the surrounding environments during uptake (Dawson et al.; 2002; Dijkstra et al., 2003; Fry, 2006). The fractionation of stable isotopes is highly affected by the limitation of the sources, usually occurring if the source is abundant, however, it is suppressed with the limited sources. For example, a shortage of carbon dioxide limits the fractionation of ^{12}C and ^{13}C, thereby incorporating a larger concentration of ^{13}C in the plants. Uptake of HCO_3, which contains relatively high fraction of ^{13}C, further increases the $\delta^{13}C$ of the plants. When the nitrogen source contains abundant ammonium, highly fractionated ^{14}N with respect to ^{15}N in the plants is observed (Yoneyama et al., 2001). Thus, the stable isotope ratio is an efficient indicator of the abundance of source carbon and nitrogen for plants (Nichols & Keeney, 1976; France, 1995).

The present study was carried out in Myall Lake, an oligohaline lake in NSW, Australia, that is highly isolated from both other water bodies and terrestrial nutrient sources. The bottom of almost the entire lake is covered with a dense charophyte (*Chara fibrosa* var. *fibrosa* (A. Br.) and *Nitella hyalina* (DC.) Ag.) mat, of which *Najas marina* L. comprises a substantial amount in autumn (Shilla et al., 2006). A thick layer of organic sediment, *gyttja* (Wetzel, 2001; Dasey et al., 2005), produced autochthonously by the decomposition of the plant tissues, has accumulated underneath the plant beds throughout the lake. The substrate is soft, anoxic and contains a high concentration of ammonium in the interstitial water. Thereby, the source and its abundance of nitrogen can be identified by analyzing nitrogen stable isotope ratios of the plant species. In addition, intensive growth of *N. marina* in autumn could consume a significant portion of available carbon dioxide, possibly increasing the proportion of ^{13}C incorporated in the plant tissues. We, therefore, analyzed the carbon and nitrogen stable isotope ratios of charophytes and *N. marina* from various points in Myall Lake to elucidate the possible nutrient sources for plants at various sampling points in different conditions. We also investigated the preferential uptake of lighter isotopes by plants and the effect of different morphological characteristics of the plant species on nutrient uptake.

2. Materials and methods

Study area

Myall Lake (Fig. 1) is an oligohaline lake with a salinity 2.2~2.8 and an area of 64 km^2, and situated about 75 km north of Newcastle on the central coast of NSW, Australia (152°E 32°S). The average depth of the lake is about 2.8 m, and the deepest part is approximately 4.5 m deep.

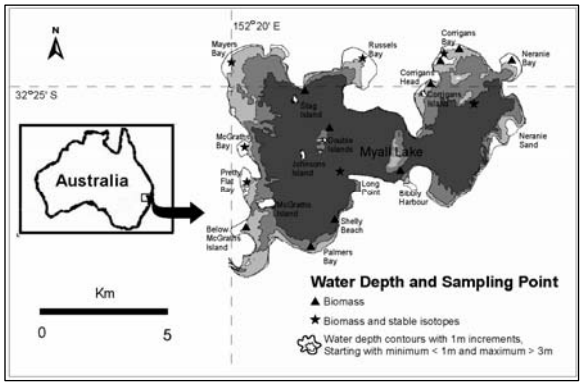

Fig. 1. Map of Myall Lake showing water depth in 1 m increment contours and sampling locations for biomass and stable isotopes.

Myall Lake is located at the upstream end of a series of four lakes, Myall Lake, Mid-Lakes (Boolambayte Lake and Two-mile Lake), and Bombah Broadwater, which are then connected to the Pacific Ocean by a 20 km long Lower Myall River. Most of the catchment drains into Bombah Broadwater through the Upper Myall River, while a small creek flows into the downstream end of the Boolambayte Lake. The catchment area of Myall Lake is twice as large as the lake area and yet both flushing by runoff and exchange with the downstream lakes are limited (Sanderson, unpubl. data). The shoreline of the northern and western bays is fixed in spite of a fluctuation of about 30 cm in the water level after heavy rainfalls during rainy season and the maximum tidal variation is only in the range of 2 cm (Sanderson, unpubl. data). Only the edge of a

southern bay in the eastern section is inundated at flooding times, flushing terrestrial materials into the lake. Therefore, Myall Lake has is highly isolated from both terrestrial run-off as well as from other lakes and rivers.

Sample collection

From 2003 to 2005, plant biomass, bottom sediment, and water samples were collected at 18-20 locations in Myall Lake (Fig. 1) at four- to seven-week intervals, together with in situ measurements of vertical distributions of water temperature, light intensity, dissolved oxygen concentration, pH, salinity, and water and *gyttja* depths. These parameters were sometimes also measured at many additional locations for comparative purposes.

At each sampling location, plants were collected from 3 to 10 quadrats by scraping with a 30 cm-wide rake for a distance of 1 or 2 m. *Gyttja* is extremely soft, so the depth of the *gyttja* layer was determined by pushing a pole downwards until it reached the more resistant underlying sediment (often a sand layer). The layer identified as *gyttja* comprised more than 50% organic matter as determined by weight-loss on ignition (LOI) at 550^{o}C for two hours (Ball, 1964).

Sampled plants were washed and immediately sorted by genus and species: *Chara fibrosa*, *Nitella hyalina*, *Najas marina*, *Myriophyllum salsugineum*, and *Vallisneria gigantea*. All the samples were then oven-dried until they reached a constant weight to obtain the dry weight and for further analyses including the analysis for carbon and nitrogen stable isotope ratios.

Stable isotope ratios

Carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) were determined for subsamples of charophytes and *Najas marina*. 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 elemental analyzer (model: EA1108, Fisons Instruments, Milan, Italy). The combustion products (CO_2 and N_2) were introduced into an isotope-ratio mass spectrometer (model: Delta Plus, Finigan, Bremen, Germany) in a continuous flow using a He 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. The ratios ^{13}C:^{12}C and^{15}N:^{14}N were calculated as

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

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

Machine drift during analyses was checked with L-*a*-alanine ($\delta^{13}C$ = -20.93‰, $\delta^{15}N$ = 7.61‰) every five samples. The accuracy of values was determined using interlaboratory-determined proline following the method of Minagawa et al. (1984) for $\delta^{13}C$ (-13.81‰) and $\delta^{15}N$ (-8.52‰). All samples were tested at least in triplicate with standard deviations (S.D.) being < 0.5‰ for $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰).

Statistical analysis

The data were analyzed using the SPSS for Windows (release 11, SPSS Inc., Chicago, IL) statistical software package, and an unpaired *t*-test was used to evaluate differences between two independent means. Pearson's test was used to verify correlation between variables.

3. Results and Discussion

Water quality

The 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, there was essentially no formation of a thermocline at any time of the year, in part due to the shallowness of Myall Lake with respect to surface area and a strongly windy climate. Water transparency has been always high (more than 4.5 m Secchi depth) even on very windy days.

Habitat characteristics

Figures 2 (a) and (b) show the relationship of the biomass of plant species with the water depth and the *gyttja* layer thickness, respectively while Fig. 2 (c) illustrates the variation of *gyttja* layer thickness against water depth. Charophytes were partially covered with dead and decomposing tissues, which were in the process of becoming *gyttja*. Thus, most *gyttja* likely originates from charophytes, although planktonic and benthic algal cells are also trapped.

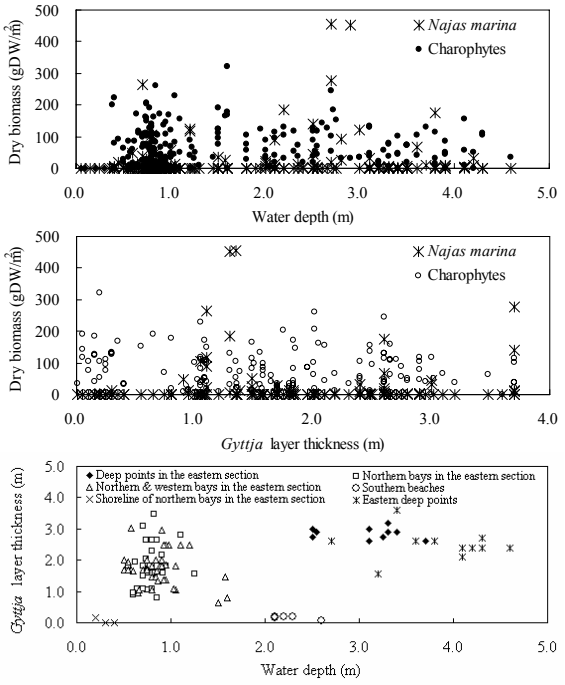

Fig. 2. The relationship of the biomass of plant species with (a) the water depth and (b) the *gyttja* layer thickness, and (c) the relationship of the *gyttja* layer thickness with the water depth.

Charophytes and *N. marina* require only a weak

anchor due to their relatively low buoyancy, and are thereby capable of growing on the surface of a thick *gyttja* layer, even if it is extremely soft. The habitats of *N. marina* are restricted to the *gyttja* surface in relatively calm water, such as below water deeper than 1.5 m or in sheltered bays (Fig. 2(b)). The occurrence of other species was limited to local areas such as rocky bottoms (*M. salsugineum*) or sandy beaches (*V. gigantea*).

Growth rate of plants

Charophytes (*C. fibrosa* and *N. hyalina*) grew through most of the year, although the growth rate varied seasonally. *Najas marina* grew vigorously from February to July, but it did not grow in other seasons.

The relative net growth rates, RGR, of charophytes and *N. marina* were obtained by,

$$\mu = \ln\left(B(t) \Big/ B(0) \right) \Big/ t$$

where $B(t)$ is the dry mass on t days after the commencement of growth, $B(0)$ is the dry mass at the starting time, μ is the growth rate, and t is the number of days from the start of growth (Kerbs, 1985).

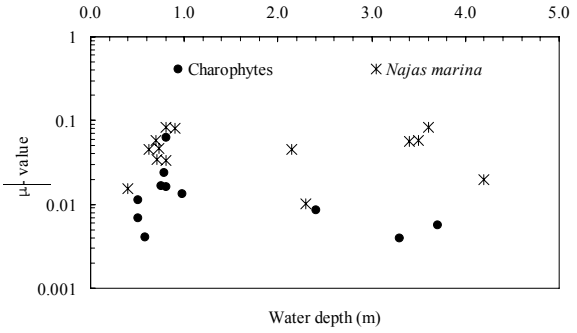

Fig. 3. The calculated net growth rates of charophytes and *Najas marina* in relation to water depth.

Figure 3 presents the RGRs of charophytes and *N. marina* in terms of water depth. The net growth rate of charophytes was low in very shallow water, increased at 0.9 m deep and thereafter, but started to decline at 2.5 m deep.

Because the growth of *N. marina* was limited to autumn, all these RGRs correspond to their intensive growth in autumn. The growth rate of *N. marina* was low in the very shallow water, then remarkably increased at 0.9 m deep or more, with an RGR of about 10 times larger than that of charophytes.

$\delta^{15}N$ signature

Figures 4 (a) and (b) show plant $\delta^{15}N$ as a function of water depth. Values at about 4 m deep belong to samples collected at the deepest western points; however, those at 2.5 to 3.5 m deep range correspond to the samples taken at the eastern deep zone, those at 20 cm to 1 m are the samples in the bays, and those at less than 10 cm are shoreline samples. Most of $\delta^{15}N$ values observed were negative and much lower than those of previous studies (Jones et al., 2004), where most of $\delta^{15}N$ values were positive.

The $\delta^{15}N$ of *C. fibrosa* and *N. hyalina* had nearly the same trend (*t*-test, all $p>0.05$), ranging from -1‰ to -9‰, mostly at less than 1 m deep, to 0‰ to -1‰ at 3 m depth in the eastern deep zone, and to positive values to -4‰ at 4 m depth of deepest western points. However, at the shoreline, where a less than 10 cm thick *gyttja* layer was present overlying the sandy bottom (Fig. 2(c)), the values were nearly zero. Likewise, the $\delta^{15}N$ of attached algae was also slightly positive.

The $\delta^{15}N$ values of *N. marina* were lower than those of charophytes (*t*-test, $p<0.05$). At about 1 m depth, $\delta^{15}N$ was mostly as low as -10‰, while it was as high as -4‰ at about 3 m depth in the eastern deepest points, then was -1.5‰ to -6‰ at 4 m deep of the western deepest points.

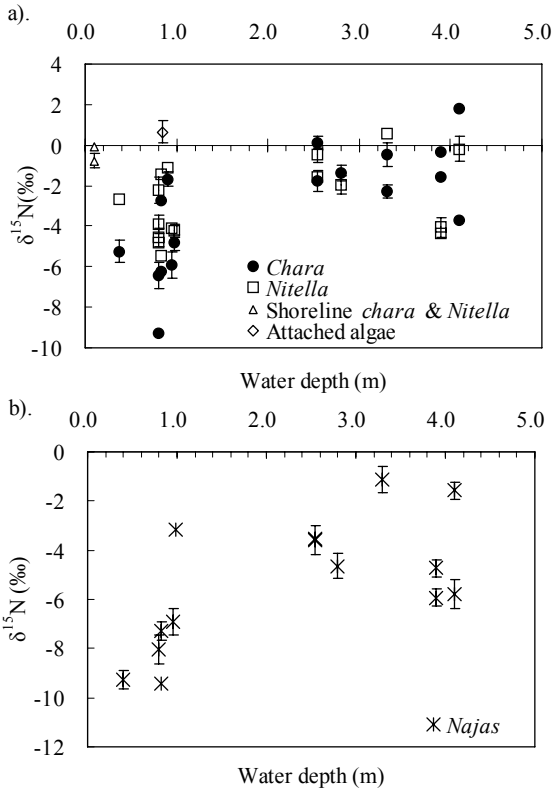

Fig. 4. The $\delta^{15}N$ stable isotope fraction plotted against water depth for (a) charophytes and (b) *Najas marina* (±1SD).

$\delta^{13}C$ signature

The $\delta^{13}C$ values are shown with respect to the water depth in Fig.s 5 (a) and (b). Unlike the $\delta^{15}N$, the $\delta^{13}C$ values of charophytes and *N. marina* were significantly different (*t*-test, $p<0.01$), while the values of two charophyte species were nearly identical (*t*-test, $p>0.05$). The $\delta^{13}C$ values of charophytes, which were -10‰ to -14‰, did not depend much on water depth for depths between 1 m to 3 m; however, shoreline and deep samples in the western end had much lower values than the others (*t*-test, $p<0.01$), being -17‰ for shoreline samples and -16‰ for deep samples. The $\delta^{13}C$ values of *N. marina* were generally higher than those of charophytes, and relatively constant at about -7‰, in spite of some low values at less than 1 m deep and more than 4 m deep.

The correlation between $\delta^{13}C$ and $\delta^{15}N$ values is shown in Fig. 6. In spite of extensive scattering, the $\delta^{13}C$ and $\delta^{15}N$ values of *C. fibrosa* and *N. hyalina* seem to be negatively correlated (Pearson r=-0.56 and -0.54 for *Chara* and *Nitella*, respectively, both $p<0.05$); however, consistently with each other species. *Najas marina* had higher $\delta^{13}C$ values (*t*-test,

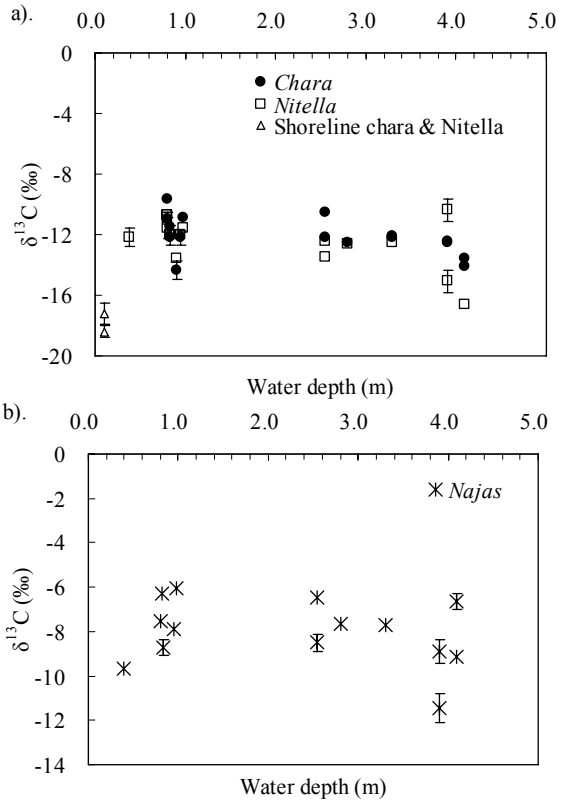

Fig. 5. The $\delta^{13}C$ stable isotope fraction plotted against water depth for (a) charophytes and (b) *Najas marina* (±1SD).

$p<0.01$) and lower $\delta^{15}N$ values than charophytes (*t*-test, $p<0.05$).

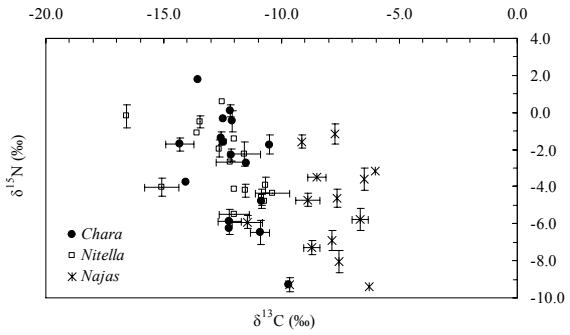

Fig. 6. The correlation between $\delta^{13}C$ and $\delta^{15}N$ for charophytes and *Najas marina*. Error bars indicate standard deviations (±1SD).

Figures 7 (a) and (b) show $\delta^{13}C$ and $\delta^{15}N$ as a function of C/N ratio. The $\delta^{15}N$ did not have any correlation with C/N (Pearson r=0.14~0.18 for all species, all $p>0.05$), while $\delta^{13}C$ consistently correlated positively with C/N among species (Pearson r=0.54~0.58 for all species, all $p<0.05$).

4. Conclusive Remarks

Sampaklis (2003) reported that the *gyttja* up to 50 cm thick in Myall Lake contained more than 80% water regardless of the location, if the *gyttja* layer was sufficiently thick. Also, the ammonium-nitrogen concentration of the water was about 250 μM NH_4 at the topmost *gyttja* layer (0 to 25 cm deep) and approximately 600 μM NH_4 in the middle layer (25 to 50 cm deep) throughout Myall Lake. These values were much higher than those in the overlying water (less than 0.7 μM). In contrast, the nitrite/nitrate concentration in the interstitial water was only 0.2 to 0.4 μM. Therefore, the dissolved inorganic nitrogen in the interstitial water of *gyttja* was mostly ammonium, and the concentration was about 300~600 times higher than that in the overlying water. Because the root and rhizoid systems of *N. marina* and charophytes are highly developed in the *gyttja* layer, the nitrogen source is likely the interstitial ammonium rather than uptake from the overlying water.

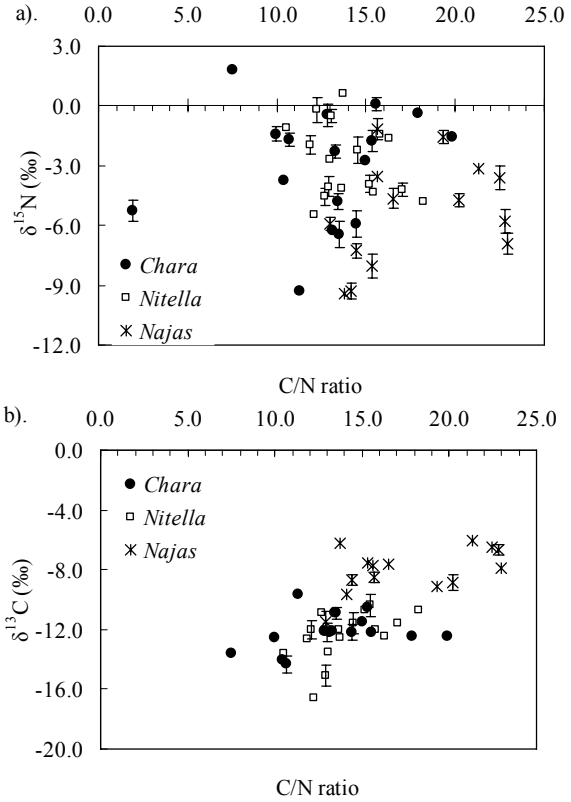

Fig. 7. The correlation of (a) $\delta^{15}N$ and (b) $\delta^{13}C$ plotted against C/N ratio for charophytes and *Najas marina* (±1SD).

Phosphorus concentration in the interstitial water was, on the other hand, ca. 0.8 μM, which was ~400 times lower than the ammonium concentration. As the ratio of nitrogen and phosphorus concentration in plant tissues was 20 to 45 (Shilla et al., 2006), thereby the growth of the plant was limited by the low phosphorus concentration and ammonium was taken up excessively from the far abundant ammonium stock, compared with the actually required amount for the growth. Therefore,

ammonium incorporating lighter nitrogen is likely fractionated by each plant species during uptake from the source with an infinite amount of ammonium, leading to the markedly low $\delta^{15}N$ values in the plants