Vol. 361: 69–82, 2008
doi: 10.3354/meps07405

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published June 9

^{13}C and ^{15}N translocation within and among shoots in two *Posidonia* species from Western Australia

Patricia Prado[1,*], Catherine J. Collier[2], Paul S. Lavery[3]

[1]Centre d'Estudis Avançats de Blanes, CSIC, Ctra. Acceso Cala St. Francesc 14, 17300 Blanes, Girona, Spain
[2]School of Marine and Tropical Biology, James Cook University, Townsville, Queensland 4811, Australia
[3]School of Natural Sciences, Edith Cowan University, 100 Joondalup Drive, Joondalup, Perth, Western Australia 6027, Australia

ABSTRACT: Translocation of ^{13}C and ^{15}N was investigated at the spatial scales of within-shoot (i.e. the seagrass clonal unit including leaves and associated vertical rhizome) and among-shoots in a mixed meadow of *Posidonia sinuosa* and *P. australis*. Incubation with ^{13}C and ^{15}N was conducted in either the oldest leaf of a shoot (i.e. within-shoot scale) or in the first shoot on the 4th or 5th branch of the main axis (i.e. among-shoots scale) and collected several times within a 1 mo period. We tested the following hypotheses: (1) developmental features in *P. australis* such as thicker and more open vascular system, higher primary production but lower leaf lifespan cause higher translocation in this species than in *P. sinuosa*, (2) translocation of ^{15}N and ^{13}C are largely influenced by source–sink organ relationships resulting in higher partitioning of C to rhizomes, whereas N is preferentially moved away to leaves, and (3) ^{15}N and ^{13}C transport towards the apical region is more dominant in *P. australis* than in *P. sinuosa*. As predicted, higher isotope content was found at both spatial scales in *P. australis* but differences were related to enhanced incorporation during incubation in this species. When both spatial scales were compared, both species showed higher ^{15}N translocation to young leaves within the same shoot, whereas in the among-shoots experiment most of the material remained within the leaves of the incubated shoot. In contrast, translocation of ^{13}C occurred mainly to rhizomes and tended to be higher at the among-shoots scale, particularly in *P. sinuosa*. No directionality was detected for either *P. australis* or *P. sinuosa*, possibly as a result of the low rates of N translocation at the among-shoots scale and the morphology of the vascular system allowing the integration of neighbouring plant parts for C requirements. Unlike for Western Australian species, the available literature on *P. oceanica* indicates patterns of among-shoots N distribution that are similar to those of C, which suggests that species are adapted to distinctive ambients.

KEY WORDS: *Posidonia* spp. · ^{15}N and ^{13}C translocation · Stable isotopes · Plant scale · Vascular architecture · Source–sink relationships · Western Australia

INTRODUCTION

Plant communities are open systems in which biogeochemical functions consist of nutrient inputs from various sources, outputs to sinks, and a variable extent of recycling (Vitousek & Reiners 1975). Thus, plants have developed strategies to cope with a spectrum of environmental conditions and maintain an adequate nutrient balance. In particular, seagrasses may obtain up to 60 to 70% of their total N uptake through leaves (Hemminga et al. 1991) and so are particularly sensitive to changes in nutrient availability in the water column (Touchette & Burkholder 2000). However, as seagrasses often inhabit nutrient-limited waters, they display mechanisms such as clonality to reduce dependence on uptake from external media. Being clonal, shoots are integrated through a below-ground rhizome mat from which nutrients and photosynthetic products can be remobilised (Fourqurean & Zieman 1991, Alcoverro et al. 2001) and transported long distances

*Email: patricia@ceab.csic.es

(Harrison 1978, Libes & Boudouresque 1987, Marbà et al. 2002). The internal distribution of nutrients is affected by the developmental morphology of the clonal growth including branching patterns, distance between meristems, and the presence of dormant modules (Lovett-Doust 1981, de Kroon & Schieving 1990, Duarte 1991). Hence, apical dominance may be essential for ecosystem development because it determines the capacity of a species to quickly colonise new substrate, while investment of nutrients into lateral or axillary meristems may produce denser infilling of a meadow and an enhanced capacity for out-competing other species (Tomlinson 1974).

Plants can also meet physiological requirements by redistributing nutrients preceding leaf abscission or by increasing the residence time within living tissues. Nutrient resorption is widespread among plants (Jonasson & Chapin 1985, Saur et al. 2000), including seagrasses (Alcoverro et al. 2001, Lepoint et al. 2002), where it may provide up to 10–15% of the N and P for new growth (Stapel & Hemminga 1997). Leaf longevity influences the retention time of nutrients in plant tissues (Escudero et al. 1992), and fast-growing species or those with a higher number of sequential leaves may have much higher external requirements than slow-growing species (Jonasson & Chapin 1985). Therefore, the amounts of internal nutrients and free metabolites that are transported from one part of the plant to another (i.e. N and C translocation; Harrison 1978, Libes & Boudouresque 1987, Marbà et al. 2002) relies on the ability to optimise, in both time and space, possible adaptive mechanisms operating at different spatial scales and in different plant organs. This includes the redistribution of already invested products in leaves (i.e. leaf resorption) and/or in storage organs and the transport of new uptake material to meet growth demands.

Among seagrass species, the genus *Posidonia* has adopted a strategy of resource exploitation based on very long leaf lifespans (mean ~170 d) and thick carbohydrate-storing rhizomes with low rates of horizontal expansion (Hemminga et al. 1999, Alcoverro et al. 2000). Nonetheless, *Posidonia* spp. display differences in terms of leaf productivity (i.e. leaf longevity, growth and sequential development) which could influence N and C requirements. For instance, *P. sinuosa* has a horizontal rhizome with condensed semi-vertical axes with up to 2000 shoots m^{-2} at the shallowest sites (Cambridge & Hocking 1997). Meadow biomass is maintained by slow leaf replacement (~4 to 5 leaves $shoot^{-1}$ yr^{-1}; Cambridge 1999) and a reduced number of long-lived leaves (usually no more than 2 per shoot and surviving up to 245 d each; Marbà & Walker 1999). In contrast, shallow shoot densities in *P. australis* vary from 600 to 800 shoots m^{-2} (Cambridge & Hocking 1997). Rhizome morphology is more robust and open-branched than in *P. sinuosa*, and shoots support 3 to 4 comparatively shorter living leaves (~70 d; Cambridge 1996), which are replaced at a higher rate (up to 7 leaves $shoot^{-1}$ yr^{-1}; Cambridge 1999). The slower but more compact growth of *P. sinuosa* may allow it to exclude other species, accounting for the formation of single-species stands and a clear dominance when coexisting with *P. australis*, which might be restricted to areas with higher N availability (Cambridge 1996, 1999). In fact, the mean residence time of N within the leaf canopy is higher in *P. sinuosa* than in *P. australis* (Cambridge 1996), which further suggests that the overall mechanisms involved in translocation may differ between species.

Until now, no research has been conducted to examine C and N translocation within or among shoots in *Posidonia* spp. from Western Australia, though patterns of clonal integration (Marbà et al. 2002) and leaf resorption (Alcoverro et al. 2000) for their Mediterranean counterpart *P. oceanica* suggest that internal distribution to demand tissues could be an important mechanism to improve the efficiency of resource exploitation. The objective of the present study was to investigate the patterns of ^{15}N and ^{13}C translocation at the within- and among-shoots spatial scales in *P. sinuosa* and *P. australis*. Specific hypotheses were (1) the slower growing *P. sinuosa* has lower N and C requirements and translocates lower amounts to other leaves and to neighbouring shoots than faster growing *P. australis*, (2) since N content for both species is higher in leaves and C is stored in below-ground organs, more N acquired from external sources will be translocated to leaves, whereas more C will be translocated to rhizomes, and (3) both seagrass species will show preferential directionality towards the apical shoot, but this is more apparent in *P. australis* due to its faster growth exerting a higher apical demand.

MATERIALS AND METHODS

Translocation of C and N was examined near the lower limit (7 to 8 m depth) of a mixed *Posidonia sinuosa* and *P. australis* meadow where it was reasonable to expect favouring of apical over lateral transport in order to support growth at the light-limited deep edge. The site, located in the northeast of Garden Island, Cockburn Sound, Perth, Western Australia (37.5° 47.2′ E, 64.4° 09.73′ N), was visited for sampling from mid-December 2004 to mid-January 2005. Nutrient conditions at the study site are slightly oligotrophic with total annual concentrations ranging between 0.3 and 0.7 µM dissolved inorganic N, 0.4 and 1.3 µM inorganic P in the water column (Simpson et al. 1996)

and in the order of 57 µM total dissolved N in the sediment pore water (Collier 2006, P. S. Lavery unpubl. data).

Within-shoot translocation. ***Experiment design, isotope incubation and sample processing:*** The within-shoot (i.e. the seagrass clonal unit including leaves and associated vertical rhizome) experiment consisted of 6 replicate plots (4.5 × 3 m) containing *Posidonia sinuosa* and *P. australis*. Within each plot, 4 shoots of each species were selected and their oldest leaf incubated with ^{15}N and ^{13}C. Despite similar summer leaf area index (LAI) values for both species (Cambridge 1996), shoots were always selected with the same number of leaves (2 and 3 leaves for *P. sinuosa* and *P. australis* respectively) as their commonest natural rank (Marbà & Walker 1999) to minimise possible differences in ^{15}N and ^{13}C incorporation between replicates. Also in both experiments, the labelled shoot and as many shoots identifiably belonging to the same plant as possible were marked with a needle for leaf production measures according to Short & Duarte (2001). Shoots with older leaves that could potentially persist throughout the experiment (i.e. absence of necrosis and high leaf pigmentation) were selected.

The method used for incubation was similar to that described in Marbà et al. (2002). The oldest leaf (within-shoot experiment) or a shoot (among-shoot experiment) was enclosed within a plastic bag fitted with a filter cassette and a plastic tap that could be closed after isotope injection. Leakage was tested prior to the beginning of experiments by using a natural dye. In both cases, the injected solution contained $NaH^{13}CO_3$ and $^{15}NH_4Cl$ to achieve a final concentration of 300 µM $NaH^{13}CO_3$ and 40 µM $^{15}NH_4Cl$ (Marbà et al. 2002). N was supplied as NH_4 since uptake affinities are higher than for NO_3 (Touchette & Burkholder 2000).

The oldest leaf was enclosed within a 0.5 l plastic bag filled and injected with a known volume of seawater and with the isotopic solution and left to incubate for 2 h. One incubated shoot including leaves and vertical rhizome was immediately collected from each replicate plot and those remaining were subsequently harvested at 1, 3 and 4 wk after incubation (i.e. 1 of the 4 shoots per plot each time).

In the laboratory, plant parts (i.e. leaves and vertical rhizome) of collected shoots were separated into labelled leaf and young leaf/leaves, and their length and width measured. Epiphytes were scraped off leaves using a razor blade and leaves were then dried at 60°C for 48 h and weighed. Leaf production was assessed according to Short & Duarte (2001). Growth increments were measured, dried and weighed (g DW d^{-1}) and then combined with the remaining leaf for grinding in a Retsch mixer mill (MM 200).

Carbohydrate contents (starch and total soluble sugars) from ground samples were determined as in Dubois et al. (1956). $\delta^{13}C$ and $\delta^{15}N$ isotope values of samples were determined using an ANCA-NT (Europa Scientific) interfaced with a 20-20 isotope ratio mass spectrometer (Europa Scientific). $\delta^{15}N$ and $\delta^{13}C$ were determined as:

$$\delta_{sample-standard} = [(R_{sample} - R_{standard})/(R_{standard})] \times 1000 \quad (1)$$

where R_{sample} is ^{13}C:^{12}C in the sample; $R_{standard}$ is ^{13}C:^{12}C in the working reference gas ($CaCO_3$ from a calcium carbonate standard [PBD] and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$ measurements, respectively) which is calibrated against an internal standard (Atropina, International Atomic Energy Agency, IAEA, and/or US Geological Survey, USGS) and $\delta_{sample-standard}$ is the difference in isotope composition of the sample relative to that of the reference, expressed in per mille (‰).

$\delta^{15}N$ and $\delta^{13}C$ in leaves and rhizome samples were first converted to atom % ^{15}N and atom % ^{13}C following the equations (Gonfiantini et al. 1995):

$$\text{Atom \% } {}^{15}N_i = 100/\{272/[1 + (\delta^{15}N_{i/air}/1000)] + 1\} \quad (2)$$

$$\text{Atom \% } {}^{13}C_i = 100/\{89/[1 + (\delta^{13}C_{i/standard}/1000)] + 1\} \quad (3)$$

in which 272 and 89 are the ^{15}N:^{14}N and ^{13}C:^{12}C ratios in international standards, N_2 and Vienna PDB, respectively, for N and C. $\delta^{15}N_{i/air}$ is the $\delta^{15}N$ value of an unknown, i, expressed relative to atmospheric N_2. $\delta^{13}C_{i/standard}$ is the $\delta^{13}C$ value of an unknown, i, expressed relative to the PDB standard.

Atom % excess of $\delta^{13}C$ and $\delta^{15}N$ in the material was calculated by subtracting atom % values from reference leaves and rhizomes from the study site from the atom % of samples. They were then transformed into isotopic mass and concentrations.

Data analysis: Differences in ^{15}N and ^{13}C mass (µg) in young leaves and rhizomes were investigated with 2-way orthogonal ANOVA with Species and Time (constrained by a time period not exceeding excision of the older leaf) as fixed factors.

The content of mobile nutrients in senescent leaves is partly given by uptake rates and partly by leaf resorption. N and C were supplied as bioavailable isotopes that can be rapidly incorporated by the leaf and moved across to sink tissues; therefore, isotope translocation partly depended on incorporation rates during incubation. We investigated resorption from the double perspective of the total N and C pool (i.e. $^{14+15}N$ and $^{12+13}C$) and the single isotope pool to evaluate possible deviations in patterns of translocation. The first approach relied upon the supply of bioavailable isotopes. The following sets of calculations were used:

$$\%D_i = [^{15}N, {}^{13}C\ L2_{4wk}/^{15}N, {}^{13}C\ L1_{4h}] \times 100 \quad (4)$$

where $\%D_i$ is the percentage decrease in isotope content in the old leaf, $L1_{4h}$ is the mass of ^{15}N or ^{13}C (mg) in the old leaf immediately after incubation (mean of all shoots, n = 6) and $L2_{4wk}$ that in the young leaf at the end of the experiment. Then, the demand for new growth was estimated as if the decrease in isotope content was representative of the whole N and C pool:

$$N, C \text{ in } D = \%D_i\,[N, C\ L1_{4h}]/aL1_{4h} \times 100 \quad (5)$$

$$\text{'new' } N, C = [N_{4wk}, C_{4wk}\ L1_n]/aL1_{n\ 4wk} \quad (6)$$

$$\%ND_i = [\text{'new' } N, C\ L1_n/N, C \text{ in } D] \times 100 \quad (7)$$

where N, C in D is the mass of $^{14+15}N$ or $^{12+13}C$ in the decrease (mg cm^{-2}), $aL1_{4h}$ is the area of the old leaf immediately after incubation, 'new' N, C is the mass of $^{14+15}N$ or $^{12+13}C$ produced throughout the experiment (mg cm^{-2}), $aL1_n$ is the area of all the leaves which showed elongation after the 4 wk period and $\%ND_i$ is the percentage of N and C demand for the new growth estimated from the decrease in isotope content.

Secondly, the decrease in the total N and C pool with leaf senescence (i.e. the resorption efficiency) was adjusted from Shaver & Melillo (1984) and calculated as:

$$\%D = [(N, C\ L2_{4wk} - N, C\ L1_{4wk})/N, C\ L2_{4wk}] \times 100 \quad (8)$$

in which $\%D$ is the percentage of the decrease in N and C content, $L1$ is the mass of $^{14+15}N$ or $^{12+13}C$ (mg cm^{-2}) in the second oldest leaf (to minimise the dilution effect caused by the addition of structural material; Stapel & Hemminga 1997) in *Posidonia australis* or in the oldest leaf in *P. sinuosa* at the last time of the experiment (i.e. maximum senescence) and $L2$ is the mass of $^{14+15}N$ or $^{12+13}C$ (mg cm^{-2}) in the younger leaf/leaves. The N and C demand during the experiment was then calculated as above (Eqs. 5 to 7) from the decrease in the $^{14+15}N$ or $^{12+13}C$ content ($\%D$). All calculations were based on changes in N and C content per unit area, to avoid potential problems arising when comparing leaves that are not yet fully developed and to minimise deviations in decrease estimates due to leaf breakage during the experiment.

Among-shoots translocation. ***Experiment design, isotope incubation and sample processing:*** For the among-shoots experiment, the same 6 replicate plots were used as in the within-shoot study. In each plot, 4 plants (i.e. shoots connected by below-ground rhizomes) of *Posidonia sinuosa* and *P. australis* per replicate plot were selected following gentle rhizome exposure to confirm the position of the shoots. In each plant, 1 shoot (usually the first on the 4th or 5th branch along the main axis) was selected for incubation with ^{15}N and ^{13}C and marked for later retrieval. The same incubation method was used but for the whole shoot. Selected shoots (i.e. the first within the 4th or 5th branch from the rhizome apex) were incubated for 4 h within 1.5 l plastic bags. Following incubation, the bag was slid from the leaf—retaining the solution within it—and the water around the leaf fanned away. Immediately after incubation, the single labelled shoot was collected from each replicate plot. The remaining plants were sampled at 1, 2 and 4 wk after incubation and care was taken to harvest at least 9 branch insertions along the main axis (the labelled shoot plus the next 4 towards and away from the apex; Fig. 1) but on occasion, the size of the plant was reduced due to rhizome breakage. Samples were carefully placed into plastic bags and transported in an icebox for further processing in the laboratory. Shoots adjacent to the one labelled (approx.

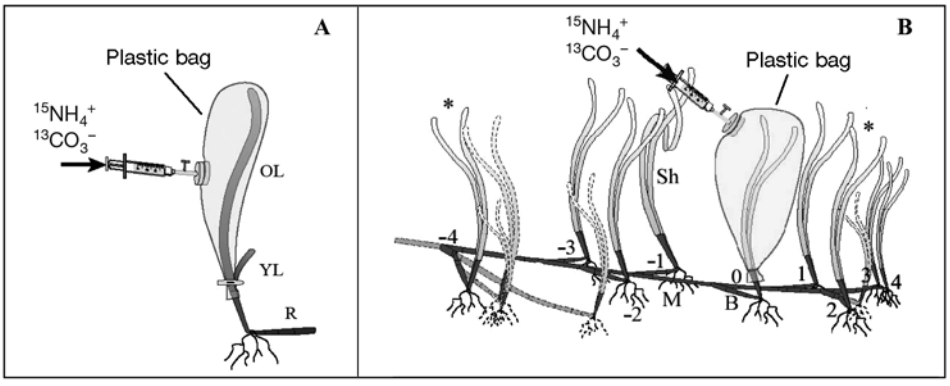

Fig. 1. Isotope incubation method in the (A) within-shoot (i.e. the oldest leaf is labelled) and (B) the among-shoots experiment (i.e. the first shoots located on the 5th branch position along the main axis are labelled; see 'Materials and methods; Among-shoots translocation' for further details). OL: old leaf; YL: young leaf; R: rhizome; Sh: shoot; B: rhizome branch; M: main axis; *: selected shoot at the branch position

5 to 10 cm away) were also collected to confirm the absence of leakage and some non-labelled shoots of *P. sinuosa* and *P. australis* were collected to record ambient $\delta^{15}N$ and $\delta^{13}C$ at the site during the experiment.

For each plant, the most recently divided shoot at the most terminal point of the main branch axis was considered the apex. The first shoot encountered within each division of the main axis (up to 9 when possible, see above) was retained for isotope analyses. These shoots are referred to hereafter as 'shoot position' running towards the apex (i.e. positions 1 to 4) or towards the back of the plant (i.e. positions –1 to –4) from the labelled shoot (Fig. 1). The use of only the first shoot was intended to minimise variation due to extremely large differences in branch size and the number of supporting shoots, particularly in *Posidonia australis*. However, since an important part of each branch was discarded (i.e. all material past the first shoot on each branch), this sampling method cannot account for all the ^{13}C and ^{15}N incorporated by the incubated shoot.

Rhizome branches (i.e. the section of the original branch from its insertion point in the main axis up to the rhizome of the first shoot; Fig. 1) and main axes sections (i.e. distance between adjacent shoot positions) were stripped of remnant sheath, and their length and diameter were measured. Leaves were scraped free of epiphytes, the length and width of the new growth measured and then dried together with the remainder of the leaves and the rhizome at 60°C for 48 h. Plant parts including leaves, main axes and rhizome branches were weighed and ground separately for isotope and carbohydrate analyses, as described in the previous section.

Data analysis: The 28 d plant sample for the among-shoots experiment was removed from the analyses due to difficulties collecting plants with up to 9 shoot positions. Morphological and ^{15}N and ^{13}C measures were averaged over the 8 and 15 d sampling times and differences between species assessed using *t*-tests.

Differences in the mass (µg) of ^{15}N and ^{13}C in labelled shoots were tested with 3-way orthogonal ANOVA with Time, plant Part (leaves and vertical rhizome) and Species as fixed factors. Equally, patterns of ^{15}N and ^{13}C contents (µg) within collected plants (i.e. the 9 branch insertions along the main axis; labelled shoot excluded) were assessed with 3-way orthogonal ANOVA with Species, sampling Time (8 and 15 d), and plant Part (leaves and vertical rhizome) as fixed factors.

Apical dominance and the influence of plant biomass on the directionality of N and C translocation were investigated by comparing patterns of isotope distribution in terms of both mass and concentration. For each shoot position (4 forward and 4 backward from the labelled shoot) isotope mass was the sum of contents within leaves, rhizome branches and main axes. Isotope concentration was determined by dividing the previously calculated isotope mass by the total DW of leaves, rhizome branches and main axes at each shoot position.

At each sampling time (8 and 15 d), patterns of ^{13}C and ^{15}N distribution (mass and concentration) were investigated with 2-way orthogonal ANOVA with Species and Direction (forward vs. backward) as fixed factors. For all ANOVAs, data were first tested for homogeneity of variances (Cochran's test) and normality (Kolmogorov-Smirnov distribution-fitting test of the residuals). Student-Newman-Keuls post hoc comparisons were used when necessary to investigate the existence of significant groups.

RESULTS

The 2 *Posidonia* species exhibited significant differences in morphological and growth characteristics as well as in N and C decrease within leaves and in patterns of isotope translocation at both spatial scales investigated. Nearly all morphological and growth features (leaf production, number of leaves per shoot, shoot weight, leaf width, branching distance and branch length) were higher in *P. australis* than in *P. sinuosa*, whereas shoot height and rhizome diameter did not differ between species (Table 1). Physiological features, including % N in leaves and total soluble sugars, were higher in *P. australis*, while C:N ratios and starch did not differ between species. Ambient $\delta^{15}N$ and $\delta^{13}C$ values in leaves were similar in both species,

Table 1. *Posidonia sinuosa* and *P. australis*. Morphological features. Values are mean ± SE per shoot (n = 9 shoot positions), pooled over the 6 replicate plots and sampling times. Significant differences between species: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Morphological feature	*P. sinuosa*	*P. australis*
Leaf productivity (mg DW shoot^{-1} d^{-1})	1.03 ± 0.2	4.30 ± 0.54**
Shoot weight (g shoot^{-1})	0.16 ± 0.02	0.36 ± 0.04***
Number of leaves shoot^{-1}	1.88 ± 0.05	2.71 ± 0.20**
Leaf height (cm)	29.53 ± 2.4	31.17 ± 2.14
Leaf width (cm)	0.73 ± 0.03	1.14 ± 0.04***
Branching distance (cm)	5.53 ± 1.12	10.22 ± 1.3*
Branch length (cm)	4.02 ± 0.7	9.92 ± 1.3**
Distance to last shoot (cm)	42.05 ± 8.8	68.37 ± 5.16
Rhizome branch area (cm^2)	0.09 ± 0.01	0.14 ± 0.01
Rhizome axis area (cm^2)	0.11 ± 0.01	0.18 ± 0.01
Rhizome distance to furthest shoot (cm)		
Forward (shoot positions 1 to 4)	13.8	29.3
Backward (shoot positions –1 to –4)	32.6	52.3

Table 2. *Posidonia sinuosa* and *P. australis*. Physiological variables. Values are mean ± SE per shoot (n = 9 shoot positions), pooled over the 6 replicate plots and sampling times. Carbohydrate values are mean ± SE of labelled shoots. L = leaf, R = rhizome, Sh = shoot. Significant differences between species: *p < 0.05

Physiological variable	Plant part	*Posidonia sinuosa*	*Posidonia australis*
N (%)	L	1.44 ± 0.05	1.63 ± 0.05*
	R	0.76 ± 0.1	0.60 ± 0.06
C:N	L	27.06 ± 0.9	25.49 ± 0.9
	R	67.95 ± 9.8	73.67 ± 6.5
Ambient $\delta^{15}N$	L	2.03	3.08
	R	2.75	2.38
Ambient $\delta^{13}C$	L	–10.14	–9.46
	R	–9.95	–10.82
Labelled $\delta^{15}N$	L	1024	1100
	Sh	1831	2580
Labelled $\delta^{13}C$	L	9.5	18.1
	Sh	0.95	11.28
Shoot beside labelled $\delta^{15}N$		15.3	11.4
Shoot beside labelled $\delta^{13}C$		–8.7	–7.4
Soluble sugars	L	56.5 ± 4.7	59.6 ± 1.4
(mg gDW^{-1})	R	89.2 ± 2.7	225.4 ± 35.9*
Starch (mg gDW^{-1})	L	65.2 ± 16.3	52.7 ± 18.6
	R	85.1 ± 2.6	84.4 ± 4.8
Total carbohydrates	L	121.7 ± 20.1	101.9 ± 18.8
(mg gDW^{-1})	R	174.4 ± 5.3	309.7 ± 40.5*

with values ranging from 2.03 to 3.08‰ and –9.46 to –10.82‰ for ^{15}N and ^{13}C, respectively (Table 2).

Within-shoot translocation

Labelled leaves

Initial isotope content in the labelled leaf was ~1.5 times higher in *Posidonia australis* than in *P. sinuosa* but showed similar masses for ^{15}N and ^{13}C. No temporal changes were detected in ^{15}N content for either species, whereas a decrease in ^{13}C was observed between the second and third sampling times (i.e. 8 to 21 d; Table 3, Fig. 2). However, differences in ^{15}N content between the labelled leaf and the rest of the shoot (i.e. younger leaves and rhizome) decreased from about 8 and 26 times higher (4 h after incubation) in *P. australis* and *P. sinuosa*, respectively, to about 3.5 times higher by the end of the experiment in both species, due to increased isotope content in young leaves and rhizomes. Also for ^{13}C content, initial differences between the labelled leaf and the rest of the shoot decreased from ~3 and 43 times higher in *P. australis* and *P. sinuosa*, respectively, to only ~0.4 at the last sampling.

Table 3. *Posidonia sinuosa* (P. s) and *P. australis* (P. a). 2-way ANOVA and Student-Newman-Keuls (SNK) tests on ^{15}N and ^{13}C (µg) in labelled leaf, young leaves (i.e. all non-labelled leaves pooled) and rhizome at 4 h and 8, 21 and 28 d after the beginning of the within-shoot experiment. Sp = species, Ti = time, *C* = Cochran's test. Significant differences are in **bold**

ANOVA		^{15}N								^{13}C							
		Labelled leaf			Young leaves			Rhizome			Labelled leaf			Young leaves			Rhizome	
	df	MS	*F*	p	MS	*F*	p	MS	*F*	p	MS	*F*	p	MS	*F*	p	MS	*F*	p
Source of variation																			
Sp	1	154.43	2.660	0.1121	1.8242	31.359	**0.0000**	0.7770	14.399	**0.0050**	158.10	1.5842	0.2167	23.272	33.652	**0.0000**	37.865	76.880	**0.0000**
Ti	3	27.99	0.482	0.6968	0.7038	12.099	**0.0000**	0.2095	3.882	**0.0160**	462.28	4.6322	**0.008**	8.657	12.518	**0.0000**	5.989	12.160	**0.0000**
Sp × Ti	3	34.65	0.595	0.6223	0.0482	0.828	0.4866	0.0643	1.192	0.3254	29.17	0.2923	0.8307	0.152	0.220	0.8822	0.140	0.205	0.8357
		Transf: –; *C* = 0.372			Transf: √√x; *C* = 0.292			Transf: √√x; *C* = 0.316			Transf: –; *C* = 0.488			Transf: √x; *C* = 0.274			Transf: √x; *C* = 0.24	
					SNK (Sp)			SNK (Sp)						SNK (Sp)			SNK (Sp)		
					P. s < P. a			P. s < P. a						P. s < P. a			P. s < P. a		
					SNK (Ti)			SNK (Ti)			SNK (Ti)			SNK (Ti)			SNK (Ti)		
					4 h = 8 d < 21 d = 28 d			4 h < 8 d = 21 d = 28 d			4 h = 8 d < 28 d = 21 d			4 h = 8 d = 21 d < 28 d			4 h < 21 d ≤ 8 d = 28 d		

^{15}N and ^{13}C in young leaves and rhizomes

Young leaves and rhizomes (all shoots from all times pooled) of *Posidonia australis* had more than 3 and 5 times higher ^{15}N content, respectively than *P. sinuosa*. However, both species displayed consistently higher ^{15}N content in young leaves than in rhizomes (~3 times) (Fig. 2, Table 3). Most of the ^{15}N translocation to young leaves occurred between 1 and 3 wk after the beginning of the experiment whereas that to rhizomes occurred earlier (Fig. 2A, Table 3).

Posidonia australis had more than 3 times the ^{13}C content of *P. sinuosa*, but within each species, there was similar mass in young leaves and rhizomes (Fig. 2B, Table 3). ^{13}C content in young leaves showed no significant increase until the last week of the experiment (i.e. 28 d; $p < 0.001$) whereas that in rhizomes increased 1 and 3 wk after the start of the experiment (Fig. 2B, Table 3).

N and C decrease and demand

The decrease of isotope in labelled leaves amounted to ~23.7 and 27.8% of ^{14}N and ~32.7 and 56.9% of ^{13}C, respectively, in *Posidonia sinuosa* and *P. australis*. Estimates of concentration decrease for the whole N and C pool ($^{14+15}N$ and $^{12+13}C$) were higher for N (~23.2% in *P. sinuosa* and 14.5% in *P. australis*) than for C (~2.6% in *P. sinuosa* and 3.7% in *P. australis*). Assuming that the reduction in the oldest leaf reflects demand by the youngest leaves, the 2 approaches to N and C decrease resulted in very different demands for the new growth. Isotope-based estimates yielded demands of ~30 and 43% of the N and 45.8 and 107% of the C in *P. sinuosa* and in *P. australis*, respectively. In contrast, N and C decrease in ageing leaves (based on changes of total contents within leaves) was ~29.3 and 14% of the N and 3.7 and 5% of the C in *P. sinuosa* and in *P. australis*, respectively.

Among-shoots scale

Labelled shoots

Isotope incubation of whole shoots resulted in ^{15}N and ^{13}C contents that were within the same order of magnitude in both species. ^{15}N content in *Posidonia australis* was ~3.5 times higher than in *P. sinuosa* and ~30 times higher in leaves than in rhizomes. Higher ^{15}N content in leaves of *P. sinuosa* 4 h after incubation (~2.7 times; $p < 0.001$) caused significant Time effects but differences were not significant thereafter (Fig. 3A, Table 4). ^{13}C was also consistently higher in *P. australis*

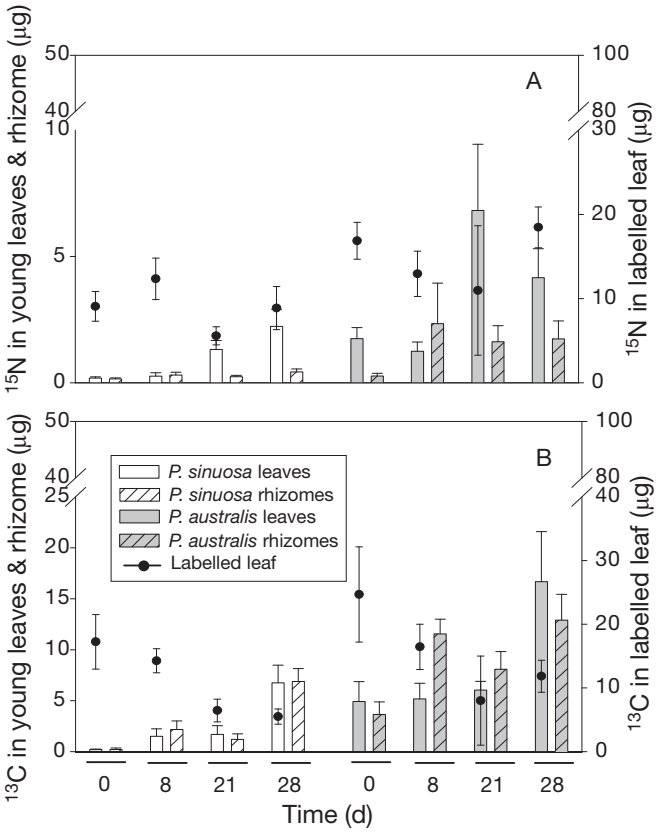

Fig. 2. *Posidonia sinuosa* and *P. australis*. (A) ^{15}N and (B) ^{13}C mass ± SE (µg) in labelled leaves, young leaves and rhizomes at the 4 sampling times during the within-shoot experiment

shoots than in *P. sinuosa* (~4.5 times) and higher in leaves than in the vertical rhizomes of the incubated shoot (~3.5 times) but there was also a significant Species × plant Part interaction. Initial ^{13}C content in leaves tended to decrease with time whereas that in rhizomes increased slightly, causing significant Time × plant Part interaction (Fig. 3B, Table 4).

Differences among plant parts

Translocation of isotopes to other plant parts was 1 order of magnitude lower for ^{15}N than for ^{13}C. *Posidonia australis* showed ~2.5 times higher ^{15}N content than *P. sinuosa* but differences were not significant when expressed as concentrations. There was a significant increase in the ^{15}N in plants (labelled shoot excluded) from 8 to 15 d after enrichment in terms of both mass and concentration (Table 5, Fig. 3C,D).

^{13}C content in *Posidonia australis* was ~4.2 times higher than in *P. sinuosa* and significantly higher in rhizome branches and main axis than in leaves (~5 times). Similar patterns were observed when data were expressed as concentrations but there was also a significant decrease over time (Table 5, Fig. 3E,F).

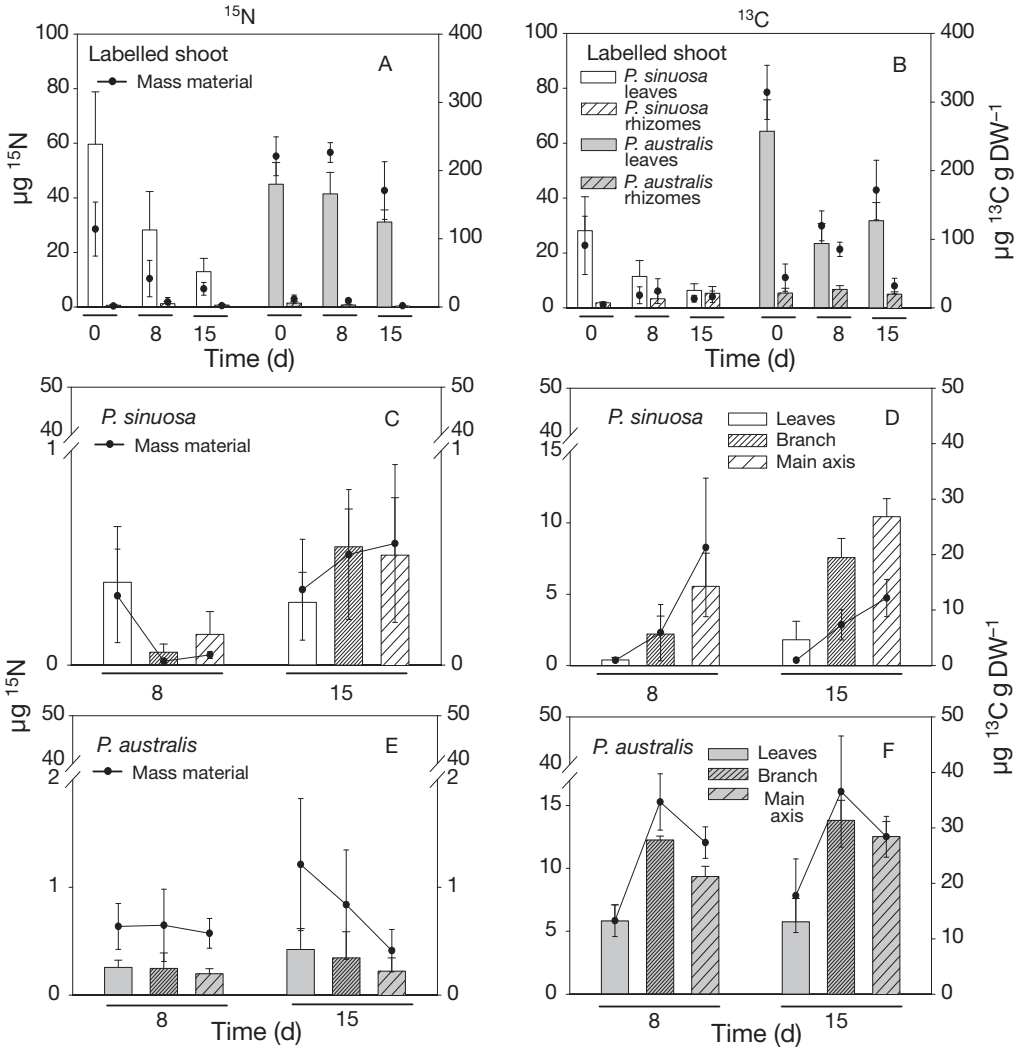

Fig. 3. *Posidonia sinuosa* and *P. australis*. (A,B) ^{15}N and ^{13}C (µg and µg g DW^{-1}) in labelled shoots at 0, 8 and 15 d after the beginning of the among-shoots experiment. (C–F) ^{15}N and ^{13}C (µg and µg g DW^{-1}) in plant parts (leaves, branches and main axis) 8 and 15 d after enrichment. Error bars are SE

Forward versus backward transport

Neither *Posidonia australis* nor *P. sinuosa* displayed directionality in patterns of isotope translocation along the main axis (Fig. 4). As indicated for previous analyses, there were significant differences between species. Eight days after incubation, the content of ^{15}N in *P. australis* (labelled shoot excluded) was ~4 times higher than in *P. sinuosa*, whereas differences were not significant when expressed as concentrations, revealing the influence of the larger plant biomass of *P. australis*. In contrast, ^{13}C was consistently higher in *P. australis* than in *P. sinuosa* in terms of both mass (~7 times) and concentration (~4 times) (Table 6, Fig. 4). Fifteen days after incubation, there were still differences in ^{13}C between species (~5 and 2 times higher in *P. australis*, for mass and concentration, respectively) but no effects were detected for ^{15}N (Table 6).

DISCUSSION

The translocation of incorporated ^{13}C and ^{15}N displayed similar patterns for *Posidonia australis* and *P. sinuosa*. However, there were differences in the amount of isotope assimilated during incubation and subsequent redistribution, indicating that both species have different mechanisms of resource exploitation. Consistently higher ^{15}N and ^{13}C content and leaf productivity in *P. australis* suggests that this species has achieved a faster uptake capacity than *P. sinuosa* (LAI values are similar in summer; Cambridge 1996) to sat-

Table 4. *Posidonia sinuosa* (P. s) and *P. australis* (P. a). 3-way ANOVA and Student-Newman-Keuls (SNK) tests on patterns of ^{15}N and ^{13}C (µg) in leaves (L) and rhizomes (R) of labelled shoots at 4 h, 8 d and 15 d after the beginning of the among-shoots experiment. Sp = species; Ti = time, P = plant part, *C* = Cochran's test. Significant differences are in **bold**

ANOVA	Isotope mass in labelled shoots						
		^{15}N			^{13}C		
	df	MS	*F*	p	MS	*F*	p
Source of variation							
Sp	1	5.759	43.01	**0.0000**	120.64	54.217	**0.0000**
Ti	2	0.599	4.47	**0.0185**	5.36	2.407	0.1045
P	1	23.289	173.91	**0.0000**	57.60	25.887	**0.0000**
Sp × Ti	2	0.337	2.51	0.0951	0.35	0.158	0.8546
Sp × P	1	0.526	3.92	0.0553	17.87	8.032	**0.0075**
Ti × P	2	0.046	0.34	0.7136	15.18	7.046	**0.0026**
Ti × Sp × P	2	0.248	1.85	0.1714	2.69	1.209	0.2873
		Transf: √√x; *C* = 0.212			Transf: √x; *C* = 0.346		
		SNK (Sp)			SNK (Ti × P)		
		P. s < P. a			R (4 h) ≤ R (15 d) = R (8 d) = L (15 d) = L (8 d) < L (4 h)		
		SNK (Ti)			SNK (Sp × Ti)		
		15 d = 8 d < 4 h			P. s (15 d) = P. s (8 d) < P. s (4 h) <		
		SNK (P)			P. a (15 d) = P. a (8 d) < P. a (4 h)		
		R < L					

isfy greater nutrient demands for growing tissues. Therefore, our first hypothesis predicting higher growth rates and isotope translocation in this species was upheld, except for ^{15}N at the among-shoots scale (Table 7). When expressed as a fraction of initially incorporated material, translocation was only higher in *P. australis* at the within-shoot scale. At this spatial scale, demands for N and C translocation in *P. australis* may be increased by the lower leaf lifespan and higher leaf production reported for this species (Cambridge 1996, Cambridge & Hocking 1997). Compared to terrestrial plants, the constraints imposed by the marine environment (e.g. leaf loss due to wave action) may act by favouring nutrient uptake over the development of efficient nutrient conservation strategies (Hemminga et al. 1999). Yet our findings suggest that the more efficient conservation strategy of *P. sinuosa* may be connected to its ability to colonise sites with low nutrient conditions, whereas *P. australis* is found in environments subjected to higher nutrient availability (Cambridge 1996, 1999) where physiological demands are met through concomitant rates of nutrient uptake and translocation.

Estimates of isotope and N and C decrease within leaves were affected by differences in the mobility of material recently taken up compared with the invested N and C components, thus causing disparities between the 2 approaches used to estimate nutrient demands. The N and C content in ageing leaves decreased by ~23.2 and 14.5% for N and ~2.6 and 3.7% for C per unit area in *Posidonia sinuosa* and *P. australis*, respectively. These values are comparable to those found in

Table 5. *Posidonia sinuosa* (P. s) and *P. australis* (P. a). 3-way ANOVA and Student-Newman-Keuls (SNK) tests on patterns of ^{15}N and ^{13}C (mass, µg and concentration, µg g^{-1} DW) 8 and 15 d after the beginning of the among-shoots experiment (labelled shoot excluded. Sp = species, Ti = time, P = plant part, L = leaves, RB = rhizome branches, M = main axis. *C* = Cochran's test. Significant differences are in **bold**

ANOVA	^{15}N						^{13}C					
	Mass			Concentration			Mass			Concentration		
	df	MS	*F*	p	MS	*F*	p	MS	*F*	p	MS	*F*	p
Source of variation													
Sp	2	1.4536	10.904	**0.0022**	0.0152	0.0345	0.8536	127.76	86.12	**0.0000**	140.87	43.490	**0.0000**
Ti	1	0.6245	4.684	**0.0371**	2.8580	6.4849	**0.0153**	6.48	4.366	0.0538	41.47	12.803	**0.0010**
P	1	0.1074	0.806	0.4545	0.7961	1.8064	0.1788	14.72	9.922	**0.0004**	43.50	13.431	**0.0000**
Sp × Ti	2	0.2227	1.671	0.2040	1.5391	3.4924	0.0698	2.10	1.414	0.2421	9.98	3.081	0.0877
Sp × P	2	0.0537	0.403	0.6716	0.1353	0.3069	0.7376	3.05	2.059	0.1424	7.91	2.442	0.1013
Ti × P	1	0.1140	0.855	0.4337	0.6713	1.5232	0.2317	0.51	0.344	0.7115	5.95	1.837	0.1740
Sp × Ti × P	2	0.2072	10.554	0.2252	0.7890	1.7904	0.1814	0.69	0.467	0.6309	1.64	0.505	0.6078
		Transf: √x; *C* = 0.252			Transf: √x; *C* = 0.164			Transf: √x; *C* = 0.182			Transf: √x; *C* = 0.182		
		SNK (Sp)						SNK (Sp)			SNK (Sp)		
		P. s < P. a						P. s < P. a			P. s < P. a		
		SNK (Ti)			SNK (Ti)						SNK (Ti)		
		8 d < 15 d			8 d < 15 d						8 d < 15 d		
								SNK (P)			SNK (P)		
								L < RB = M			L < M = RB		

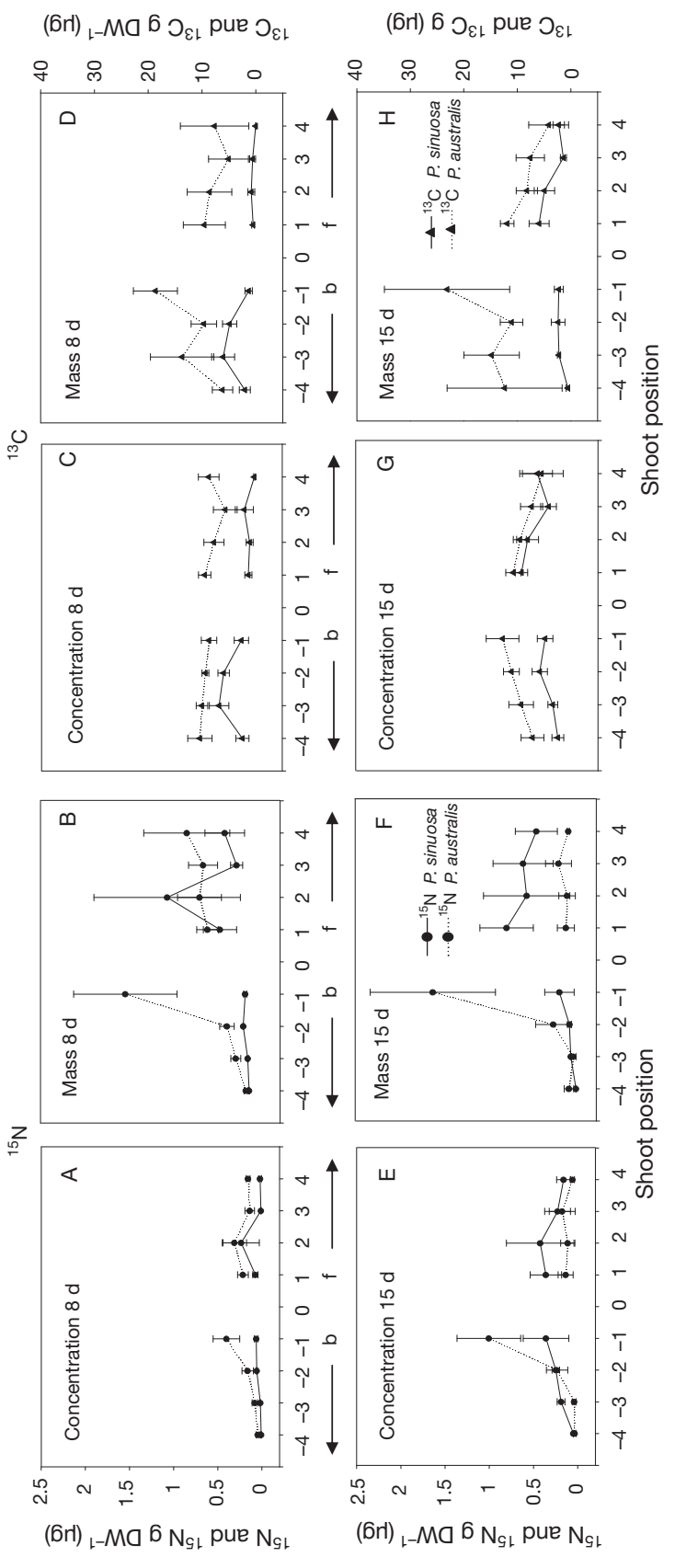

Fig. 4. *Posidonia sinuosa* and *P. australis*. ^{15}N and ^{13}C (µg and µg g^{-1} DW) in the forward (f) and backward (b) direction from the labelled shoot (not included). For each shoot position, values refer to total contents in leaves, rhizome branches and main axes to include differences in allocation among plant parts. Data are indicated at 8 d (A–D) and 15 d (E–H) after the beginning of the among-shoots experiment

other seagrass species and support the finding that C is not effectively resorbed (Stapel & Hemminga 1997). ^{13}C decrease (~32.7% in *P. sinuosa* and 56.9% in *P. australis*) notably exceeded that of leaf C pools, which are mostly comprised of refractory material (insoluble carbohydrates, lignin, cellulose; Romero et al. 1992, Cebrian 1999). This indicates that translocation of C from leaves mainly consists of material recently taken up from the environment, rather than refractory material. Larger but very variable rates have been reported for decreases in leaf N across seagrass species (e.g. Pedersen & Borum 1993: 20 to 80%; Stapel & Hemminga 1997: 19 to 58%; Alcoverro et al. 2000: 25 to 40%). Conversely, the similarity of ^{15}N (~23.7% in *P. sinuosa* and 27.8% in *P. australis*) and leaf N decrease in both species suggests that ^{15}N is largely incorporated by incubated tissues and resorbed subsequently with previously invested pools (e.g. structural proteins; Kraemer et al. 1998, Alcoverro et al. 1999). Differences between isotope and N and C decrease within leaves were, however, higher in *P. australis*, apparently due to enhanced translocation during the incubation process as evidenced by records of ^{13}C and ^{15}N in young leaves and rhizome immediately after labelling. Although mature tissues are considered susceptible to leaching (Tukey 1970), the low isotope decrease following incubation suggests that this was not the case in our study.

^{15}N translocation in both *Posidonia australis* and *P. sinuosa* was higher to young leaves (i.e. within-shoot experiment) but displayed very low rates from the labelled shoot to leaves in adjacent shoots (i.e. among-shoots experiment). Therefore, the first component of our second hypothesis, predicting greater ^{15}N translocation to leaves due to higher N requirements, was rejected. The second component, predicting higher ^{13}C translocation to storage in rhizomes, was retained, as there was more ^{13}C recovered in rhizomes than in leaves. Differences in ^{15}N and ^{13}C contents within a shoot as opposed to among shoots supports the notion that source–sink relationships and the possible constraints

Table 6. *Posidonia sinuosa* (P. s) and *P. australis* (P. a). 2-way ANOVA and Student-Newman-Keuls (SNK) tests on patterns of isotope directionality in the among-shoots experiment (mass, µg and concentration, µg g DW^{-1}) showing ^{15}N and ^{13}C (8 d and 15 d, in 4 forward and 4 backward shoot positions. Sp = species, D = direction *C* = Cochran's test. Significant differences are in **bold**

ANOVA		^{15}N (8 d)						^{13}C (8 d)					
		Mass			Concentration			Mass			Concentration		
	df	MS	*F*	p	MS	*F*	p	MS	*F*	p	MS	*F*	p
Source of variation													
Sp	1	0.3186	14.104	**0.0027**	0.0315	0.6019	0.4529	269.08	18.325	**0.011**	167.12	30.682	**0.001**
D	1	0.0062	0.273	0.6107	0.1798	3.4354	0.0885	44.59	3.037	0.1070	15.32	2.813	0.1193
Sp × D	1	0.398	1.764	0.2088	0.0086	0.1642	0.6924	10.90	0.742	0.4059	0.03	0.005	0.9450
		Transf: – ; *C* = 0.507			Transf: √x; *C* = 0.716			Transf: – ; *C* = 0.731			Transf: – ; *C* = 0.474		
		SNK (Sp)						SNK (Sp)			SNK (Sp)		
		P. s < P. a						P. s < P. a			P. s < P. a		
		^{15}N (15 d)						^{13}C (15 d)					
Sp	1	0.1989	1.7884	0.2059	0.0097	0.0532	0.8214	407.54	14.024	**0.0028**	89.356	12.198	**0.0044**
D	1	0.0558	0.5108	0.4922	0.0288	0.1584	0.6977	40.86	1.406	0.2587	5.615	0.766	0.3985
Sp × D	1	0.5132	4.0131	0.0528	0.6416	3.5229	0.0850	101.15	3.481	0.0867	28.095	0.3835	0.0738
		Transf: – ; *C* = 0.517			Transf: – ; *C* = 0.501			Transf: √x; *C* = 0.512			Transf: – ; *C* = 0.351		
								SNK (Sp)			SNK (Sp)		
								P. s < P. a			P. s < P. a		

Table 7. *Posidonia australis, P. sinuosa* and *P. oceanica*. Structural, physiological and functional aspects related to the use of N and C. TNC = Total Non-structural Carbohydrates, APP = Aboveground Primary Production. (–) = no further data available

	P. australis	*P. sinuosa*	*P. oceanica*	Source
Leaf N (%)[a]	1.63	1.44	1.47	Present study, Alcoverro et al. (2001)
Leaf C (%)[a]	38.3	38.31	35.8	Present study, Alcoverro et al. (1995)
TNC in rhizomes (mg g^{-1} DW)[a]	309.4	174.3	160.2	Present study, Pirc (1985)
TNC in leaves (mg g^{-1} DW)[a]	112.6	121.2	49.9	Present study, Pirc (1985)
Leaves' production (no. $shoot^{-1}$ yr^{-1})	7	5	5.7-8.9	Cambridge (1999), Marbà et al. (1996)
APP (mg DW $shoot^{-1}$ d^{-1})	4.3	1.03	6.5	Present study, Alcoverro et al. (2000, 2001)
Shoot density (no. m^{-2})	600–800	900–1900	500–900	Cambridge & Hocking (1997), Alcoverro et al. (2001)
No. leaves $shoot^{-1}$	3–4	1–2	5–7	Cambridge (1996), Marbà & Walker (1999), Bay (1984)
Leaf longevity (d)	140	245	150–300	Marbà & Walker (1999), Romero (1989)
Mean N residence time (MRT) (yr)	0.25	0.40	0.20	Cambridge (1996), Lepoint et al. (2002)
Canopy losses (mg N $shoot^{-1}$ d^{-1})[c]	0.043	0.016	0.083	Cambridge & Hocking (1997), Alcoverro et al. (2000)
Within-shoot N (% translocated ^{15}N $shoot^{-1}$ d^{-1})[b]	1.32	0.97	–	Present study, –
Among-shoots N (% translocated ^{15}N $shoot^{-1}$ d^{-1})[b]	0.23	0.38	2.94	Present study, Marbà et al. (2002)
Within-shoot C (% translocated ^{13}C $shoot^{-1}$ d^{-1})[b]	3.41	1.92	–	Present study, –
Among-shoots C (% translocated ^{13}C $shoot^{-1}$ d^{-1})[b]	3.55	4.31	3.89	Present study, Marbà et al. (2002)

[a]Values are indicated for early summer
[b]Values estimated as the % of the total incorporated ^{15}N and ^{13}C translocated to young leaves and rhizomes (within-shoot) and other shoots (among-shoots experiment) per day
[c]Annual estimates from monthly averages

given by vascular connections and sink size are important factors shaping patterns of translocation (Bledsoe & Orians 2006). Hence, leaves acting both as sink and source may explain opposite patterns of ^{15}N translocation between plant scales and the retention of N by growing tissues where it is needed, particularly in *P. australis* (Table 7). Yet, given that only the first shoot emerging at each division of the main axis (shoot posi-

tion) was analysed, sharing patterns within branch hierarchies could not be assessed in the present study. Semiautonomous functioning is, however, a common feature in plants with morphological subunits (Watson & Casper 1984) and could partly explain low N translocation among shoots. Patterns of N distribution in clonal plants may also be influenced by root uptake from available sediment pools. However, NH_4 concentrations in the pore water at the Garden Island site (9 µM) were lower than those reported in other studies of translocation among shoots (Marbà et al. 2002) and much lower than those at the *P. oceanica* site (see also Alcoverro et al. 1995 for details). In addition, higher concentrations of dissolved inorganic N in pore water appear to be necessary to achieve significant effects in plant processes under equal availability in the water column (Lee & Dunton 1999).

Conversely, translocation of C was more similar at both plant scales but tended to be higher among shoots, particularly in *Posidonia sinuosa* (Table 7). In fact, in clonal plants featuring specialised organs for C storage, the main axis may behave as a single individual allowing growth and respiration of the whole plant and supported by leaf tissues (Chapman & Robson 1991).

Neither species displayed any pattern of directionality, possibly due to retention of ^{15}N within shoots and diversion of ^{13}C towards the rhizome structure. Therefore, our third hypothesis stating that higher growth in *Posidonia australis* causes greater apical demand in this species was rejected. In fact, the presence of directionality seems to be connected to the developmental morphology of the vascular system rather than to differences in nutrient requirements for new growth (reviewed by Vuorisalo & Hutchings 1996). For instance, directionality is found in species such as *Cymodocea* that produce erect axes by continuous branching (Terrados et al. 1997a,b, Marbà et al. 2002). Conversely, both *P. australis* and *P. sinuosa* have diffuse branching patterns and numerous apical meristems which may explain the absence of directionality and a more even distribution of resources such as already evidenced in *P. oceanica* (Marbà et al. 2002). Collectively, our data suggest that despite a high degree of physiological integration, shoots may compete with their neighbours for N sources in the water column (i.e. 'selfish' behaviour sensu Vuorisalo & Hutchings 1996). In contrast, since more C was translocated to connected neighbours through the belowground rhizome mat, patterns of distribution were 'cooperative' (sensu Vuorisalo & Hutchings 1996), and may contribute to enhancing the overall fitness of the plant (Hamilton 1964).

In order to improve our understanding of the strategies of N and C exploitation from the water column in *Posidonia* spp., we compared patterns of N and C translocation (among-shoots) as well as morphological, physiological and leaf production features recorded for Western Australian species to similar information available for *P. oceanica* (Table 7). Contents of ^{13}C and ^{15}N in plant parts of *P. oceanica* from Marbà et al. (2002) were expressed as percentage of translocated mass shoot^{-1} d^{-1}. In contrast, with the higher among-shoots translocation of C observed in the present study (~11 times higher in *P. sinuosa* and 15 times higher in *P. australis*), *P. oceanica* displayed similar patterns of translocation for both N and C. Estimates on a per-shoot basis indicate that *P. oceanica* may translocate ~8 to 13 times more N than the Western Australian species, allowing a more equitative sharing of resources. Alternatively, given that *P. oceanica* was investigated at a larger plant scale, observed patterns for this species may be evidence of the existence of higher N translocation at higher branching hierarchies.

At the within-shoot scale, no similar data is available for *Posidonia oceanica* and so we cannot evaluate differences among the species. Instead, the mean residence time (MRT) of N within the leaf canopy has been assessed for all 3 species and may also clarify differences in nutrient use (Table 7). Higher MRT can be achieved through leaf persistence (Escudero et al. 1992), which is negatively associated with leaf formation (Hemminga et al. 1999) and/or leaf resorption. Among the 3 species, *P. oceanica* has the longest leaf lifespan and the highest among-shoots translocation and leaf N and C decrease but similar leaf production rates compared to *P. australis* (Alcoverro et al. 2000). However, it has the shortest MRT of N in the leaf canopy (Cambridge 1996, Lepoint et al. 2002), which suggests that the system may be subject to larger N losses. Plants can modify patterns of translocation in response to environmental conditions (Evans 1991, Birch & Hutchings 1994) including herbivory (Thomas & Watson 1988, Shea & Watson 1989). Indeed, leaf losses to herbivores in *P. oceanica* can reach about 57% of the annual leaf production (Prado et al. 2007), whereas herbivory is uncommon and largely restricted to mesograzers in Western Australian species (Jernakoff & Nielsen 1997, Keuskamp 2004). Hence, higher N translocation and longer leaf lifespan of *P. oceanica* could be mechanisms that allow this species to offset herbivory losses.

To conclude, the present study shows that the faster growing *Posidonia australis* has higher ^{15}N incorporation and translocation than *P. sinuosa*; ^{15}N is distributed more to leaves within the same shoot, while for ^{13}C there is high translocation to rhizomes, where it is likely stored; and neither species has distinct directionality in translocation, presumably due to the presence of diffuse branching patterns. Other than these physio-

logical and structural adaptations to the physical environment, our results also offer a comparative ground with *P. oceanica*, suggesting that herbivory may influence the adaptative capacity of N and C nutrient translocation in seagrasses. It is essential that future investigations dealing with patterns of distribution in seagrass ecosystems be performed with increasing detail. Similar species may optimise nutrient conservation, direct uptake from the water column, or other strategies (e.g. uptake by roots; Lee & Dunton 1999) to overcome the constraints imposed by biotic and abiotic conditions and meet their nutrient requirements.

Acknowledgements. This study was funded by a scholarship from the Strategic Research Fund for the Marine Environment awarded to C.J.C. P.P. was supported by a short-term outgoing scholarship from the Catalonian Government (UNI/2182/2003). We also thank J. Eyres, J. How and P. Quintana for field assistance, J. Tranter for advice on isotopic issues and M. A. Mateo for constructive comments on the manuscript.

LITERATURE CITED

Alcoverro T, Duarte CM, Romero J (1995) Annual growth dynamics of *Posidonia oceanica*: contribution of large-scale versus local factors to seasonality. Mar Ecol Prog Ser 120:203–210

Alcoverro T, Zimmerman RC, Kohrs DG, Alberte RS (1999) Resource allocation and sucrose mobilization in light-limited eelgrass *Zostera marina.* Mar Ecol Prog Ser 187: 121–131

Alcoverro T, Manzanera M, Romero J (2000) Nutrient mass balance of the seagrass *Posidonia oceanica*: the importance of nutrient retranslocation. Mar Ecol Prog Ser 194: 13–21

Alcoverro T, Cebrián E, Ballesteros E (2001) The photosynthetic capacity of the seagrass *Posidonia oceanica*: influence of nitrogen and light. J Exp Mar Biol Ecol 261: 107–120

Bay D (1984) A field study of the growth dynamics and productivity of *Posidonia oceanica* (L.) Delile in Calvi Bay, Corsica. Aquat Bot 20:43–64

Birch CPD, Hutchings MJ (1994) Exploitation of patchily distributed soil resources by the clonal herb *Glechoma hederacea.* J Ecol 82:653–664

Bledsoe TM, Orians CM (2006) Vascular pathways constrain ^{13}C accumulation in large root sinks of *Lycopersicon esculentum* (Solanaceae). Am J Bot 93:884–890

Cambridge ML (1996) Is habitat separation between two species of *Posidonia* from Western Australia due to differences in nutrient use efficiency? In: Kuo J, Phillips RC, Walker D, Kirkman H (eds) Seagrass biology: Proceedings of an International Workshop, Rottnest Island, Western Australia. The University of Western Australia, Nedlands, p 173–180

Cambridge ML (1999) Growth strategies of Rottnest Island seagrasses. In: Walker DI, Wells FE (eds) The seagrass flora and fauna of Rottnest Island, Western Australia. Western Australian Museum, Perth, p 1–24

Cambridge ML, Hocking PJ (1997) Annual primary production and nutrient dynamics of the seagrasses *Posidonia sinuosa* and *Posidonia australis* in south-western Australia. Aquat Bot 59:277–295

Cebrian J (1999) Patterns in the fate of production in plant communities. Am Nat 154:449–468

Chapman DF, Robson MJ (1991) Tissue partitioning during leaf development in ornamentally-grown *Frithia pulchra* (Mesembryanthemaceae), a 'Window plant'. Ann Bot (Lond) 67:303–308

Collier C (2006) Characterizing responses of the seagrass *Posidonia sinuosa* to changes in light availability. PhD thesis, Edith Cowan University, Perth

de Kroon H, Schieving F (1990) Resource allocation patterns as a function of clonal morphology: a general model applied to a foraging clonal plant. J Ecol 79:519–530

Duarte CM (1991) Allometric scaling of seagrass form and productivity. Mar Ecol Prog Ser 77:289–300

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric determination of sugars and related substances. Anal Chem 28:350–356

Escudero A, del Arco JM, Sanz IC, Ayala J (1992) Effects of leaf longevity and retranslocation efficiency on the retention time of nutrients in the leaf biomass of different woody species. Oecologia 90:80–87

Evans JP (1991) The effect of resource integration on fitness related traits in a clonal dune perennial, *Hydrocotyle bonariensis.* Oecologia 86:268–275

Fourqurean JV, Zieman JC (1991) Photosynthesis, respiration and whole plant carbon budget of the seagrass *Thalassia testudinum.* Mar Ecol Prog Ser 69:161–170

Gonfiantini R, Stichler W, Rozanski K (1995) Standards and intercomparison materials distributed by the International Atomic Energy Agency for stable isotope measurements. IAEA-TECDOC-825. Available at: www-pub.iaea.org/MTCD/publications/pdf/te_825_prn.pdf

Hamilton WD (1964) The genetical evolution of social behaviour I–II. J Theor Biol 7:1–52

Harrison PG (1978) Patterns of uptake and retranslocation of ^{14}C by *Zostera americana* den Hartog in the laboratory. Aquat Bot 5:93–97

Hemminga MA, Harrison PG, van Lent F (1991) The balance of nutrient losses and gains in seagrass meadows. Mar Ecol Prog Ser 71:85–96

Hemminga MA, Marbà N, Stapel J (1999) Leaf nutrient resorption, leaf lifespan and the retention of nutrients in seagrass systems. Aquat Bot 65:141–158

Jernakoff P, Nielsen J (1997) The relative importance of amphipod and gastropod grazers in *Posidonia sinuosa* meadows. Aquat Bot 56:183–202

Jonasson SJP, Chapin FS III (1985) Significance of sequential leaf development for nutrient balance of the cotton sedge, *Eriophorum vaginatum* L. Oecologia 67:511–518

Keuskamp D (2004) Limited effects of grazer exclusion on the epiphytes of *Posidonia sinuosa* in South Australia. Aquat Bot 78:3–14

Kraemer GP, Kohrs D, Alberte RS (1998) Developmental changes of sucrose synthase activity and abundance in root and leaf tissues of *Zostera marina.* Aquat Bot 62: 189–198

Lee KS, Dunton KH (1999) Influence of sediment nitrogen-availability on carbon and nitrogen dynamics in the seagrass *Thalassia testudinum.* Mar Biol 134:217–226

Lepoint G, Defawe O, Gobert S, Dauby P, Bouquegnau JM (2002) Experimental evidence for nutrient recycling in the leaves of the seagrass *Posidonia oceanica.* J Sea Res 48: 173–179

Libes M, Boudouresque CF (1987) Uptake and long-distance transport of carbon in the marine phanerogam *Posidonia*

oceanica. Mar Ecol Prog Ser 38:177–186

Lovett-Doust L (1981) Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*). I. The dynamics of ramets in contrasting habitats. J Ecol 69: 743–755

Marbà N, Walker DI (1999) Growth, flowering, and population dynamics of temperate Western Australian seagrasses. Mar Ecol Prog Ser 184:105–118

Marbà N, Cebrián E, Enríquez S, Duarte CM (1996) Growth patterns of Western Mediterranean seagrasses: species-specific responses to seasonal forcing. Mar Ecol Prog Ser 133:203–215

Marbà N, Hemminga MA, Mateo MA, Duarte CM, Mass YEM, Terrados J, Gacia E (2002) Carbon and nitrogen translocation between seagrass ramets. Mar Ecol Prog Ser 226:287–300

Pedersen MF, Borum J (1993) An annual nitrogen budget for a seagrass *Zostera marina* population. Mar Ecol Prog Ser 101:169–177

Pirc H (1985) Growth dynamics in *Posidonia oceanica* (L) Delile. I. Seasonal changes of soluble carbohydrates, starch, free amino acids, nitrogen and organic anions in different parts of the plant. PSZN I: Mar Ecol 6:141–165

Prado P, Tomas F, Alcoverro T, Romero J (2007) Extensive direct measurements of *Posidonia oceanica* defoliation confirm the importance of herbivory in temperate seagrass meadows. Mar Ecol Prog Ser 340:63–71

Romero J (1989) Seasonal pattern of *Posidonia oceanica* production: growth, age and renewal of leaves. In: Boudouresque CF, Meinesz A, Fresi E, Gravez V (eds) International workshop on *Posidonia* beds, Vol 2. GIS Posidonie, Marseille, p 63–67

Romero J, Pergent G, Pergent-Martini C, Mateo MA, Regnier C (1992) The detritic compartment in a *Posidonia oceanica* meadow: litter features, decomposition rates and mineral stocks. PSZN I: Mar Ecol 13:73–83

Saur E, Nambiar EKS, Fife DN (2000) Foliar nutrient retranslocation in *Eucalyptus globulosus*. Tree Physiol 20: 1105–1112

Shaver GR, Melillo JM (1984) Nutrient budgets of marsh plants: efficiency concepts and relation to availability. Ecology 65:1491–1510

Shea MS, Watson MA (1989) Patterns of leaf and flower removal: their effect on fruit growth in *Chamaenerion angustifolium* (tireweed). Am J Bot 76:884–890

Short FT, Duarte CM (2001) Methods for the measurement of seagrass growth and production. In: Short FT, Coles RG (eds) Global seagrass research methods. Elsevier, Amsterdam, p 155–182

Simpson CJ, Burt JS, Cary JL, D'adamo N, Masini RJ, Mills DA (1996). Southern metropolitan coastal waters study (1991–1994). Final report 17. Department of Environmental Protection, Perth

Stapel J, Hemminga MA (1997) Nutrient resorption from seagrass leaves. Mar Biol 128:197–206

Terrados J, Duarte CM, Kenworthy WJ (1997a) Experimental evidence for apical dominance in the seagrass *Cymodocea nodosa*. Mar Ecol Prog Ser 148:263–268

Terrados J, Duarte CM, Kenworthy WJ (1997b) Is the apical growth of *Cymodocea nodosa* dependent on clonal integration? Mar Ecol Prog Ser 158:103–110

Thomas LP, Watson MA (1988) Leaf removal and the apparent effects of architectural constraints on development in *Capsicum annuum*. Am J Bot 75:840–843

Tomlinson PB (1974) Vegetative morphology and meristem dependence—the foundation of productivity in seagrasses. Aquaculture 4:107–130

Touchette BW, Burkholder JM (2000) Review of nitrogen and phosphorus metabolism in seagrasses. J Exp Mar Biol Ecol 250:133–167

Tukey HB (1970) The leaching of substances from plants. Annu Rev Plant Physiol 21:305–324

Vitousek PM, Reiners WA (1975) Ecosystem succession and nutrient retention: a hypothesis. Bioscience 25:376–381

Vuorisalo T, Hutchings MJ (1996) On plant sectoriality, or how to combine the benefits of autonomy and integration. Plant Ecol 127:3–8

Watson MA, Casper BB (1984) Morphogenetic constraints on patterns of carbon distribution in plants. Annu Rev Ecol Syst 15:233–258

Editorial responsibility: Kenneth Heck, Dauphin Island, Alabama, USA

Submitted: April 23, 2007; Accepted: January 15, 2008
Proofs received from author(s): May 25, 2008