Effect of ammonium on fractionation of stable isotope of nitrogen and growth performance in *Chara fibrosa*

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Abstract: *Chara fibrosa* was grown hydroponically under various conditions with respect to different nitrogen concentrations (0, 0.2, 1.0, 5.0, and 10.0 ppm NH_4^+-N) to observe the variation of fractionation of N stable isotope and growth performance. There was a significant variation in fractionation of nitrogen isotope during uptake from different concentration of N by *Chara fibrosa*. When plant absorbed a large amount of NH_4^+-N from the solution, the high negative value of $\delta^{15}N$ was observed in *Chara* and the negative value of $\delta^{15}N$ was increased with the increase of nitrogen concentration in the medium. The shoot length and both chlorophyll a and b content was increased with the increase of nitrogen concentration in the medium.
Key words: *Chara fibrosa;* fractionation; nitrogen isotopes.

Introduction

Nitrogen (N) nutrition of plants mainly depends on the availability of different inorganic N forms in the environment . Ammonium (NH_4^+) is one of the most important inorganic nitrogen sources for plants. Since the assimilation of NH_4^+ requires less energy than that of NO_3^-, many plants prefer the former source .

When NH_4^+ is supplied as the exclusive N source at high concentrations, it is toxic and impairs plant growth . Some emergent wetland species dominating lake littorals, such as common reed (*Phragmites australis*), can tolerate a high concentration of NH_4^+, whereas others, such as reed sweet-grass (*Glyceria maxima*), tend to avoid high NH_4^+ concentrations by shallow rooting . There are some literatures about the preferable form of nitrogen for the emergent and free floating aquatic macrophytes but about *Chara fibrosa* these type of literatures are very scare.

The natural variation in stable isotopes has been shown to be a powerful tool in several studies of plant ecosystem N dynamics .The natural abundance of ^{15}N ($\delta^{15}N$) of plant biomass has been shown to vary as a function of isotopic composition of primary N source (soil, fertilizer, N_2) and the isotope ratio of the source is preserved during N absorption, assimilation and translocation . However, physico-chemical processes in soil like N-uptake, denitrification by bacteria, leaching and volatilization of organic nitrogen compounds and physiological processes within plant like assimilation through distinct pathways can discriminate against ^{15}N . Therefore, $\delta^{15}N$ of plants reflects the net effect of a range of processes .

New insights about the net discrimination that occurs during N-assimilation have been obtained by growing plants hydroponically in the presence of a single inorganic N-source. When NH_4^+ is the sole source of N, there is little, if any, discrimination if the N concentration is limiting . But there may be large whole plant depletion in ^{15}N at high N concentrations . Remarkably little is known about whether fractionation occurs during the uptake and assimilation of N from different concentration by macro algae *Chara*. In the present study, we hypothesized that there would be no discrimination against ^{15}N in *Chara fibrosa* fed with different levels of NH_4^+-N. Therefore, fractionation of stable N isotopes, if any, will provide insight into the response of this species to different levels of N. The objective of this study was therefore, to investigate the fractionation of stable N isotopes as a function of levels of N.

Materials and Methods

Experimental setup: The plants were grown in 2 L glass beaker with 5 concentration levels viz. 0, 0.2, 1, 5, 10 ppm. The sources of N was $(NH_4)_2SO_4$ (the $\delta^{15}N$ value was -9.95±0.06). Approximately 400g of commercially available river sand (90% < 1 mm) (DIY, Doit, Japan) per beaker was used as substrate. Before using as substrate, sand was washed thoroughly with tap water to remove dust particles until the supernatant was found to be clear. Finally it was washed with distilled water. Washing of substrate ensured that it did not provide any nutrient to interfere the effect of supplied N on the growth of Chara fibrsa in the beaker.

Modified Forsberg solution (Table 1) was used as cultural media where, $Ca(NO_3)_2$ was substituted by $CaCl_2.2H_2O$, as N was used as treatment. 2 L of culture media was used in each tank and the quantity was adjusted biweekly with deionized distilled water to compensate evapotranspiration. The pH of the solutions maintained 7.0 ± 0.5 by adding HCl or NaOH. The experiment was conducted for a period of 5 weeks .All beakers were placed in a growth incubator, at a temperature of 25 ± 1 °C, the illumination at the water surface was 35–45 μmol m^{-2} s^{-1} (PAR-06, Prede Co. Ltd., Japan) and the photoperiod was 12:12 h (light:dark). Before transplanting length of each shoot was measured and recorded.

Plant analyses: Shoot elongation was calculated as the percent increment of shoot length relative to the initial length of corresponding shoot at the time of transplanting (Eq. [1]). $E_t=(L_t-L_0)\times100/L_0$. Where, E_t is the shoot elongation (% relative to the initial length) at tth DAT, L_t is thecumulative shoot length in the tth DAT, and L_0 is the initial shoot length of the plant.Chlorophyll a and chlorophyll b content of fresh T. natans leaves were analyzed at each harvesting. For measuring chlorophyll content, chlorophyll was extracted from leaves by N,N-dimethylformamide (DMF) . Tips (around 2cm) were cut and weighed and then placed in 5 mL DMF in a test tube. The test tube was then incubated overnight in refrigerator (4° C) for chlorophyll extraction. The absorption of extracts was read at 647 and 663 nm and chlorophyll a and

chlorophyll b contents were calculated using the formula and the extinction coefficients given by .

After the above measurements, plants were dried at 60 °C to constant weight in an oven. Following drying, samples were re-weighed (for dry weight), homogenized by grinding into fine powder using a mortar and pestle. Powdered samples were stored in air-tight vials for subsequent analyses. Total nitrogen (TN) of powdered plant samples were measured by an auto analyzer (VarioMICROcube, Elementar Analysensysteme GBbH, Germany). The $\delta^{15}N$ was analyzed by an auto analyzer (Isoprime, Micromass, UK)). All analyses were done in five replicates.

Statistical analysis: All data are presented in the paper as mean with Standard Error (SE).Differences among treatments were analyzed by one-way ANOVA to check the significant differences ($P < 0.05$) with a post hoc Turkey test. For this purpose SPSS for windows (release 13, SPSS INC., Chicago, IL) statistical software package was used.

Results and Discussion

Experiment with charophyte showed that there was a significant difference in shoot length of *Chara fibrosa* when they were grown in different concentration of nitrogen. It was observed that shoot length increased with the increase of nitrogen concentration in the medium (Fig. 1). Significant differences of chlorophyll content were observed in *Chara fibrosa* when we applied different concentration of NH_4-N. Chlorophyll content was increased with the increase of nitrogen in solution and highest amount of chlorophyll a &b was obtained in the highest concentration (10 ppm) of NH_4^+-N. It was observed that both chlorophyll a & b content was increased with the increase of nitrogen concentration in the medium (Fig. 2).

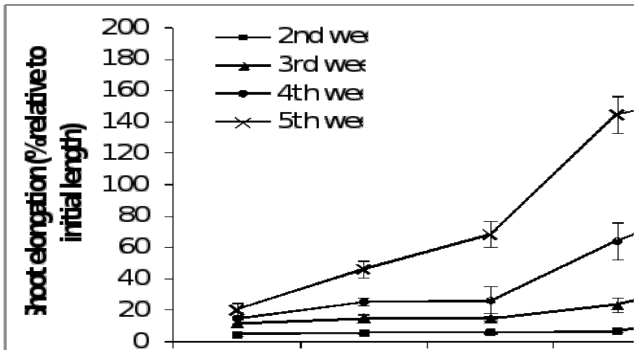

Fig.1. Effect of NH_4^+-N on shoot elongation in *Chara fibrosa*

The N content was increased with the increase of N concentration in the medium. There was a negative correlation between the N concentration in plant and $\delta^{15}N$ values (values are not shown) and in the same way negative correlation was found between nitrogen concentration of medium and $\delta^{15}N$ value in plant ($r^2 = -0.85$, $p < 0.01$). We found a positive correlation between the calculated fractionation factor (ε) and the $\delta^{15}N$ value of plant ($r^2 = 0.924$, $p < 0.001$). The effect of nitrogen on $\delta^{15}N$ values are shown in Fig. 3. There was a significant effect on NH_4-N on fractionation of nitrogen isotopes in plants. In control treatment all plant parts showed the highest positive value of $\delta^{15}N$.

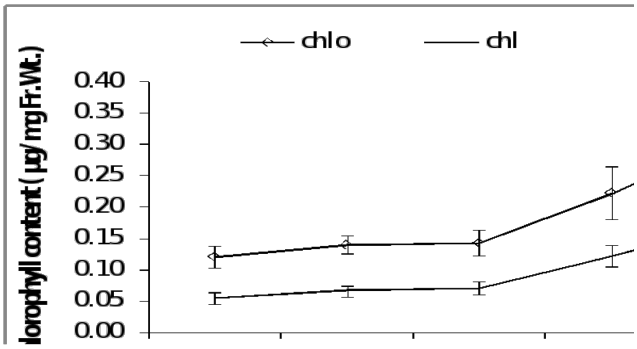

Fig. 2. Effect of NH_4^+-N on chlorophyll content and Fv/Fm value in *Chara fibrosa*

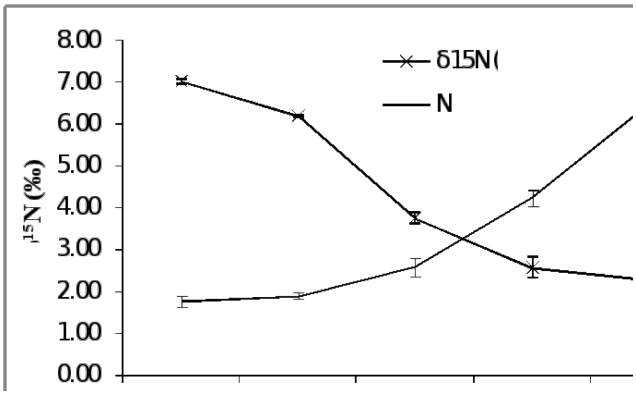

Fig. 3. Effect of NH_4^+-N on nitrogen uptake and fractionation in *Chara fibrosa*

Ammonium is an important nitrogen sources for plant growth and the preference for a particular ion is an important factor affecting plant community composition . Ammonium nitrogen can cause toxicity symptoms in plants as NH_4^+ is [the] exclusive N source. The assimilation of NH_4^+ has lower energy costs, however, many plant species show reduced growth under strict NH_4^+ nutrition and develop NH_4^+ toxicity syndrome, which is associated with an accumulation of NH_4^+ in tissues . In this study, *Chara fibrosa* grew well in 10 ppm NH_4^+-N. Litav and Lehrer, showed that almost all leaves of *Potamogeton lucens* rotted and died at NH_4^+ concentration of 3 mM. Many NH_4^+-N tolerant plant species have been identified by

researchers. Several species of duckweeds (e.g., *Lemna gibba*) , tropical wetland varieties of rice (e.g., *Oryza sativa*) and the wetland species common reed (*Phragmites australis*) and *Sesbania natans* are NH_4^+-N tolerant. We also found that *Chara fibrosas* can tolerate a high concentration of ammonium nitrogen and it was grown well in 10 ppm NH_4^+-N. The concentrations of nitrogen at which the symptoms of toxicity appear differ widely among plant species. Sensitive species can show chlorosis of leaves and suppression of growth at external NH_4^+ concentrations above 0.1–0.5 mM. For example, the growth rate of the floating aquatic fern *Azolla filiculoides* decreased and the plants had root damage when grown in polluted water with a concentration of NH_4^+ above 0.1 mM . However, duckweed (*Lemna minor*) grew well at concentrations of NH_4^+ as high as 4 mM . Litav and Lehrer, also showed that almost all leaves of *Potamogeton lucens* rotted and died at NH_4^+ concentration of 3 mM. When *Chara* was cultured with tip cuttings growth was increased in higher. The concentration of chlorophyll a and b was also increased in higher concentration of $NH4^+$-N. So, it can be concluded that *Chara fibrosa* can grow a high concentration of ammonium.

NH_3 is lost by stomata at high concentration of nitrogen in NH_4^+-fed plants which may favor the enrichment of ^{15}N in plant . Nitrogen isotopic fractionation against ^{15}N was caused by volatilization of NH_3 in the in plant . For *Oryza sativa* L., the fractionation was dependent on the external NH_4^+ concentration, which ranged from -7.8 to -18 ‰ when the external $NH4^+$ concentrations ranged from 0.4 to 7.2 mM .We found that the $\delta^{15}N$ values were decreased with the increase of N concentration. The $\delta^{15}N$ values in plant were closer to the source $\delta^{15}N$ in low N availability conditions (at low N concentrations). Likewise, when the N concentration increased, the amount of substrate became unlimited and the isotope effect was observed .

The $\delta^{15}N$ values of tissue were inversely correlated with the concentrations of tissue nitrogen.

It can be concluded that there was a significant fractionation was occurred during uptake of N by *Chara fibrosa* which depended on concentration (fractionation increased with the increase of concentration on nitrogen in the medium) and form of nitrogen.

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