NITROGEN UPTAKE AND LEAF NITROGEN CONTENT OF PHRAGMITES JAPONICA

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Plants require nitrogen (N) within the soil to produce high yields. However, the transport mechanism of nutrients from root medium to plant and the response of plant nitrogen tissue to nitrogen uptake have not been identified so far. The experiments in this study were performed in summer of 2005 and 2006 in order to define the mechanism of nutrient uptake and specify the response of plant nutrient content in *Phragmites japonica*.

The results suggest that the mayor part of nitrogen absorption is due to an active uptake process. The influx is subject to negative feedback on high external concentration and reaches a maximum uptake rate after 6-8 hours of exposure. The response of leaf N content to NO_3^- uptake not only proves the uptake process based on plant demand but also appears to be independent with external concentration and uptake rate. The results also imply the fact that plants may have sufficient capacity to store nutrients.

Key Words**:** *nitrate, active uptake, leaf nitrogen content, Phragmites japonica*

1. INTRODUCTION

Plants acquire most of constituents of their tissue from their root environments. Nutrient and water in the soil, in particular, governs plant growth. Nutrient and water are indispensable and interrelated components for producing high yields in plant.

In recent decades, it has been reported that the number of bare bar transformed to vegetated bar has been gradually increasing on Japanese sand-bed rivers. Riparian vegetation, however, is commonly found on sandbar with low moisture, extremely low subsurface water level and a considerable distance from the river edge which suggests limited access to water and nutrients. Moreover, nitrate and other mineral nutrients required for optimal plant growth frequently exist at relatively low concentration in natural soil[1)]. As a result, nutrient-limited growth is the usual state for most organisms of the natural environments. Nevertheless, the vegetation community in fact has still been growing vigorously; the increased vegetated areas are typical evidence of this kind of development. To thrive on these dilute nutrients, it is supposed that plants may possess a specific uptake mechanism system in their root cells.

This article believes that ion fluxes from the soil solution to plant root may result from passive or active forces. Transport is thought of as a passive force, if the flux is driven by energy associated with any concentration and/or pressure gradient. Passive transport occurs by mass flow in which solutes are transported with the convective flow of water from the soil to plant roots. As plant solution concentration of many macronutrients typically is larger than in soil solution, active uptake must have occurred[2)]. Active transport is by definition a process in which energy, provided by respiration is expended for moving ions from a zone of lower to higher electrochemical concentration. The intrinsic difference between passive and active uptake leads to different nutrient concentrations in soil solution. Specifically, passive nutrient uptake does not alter the soil solution concentration, whereas active uptake reduces the nutrient concentration in the soil solution.

In order to inspect the uptake mechanism of plants living on riparian bars, among the perennial

reeds, *P. japonica* is chosen due to a widely distribution in Japanese river zone. *P.japonica* can survive until the following year, has a remarkable ability to adapt and grow in soils containing widely different levels of ions and nutrients [3].

The intimate relationship between *Phragmites spp* and nutrient transport such as nutrient removal are currently being examined more closely[4),5),6),7),8)]. At the same time several mathematical models have been developed to simulate their growth[4),9),10)]. However, within the literature, the mayor part of recent researches related to nutrient-limited growth has not paid attention to nutrient uptake mechanism. Due to the fact that nutrient uptake requirements and thereby the amount of mineral nutrients adsorbed by the roots have been less well quantified, current researches of *Phragmites* spp. are insufficient to account for growth differences attributable to nutrient conditions[11)].

The article aims to (a) to specify the primary mechanism of nutrient uptake of *P. japonica* (b) to assess the response of plant nutrient concentration to nutrient uptake.

2. METHODOLOGY OF EXPERIMENT

Experiments were divided in two parts. At first, passive and active uptake characteristic were examined. Secondly, mechanism of active uptake process and response of plant nitrogen concentration were investigated.

(1) Plant sampling

P.japonica was sampled by removing clods of soil containing the roots of shoot clusters from the Yahagi river twice during both the summer of 2005 and 2006 and then transferred to laboratory. Plants were pretreated with tap water. The plants were separated into multi-shoot clusters. Roots were washed with a phosphorous-free detergent, and rinsed with tap water. The plants were then placed in polyethylene jars containing 2 L of culture solution covered with aluminum foil to protect roots from sunlight.

(2) Plant culture and measure techniques

Of all the mineral elements required by the plant's demand, inorganic nitrogen is needed in the greatest amount and most significantly affected the growth of *P. australis* significantly more than phosphorus[12)]. The ratio of nitrogen and phosphorus (N:P) has been widely used as a indicator factor to determine which nutrient may limit growth. It is also claimed N:P ratio in nutrient source in range of 4 and 14 on weight basis (the equivalent of 10 and 33 on molar basic) produce a maximized growth[12)]. Furthermore Koerselman and Meuleman[13)], strengthened the fact that the N:P ratio less than 14 should indicate nitrogen limitation on community level. In the present study, the N:P ratio on weigh basic in subsurface water results from field observations is smaller than 10, which according to the relationship above would be indicative of nitrogen limitation. As NO_3^- is the major N which plant root uses the most, from now on, NO_3^- is mainly considered in the hydroponic experiment.

a) Experiment on "Active-Passive uptake"

The composition of solution was as follows: K_2HPO_4 0.19mM and KNO_3 0.33mM. Fifteen clusters of *P.japonica* were inserted into fifteen polyethylene jars filled with the culture solution at 22:00 October 20, 2005 exposed to natural weather conditions. After 2 hours acclimation, the process of measurement was started as follows: weight was measured before and after each interval and before and after sampling so that nutrient uptake values could be adjusted by the amount of sample that was taken. Samples of 20-30 mL of solution were taken at 8-hours intervals from 0:00 to 24:00 October 21, 2005.

The culture solutions were not renewed and so in all experiments the nitrate and phosphorus concentration of the medium decreases progressively. In the experiment, the solution were not aerated in order to quantify amount of oxygen was depressed. Dissolved oxygen (DO) was measured at the same time as sampling. Water quality analysis was conducted with ion chromatography (TOA-DKK IA-200). At the end of experiment, roots were thoroughly washed with tap water and then dried for 2 hours at 105^0C. Plants were divided and weighed into root, stem, and leaf.

Passive uptake defined by transpiration was calculated by weighing amount of water change during each interval. Total amount of nutrient uptake was defined by the difference in amount before and after each interval. The amount of passive nutrient uptake was calculated as the product of mean concentration and water's weight change. Remaining uptake was considered active uptake.

Root respiration was defined as oxygen consumption during incubation and calculated from initial and final oxygen concentrations.

b) Experiment on "Active uptake mechanism"

Active uptake was only concerned in this part. Two set levels of supply solution, KNO_3 0.22mM, K_2HPO_4 0.046mM and KNO_3 0.406mM, K_2HPO_4 0.097mM were established. The N:P ratio was 5 so that only N would be a growth-limiting nutrient.

At each level of supply solution, three clusters of plants were pretreated with tap water in 3 days and inserted into three jars filled with the culture solution at 07:00 August 11, 2006 exposed to natural weather conditions. After 1 hour acclimation, the process of measurement was started as follows: every 30 minutes from 8:00 to 17:00, every 1 hour from 17:00 to 20:00 and every 3 hours from 20:00 to 8:00 in next day, samples of 20mL of solution were taken. The culture solutions were renewed every 3 hours. Net uptake is measured by difference between initial and final ion content in the nutrient medium.

Following the water sampling, the 2-3 leaves were harvested every 3 hours, dried in 2 hours at 105^0C and subsequent weighing. They were ground with a mortar and pestle. Leaf nitrogen contents were measured on separate subsample. 5.0 mg dried sample was analyzed by using Portable Colorimeter (HACH DR/890) for total nitrogen (TN). Water quality analysis was conducted with ion chromatography. At the end of experiment, plant was dried and root, stem, and leaf were weighted.

3. RESULTS AND DISCUSSION

(a) Active – Passive uptake

Fig. 1 shows the nutrient concentrations at the beginning and end of the experiment. The final combined NO_2-N and NH_4-N concentration is only 6% of the total reduction in NO_3-N concentration during the course of the experiment, so it is clear that NO_3-N was not merely transformed to another N form but used in plant uptake. The 81.4% reduction in NO_3-N uptake was considerable greater than the 15.6% reduction in PO_4-P uptake, so it can also be concluded that NO_3-N uptake was not limited by P availability.

At first, active and passive forces are examined to find out which can play a key role in uptake process. **Fig. 2a** and **Fig. 2b** shows the passive and active uptake of NO_3^- and PO_4^- during the experiment. Passive uptakes of NO_3^- and PO_4^- for the whole day were only about 1.5% and 11.2% of total uptake respectively. Proportion of passive uptake declines during the 24-hours period as shown in **Fig.2a** and **Fig. 2b**. These evidences support the fact that the transpiration flux or passive uptake is not essential to ion uptake in plants. Le Bot et al.[14] also has recommended the same judgment and stated that it is necessary to dissociate mineral fluxes from water fluxes in developing modeling of plant nutrient uptake. The percentage of transpiration in the first interval in both of NO_3^- and PO_4^- is higher than others which may indicate the previous dry condition in transferring plant clusters from field to

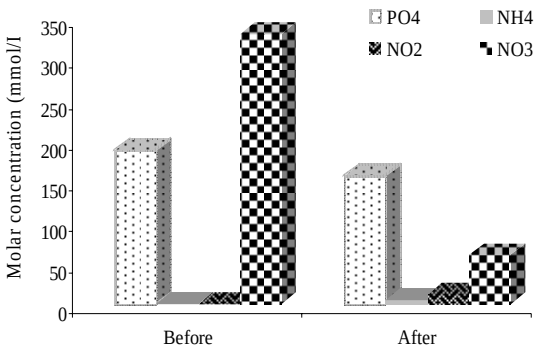

Fig. 1 Concentration of rooting medium

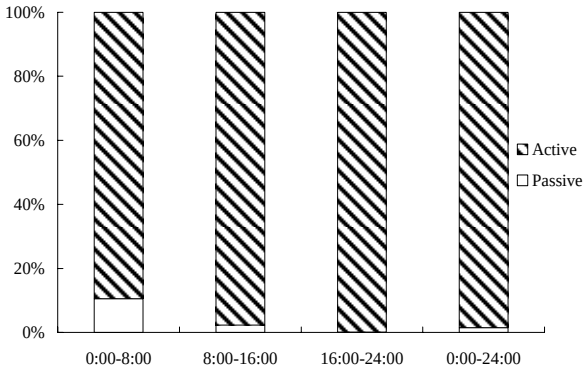

Fig. 2a Active and passive uptake of NO_3^-

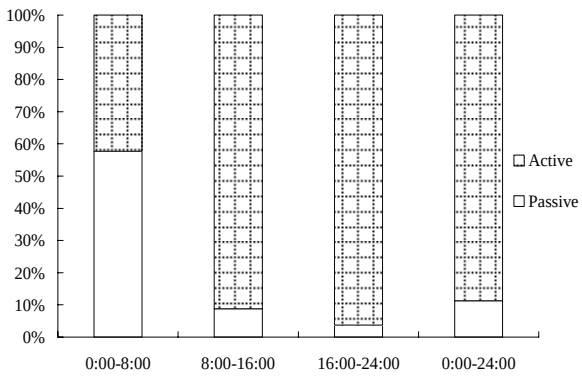

Fig. 2b Active and passive uptake of PO_4^-

laboratory.

It can also be seen that only active process accounts for almost the entire amount of nutrient uptake in plants, in particular in the nighttime (**Fig.2a** and **Fig. 2b**). In order to move ions from external medium to plants, energy which is provided by respiration must be expended by reducing oxygen concentration as showed in **Fig.3**.

It has been indicated that when the NO_3^- and PO_4^- concentration of the cell is higher than the outer medium, active uptake has occurred. During the uptake period until 16:00, NO_3^- active uptake

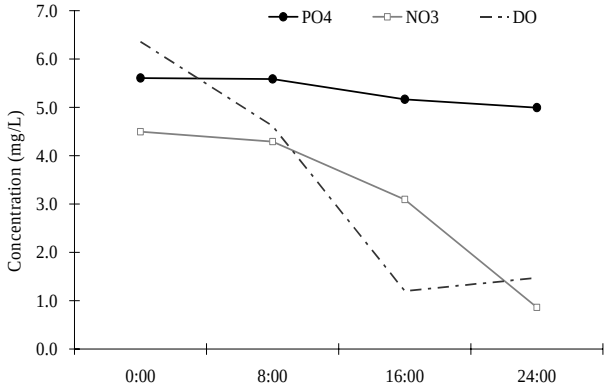

Fig. 3 Changes of concentration in culture solution

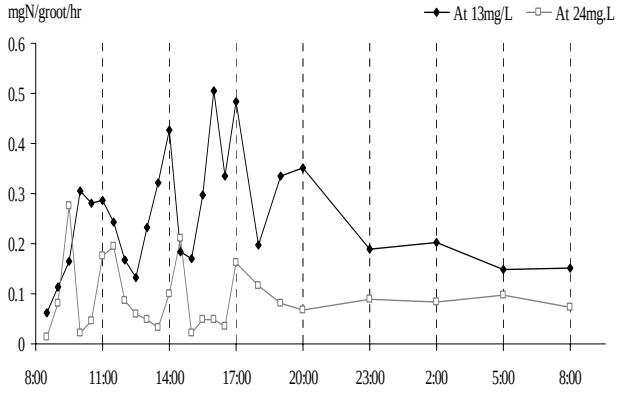

Fig. 4a Mean active uptake rate at each interval time

was directly proportional to root respiration, as represented by DO consumption in two first intervals (**Fig.3**). NO_3^- uptake increased rapidly in time, most of the NO_3^- was removed from solution. Within 24 hours period, however only approximately 1mg/L of PO_4^- was removed from external medium (**Fig.3**). By comparing **Fig.2 a**, **Fig.2b** and **Fig.3**, it has been suggested that plant proceed the active uptake of NO_3^- higher than PO_4^-. Indeed, unlike other ions so far studied, NO_3^- transport system is unique by itself possessing an induced uptake, in part, by contact with the substrate ion [15),16)].

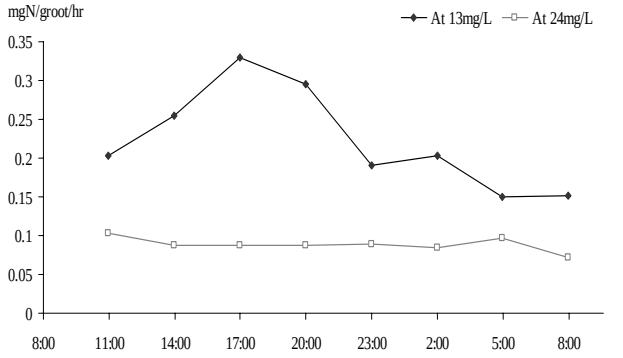

Fig. 4b Mean active uptake rate at every 3 hours

(b) Active uptake mechanism

From the result of the "Active-Passive uptake" experiment, active uptake process greatly contributes to the quantities of nutrient absorbed in plant root. Therefore, from now on, only active uptake was considered.

In **Fig.4a** and **Fig.4b**, the black and grey line represent the active uptake rate of 13mg/L, 24mg/L NO_3^- in culture solution, respectively. **Fig.4a** shows the real uptake rate at each interval time. Meanwhile, **Fig.4b** expresses the mean active uptake at every 3 hours by summing up the active uptake in each batch of culture solution. Siddiqi et al.[16)] suggested that plants which have not been pretreated with NO_3^-, show low rate of NO_3^- uptake. However, following exposure to NO_3^-, NO_3^- uptake increase significantly with time. In this experiment, NO_3^- influx was small in first 1 hour after exposure to NO_3^- (**Fig.4a**). Following that, NO_3^- uptake rate increased for the first 9 hours (at 13mg/L NO_3^- of rooting medium) and 6 hours (at 24mg/L NO_3^- of rooting medium) of exposure and then declined to a relatively steady value, reached after 12 hours.

The time taken to attain maximum uptake rate varied with NO_3^- in rooting medium (**Fig.4a** and **Fig.4b**). The starving conditions created by pre-treating of plant with tap water (NO_3^- is nearly

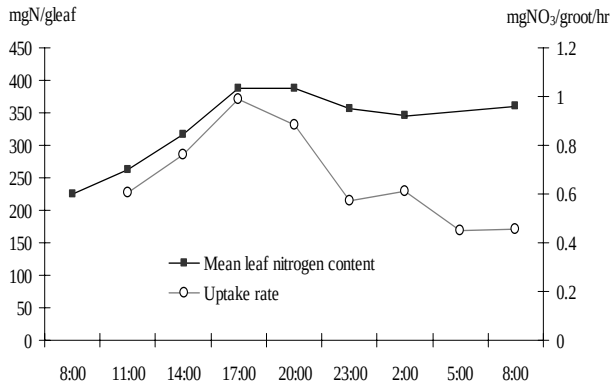

Fig. 5a Mean leaf N content and N uptake at 13 mg/L NO_3^-

zero) in this study caused NO_3^- influx to increase by factor of two-to-fivefold compared with the initial 30-minutes uptake flux (**Fig.4a**).

Maize roots reached maximum uptake rates after 6-8 hours of exposure to solutions of various NO_3^- solutions[15)]. Siddiqi et al.[16)] found in barberly that lower concentrations of NO_3^- used requires longer periods of contact to achieve maximum influx of NO_3^- compared to higher concentration. In the current experiment, at 13mg/L NO_3^- in culture solution, uptake rate reach maximum value after 8 hours of exposure whereas 6 hours of exposure at 24

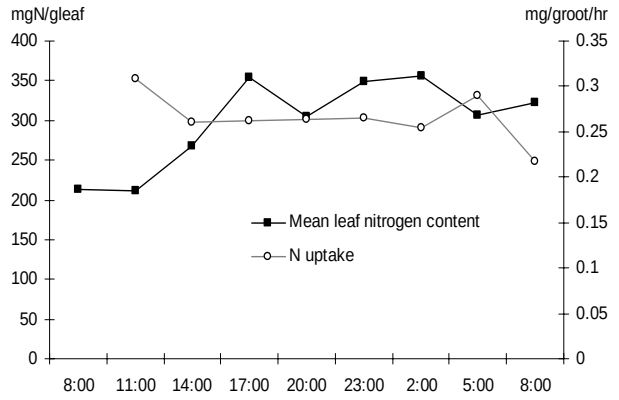

Fig. 5b Mean leaf N content and N uptake at 24mg/L NO_3^-

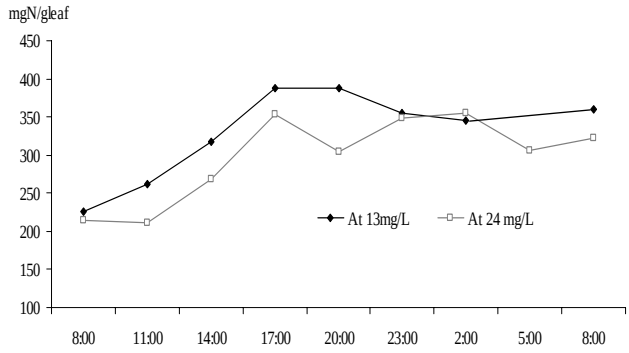

Fig. 6 Mean leaf nitrogen content

mg/L NO_3^- (Fig.4a). In both Fig.4a and Fig.4b, the uptake rate in experiment shows higher value at lower external NO_3^- concentration (13mg/L) than its at higher NO_3^- concentration (24mg/L). Yajnik et al.[17] also asserted the sensitivity of the uptake of NO_3^- to concentration of NO_3^- is high for very low concentration and decreases rapidly as these concentration increases.

Imsandem et al.[1] and Crawford et al.[18] suggested NO_3^- itself acts as the primary signal to regulate uptake processes in overcoming supply deficiencies which is based on total demand regardless of the concentration in the rooting medium. Forde et al.[19], however, believed that the NO_3^- uptake system is stimulated by both the external NO_3^- supply and feedback regulated according to the internal N status of the plant, a means of coordinating uptake rates with the plant's demand for N.

The demand approach to nutrient uptake is based on the assumption that actual plant nutrient uptake is ultimately driven by nutrient demand and luxury consumption term. When plants take up more nutrients than needed for requirements, it is called luxury consumptions of nutrients. Among them, luxury consumption term serves as a symbol of plant nutrient status signal[11]. Even though the demand approach is now widely used to explain the negative correlation of NO_3^- uptake and external NO_3^- concentration but still have not been proven clearly.

However, in the case of 13mg/L of external NO_3^- concentration, within this experiment, a rapid and lengthy induction period (up to the time when influx reach peak value) may result from long starvation condition (3 days) and nutrient deficiency of demand. In other words, a starvation condition results in the absence of satiety signals, so upon exposure to NO_3^-, plants uptake rapidly until satiety is signaled. In contrast, in case of 24mg/L of external NO_3^- concentration, lower rate and shorter induction period was recorded. It may expect in this case that plant N status satiety was identified after 3-6 hours exposure (**Fig.4a** and **Fig.5b**).

Fig.5a and **Fig.5b** show mean leaf nitrogen content and uptake rate in both of 13mg/L and 24mg/L NO_3^- of rooting medium. Siddiqi et al.[16] claimed that plants have not been pretreated with NO_3^-, show low levels of N in plant tissue and low rate of NO_3^- uptake. In this study, prior the exposure to NO_3^-, plants were grown in starving conditions for 3 days (nitrate content is nearly zero). It results in the initial leaf N content at 8:00 which was quite low. During the induction period (up to the time when influx reach peak value), uptake rate was positively correlated with the mean leaf N content and subsequently, uptake rate declined while leaf N content showed little changes at 13mg/L NO_3^- (**Fig.5a**). However, uptake rate does not clearly correlate with the leaf N content at 24mg/L external NO_3^- (**Fig.5b**).

From reviews of hydroponics, it also has been found that starving condition would induce the uptake rate increases for the first period of expose to medium, and then declined after root N concentration has reached a critical value [15),16),18)]. In this experiment, it is supposed that root N content had reached a critical value after 9 hours and 6 hours exposure in 13mg/L and 24mg/L NO_3^- respectively. Even though root N concentration was not monitored in this study, leaf N content can be used to assess the root N content.

Obviously, plant N content was still maintained at relatively steady value when uptake rate decreased (**Fig. 5a**). This is clear evidence for NO_3^- uptake based on their demand. This result may propose that luxury consumption of N occurs in the first duration of exposure which causes an increasing of plant N concentration. Besides, it also implies storage depletion may occur afterwards to maintain steady leaf N content.

Fig.6 gives an interesting point about leaf N content. It shows that leaf N content increase in

luxury consumption period and there seems to maintain steady leaf N content no matter what the variation of uptake rate and rooting medium concentration. This evidence again strongly proves that NO_3^- uptake mechanism based on plant demand. It also supports the fact that the existences of luxury comsumption and storage depletion are real.

4. CONCLUSIONS

The experiment was performed to define the vital mechanism of nutrient uptake of *P. japonica* and specify the response of plant nutrient concentration to nutrient uptake.

The results suggested that active uptake process constitutes the mayor part of nutrient absorbed. It is proposed the fact that plants proceed the active uptake of NO_3^- higher than PO_4^-.

The present results show that in *P.japonica*, NO_3^- influx is subject to negative feedback from higher external concentration and plants reach a maximum uptake rate after 6-8 hours of exposure. The response of leaf N content to NO_3^- uptake in the experiment gives the matter of great interest which not only proves the presence of uptake mechanism based on plant demand but also appear to be independent from rooting medium concentration and uptake rate. The existence of luxury uptake and storage deletion implies that plants must have sufficient capacity to store nutrients.

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