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Experimental evidence for N recycling in the leaves of the seagrass *Posidonia oceanica*

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Abstract

A one-year in situ experiment using ^{15}N as a tracer was designed to assess the N recycling in the leaves of the seagrass *Posidonia oceanica* (L.) Delile. *P. oceanica* was shown to partly recycle the internal nitrogen pool of its leaves in order to contribute to new leaf growth. The leaves sampled in June 1999 contained 20% of the quantity of ^{15}N found in June 1998. N recycling caused a difference between N and biomass turnover rate (0.8 vs 1.3 y^{-1}) of *Posidonia* leaves. This 40% difference should correspond to the contribution of recycled N to the annual N requirement of *Posidonia* leaves. The N recycling appears to be insufficient to significantly reduce the quantitative impact of N loss due to autumnal leaf fall. However, new leaf growth between June and October is mainly sustained by this recycling because the tracer concentration in new leaves was the same as in the other leaves. By contrast, tracer concentration decreased drastically between October 1998 and June 1999, showing the more important contribution of N uptake during winter and spring. Nevertheless, recycling occurs throughout the year as demonstrated by the presence of tracer in the youngest leaves of shoots sampled one year after the tracer addition.

Keywords: Nutrient recycling; ^{15}N; Seagrasses; *Posidonia;* Mediterranean Sea

1. INTRODUCTION

The occurrence of seagrass meadows in nutrient-poor coastal waters reflects the high efficiency of marine phanerogams to take up and/or retain nutrients, within the plant or the meadow (Hemminga et al., 1991). The re-use of endogenous nutrients, re-mobilised from old leaves before abscission and translocated to growing tissues could be an important process to meet the nitrogen requirements of seagrass shoots (Patriquin, 1972), and to reduce the dependence of seagrass on exogenous nitrogen sources (Borum et al., 1989). However, Hemminga et al. (1999) estimated that, although many species grow in nutrient-poor waters, the nutrient conservation strategies were paradoxically not strongly developed in most seagrasses. On average, this process meets only 10 to 20% of the annual N needs of most seagrass species (Pedersen and Borum, 1992; Stapel and Hemminga, 1997).

Hemminga et al. (1999) pointed out the lack of experimental measurements of nutrient recycling. In most studies, nutrient recycling is calculated from the decrease in leaf nutrient concentration (e.g. Shaver and Melillo, 1984; Stapel and Hemminga, 1997; Alcoverro et al., 2000). ^{15}N tracer experiments offer a good tool to follow the internal dynamics of nitrogen in seagrasses (Borum et al., 1989; Pedersen et al., 1997; Stapel et al., 2001). For example, short-term (4-week) aquarium experiments on *Zostera marina* show that 90% of the N lost from old leaves was recovered by other parts of the plant (Borum et al., 1989). In situ, this process could contribute up to 12% of the annual N demand of the leaves (Pedersen and Borum, 1992).

Posidonia oceanica (L.) Delile is a large and long-living seagrass which shows a seasonal biomass cycle with an early summer maximum and a winter minimum (Bay, 1984; Romero, 1989; Gobert et al., 1995). This cycle and primary production are mainly controlled by light and water temperature (Marba et al., 1996). Nutrient availability acts as a local and seasonal limiting factor: shoot growth is generally nutrient limited where the interstitial and column waters are nutrient depleted in summer (Alcoverro et al., 1995).

The aim of this long-term ^{15}N tracer experiment was to establish in situ to what extent N re-mobilisation from adult *P. oceanica* leaves contributes to the growth of young leaves.

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2. MATERIAL AND METHODS

2.1. Study location

The experiment was conducted in the *Posidonia oceanica* seagrass meadow of the Revellata Bay (Gulf of Calvi, Corsica, France) (Fig. 1), close to the marine research station STARESO, from June 1998 to June 1999. The growth dynamics and the ecological characteristics of this meadow have been studied since the 1970s (e.g. Bay, 1984; Frankignoulle and Bouqueg-neau, 1990; Pergent-Martini et al., 1994; Havelange et al., 1997; Dalla Via et al., 1998; Gobert et al., 2001). The seagrass bed covers about 75% of the Revellata Bay soft bottom (i.e. 14 hectares), extending from 0 to 38 m depth.

2.2. Experimental design

In June 1998, 40 shoots of *P. oceanica* were marked with a small float and isolated in plastic bags.

They were spaced 2 m apart along the 10 m depth isobath. Five cm of a ^{15}N labelled ammonium sulphate solution (10 mM; 99.0%^{15}N) (Eurisotop, France) were added in each plastic bag with a syringe. After two days, the plastic bags were removed and the leaves of four marked shoots were collected (= T_0). The leaves of the other shoots were punched with a hypodermic needle just above the ligula of the outermost leaf (Zieman, 1974). For the following samplings ($T \Rightarrow T_4$) (Table 1), the leaf material comprised between the ligula and this mark was considered to be the tissue formed after tracer addition and was named 'new tissue'. The whole leaf was considered to be new tissue when the mark was no longer present. At the end of this experiment, 21 marked shoots (i.e. 50%) were not recovered due to float losses.

Fig. 1. *Location of the experiment site in front of the oceanographic station STARESO in Revellata Bay (Gulf of Calvi, Corsica) (8°45'E, 42°35'N).*

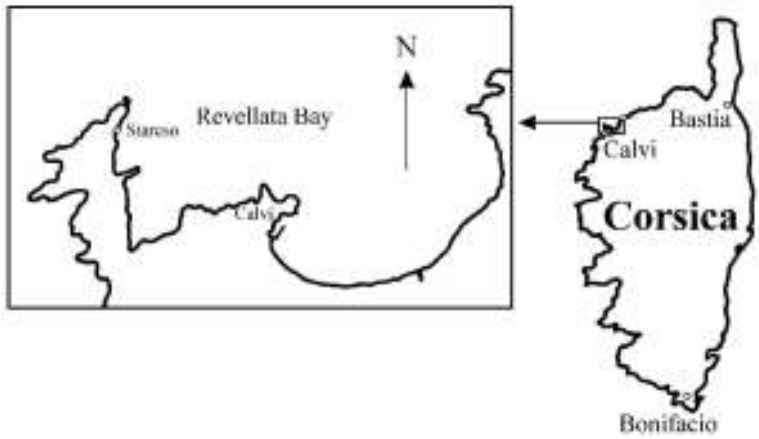

Table 1*. Sampling dates, number of shoots (n), mean values (± sd) of shoot weight, N content, N concentration, N abundance and percentage of new tissues reported to T_0 in Posidonia oceanica shoots sampled during a N tracer experiment*

Date	n	Shoot dry weight (mg_{dw})	N content (mgN $shoot^{-1}$)	N concentration ($\%_{dw}$)	N abundance (Atom $^{15}N\%$)	New tissues (%dw)
To 05.06.1998	4	1218 ± 457	11 ± 4	0.9 ± 0.1	4.47 ± 0.89	0
T_1 13.06.1998	5	1652 ± 176	17 ± 4	1.0 ± 0.1	4.19 ± 1.16	1 ± 0
T_2 25.08.1998	4	702 ± 269	5 ± 3	0.7 ± 0.2	4.35 ± 1.62	32 ± 5
T_3 29.10.1998	3	497 ± 96	4 ± 1	0.8 ± 0.1	6.23 ± 1.29	72 ± 8
T_4 08.06.1999	3	1047 ± 131	16 ± 3	1.5 ± 0.1	1.08 ± 0.32	100 ± 0

After collection, the *P. oceanica* leaves were scraped with a razor blade to remove the leaf epiphytes (Dauby and Poulicek, 1995). The leaves of each plant were separated and numbered from the youngest to the oldest. New and old tissues (see above) were separated. Each leaf fraction was dried for 48 h at 50 °C and weighed (± 0.1 mg). New and old materials were pooled per individual leaf and finely ground.

In the pooled samples, isotopic and elemental measurements were performed in triplicates with an Optima mass spectrometer (Micromass, UK) coupled to a C-N-S elemental analyser (Carlo Erba, Italy). The abundance of ^{15}N in *Posidonia* leaves was expressed in atom $\%^{15}N$. 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 calculated from the formula describing the isotopic mixing (Eq. (1)) the expected ^{15}N abundance in leaves without any N recycling. If no N recycling occurs, new tissues will have a natural N abundance and old tissues conserve the ^{15}N abundance of T_0 samples (i.e. 4.469 $\%^{15}N$). In Revellata Bay, the natural abundance of ^{15}N in *Posidonia* leaves is 0.367 ± 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).

$$A_{exp} = A_{nat} \cdot \%_{new} + A_{ini} \cdot (1 - \%_{new}) \qquad (1)$$

with: A_{exp}: ^{15}N abundance expected in *Posidonia* leaves without any N recycling (atom$\%^{15}N$); A_{nat}: natural N abundance (atom% N); A_{ini}: initial N abundance (atom$\%^{15}N$) (T_0, Table 1). $\%_{new}$: percentage of new tissues relative to total dry weight.

For this calculation, we assume that there was no N transfer between the leaves and rhizomes. As the rhizomes were not sampled in this study, the quantity of tracer stored there is unknown. However, as the ^{15}N content in leaves of T_0 and T_1 samples (i.e. initial and samples collected after one week) were not significantly different (Mann-Whitney test, $p>0.2$), we suggest that N storage in rhizomes was not important at this time and that the quantity of tracer contained in rhizomes was low compared to the ^{15}N pool of the leaves.

3. RESULTS

Of the four periods investigated in this study, the weight and N content of leaves were highest in June 1998 and lowest in October 1998 (Table 1). The N concentration in leaves was highest in June 1999 and lowest in August 1998. After the addition of the ^{15}N tracer, the ^{15}N abundance in *Posidonia* leaves was higher than the natural ^{15}N abundance (Table 1). This remained detectable one year after the ^{15}N enrichment when all the leaf biomass was renewed.

The mean ^{15}N abundance did not show significant variations between T_0, T_1, T_2 and T_3 (Mann-Whitney test, $p > 0.3$). The leaves sampled in June 1999 (T_4) were significantly impoverished in ^{15}N compared to the other sampling dates (Mann-Whitney test, $p <0.05$). Nevertheless, the N abundance in T4 samples still represented three times the natural ^{15}N abundance in *Posidonia* leaves. The proportion of new tissue increased from T_0 to T_4. In June 1999, all tissues were newly formed.

The leaf N content (mg N $leaf^{-1}$) was highest in June 1999 and lowest in October 1998 for each leaf rank (Fig. 2A-E). The distribution pattern of N content was similar in June 1998 and 1999 (T_0, T_1 and T_4) with the highest N content in the third or fourth leaf. In August and October, the N leaf contents were lower.

The leaf N concentration ($\%_{dw}$) decreased from the youngest leaf (rank 1) to the oldest (Fig. 2A-E). This decrease was slight from rank 1 to 3 and more pronounced from rank 3 to the last one. The greatest difference between minimum and maximum N concentrations was observed in October 1998, when leaf rank 1 was $1.3\%_{dw}$ and leaf rank 8 was $0.3\%_{dw}$. Individually, each leaf was always enriched in ^{15}N, even in June 1999 (Fig. 2F-J).

According to the increase of the new tissue proportion, the expected ^{15}N abundance decreases from T_0 to T_4 (Fig. 3). The measured abundance was equal to or higher than expected in the absence of N recycling. However, the measured ^{15}N abundance decreased drastically between October 1998 and June 1999, tending towards the expected ^{15}N abundance.

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Fig. 2. *Mean values (± s.d.) of N content (histogram, A-E), N concentration (black square, A-E), ^{15}N abundance (F-J, open circles) and the proportion of new tissues (F-J, black circles), in relation to the leaf rank of Posidonia oceanica shoots sampled on five occasions after the addition of a ^{15}N tracer solution (5 June 1998). Leaves are ranked from the youngest to the oldest.*

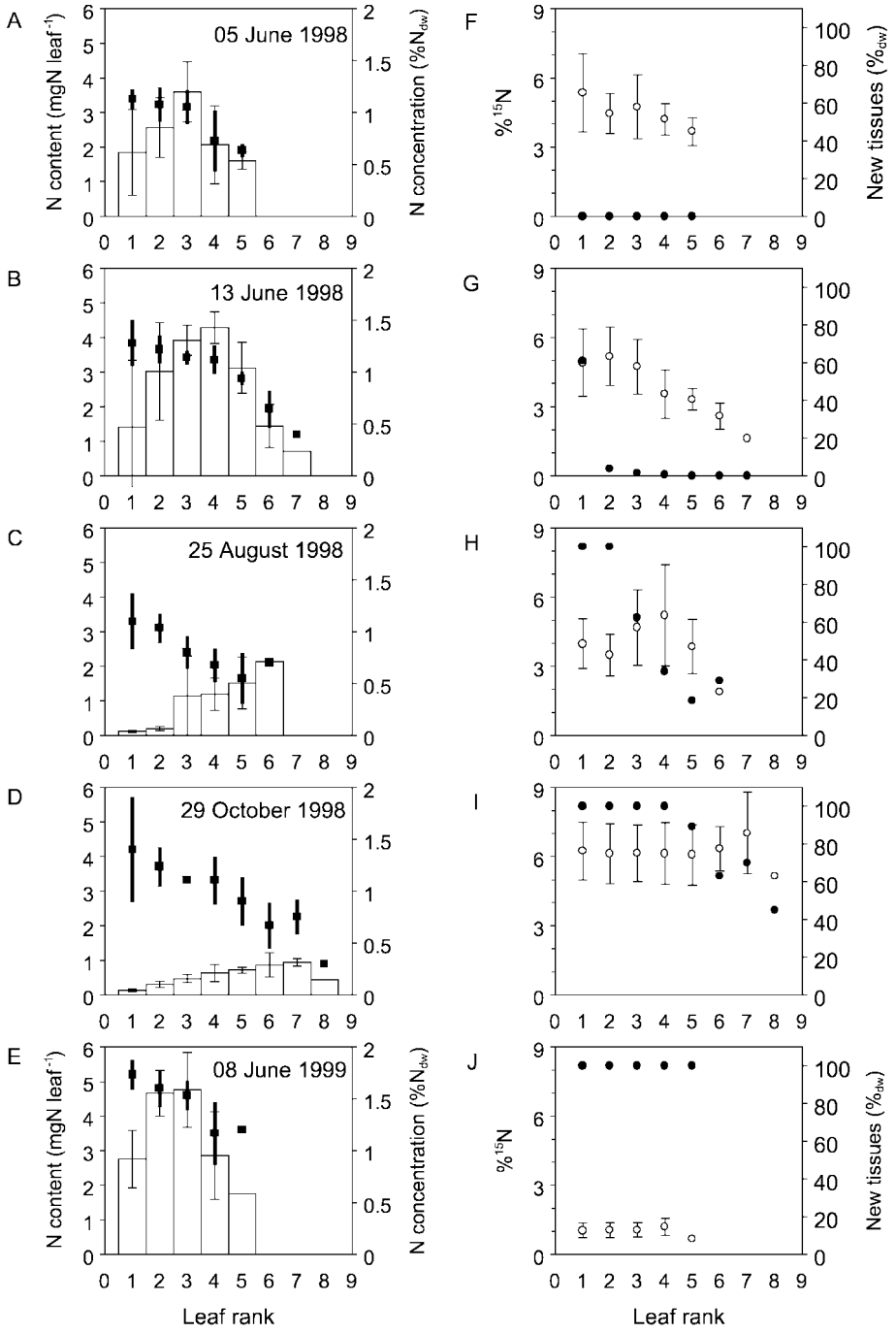
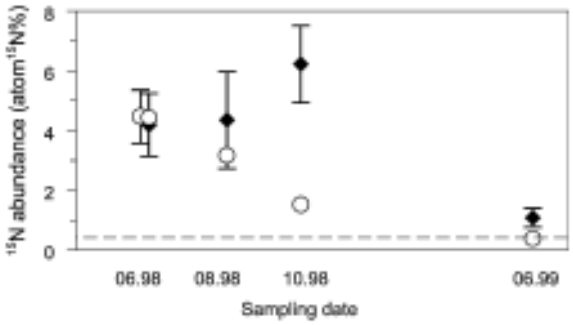


Fig. 3. *Expected values (circles) and mean (± s.d.) measured values (diamonds) of ^{15}N abundance in Posidonia leaves of shoots sampled on five occasions after the addition of a N tracer solution. Interrupted line: natural abundance of ^{15}N in P. oceanica leaves (i.e. 0.3673%^{15}N) (Lepoint et al, 2000).*



4. DISCUSSION

The leaf N concentration decreased with increasing leaf age, which is common in many seagrasses (Borum et al., 1989; Kraemer and Mazzella, 1999; Alcoverro et al., 2000). This pattern does not only represent the N resorption and recycling processes (Stapel and Hemminga, 1997), but also corresponds to the dilution of the nitrogen pool by the addition of structural components such as lignin (Klap et al., 2000) or, during senescence, the resorption of other elements (e.g. C, P, Mg) (Hemminga et al., 1999). The ^{15}N methodology offers the possibility to trace directly and specifically the internal N recycling.

Although their leaf biomass was entirely renewed, the leaves sampled in June 1999 contained 20% of the ^{15}N incorporated in June 1998 (Table 1, Fig. 2). This experimentally demonstrates the re-use of the N from old leaves to partly ensure the growth of young or new leaves. The difference between this measured N turnover and biomass turnover measured by Bay (1984) and Pergent-Martini et al. (1994) is about 40% (i.e. 0.8 y^{-1} vs 1.3 y^{-1}*),* which should correspond to the contribution of recycling in the annual N requirement of *P. oceanica* leaves. This matches the calculations of Alcoverro et al. (2000) but is higher than for other seagrasses (10-20%) (e.g., Pedersen and Borum, 1992; Stapel and Hemminga, 1997; Hemminga et al., 1999). Alcoverro et al. (2000) hypothesised that the long lifespan of *P.oceanica* leaves (longer than those of other seagrasses: on average 150 vs 88 days, Hemminga et al., 1999) allows increased N retention time and thus enhances the efficiency of N resorption.

Between June and October, the leaf weight and the leaf N content decreased drastically, as a consequence of the autumnal leaf fall (Table 1). The N recycling appears to be insufficient to significantly reduce the quantitative impact of this loss as N content and leaf weight decreased in the same way. However, in August and October, the ^{15}N abundance in new leaves (rank 1 to 4 in October) was not significantly different from those of old leaves (Fig. 2) and measured ^{15}N abundance was higher than expected in the absence of N recycling (Fig. 3). Therefore, new leaf growth between June and October was mainly sustained by internal N recycling. This period corresponds to a nutrient depletion period of the water column and, probably, to a nutrient growth limiting and nutrient imbalance period for *Posidonia* shoots (Alcoverro et al., 1995). The re-use of the internal N pool reduces this seasonal nutrient imbalance for *Posidonia* shoots (Alcoverro et al., 2000), but is insufficient to significantly decrease the impact of leaf fall. Consequently, the need of external N to replenish autumnal loss and to ensure leaf growth is important. The decrease of tracer abundance between October (T_3) and June (T_4) (Figs. 2 and 3) is the result of dilution of the tracer pool by an external ^{14}N source. This demonstrates that N uptake is the main N source for leaf growth during this period. As measured by in situ ^{15}N methodology, N uptake by leaves occurs the year round, but is very important during winter and early spring when the nitrate concentrations are highest in the water column of Revellata Bay (Lepoint et al., 2002). During this period, the N uptake exceeds the N requirements and incorporated N is transiently stored in leaves and rhizomes (Alcoverro et al., 2000). Nevertheless, the presence of ^{15}N tracer in the youngest leaves of June 1999 demonstrates that N re-sorption and recycling also occur throughout the year. This explains the importance of this process on the annual scale.

In conclusion, this experiment proves that N recycling occurs in the leaves of *P.oceanica.* This recycling is unable to decrease significantly the quantity of N lost due to autumnal leaf fall. However, this process ensures the N need of leaves between early summer and autumn, which is a nutrient-depleted period in Revellata Bay. We estimate that N re-use could contribute up to 40% of the annual N requirements of *P. oceanica.* This constitutes a higher contribution than in N budgets of other seagrasses.

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