Temperature effects on decomposition of a *Posidonia oceanica* mat

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ABSTRACT: *Posidonia oceanica* mats act as an important carbon (C) sink in the Mediterranean by accumulating roots, rhizomes and leaf sheaths, and allochthonous organic matter over millennia due to low rates of decomposition of organic matter. Thus far, rates of decomposition have only been modeled, whereas few attempts have been made to measure decomposition rates under *in situ* conditions in *P. oceanica* mats. We studied decomposition of *P. oceanica* mats by simulating *in situ* conditions at different temperatures. Samples from 5 different depths in a 3 m thick vertical profile of a *P. oceanica* mat were each incubated under anoxic conditions for 7 mo at 5 different temperatures (15, 20, 25, 30, and 35°C). Our measured low mat mineralization rates confirm slow decomposition and support that *P. oceanica* mats act as important long-term C sinks in the Mediterranean. However, between 15 and 25°C, both C and nitrogen (N) mineralization increased with increasing temperature, and generally declined above 25°C, probably due to unbalanced bacterial growth in the mat. Relatively, N mineralization was stimulated more than CO_2 release (i.e. up to 14.3 and 4.5 times, respectively), suggesting that regeneration of N in *P. oceanica* mats is enhanced with increasing temperatures up to 25°C. This may affect the nutrient dynamics in this oligotrophic ecosystem. Our data suggest that CO_2 release due to decomposition of the mat is potentially enhanced under a scenario of climate change, thus reducing the C sequestration capacity of the mats during this century.

KEY WORDS: Mineralization · Carbon sink · Nitrogen · Seagrass · Mediterranean Sea

INTRODUCTION

The detritus of roots, rhizomes, and leaf sheaths of the Mediterranean endemic seagrass *Posidonia oceanica* can accumulate in large peat-like structures called 'mattes' or mats (Boudouresque & Meinesz 1982). The age of the detritus dated so far is as old as 6000 yr BP (Lo Iacono et al. 2008). This is due to unusually slow decomposition of plant belowground detritus after an initial rapid decay of labile organic matter (OM) within the first 3 to 5 yr after plant death (Romero et al. 1992), and the detritus loses a large part of its constituents (organic and mineral elements;

Mateo et al. 2006). The mats are important carbon (C) sinks in the Mediterranean, with an estimated organic C long-term accumulation rate between 19 and 191 g m^{-2} yr^{-1} (Romero et al. 1994, Mateo et al. 1997, 2006, Lo Iacono et al. 2008) burying about 25 to 35% of *P. oceanica* primary production (Romero et al. 1994) and accumulating an organic C stock of 4×10^4 to 16×10^4 g C m^{-2} (Mateo et al. 1997). Assuming coverage of *P. oceanica* of 2% of the Mediterranean seafloor (Béthoux & Copin-Montégut 1986), the total C sink may range from 2.0×10^{15} to 8.1×10^{15} g C (Mateo et al. 1997, Lo Iacono et al. 2008). This C burial is high for the Mediterranean, but small com-

pared to more important global C sinks in peat and coral reefs (450×10^{15} g C and 100×10^{15} to 400×10^{15} g C, respectively; Mateo et al. 1997).

Posidonia oceanica, together with *P. australis* and *Thalassodendron ciliatum*, are the only seagrasses known to store and accumulate substantial amounts of highly decay-resistant organic material (Mateo et al. 2006). A combination of factors has been suggested for the massive accumulation of below-ground organs of *P. oceanica* (Mateo et al. 2006). The rhizomes can attain considerable lengths (over 1 m) and grow both horizontally (plagiotropic) and vertically (orthotropic, Pergent 1990) resulting in an extensive web of plant tissues which is both scarcely accessible, and due to its chemical composition, not attractive to herbivores, leading to accumulation of detritus after plant death (Mateo et al. 2006). The leaf sheaths, which are lignified and fibrous (Kuo 1978), remain attached to the rhizome after leaf-blade abscission (Tomlinson 1972, Pergent 1990) and persist in the sediments together with the lignified and tannin-containing epidermis of the rhizomes (Kuo 1978, Kuo & Cambridge 1978, Mateo et al. 2006). Inside the mat, rhizomes, roots, and sheaths decay little (Serrano 2011). The slow decomposition of *P. oceanica* below-ground parts can be related to its high content of lignin, cellulose, and phenolic compounds (Harrison 1989, Klap et al. 2000), which are not readily degraded by microbes (Godshalk & Wetzel 1978), while the highly anoxic conditions in the mat also contribute to its preservation (Mateo et al. 1997, 2006).

Microorganisms decompose organic material to gain energy and substrates for maintenance and growth. Mineralization of OM produces TCO_2 ($TCO_2 = CO_2 + HCO_3^- + CO_3^{2-}$) and NH_4^+. TCO_2 is the end product of terminal mineralization, whereas NH_4^+ is produced during the initial hydrolysis and fermentation of macromolecules into low-weight dissolved organic C (DOC) and is decoupled from the terminal mineralization (Kristensen & Hansen 1995). The NH_4^+ produced can be re-assimilated by bacteria as essential nutrients for cell growth and metabolism and thereby immobilized (Kristensen & Hansen 1995). Assuming a bacterial C:N ratio of about 5 (Goldman et al. 1987, Fagerbakke et al. 1996) and a C:N ratio for plant detritus >25, all NH_4^+ from hydrolysis and fermentation can potentially be re-assimilated by bacteria (Blackburn 1979). Mineralization of OM may be limited in any of the steps in the decay pathway (Arnosti et al. 1998, Robador et al. 2010), and temperature is one factor that affects the bacterial activity. Bacteria typically respond to temperature with a Q_{10} of about 2 to 3 up to the optimum temperature for growth (Pomeroy & Wiebe 2001). Bacteria continue metabolizing at temperatures beyond optimal temperatures, allowing them to remain functional, though without growing and dividing (Isaksen & Jørgensen 1996). Under temperate conditions, mesophilic bacteria are the most abundant in marine sediments with optimal growth at 25 to 30°C and metabolic activity from 0 to 35°C (Isaksen & Jørgensen 1996).

While mat accumulation rates (dry mass, C, N, and phosphorus) and decay rates of 'recent' leaves, roots, and rhizomes have been estimated in previous studies (Romero et al. 1992, 1994, Mateo & Romero 1997, Mateo et al. 1997, 2003, 2006), to our knowledge no studies have investigated decomposition of old parts of the mat apart from the estimates modeled by Mateo et al. (1997). Furthermore, none of these studies examined possible effects of increasing temperatures on mat dynamics. The global average surface temperature is modeled to increase by 1.1 to 2.9°C in the 21st century in the best-case scenario and by 2.4 to 6.4°C in the worst (IPCC 2007), increasing more in winter than in summer (Alcamo et al. 2007). High-resolution regional ocean models estimate a 3.1°C rise in the sea surface temperatures of the Mediterranean Sea by 2099 (Somot et al. 2006). The reported warming trend of the NW Mediterranean during the period 1974 to 2001 was 0.04°C yr^{-1} for surface waters (Coma et al. 2009). Global environmental change is expected to lead to a higher frequency and shorter return time of extreme events (IPCC 2007). Climatic conditions in the Mediterranean in 1999 and 2003 included temperatures ca. 3 to 4°C above average and prolonged water column stability in late summer (Cerrano et al. 2000, Sparnocchia et al. 2006).

In this study, we investigated the decomposition rates of a *Posidonia oceanica* mat, including the C:N stoichiometry of the mineralization process to explore the possible bacterial substrates during degradation of the mat. We examined whether the decomposition process is affected by temperature under the hypothesis that an increase in temperature causes an increase in decomposition rates and consequently an increase in CO_2 emission from the mat. We also hypothesized that the temperature effect on decomposition rates decreases as the age of the detritus increases down in the mat owing to an increase in the relative content of refractory compounds. To explore these relationships, mat material from different depths in a *P. oceanica* mat in Ibiza (Balearic Islands, Spain) was incubated in anoxic jars for 7 mo in a temperature gradient (15 to 35°C). Decomposition of plant detritus and mineralization rates were assessed

as loss of plant material and accumulation of mineralization products (TCO$_2$, NH$_4^+$) over time.

MATERIALS AND METHODS

Study site

Sampling was performed during July 2008 in a *Posidonia oceanica* meadow growing in Talamanca Cove, Ibiza (Balearic Archipelago, Spain; 38° 54.8′ N, 1° 27.3′ E), from the surface (>1 m) and extending into deep waters (Fig. 1). Talamanca Cove is located in a tourist area on the south coast of Ibiza, delimited by Tabernera and s′Andreus Points and open to the SE by a ~2 km wide mouth. At the study site, *P. oceanica* meadows form an irregular landscape due to natural erosion processes exposing walls of the *P. oceanica* mat. The top of the studied wall was located at 2 m water depth and continued to about 5 m depth. The temperature at the location varies between 13 and 25°C, with an annual average of ~17°C, and the annual average salinity is 37.5‰ (Monserrat et al. 2008).

Field sampling

Cores were taken horizontally by SCUBA diving from an exposed vertical mat wall using hand-operated 90 cm long, 67 mm inner diameter PVC corers fitted with a serrated cutting edge. Two to 3 replicate horizontal cores were sampled at 5 depths (hereafter referred to as Levels L1 to L5) down the mat wall at 54, 108, 162, 216, and 270 cm from the top (Fig. 2). The outermost 10 cm of each core were discarded to eliminate interference from solute exchange with ambient water. Immediately after sampling, the material from each level was homogenized in a bucket and transferred to 20 ml glass vials with gas-tight lids. Homogenizing of mat material affects the natural chemical, physical, and biological conditions in the mat, but only in the short term, i.e. for several days (Aller & Mackin 1989, Sun et al. 1991). Hence, possible artifacts of homogenisation are considered insignificant for this experiment, which ran for 7 mo (see 'Discussion').

Experimental setup

Vials were incubated at 5 temperatures (15, 20, 25, 30, 35°C) for 7 mo, with monthly sampling (S0 to S6)

following the procedures of Kristensen & Hansen (1995). The vials were closed with tight lids and sealed with tape. The closed vials were buried in anoxic sediment as an additional protection measure to avoid any oxygen leaking into the vials during the very long incubation period. The sediment with the vials was submerged in water, and temperature was controlled by heaters and circulation pumps to secure constant conditions (temperature variation <0.4 to 0.8°C).
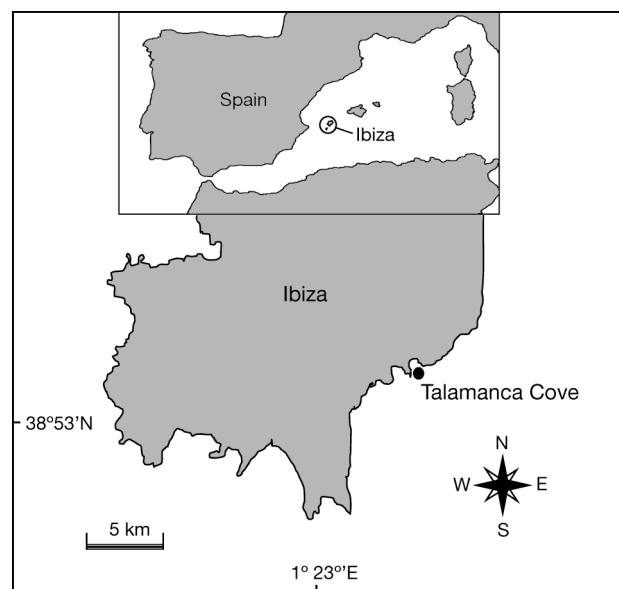

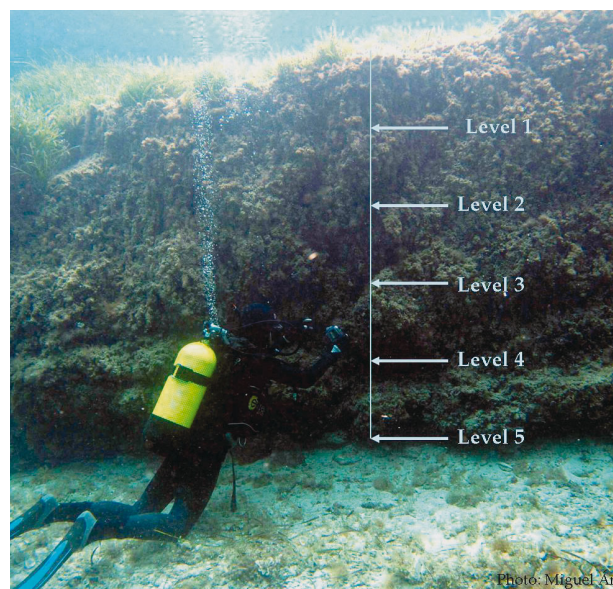Fig. 1. Study site in Talamanca Cove, Ibiza, Balearic Islands, Spain

Fig. 2. *Posidonia oceanica*. Mat similar to the one investigated, showing the 5 levels in the seagrass mat

Each month, 5 replicate vials from each temperature and mat level were sampled for measuring pH, concentrations of TCO_2, ammonium (NH_4^+), and DOC, together with particulate organic C and nitrogen (POC and PON, respectively) in the particulate mat fraction (coarse and fine, see 'Analyses' below). The initial samples (S0) were taken right after returning to the laboratory, no more than 7 d after filling the vials. Pore water was extracted by centrifugation at $1500 \times g$ for 10 min, replacing the lid with a new lid with 5 holes and mounted with a 25 mm GF/C filter. The vial was placed upside down and centrifuged once for <1 min to obtain pore water for pH and TCO_2 analysis, and then 10 min for analysis of NH_4^+ and DOC in the porewater samples. Samples for TCO_2 (1 ml) were fixed with 15 µl 20% $HgCl_2$ and stored at room temperature until analysis within 4 mo. Samples for NH_4^+ and DOC were stored frozen. The particulate material remaining in the vial was stored frozen and later separated into coarse and fine fractions. Samples for density and porosity (n = 10) were collected from each mat level to be able to calculate volumetric process rates.

Analyses

TCO_2 was determined by flow injection and the diffusion cell principle (Hall & Aller 1992), and NH_4^+ was measured using the colorimetric method of Bower & Holm-Hansen (1980). DOC concentrations were determined by a Shimadzu total organic C analyzer (TOC 5000). The number of replicates for each analysis varied from 3 to 5. Density of the mat material in the vials was determined by the wet weight of the content of 10 vials divided by the volume of the vials. Porosity was determined from the water content obtained after drying the mat material at 105°C for 24 h. The mat material was separated into coarse mat/plant detritus (coarse particulate OM, CPOM, >1 mm) and fine plant/sediment fraction (FPOM, <1 mm) by dry sieving, and quantified by weight after drying both fractions (105°C for 24 h). POC and PON in coarse and fine fractions were determined on a Carlo Erba CHN elemental analyzer EA 1108 by the method of Kristensen & Andersen (1987).

Calculations and statistics

The mineralization rates (µmol cm^{-3} d^{-1}) of organic C (OC) and organic N (ON) in the mat were determined from linear regressions of the change in pore-

water concentration of their products TCO_2 and NH_4^+, respectively, versus the incubation time (only regressions with p < 0.05 were used, n = 21 to 28), and the porosity for each level. The accumulation of TCO_2 and NH_4^+ occurred in 2 steps, and an average rate was calculated for the entire period. The loss or gain in POC and PON between S0 and S6 for both coarse (ΔCPOC, ΔCPON) and fine fractions (ΔFPOC, ΔFPON, µmol cm^{-3}) were calculated by dividing the weight of the coarse or fine fraction (g dry weight, DW) by the vial volume (cm^3) and multiplying this with the loss or gain in POC or PON content in the coarse and fine fraction (µmol g^{-1} DW), respectively.

All data used for statistical analysis were checked for normality (Kolmogorov–Smirnov test) and for variance homogeneity (Levene's test). The effect of level on mat characteristics was tested by a 1-way analysis of variance (ANOVA), and the effect of level and temperature on mineralization rates was tested by a 2-way ANOVA. A Holm-Sidak multicomparison test was applied post hoc in case of significant differences. If the equal variance test failed, a nonparametric Kruskal-Wallis ANOVA on ranks was performed followed by Tukey's post hoc test. Significance was established when p < 0.05. SigmaStat (ver. 3.1) was used for the statistics.

RESULTS

Mat characteristics

The pore water in the mat in Talamanca Cove taken from initial samples (S0) showed decreasing TCO_2 and nutrient concentrations with depth (Table 1). The TCO_2 concentration was between 2.68 and 4.35 mM, with the highest concentrations in L1 and L2 (p < 0.01, Table A1 in Appendix 1). The NH_4^+ concentration ranged from 79 to 486 µM, with the highest concentrations in L1 and L2 (p < 0.01, Table A1 in Appendix 1), whereas there was no significant change in the DOC concentration with depth (0.73 to 1.39 mM, p = 0.14, Table A1 in Appendix 1).

The particulate pools of C and N showed less variability along the vertical profile (Table 2). Only POC in the coarse fraction (CPOC) decreased with mat depth from 37 to 24% DW, with L5 significantly lowest (p = 0.02), while CPON, FPOC, and FPON did not vary significantly with depth. Only FPOC in L2 was significantly higher than the other levels (p < 0.001, Table 2). Due to the decrease in CPOC, the C:N ratio for coarse detritus decreased down through the mat (from 142 to 84), with L5 significantly lower than L2

Table 1. Nutrient and carbon concentrations in pore waters in the 5 levels of the *Posidonia oceanica* mat at Talamanca Cove obtained from the initial samples (S0). Average TCO_2, NH_4^+, and dissolved organic carbon (DOC) concentrations (in mM or μM) are given (± SEM, n = 3–5). Shown are results from Holm-Sidak pairwise multicomparisons for differences between levels. 'Level ≠' columns give the levels which are significantly different (p < 0.01) from a given level, ns: no significant differences between levels

Level	Depth within mat (cm)	TCO_2 (mM)	Level ≠	NH_4^+ (μM)	Level ≠	DOC (mM)	Level ≠
1	52	4.3 ± 0.2	L3,4,5	463[a]	L2,3,4,5	1.38[a]	ns
2	108	4.4 ± 0.2	L3,4,5	278 ± 14	L1,3,4,5	1.39 ± 0.2	ns
3	162	2.7 ± 0.1	L1,2	137 ± 39	L1,2,5	0.97 ± 0.2	ns
4	216	2.9 ± 0.3	L1,2	89 ± 14	L1,2	1.13 ± 0.4	ns
5	270	3.1 ± 0.1	L1,2	78 ± 14	L1,2,3	0.73 ± 0.1	ns

[a]n = 1

(p = 0.01, Table A1 in Appendix 1). The C:N ratio in the fine fraction was much lower (13 to 25) with significantly higher C:N for L2 compared to L1, L3, and L4 (p < 0.005, p < 0.0007, and p < 0.002, respectively; Table A1 in Appendix 1).

Mineralization rates

The mat decomposition experiment demonstrated an accumulation of mineralization products (TCO_2 and NH_4^+), although the changes over time showed variable linearity (linear regression, R^2 = 0.36 to 0.85 and 0.003 to 0.95, respectively). Changes with time are given for L5 (20 and 35°C) as an example (Fig. 3), showing TCO_2 increase from 2.8 to 12.5 mM and 12.4 mM, respectively. For NH_4^+, there was an increase from 57 to 2.3 mM and 3.3 mM, respectively. C and N mineralization rates, calculated from the accumulation of TCO_2 and NH_4^+, respectively, were obtained for all levels and temperatures (Fig. 4). C

mineralization rates varied from 19 to 44 nmol cm^{-3} d^{-1} at 15°C and increased to a maximum at 25°C (53 to 93 nmol cm^{-3} d^{-1}), declining thereafter to 25 to 40 nmol cm^{-3} d^{-1} at 35°C (Fig. 4A). An exception was L4, with maximum mineralization rates at both 25 and 30°C (68 and 69 nmol cm^{-3} d^{-1}, respectively). The effect of level differed for each temperature, but generally L1 attained higher and L5 lower rates when compared across levels. The N mineralization rates varied from 1.8 to 7.1 nmol cm^{-3} d^{-1} at 15°C and attained a maximum between 9.4 and 16.9 nmol cm^{-3} d^{-1} at 25 to 30°C and declined to 10.5 to 13.7 nmol cm^{-3} d^{-1} at 35°C (Fig. 4B). The effect of level was variable and differed between temperatures.

The C:N product ratios of the mineralization rates did not differ between levels and were generally low (1.6 to 8.6) with only one exception (L4 with a C:N = 17 at 15°C, Fig. 4C), which was treated as an outlier. C:N ratios decreased with increasing temperatures, and all levels attained lowest C:N ratios at 30 to 35°C. There were no significant changes in DOC concentrations in any of the incubations (linear regressions), and the DOC concentration remained around 1 mM in all levels and at all temperatures (data not shown).

Decomposition of plant and sediment OM

Based on the initial (S0; Tables 2 & A2 in Appendix 1) and final (S6) POC and PON contents in coarse and fine detritus fractions (Tables 3 & A2 in Appendix 1), the change in OC and ON pools were calculated for the 7 mo duration of the incubation (Tables 4 & A3 in

Table 2. *Posidonia oceanica*. Nitrogen and carbon pools in the coarse detritus (>1 mm) and fine fraction (<1 mm) in the 5 levels of the seagrass mat at Talamanca Cove. Average coarse particulate organic carbon and nitrogen (CPOC and CPON, respectively) and fine POC and PON (FPOC and FPON) are given (±SEM, n = 3–5) as well as molar C:N ratios of coarse (C C:N) and fine (F C:N) organic matter. Shown are results from Holm-Sidak multicomparison tests for differences between levels 'Level ≠' columns give the levels which are significantly different (p < 0.01) from a given level, DW: dry weight, ns: no significant differences between levels

| Level	CPOC (% DW)	Level ≠	CPON (% DW)	Level ≠	C C:N molar	Level ≠	FPOC (% DW)	Level ≠	FPON (% DW)	Level ≠	F C:N molar	Level ≠
1	37.5 ± 0.2	L5	0.34 ± 0.02	ns	128.7 ± 9.6	ns	1.08 ± 0.11	L2	0.08 ± 0.01	ns	16.2 ± 0.6	L2
2	37.3 ± 2.9	L5	0.31 ± 0.03	ns	142.0 ± 9.2	L5	1.62 ± 0.06	L1,3,4,5	0.08 ± 0.01	ns	25.3 ± 2.1	L1,3,4
3	34.4 ± 0.6	ns	0.38 ± 0.03	ns	108.4 ± 11.0	ns	0.97 ± 0.04	L2	0.09 ± 0.01	ns	13.2 ± 1.5	L2
4	29.9 ± 0.8	ns	0.34 ± 0.02	ns	103.1 ± 9.7	ns	1.20 ± 0.07	L2	0.09 ± 0.00	ns	15.2 ± 1.1	L2
5	24.7 ± 0.6	L1,2	0.35 ± 0.02	ns	83.8 ± 7.1	L2	1.11 ± 0.06	L2	0.08 ± 0.01	ns	17.7 ± 2.7	ns
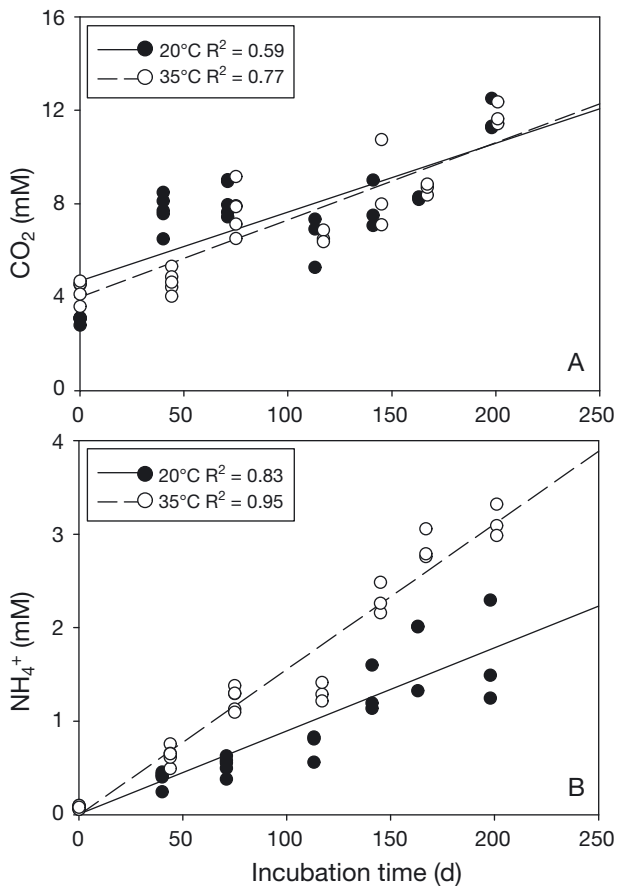
Fig. 3. *Posidonia oceanica*. Accumulation of the mineralization products (A) TCO_2 and (B) NH_4^+ over time in Level 5 at 2 temperatures (20 and 35°C). Linear regression lines are given, including R^2 values
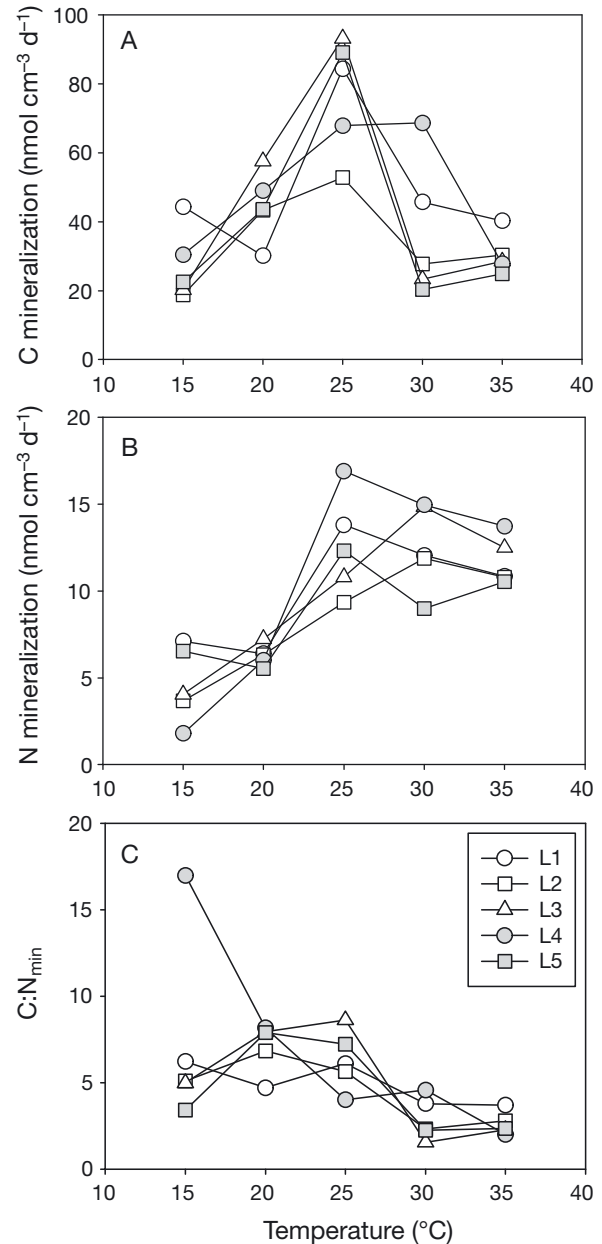

Fig. 4. *Posidonia oceanica*. Change in mineralization rates in (A) C mineralization, (B) N mineralization, and (C) C:N ratio in mineralization products (C:Nmin) with temperature in the 5 seagrass mat levels. C and N mineralization rates were calculated from changes in TCO_2 or NH_4^+ concentration, respectively, over time (n = 3–5), and the C:N ratio was calculated by division of the rates

Appendix 1). In L1, L2, and L3, ΔCPOC decreased, with the largest loss in L3 (6 to 17% of initial pool), followed by L2 (2 to 12%) and L1 (3 to 9%). In L4, the ΔCPOC decreased at 3 out of 5 temperatures, and increased at the remaining 2 (25 and 30°C), whereas the ΔCPOC increased at all temperatures in L5. Similar to ΔCPOC, the ΔCPON decreased significantly in the 4 upper levels at 15 to 25°C, except in L3, where ΔCPON increased at 15°C (Tables 4 & A3 in Appendix 1). The decrease in ΔCPON in the 4 upper levels was largest in L1 (~30%), and up to 25% was lost in the other levels. At temperatures >25°C, ΔCPON increased in all levels. The C:N ratios in coarse detritus were higher after 7 mo of decomposition than the initial ratios (Table 2, up to 23%) and attained a maximum at 25°C (Table 3). Exceptions were at higher temperatures (>25°C), where ratios decreased below the initial value, up to 32%.

In contrast to coarse detritus, there was a consistent increase in ΔFPOC and ΔFPON content for all levels and all temperatures from the initial (Table 2) to final samples (Table 3). The increases were highest at the lower temperatures for all levels (up to 2 times higher), and this pattern was the same for all levels, except for L4, with no major changes in ΔFPON between temperatures.

Table 3. *Posidonia oceanica.* Nitrogen and carbon pools in the coarse (>1 mm) and fine fraction (<1 mm) in the 5 levels of the seagrass mat at Talamanca Cove after 7 mo of incubation at 5 different temperatures. Average coarse particulate organic carbon and nitrogen (CPOC and CPON, respectively) and fine POC and PON (FPOC and FPON) are given in % dry weight (DW, $\pm$SEM, n = 3) as well as molar C:N ratios of coarse (C C:N) and fine fractions (F C:N)

Level	Temp (C°)	CPOC %DW	CPON %DW	C C:N molar	FPOC %DW	FPON % DW	F C:N molar
1	15	34.4 ± 2.0	0.33 ± 0.01	122.2 ± 4.4	2.47 ± 0.31	0.16 ± 0.03	18.0 ± 0.8
	20	33.9 ± 1.3	0.24 ± 0.01	166.0 ± 10.3	2.79 ± 0.57	0.16 ± 0.04	20.5 ± 1.0
	25	34.1 ± 0.5	0.24 ± 0.02	172.8 ± 19.5	0.92 ± 0.19	0.09 ± 0.01	11.9 ± 2.3
	30	36.1 ± 0.2	0.45 ± 0.03	94.9 ± 5.9	1.84 ± 0.12	0.10 ± 0.01	22.4 ± 0.9
	35	33.8 ± 2.0	0.44 ± 0.01	90.7 ± 7.4	1.64 ± 0.19	0.06 ± 0.01	30.7 ± 1.6
2	15	36.5 ± 1.6	0.29 ± 0.02	149.0 ± 9.7	2.54 ± 0.30	0.12 ± 0.02	24.6 ± 1.7
	20	32.7 ± 2.4	0.23 ± 0.02	166.4 ± 18.1	2.40 ± 0.23	0.13 ± 0.02	22.4 ± 1.6
	25	33.0 ± 0.5	0.23 ± 0.02	174.3 ± 17.1	2.11 ± 0.15	0.08 ± 0.01	29.7 ± 2.7
	30	36.2 ± 1.0	0.40 ± 0.02	104.6 ± 2.3	1.87 ± 0.05	0.06 ± 0.01	33.9 ± 1.0
	35	34.9 ± 1.1	0.43 ± 0.02	94.0 ± 0.2	2.40 ± 0.29	0.05 ± 0.01	59.9 ± 2.1
3	15	32.4 ± 3.4	0.46 ± 0.04	82.0 ± 5.4	2.02 ± 0.20	0.14 ± 0.02	17.1 ± 0.6
	20	29.3 ± 1.9	0.28 ± 0.02	121.2 ± 5.4	1.64 ± 0.05	0.13 ± 0.01	15.0 ± 1.0
	25	30.1 ± 1.8	0.28 ± 0.05	132.7 ± 20.0	1.51 ± 0.14	0.09 ± 0.01	19.4 ± 2.8
	30	31.8 ± 3.2	0.47 ± 0.05	78.7 ± 6.3	1.10 ± 0.10	0.06 ± 0.01	24.7 ± 3.3
	35	28.5 ± 0.3	0.42 ± 0.01	79.4 ± 1.6	1.57 ± 0.23	0.06 ± 0.01	32.3 ± 4.3
4	15	29.3 ± 1.6	0.32 ± 0.03	107.1 ± 7.1	1.83 ± 0.11	0.12 ± 0.01	18.6 ± 1.0
	20	27.8 ± 0.6	0.30 ± 0.04	109.4 ± 11.0	1.44 ± 0.13	0.11 ± 0.01	16.1 ± 1.0
	25	32.2 ± 1.3	0.34 ± 0.02	111.8 ± 11.0	2.26 ± 0.61	0.14 ± 0.05	19.8 ± 1.4
	30	32.7 ± 0.8	0.47 ± 0.02	81.8 ± 3.6	1.62 ± 0.17	0.06 ± 0.01	32.1 ± 3.4
	35	29.3 ± 0.3	0.49 ± 0.01	70.3 ± 1.1	1.31 ± 0.11	0.10 ± 0.02	16.6 ± 1.8
5	15	34.2 ± 0.5	0.45 ± 0.02	89.5 ± 3.7	1.82 ± 0.17	0.14 ± 0.01	15.6 ± 0.3
	20	30.9 ± 0.4	0.35 ± 0.02	103.9 ± 7.2	1.66 ± 0.20	0.16 ± 0.03	12.4 ± 1.2
	25	31.9 ± 2.9	0.50 ± 0.06	74.7 ± 5.5	1.52 ± 0.04	0.09 ± 0.01	19.9 ± 0.6
	30	30.2 ± 1.4	0.47 ± 0.02	75.5 ± 1.1	1.41 ± 0.08	0.06 ± 0.01	30.7 ± 5.6
	35	31.1 ± 1.1	0.47 ± 0.01	76.9 ± 1.9	1.16 ± 0.24	0.11 ± 0.02	12.7 ± 1.0

DISCUSSION

The nutrient concentrations and OM pools in the mat in Talamanca Cove are comparable to other studies of *Posidonia oceanica* mats showing high OM pools with depth (Mateo et al. 1997). Both dissolved and particulate nutrient pools showed maximum values at ~1 m depth and decreased at greater depths, suggesting accumulation of OM until ~1 m followed by net decomposition in the deeper parts of the mat. The relative N loss from the particulate matter was less than the relative C loss resulting in decreasing C:N ratios in the oldest parts of the mat. Decreasing concentrations of dissolved N (NH_4^+) suggest that N was immobilized from the interstitial water by bacteria, resulting in N enrichment of the mat detritus through bacterial colonization (Mateo et al. 1997). Similar observations of declining C:N ratios with increasing age of the detritus has been observed in terrestrial (Rumpel & Kogel-Knabner 2011) and marine ecosystems (Mateo et al. 1997, Holmer & Olsen 2002).

These *in situ* observations in Talamanca Cove are consistent with the experimental results of this study.

Microbial metabolites (TCO_2 and NH_4^+) accumulated and particulate organic pools decreased with incubation time, reflecting a net decomposition of mat material. The C:N stoichiometry did not follow the observations from the field site, as the C:N ratio of mat material increased during the 7 mo incubation period, which contradicts the observed decline in C:N ratios in the oldest parts of the mat. Increasing temperature increased the net decomposition until an upper limit of 25 to 30°C, after which decomposition ceased.

Mineralization rates in mat material

Low rates of mat decomposition under *in situ* conditions are controlled by low availability of oxygen and a high content of refractory compounds in the mat material (Hedges et al. 2000, Mateo et al. 2006). In the experiment, oxygen was eliminated to reflect *in situ* conditions, and the presence of refractory compounds was confirmed by high C:N ratios in the mat (84 to 142). The experimental mineralization rates of the mat material were similar to rates found

Table 4. *Posidonia oceanica*. Changes in carbon (C) and nitrogen (N) during the incubation based on accumulation of mineralization products and net change in coarse and fine particulate organic carbon (POC) and particulate organic nitrogen (PON) pools. Shown are the production of C and N from mineralization (ΔDIC and ΔDIN), change in ΔCPOC and ΔCPON, or ΔFPOC and ΔFPON increase (positive value) or depleted (negative value) in the coarse or fine fraction (μmol cm^{-3}). Values are average ± SEM for ΔCPOC/N and ΔFPOC/N (n = 3); for ΔDIC/DIN, accumulation was calculated from the slope of regression lines (see Fig. 4)

Level	Temp (°C)	ΔDIC (μmol cm^{-3})	ΔCPOC (μmol cm^{-3})	ΔFPOC (μmol cm^{-3})	ΔDIN (μmol cm^{-3})	ΔCPON (μmol cm^{-3})	ΔFPON (μmol cm^{-3})
1	15	8.7	−145 ± 96	708 ± 161	0.8	−0.6 ± 0.4	36.5 ± 11.4
	20	5.9	−169 ± 61	873 ± 295	1.2	−4.2 ± 0.3	36.2 ± 17.6
	25	16.5	−159 ± 22	−88 ± 99	2.7	−4.3 ± 1.0	4.6 ± 1.4
	30	8.9	−63 ± 7	383 ± 60	2.3	4.3 ± 1.3	7.3 ± 2.9
	35	7.8	−174 ± 93	281 ± 98	2.1	3.8 ± 0.5	−7.2 ± 4.1
2	15	3.7	−29 ± 66	492 ± 160	0.7	−0.7 ± 0.6	21.8 ± 9.8
	20	5.5	−187 ± 100	417 ± 123	1.3	−2.7 ± 0.8	23.9 ± 10.3
	25	6.7	−173 ± 22	262 ± 77	1.9	−2.9 ± 0.9	3.7 ± 2.9
	30	5.5	−44 ± 39	135 ± 25	1.3	3.4 ± 0.7	−5.2 ± 1.3
	35	6.0	−95 ± 45	420 ± 155	2.1	3.4 ± 0.5	−12.9 ± 4.4
3	15	4.0	−75 ± 127	516 ± 101	0.8	2.7 ± 1.3	21.4 ± 6.4
	20	7.3	−191 ± 72	331 ± 27	1.4	−3.0 ± 0.6	17.3 ± 1.9
	25	11.8	−162 ± 66	265 ± 67	2.2	−3.1 ± 1.7	2.4 ± 4.0
	30	4.6	−99 ± 119	62 ± 50	1.3	3.1 ± 1.6	−13.7 ± 5.3
	35	5.7	−219 ± 13	293 ± 115	2.5	1.4 ± 0.2	−13.1 ± 0.4
4	15	6.1	−22 ± 53	298 ± 53	0.4	−0.6 ± 0.9	9.1 ± 1.0
	20	6.2	−74 ± 20	110 ± 63	1.2	−1.2 ± 1.1	5.0 ± 4.5
	25	8.6	77 ± 43	499 ± 290	3.4	−0.1 ± 0.6	19.4 ± 20.3
	30	13.7	95 ± 26	197 ± 60	1.3	3.7 ± 0.6	−13.0 ± 4.0
	35	5.6	−23 ± 9	52 ± 53	2.7	4.2 ± 0.2	1.1 ± 6.7
5	15	4.5	669 ± 32	307 ± 71	0.8	5.8 ± 1.0	22.4 ± 5.3
	20	8.8	439 ± 28	236 ± 84	1.1	−0.02 ± 1.3	30.7 ± 9.2
	25	11.2	510 ± 204	178 ± 15	2.5	9.2 ± 3.3	5.0 ± 1.2
	30	4.1	389 ± 98	128 ± 36	1.1	7.0 ± 1.1	−7.3 ± 2.2
	35	5.0	450 ± 80	21 ± 104	2.1	7.3 ± 0.7	10.8 ± 5.9

in coastal sediments, although the OM content is much lower in coastal sediments (1 to 5% DW Canfield et al. 1993, Holmer et al. 2003) indicating slow decomposition of the mat material when considering the high organic content (>30% DW).

Temperature affected the C and N mineralization rates very strongly, as C mineralization was stimulated up to 4.5 times and N mineralization up to 14.3 times by an increase in temperature of 10°C. This is a higher increase than that found in most studies of marine sediments. Studies of temperature responses of microbial processes in marine sediments have shown that rates of both hydrolysis and fermentation exceed the terminal mineralization at higher temperatures compared to normal conditions (Weston & Joye 2005, Robador et al. 2010). As a result, dissolved OM (DOM) may accumulate in the pore waters at higher temperatures, as the sulfate-reducing bacteria are not able to keep up with the production (Robador et al. 2010). In our experiment, however,

we neither observed detectable changes in the DOC pools during the duration of the experiment, nor did we observe that DOC accumulation was affected by temperature. This suggests that, in the mat incubations, the sulfate-reducing bacteria consumed all of the organic C substrates produced by hydrolysis and fermentation. This is probably due to the low availability of OM in the mat with sulfate-reducing bacteria controlled by the production rate of small organic substrates. The high temperature response for NH_4^+ mineralization rate indicates that deamination was stimulated more strongly than C oxidation. To our knowledge, no studies are available on temperature effects on decoupling of C and N mineralization. However, as deamination occurs during all steps in the decomposition pathway, NH_4^+ production may be enhanced without accumulating DOM in the pore waters. Both C and N mineralization decreased at temperatures above 25 to 30°C, and a similar temperature response has been found for sulfate reduction

rates in temperate sediments (Widdel & Hansen 1992, Isaksen & Jørgensen 1996). This is attributed to the presence of mesophilic bacteria that show optimal growth at relatively low *in situ* temperatures (20 to 25°C, Isaksen & Jørgensen 1996). At temperatures above optimal growth, high requirements for maintenance for the slow-growing bacteria in the mat result in negative growth rates and overall decrease in mineralization rates. Presence of mesophilic sulfate-reducing bacteria is known from *Posidonia oceanica* meadows, where they are isolated from the living parts of the plants (García-Martínez et al. 2009). Their optimal growth temperature was 28 to 34°C, suggesting that bacteria capable of growing at the experimental temperatures are present in the mat, but they may require longer time for adaptation to higher temperatures than the duration of this experiment. Also, very slow-growing bacteria may be present in the dead mat, where temperature effects on maintenance requirements may strongly perturb the balanced *in situ* slow growth rates. The unbalanced growth rates may explain why a relatively low increase in temperature results in increased mineralization rates while higher temperatures result in reduced rates, as the bacteria can no longer cope with their maintenance requirements and have negative growth rates.

In contrast to the temperature response, there were no consistent effects of the age of the mat material on the C and N mineralization rates. Within each temperature, mineralization rates varied up to a factor of 5 between levels, but the variability was not consistent between temperatures. This is in contradiction to observations of OM decomposition in sediments, where the microbial activities typically decrease with increasing age of macrophyte OM (Harrison 1989) and decrease with increasing C:N ratios in macrophyte OM (Kristensen & Hansen 1995). As the first level in the mat was sampled at 54 cm, this material was already several hundred years old, and beyond the period of fast decay (Romero et al. 1992, Mateo & Romero 1997, Mateo et al. 2006). The lack of age effect on C and N mineralization rates could be due to low pools of labile organic compounds present throughout the mat sampled.

C:N stoichiometry in mat decomposition

C and N stoichiometry of the accumulating dissolved mineralization products has been used to determine the source material under decomposition (Kristensen & Hansen 1995). In this experiment, the C:N ratios in the mineralization products were consistently lower (<8) than C:N ratios in the particulate coarse (CPOM C:N = 84 to 142) and fine OM (FPOM C:N = 13 to 25). Discrepancy between the C:N ratio of mineralization products and the OM under decomposition is commonly observed in marine sediments and attributed to the decoupling between C and N mineralization, where uptake of N into bacteria results in higher C:N ratios in the mineralization products compared to the source material (Kristensen & Hansen 1995). In this experiment, however, the C:N ratios remained lower than the mat material throughout the incubation (4 to 8, Fig. 3) with ratios closer to bacterial C:N ratios (~5).

Based on C and N mineralization rates, it is possible to estimate the C:N ratio in the substrate ($1/N_s$) under decomposition from Lomstein et al. (1998). First, bacterial N incorporation was estimated using the equation:

$$i = C_O E N_c / (1 - E) \qquad (1)$$

where C_O is the C oxidation estimated from C mineralization rates during the decomposition period (nmol C cm^{-3}d^{-1}); E is the C incorporation efficiency (no unit), and N_c is the ratio between N and C (mol mol^{-1}) in bacterial cells. E was assumed to be 0.19 (Pedersen et al. 1999) and N_c was 0.2 mol mol^{-1} (Fagerbakke et al. 1996). Then N_s was calculated:

$$N_s = E N_c d / i \qquad (2)$$

where E, N_c, and i are known from Eq. 1, and d is the gross NH$_4^+$ mineralization (nmol N cm^{-3} d^{-1}) calculated from the increase in N mineralization products. These calculations show that C:N ratios in the substrate used by the bacteria ranged between 0.6 and 54 with an average of 7.6, which is well below the mat material (84 to 142) but higher than bacterial cells (~5), suggesting that the OM under decomposition was a mix of mat material, sediment OM (including non-seagrass sources), and bacterial cells dominated by the last component. The loss of OC and ON from the coarse fraction during the experiment, with relatively larger loss of N at temperatures up to 25°C, supports that mat material was a source of OM for the bacteria. At temperatures above 25°C, no net loss in C and N was detected from the coarse fraction, which could be due to colonization of this fraction by bacteria. At the same time, the C:N ratio increased in the fine fraction, more so than observed at the lower temperatures, suggesting a relocation of the high C:N OM from the coarse to the fine fraction. Further studies are needed to fully understand the C:N stoichiometry at high temperatures.

Temperature effects on mat decomposition

Compared to C and N turnover of mat and macrophyte detritus in general (Table 5), the C and N turnover found in Talamanca Cove at 15°C (closest to annual average water temperature of 17°C) is low. The C ($<0.016\%$ C d^{-1}) and N turnover ($<0.0001\%$ N d^{-1}) were 1 to 3 orders of magnitude lower than observed elsewhere (Table 5). The results from Talamanca Cove confirm that the mat material is highly resistant to microbial decomposition, and the rates compare well with modeled values (Mateo et al. 1997). The non-linear response of mineralization rates to age of the mat and temperature complicates the prediction of the C sink capacity of *Posidonia oceanica* mat in a future warmer climate. Temperature generally enhanced mineralization rates down through the mat attaining maximum rates at 25 to 30°C, with declining rates at higher temperatures. If an extrapolation from this experiment to an *in situ* scenario is possible, a rise in ocean temperature may decrease the capability of the mat to act as a C sink up to 25 to 30°C and with unknown response above this temperature. At present, the water temperature in the NW Mediterranean ranges from 15 to 26°C based on monthly averages, and increasing temperatures may increase C mineralization in the mat and thereby enhance CO_2 emission to the atmosphere. The temperature response may be buffered by a lower temperature inside the mat, as Frost (1969) noted a lower temperature inside the mat compared to surrounding waters, which may be similar to observations in peat (A. Martínez-Cortizas pers. comm.). Temperature will increase the release of NH_4^+ more than CO_2, and higher temperatures may thus not only increase emissions of CO_2 but also the regeneration of nutrients in *P. oceanica* mats, which could affect the delicate nutrient balance in these oligotrophic ecosystems. Furthermore, temperature limits for the growth of living *P. oceanica* on top of the mat should be taken into consideration as it affects the input of detritus to the mat. *P. oceanica* can live within a broad temperature range of 9 to 29°C (Gobert et al. 2006), but if the ocean temperature increases above the 29°C, even just for short periods, plant mortality increases (Marbà & Duarte 2010) and accumulation of detritus could be drastically reduced.

CONCLUSION

This work is the first to investigate the mechanisms leading to the millenary accumulation of C and nutrients occurring within the very atypical marine sediment represented by *Posidonia oceanica* mats. The study confirms low mineralization of mat detritus resulting in major storage of C and nutrients in the *P. oceanica* ecosystems in the Mediterranean. Further work in this field should aspire to (1) a more detailed description of the bacterial processes within the mat aiming to accurately track the sources and fates of the various C fractions, and (2) to the integration of

Table 5. Carbon (C) and nitrogen (N) turnover rate (% d^{-1}) for the investigated *Posidonia oceanica* mat and other macrophytes from the literature. –: no data

Species	C turnover rate (% C d^{-1})	N turnover rate (% N d^{-1})	Material/notes	Source
Posidonia oceanica	0–0.016	0–0.00010	Mat (15°C)	Present study
P. oceanica	0.012	–	Leaf 20 m depth	Romero et al. (1992)
P. oceanica	0.01–0.03	–	Old leaf (15–24°C)	Romero et al. (1992)
P. oceanica	0.04–0.05	0.0002	Leaf 5 and 13 m depth	Mateo & Romero (1997)
P. australis	0.458, 0.505	–	Leaf experiment 1 and 2 (seagrass)	Walker & McComb (1985)
Zostera marina	0.2–0.3	–	Fresh root and rhizome (seagrass)	Harrison (1989)
Halophila decipiens	7.1–11.0	–	Fresh root and rhizome (seagrass)	Harrison (1989)
Thalassia testudinum	0.04–0.8	–	Fresh root and rhizome (seagrass)	Harrison (1989)
Rhizophora apiculata	1.4	–	Leaf, seagrass sediment (mangrove)	Holmer & Olsen (2002)
Enhalus acoroides	2.1	–	Leaf, sediment (seagrass)	Holmer & Olsen (2002)
Ulva lactuca	0.47	0.37	Leaves, aerobic slurry (green algae)	Kristensen (1994)
Fucus vesiculosus	0.63	0.42	Leaves, aerobic slurry (brown algae)	Kristensen (1994)
Zostera marina	0.41	0–0.01	Leaves, aerobic slurry (seagrass)	Kristensen (1994)
Ruppia maritima	0.47	0.14	Leaves, aerobic slurry (seagrass)	Kristensen (1994)
Rhizophora apiculata	0.11	0–0.01	Leaves, aerobic slurry (mangrove)	Kristensen (1994)
Fagus silvatica	0	0–0.01	Leaves, aerobic slurry (beech)	Kristensen (1994)

the complex effects that temperature has on the C dynamics at plant and ecosystem levels.

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Appendix 1. Analysis of variance (ANOVA) results for Tables 1 to 4. DOC: dissolved organic carbon, CPOC (FPOC): coarse (fine) particulate organic carbon, CPON (FPON): coarse (fine) particulate organic nitrogen, C C:N (F C:N): molar C:N ratios of coarse (fine) organic matter, DW: dry weight, ΔDIC (ΔDIN): production of C (N) from mineralization

Table A1. One-way ANOVA results for the effect of level (Tables 1 & 2). Significant values are shown in **bold**

Dependent variable	df	SS	MS	F	p
CO_2	4	10.499	2.625	18.917	**<0.001**
NH_4^+	4	0.0986	0.0329	61.091	**<0.001**
DOC	3	0.812	0.271	2.837	0.116
CPOC[a]	4	–	–	–	**0.020**
CPON	4	0.00708	0.00177	0.643	0.644
C C:N	4	6201.480	1550.370	5.844	**0.011**
FPOC	4	0.733	0.183	11.924	**<0.001**
FPON	4	0.000688	0.000172	0.895	0.502
F C:N	4	259.132	64.783	6.820	**0.006**

[a]ANOVA on ranks

Table A2. Two-way ANOVA results for the data presented in Table 3. Significant values are shown in **bold**

	df	SS	MS	F	p
CPOC %DW					
Level	4	278.124	69.531	8.696	**<0.001**
Temperature	4	73.315	18.329	2.292	0.072
Level × temp	16	89.631	5.602	0.701	0.780
CPON %DW					
Level	4	0.153	0.0383	17.114	**<0.001**
Temperature	4	0.355	0.0886	39.653	**<0.001**
Level × temp	16	0.123	0.00771	3.451	**<0.001**
C C:N					
Level	4	31853.897	7963.474	29.888	**<0.001**
Temperature	4	35642.335	8910.584	33.443	**<0.001**
Level × temp	16	12391.963	774.498	2.907	**0.002**
FPOC %DW					
Level	4	5.705	1.426	8.135	**<0.001**
Temperature	4	3.829	0.957	5.459	**<0.001**
Level × temp	16	7.223	0.451	2.575	**0.006**
FPON %DW					
Level	4	0.00649	0.00162	1.673	0.171
Temperature	4	0.0657	0.164	16.931	**<0.001**
Level × temp	16	0.0219	0.00137	1.410	0.176
F C:N					
Level	4	2372.946	593.236	41.189	**<0.001**
Temperature	4	2208.552	552.138	38.335	**<0.001**
Level × temp	16	2869.426	179.341	12.452	**<0.001**

Table A3. Two-way ANOVA results for data presented in Table 4 (general linear model). Significant values are shown in **bold**

	df	SS	MS	F	p
ΔDIC (μmol cm^{-3})					
Level	4	94.837	23.709	3.796	**0.024**
Temperature	4	47.848	11.962	1.915	0.157
ΔCPOC (μmol cm^{-3})					
Level	4	45171.319	11292.830	2.711	0.067
Temperature	4	1465711.117	366427.788	87.976	**<0.001**
ΔFPOC (μmol cm^{-3})					
Level	4	315707.919	78926.980	2.068	0.133
Temperature	4	199075.255	49768.814	1.304	0.310
ΔDIN (μmol cm^{-3})					
Level	4	10.358	2.589	10.463	**<0.001**
Temperature	4	1.292	0.323	1.306	0.310
ΔCPON (μmol cm^{-3})					
Level	4	154.119	38.530	9.426	**<0.001**
Temperature	4	127.622	31.905	7.805	**0.001**
ΔFPON (μmol cm^{-3})					
Level	4	3873.735	968.434	12.093	**<0.001**
Temperature	4	586.381	146.595	1.831	0.172