

Nitrogen dynamics of the surfgrass *Phyllospadix iwatensis*

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ABSTRACT: Nitrogen uptake by leaves and roots of the surfgrass *Phyllospadix iwatensis* Makino, growing on the subtidal rocky shore of Akkeshi Bay, Japan, was assessed by experimental measurements for dissolved inorganic nitrogen (DIN) uptake and field measurements for plant biomass and DIN concentrations in both the water column and sediments. Mainly, leaves of *P. iwatensis* contributed to total nitrogen uptake in fall and winter when DIN concentration in the water column was high. Otherwise, contributions of roots were higher than those of leaves in spring and summer when DIN in the water column was low. Both leaves and roots contributed to the nitrogen acquisition. However, the nitrogen uptake from external media would not be enough for nitrogen incorporation in the highly productive season. Internally recycled nitrogen may play an important role in this season.

KEY WORDS: Seagrass · *Phyllospadix iwatensis* · Nitrogen uptake · Retention

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INTRODUCTION

Seagrasses achieve high production and play important roles in the material dynamics of the coastal ecosystems. The high productivity requires high nutrient incorporation, and limited productivity due to shortages of nutrients has been reported in many seagrass populations (Short 1987, Duarte 1990, Bulthuis et al. 1992, Kenworthy & Fouseca 1992, Alcoverro et al. 1997, Udy & Dennison 1997, Lee & Dunton 1999). Seagrasses can use both internal and ambient nitrogen sources. The use of an internal nitrogen source requires the nitrogen translocation from old or storage organs to young growing organs through the developed vascular system. Nitrogen translocation plays an important role in nitrogen economics of terrestrial angiosperm; however, it has been suggested not to be strongly developed in seagrasses (Hemminga et al. 1999). In contrast to terrestrial vascular plants, aquatic vascular plants including seagrasses take up dissolved inorganic nitrogen (DIN) not only from sediment pore-

water, but also from the water column, through below- and aboveground routes, respectively (Iizumi & Hattori 1982, Thursby & Harlin 1982, Short & McRoy 1984, Stapel et al. 1996, Lee & Dunton 1999). DIN uptake rate has been suggested to depend on ambient DIN concentration, and the Michaelis-Menten model has been commonly used to describe DIN uptake kinetics in seagrasses (Thursby & Harlin 1984, Stapel et al. 1996, Pedersen et al. 1997, Terrados & Williams 1997). Nitrogen uptake kinetics are highly variable among species, habitats and organs (Touchette & Burkholder 2000). Due to high ammonium (NH_4^+) concentration in sediment porewater, nitrogen uptake through roots was considered the main pathway of acquiring nitrogen from ambient sources (Short & McRoy 1984, Thursby & Harlin 1984). Nevertheless, it has been suggested that the nitrogen uptake rate through aboveground parts is higher than through belowground parts, and a considerable contribution of aboveground parts to the total nitrogen uptake has been indicated (Touchette & Burkholder 2000). The aboveground

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nitrogen uptake ratio has been reported to contribute 40 to 96% of total nitrogen acquisition in *Zostera marina* L. (Iizumi & Hattori 1982, Pedersen & Borum 1992, Hemminga et al. 1994) and 52% in *Thalassia testudinum* Banks ex König (Lee & Dunton 1999). Particularly, in the case of the surfgrass *Phyllospadix torreyi* Watson, which grows on rocks, belowground nitrogen uptake has not been detected (Terrados & Williams 1997). However, another rocky seagrass, *Amphibolis antarctica* (Labill.) Sander et Aschers ex Aschers, can uptake nitrogen from sediment porewater despite the low uptake kinetics of the belowground parts (Pedersen et al. 1997).

Phyllospadix iwatensis Makino is a surfgrass which is distributed on intertidal and subtidal rocky shores in the North West Pacific (Yabe et al. 1996). *P. iwatensis* grows in dense colonies and forms a thick belowground anaerobic mat with sediments and detritus (Yabe et al. 1996). Generally, in the anaerobic sediment mat, there is abundant NH_4^+ that is a useful nitrogen source for seagrasses. *P. iwatensis* allocates much biomass into belowground parts (Yabe et al. 1995). The belowground parts of *P. iwatensis* are likely to be useful for attachment and nutrient storage as in *P. torreyi*. Moreover, NH_4^+ uptake by belowground parts may play an important role in nitrogen acquisition in *P. iwatensis*.

This study aims to assess the contribution of nitrogen uptake of each part of *Phyllospadix iwatensis* from ambient nitrogen sources, and the translocation of internal nitrogen in *P. iwatensis*. To address these aims, we estimated the seasonal changes of nitrogen uptake and nitrogen requirement (the nitrogen required for growth) of each part of *P. iwatensis* through field and laboratory measurements and experiments.

MATERIALS AND METHODS

Field site and nitrogen environments. Field studies were conducted in a surfgrass bed on the rocky shore of Aikap Cape, Akkeshi Bay, Hokkaido, northern Japan, facing the Pacific Ocean ($43^\circ 00' \text{N}$, $144^\circ 50' \text{E}$, Fig. 1). This site is exposed to high waves. *Phyllospadix iwatensis* formed dense beds in both intertidal and subtidal zones, but this study was only conducted on the population in the highest subtidal zone.

All field research was conducted from November 2000 to October 2002. Water temperature in the study site was taken from the report of Akkeshi Marine Station (AMS), Hokkaido University from 500 m north of the study site (Nakamura et al. 2001, 2003). All sampling was done at low tide of spring tide. Measurements of DIN concentration in the water column and NH_4^+

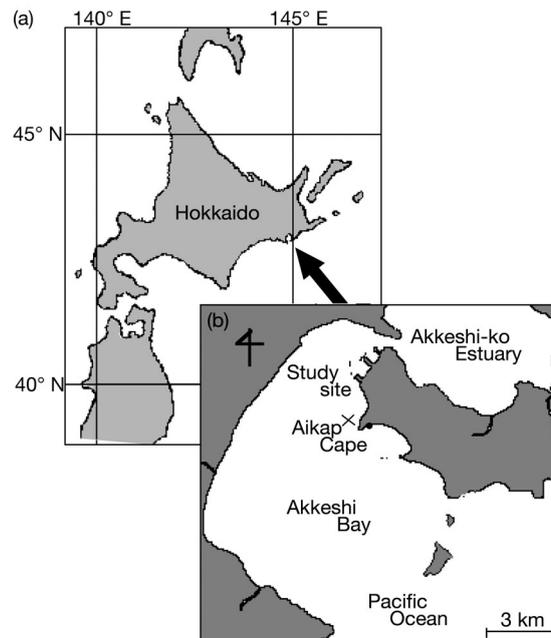


Fig. 1. (a) Map of Hokkaido, Japan. (b) Map of the study region

concentrations in sediment porewater were performed monthly and bimonthly, respectively. Five samples were collected from the surface water at each sampling and stored in ice. Within 2 h of collection, these samples were filtered through glass filters (Whatman GF/C). Sediment was collected with a 10 ml syringe to a depth of 3 cm. Three sediment samples were mixed into 1 centrifugation bottle as a single sample and the porewater was extracted by centrifugation ($3000 \times g$ for 15 min) based on the methods of Dennison et al. (1987). Five samples of the porewater were collected. Ammonium concentration was determined by using the phenol hypochlorite method, immediately within 4 h of collection (Strickland & Parsons 1968). Nitrate + nitrite concentrations of water sample were determined with an autoanalyzer (BRAN+LUEBBE, TRAACS800, Cd-Cu method).

Biomass. Plant biomass was measured from 5 samples almost bimonthly with a 10×10 cm quadrat, including sediment-belowground mat complex. After cleaning, shoot number was counted and the plants were separated into aboveground parts (leaf blade and leaf sheath) and belowground parts (root and rhizome). Ash free dry weight (AFDW) was estimated from the difference between dry weight (desiccation at 80°C for 48 h) and ash weight (combustion at 500°C for 4 h).

Uptake experiments. Ammonium (NH_4^+) and nitrate + nitrite ($\text{NO}_3^- + \text{NO}_2^-$) uptake by aboveground parts of *Phyllospadix iwatensis* and NH_4^+ uptake by belowground parts were measured by the incubation experiments. Although in terrestrial plants $\text{NO}_3^- + \text{NO}_2^-$

uptake by belowground parts is the primary inorganic nitrogen source, belowground parts of seagrasses mainly take up NH_4^+ , because of high NH_4^+ concentration and low $\text{NO}_3^- + \text{NO}_2^-$ concentrations in porewater of anaerobic sediment (Touchette & Burkholder 2000). In this study, $\text{NO}_3^- + \text{NO}_2^-$ uptake by belowground parts was not measured, because of the low ratio of $\text{NO}_3^- + \text{NO}_2^-$ to total DIN concentration in the porewater of this study site (5.9 to 13.5%).

An incubation chamber for aboveground nitrogen uptake experiments was constructed from 50.0 cm long, 3.6 cm diameter cylindrical plexiglass (Fig. 2a). During the incubation, both ends of the cylinder were capped with silicone stoppers. Belowground parts of *Phyllospadix iwatensis* shoot were fixed in 8 ml plastic tubes filled with filtered seawater (Whatman GF/C) by a stopper, and a magnetic stir bar was fixed to another stopper for stirring the water in the plexiglass chamber.

A 100 ml glass flask was used for the belowground uptake experiments (Fig. 2b). To fix the belowground parts in the flask, plants were put through the silicone stopper. The gap between the plant and the hole was filled up with silicone grease.

Plants used in the experiments were collected from the field site about 1 wk before the experiment. The rhizome of each shoot was cut to 3 cm, and incuba-

ted with the sediments in the outdoor tank (1.5 m long, 1.0 m wide, 0.5 m deep), with running seawater (0.6 t h^{-1}) in front of the AMS. A day before the experiments, these plants were moved to the 20 l aquarium filled with filtered seawater, which was aerated and controlled at the same water temperature as the experiment. At this time, the shoots for aboveground experiments were cut to 50 cm and gently cleaned of epiphytes using a wet paper towel.

Uptake experiments were performed in a water bath, in which water temperature was controlled. The chamber for aboveground experiments was placed horizontally in the bath, and the chamber for belowground experiments was placed in a 20 l aquarium filled with filtered seawater sunk in the water bath. From 2 h before and during the experiments, the shoots' light exposure was controlled at about $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ by a cool fluorescent light. The chamber for the belowground experiments was covered by an aluminium film.

The uptake rate of DIN was assumed to be represented by a decreasing rate in nutrient concentration in the chamber from the beginning (0 h) to the end of the incubation (2 or 4 h). After the experiments, plants were separated into aboveground and belowground parts, and the AFDW weighed. The uptake rate was standardized by biomass (g AFDW) and time (h).

Since N uptake kinetics show seasonal variation (in *Thalassia testudinum*; Lee & Dunton 1999), uptake experiments were performed in 5 different seasons: February (0°C), March (3°C), May (8°C), August (18°C) and October (13°C) 2001. The experiments were also conducted with 5 or 6 different grades of DIN concentrations. DIN concentrations were adjusted by adding small amounts of concentrated NH_4Cl or KNO_3 solution. NH_4^+ and $\text{NO}_3^- + \text{NO}_2^-$ uptakes by aboveground parts were measured at initial NH_4^+ and $\text{NO}_3^- + \text{NO}_2^-$ concentrations of 2, 5, 10, 20 and $40 \mu\text{M}$. NH_4^+ uptake by belowground parts was measured at initial NH_4^+ concentrations of 20, 50, 80, 100, 150 and $200 \mu\text{M}$.

Uptake rates were also plotted against the respective nutrient concentration and fitted to the Michaelis-Menten model $V = V_{\text{max}} \times S / (S + K_m)$, where V is uptake rate, S nutrient concentration, V_{max} maximum uptake rate, and K_m the half saturation constant using non-linear least square regression (Stapel et al. 1996, Pedersen et al. 1997). This model is commonly used to describe nutrient uptake kinetics of aquatic plants. Hanes-Woolf plots of uptake rates were illustrated to examine the application of the Michaelis-Menten model (Lee & Dunton 1999).

Estimation of nitrogen acquisition. Daily nitrogen acquisition ($\mu\text{g N shoot}^{-1} \text{ d}^{-1}$) by above- and belowground parts of the surfgrass was estimated from nitrogen uptake rate and above- or belowground biomass

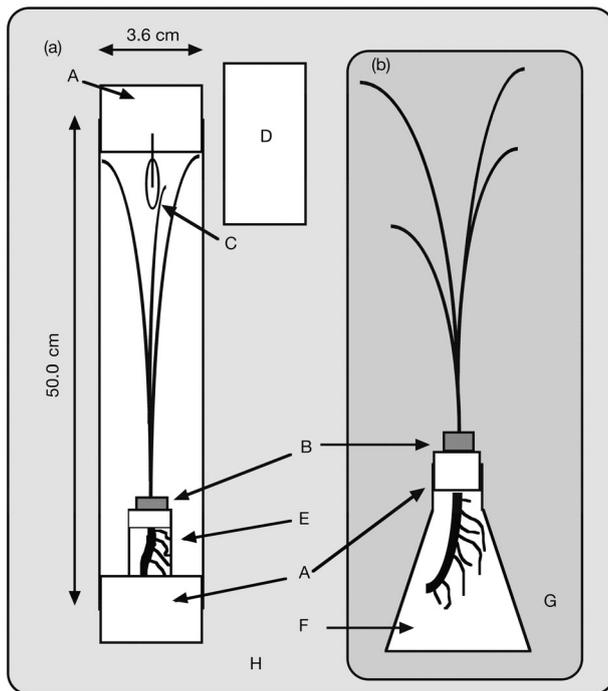


Fig. 2. Uptake experiment chambers. (a) Chamber for DIN uptake experiments by the aboveground part of *Phyllospadix iwatensis*. (b) Chamber for NH_4^+ uptake experiments by the belowground part of *P. iwatensis*. A: silicone stopper; B: silicone grease; C: magnetic stirring bar; D: magnetic stirrer; E: 8 ml tube; F: 100 ml flask; G: aquarium; H: water bath

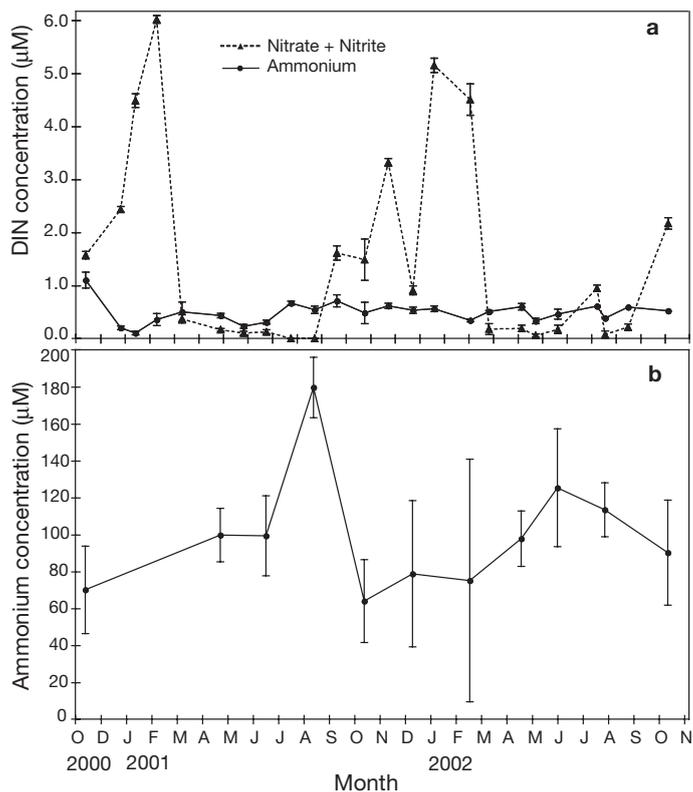


Fig. 3. (a) Water column NH_4^+ and $\text{NO}_3^- + \text{NO}_2^-$ concentrations, and (b) porewater NH_4^+ at Aikap Cape. Vertical bars represent ± 1 SD ($n = 4$ or 5)

(Lee & Dunton 1999). Parameters in uptake kinetics for each season were applied to the calculations of monthly nitrogen uptake rates at ambient DIN concentration. In particular, porewater NH_4^+ concentration in February 2002 was applied to the estimation of the belowground nitrogen acquisition in January 2001. The contribution of nitrogen uptake by each part of the surfgrass to total nitrogen acquisition was regressed on DIN concentration. The significance of regressions was tested after the contribution data was arcsine-transformed.

Estimating nitrogen requirement. Aboveground production ($\mu\text{g DW shoot}^{-1} \text{d}^{-1}$) and nitrogen requirement ($\mu\text{g N shoot}^{-1} \text{d}^{-1}$) were estimated respectively from multiplying the leaf growth rate of shoots ($\text{cm}^2 \text{shoot}^{-1} \text{d}^{-1}$) by specific leaf weight (g DW cm^{-2}), and aboveground production by nitrogen content in the youngest leaf (% DW). Leaf growth rate of a shoot was measured from the leaf length changes during the incubation experiments (18 to 25 d, 20 to 30 shoots) in the outdoor aquarium. The experiments were carried out 7 times between March 2001 and February 2002. Surfgrasses with sediments collected from the study site were used for the experiments. Specific weight and nitrogen content in the leaf were measured for the surfgrasses collected monthly from January 2001 to December 2001. Specific leaf

weight was determined by dividing the dry weight by the total leaf area of a shoot ($n = 15$). Nitrogen content was measured using a CHN analyzer (Yanaco, MT-5) after drying at 80°C for 48 h ($n = 5$). Specific leaf weight and nitrogen content in each month were applied to the monthly calculation.

Belowground production ($\mu\text{g DW shoot}^{-1} \text{d}^{-1}$) and nitrogen requirement ($\mu\text{g N shoot}^{-1} \text{d}^{-1}$) were estimated by the method of Pedersen & Borum (1993) in the incubation experiment from September to October 2001. In *Phyllospadix* spp., a rhizome segment is produced with every new leaf production (Short & Duarte 2001). Based on this relationship, belowground production was calculated as the product of average rhizome internode production (reciprocal number of leaf plastochrone interval, d^{-1}) and weight of full-grown rhizome internodes with roots ($\mu\text{g DW internode}^{-1}$). The belowground nitrogen requirement was calculated as the production multiplied by the nitrogen content of the rhizome with roots (% DW). Weight and nitrogen content of rhizome internode with roots were determined at the third youngest internode and the youngest rhizome internode, respectively. The ratio of shoot nitrogen requirement in above- and belowground parts to the aboveground part in this experiment was applied in estimating the shoot nitrogen requirement in other incubation experiments.

RESULTS

Environmental nutrients

Monthly averaged water temperature changed seasonally from -0.9 to 16.7°C . The NH_4^+ concentration in the water column was less than $1.1 \mu\text{M}$ throughout the

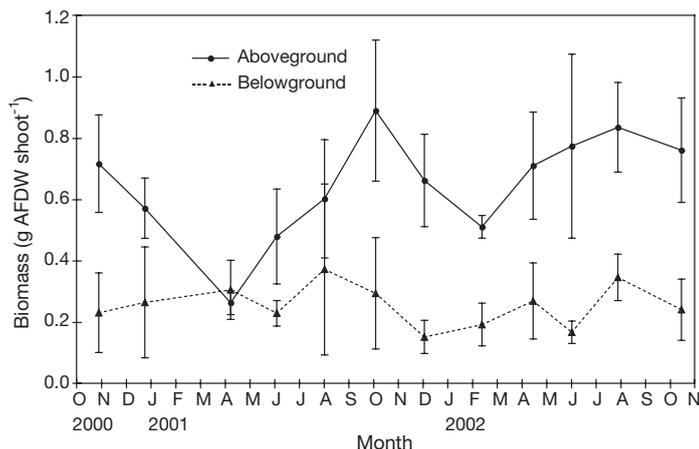


Fig. 4. *Phyllospadix iwatensis*. Seasonal changes in the average weight of aboveground and belowground parts. Vertical bars represent ± 1 SD ($n = 3$ or 5)

year (Fig. 3a). The $\text{NO}_3^- + \text{NO}_2^-$ concentration in the water column changed seasonally from 0 to $6.01 \mu\text{M}$, increased in fall and winter, and remained lower than $0.2 \mu\text{M}$ in spring and summer. The NH_4^+ concentration in sediment porewater varied within a higher range (64 to $180 \mu\text{M}$) than in the water column (Fig. 3a,b). The NH_4^+ concentration in porewater increased in summer. The $\text{NO}_3^- + \text{NO}_2^-$ concentration in porewater ($5.5 \pm 3.1 \mu\text{M}$, $n = 5$) was lower than the NH_4^+ concentration in porewater ($99.3 \pm 39.6 \mu\text{M}$).

Biomass

Aboveground biomass varied from 0.26 to $0.89 \text{ g AFDW shoot}^{-1}$, increasing in summer and decreasing in winter (Fig. 4). From January to April 2001, a drastic decrease occurred due to scouring by drift ice in February 2001. Otherwise, drift ice did not flow into Akkeshi Bay in winter 2002 and there was no damage to surfgrass by ice scouring. Belowground biomass ranged from 0.15 to $0.37 \text{ g AFDW shoot}^{-1}$ and a seasonal trend was not apparent.

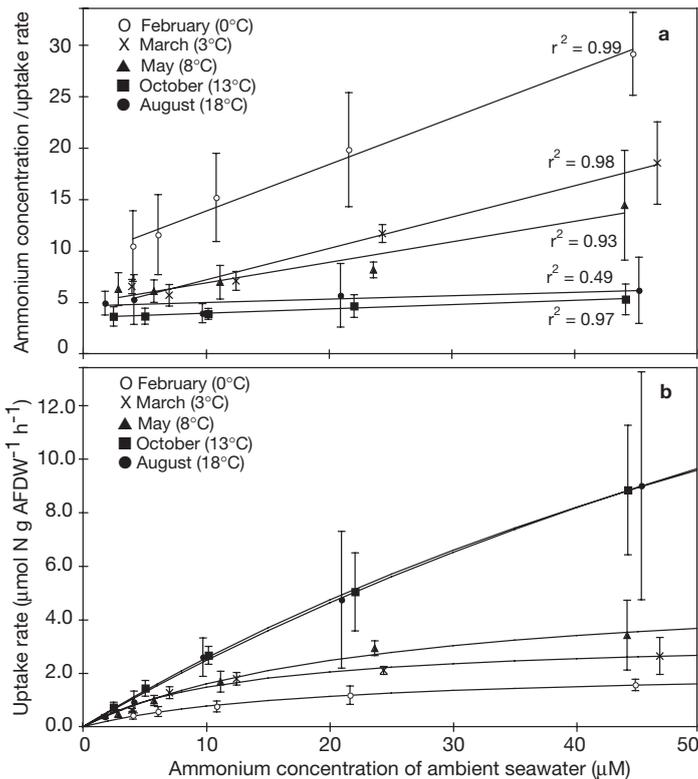


Fig. 5. *Phyllospadix iwatensis*. Aboveground NH_4^+ uptake. (a) Hanes-Woolf plots. The regression lines were obtained from each temperature. (b) Uptake rates at different NH_4^+ concentrations of ambient seawater. The curves represent the best fits of the Michaelis-Menten models obtained from each temperature. Vertical bars represent $\pm 1 \text{ SD}$ ($n = 3$ or 4)

Uptake experiments

On aboveground NH_4^+ and $\text{NO}_3^- + \text{NO}_2^-$ uptake, the regression coefficients of the Hanes-Woolf plot were high ($r^2 > 0.92$) and significant ($p < 0.01$), except for the aboveground NH_4^+ uptake experiment in August (18°C) ($r^2 > 0.49$, $p = 0.19$) (Figs. 5a & 6a). The Michaelis-Menten model was applied to the aboveground DIN uptake (Figs. 5b & 6b). However, aboveground NH_4^+ uptake rates in October (13°C) and August (18°C) were not saturated within the range of NH_4^+ concentrations used in the experiments. The saturation rate of aboveground NH_4^+ uptake increased with water temperature from 2.2 to $35.5 \mu\text{mol g AFDW}^{-1} \text{ h}^{-1}$ (Table 1). The saturation $\text{NO}_3^- + \text{NO}_2^-$ uptake rate in the aboveground parts increased slightly with water temperature, and it was lower than aboveground NH_4^+ uptake rate (Table 1). For belowground parts, NH_4^+ uptake in February (0°C) was significant as shown by linear regression of the Hanes-Woolf plot ($r^2 = 0.90$, $p < 0.01$) (Fig. 7a). The Michaelis-Menten model was, therefore, applied to the belowground NH_4^+ uptake rate in February (0°C) in the same way as

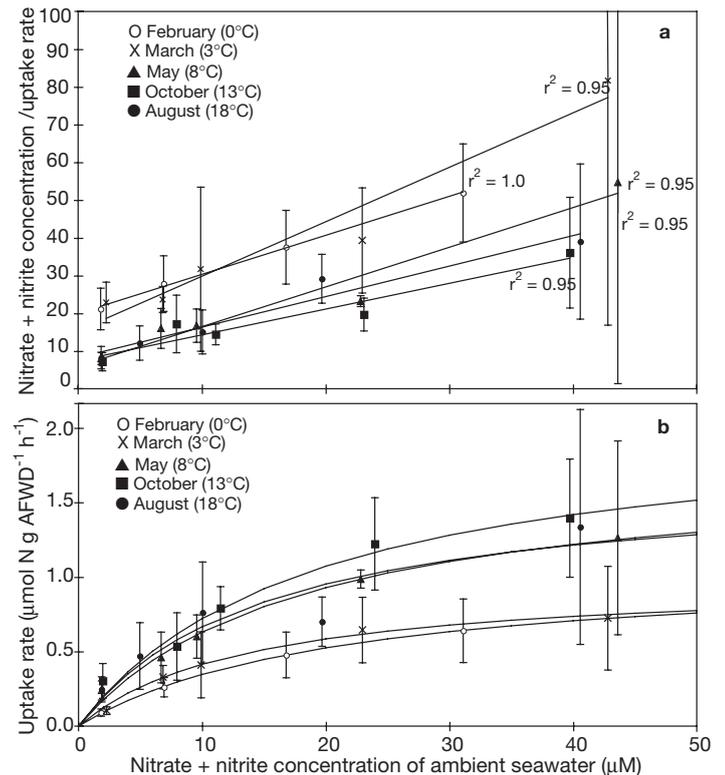


Fig. 6. *Phyllospadix iwatensis*. Aboveground $\text{NO}_3^- + \text{NO}_2^-$ uptake. (a) Hanes-Woolf plots. The regression lines were obtained from each temperature. (b) Uptake rates at different $\text{NO}_3^- + \text{NO}_2^-$ concentrations of ambient seawater. The curves represent the best fits of the Michaelis-Menten models obtained from each temperature. Vertical bars represent $\pm 1 \text{ SD}$ ($n = 3$ or 4)

Table 1. *Phyllospadix iwatensis*. Parameters of Michaelis-Menten model for nitrogen uptake. V_{\max} : maximum uptake rate; K_m : half-saturation constant; α : uptake affinity (V_{\max}/K_m)

	V_{\max} ($\mu\text{mol N g AFDW}^{-1} \text{ h}^{-1}$)	K_m	α (μM)	n (V_{\max}/K_m)	r^2
Aboveground NH_4^+ uptake					
February (0°C)	2.2	18.7	0.12	5	1.00
March (3°C)	3.3	12.7	0.26	5	0.97
May (8°C)	5.5	24.1	0.23	5	0.99
October (13°C)	30.6	109.2	0.28	5	1.00
August (18°C)	35.5	133.5	0.27	5	1.00
Aboveground $\text{NO}_3^- + \text{NO}_2^-$ uptake					
February (0°C)	1.1	21.1	0.05	5	1.00
March (3°C)	1.0	13.9	0.07	5	0.99
May (8°C)	1.8	18.1	0.10	5	0.99
October (13°C)	2.1	19.0	0.11	5	0.97
August (18°C)	1.7	15.1	0.11	5	0.86
Belowground NH_4^+ uptake					
February (0°C)	0.5	61.3	0.01	6	0.82

the aboveground DIN uptake rate (Fig. 7b, Table 1). However, the regression in March (3°C), May (8°C), October (13°C) and August (18°C) was not significant ($r^2 = 0.50, 0.15, 0.63$ and 0.02 , respectively, $p > 0.05$) (Fig. 7a), and the Michaelis-Menten model was not applied to the belowground NH_4^+ uptake in these seasons. The belowground NH_4^+ uptake rate in March (3°C), May (8°C), October (13°C) and August (18°C) increased with NH_4^+ concentration and saturated twice (Fig. 7b). Moreover, belowground NH_4^+ uptake kinetics increased with water temperature (0 to 18°C). With the exception of the uptake rate in February (0°C), field belowground NH_4^+ uptake rate was estimated from the linear relationship between NH_4^+ concentration and NH_4^+ uptake rate

in experiments at just above and below the field NH_4^+ concentration.

Nitrogen acquisition

Daily total nitrogen acquisition ranged from 57.3 to 250.2 $\mu\text{g N shoot}^{-1} \text{ d}^{-1}$ and average acquisition was $108.2 \pm 57.3 \mu\text{g N shoot}^{-1} \text{ d}^{-1}$ (Fig. 8). Nitrogen acquisition from water column NH_4^+ ranged from 2.2 to 73.8 $\mu\text{g N shoot}^{-1} \text{ d}^{-1}$, and seasonal fluctuation was not observed. Aboveground acquisition from water column $\text{NO}_3^- + \text{NO}_2^-$ ranged from 0 to 55.0 $\mu\text{g N}^{-1} \text{ shoot}^{-1} \text{ d}^{-1}$, and increased in fall and winter, but was very low in spring and summer. Belowground nitrogen acquisition from porewater NH_4^+ was highly variable, ranging from 13.9 to 221.4 $\mu\text{g N shoot}^{-1} \text{ d}^{-1}$. The contribution of aboveground nitrogen acquisition from water column NH_4^+ ranged from 3.5 to 47.4% of total nitrogen acquisition, but was not seasonal (Fig. 9). The contribution of aboveground acquisition from water column $\text{NO}_3^- + \text{NO}_2^-$ clearly shows seasonal fluctuation, being high (24.9 to 58.6%) in fall and winter but low (0 to 5.3%) in spring and summer. In contrast, belowground nitrogen uptake from porewater contributed greatly in spring and summer (57.2 to 88.5%), but the contribution decreased in fall and winter (22.1 to 38.9%). The contribution of each nitrogen acquisition was correlated with each nutrient concentration (Fig. 10). Furthermore, contribution of aboveground $\text{NO}_3^- + \text{NO}_2^-$ uptake and belowground NH_4^+ uptake decreased with increasing NH_4^+ in porewater and with increasing $\text{NO}_3^- + \text{NO}_2^-$ in the water column, respectively.

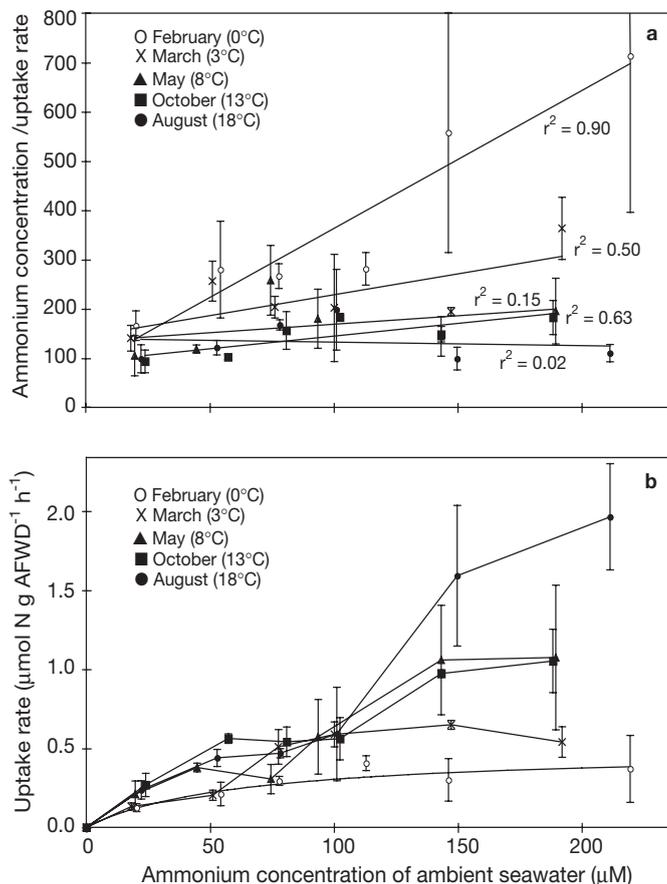


Fig. 7. *Phyllospadix iwatensis*. Belowground NH_4^+ uptake. (a) Hanes-Woolf plots. The regression lines were obtained from each temperature. (b) Uptake rates at different NH_4^+ concentrations of ambient seawater. The curves represent the best fits of the models obtained from each temperature. Vertical bars represent ± 1 SD ($n = 3$ or 4)

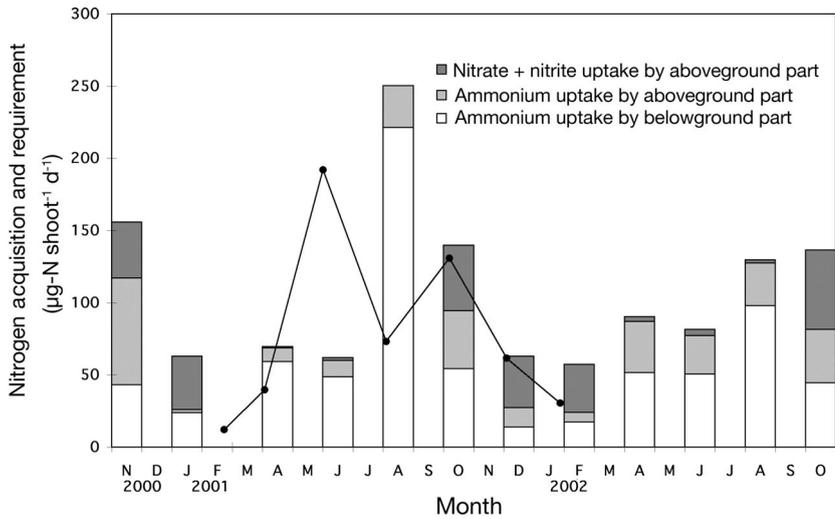


Fig. 8. *Phyllospadix iwatensis*. Seasonal changes in nitrogen acquisition and requirement. Bars represent nitrogen acquisition. Solid circles represent nitrogen incorporations

Nitrogen requirement

Leaf growth rate reached a maximum ($0.97 \pm 0.20 \text{ cm}^2 \text{ shoot}^{-1} \text{ d}^{-1}$) in the incubation experiment between May and June, and a minimum ($0.05 \pm 0.03 \text{ cm}^2 \text{ shoot}^{-1} \text{ d}^{-1}$) between February and March (Fig. 11). Specific leaf weight ranged from $638 \pm 73 \text{ } \mu\text{g DW cm}^{-2}$ in December to $1019 \pm 40 \text{ } \mu\text{g DW cm}^{-2}$ in June. Aboveground production showed seasonal fluctuation, ranging from $409 \text{ } \mu\text{g DW shoot}^{-1} \text{ d}^{-1}$ between February and March to $9919 \text{ } \mu\text{g DW shoot}^{-1} \text{ d}^{-1}$ between May and June. The nitrogen concentration in the youngest leaf was more than 2% DW during winter and spring but rapidly decreased after May and reached a minimum (1.26% DW) in

DISCUSSION

Many seagrass species can take up nitrogen through both leaves and roots (Iizumi & Hattori 1982, Thursby & Harlin 1982, 1984, Short & McRoy 1984, Stapel et al. 1996). Nevertheless, it is known that most of the nitrogen acquired by surfgrass must be channeled through the leaves, while surfgrass roots function primarily in attachment. This is due to the poor nitrogen source around the roots as well as to undetected root nitrogen uptake (Terrados & Williams 1997). However, in some *Phyllospadix iwatensis* beds, plants trapped sediments and reformed their habitat to a sedimentary one, even on high-energy rocky shores (Yabe et al. 1996). The present study shows that the *P. iwatensis* bed in Akkeshi Bay had nitrogen-rich sediment around the roots. Furthermore, nitrogen uptake kinetics of both leaves and roots were detected.

Nitrogen uptake by *Phyllospadix iwatensis* varied according to season (Figs. 5 to 7). Lee & Dunton (1999) also reported seasonal changes of nitrogen uptake kinetics in the turtlegrass *Thalassia testudinum*. Topinka (1978) observed temperature-dependent NH_4^- and NO_3^- uptake by brown algae. Touchette & Burkholder (2000) revealed that glutamine synthetase activity in the eelgrass *Zostera marina* L. is influenced by water temperature. Water temperature is likely to be one of the important factors involved in the observed seasonal change of nitrogen uptake by *P. iwatensis*. Moreover, the direct or indirect connection between the photosynthetic and nitrogen assimilation suggests that seasonal changes in light conditions also affect nitrogen

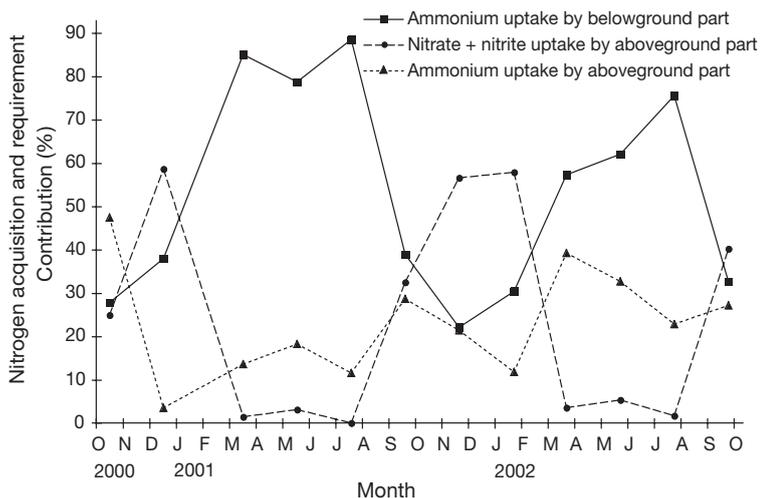


Fig. 9. *Phyllospadix iwatensis*. Seasonal changes in the contributions of each source of nitrogen acquisition to total nitrogen acquisition

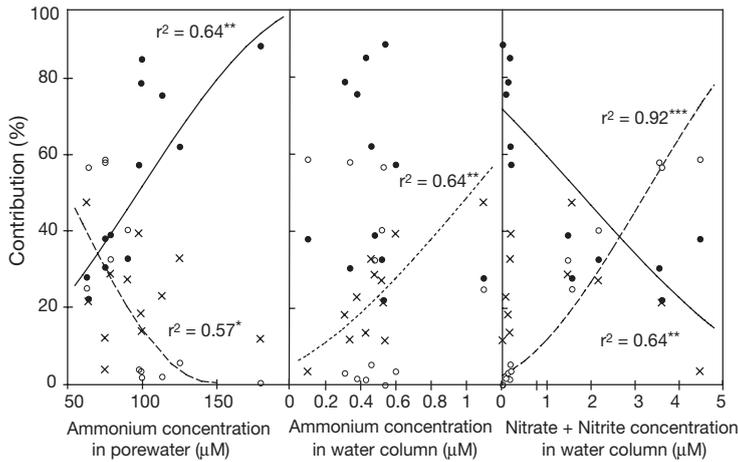


Fig. 10. *Phyllospadix iwatensis*. Correlation between the contribution of nitrogen uptake by each part to total nitrogen acquisition and DIN concentration. ●: NH_4^+ uptake by belowground part; ○: NH_4^+ uptake by aboveground part; ×: $\text{NO}_3^- + \text{NO}_2^-$ uptake by the aboveground part. Solid lines: significant regressions on NH_4^+ uptake by the belowground part; dotted line: NH_4^+ uptake by the aboveground part; dashed lines: $\text{NO}_3^- + \text{NO}_2^-$ uptake by aboveground part (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

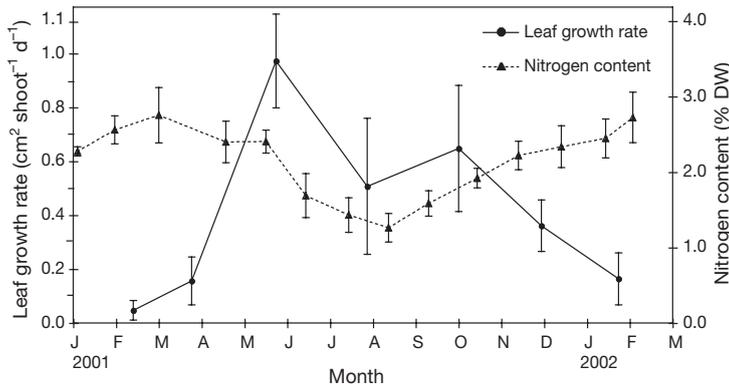


Fig. 11. *Phyllospadix iwatensis*. Seasonal changes in leaf growth rate and nitrogen content in the youngest leaf tissue. Vertical bars represent ± 1 SD (elongation rate: $n = 20$ to 30 , nitrogen content: $n = 5$)

uptake. However, there is no evidence for light conditions affecting nitrogen uptake in seagrass species. In *Z. marina*, diurnal variation has not been observed for nitrogen uptake (Iizumi & Hattori 1982), and high nitrate reduced activity was maintained even during dark periods (Touchette & Burkholder 2000). These reports suggest that short-term changes in light conditions (such as diurnal variation) do not affect seagrass nitrogen uptake, and that the light conditions in the experiments of this study (kept uniform at $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in all experiments) would affect neither the measurement of nitrogen uptake kinetics nor the estimated nitrogen budget of *P. iwatensis*.

Both aboveground leaves and belowground roots of *Phyllospadix iwatensis* can function as nitrogen uptake organs; however, their contribution to nitrogen acqui-

sition from each nitrogen source changed seasonally (Fig. 9). During spring and summer, $\text{NO}_3^- + \text{NO}_2^-$ concentration in the water column was very low ($< 0.2 \mu\text{M}$), but NH_4^+ concentration in sediment porewater was high ($> 100 \mu\text{M}$). Thus, nitrogen acquisition from water column $\text{NO}_3^- + \text{NO}_2^-$ accounted for only a small percentage of total nitrogen acquisition. Porewater NH_4^+ uptake by belowground parts was the main pathway from ambient nitrogen sources (57 to 89%). In temperate coastal marine environments, including this study site, DIN concentration in the water column usually remains low after a spring bloom of phytoplankton. During this low nutrient season, seagrasses, including *P. iwatensis*, are at a greater advantage than other marine primary producers such as phytoplankton, epiphytes and seaweeds, because of the possibility of nitrogen acquisition from porewater NH_4^+ . In contrast, from fall to winter, $\text{NO}_3^- + \text{NO}_2^-$ concentration in the water column increased to $6 \mu\text{M}$ after loss of water-column stratification; however, the range of NH_4^+ concentration in sediment porewater was lower than in spring and summer. $\text{NO}_3^- + \text{NO}_2^-$ uptake from the water column, therefore, played a more important role in nitrogen acquisition (25 to 59%), and the contribution of belowground nitrogen uptake decreased to about 30%. Zimmerman et al. (1987) showed that the contributions of leaf and root nitrogen uptakes in *Zostera marina* varied as a function of ambient DIN concentration, and Lee & Dunton (1999) demonstrated that contributions of leaf and root to the total nitrogen budget of *Thalassia testudinum* varied seasonally. This study reveals that the contributions of nitrogen acquisition from water column $\text{NO}_3^- + \text{NO}_2^-$ and porewater NH_4^+ changed seasonally, mainly depending on the large seasonal fluctuation of the ambient nitrogen sources (Fig. 10). Otherwise, the contribution of nitrogen acquisition from water column NH_4^+ did not change seasonally, as there was no seasonal fluctuation of NH_4^+ concentration in the water column. Although the NH_4^+ concentration in the water column was $< 1 \mu\text{M}$ in most seasons, water column NH_4^+ was the important ambient nitrogen source since a *P. iwatensis* leaf has a higher uptake affinity for NH_4^+ than for $\text{NO}_3^- + \text{NO}_2^-$. As a nitrogen source, seagrasses prefer NH_4^+ to $\text{NO}_3^- + \text{NO}_2^-$, which is energetically expensive to assimilate (Touchette & Burkholder 2000). Moreover, aquatic plants have been suggested to take advantage of this higher uptake affinity to use the pulsed NH_4^+ in the water column (Pedersen et al. 1997).

The nitrogen requirement in a shoot changed seasonally and was not always synchronized with the

change of nitrogen acquisition (Fig. 8). From spring to summer, the nitrogen requirement increased dramatically depending on the increase of production, yet the nitrogen acquisition from the ambient source did not increase. The nitrogen acquisition in summer could, therefore, not satisfy the shoot nitrogen requirement. Particularly, nitrogen acquisition in a shoot in June 2001 supplied only 25% of the nitrogen requirement for growth of a shoot between May and June 2001, because the aboveground biomass during this season was suppressed by the ice scouring in the winter. In winter, the nitrogen requirement was lower than the nitrogen acquisition from ambient nitrogen. These results suggest that nitrogen is supplied not only from ambient nitrogen uptake but also from another nitrogen source during the high productive season, and that there is extra uptake of nitrogen in the low productive season. The shortage and excess of nitrogen would be offset by the internal nitrogen retention system of seagrass. Nutrient retention is a common phenomenon in vascular plants and the use of an internal nitrogen source would reduce the dependence on nitrogen uptake from ambient sources. Yet, Hemminga et al. (1999) concluded that seagrasses have comparatively poorly developed nutrient conservation strategies, and retranslocation represents only 15% of the nitrogen requirement. Otherwise, it has been suggested that nitrogen translocation accounts for 40% of the nitrogen requirement on an annual basis (e.g. *Posidonia oceanica*; Alcoverro et al. 2000). In *Phyllospadix iwatensis*, nitrogen translocation from an internal source through the retention system could play an important role in the growth of shoots as in *P. oceanica*, particularly in the high productive season (Fig. 12). Excess nitrogen during winter could be stocked in the storage organs such as rhizome and leaf sheath, which have been reported to function as carbon sources (Kuo et al. 1988). *P. iwatensis* has longer leaf longevity (166 d; Hamamoto & Mukai 1999) than other seagrasses. An extended leaf life span is known to be an important mechanism in conserving nutrients and in reducing nutrient demands (Escudero et al. 1992). The long life span allows the seagrass to keep the nitrogen rich leaves made in winter on a shoot until summer and to function as a nitrogen storage organ.

Nitrogen content in plant tissue has been considered an index of plant nutrient status (Touchette & Burkholder 2000). Short (1987) and Duarte (1990) reported that seagrass growth is nitrogen-limited when nitrogen content of leaves is lower than 1.8% DW. Nitrogen content of the youngest leaves of *Phyllospadix iwatensis*, which are generally the most nitrogen-rich, decreased to 1.3% DW (Fig. 11). Nitrogen supply by internal nitrogen translocation would relax the nitrogen limitation of production.

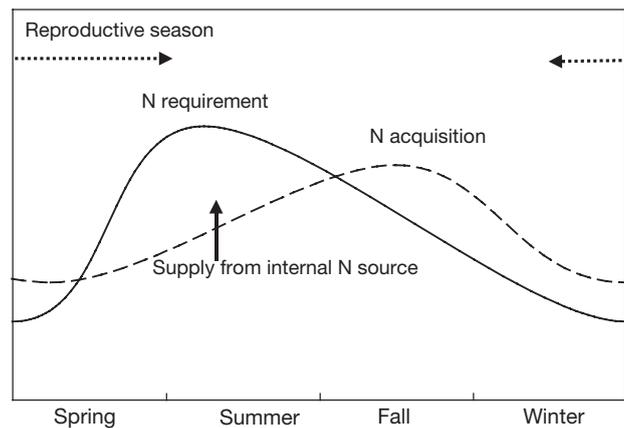


Fig. 12. *Phyllospadix iwatensis*. A model of seasonal change for nitrogen acquisition and requirement

Phyllospadix iwatensis develops the flowering spathe in early spring (Yabe et al. 1995, 1996). *Phyllospadix torreyi* has a higher nitrogen content in the spadix (3.3% DW, cf. Williams 1995) than in the leaf (2.4% DW; cf. Ramirez-Garcia et al. 2002). Flower formation represents a nitrogen sink, and the excess nitrogen in this season could contribute to the development of *P. iwatensis* flowers (Fig. 12).

In conclusion, *Phyllospadix iwatensis* can acquire nitrogen effectively from spatially different ambient sources (water column and porewater), which show different seasonal fluctuations. Seasonal differences between nitrogen demand and acquisition could be offset by the retention system. Differences in the development of belowground nutrient uptake and in the nutrient retention system in seagrass species could be the result of adaptation to coastal ecosystems, which have variable nutrient environments.

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