On the use of sediment fertilization for seagrass restoration: a mesocosm study on *Zostera marina* L.

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Abstract

The use of nitrogen and phosphorus sediment fertilization for seagrass restoration is explored. Special attention was given to the effects of nitrogen sediment fertilization. The sediment fertilization treatment combined different levels of nitrogen (0, 30, 500 mg N g DW^{-1}) with sufficient phosphorus to avoid P-limitation (fertilizer N:P ratio < 0.25). Using indoor mesocosms, we studied the effects of sediment fertilization, and its interactions with light availability (55, 200 μmol m^{-2} s^{-1}) and sediment redox conditions (300, -100 mV), on *Zostera marina* L. We assessed (1) treatment effects on growth and plant biomass distribution, (2) the capacity of *Z. marina* roots to meet the plant nutrient demand, (3) plant tolerance to high nutrient porewater concentration, and (4) pro's and con's of use NH_4NO_3 as the N source in sediment fertilization for seagrass restoration. Plant biomass, growth and leaf turnover rate were stimulated by light and sediment fertilization. Biomass partitioning was not affected by light availability, whereas the relative root production was decreased in fertilized sediments. Root uptake following fertilization met nutrient plant demand. After high sediment fertilization, ammonium porewater concentration was high (30 mM) regardless of redox conditions. On the other hand, nitrate availability was also high, but 80% lower in reduced sediments (0.7–4 mM) compared to non-reduced ones (20 mM). Plants of *Z. marina* exhibited a remarkable tolerance to high N + P sediment fertilization. However, plant inhibition (reduction in plant weight, leaf growth and leaf turnover rate) was detected when porewater N concentrations exceeded 30 mM. The effects of phosphorus and ammonium toxicity were discarded because availability was similar for both inhibited and non-inhibited plants. We attributed the *Z. marina* inhibition to the extra

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porewater nitrogen available as nitrate (20 mM). Experimental treatments did not inhibit the photosynthetic apparatus of *Z. marina*. The mechanisms of inhibition might be related with deficiencies in energy or C-skeletons, since inhibitory effects were buffered when saturating irradiance and/or nitrate levels decreased in reduced sediments. In conclusion, we consider that the combined N + P sediment fertilization, with NH_4NO_3 as N source and high P supply, is highly beneficial for *Z. marina* restoration. This species has positive response to N + P sediment fertilization, high tolerance to the extensive porewater enrichment, and bacterial metabolism may reduce the porewater nitrate availability in anoxic seagrass sediments. However, for adequate sediment fertilization for restoration purposes, several precautions are suggested.

1. Introduction

The decline of seagrasses has a negative effect on worldwide coastal biodiversity (Orth and Moore, 1983; Giesen et al., 1990; Short and Wyllie-Echeverria, 1996). Light reduction, especially as a consequence of anthropogenic activities, is considered the main cause of the increase in seagrass decline (Dennison et al., 1993; Short and Wyllie-Echeverria, 1996). Because of the ecological importance of seagrass habitats, many efforts were aimed at conserving existing communities and restoring the lost ones (Fredette et al., 1985; Fonseca et al., 1986, 1998; Lewis, 1987). In drastically affected areas, such as the Wadden Sea Coasts (NW Europe), the chance of natural revegetation is low, making active seagrass restoration the only alternative to recover the lost communities (van Katwijk et al., 2000). However, most seagrass restoration projects had only limited success (Merkel, 1990; Zimmerman et al., 1995; Sheridan et al., 1998). Transplanting constitutes a perturbation to the plant, especially in case of bare-root plantings, and may delay the normal growth until a favourable rhizosphere is established (Kenworthy and Fonseca, 1992). In addition, relatively small root systems upon transplanting may cause weak anchorage and insufficient nutrient uptake. Sediment fertilisation has been suggested as method to enhance successful growth of *Zostera marina* transplantings over opportunistic alga (Kenworthy and Fonseca, 1992; Sheridan et al., 1998), reducing the chance of negative effects on light as caused by water column fertilisation.

Previous work on *in situ* sediment enrichment had reported only positive effects of sediment fertilisation on seagrass growth (Orth, 1977; Kenworthy and Fonseca, 1992; Udy and Dennison, 1997; Lee and Dunton, 1999; Touchette and Burkholder, 2000). Studies on column water enrichment are agreed with the positive effects of phosphorus enrichment for seagrasses (Touchette and Burkholder, 2000). However, an in-depth study on sediment fertilisation is necessary to discard inhibitory effects of N sediment fertilisation on seagrass growth, which have been frequently described for column water enrichment (van Katwijk et al., 1997; Burkholder et al., 1992, 1994; Touchette and Burkholder, 2000).

In the previous studies on sediment fertilisation, porewater nitrogen concentrations (<2 mM NH_4^+) were within the same range as can be present in eutrophic sediments of coastal systems (<7 mM, Murray et al., 1978; Hansen and Kristensen, 1998; Hopkinson

et al., 1999). Although N fertilisation is frequently applied as NH_4NO_3, the positive effects of such sediment N-enrichment on seagrass growth were ascribed to the increase of porewater ammonium. In anoxic seagrass sediments, the availability of NO_3^- may be reduced (Udy and Dennison, 1997; Worm and Reusch, 2000; Worm et al., 2000) and its uptake inhibited by ammonium availability (Iizumi and Hattori, 1982; Zimmerman et al., 1987). Interactions with external variables, such as light availability, which meet the C-demand for N-assimilation (van Katwijk et al., 1997), or the sediment redox conditions, that affects the available N form (Anschutz et al., 2000), are generally not considered. Hence, mechanistic questions on (1) the interaction of sediment fertilisation with other external variables affecting the nutrient uptake, such as light and redox sediment condition, and (2) the dependence of such interactions on N-level and N-form, remain unexplored.

Mesocosms offer and ideal model system for answering these mechanistic questions, by allowing wide ranges of external variables, while minimising the variability of other conditions (Odum, 1984; Short, 1985, 1987). Mesocosm studies on effects of water column enrichment on seagrass growth revealed that high water column concentrations of ammonium and nitrate may inhibit seagrass growth (Short et al., 1995; Burkholder et al., 1992, 1994; van Katwijk et al., 1997; Brun et al., 2002). Whereas high ammonium uptake may inhibit seagrass photosynthesis (van Katwijk et al., 1997), negative effects of high nitrate appear to be related with deficiencies in C-skeletons (Burkholder et al., 1992, 1994). The N inhibitory threshold seems depend on the plant capacity to assimilate the N uptake (van Katwijk et al., 1997). Although the main differences have been attributed to inter-specific divergences (Touchette and Burkholder, 2000), external variables, as light and phosphorus availability, may also affect seagrass assimilation capacity, limiting the availability of C−skeletons and/or energy for N assimilation (van Katwijk et al., 1997; Brun et al., 2002). In contrast to the deep knowledge about the effects of water column enrichment, none of the previous mesocosm studies have focused on the effects of sediment fertilisation on seagrass growth. As far as we know, only one study dealt with the relative effects of porewater NH_4^+ availability on *Z. marina* growth, after water column enrichment (van Katwijk et al., 1997). Plants exhibited a higher tolerance to ammonium porewater concentrations (with maximum concentrations $<250\,\mu M$) than to water column levels ($<125\,\mu M$). Effects of higher porewater nutrient concentrations and their interaction with other external variables (e.g. light or redox sediment conditions), as referred above, remain unexplored (Hemminga, 1998). Mechanistic studies on the interaction between seagrass rhizome/root system and sediment environment are needed to achieve the optimum use of sediment fertilisation in restoring seagrass beds in a wide range of abiotic variables.

In this experiment, we worked with a broad range of nitrogen sediment fertilisation ($40\,\mu M$ to $35\,mM$), supplying enough phosphorus to avoid negative consequences of P-limitation (fertiliser N:P ratio < 0.25). We studied the effects of sediment fertilisation on growth and biomass partitioning of *Zostera marina*. Special attention was given to the effects of N form (NH_4^+ versus NO_3^-), and interactions with light availability (55 versus $200\,\mu mol\,m^{-2}\,s^{-1}$) and sediment redox conditions (300 versus $-100\,mV$). That is, using laboratory mesocosms, we assessed (1) treatment effects on growth and biomass partitioning of *Z. marina*, (2) the capacity of *Z. marina* roots to meet the plant N-demand, (3) the tolerance of *Z. marina* to high nutrient porewater concentration, and (4) possible benefits and limitations of using NH_4NO_3 as N source in N + P sediment fertilisation for restoration purposes.

2. Materials and methods

2.1. Plant material

Zostera marina plants were collected from an annual population in the intertidal of Zandkreek (Zealand, The Netherlands). For the experiment, only young plants with one developed shoot were selected.

2.2. Experimental design

The effects of sediment fertilisation, light availability and soil redox conditions on *Z. marina* were determined using a factorial design 2 (light) × 3 (nutrients) × 2 (redox conditions, only for the highest nutrient treatment) in a mesocosm experiment with six replicates. Individual plants were transplanted in cylindrical pots (85 mm diameter; 250 mm height). All pots were submersed in a single aquarium (2000 mm × 600 mm × 600 mm) and cultivated during 2 months. The water column was homogenised with a multi-pump system and continuously flushed ($100 \, l \, h^{-1}$) with filtered seawater from Oosterschelde estuary (salinity 28–29).

Half of pots ($n = 24$) were exposed to saturating light levels (high irradiance, HI: $200 \, \mu mol \, m^{-2} \, s^{-1}$) and the other half to a subsaturating light (low irradiance, LI: $55 \, \mu mol \, m^{-2} \, s^{-1}$). Light was supplied with five sodium lamps (type SGR 102–400, $1 \times$ SON-T 400 W) and five halure ones (type MGR 102–400, $1 \times$ HPI-T 400 W). The light levels were achieved by interposing neutral density covers between the light source and the aquarium. Within each light treatment, pots were moved twice a week to avoid any potential heterogeneity in the light field. Plants were maintained at $18 \, °C$ under an 18 h light- and 6 h dark-cycle.

Contrasting sediment conditions were obtained by supplementing low nutrient sandy sediment (1.9 kg per pot) with a mixture of slow release fertilisers (N–P–K, Osmocote®) and organic matter. The experimental levels simulated the N availability for pristine (0F, $0 \, mg \, N \, g \, DW^{-1}$), low fertilised (LF, $30 \, mg \, N \, g \, DW^{-1}$) and high fertilised (HF, $500 \, mg \, N \, g \, DW^{-1}$) sediments. The N fertilisation level in the LF sediment is comparable to porewater N availability in eutrophic systems (Hansen and Kristensen, 1998; Clavero et al., 2000), while the level of N fertilisation in HF sediments is only possible by active fertilisation. To avoid effects of P limitation, all pots were highly P enriched (fertiliser N:P ratio < 0.25) with 0.3, 0.9 and $2 \, mg \, P \, g^{-1}$ DW for 0F, LF and HF treatments, respectively. For the high fertilisation level (HF), we also manipulated soil redox conditions, which affected the availability of nitrate versus ammonium. Reduced and fertilised sediments (HF-R level) were obtained by adding organic matter (1 g grounded plant tissues + 1 g cellulose per pot) to an additional group of the HF-pots. To induce nutrient release from the fertiliser and the degradation of the organic matter, all pots were submerged in seawater 2 weeks before transplanting.

2.3. Plant growth and biomass partitioning

Length and width of individual leaves were measured every 12 days for each plant. The recruitment and morphometry of new leaves were also quantified. At the end of the

experiment, plants were retrieved and individual leaves, spathe peduncles, rhizomes and roots were oven-dried (48 h at 60 °C) and weighted. The leaf tissues were analysed for C and N content (Carlo-Erba NA 1500 CHN-analyser). The leaf growth rate on a dry weight base (LGR, mg DW per plant per day; Eq. (1)) was obtained as product of the leaf elongation rate (LER, centimetre per plant per day; Eq. (2)) by the ratio between leaf dry weight (DW, g) and the leaf length (LL, cm). We also quantified the leaf turnover rate (LTR, per day; Eq. (3)) and the leaf loss rate (LLR, centimetre per plant per day; Eq. (4)).

$$LGR = LER \times \frac{DW}{LL} \tag{1}$$

$$LER = \frac{\sum_{i=1}^{n}[(LL_{i,f} - LL_{i,o}) > 0]}{t_f - t_o} \tag{2}$$

$$LTR = \frac{LER}{\sum_{i=1}^{n} LL_{i,o}} \tag{3}$$

$$LLR = \frac{\sum_{i=1}^{n}|(LL_{i,f} - LL_{i,o}) < 0| + \sum_{i=1}^{m} LL_{i,o}}{t_f - t_o} \tag{4}$$

where $LL_{i,o}$ and $LL_{i,f}$ are the length of each individual leaf (subscript i) at the beginning (subscript o) and at the end (subscript f) of the study period (t_f–t_o), respectively; the subscript n indicates the total number of standing leaves in each plant at the end (t_f) of the study period; the subscript m indicates the number of leaves lost at the end of the study period.

2.4. Photosynthetic response

Prior to sampling of plants, variable chlorophyll-fluorescence of PS II was measured using a PAM-2000 fluorometre (Walz, Effelfrich, Germany) to evaluate the conditions of the photosynthetic apparatus. In a climated room (18 °C), fluorescence measurements were performed in the basal portion (4 cm over sheath) of the youngest completely developed leaves. Each leaf was placed underwater at 4 mm from the fiberoptics. Optimum quantum yield (F_v/F_m) was calculated as the ratio of variable to maximum fluorescence of the dark-acclimated leaf (darkness for 5 min). To monitor changes in photosynthetic parameters, photosynthesis versus irradiance curves were determined. Samples were exposed to increasing irradiances of actinic red light (650 nm, 15–550 µmol m^{-2} s^{-1}). Since the specific absorption coefficient for chl a was not estimated, the photosynthetic response was expressed in terms of relative electron transport rate (rel.ETR; Schreiber et al., 1994). Although this estimation differs from the absolute electron rate, the rel.ETR versus light curve may be used to detect stress indications in the photosynthetic apparatus (Hemminga and Duarte, 2000). The rel.ETR values were calculated by multiplying the quantum yield in light ($\Delta F/F'_m$) and photosynthetically active photon flux density (PPFD, mmol photons m^{-2} s^{-1}), i.e. rel.ETR $= (\Delta F/F_m)' \times$ PPFD. The photosynthetic parameters, maximum photosynthetic capacity (rel.ETR$_{max}$), photosynthetic efficiency (α) and a photoinhibition slope (β), were calculated by fitting the rel.ETR versus irradiance curves to a photoinhibition model modified from the one described by Platt et al. (1980) for marine phytoplankton.

2.5. Sediment conditions

Every 12 days, the redox potential of the HF and HF-R sediments was registered at 100 mm depth, using home-made platinum electrodes with a Ag/AgCl double junction reference electrode. All readings (MB 11 mV, Microscale Measurements, The Hague, The Netherlands) were converted to values for a standard Hydrogen reference electrode (i.e. adding 210 mV). To ascertain a stable signal, the electrodes were always allowed to stabilise for 2 h before taking the readings. Samples of porewater from 2 to 12 cm depth were collected with soil moisture samplers (RHIZON SMS-10 cm, Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) and kept frozen until analysis. The concentrations of nitrite and nitrate, and ammonium in porewater were analysed with a Skalar 5100 autoanalyser according to Strickland and Parsons (1972).

2.6. Statistical analysis

The experiment had a block design with six replicates per experimental case. Light and sediment effects were contrasted using a two-way ANOVA (Zar, 1984), followed by the Tukey test for multiple comparisons in case of a significant ANOVA result. Heterocedastic data were log transformed. In all cases, the significance level was set at 5% probability.

3. Results

3.1. Sediment conditions

Present sediment treatments were successful in creating significant differences in (a) NO_3^- and NH_4^+ porewater concentrations among fertilisation levels and (b) redox potentials among sediments with contrasting amounts of organic matter (Table 1). That is, the NO_3^- and NH_4^+ concentrations in the porewater varied by a factor 10,000 and 1000, respectively (Table 2). Whereas the addition of organic matter to the fertilised sed-

Table 1

Statistical analysis for the effects of light and redox treatments on sediment conditions (sediment redox potential and concentrations of porewater nitrogen)

Variable	Time (days)		Variation sources		
			Light	Redox	Light × redox
Sediment potential	0		–	**	–
Sediment potential	50		–	**	–
Nitrite and nitrate	50	T	*	**	*
Ammonium	50	T	*	**	**

T indicates log-transformed data.

 * $P < 0.05$.

 ** $P < 0.001$.

Table 2
Final concentrations of porewater nitrogen in function of light availability and sediment conditions

Porewater	Sediment	Light	
		LI	HI
Nitrite + nitrate (mM)	0F	$3 \times 10^{-3} \pm 1 \times 10^{-3}$ a	$8 \times 10^{-3} \pm 2 \times 10^{-3}$ b
	LF	$2 \times 10^{-3} \pm 4 \times 10^{-4}$ a	$8 \times 10^{-3} \pm 2 \times 10^{-3}$ b
	HF	22.2 ± 3.6 d	18.3 ± 2.5 d
	HF-R	4.1 ± 3.2 c	0.7 ± 0.3 c
Ammonium (mM)	0F	0.18 ± 0.04 b	0.04 ± 0.01 a
	LF	2.2 ± 0.2 c	1.6 ± 0.2 c
	HF	35.4 ± 3.3 d	30.0 ± 5.0 d
	HF-R	32.9 ± 4.1 d	31.9 ± 6.0 d

The values represent the mean and the S.E. ($n = 6$). For each variable (nitrite + nitrate or ammonium concentrations), different letters in the alphabetic codes mean significant differences between experimental cases (see Table 1).

iments (HF-R) had no effect on the porewater NH_4^+ (ca. 30 mM for NH and NH-R cases), porewater nitrite + nitrate concentrations were reduced by approximately 80% (Tables 1 and 3), regardless of the light level.

Sediments supplied with organic matter (HF-R) maintained a redox potential close to -100 mV, revealing an effective reduction of the oxygen levels throughout the experiment (Table 3). The redox potential of the high fertilised sediments without addition of organic matter (HF) was much higher: around 300 mV at the start of the experiment, which then gradually decreased to just above 0 mV after 50 days. Sediment redox potential was unaffected by light treatment (Table 1).

3.2. Plant responses

Plant dry weight (DW_t) was affected significantly by light and sediment conditions (Fig. 1A, Table 4). Plants under subsaturating light accumulated lower biomass than those grown at saturating level ($P < 0.05$) irrespective of the sediment enrichment conditions.

Table 3
Sediment redox potential in high fertilised sediments in function of light and organic matter addition

Experimental time (day)	Sediment	Light	
		LI	HI
0	HF	273.4 ± 15.6	236.8 ± 27.5
	HF-R	-107.7 ± 14.6	-64.2 ± 35.5
50	HF	66.7 ± 29.8	23.5 ± 45.1
	HF-R	-98.5 ± 35.8	-126.2 ± 26.9

HF: without organic matter addition, HF-R: with organic matter addition. The values represent the mean and the S.E. ($n = 6$).
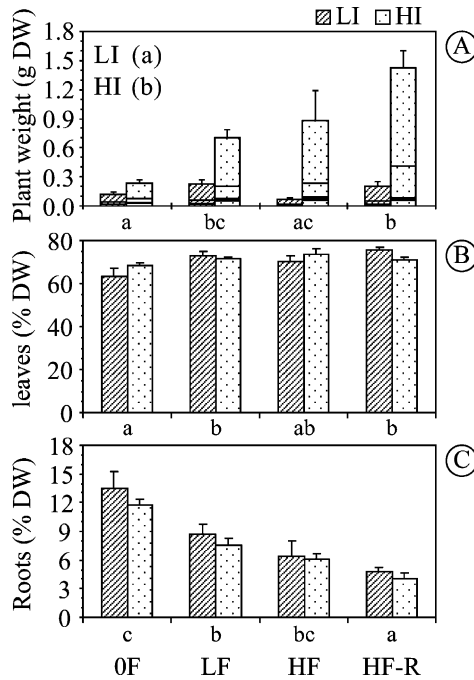
Fig. 1. Plant weight (A) and percentage of leaf (B) and of root (C) biomass in function of light availability and sediment conditions. In part A, the columns are divided from top to down, in leaves, spathe peduncles, rhizomes (on black) and roots, respectively. Codes with the same letter means not significant differences between light (A) or sediment levels (A–C; $P < 0.05$). Error bars represent S.E. (n=6).

Table 4
Statistical analysis for the effects of light and sediment treatments on biological variables of *Z. marina*

Variable		Variation sources		
		Light	Sediment	Light × sediment
Plant weight		**	**	–
Leaves (% DW)		–	*	–
Roots (% DW)		–	**	–
Leaf carbon content		–	*	–
Leaf nitrogen content		–	**	–
Leaf growth rate	T	**	**	*
Leaf turnover rate		–	**	–
Leaf loss rate		**	–	–
F_v/F_m		–	*	–
rel.ETR$_{max}$		–	–	–
α		–	–	–
β	T	–	–	–

T indicates log-transformed data.
   * $P < 0.05$.
   ** $P < 0.001$.

Table 5
Leaf carbon and nitrogen content of *Z. marina* plants in function of light availability and sediment conditions

Sediment	Light	
	LI	HI
Leaf carbon content (% DW)		
0F	27.5 ± 0.9	26.1 ± 0.9 a
LF	27.9 ± 0.9	29.6 ± 0.8 b
HF	28.4 ± 0.5	26.6 ± 0.8 a,b
HF-R	27.4 ± 0.9	28.0 ± 0.5 a,b
Leaf nitrogen content (% DW)		
0F	$1.65 \pm 0.04^*$	$1.63 \pm 0.06^*$ a
LF	1.81 ± 0.08	1.82 ± 0.07 b,c
HF	$1.49 \pm 0.17^*$	1.84 ± 0.14 a,b
HF-R	1.92 ± 0.09	1.92 ± 0.13 c

The values represent the mean and the S.E. ($n = 6$). Different letters in the alphabetic codes in the last column of every constituent indicate significant differences between sediment levels.

  * *N*-values below the critical 1.8% DW for seagrass leaves (Duarte, 1990).

Although P was highly supply in all pots, plants grown in non N fertilised sediments (0F) accumulated less biomass than those cultured in low (LF) or high (HF) N fertilised ones ($P < 0.05$). However, plants growing at HF sediments under subsaturating light (LI) formed an exception to this trend. The HF-LI conditions were apparently stressful for *Zostera marina*, since these plants exhibited the lowest weight values. Biomass partitioning was affected by the sediment treatments, but not by light (Fig. 1B and C, Table 4). In non N fertilised sediments (0F), the percentage of plant biomass allocated into leaves was comparatively low, yielding a high relative biomass in the roots. With increasing sediment fertilisation, we found a sharp decrease in relative root weight (i.e. from 14% for 0F to 4% for HF sediments; Fig. 1C). The percentage of rhizome ($<5\%$ DW_t) and spathe peduncle biomass ($<25\%$ DW_t) were unaffected by the experimental treatments (data not shown). Most of the plants developed generative shoots ($>90\%$), but there was no effect of experimental conditions ($P > 0.05$).

The leaf N and C contents were affected by sediment conditions ($P < 0.05$), but not by light (Table 4). Plants growing in low N sediments (0F) may be N-limited, since the leaf N content was below the critical value in plants that accumulated lower biomass that in other treatments (Table 5, see above). The N-limitation may also occur in plants cultured in fertilised sediments without organic matter supply (HF) but under subsaturating light, with the lowest weight values (see above).

The leaf growth rate (LGR) varied from 1 to 48 mg DW per plant per day (Fig. 2A). Similar to the plant weight pattern (Fig. 1A), the LGR was affected by light and sediment treatments and their interaction (Table 4, Fig. 2A). The LGR values were lower under subsaturating light than under saturating one, regardless of the sediment treatment. Plants grown in non N fertilised sediments (0F) had lower LGR values than those cultured in fertilised ones (LF and HF), especially when grown under saturating light. Once more, stress indications were detected in plants growing at high fertilised sediments (HF)
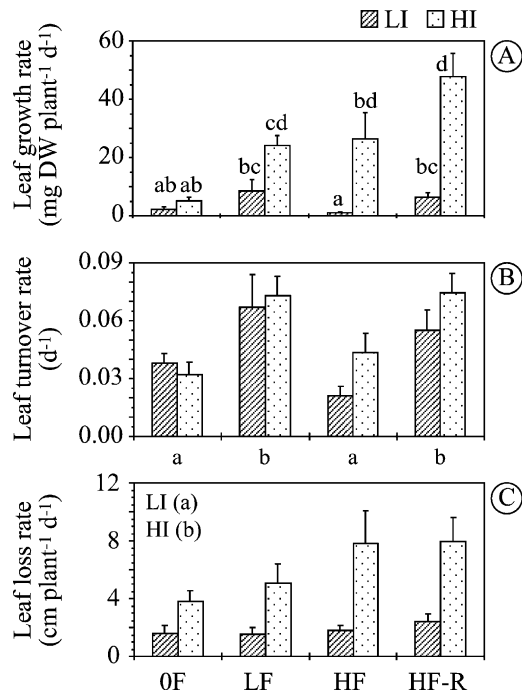
Fig. 2. Leaf growth rate (A), leaf turnover rate (B) and leaf loss rate (C) of *Z. marina* plants in function of light availability and sediment conditions. Codes with the same letter means not significant differences between experimental cases (A), sediment (B) or light levels (C; $P < 0.05$). Error bars are S.E. ($n = 6$).

under subsaturating light (LI), recording the lowest LGR values (1.1 mg DW per plant per day).

Although DW_t and LGR were significantly affected by light and sediment conditions, the leaf turnover rate (LTR) was only affected by the sediment treatments (Table 4, Fig. 2B). The lowest LTR (ca. 3% per day) were found in plants grown in non N fertilised sediments (0F) as well as in high fertilised ones (HF). In contrast, the leaf loss rates (LLR) were only affected by the light treatment (Table 4, Fig. 2C). The highest LLR values were recorded in plants grown under saturating light levels (HI) ($P < 0.05$).

Negative effects of the experimental treatments on photosynthetic apparatus were not detected. Although *Z. marina* plants appearing sensitive to high irradiances with positive photoinhibition coefficients (Table 6), no significant differences in any photosynthetic parameter (rel.ETR$_{max}$, α, β) were detected between light and/or sediment treatments (Table 4). However, the results could differ significantly if the specific absorption coefficient for chl a changes among treatments. The optimum quantum yield (F_v/F_m) was significantly affected by the sediment treatment, but not by the light one (Table 4). The plants from LF sediments showed significantly higher F_v/F_m values than those from HF-R ones (Table 6). However, this variation was small (c.v. < 15%) within a range of high values (0.7–0.6).

Table 6
Optimum quantum yield (F_v/F_m) and photosynthetic parameters (rel.ETR$_{max}$: photosynthetic capacity, α: photosynthetic efficiency, β: photoinhibition coefficient) of *Z. marina* plants in function of light availability and sediment conditions

Sediment	Light	
	LI	HI
F_v/F_m		
0F	0.70 ± 0.01	0.62 ± 0.03 b
LF	0.68 ± 0.02	0.70 ± 0.01 b
HF	0.65 ± 0.05	0.61 ± 0.01 a,b
HF-R	0.62 ± 0.03	0.59 ± 0.03 a
rel.ETR$_{max}$		
0F	44.4 ± 4.3	46.2 ± 9.7
LF	53.1 ± 4.1	55.9 ± 6.4
HF	39.7 ± 9.0	53.3 ± 6.3
HF-R	46.5 ± 5.0	47.4 ± 6.2
α		
0F	0.68 ± 0.15	0.65 ± 0.10
LF	0.41 ± 0.11	0.71 ± 0.13
HF	0.67 ± 0.08	0.59 ± 0.11
HF-R	0.57 ± 0.10	0.56 ± 0.13
β		
0F	0.29 ± 0.28	0.94 ± 0.92
LF	0.51 ± 0.33	0.99 ± 0.94
HF	0.02 ± 0.00	0.95 ± 0.92
HF-R	0.44 ± 0.43	0.93 ± 0.89

The values represent the mean and the S.E. ($n = 5$). Different letters in the alphabetic codes in the last column indicate significant differences between sediment levels.

4. Discussion

4.1. Growth and biomass partitioning

Present results show that light reduction affects the growth of photosynthetic as well as non-photosynthetic tissues. As previously described (Setchell, 1929; Phillips and Lewis, 1983; Backman, 1991), plants remain smaller under subsaturating light (LI) than under saturating levels (HI). However, the biomass partitioning among leaves, roots and rhizomes remained similar in both cases. Porewater nitrogen availability also affects *Zostera marina* growth. Although sediment was not P limited, growth rate and biomass were lower for plants growing in non N fertilised sediments (0F) than for those growing under N fertilised conditions (LF or HF). The sediment fertilisation treatment affected the biomass partitioning between leaves and belowground modules. In non-N fertilised sediments (0F), plants allocated higher biomass percentages to the roots, but lower to the leaves, than those grown in N fertilised (LF or HF) soils. This pattern has been previously observed as response to nutrient limitation in terrestrial plants (Brouwer, 1962, 1963; Gleeson, 1993) and in

seagrasses (Lee and Dunton, 1999; Hemminga and Duarte, 2000). As phosphorus limitation was discarded in this experiment, the present results suggest that, for *Z. marina*, this response may be attributed mainly to nitrogen limitation. The highest percentage of root biomass (>10% DW) was found in non N fertilised sediments, where plants had lower plant weight and growth rate than those from fertilised soils and leaf N contents were below critical value gave for seagrass leaves (1.8% DW, Duarte, 1990). Such evidence of N limitation in plants growing in non N fertilised sediments, reinforce the contention that, in pristine systems, nitrogen levels are insufficient to meet the N demand of *Z. marina* (Short, 1983; Zimmerman et al., 1987; Touchette and Burkholder, 2000). Differences on biomass partitioning between leaves and roots of *Z. marina* might favour plant acclimation to N deficiencies, increasing root uptake surface and decreasing the whole-plant N-requirements by reducing the percentage of leaf biomass.

4.2. N-uptake by the roots

Present results confirm that *Zostera marina* roots may not supply the N plant demand in pristine systems, but may do in nutrient rich sediments. Although leaves contribute to nutrient uptake, *Z. marina* showed a leaf N deficiency in 0F sediments, not observed in N enriched ones. This N deficiency could be based on either (1) a reduced root uptake capacity or (2) a limited diffusion from porewater to the root surface. The first hypothesis is disproved by the N sufficiency observed in plants from N enriched sediments. This N sufficiency supports the conclusion of high root uptake capacity, previously described for *Z. marina* roots (Iizumi and Hattori, 1982; Short and McRoy, 1984). Hence, the main factor controlling the N uptake by *Z. marina* roots in poor nutrient sediments seems to be the nutrient diffusion, as was also deduced for *Thalassia hemprichii* (Stapel et al., 1996). In sediments with high N porewater availability (LF and HF sediments), a sharper concentration gradient around the root surface is expected. This would increase the ammonium supply towards the root surface according to the Fick's first law, explaining the positive responses of *Z. marina* in most of the N fertilised sediments.

4.3. Tolerance of Zostera marina to high sediment fertilisation

Zostera marina plants exhibited a remarkable tolerance to high sediment fertilisations. This species was positively stimulated by porewater N concentrations 1000 higher (30 μM) than those considered inhibitory in the water column (>35 μM ammonium, van Katwijk et al., 1997, >10 mM nitrate, Burkholder et al., 1992). The exceptional tolerance of *Z. marina* to high N porewater availability may be explained by a reduced risk of damaging the photosynthetic machinery and suitable conditions for a high ammonium assimilation in roots. Ammonium assimilation in roots, as generally found in angiosperms (Goodwin and Mercer, 1983), might prevent photosynthetic inhibition (Tables 2 and 5), which is considered to be the main mechanism of ammonium toxicity for seagrasses (van Katwijk et al., 1997). The high availability of phosphorus in the sediment may also help to increase the tolerance to porewater nitrogen (Brun et al., 2002), because P is needed for nitrogen assimilation (Turpin, 1991; Penhale and Thayer, 1980; Pellikaan and Nienhuis, 1988; Udy and Dennison, 1997). In addition, long photoperiods, such as the 18 h light per day used in this experiment,

will supply enough energy and C-skeletons, needed for nitrogen assimilation by *Z. marina* (Dennison and Alberte, 1986; Zimmerman et al., 1989; Kraemer and Alberte, 1993).

Although with high tolerance, plant inhibitory effects (i.e. reduction in plant weight, leaf growth and leaf turnover rate) were detected in part of the high fertilised sediments (HF, 0.5 mg N g^{-1} DW, 2 mg P g^{-1} DW). Negative effects of P sediment fertilisation were discarded because the P sediment fertilisation was similar for inhibited and non-inhibited plants (i.e. HF and HF-R). The threshold of *Z. marina* tolerance to sediment fertilisation was detected at N porewater concentration of 30 mM. Ammonium toxicity was not probable because negative consequences for photosynthetic apparatus were absent (Tables 2 and 5) and because the porewater ammonium concentration was similar for inhibited and non-inhibited plants (in HF and HF-R sediments). The main difference between high fertilised sediments with (HF-R) and without (HF) organic matter supply, in addition to redox potential divergences, was the loss of nitrate availability in HF-R sediments (reduction of 80%). The decrease in nitrate concentration was attributed to anaerobic bacterial metabolism (i.e. denitrification; Valiela, 1995). The inhibitory effects were attributed to the extra 20 mM of nitrogen, available as nitrate in the porewater. The mechanisms of such inhibition are probably related to energy or C-skeleton deficiencies, since saturating light counteracted the negative outcome of the high N sediment fertilisation (e.g. HI-HF versus LI-HF, Figs. 1 and 2).

4.4. Pro's and con's of NH$_4$NO$_3$ use as N source in sediment fertilisation for restoration

After these results, we concluded that the use of N + P sediment fertilisation, with NH$_4$NO$_3$ as N source and high P supply, can be highly beneficial in restoration of *Zostera marina* meadows. This species has (1) positive growth responses to porewater nutrient enrichment, (2) high tolerance to extensive sediment fertilisation and, in addition, (3) bacterial metabolism may reduce the porewater NO$_3^-$ availability in anoxic sediments of seagrass beds.

However, to obtain positive results on *Z. marina* growth after sediment fertilisation, several precautions must be taken. Firstly, the phosphorus supply has to be enough to balance the NH$_4$NO$_3$ sediment enrichment. Secondly, the quantity of fertilisers should be adjusted to the light availability for production of energy and C-skeletons, as excessive porewater N concentration may inhibit *Z. marina* growth. Thirdly, sediment fertilisation should be adjusted to sediment redox conditions, since sediment processes, e.g. denitrification, may reduce the concentration of N available forms. Finally, sediment fertilisation should allow the sediment redox and water quality re-establishment before nutrient release starts (i.e. retarded release fertilisers).

Acknowledgements

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