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FIELD MEASUREMENTS OF INORGANIC NITROGEN UPTAKE BY EPIFLORA COMPONENTS OF THE SEAGRASS *POSIDONIA OCEANICA* (MONOCOTYLEDONS, POSIDONIACEAE)

Gilles Lepoint
Laboratoire d'Océanologie, MARE Centre, Institut de Chimie, Bât. B6, Université de Liège, 4000 Liège, Belgique

Julien Jacquemart
MARE Centre, Algologie, Mycologie et Systématique Expérimentale, Bât. B22, Boulevard du Rectorat 27, 4000 Liège, Belgique

Jean-Marie Bouquegneau
Laboratoire d'Océanologie, MARE Centre, Institut de Chimie, Bât. B6, Université de Liège, 4000 Liège, Belgique

Vincent Demoulin
MARE Centre, Algologie, Mycologie et Systématique Expérimentale, Bât. B22, Boulevard du Rectorat 27, 4000 Liège, Belgique

and Sylvie Gobert
Laboratoire d'Océanologie, MARE Centre, Institut de Chimie, Bât. B6, Université de Liège, 4000 Liège, Belgique

Abstract

Crustose corallines, crustose and erect brown algae, and sessile animals are major components of the epiphytic community of the Mediterranean seagrass *Posidonia oceanica* (L.) Delile. Production, biomass, and specific composition of this epiphyte-seagrass association are impacted by anthropogenic increase of nutrient load in this oligotrophic area. In this context, nitrogen uptake by *P. oceanica* and its epiflora was measured using the isotope ^{15}N at a 10 m depth in the Revellata Bay (Corsica, Mediterranean Sea). Epiflora components showed various seasonal patterns of biomass and abundance. The epiphytic brown algae appeared at the end of spring, later than the crustose corallines, and after the nitrate peak in the bay. Because of their later development in the season, epiphytic brown algae mostly rely on ammonium for their N needs. We hypothesize that the temporal succession of epiphytic organisms plays a crucial role in the N dynamics of this community under natural conditions. The epiphytic brown algae, which have a growth rate one order of magnitude greater than that of crustose corallines, showed lower N-uptake rates. The greater N-uptake rates of crustose corallines probably reflect the greater N requirements (i.e., lower C/N ratios) of red algae. We determined that the epiflora incorporated ammonium and nitrate more rapidly than their host. Nevertheless, when biomass was taken into account, *P. oceanica* was the most important contributor to N uptake from the water column by benthic macrophytes in this sea-grass bed.

Key words: ^{15}N tracer; corallines; eutrophi-cation; Mediterranean sea; nitrogen; seagrass; stable isotopes

Eutrophication of coastal waters has complex consequences on benthic and pelagic ecosystems (Cloern 2001). Eutrophication pressure involves changes in primary production, species composition, and, more generally, ecosystem processes. Classically, eutrophication effects on benthic ecosystems are described as sequential events where the original macrophyte communities (i.e., marine phanerogams and perennial macroalgae), which are adapted to low dissolved nutrient concentrations, are gradually replaced by more ephemeral macrophytes or planktonic algae (Munda 1993, Duarte 1995, Pedersen 1995, Mac Clelland et al. 1997, Cloern 2001).

In nutrient-poor areas, such as the Mediterranean Sea, climax macrophytes like the marine monocotyledon *Posidonia oceanica* are well adapted to low ambient concentrations of nutrients. Direct effects of anthropogenic nutrient enrichment on this species are assumed to be negative because it is not primarily limited by nutrient availability in most of its repartition area (Alcoverro et al. 1995, 1997b).

Seagrasses with a long life span, such as *P. oceanica,* support a complex community of epiphytic organisms. Epiflora components of the epiphytic community might be more nutrient limited than their host plant because

they have a higher nutrient demand (i.e., lower C/N/P ratio) (Pedersen and Borum 1997). They have less possibility of mitigating nutrient depletion because they have access to water-column nutrients only. Moreover, epiphytic macroalgae are too thin to have great amounts of nutrient, and such reserves may also be of limited use with the life span of epiphytes being limited to 50-200 d because of the leaf turnover of their host (Duarte and Chiscano 1999).

Therefore, they are assumed to react more rapidly than their host to an increase in nutrient load. The effects of increased nutrient load on the diverse seagrass ecosystems in the world can affect epiphyte spatial heterogeneity, increase epiphytic biomass, and lead to epiphytic species shifts (Borum 1985, Silberstein et al. 1986, Short et al. 1995, Tomasko et al. 1996, Franko-vich and Fourqurean 1997, Wear et al. 1999, Leoni et al. 2006). These different effects have been shown for *P. oceanica* meadows subject to mariculture loadings (Ruiz etal. 2001, Dimech et al. 2002, Holmer et al. 2003) or exposed to urban and industrial sewage (Piazzi et al. 2004). Nevertheless, eutrophication is a complex phenomenon, and the effects described above may be masked by alteration of other ecosystem processes or characteristics, such as the distribution of macroinvertebrates (Dimech et al. 2002) or an increase in herbivores (Ruiz et al. 2001).

Biomass increase or a species shift in an epiphytic community may have dramatic effects on the host plant by reducing the amount of light reaching the host (Drake et al. 2003). Therefore, epiphytic community changes are often thought to be one of the factors responsible for seagrass decline under eutrophication pressure (Wear et al. 1999, Hauxwell et al. 2003). However, the epiphytic community can play multiple roles in seagrass ecosystems by constituting a pelagic-benthic coupling (Lemmens et al. 1996), contributing to sediment dynamics (Perry and Beavington-Penney 2005), or supporting most of the trophic web associated with the *P. oceanica* meadow community (Mazzella et al. 1992, Lepoint et al. 2000).

Epiphytic communities on seagrass leaves are very complex components of the ecosystem, involving numerous species, especially in the case of seagrasses with long life spans, such as *P. oceanica* (Kerneis 1960, Van der Ben 1971, Boero et al. 1985, Mazzella et al. 1989). Seagrass epiflora communities are often dominated by red crustose corallines (calcareous red algae) or red filamentous algae (Trautman and Borowitzka 1999, Leliaert et al. 2001, Lavery and Vanderklift 2002, Borowitzka et al. 2006). These groups are also present on *P. oceanica* leaves, but it has been recognized that a characteristic assemblage of brown algae colonizes its leaves (Péres and Picard 1964). In the Calvi area, these brown algae can represent an important biomass, especially in the late spring and summer and when anthropogenous nutrients are present. The epiphytes of *P. oceanica* constitute a multistratified community, resulting from sequential colonizations on growing *P. oceanica* leaves (Van der Ben 1971). Diatoms and other microorganisms are epiphytic pioneers but also occur as secondary epiphytes (Mazzella and Russo 1989). Crustose corallines or the crustose brown alga *Myrionema orbiculare* J. Agardh, a characteristic species of *P. oceanica* epiflora, form a first layer of macroepiphytes. This layer is complemented by sessile animals, such as the mono-layered bryozoan *Electra posidoniae* (Gautier), and highly characteristic erect photophilous brown algae, such as *Cladosiphon* spp. and *Giraudya sphacelarioides* Derbès et Solier (Van der Ben 1971). The filamentous red algae appear generally later in summer, at which time they can constitute the highest number of species; however, they are mostly very thin, small algae representing a limited biomass and cover (Van der Ben 1971, fig. 17).

The spatial structure of the *P. oceanica* epiphytic community occurs at different scales in relation to bathymetry (10 m scale), meadow patchiness (m scale), patch structure (10 cm scale), and, finally, the shoot itself (cm scale) (Van der Ben 1971, Mazzella et al. 1989, Dalla Via et al. 1998, Cebrian et al. 1999). Light plays a strong role in this pattern (Dalla Via et al. 1998), restricting epiphytic brown algae in their spread both spatially and seasonally. As a result of their photophilous character, the depth range where these brown algae occur is often more limited than that of crustose corallines (Van der Ben 1971, Mazzella et al. 1989). Moreover, they often occur on the apex of *P. oceanica* leaves or on leaves exposed to greater light (Mazzella et al. 1989, Dalla Via et al. 1998, Cebrian et al. 1999). In contrast, the tolerance of crustose corallines to light-level variability is well known (Figueiredo et al. 2000, Ichiki et al. 2000), and these species may colonize the entire length of *P. oceanica* leaves across the complete depth range of the meadow (Mazzella et al. 1989, Dalla Via et al. 1998, Cebrian et al. 1999). On apex or light-exposed leaf faces, photophilous brown algae can sometimes overgrow the crustose corallines and the animals as they have a growth rate of about one order of magnitude greater than that of crustose corallines (Cebrian et al. 1999).

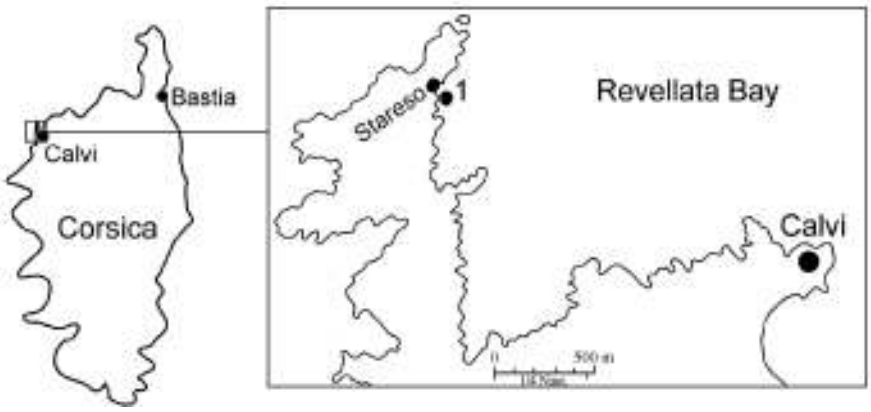
We hypothesize that the various components of the epiphytic community of *P. oceanica* react differently to a moderate increase in nutrient load based on their different growth and nutrient-uptake rates. The relatively fast-growing brown algal species could have an advantage relative to the other functional components of the epiphytic community. The first step to test this hypothesis is to assess *in situ,* at ambient nutrient concentrations,

the N uptake of the epiflora components of *P. oceanica* and of their host. To our knowledge, field measurements of epiphytic N uptake are restricted to measurement at the community level of *Thalassia testudinum* Banks ex K. D. König or *P. oceanica* (Cornelisen and Thomas 2002, 2006, Lepoint et al. 2004b) and do not take place on the different components of this complex association.

MATERIALS AND METHODS

Study site. All sampling and measurements were performed in the Revellata Bay (Gulf of Calvi, Western Corsica, France), near the marine research centre STARESO (42°35' N, 8°43' E) (Fig. 1). *A P. oceanica* seagrass meadow grows here to 40 m depth, which is close to the deepest limit recorded for this species in the western Mediterranean. This meadow, which is one of the most productive *P.oceanica* beds of the NW Mediterranean (Pergent-Martini et al. 1994), covers 70% of the seafloor of the Revellata Bay. Current speeds in Revellata Bay vary between 1 and 25 $cm.s^{-1}$ at 10 m depth above the *P. oceanica* canopy (Norro 1995). In the absence of significant tide, water flow above the canopy is driven by local wind conditions and water circulation outside the bay (Skliris et al. 2001). For average wind conditions, water speed at 10 m depth above the *P. oceanica* canopy is about 5 $cm.s^{-1}$ at our study site (Norro 1995).

***FIG. 1**. Experimental site (1) was located in front of STARESO in the Revellata Bay (Gulf of Calvi, Western Corsica, France) at 10m depth.*



Specific composition and temporal evolution of epiflora. In order to assess the temporal evolution of the epiphytic flora composition, samples were taken by scuba diving at 15 m depth, between April 2001 and April 2002. The sampling site was visited twice a month on average, but no samples were taken in September and October 2001, or in February and March 2002. On each visit, five external *Posidonia* leaves were sampled from randomly selected shoots to examine the epiphytic assemblages. We selected the oldest leaves, as a pilot study had shown that younger leaves usually did not present characteristic taxa. This pilot study, following Kendrick and Lavery's (2001) specifications, and based on species-area curves, also showed that the apical parts of the oldest leaves are the most appropriate to present mature epiphytic assemblages. For this reason, only the apical parts (the first 5 cm from the tips) and the external face of each leaf were observed, using a stereomicroscope (Stemi 200, Zeiss, Zurich, Switzerland). Epiphyte taxa were identified to species level where possible and were counted. Scores obtained for five leaves were obtained for each sample. Species, like the brown alga *M. orbiculare* and corallines that form crusts on leaves, were quantified using percentage cover estimates. In this paper, we present the temporal composition of dominant epiphytic species in relation to ^{15}N-uptake measurements. A more complete description of epiflora assemblage in the Revellata Bay is available in Jacquemart (2003).

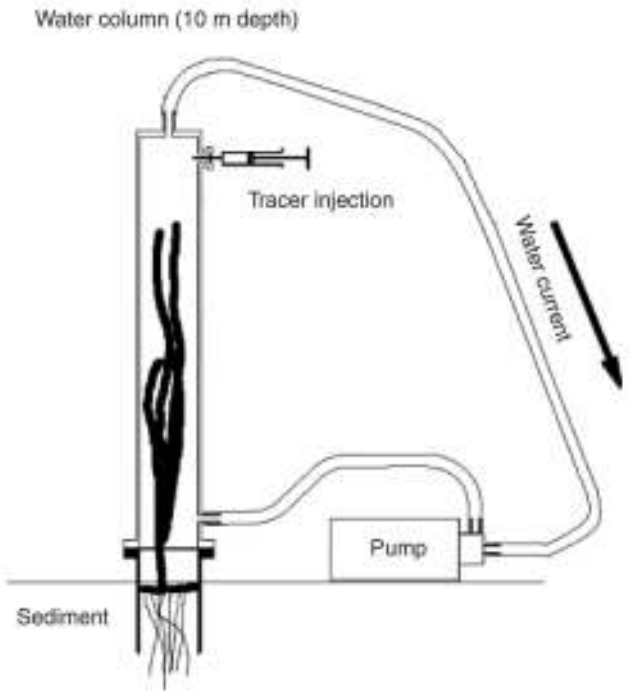
Experimental design and protocol. All experiments were conducted in the seagrass meadow at a 10 m depth by scuba diving. Nitrate and ammonium uptake were measured for the *P.oceanica* leaves and their algal epiphytes. The epiflora was separated according to the ecological characteristics of the different assemblages (i.e., erect and crustose photophilous brown algae or crustose corallines). Experiments were performed in 2003 and 2004 during the months of March, June, and November. Between 12 and 16 incorporation experiments were performed for each experimental period (n_{tot} = 68).

The custom-made experimental device was composed of one transparent Plexiglas cylinder and a submerged pump used to ensure the homogenization of water content (~4 L) (Fig. 2) and flow of ~2$cm \cdot s^{-1}$, a value commonly encountered in the *P.oceanica* canopy of Revellata Bay (Van Keulen and Borowitzka, 2002).

Nevertheless, imposed water flow had a vertical component, which was probably greater than the natural mean secondary vertical flow, although it is possible to observe such flows in a seagrass canopy (Nepf and Koch 1999). A PVC base was driven into the sediment (25 cm depth) 24 h before the experiment, isolating one shoot of *P.oceanica* (Fig. 1). A Plexiglas cylinder was placed vertically onto the PVC base. A rubber membrane separated the cylinder and the PVC base, isolating a leaf and a root chamber.

All experiments had a duration of 1 h and were begun between 9:00 and 10:00 a.m. A longer time would have led to nutrient depletion effects, due to the low concentrations present (generally <0.5µM). The ^{15}N tracers were solutions of ammonium sulfate (99.0% ^{15}N) or nitrate sodium (99.0% ^{15}N) (Eurisotop, Saint-Aubin, France). Water samplings were done in the cylinder before and 5 min after the tracer addition and at the end of the experiment for nutrient concentration measurements. Tracer additions represented on average 0.15 ± 0.14 µM (n = 31) for ammonium and 0.09 ± 0.06 µM (n = 33) for nitrates (Fig. 3). At the end of the experiment, benthic primary producers were collected in plastic bags.

FIG. 2. *Experimental device used for ^{15}N-tracer experiment.*

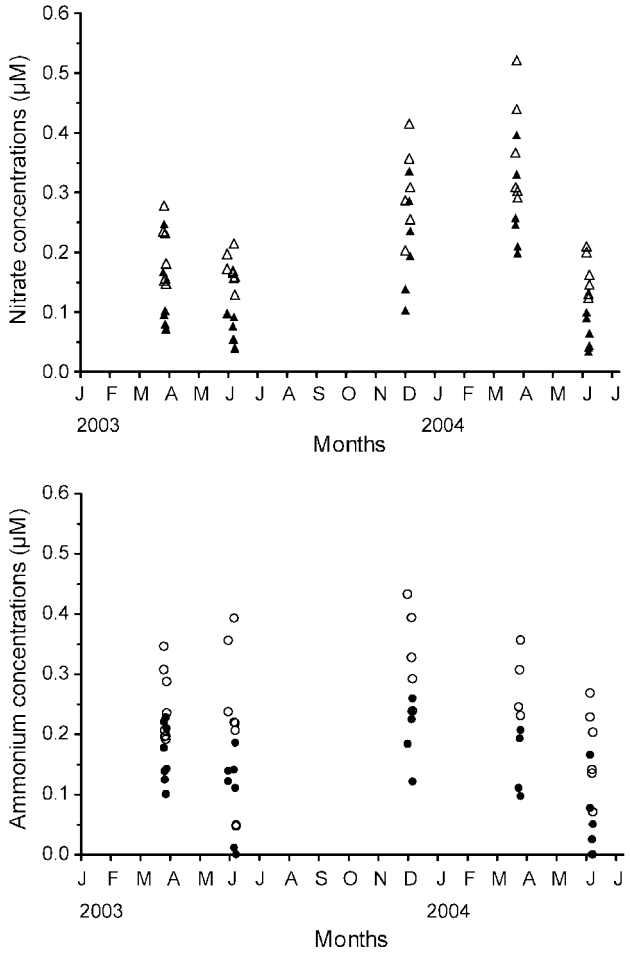


Sample treatment and measurements. Posidonia oceanica leaves were scraped under a stereomicroscope with a razor blade to separately remove different components of the epiphyte community: first, fixed animals (mainly the bryozoan *E. posidoniae);* second, crustose corallines; and third, brown photophilous algae, both erect [*Cladosiphon* spp., *G. sphacelarioides*, and *Sphacelaria cirrosa* (Roth) C. Agardh.] and crustose (e.g., *M. orbiculare*). Leaves and epiphytes were oven-dried at 60°C for 48 h and then weighed.

All samples were finely ground using mortar and pestle or a microgrinding device (MM301, Retch, Haan, Germany) for spectrometric and elemental analysis. Isotopic and elemental measurements were performed with an isotopic ratio mass spectrometer (Optima, GV Instruments, Manchester, UK) coupled to a C-N-S elemental analyzer (NA1500, Carlo Erba, Milan, Italy). Elemental results were expressed as a percentage of the considered element relative to the total dwt (% dwt). These data were used to calculate specific uptake rates and uptake fluxes.

Nitrate and ammonium concentrations in water samples were measured with an analytical automated chain (Skalar, Breda, the Netherlands), using a method adapted for oligo-trophic water analysis. Analytical precision, based on repetitive measurement of a natural sample (n=l2), was 0.02 and 0.1 µM for nitrate and ammonium, respectively. The detection limit, measured as the difference between milliQ water and nutrient-exhausted seawater, was ~0.002 and 0.01 µM for nitrate and ammonium, respectively.

FIG. 3. *Comparison of ammonium and nitrate concentrations before (black symbols) and after (open symbols) the ^{15}N-tracer additions at the beginning of uptake experiments.*



Nitrogen-uptake calculations. Specific uptake rates (V) (µg N·g·N^{-1}·h^{-1}) were calculated according to the following equation (adapted from Collos 1987):

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

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, and t is the duration of the experiment. To facilitate comparison with data in the literature, V was converted into µg N·g$^{-1}_{\text{ash-free dwt}}$·h^{-1} using N concentration in each sample (measured simultaneously with isotopic abundance) and mean ash-free content. Ash-free contents of *P. oceanica* leaves, crustose corallines, and epiphytic brown algae were measured by combusting random subsamples (n = 12 for each) at 450°C for 48 h. Ash-free contents were 87 ± 5, 48 ± 5, and 27 ± 2% dwt for *P. oceanica* leaves, brown epiflo-ra, and crustose corallines, respectively.

Uptake fluxes *(R)* (g N·m^{-2}·h^{-1}) were calculated for each experiment according to the following equation:

$R = V$ x biomass N (2)

For each experiment, the biomass N was calculated following the equation:

Biomass N_{sample} = % N_{sample} x shoot dwt
x average shoot density (3)

The mean shoot density was measured (259 counts) using a circle with a diameter of 40 cm randomly launched in the meadow. We observed no significant seasonal change of density in our study of the Revellata Bay over 10

years (S. Gobert, personal observation) (402 ± 140 shoot · m^{-2}). The N concentration (% N_{sample}) of each sample was measured simultaneously with isotopic abundance.

In *P. oceanica* shoots, the natural abundance of ^{15}N (A_0) was 0.3673 ± 0.0005% ^{15}N (i.e., +2.6‰ in δ notation) (Lepoint et al. 2000). The ^{15}N natural abundance for epiphytes was within the same range. We did not find any seasonal variation of this value in the Revellata Bay (Lepoint et al. 2003), but Vizzini et al. (2003) detected a variation of ± 2‰ in their study. To encompass the natural variation of A_0, the experimental particulate material was considered as enriched in ^{15}N relative to ^{15}N natural abundance *when* $A_f - A_0$ was $\geq$ 0.001% ^{15}N. This natural variation *of* A_0 leads to possible uncertainty in uptake rate calculations.

A_d was considered to be constant during the experiment duration (1 h) and was calculated according to the isotopic mixing equation:

$$C_d \, xA_d = A_{d0} \times C_{d0} + A_t \times C_t \qquad (4)$$

C_d is the concentration of ammonium or nitrate in the dissolved phase after the addition of ^{15}N tracer. A_{d0} and C_{d0} are, respectively, the natural ^{15}N abundance and the initial concentration of nitrate or ammonium in the dissolved phase. A_t and C_t are, respectively, the ^{15}N abundance (99.0% ^{15}N) and the concentration of the added tracer. A_{d0} was fixed at 0.37% ^{15}N, as the importance of the natural variation of this term is small compared with the variations of the tracer terms. Calculated A_d varied between 42.6% and 99% ^{15}N.

For A_d calculation when initial nutrient concentrations were undetectable, we considered C_{d0} to be equal to the analytical quantification limit. The quantification limit was estimated to be 0.01 and 0.05 µM for nitrate and ammonium, respectively (i.e., five times the value of the respective analytical detection limits).

RESULTS

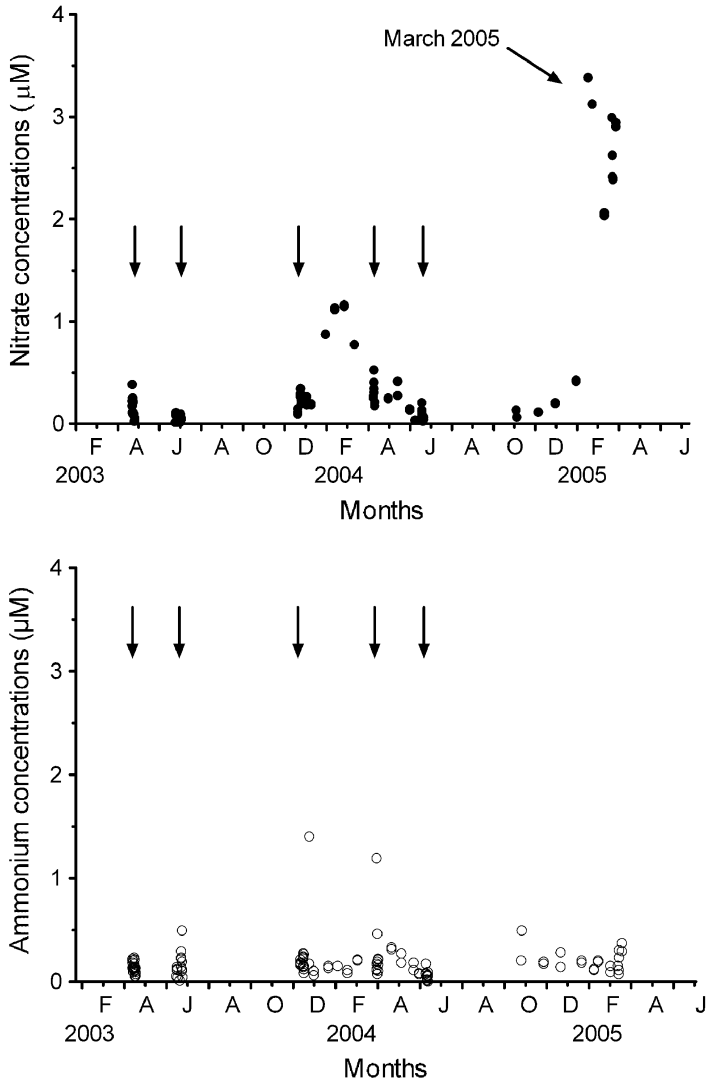
Nutrient concentrations in the Revellata Bay ranged between nondetectable and 2 µM for ammonium, and between nondetectable and 3.5 µM for nitrate (Fig. 4). There was no clear seasonality of ammonium concentrations. By contrast, nitrate concentrations showed a strong seasonal pattern, being at a maximum during a few weeks in early spring.

The temporal evolutions of the percentage of leaf surface covered by the brown algae *M. orbiculare* and the crustose corallines are shown in Fig. 5a. These two major components of the *P. oceanica* leaf epiflora showed a strong seasonal pattern. Maximum cover values were reached in May and June. In May, percentage covers reached 33% and 60% for *M. orbiculare* and for the crustose corallines, respectively. In summer, the values were 15% and 30%, respectively. J. Jacquemart's unpublished data also show that summer (July and August) was the period of maximal epiphytic diversity with the development of several species of erect red algae, mostly Ceramiales. These algae are, however, usually small and less important than brown algae in terms of cover and biomass. Nonetheless, it would be interesting to include them in a future study of the peculiar summer situation (a very low natural level of nutrients, but with the possibility of local eutrophication linked to tourism). The latter group is well represented between April and August, but almost completely lacking from November to February. The percentage cover values reached by the crustose corallines during this period were very low, ranging from 0.1% to 1%.

The temporal evolution of the total number of the main epiphytic erect brown algae is shown in Fig. 5b. This group included the following species: *Dictyota dichotoma* (Huds.) J. V. Lamour., *Cladosiphon* spp., *G. sphacelarioides,* and *S. cirrosa.* These taxa, considered here as a whole, showed strong quantitative seasonal variations. In spring, between April and June, the values appeared to be largely variable, ranging from eight to 58 individuals per sample. The number of individuals reached their maximum in late summer, with 103 counted in August. Between November and February, erect brown algae were virtually absent. This group continued to be poorly represented until April.

Leaf dwt was minimal in December 2003 and maximal in June 2004 (Fig. 6a), as typically observed for this species (Gobert et al. 2006). Epiphyte dwt also presented minimal values in December 2003 and maximal values in June 2003. Our sampling did not include the summer maximum of epiphyte diversity on *P. oceanica* leaves, which occurs during July or August.

FIG. 4. *Nitrate and ammonium concentrations in the water column at 10 m depth in the Revellata Bay between March 2003 and March 2005. Black arrows indicate experimental campaigns (n = 5).*

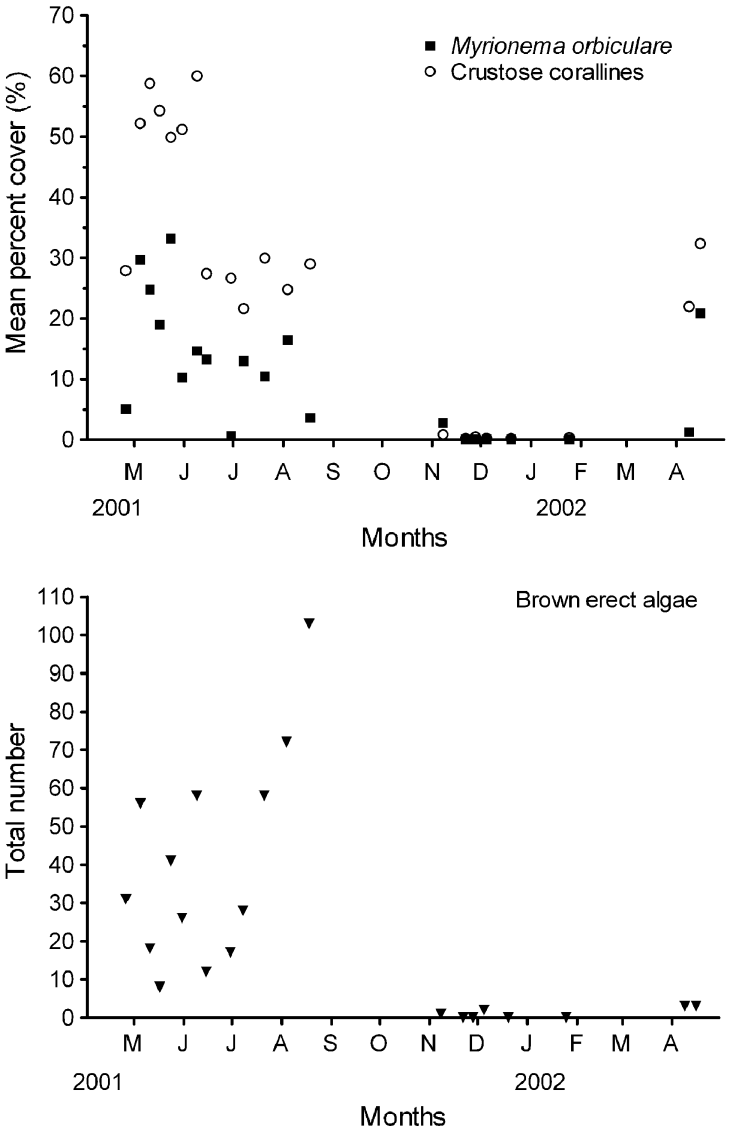


Total dwt was minimal in December for the three epiphytic components (Fig. 6b). Epifauna was mainly represented by *E. posidoniae,* a slightly calcified bryozoan, which is almost restricted to *P. oceanica* leaves (Gautier 1954). Fauna represented on average 39% of total epiphyte weight, with a contribution ranging between 16% and 63% in December 2003 and March 2003, respectively. Crustose corallines contributed on average 49% of total epiphyte weight, ranging from 30% (March 2003) to 84% (December 2003). Photophilous algae were almost completely absent in December 2003. Other potential macroepiphytic groups (mainly Ceramiales) were almost absent from our samples, as they mostly develop during the summer months.

Mean nitrogen concentrations of flora showed that *P. oceanica* had the highest N concentration when expressed relative to total dwt (Table 1). However, when data were expressed relative to ash-free dwt, N concentration in crustose corallines was higher than that of brown algae, and both were higher than the N concentration in *Posidonia* leaves.

Average and median values of ammonium and nitrate biomass-specific uptake, respectively $V_{NH4}$and V_{NO3} , by *P. oceanica* leaves and different components of the epiphytic community are presented in Table 1. For *P. oceanica* leaves or crustose corallines, V_{NO3} did not significantly differ from V_{NH4} (*P>0.1, t-test*). By contrast, the V_{NO3} and V_{NH4} of brown algae differed significantly (P = 0.0123, *t-test*).

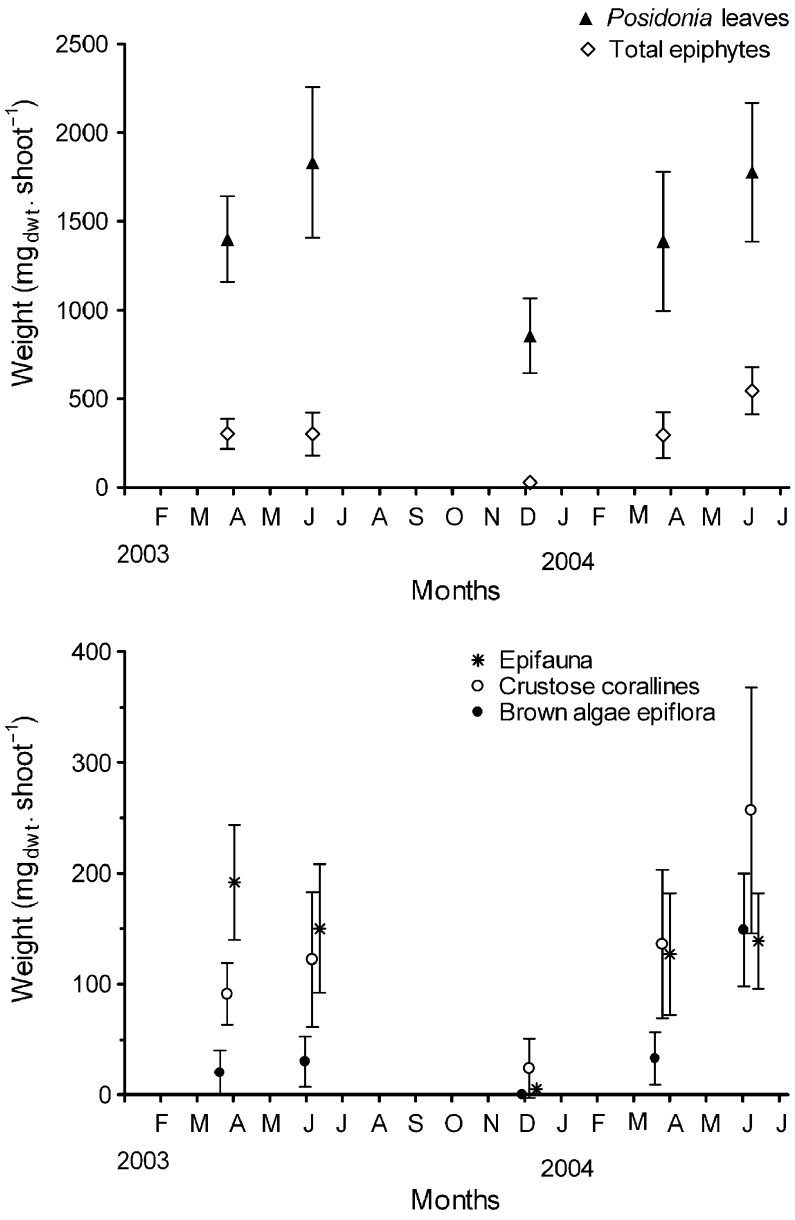
FIG. 5. *Temporal evolution of mean cover of crustose coralline and crustose brown (Myrionema orbiculare) epiphytes (upper) and of the number of erect brown epiflora on the seagrass Posidonia oceanica at 15 m depth (lower) in front of STARESO in the Revellata Bay.*



The V_{NO3} and V_{NH4} of both epiflora components were never close to 0, except in December when brown algae were absent (Fig. 7). By contrast, the V_{NH4} of *P. oceanica* was sometimes close to the detection limit in June and March. No clear seasonal pattern emerged from NH_4-uptake measurements. The ANOVA tests showed a significant effect of flora type on V_{NH4} (Table 2), but not of sampling date. The V_{NH4} of *P. oceanica* was significantly lower than that of epiflora components (*post hoc* Tukey's test, P<0.001). The V_{NH4} of brown epiphytic algae was significantly lower than that of crustose corallines *(post hoc* Tukey's test, P<0.001).

No clear temporal pattern was evident for nitrate uptake by the epiphytes, except for a maximum V_{NO3} measured for crustose corallines in December 2003 (Fig. 7). Despite great variation, the V_{NO3} of the epiflora was never equal to 0, except in December 2003 for brown algae. By contrast, the V_{NO3} of *P.oceanica* was often not detectable in June when $N0_3$ concentrations were close to the detection limit. Analysis of variance (ANOVA) tests showed a significant effect of flora type on V_{NO3} (Table 2), but not of sampling date. The V_{NO3} of *P. oceanica* leaves was significantly lower than that of crustose corallines *(post hoc* Tukey's test, P<0.005) and brown algae (*post hoc* Tukey's test, P<0.005). The V_{NO3} of the two epiphytic components also differed significantly (*post hoc* Tukey's test, P<0.005).

FIG. 6. *Temporal evolution of average (± SD) weights of Posidonia oceanica leaves, of total epiphytes (a) and of sessile epi-fauna, crustose coralline, and brown photophilous (erect and crustose) epiflora (b) at 10m depth, between March 2003 and June 2004 in front of STARESO in the Revellata Bay.*



Average uptake fluxes of nitrate (μg $N \cdot m^{-2} \cdot h^{-1}$) by the different primary producers did not differ significantly from the ammonium uptake fluxes of each producer (Table 1). Nitrate-uptake fluxes of *P. oceanica* leaves were very variable and showed minimal values in June 2003 (Fig. 8). These nitrate uptake fluxes were generally greater than those of the epiflora, except in June. Uptake fluxes by crustose corallines exhibited minimal values in December but were generally greater than those of brown algae. Nitrate-uptake flux by this last group was only significant in March and June 2004. The ANOVA tests showed a significant effect of flora type on NO_3-uptake fluxes (Table 3). *Posidonia oceanica* uptake was significantly higher than that of epiphytic flora components (*post hoc* Tukey's test, $P<0.01$ and <0.001 for crustose corallines and epiphytic brown algae, respectively). There was no significant difference between the NO_3-uptake fluxes of the two epiphytic components.

There was no apparent seasonal variation in ammonium-uptake fluxes (Fig. 8), but measurements showed important variability, for both *Posidonia* leaves and crustose corallines. Uptake fluxes by brown algae were only significant in June 2004. The ANOVA tests showed a significant effect of flora type on NH_4-uptake fluxes (Table 3). *Posidonia oceanica* uptake was significantly higher than that of epiphytic flora components (*post hoc* Tukey's test, $P<0.05$ and $P<0.001$ for crustose corallines and epiphytic brown algae, respectively).

There was no significant difference between the NH_4-uptake fluxes of the two epiphytic components.

FIG. 7. *Nitrate- and ammonium-specific uptake rates (µg $N \cdot g^{-1}_{ash\text{-}free\ dwt} \cdot h^{-1}$) by crustose corallines, brown photophilous epiflora, and Posidonia oceanica leaves at 10 m depth, between March 2003 and June 2004 in front of STARESO in the Revellata Bay.*

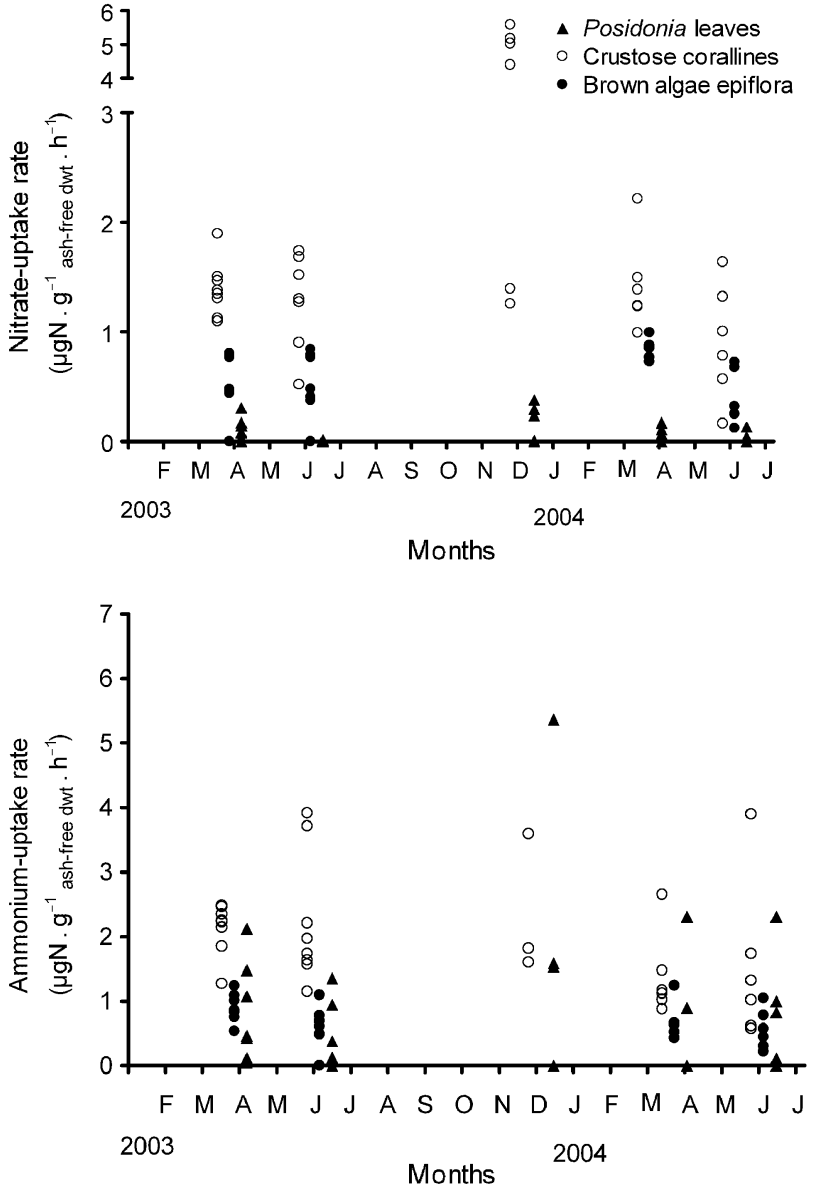


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***TABLE 1**. Mean values ± SD (median) of N concentrations (% dwt and % ash-free dwt), specific uptake rate (V), and uptake fluxes (R) for ammonium and nitrate incorporation by Posidonia oceanica and its epiphytes at 10 m depth in the Revellata Bay (Calvi, Corsica)*

	% N_{dwt}	% $N_{ash\text{-}free\ dwt}$	V_{NH4} µgN·gN^{-1}·h^{-1}	V_{NH4} µg N·g$^{-1}_{ash\text{-}free\ dwt}$·h^{-1}	V_{NO3} µgN·gN^{-1}·h^{-1}	V_{NO3} µg N·g$^{-1}_{ash\text{-}free\ dwt}$·h^{-1}	R_{NO4} µg N.m^{-2}.h^{-1}	R_{NO3} µg N·m^{-2}·h^{-1}
Posidonia leaves	1.4 ± 0.3	1.6 ± 0.3 *n* = 68	7.1 ± 11.6(2.5) *n* = 31	0.72 ± 1.1 (0.12) *n* = 31	5.5 ±8.1 (2.1) *n* = 33	0.09 ±0.11 (0.03) *n* = 33	51.3 ±65 (23.6) *n* = 31	38.2 ±47.9 (15.5) *n* = 33
Crustose corallines	0.9 ± 0.2	3.5 ± 0.8	86.9 ± 101.8 (55.2) *n* = 31	1.91 ±0.91 (1.73) *n* = 31	55.8 ± 59.3 (34.4) *n* = 33	1.72 ± 1.32 (1.35) *n* = 33	27.6 ±31.2 (20.4) *n* = 31	15.7 ± 11.8 (12.1) *n* = 33
Brown epiphytic algae	1.05 ± 0.3 *n = 49*	2.1 ± 0.7 *n = 49*	38.3 ± 3.0(38.1) *n* = 23	0.70 ± 0.3 (0.68) *n* = 23	28.7 ± 12.0 (26.8) *n* = 23	0.54 ± 0.30 (0.57) *n* = 23	6.4 ± 8.7 (3.8) *n* = 23	4.2 ± 5.0 (2.9) *n* = 23

Ash-free dwt has been measured on 12 samples by fire-loss at 450°C. *n* = number of samples.

***TABLE 2.** Summary of ANOVA results.*

	V_{NH4}			V_{NO3}		
Source of variation	MS	*F*	*P*	MS	*F*	*P*
Dates	0.58	$F_{3,90}$=1.93	NS	0.39	$F_{3,89}$=1.23	NS
Flora types	49.11	$F_{1,90}$ =163.05	<0.001	45.59	$F_{1,89}$= 142.27	<0.001
Flora types x dates	0.58	$F_{7,90}$ = 0.35	NS	6.08	$F_{7,89}$ = 6.52	<0.001

MS, mean square; *F,* Snedecor's *F; P,* significance level; NS, nonsignificant difference (P>0.05). Flora types: *Posidonia oceanica* leaves, epiphytic crustose corallines, epiphytic brown algae.

***TABLE 3.** Summary of ANOVA results.*

	R_{NH4}			R_{NO3}		
Source of variation	MS	*F*	*P*	MS	*F*	*P*
Dates	1231.2	$F_{3,90}$ = 0.68	NS	1584.	$F_{3,89}$ = 2.17	NS
Flora types	1256.1	$F_{1,90}$=6.91	<0.01	9265.3	$F_{1,89}$= 12.68	<0.001
Flora type x dates	254.35	$F_{7,90}$=0.14	NS	2489.5	$F_{7,89}$ = 3.41	<0.005

MS, mean square; *F,* Snedecor's *F; P,* significance level; NS, nonsignificant difference (P>0.05). Flora types: *Posidonia oceanica* leaves, epiphytic crustose corallines, epiphytic brown algae.

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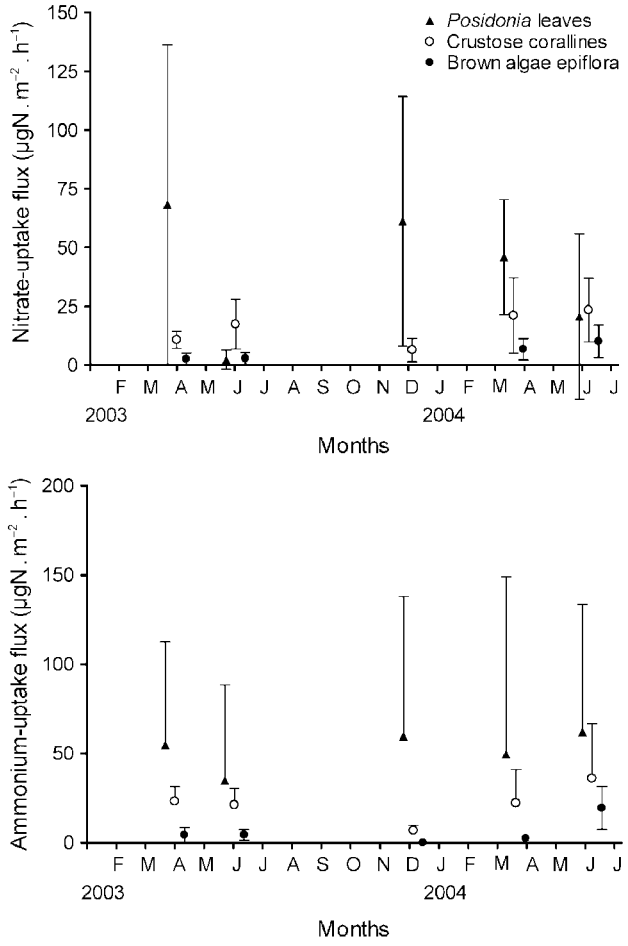
DISCUSSION

We measured simultaneously *in situ* the N-uptake rates ($gN \cdot gN^{-1} \cdot h^{-1}$ or $g\ N \cdot g^{-1}_{ash\text{-}free\ dwt} \cdot h^{-1}$) and calculated fluxes ($gN \cdot m^{-2} \cdot h^{-1}$) of *Posidonia* leaves and of its different epiflora components. Our measurements were made at ambient nutrient concentrations, which, being close to our analytical limit, resulted in a significant variability in the calculated uptake rates. This variability was greater for ammonium uptake than for nitrate uptake due to a higher limit of detection and a lower measurement precision of ammonium concentrations. Nevertheless, we estimate that our measurements provide a good estimate of N uptake by flora at natural nutrient concentrations. Uptake rates were not correlated to external nutrient concentrations. However, our experiments were performed in a relatively narrow range of concentrations (0-0.5 and 0-1 μM, for nitrate and ammonium, respectively), representative of natural nutrient occurrence, but far below concentrations generally used to assess kinetic parameters (0-10 mM). It is possible that this relationship was encompassed by experimental variability, as described above.

Another cause of natural variability for ammonium uptake is the variability of water flow speed. Generally, uptake rates for ammonium by seagrasses and their epiflora increase with an increase in water flow (Cornelisen and Thomas 2002, 2006). This increase in relation to water flow is also observed in seaweed ecosystems (Wheeler 1988, Hurd et al. 1996) and is linked to the influence of water flow on the thickness of the diffusion boundary layer. In studied seagrass systems, nitrate uptake seems to be less affected by water flow because it is more biologically limited by the nitrate reductase activity. In our experiment, water flow was not responsible for uptake variability because this was an invariant factor in our experimental setting. It was representative of water flow speed occurring in the *P. oceanica* canopy of Revellata Bay in mean condition of wind (i.e., a water flow of about $2cm \cdot s^{-1}$*).* Attenuation of water current by a seagrass canopy, such as *P. oceanica,* is a well-established process (Van Keulen and Borowitzka 2002, Koch et al. 2006). This attenuation may lead to a local and transient nutrient gradient in *P. oceanica* canopy water from sediment to overlying water (Gobert et al. 2003). In the Revellata Bay, this occurs particularly on calm days in late spring and summer, a period of nutrient exhaustion in the water. All investigated flora took up both nitrate and ammonium at concentrations close to our analytical limit (i.e., 0.002 and 0.01 μM for nitrate and ammonium, respectively). Therefore, we can state that *P. oceanica* and its natural epiflora assemblage are well adapted to the very low nutrient concentrations of their environment. Epiphytic colonization on *P. oceanica* leaves is structured, both spatially and temporally (Mazzella et al. 1989, Dalla Via et al. 1998). In agreement with Mazzella et al. (1989), we observed that crustose corallines are present early in the season on the *Posidonia* leaf surface, forming a first layer of macroepiphytes. Therefore, crustose coralline might benefit from the greater nitrate concentrations in the water column during winter and spring. They also take up ammonium throughout the year. By contrast, the brown algae of the epiflora generally developed at the end of spring, later than the crustose corallines and after the peak in nitrate concentrations. Because of that timing of events, epiphytic brown algae and also erect red algae, which develop in summer, would depend more on ammonium to ensure their N needs than would crustose corallines. The temporal pattern of epiphytic colonization and its correlation (or lack thereof) with nitrate peak occurrence strongly determine the potential N sources that the different epiflora components may take up.

The biomass dynamics of *P.oceanica* and epiphytes are primarily determined by solar cycle through light availability and temperature evolution (Alcoverro et al. 1995, 1997a). Nutrient availability or grazing is thought to explain local variability in biomass dynamics (Alcoverro et al. 1997b). *Posidonia oceanica,* like many other seagrasses, has a very complex nutrient budget, which enables it to meet its nutrient requirements in nutrient-poor conditions (Gobert et al. 2006). Epiphytic macroalgae probably have less possibility of mitigating the seasonal nutrient poverty in the water column of this nutrient-poor coastal area. Owing to the renewal of host leaves, the life span of epiflora on their host is *de facto* limited to a time ranging between 50 and 200d according to leaf turnover (Cebrian et al. 1999). This leaf turnover is an important factor for epiphyte dynamics (Mazzella et al. 1989). Because of their short lifetime and the temporal structure of epiphytic colonization, nutrient storage strategies are probably limited for epiflora. Moreover, they cannot take up nutrients directly from the sediment as can their host, and they are in competition with both their host and other epiphytes and phytoplankton for water-column nutrients. For these reasons and, although crustose corallines and epiphytic brown algae take up nutrients at very low concentrations, epiflora might be more limited than their host by nutrient availability. In this context, as in other macrophyte epiphytic communities (Hurd et al. 2000, Bracken 2004), there are possible interactions between epifauna and epiflora. Indeed, considering the high N content of epiphytic animals compared with epiphytic algae (about 10% dwt vs. l%-5% dwt), N excretion by epifauna, which represents between 16% and 63% (mean 39%) of epiphyte biomass, could constitute an important N source for epiphytic algae.

FIG. 8. *Mean values (± SD) of nitrate- and ammonium-uptake fluxes (µg $N.m^{-2}.h^{-1}$) by crustose corallines, by brown photophilous epiflora and by Posidonia oceanica leaves at 10 m depth, between March 2003 and June 2004 in front of STARESO in the Revellata Bay.*



Photophilous brown algae growing on *P. oceanica* leaves have a growth rate (in terms of biomass increase) one order of magnitude greater than that of crustose corallines (0.120 vs. $0.017d^{-1}$) (Cebrian et al. 1999). According to Pedersen and Borum (1997), photophilous brown algae epiflora components would display higher N-uptake rates compared with crustose corallines in order to match the N needs required for their higher growth rate. Nonetheless, our field measurements demonstrated that crustose corallines had a higher average N-uptake rate than did epiphytic brown algae (0.0021 vs. $0.0009d^{-1}$ and 0.0013 vs. $0.0007d^{-1}$ for NH_4 and NO_3 uptake, respectively). Pedersen and Borum (1997) based their conclusion on the fact that ephemeral algae have a higher growth rate and higher N concentrations in their tissues than do slow-growing algae. In our study, crustose corallines displayed higher N concentrations in their tissues than did fast-growing brown algae (3.5% vs. 2.1% of ash-free dwt) (Table 1). This higher N content was probably due to the use of phycobiliproteins as major pigments by red algae (Rico and Fernandez 1996). Nevertheless, it does not explain entirely the difference observed between growth rates and N-uptake rates.

Fertilization experiments or studies on the impact of aquaculture on *P. oceanica* meadows have shown that epiphyte biomass increases with nutrient increase (Cancemi et al. 2003, Holmer et al. 2003, Leoni et al. 2006), a potential cause of seagrass decline (Kemp 2000), though in some circumstances the epiphyte load can be mitigated by herbivory (Ruiz et al. 2001). At ambient concentrations, crustose corallines take up both nitrate and ammonium more quickly than erect photophilous algae. The only published value we found for a crustose coralline was for one from Japan (Ichiki et al. 2000) with a K_m for nitrate uptake of about 4 µM, while K_m for rates of ammonium uptake by algae are generally higher than 10 µM (Rees 2003). It is thus probable that nutrient uptake by the epiflora is below the saturation point, but it is difficult to predict which ecological group would be favored by a moderate nutrient increase. Wear et al. (1999) observed during their fertilization experiment on different monospecific seagrass beds from Florida that an increase in epiphyte biomass was due to the development of two filamentous species (a brown and a red), already present in the control group, but no

increase of crustose corallines. A similar situation may apply in our area, because higher nitrogen uptake may only be used for phycoerythrin synthesis and crustose coralline growth is limited by substrate availability (i.e., seagrass leaf area). On the other hand, erect photophilous brown algae, and possibly red filamentous algae in the summer, may increase in size without much extension of the occupied leaf surface.

The average contributions of epiphytes and *Posidonia* leaves to N fluxes (NH_4 + NO_3 uptake fluxes) calculated from our uptake measurements were 30% and 70%, respectively. These contributions showed great seasonal variations, and epiphytes contributed between 8% and 51% of the leaf community (i.e., epiphytes plus *Posidonia* leaves) uptake, respectively, in December 2003 and in June 2003 and 2004. Relative biomass of epiphytes and *Posidonia* leaves is determinant for this seasonal variation and for the relative importance of uptake fluxes. The contribution of epiphytes may thus appear relatively modest at the scale of a *Posidonia* shoot, but it represents a significant transfer of nitrogen from the water column to the benthic compartment.

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