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Nitrogen dynamics in *Posidonia oceanica* cuttings: implications for transplantation experiments

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Abstract

A ^{15}N tracer study was performed during an experimental transplantation trial of natural *Posidonia oceanica* cuttings. The experiment was done in situ at 17 m depth in the Revellata Bay (Calvi, NW Corsica, France). Despite high survival rates of transplants (>90%) after one year, the weight and the N content of transplants are significantly lower than those of reference plants. In absence of roots, the transplants are not able to meet their N requirement because, leaf uptake is insufficient to replenish the N lost during the natural leaf decay. This could constitute a major cause of long-term failure for transplantation experiments or natural re-colonisation processes. The increase of the ^{15}N content in the roots shows that the plant re-allocates the nitrogen of one organ (i.e. leaves, rhizomes) to ensure the growth of another (i.e. roots).

Keywords: *Posidonia;* Seagrass; Transplantation; Nitrogen budget; ^{15}N tracer; NW Mediterranean

1. Introduction

The *Posidonia oceanica* seagrass meadow is a major ecosystem of the Mediterranean coastal zone. The occurrence of the flowering and success of its sexual reproduction are low (e.g. Gobert et al., 2001; Balestri and Cinelli, 2002). Therefore, the vegetative reproduction from natural *P. oceanica* cuttings represents a major way of spreading for *P. oceanica.* The success of this process is low due to the difficulty of rooting and anchoring for cuttings but allows lost *P. oceanica* meadows to be recovered albeit at very low rates (Pergent-Martini and Pasqualini, 2000).

This natural mode of re-colonisation has been used in many transplantation experiments which aimed to restore destroyed *P. oceanica* meadows (e.g. Meinesz et al, 1992; Molenaar et al., 1993). But the survival of *P. oceanica* transplants is often low at long term, even though they are artificially fixed on the sediment. Genot et al. (1994) point out the importance of carbohydrates reserves and chlorophyll content for the transplantation success. The acquisition of nutrients by transplants is probably also a critical factor for long term survival of transplants (Vangeluwe et al., submitted for publication).

P. oceanica beds are settled in coastal areas where nutrient availability is possibly a limiting factor for plant growth (Alcoverro et al., 1995). Like other seagrass species, *P. oceanica* has a complex nitrogen budget in order to meet its nitrogen requirement (Lepoint et al., 2002b). This budget involves leaf and root N uptake, N storage strategies and N recycling (Alcoverro et al., 2000; Invers et al., 2002; Lepoint et al, 2002a,b). Moreover, Marba et al. (2002) show a strong physiological integration of *P. oceanica* ramets, with a transfer of nitrogen from parent shoot to new shoot. This ramet integration seems to play an important role for the initial N acquisition by new shoots (Marba et al., 2002). Indeed, the N requirement of expanding ramets is high due to the development of organs of new shoots. But, their N uptake capacities are low because the root development is delayed relatively to rhizome and leaf developments (e.g. Molenaar et al., 1993).

For these reasons, we guess that the differences between N demand and N acquisition capabilities of *P. oceanica* cuttings could constitute a major cause for the failure of transplantation experiments or natural colonisation processes, in addition to anchoring difficulties. To test this hypothesis, we have performed a long term ^{15}N tracer experiment using natural cuttings of *P. oceanica* in the context of a transplantation experiment. This ^{15}N labelling experiment allows us to follow the transfer of nitrogen between the different plant part (i.e., roots, leaves, rhizomes and scales) (Marba et al., 2002) but also to distinguish between N sources involved in the N budget of *P. oceanica* cuttings (i.e., internal re-mobilisation vs. external uptake) (Lepoint et al., 2002a). In this

study, we compare the N dynamics of *P. oceanica* cuttings to the N dynamics of *P. oceanica* in its natural environment (e.g., Alcoverro et al., 2000; Lepoint et al., 2002b).

2. Material and method

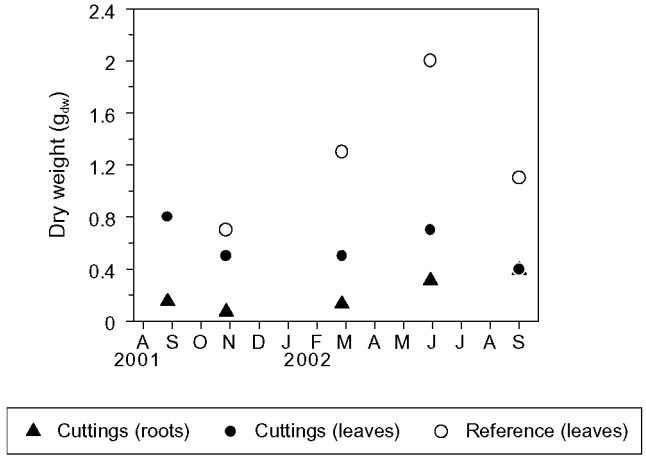
Experimental work was done in the Revellata Bay in front of the oceanographic station STARESO (Calvi, Western Corsica, France) from September 2001 to September 2002 on a sandy patch at 17 m depth. The transplantation experiment was performed using naturally uprooted shoots of *P. oceanica.* These cuttings were collected in the meadow of the Revellata Bay between 5 and 15 m depth. Naturally uprooted cuttings were use in order to minimise the impact of transplant collection on the *P. oceanica* meadow (Augier et al., 1996).

Cuttings were kept in an aquarium for three days, and then labelled during two days with repeated additions of a ^{15}N labelled ammonium sulphate solution (99.0% ^{15}N) (Eurisotop, France). One hundred shoots with orthotropic rhizomes (maximum 10 cm) were attached with natural links (sisal) on woven bamboo grids (1 m^2). Five shoots were sampled on the grid and on a reference site in September and November 2001, in March, June and September 2002.

The sampled leaves were scraped to remove epiphytes. Leaves, scales, roots and rhizomes were lyophi-lised for 48h and weighed. Isotopic and elemental measurements were performed with an Optima mass spectrometer (Micromass, UK) coupled to a C-N-S elemental analyser (Carlo Erba, Italy). The abundance of ^{15}N in *P. oceanica* organs was expressed in ^{15}N atom%. This represents the proportion of ^{15}N atoms relative to the total N atoms ($^{14}N + {}^{15}N$). Two units are used to express the elemental composition: the N content which is expressed in mg N (per leaf or per shoot) and the N concentration which is expressed in percent relative to the total dry weight ($\%_{dw}$)·

We calculate the ^{15}N in excess in the labelled *P. oceanica* by subtracting the natural ^{15}N abundance from the measured ^{15}N abundance. In the Revellata Bay, the natural ^{15}N abundance in *P. oceanica* leaves is 0.36 ± 0.005% ^{15}N (i.e. +2.6‰ in $\delta^{15}N$ notation) and does not show a clear seasonal pattern (Lepoint et al., 2000). We calculate the ^{15}N content in excess (µg $^{15}N_{excess}$ $shoot^{-1}$) using the ^{15}N abundance in excess and N content.

Fig. 1*. Temporal evolution of dry weight of leaves and roots of Posidonia oceanica cuttings and reference shoots from September 2001 to September 2002. Number of samples = 5.*



3. Results

The survival rate of transplants is about 90% after one year. Few shoots divide and few shoots change their growth direction (i.e. tend to grow horizontally).

Leaf dry weight of transplants is always lower than those of reference samples (Fig. 1) (Mann-Whitney test, all $p \leq 0.01$). Leaf dry weights of reference shoots show a seasonal variation which is well described in the literature (e.g. Buia et al., 1992) with a maximum in June 2002 and a minimum in November 2002. Leaf dry weight of transplants shows the same temporal variation, except that the maximum was in September 2002. Root dry

weight of transplants increases significantly from November 2001 to June 2002 (Fig. 1) (Mann-Whitney test, all $p \leq 0.05$).

N concentrations of transplant and reference leaves ($\%_{dw}$) vary in the same way with the time, showing a maximum in March 2002 and a minimum in September 2002 for both transplants and references (Fig. 2). N concentrations of reference leaves are significantly higher than those of transplant leaves (Mann-Whitney test, $p \leq 0.001$), except in September 2002.

N contents in leaves (mg N $shoots^{-1}$) of transplants and references change with the time (Fig. 2) but do not show the same temporal trends. Leaf N contents of reference samples are the highest in March 2002 and the lowest in September 2002. Transplants dispose the highest values in September 2001 when they were attached to the bamboo grids and the lowest values in September 2002. Temporal variation of N contents in transplants is less pronounced than in reference samples. Leaf N contents of references are significantly higher than those of transplants (Mann-Whitney test, all $p \leq 0.005$).

N concentrations in rhizomes of transplants and references vary between 0.8 and 1.3%$_{dw}$· They are not significantly different (Mann-Whitney test, all $p > 0.1$). They do not show any significant seasonal variation (Mann-Whitney test, all $p > 0.1$).

N contents of rhizomes represent between 40% and 50% of the total nitrogen content of the transplants, constituting the most important stock of nitrogen in the plant (Fig. 3). N contents of rhizome tend to decrease from September 2001 to September 2002. This trend is only significant when samples from September 2001 and 2002 are compared (Mann-Whitney test, $p \leq 0.01$).

Roots represent the less important stock of nitrogen from September 2001 to June 2002 when leaf and root N contents are of the same importance. N contents of roots tend to increase from November 2001 to June 2002 (Mann-Whitney test, $p \leq 0.05$), but the N concentrations in roots do not show a significant temporal variation (Mann-Whitney test, all $p > 0.1$).

N contents of scales and leaves are of intermediary importance when compared to rhizomes and roots N stock. The N contents of scales do not a show significant temporal variation.

All organs of the transplants were enriched in ^{15}N compared to the natural ^{15}N abundance in *P. oceanica* (Fig. 4). This enrichment remains measurable in all organs one year after tracer addition. The leaves are significantly more enriched in ^{15}N than the scales, the roots and the rhizomes (Mann-Whitney test, $p \leq 0.000$). The ^{15}N abundance in leaves shows a significant decrease from November sampling to March sampling (Mann-Whitney test, $p \leq 0.001$), staying quite stable in the following ones (Mann-Whitney test, all $p > 0.1$). The ^{15}N abundance in scales, roots and rhizomes does not show a significant temporal variation (Mann-Whitney test, all $p > 0.1$), except the ^{15}N abundance of rhizome between June 2002 and September 2002 (Mann-Whitney test, $p \leq 0.05$).

In September 2001, the leaves contain the greatest quantity of added ^{15}N (μg $^{15}N_{inxcess}$ $shoot^{-1}$) (Fig. 5). These ^{15}N contents decrease drastically between November and March samplings (Mann-Whitney test, $p \leq 0.001$), and continues to decrease slightly till September 2002 (Mann-Whitney test, $p \leq 0.01$). On the contrary, roots ^{15}N contents tend to increase from September 2001 to September 2002 (Mann-Whitney test, $p \leq 0.05$). The ^{15}N contents of rhizomes and scales do not show a significant temporal variation (Mann-Whitney test, $p > 0.1$). In September 2002, the ^{15}N contents of rhizomes are the most important stock of ^{15}N in the plant.

***Fig. 2**. Temporal evolution of nitrogen contents (bars) and nitrogen concentrations (circles) in leaves of Posidonia oceanica cuttings and reference shoots from September 2001 to September 2002. Number of samples = 5.*

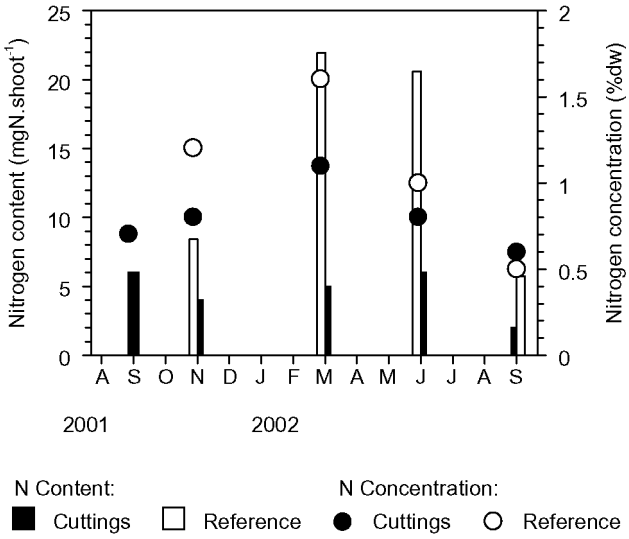
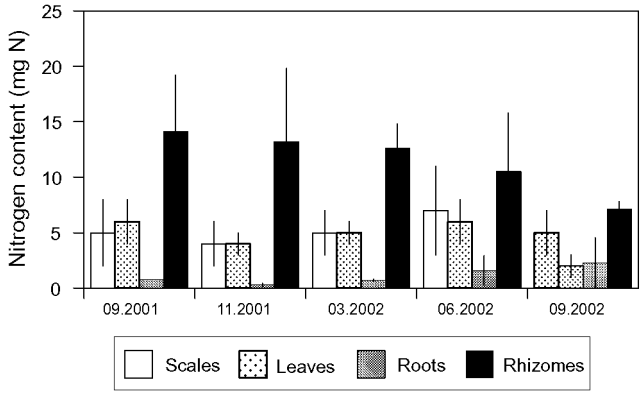


***Fig. 3.** Temporal evolution of mean values (±standard deviation) of nitrogen contents in scales, leaves, roots and rhizomes of Posidonia oceanica cuttings from September 2001 to September 2002. Number of samples = 5.*



4. Discussion

Although the survival rates of transplants are quite high (>90%) after one year, the weight and the elemental composition of transplants are significantly lower than those of reference plants (Fig. 1). Moreover, leaf weight and N contents of transplants in September 2002 are significantly lower than those of initial cuttings (Mann-Whitney test, $p \leq 0.001$). This is partly due to a change in growth orientation and the morphology of transplants, i.e. the progressive transition from a vertical (orthotropic) to an horizontal orientation (plagiotropic) which is the colonising form of *P. oceanica* (Meinesz et al., 1993). Plagiotropic shoots generally have a lower leaf biomass than orthotropic shoots (Caye, 1980). However, only a few shoots showed this change of growth orientation. This decrease of biomass and N content and this difference between the N content of transplant and reference shoots are probably due to the difficulty for transplants to meet their nutrient demand. Transplants are unable to replenish their nutrient reserves in spring, in contrast to reference shoots (Fig. 2). From November 2001 to March 2002, the leaf N contents of reference shoots increase significantly (Mann-Whitney test, $p \leq 0.01$), but not the leaf N contents of transplants (Mann-Whitney test, $p > 0.1$). This difficulty is not a consequence of a low nutrient availability as the concentrations of nutrient (at least N and P) in interstitial water of the sand patch are ten times higher than those in interstitial water of the meadow (Gobert et al., 2003). The physico-chemical properties of the interstitial water in the sand patch, i.e. pronounced anoxia and high sulphides contents, could be responsible for these difficulties. More likely, it is the inadequacy of uptake capabilities of transplants to plants N needs which are responsible of the difficulties to meet the nutrient demand. Indeed in absence of roots, the

leaves alone are unable to furnish enough N to ensure the N demand of the transplant. Normally, roots uptake of nutrients represent about 35% of the annual N need of *P. oceanica* and at least 50% of the nitrogen coming from the external environment (Lepoint et al., 2002b). Belowground biomass constitutes at least two thirds of the total biomass of *P. oceanica* (Duarte and Chiscano, 1999). In the Revellata Bay, this proportion increases to four fifths probably because of the very low nutrient concentration in this bay (Lepoint et al., 2002b). An important part of the energy of transplants is allocated to root formation and, after one year, root biomass is three time higher than that of initial cuttings which constitutes a significant increase (Mann-Whitney test, $p \leq 0.05$).

***Fig. 4**. Temporal evolution of mean values (±standard deviation) of the ^{15}N abundance in scales, leaves, roots and rhizomes of Posidonia oceanica transplants from September 2001 to September 2002. The natural abundance of ^{15}N in P. oceanica is 0.367 ± 0.005 ^{15}N% (Lepoint et al., 2000). Number of samples = 5.*

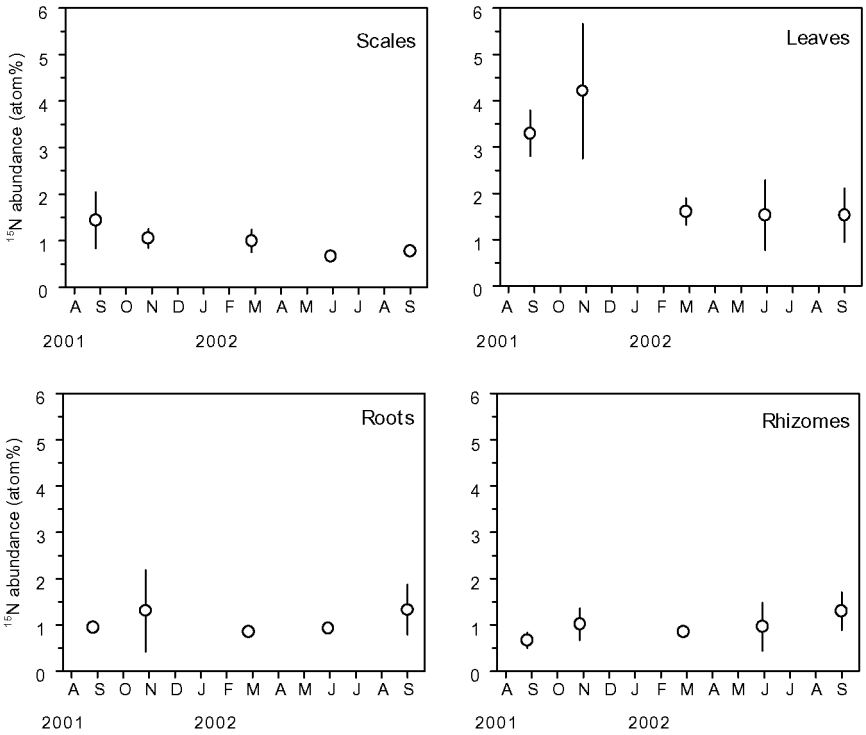
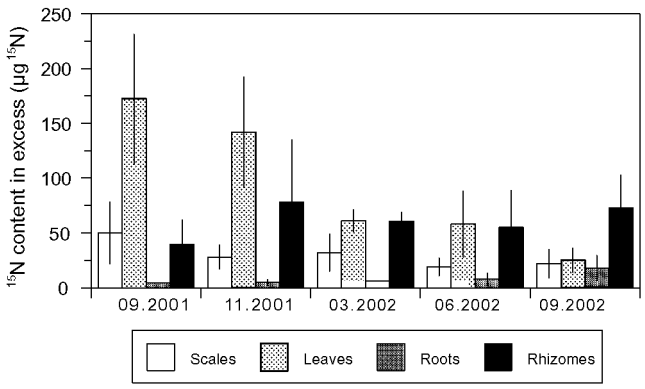


***Fig. 5**. Temporal evolution of mean values (±standard deviation) of ^{15}N contents in excess in scales, leaves, roots and rhizomes of Posi-donia oceanica cuttings from September 2001 to September 2002. Number of samples = 5.*



All organs of transplants contain ^{15}N in excess to the natural abundance of this isotope (Fig. 3). This is astonishing in the case of scales which are dead tissues constituted by the dead sheathes, remaining after the leaf decay. The ^{15}N measured in scales was probably passively absorbed during the labelling by the same mechanism involved as for Fe accumulation (Roméo et al., 1995). The ^{15}N abundance in scales does not show a clear temporal variation. As in the case of scales, the ^{15}N abundance in roots and in rhizomes shows little changes during this study. On the contrary, leaf ^{15}N abundance decreases drastically between November 2001 and March 2002. This is the result of the dilution of tracer pool by an external ^{14}N source. This demonstrates that the N leaf uptake is the main N source during this time for the leaf growth (Lepoint et al., 2002a). N leaf uptake is very important during winter and early spring when the nitrate concentrations are the highest in the water column of the Revellata Bay (Lepoint et al., 2002b). Normally, during this period, the N uptake exceeds the N requirements and incorporated N is transiently stored in leaves and rhizomes (Alcoverro et al., 2000). It is not the case in rhizomes of transplanted shoots as their ^{15}N abundance do not change (or slightly increase) during this period (i.e., the dilution of the ^{15}N pool of rhizome by a ^{14}N external source does not occur). This is also a sign that the N acquisition by the transplants is different from reference shoots: the storage of N in rhizomes is low, showing that incorporated N is directly allocated to leaf growth and N storage does not occur.

About 50% of ^{15}N added in June 2001 was lost, mainly through leaf decay. In terms of ^{15}N, leaves are the main stock of nitrogen at the beginning. The ^{15}N content of leaves decreases continuously, showing that the plant is unable to completely conserve its ^{15}N stock in the leaves despite occurring recycling mechanisms (Lepoint et al., 2002a). This ^{15}N is lost through the natural leaf decay, but is also partly channelled to roots development, as shown by the significant increase (four times) of the roots ^{15}N content. This demonstrates that the development of roots is of high priority for the transplant which is able to re-mobilise N from one organ to ensure the growth of another. The ^{15}N found in roots after one year represents only 7% of the ^{15}N quantity added initially. As ^{15}N abundance (^{15}N%) of roots does not change (or slightly increases), it appears that this internal re-mobilisation constitutes the main source of nitrogen for the root growth (and not an external ^{14}N source). This pleads for the existence of some N pools in the plant not mobilisable at the same time.

This experiment demonstrates that *P. oceanica* transplants are probably unable to completely meet their nutrient requirements due to the low development of their roots. Tracer experiment shows that the plant is unable to replenish its N reserve during winter, contrary to *P. oceanica* in its natural environment. On the other hand, the cuttings re-allocate a part of the N contained in leaves and rhizomes to the roots formation, which seem to be a priority of the plant development. Hereby, the availability of nutrients is not the reason for this difficulty. This suggests that the use of fertiliser is inadequate to increase the transplantation success. On the contrary, attention to root development and use of specific growth hormones should be a good method to increase the success of *Posidonia* transplantation (Balestri and Bertini, 2003).

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References

Alcoverro, T., Duarte, C.M., Romero, J., 1995. Annual growth dynamics of *Posidonia oceanica:* contribution of large-scale versus local factors to seasonality. Marine Ecology Progress Series 120, 203-210.

Alcoverro, T., Manzanera, M., Romero, J., 2000. Nutrient mass balance of the seagrass *Posidonia oceanica:* the importance of nutrient retranslocation. Marine Ecology Progress Series 194, 13-21.

Augier, H., Eugene, C, Harmand-Desforges, J.M., Sougy, A., 1996. *Posidonia oceanica* re-implantation technology of the marine gardeners is now operational on a large scale. Ocean Coastal Management 30, 297-307.

Balestri, E., Bertini, S., 2003. Growth and development of *Posidonia oceanica* seedlings treated with plant growth regulators: possible implications for meadow restoration. Aquatic Botany 76, 291-297.

Balestri, E., Cinelli, F., 2002. Sexual reproductive success in *Posidonia oceanica.* Aquatic Botany 75, 21-32.

Buia, M.C., Zupo, V., Mazzella, L., 1992. Primary production and growth dynamics in *Posidonia oceanica.* PSZNI Marine Ecology 13, 2-16.

Caye, G., 1980. Analyse du polymorphisme caulinaire chez *Posidonia oceanica* (L.) Delile. Bulletin de la Société de Botanique Française 127, 257-262.

Duarte, CM., Chiscano, C.L., 1999. Seagrass biomass and production: a reassessment. Aquatic Botany 65, 159-174.

Genot, I.,Caye, G., Meinesz, A., Orlandini, M., 1994. Role of chlorophyll and carbohydrate contents in survival of *Posidonia oceanica* cuttings transplanted to different depths. Marine Biology 119,23-29.

Gobert, S., Defawe, O., Lepoint, G., Demoulin, V., Bouquegneau, J.M., 2001. Anthesis effects on *Posidonia oceanica* (L.) Delile phenology in the Bay of Calvi (Corsica, Mediterranean Sea). Hydrobiologia 455, 121-125.

Gobert, S., Kyramarios, M., Lepoint, G., Pergent-Martini, C, Bouquegneau, J.-M., 2003. Variations à différentes échelles spatiales de l'herbier à *Posidonia oceanica* (L.) Delile; effets sur les paramètres physico-chimiques du sédiment. Oceanologica Acta 26, 199-207.

Invers, O., Pérez, M., Romero, R., 2002. Seasonal speciation in temperate seagrass *Posidonia oceanica* (L.) Delile. Journal of Experimental Marine Biology and Ecology 273, 219-240.

Lepoint, G., Nyssen, F., Gobert, S., Dauby, P., Bouquegneau, J.-M., 2000. Relative impact of a *Posidonia* seagrass bed and its adjacent epilithic algal community in consumers diet. Marine Biology 136, 513-518.

Lepoint, G., Defawe, O., Gobert, S., Dauby, P., Bouquegneau, J.-M., 2002a. Experimental evidence for N recycling in the leaves of the seagrass *Posidonia oceanica.* Journal of Sea Research 48, 173-179.

Lepoint, G., Millet, S., Dauby, P., Gobert, S., Bouquegneau, J.M., 2002b. An annual nitrogen budget of the seagrass *Posidonia oceanica* as determined by in situ uptake experiments. Marine Ecology Progress Series 237, 87-96.

Marba, N., Hemminga, M.A., Mateo, M.A., Duarte, C.M., Mass, Y.E., Terrados, J., Gacia, E., 2002. Carbon and nitrogen translo-cation between seagrass ramets. Marine Ecology Progress Series 226, 287-300.

Meinesz, A., Molenaar, H., Bellone, E., Loques, F., 1992. Vegetative reproduction in *Posidonia oceanica.* 1. Effects of rhizomes length and transplantation season in orthotropic shoots. PSZNI Marine Ecology 13, 163-174.

Meinesz, A., Caye, G., Loques, F., Molenaar, H., 1993. Polymorphism and development of *Posidonia oceanica* transplanted from different parts of the Mediterranean into the national park of Port-Cros. Botanica Marina 36, 209-216.

Molenaar, H., Meinesz, A., Caye, G., 1993. Vegetative reproduction in *Posidonia oceanica.* Survival and development in different morphological types of transplanted cuttings. Botanica Marina 36, 481-4188.

Pergent-Martini, C, Pasqualini, V., 2000. Seagrass population dynamics before and after the setting up of a wastewater treatment plan. Biologica Marina Mediterranea 7, 405-408.

Roméo, M., Gnassia-Barelli, M., Juhel, T., Meinesz, A., 1995. Memorization of heavy metals by scales of the seagrass *Posidonia oceanica,* collected in the NW Mediterranean. Marine Ecology Progress Series 120, 211-218.

Vangeluwe, D., Lepoint, G., Bouquegneau, J.M., Gobert, S., submitted for publication. Effect of experimental transplantation on the health status and C, N, P distribution in the leaves and the belowground parts of *Posidonia oceanica* (L.) Delile. Oceanologica Acta.