Annual nitrogen budget of the seagrass *Posidonia oceanica* as determined by *in situ* uptake experiments

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ABSTRACT: The uptake of nitrate and ammonium by the roots and leaves of *Posidonia oceanica* were determined between February 1997 and June 1999 by *in situ* experiments using the isotope 15 of nitrogen (^{15}N) as a tracer in a nutrient-poor coastal zone of the NW Mediterranean Sea (Revellata Bay, Corsica). Nitrate and ammonium leaf uptakes are recorded at 0.05 and 0.1 µM respectively. The high variability observed cannot be explained solely by the variation of the substrate concentrations in the water column. For leaves, mean specific uptake rates were 47 ± 45 and 43 ± 64 µg N g N^{-1} h^{-1}. Nitrate and ammonium leaf uptake fluxes (g N m^{-2} yr^{-1}) seem to have the same importance on an annual basis. Nitrate uptake occurs mainly in winter and early spring, when nitrate concentrations in the water column are highest. The uptake of N, and mainly of ammonium, is significant throughout the year with maxima at the beginning of spring, but it is insufficient to meet the annual N requirement of the plant. *Posidonia* root biomass was very high and corresponded to high specific N uptake rates by the roots. Ammonium was incorporated by the roots 6 times faster than nitrate. In the sediment, this uptake capacity is limited by the nutrient diffusion rate, and the root uptake is therefore insufficient to meet the N requirements of the plant. In fact, *P. oceanica* of Revellata Bay have a complex N budget involving uptake and recycling processes and allowing the plants to meet their N requirements in one of the most nutrient-poor areas of the NW Mediterranean Sea. We calculated that leaf and root would contribute to 40 and 60% of the annual N uptake, respectively, and 60% of the annual N requirement of the plant.

KEY WORDS: Seagrass · Nitrogen uptake · ^{15}N tracer · NW Mediterranean

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INTRODUCTION

Seagrasses are among the most productive marine primary producers per unit area (Duarte & Chiscano 1999). Therefore, the nutrient needs of seagrasses are relatively high, although their nitrogen and phosphorus contents are low compared to other marine producers (Duarte 1990, Stapel et al. 2001). Many seagrass meadows are located in nutrient-poor coastal areas, where seagrasses are good competitors compared to phytoplankton and fast-growing benthic algae (Cloern 2001).

Seagrasses have several mechanisms to satisfy their nutrient requirements. Uptake rates of inorganic N and P are significant even in very nutrient-poor environments (Stapel et al. 1996). Many species have nutrient storage strategies (i.e. uptake of nutrients when available, storage and use of reserves when environmental conditions become adequate for plant growth; e.g. Kraemer & Mazzella 1999, Alcoverro et al. 2000). This strategy allows seagrass species, in particular large species with a long life span, to 'buffer' a scarcity of nutrients in the environment (Marba et al. 1996). Internal recycling (i.e. resorption and remobilisation of nutrients from old leaves to contribute to young leaf growth) is 1 such process used by sea-
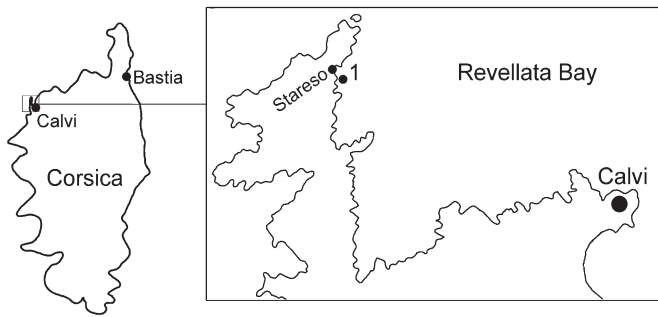
Fig. 1. Location of the experimental site (1) in Revellata Bay (Gulf of Calvi, Corsica, France) at 10 m depth in front of the oceanographic station STARESO

grasses to meet their nutrient requirements (Hemminga et al. 1999).

Some species take up inorganic nitrogen only through their leaves (e.g. *Phyllospadix torreyi*; Terrados & Williams 1997) or their roots. In *Thalassia hemperichii*, roots and leaves are of similar importance in N uptake (Lee & Dunton 1999a). In *Zostera marina*, annual populations show only uptake by the leaves, but perennial populations with greater root biomass have significant root and leaf uptakes (Pedersen & Borum 1993, Hemminga et al. 1994).

Posidonia oceanica is a large seagrass, endemic to the Mediterranean Sea and living in very nutrient-poor areas. It has a very great root biomass, and the ratio between the maximum of root biomass and leaf biomass is the highest among seagrasses (Duarte & Chiscano 1999). To determine an annual N budget for *P. oceanica*, we measured the uptake of nitrate and ammonium by the roots and the leaves of the plant. We performed *in situ* experiments, using the isotope 15 of nitrogen (^{15}N) as a tracer, in one of the most nutrient-poor areas in the NW Mediterranean (Revellata Bay, Calvi, Corsica).

MATERIALS AND METHODS

Study site. All samplings and measurements were done in Revellata Bay (Gulf of Calvi, Corsica, France) near the marine research station STARESO (Fig. 1). The bay opens to the northeast, has a surface area of approximately 245 ha and an average depth of 40 m (maximum about 60 m). The seafloor drops gradually (2% slope) from south to northeast. An extensive *Posidonia oceanica* meadow covers about 180 ha of the sandy seafloor, reaching 38 m depth (Bay 1984). The seagrass bed of Revellata Bay has been studied since the 1970s (e.g. Bay 1984, Dalla Via et al. 1998, Gobert et al. 2001).

The sea surface temperature ranges from 12°C (February-March) to 26°C (August-September) with an annual mean of 18.1°C (STARESO data, Lepoint 2001). The water column is strongly stratified from May to October, with the thermocline around 25 to 30 m depth (range: 20 to 50 m, depending on wind conditions). Water residence time in the bay varies from 5 d in winter to 10 d in summer (Norro 1995).

Uptake measurements. All experiments were carried out *in situ* at 10 m depth (Fig. 1). Nitrate and ammonium uptake by the leaves and roots of the seagrass *Posidonia oceanica* was measured in 1997 and 1998 during February, June and October, and in 1999 from February to June.

Nitrogen uptake by the leaves was measured with an experimental device composed of 2 transparent Plexiglas cylinders and a submerged pump to recirculate the water (about 8 l; Fig. 2). One cylinder was placed vertically on an open base made of PVC and driven into the sediment 24 h before the experiment to isolate a *Posidonia oceanica* shoot. A rubber membrane separated the cylinder into a leaf and a sediment chamber. Experiments were done in duplicate (i.e. addition of same nutrient at same concentration).

To measure uptake by the roots, *Posidonia oceanica* shoots were uprooted 24 h before the experiments and placed in an experimental device consisting of a leaf compartment (transparent Plexiglas bell of 4 l volume) placed on a root compartment (closed dark chamber of PVC, 2 l volume). A rubber membrane separated the 2 compartments, and the pump was connected to the root chamber.

All experiments had a duration of 1 h. The ^{15}N tracers were solutions of ammonium sulphate (99.0% ^{15}N) or sodium nitrate (99.0% ^{15}N) (Eurisotop, France). For

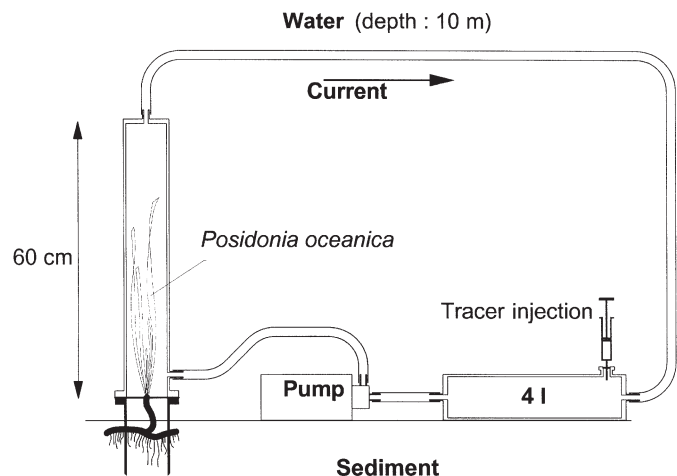Fig. 2. Experimental device used to measure the *in situ* uptake of ^{15}N by leaves

leaf uptake measurements, when the ambient concentrations of ^{15}N were near the detection limits, the tracer was added to a final concentration of about 0.05 µM. In other conditions, we tried to add a minimum of tracer which generally corresponded to 10 to 20% of the initial ambient concentration.

In root uptake measurements, tracer was added to a final concentration representative of pore water concentrations (2 to 4 µM).

Water samples for nutrient concentration measurements were taken from the cylinder before and 5 min after adding the tracer, and at the end of the experiment.

After sampling, the *Posidonia* leaves were scrapped with a razor blade to remove epiphytes. *Posidonia* organs were briefly rinsed with distilled water to remove adsorbed ^{15}N, oven-dried at 60°C for 48 h and weighed.

Samples were finely ground for spectrometric and elemental analysis. These measurements were done in triplicate for leaves, roots and rhizomes from each experiment, using an isotopic ratio mass spectrometer (Optima, Micromass) coupled to a C-N-S elemental analyser (Carlo Erba). Nitrate and ammonium concentrations in water samples were measured colorimetrically with an automated analytical chain (Technicon, or Skalar). Analytical precision was 0.01 µM for nitrate and 0.05 µM for ammonium.

Leaf biomasses in the field were measured during each campaign on the sampling site. Biomasses were calculated on the basis of density and dry weight of the shoots. The density was determined during each campaign using a 40 cm hoop randomly thrown in the meadow. We observed no significant change in density during this study (n = 259, 402 ± 140 shoots m^{-2}). Dry weight of the shoots was measured during each campaign, and monthly in 1999 (10 shoots randomly collected + experimental shoots). Belowground biomass was measured in June 1999 at 12 m depth on 4 samples of mats (30 × 30 × 30 cm). Dead and living material were separated according to the following criteria: connection or absence of connection of the roots and rhizomes to a living shoot with leaves; appearance of roots and rhizomes connected to a living shoot (dead roots and rhizomes are black, often empty or rotten inside; living rhizomes are usually pink inside and living roots are brown).

Dry weight biomass was converted to N biomass using elemental data (monthly averages).

Calculations. Specific uptake rates V (µg N g N^{-1} h^{-1}) and uptake fluxes ρ (µg N m^{-2} h^{-1}) were calculated after Collos (1987):

$$V = \frac{A_f - A_0}{A_d \times t} = \frac{\rho}{biomassN} \qquad (1)$$

where A_f is the final ^{15}N abundance measured in the plant, A_0 is the initial (= natural) ^{15}N abundance in the plant, A_d is the ^{15}N abundance in the dissolved phase at the beginning of the experiment, t is the duration of the experiment, and *biomassN* is the nitrogen biomass measured in the field. The conversion of biomasses (dry weight) to *biomassN* was done using averaged N measurements. In Revellata Bay, the natural abundance (A_0) of ^{15}N in *Posidonia oceanica* shoots was 0.36728 ± 0.0005% ^{15}N (i.e. +2.6‰ in δ notation; Lepoint et al. 2000). The experimental particulate material is considered as enriched in ^{15}N relative to natural ^{15}N concentrations when $A_f - A_0 \geq 0.001\%$ ^{15}N. A_d was considered constant during the experiment (1 h) and calculated according to the isotopic mixing equation:

$$C_d \times A_d = A_{d_0} \times C_{d_0} + A_t \times C_t \qquad (2)$$

where C_d is the concentration of ammonium or nitrate in the dissolved phase after the ^{15}N tracer addition, A_{d_0} and C_{d_0} are the natural ^{15}N abundance and the initial concentration of nitrate or ammonium in the dissolved phase, respectively, and A_t and C_t are the ^{15}N abundance (99.0% ^{15}N) and the concentration of the added tracer, respectively. A_{d_0} was set at 0.37, considering that the importance of natural variation of this term is small when compared to the variations of the tracer concentrations.

RESULTS

Nutrient concentrations

Water column concentrations of NO_3 and NH_4 were very low (Fig. 3). In 1999, a peak of nitrate was recorded at the beginning of spring, related to winter mixing of the water column and an occurrence of NE winds transporting deeper and nutrient-enriched waters from the open sea into the bay (Brohée et al. 1989, Skliris et al. 2001). The concentrations were in the range of values measured in other coastal waters of the NW Mediterranean (e.g. Delgado et al. 1994 on the Catalan coast of Spain, and Kraemer & Mazzella 1999 in Ischia Bay, Italy).

During this study, S. Gobert et al. (unpubl.) measured the concentrations of NO_3 and NH_4 in the pore water of the seagrass meadow. These concentrations were higher than those in the water column (Fig. 3). NH_4 concentrations were always higher than NO_3 concentrations. The concentrations measured in Revellata Bay were in the same range as those measured by Cancemi et al. (2000) at another site of the western Corsican coast, but much lower than pore water concentrations at other coastal sites of the NW Mediterranean (e.g. Lopez et al. 1995, Kraemer & Mazzella
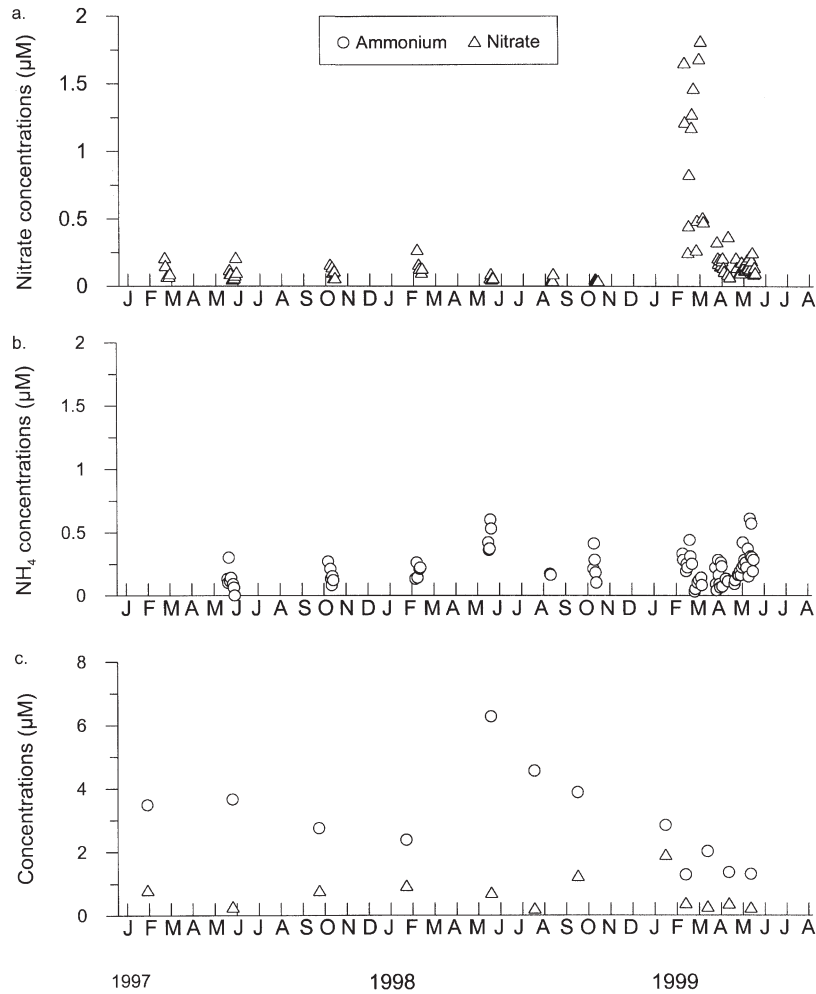
Fig. 3. Nitrate and ammonium concentrations measured in the water column (a,b) and in the pore water (c) of the seagrass bed in Revellata Bay. Water column measurements correspond to the initial samplings made in the experimental chambers used for ^{15}N uptake measurements. Pore water measurements were done by S. Gobert et al. (unpubl.) during our experimental work. Samples were taken using a 60 ml syringe with a 10 cm nonox needle. Concentrations were measured as for water column samples after filtration on GF/C
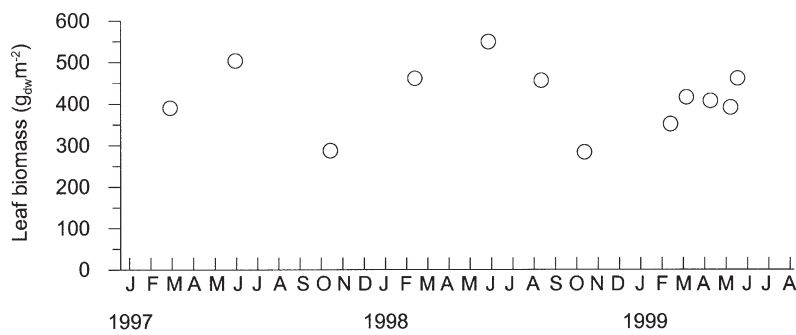

Fig. 4. *Posidonia oceanica.* Monthly averages of the leaf biomasses at 10 m depth

Table 1. *Posidonia oceanica.* Belowground (mean ± SD) and maximum (g dry wt m^{-2}) leaf biomass in Revellata Bay seagrass meadow. Belowground sampling (n = 4) done in June 1999 at 12 m depth (sample volume: 30 × 30 × 30 cm)

Living biomass	Necromass	Total	Maximum leaf biomass	Belowground/leaf biomass ratio
3068 ± 1909	9914 ± 2331	12982 ± 3340	550	5.4

1999). This low level of inorganic nitrogen in the pore water of the sediment along the western Corsican coast is a notable characteristic of the ecosystem, as the sediment is often considered an important source of nutrients for benthic and pelagic primary producers.

Posidonia oceanica leaf and belowground biomasses

Leaf biomass shows a clear seasonal variation with minima in October and maxima in June (Fig. 4). In Revellata Bay, leaf biomass is generally lowest in winter and highest in early summer (Bay 1984) which corresponds to the pattern described for this species (e.g. Alcoverro et al. 1995). The average annual leaf biomass at 10 m depth was $413 \pm 80 \ g_{dw} \ m^{-2}$.

The belowground biomass measured in Revellata Bay is very important (Table 1). In June 1999 the leaf biomass constituted less than one-sixth of the total living biomass. The belowground material consists mainly of dead and living roots of *Posidonia oceanica*. Dead and living rhizomes were only a minor part of the material collected, although the proportion of rhizomes is usually greater than the proportion of roots (Mazzella et al. 1998). Large dead leaf fragments were absent, but leaf decay could be present as detritus in the fine material and sediment filling up the spaces between the roots and rhizomes (Mateo & Romero 1997). The necromass forms two-thirds of the total biomass, which agrees with data from the literature (e.g. Mateo & Romero 1997).

The N contents of the leaves ($mg \ N \ shoot^{-1}$) show a strong seasonal pattern (Fig. 5) corresponding to the pattern described by Alcoverro et al. (1995). Values were lowest in October, and highest in June of 1997 and 1998, and March of 1999.

Relative N concentrations in the leaves (% dry wt) were also highly variable (Fig. 5), with low values in

Table 2. *Posidonia oceanica*. Mean (±SD) specific uptake rates of ammonium and nitrates (V_{NH_4} and V_{NO_3}) by the leaves. Experiments were done in Revellata Bay (Gulf of Calvi, Corsica) at 10 m depth between February 1997 and June 1999. Starting in February 1998, the leaves were classified according to Giraud (1979). n = number of experiments

Type of leaves	V_{NH_4} ($\mu g \ N \ g \ N^{-1} \ h^{-1}$)	n	V_{NO_3} ($\mu g \ N \ g \ N^{-1} \ h^{-1}$)	n
Juveniles (<5 cm)	80 ± 39	4	85 ± 132	8
Intermediates (>5 cm, without ligula)	50 ± 44	22	63 ± 82	30
Adults (>5 cm, with ligula)	59 ± 58	20	76 ± 99	33
All leaves (weighted averaged)	47 ± 45	26	43 ± 64	58

October and high values in February (i.e. before the maxima of leaf biomass and N content).

Uptake experiments

The minimum specific nitrogen uptake rate measured *in situ* in the *Posidonia oceanica* meadow was $10 \ \mu g \ N \ g \ N^{-1} \ h^{-1}$.

In *Posidonia oceanica* leaves, the average specific uptake rate of nitrate V_{NO_3} was not significantly different from that of ammonium V_{NH_4} (Table 2). During the study period, the V_{NO_3} were highest in early spring 1999 ($160 \ \mu g \ N \ g \ N^{-1} \ h^{-1}$) (Fig. 6). In some experiments, particularly in 1997, no NO_3 uptake by leaves was detected.

The V_{NH_4} were generally equal to or lower than the V_{NO_3} (Fig. 6). An uptake of NH_4 by *Posidonia oceanica* leaves was measured in all but 2 experiments (June 1997 and May 1999). The V_{NH_4} were highest in March-April 1999.

From February 1998, the specific uptake rates of the different types of leaves (Giraud 1979) were recorded separately. The average V_{NO_3} of the different leaf types are not significantly different from the V_{NH_4} (Table 2). The V_{NO_3} and V_{NH_4} of juvenile leaves were higher than those of adult and intermediate leaves (Table 2).
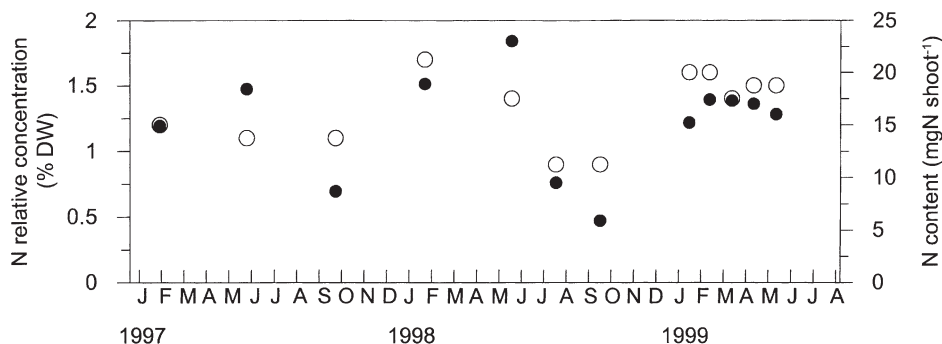

Fig. 5. *Posidonia oceanica.* Monthly averages of the relative N concentrations (●) and the N content (○)
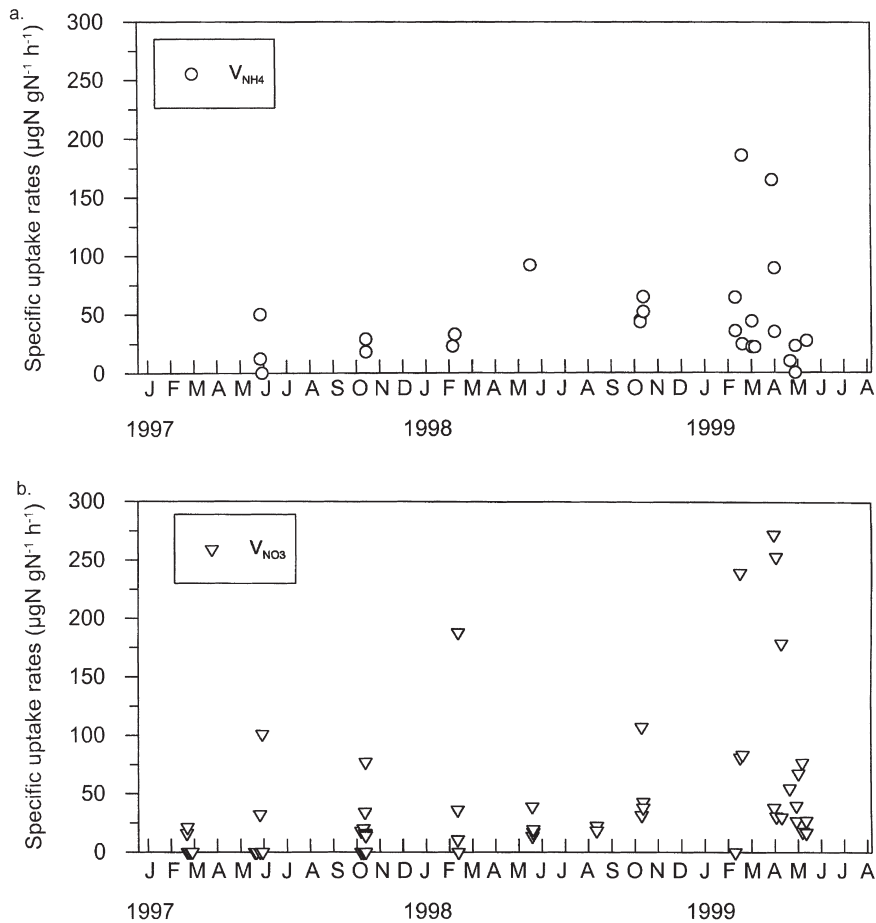
Fig. 6. *Posidonia oceanica.* Specific uptake rates of (a) ammonium and (b) nitrate by the leaves during ^{15}N tracer experiments performed at 10 m depth in Revellata Bay between February 1997 and June 1999. Each value corresponds to 1 experiment

The average specific uptake rates of *Posidonia oceanica* roots are higher than those of the leaves (Table 3). The V_{NH_4} of roots are higher than the V_{NO_3}.

DISCUSSION

The uptake rates measured in *Posidonia oceanica* are of the same order of magnitude as for other sea-grasses (e.g. Pedersen et al. 1997). However, to our knowledge this study is the first attempt to measure the *in situ* uptake rate at ambient substrate concentrations. In nutrient-poor waters, uptake rates at ambient nutrient concentrations are generally calculated from the kinetic parameters of Michaelis-Menten curves (e.g. Stapel et al. 1996, Lee & Dunton 1999a). The ^{15}N method has 2 advantages: firstly, the addition of small amounts of ammonium or nitrate allows measurement of the N uptake at quasi-ambient nutrient concentrations. Secondly, the ^{15}N measurement can be done separately by each of the producers present. It is therefore possible to discriminate between the uptake by the seagrass leaf and that of the epiphytes or other producers (such as phytoplankton), avoiding experimental

artefacts (due to scraping off of epiphytes, or seawater filtration).

However, 2 main methodological errors could affect the measurements of the uptake rates: firstly, uncertainty in the nutrient concentration measurements used to calculate the initial ^{15}N enrichment (A_d) of the dissolved phase (Eq. 2) and, secondly, the isotopic dilution which affects this enrichment during the experiment (i.e. dilution of the dissolved ^{15}N pool by ^{14}NH$_4$

Table 3. *Posidonia oceanica.* Mean (±SD) specific uptake rates of ammonium and nitrate by the leaves and roots. Nutrient concentrations are the mean concentrations measured in the root or in the leaf compartment after the ^{15}N tracer additions. n = number of experiments

		Specific uptake rates (μg N g N^{-1} h^{-1})	Nutrient concentrations (μM)	n
Roots	NH$_4$	484 ± 540	4.9 ± 6.1	20
	NO$_3$	76 ± 70	2.2 ± 2.4	27
Leaves	NH$_4$	47 ± 45	0.2 ± 0.2	26
	NO$_3$	43 ± 64	0.2 ± 0.4	58

produced by remineralisation; see Glibert et al. 1982, Dugdale & Wilkerson 1986). It is difficult to estimate the impact of these methodological biases.

The nutrient uptake by the seagrass leaves is demonstrated for most species, including those settled in nutrient-poor areas (Stapel et al. 1996, Touchette & Burkholder 2000). In this study, the leaf uptakes of NO_3 and NH_4 were recorded at 0.05 and 0.1 µM respectively.

In general, N uptake rates by seagrasses show a Michaelis-Menten relation to the concentration of substrate (Touchette & Burkholder 2000). However, this relation is often established for a large range of concentrations regardless of the ambient concentrations in nitrate or ammonium. In the nutrient concentrations measured in Revellata Bay, such a relation between substrate concentration and uptake rates is very confusing. The variability of measurements is very important, and only the highest uptake rates of nitrate by leaves are correlated to the highest nitrate concentrations in the environment (Figs. 3 & 6). This peak of nitrate is very restricted in time and, consequently, the high variability of uptake rates observed in this study is not explained by the variation of substrate concentrations. Although this variability could result from uncertainty in nutrient concentration measurements, it is more likely a biological reality, demonstrating that other environmental and physiological factors influence nutrient uptake rates (e.g. light availability and photosynthetic rate, temperature, competition with other organisms).

Using the specific uptake rates and the N biomass obtained in the field experiments, we have calculated the uptake fluxes of nitrate and ammonium (µg N m^{-2} h^{-1}) by *Posidonia oceanica* leaves (Fig. 7). Uptake values in individual shoots vary between 0 and 120 µg N shoot^{-1} d^{-1}, which is similar to the uptake fluxes determined by Alcoverro et al. (2000) (0 to 113 µg N shoot^{-1} d^{-1}), from a N balance budget.

These fluxes show a high variability linked to experimental variability in the specific uptake rate and to the seasonality of biomass. For each period of measurement, it was possible to record incorporation of N by *Posidonia oceanica* leaves. This has important effects on the N dynamics of the plant. On one hand, according to the calculations of Alcoverro et al. (1995), the incorporation during winter (a period of low growth and high nitrate) explains the N concentration and content increase during this season, when the leaves of *P. oceanica* act as a storage organ, as in other Medi-
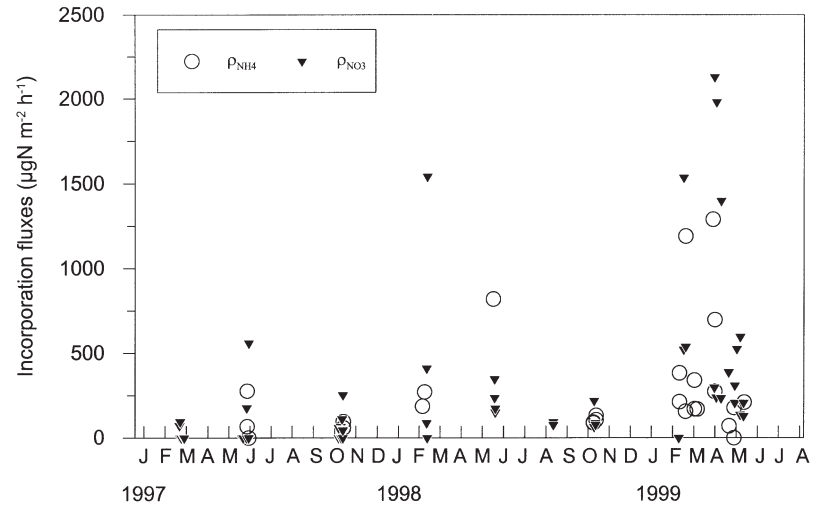

Fig. 7. *Posidonia oceanica.* Uptake fluxes of ammonium and nitrate by the leaves during ^{15}N tracer experiments performed at 10 m depth in Revellata Bay between February 1997 and June 1999. Each value corresponds to 1 experiment

terranean seagrasses such as *Zostera noltii* and *Cymodocea nodosa* (Kraemer & Mazzella 1999). On the other hand, *P. oceanica* leaves take up nutrients all year round, not merely during the nitrate-rich and low-growth period (i.e. winter and early spring).

Between October 1998 and June 1999, a mean increase of the leaf nitrogen content of 11 mg N shoot^{-1} was measured. This quantity must be considered a minimum increase, as leaf loss, grazing and N leaching are not accounted for. The uptake of N by the leaves allows to meet this quantity only if we consider that the maximum uptake rate measured in March-April 1999 is maintained during a long period (some weeks) and if night uptake of nitrate occurs. These maximum uptake rates were only recorded during 1 wk in early spring 1999, and the night uptake of nitrate is small (Lepoint 2001). Leaf uptake is probably insufficient to meet the annual N requirement of *Posidonia oceanica*.

The root uptake of nitrogen is the second process examined in this study. The possession of roots in a nutrient-poor area is a clear competitive advantage compared to non-rooted primary producers (i.e. leaf epiphytes, phytoplankton, benthic macroalgae). Indeed, rooted plants can exploit the sediment as a nutrient source inaccessible to other primary producers (Hemminga 1998). An increase of the root biomass relative to the leaf biomass augments in the uptake capacity by increasing the volume of sediment exploited by the plant and the surface involved in root uptake (Casper & Jackson 1997). However, Hemminga (1998) pointed out that high root biomass increases the oxygen needs and could drastically change the carbon

Table 4. *Posidonia oceanica.* Annual N budget (first method) in Revellata Bay at 10 m depth. Annual N requirement ($g\,N\,m^{-2}\,yr^{-1}$) is calculated from Pergent-Martini et al. (1994) (see details in the text). Annual root and leaf uptakes of ammonium and nitrate ($g\,N\,m^{-2}\,yr^{-1}$) are calculated from ^{15}N uptake and biomass measurements

N annual requirement		9.7
Root uptake	NH_4	26.0
	NO_3	4.1
Leaf uptake	NH_4	1.3
	NO_3	1.2

balance in the plants. Thus, root biomass development is closely related to the photosynthetic activity in the leaves of the plant (Hemminga 1998).

The living belowground biomass measured in Revellata Bay was almost twice as high as the average maximum belowground biomass reported in the review of Duarte & Chiscano (1999). The maximum leaf biomass is similar to the average maximum biomass reported for *Posidonia oceanica* by the same authors (550 vs 505 $g_{dw}\,m^{-2}$). Thus, the ratio between belowground and leaf biomass in Revellata Bay is higher than the average reported by Duarte & Chiscano (1999) (5.4 vs 3.2). Moreover, live roots were dominant (more than 50% of the living belowground biomass) in the samples compared to the live rhizome biomass. Generally, this proportion varies from 8 to 16% (Mateo & Romero 1997, Mazzella et al. 1998).

It is not surprising that the root biomass should be relatively high at Revellata Bay. The nutrient concentrations in the pore water characteristic of the western coast of Corsica are 10 to 100 times lower than the concentrations measured in Italian and Spanish *Posidonia oceanica* meadows (e.g. Alcoverro et al. 1995, Kraemer & Mazzella 1999). Lee & Dunton (1999b) have shown that the root biomass is relatively higher at a site with low nutrient concentrations in the sediment, compared to a high-nutrient concentration site.

We calculated a N budget for *Posidonia oceanica* in Revellata Bay (Table 4). The N requirement of *P.*

oceanica in Revellata Bay is calculated from the data of Pergent-Martini et al. (1994) obtained in this meadow at 10 m depth. We converted the leaf and belowground production data expressed in $g_{dw}\,m^{-2}\,yr^{-1}$ to $g\,N\,m^{-2}\,yr^{-1}$ using our N concentration measurements. This value is considered as the quantity of nitrogen annually required by the annual primary production as measured by Pergent-Martini et al. (1994). Annual uptake fluxes ($g\,N\,m^{-2}\,yr^{-1}$) are calculated from the average specific uptake rates and biomasses, considering that N uptake takes place an average of 12 h d^{-1}.

In fact, Table 4 shows that the root uptake calculated as described above is 3 times higher than the N required annually. The contribution of the roots to N incorporation is then clearly overestimated. As in many uptake experiments on the seagrass root, the measurements were done in the absence of sediment (e.g. Iizumi & Hattori 1982, Lee & Dunton 1999a). Moreover, the pump ensured water circulation in the chamber, and consequently nutrients were always available for root incorporation. *In situ*, this availability is mainly controlled by the diffusion rate of nutrients in the pore water (Stapel et al. 1996). In a *Thalassia hemprichii* meadow, these authors have shown that the N supplied by diffusion in 1 h represented only 5% of the uptake capacity of the roots during this time interval. They conclude that root uptake is primarily dependent on diffusion limits in the sediment and not on the uptake capacity of roots.

We have tried to calculate a second N budget considering that *Posidonia oceanica* has 3 principal mechanisms to meet its N requirement: leaf and root uptake, and internal recycling of N (Table 5). We measured the leaf uptake in this study, and the contribution of internal recycling has been estimated as 40% of the annual need by Alcoverro et al. (2000). Lepoint et al. (2002) have shown by another ^{15}N tracer experiment that this contribution is the same in Revellata Bay. This value is higher than the average contribution of 20% by internal recycling in seagrasses (Hemminga et al. 1999). This difference could be related to the long life span of the *P. oceanica* leaves (>150 d), which allows a relatively efficient resorption of the N contained in adult

Table 5. *Posidonia oceanica.* Annual N budget (second method) at 10 m depth in Revellata Bay (Corsica)

Requirement/process	Values/contribution	Source
Annual N requirement	9.7 $g\,N\,m^{-2}\,yr^{-1}$	Calculated from Pergent-Martini et al. (1994)
Annual N leaf uptake	2.5 $g\,N\,m^{-2}\,yr^{-1}$/26% of N requirement	Calculated from ^{15}N uptake and biomass measurements
N internal recycling	40% of N requirement	Alcoverro et al. (2000), Lepoint et al. (2002)
Annual N root uptake	3.3 $g\,N\,m^{-2}\,yr^{-1}$/34% of N requirement	Deducted from the contributions of N leaf uptake and N internal recycling

leaves to contribute to the growth of young leaves (Alcoverro et al. 2000).

In this second budget, the contribution of the roots is slightly more important than leaf uptake. This agrees with the root biomass measured in Revellata Bay, which partly compensates the limitation of root uptake due to diffusion rates of nutrients in the sediment.

On the other hand, although the concentrations of N in the water column are low, the leaves of *Posidonia oceanica* act as a relatively efficient organ for N uptake. This pattern is different from the pattern of photosynthetic rates. Modigh et al. (1998) have shown that the photosynthetic capacity of leaves decreases as leaf age increases. Kraemer et al. (1997) have shown that the assimilation of N (i.e. the conversion of inorganic N into amino acids) mainly occurs in the growing leaves of *P. oceanica*. Therefore, the N incorporated by the adult leaves in excess to their needs is quickly transferred to these growing tissues (Alcoverro et al. 2000). Adult leaves, although they have reduced photosynthetic activity, contribute clearly to the leaf uptake of N. Borum et al. (1989) and Pedersen et al. (1997) have experimentally demonstrated this process in *Zostera marina* and *Amphibolis antarctica*. For the latter, Pedersen et al. (1997) showed that the transfer of N incorporated in old leaves is a continuous process representing 40% of the N needs of the young leaves.

The leaf biomass fluctuates seasonally and partly determines the variation of the N quantity incorporated by the leaves. For example, the specific uptake rates measured in June and October 1998 are similar, but the leaf uptake flux measured in June is higher than in October in relation to higher leaf biomass. The variations in specific uptake rates control leaf N fluxes during high nutrient concentration events, as in March 1999.

The N budget calculated for the *Posidonia oceanica* meadow of Revellata Bay is complex: several N pools and sources are involved, and leaves as well as roots participate in the uptake. This budget could be restricted to the nutrient-poor conditions encountered in Revellata Bay. Nevertheless, Lee & Dunton (1999a,b) compared the N budget of 2 *Thalassia testudinum* meadows with different pore water nutrient concentrations and showed that the relative contribution of root and leaf uptake were the same in both meadows, although the absolute N fluxes were different. They related these observations to the modification of the ratio between root and leaf biomass, which equilibrates the respective contributions of the different organs. The fact that we measured such a modification in Revellata Bay compared to the continental meadows confirms the findings of Lee & Dunton (1999a,b). Moreover, the contribution of internal recycling of N appears to be similar in the Catalan and Revellata Bay

meadows (Alcoverro et al. 2000, Lepoint et al. 2002). Therefore, the budget elaborated for Revellata Bay meadow could apply to other meadows of the western Mediterranean, but such a generalisation needs more extensive experimental work. In this context, the ^{15}N tracer methodology appears to be an important tool.

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