Effects of plant diversity on primary production and species interactions in brackish water angiosperm communities

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ABSTRACT: Research on plant biodiversity and ecosystem functioning has mainly focused on terrestrial ecosystems, and our understanding of how plant species diversity and interactions affect processes in marine ecosystems is still limited. To investigate if plant species richness and composition influence plant productivity in brackish water angiosperm communities, a 14 wk field experiment was conducted. Using a replacement design with a standardized initial aboveground biomass, shoots of *Zostera marina*, *Potamogeton filiformis* and *P. perfoliatus* were planted on a shallow, sandy bottom in replicated monocultures and all possible species combinations. Response variables included aboveground and belowground biomass, shoot density, space occupation and porewater nutrients. To determine whether selection and/or complementarity controlled productivity, additive partitioning and D_i were calculated. Richness effects were species-specific and only increased the biomass production of *P. perfoliatus* and tuber production of *P. filiformis*, while species composition generally had a stronger effect on biomass production. Additive partitioning indicated a positive complementarity effect for the aboveground biomass production in bicultures in general, while a positive net effect was found in the *P. perfoliatus* and *P. filiformis* biculture. Despite the absence of significant results for other treatments and plant variables, a trend of positive complementarity and negative selection effects were present. Plant diversity had no clear effect on sediment porewater nutrient concentrations, indicating weak richness effects on resource use.

KEY WORDS: Seagrass · Ecosystem function · Complementarity · Selection · Resource use

INTRODUCTION

As the relationship between biodiversity and ecosystem function (BEF; usually primary production) has mainly been based on experimental work in terrestrial plant ecosystems (e.g. Hooper & Vitousek 1997, Hector et al. 1999, Loreau et al. 2001), it is still largely unknown to what extent terrestrial plant ecosystem processes are transferable to vegetated marine ecosystems (Solan et al. 2006). Experimental work manipulating different levels of diversity in marine systems have mostly shown significant effects of species richness. Specifically, these studies have shown the following: (1) in terms of primary producers and sessile animals, the effects of individual species are stronger than the role of diversity per se; (2) diverse communities tend to be less variable, with enhanced resistance and faster recovery from disturbance compared to simple communities; and (3) diverse prey assemblages show higher resistance to predation compared to simple communities (Stachowicz et al. 2007). However, within BEF research, only about 10 field studies (e.g. Stachowicz et al. 2007) manipulating submerged macrophyte species (or genotypic) richness have been published, the majority being experiments with macroalgae, and only a couple of experiments have investigated effects of angiosperm (seagrass) species richness on ecosystem functioning (Parker et al. 2001, Moore & Fairweather 2006). This is surprising given the strong research emphasis on plants within terrestrial BEF work and the similarities between aquatic and terrestrial angiosperm assemblages (Duarte 2000).

Seagrass meadows provide vital functions (primary production, nutrient cycling), goods (habitat, food resources) and services (erosion control, indicator of coastal health), but are lost at accelerating rates due to human pressures (Duarte 2000, Orth et al. 2006). Half of the documented seagrass meadows are multispecific and contain 2 to 3 co-occuring species, while Asian seagrass meadows may consist of >10 co-occuring species (Duarte et al. 2000). Interestingly, the angiosperm assemblages in the Baltic Sea have comparable plant species (>10) and functional richness (Kautsky 1988), and multispecific meadows commonly contain 3 to 6 plant species of marine and limnic origin (Boström et al. 2003). However, due to the scarcity of field manipulations, the extent to which plant species diversity influences biological processes in seagrass meadows is still largely unexplored (Williams 1990, Kautsky 1991, Duarte et al. 2000).

Because plant species affect biogeochemical processes differently, the species diversity in an ecosystem influences the processes of that system (Tilman et al. 1997, Hooper 1998). If species are complementary, communities with a large repertoire of different functional groups should either utilize available resources more efficiently and thus show greater productivity compared to simple communities, i.e. a niche-differentiation effect (Tilman 1999, Loreau 2000), or enhance ecosystem-level processes due to beneficial intra- or interspecific interactions, i.e. facilitation (Cardinale et al. 2002, Srivastava & Vellend 2005). However, while the relative role of individual species in a community may vary greatly, resource use and productivity is often expected to increase as diversity increases, simply because one or a few dominating species frequently affect a process the most, i.e. a selection effect (Hooper et al. 2005). Furthermore, species composition may affect processes in such a way that specific combinations of species have a higher impact on processes than other species combinations, though the number of species is the same (Stachowicz et al. 2007). Which of these mechanisms are important for ecosystem functioning of aquatic vegetated systems is still largely unknown (Duarte et al. 2000, Engelhardt & Ritchie 2002), thus limiting our ability to predict consequences of species loss and disturbance, as well as the resistance and recovery capacity of multispecific plant assemblages.

While work in terrestrial grasslands suggests that species with, for example, differing rooting depths and nutrient requirements should be able to coexist, exploit resources more fully and show higher productivity (Fargione & Tilman 2005), there is still limited information on the influence of plant diversity on resource use in marine environments.

The main aim of the present study was to investigate *in situ* how angiosperm species richness and composition influence plant primary production and resource use. We assembled plots of the following 3 species: *Zostera marina* L., *Potamogeton perfoliatus* L. and *P. filiformis* Pers. These species differ markedly in terms of morphological traits, biomass allocation patterns and productivity (Kautsky 1988, 1991), and are considered able to coexist in the Baltic Sea due to differential resource use (Kautsky 1991). Thus, in addition to species richness, the number of different functional types was also manipulated, which allows for a better understanding of which species traits are important in competitive and/or complementary interactions (Tilman et al. 1997). Furthermore, as all combinations of the plant species were manipulated in a replicated field experiment, the relative importance of complementarity and selection effects in angiosperm assemblages was also assessed.

We hypothesized that species complementarity would result in more efficient resource use (decreased sediment nutrient pool concentrations) and higher production in mixed cultures compared to monocultures, while a positive selection effect would be present in mixtures containing *Potamogeton perfoliatus* and/or *Zostera marina* due to their high production capacity. In terms of spatial partitioning, *Z. marina* and *P. perfoliatus* were hypothesized to show extensive lateral spread, while *P. filiformis* was expected to show moderate space occupation capacity (see Kautsky 1988).

MATERIALS AND METHODS

Study area and site characteristics. The non-tidal northern Baltic Sea is characterized by low salinity (6 to 8 PSU), strong seasonal temperature fluctuations (0 to 20°C) and mixed macrophyte communities (Kautsky 1988, Kautsky & Kautsky 2000). The present study was conducted in the Archipelago Sea, SW Finland, where submerged sandbanks support diverse macrophyte assemblages (Boström et al. 2006). The average water depth at the study site (Fårö Island, 59° 55.219′ N, 21° 47.711′ E) was 1.3 m, and the area is relatively exposed to NE winds. The nearshore area is mostly unvegetated and consists of a sediment dominated by fine (~70% 0.125 mm) to very fine (~5% 0.0062 mm) sand with low (<0.5%) organic content. A seagrass meadow dominated by eelgrass *Zostera marina*, sago pondweed *Potamogeton pectinatus* L. and perfoliate pondweed *P. perfoliatus* grows from 2 to 6 m depth. Slender-leaved pondweed *P. filiformis* and horned pondweed *Zannichellia palustris* L. grow both within the meadow and in monocultures bordering the meadow, while Eurasian water-milfoil *Myriophyllum spicatum* L. and ditchgrass *Ruppia cirrhosa* (Petagna) Grande occur patchily within the meadow. During the present study, the water temperature ranged between 10°C (October) and 20°C (July).

Experimental setup and sampling. The plant species used in the experiment were *Zostera marina*, *Potamogeton filiformis* and *P. perfoliatus*. A replacement design with a standardized aboveground biomass was used (Harper 1977). Thus, the total start aboveground biomass in all plots was 30 g wet weight (WW), i.e. 30 g of a species in monocultures, 15 g of each component species in bicultures and 10 g of each component species in the triculture. This total biomass corresponded to 21, 26 and 37 shoots for *Z. marina*, *P. filiformis* and *P. perfoliatus*, respectively. Plants were grown in all possible combinations (7 treatments) in a randomized block design with 4 replicates of each treatment. The distance between plots was 2 m and the distance between blocks was 2.5 m. Two weeks prior to the start of the experiment, scattered plants and stones were removed from the unvegetated area.

When starting the experiment, experimental plants (ramets) were collected from the study site and transplanted within 8 h. To estimate the initial dry weight (DW) of aboveground and belowground biomass, 20 to 30 randomly chosen plants of each species were set aside. To standardize plant spacing and prevent uprooting, plants were carefully tied to a flexible 30 × 30 cm plastic grid (mesh size 30 mm). In mixed plots, individual component species were tied in a non-random, even spatial pattern, avoiding grouping of conspecifics. Plants were kept submerged during the handling process. Experimental units were planted 3 to 5 cm into the sediment using SCUBA diving. In addition, empty grids used as controls for sediment nutrients were positioned randomly within each block (n = 4). The experiment ran for 14 wk (10 July to 21 October 2007), representing one growing season.

To estimate the potential influence of plant species richness on organic matter deposition and mineralization, sediment cores (diameter 2.5 cm, depth 5 cm) were taken for sediment C:N determination 1 mo prior to the termination of the experiment (11 September). The C:N samples were sieved (0.5 mm), dried (105°C, 24 h) and ground, and subsequently analyzed in a certified lab according to the method of Kristensen & Andersen (1987) with an elemental analyzer (Carlo-Erba 1100EA). Three days prior to the termination of the experiment, (i.e. after ~13 wk, when plots had reached maximum development), the space occupation capacity through clonal growth of each species in each plot was assessed by measuring the maximum spatial expansion (cm) from the initial plot edge. All plots were also sampled for sediment porewater nutrients using Rhizon soil moisture samplers (type SMS: length 100 mm, Ø 2.5 mm, Eijelkamp Agrisearch Equipment) connected to 125 ml vacuum bottles. The sampler was inserted to 10 to 12 cm depth in the center

of each plot (thus covering the entire root layer) and connected to a vacuum bottle with a PVC tube and a syringe. Porewater samples were kept in the dark and deep-frozen (–20°C) upon arrival at the laboratory, and analyzed for NH_4^+ and PO_4^{3-} in a certified lab. In addition, water column nutrient samples (total N, total P, NH_4^+, PO_4^{3-}) were taken from the experimental area on 20 August, 5 September and 2 and 21 October, and treated as outlined above.

At the termination of the experiment, all plant material including belowground parts in each plot was harvested (except for one replicate of the *Potamogeton perfoliatus* monoculture which was lost), transported to the laboratory and deep frozen (–18°C). In the laboratory, samples were thawed and plants were carefully cleansed from animals, algae, etc. The number of shoots, as well as the aboveground (shoots) and belowground (rhizomes, roots) biomass, was determined for each species. The biomass (DW) was determined on an analytical scale after drying to constant weight (48 h, 60°C). In addition, the number and biomass (DW) of overwintering organs was determined for all *P. filiformis* (tubers) and *P. perfoliatus* (turions) ramets. To compensate for the initial differences in planting biomasses, tuber and turion abundances were multiplied by 2 and 3 in bicultures and tricultures, respectively, see Engelhardt & Ritchie (2002).

Data analysis. To determine the mechanisms explaining observed patterns, additive partitioning of biodiversity effects was used to assess the importance of selection (SE), complementarity (CE) and net biodiversity effect (NE) in mixed cultures (Loreau & Hector 2001). In addition, D_i was calculated to describe how the yield of a species in polyculture differs from its expected yield (Loreau 1998):

$$D_i = (O_i - E_i)/E_i$$

where O_i and E_i are the observed and expected yields of species i, respectively.

One-way ANOVA and regression analysis were performed to detect differences in plant variables. Prior to analysis, Kolmogorov-Smirnov and Levene's tests were used to test if data fulfilled assumptions for parametric testing. In some cases data, were log-transformed to fulfill these assumptions. In case of heteroscedasticity and for calculations involving percentage values >100, a non-parametric Kruskal-Wallis test accompanied by a Mann-Whitney *U*-test was used. Additive partitioning and D_i were calculated for aboveground, belowground and total biomass production. The calculated indices were tested against a value of 0.0 with a 1-sample *t*-test. To correct for multiple comparisons, results from the *t*-tests were corrected using the Dunn-Sidák method (Sokal & Rohlf 1994). All means are given ±SE.

RESULTS

Biomass allocation patterns and species composition effects

The plant communities differed significantly from each other in terms of aboveground, belowground and total biomass ($H = 25.38$, p = 0.017; $H = 16.01$, p = 0.014 and $H = 15.44$, p = 0.017, respectively, Fig. 1). The number of shoots per plot varied between 14 ± 3 (*Potamogeton perfoliatus* monoculture) and 218 ± 31 (*P. filiformis* monoculture) (Fig. 1a). The final accumulated aboveground biomass ranged between 0.3 ± 0.1 (*P. perfoliatus* monoculture) and 4.3 ± 0.4 g DW (biculture of *Zostera marina* and *P. filiformis*), while belowground biomasses were generally higher and ranged from 1.2 ± 0.3 (*P. perfoliatus* monoculture) to 6.0 ± 1.6 g DW (*Z. marina* monoculture) (Fig. 1b,c). The total plot biomass ranged from 1.5 ± 0.3 (*P. perfoliatus* monoculture) to a maximum of 10 ± 2.6 g DW (*Z. marina* monoculture) (Fig. 1d). As root:shoot (R:S) ratios did not differ for individual species across treatments, ratios were pooled. Thus, *P. perfoliatus* and *Z. marina* invested most in belowground biomass, with R:S ratios of 2.9 ± 0.1 and 1.5 ± 0.1, respectively, while *P. filiformis* allocated most of its production to shoots (R:S ratio of 0.9 ± 0.03). For *Z. marina*, the spatial expansion in terms of

clonal growth ranged between 25.8 ± 4.2 (biculture with *P. perfoliatus*) and 32.8 ± 2.8 cm (monoculture), and for *P. perfoliatus* from 18.3 ± 4.4 (biculture with *Z. marina*) to 25.0 ± 7.6 cm (biculture with *P. filiformis*). The highest space occupation capacity was recorded for *P. filiformis* (42.3 ± 6.2 [triculture] to 62.5 ± 2.2 cm [monoculture]).

Species composition had a strong effect on the community performance. When present, *Zostera marina* significantly increased the belowground and total biomass production of the plant communities by 42 and 34%, respectively (Table 1) ($F_{1,10} = 8.793$, p = 0.014; $F_{1,10} = 7.142$, p = 0.023). Similarly, *Potamogeton filiformis* contributed significantly to the shoot density, with 160% more shoots in communities with the species present ($F_{1,10} = 73.302$, p < 0.001), while the presence of *P. perfoliatus* led to 35% lower aboveground biomasses ($F_{1,10} = 15.876$, p = 0.003).

Temporal changes in plant communities and richness effects

When comparing the performance of individual species in different mixtures, the relative (%) temporal change in shoot density (ramet production), aboveground, belowground and total biomass production
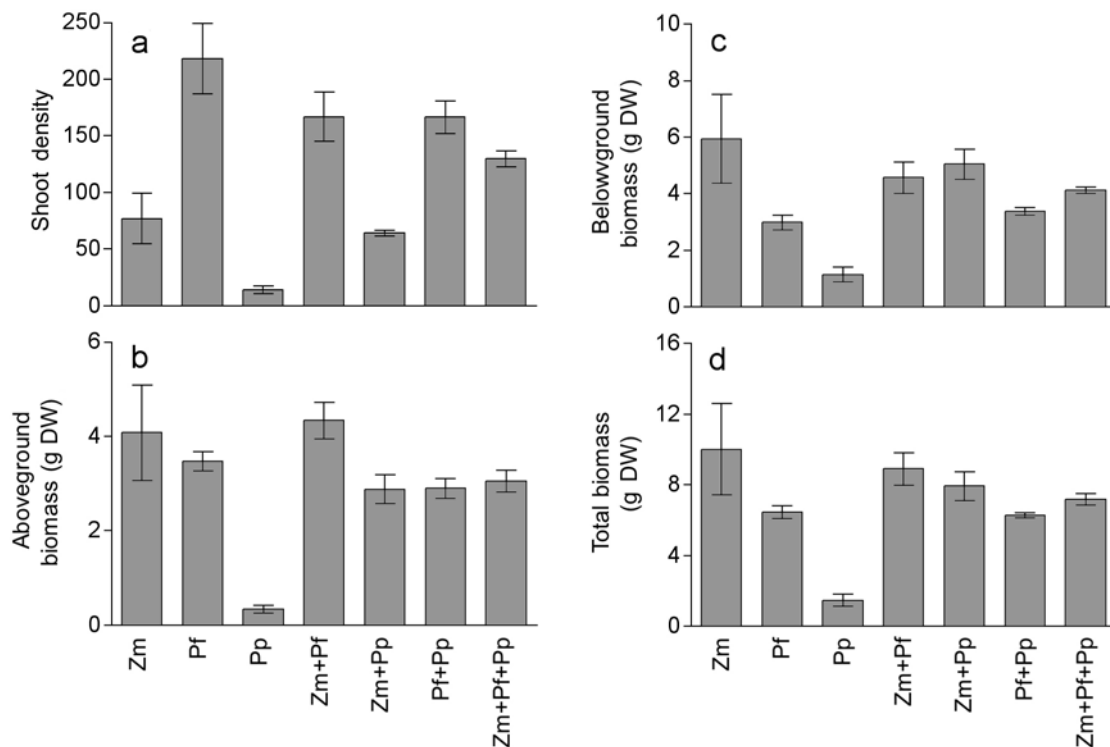

Fig. 1. *Zostera marina*, *Potamogeton filiformis* and *P. perfoliatus*. Mean (a) shoot densities, (b) aboveground biomass, (c) belowground biomass and (d) total biomass in the assembled plant communities. Zm: *Z. marina*; Pf: *P. filiformis*; Pp: *P. perfoliatus*. Error bars are ±SE

Table 1. *Zostera marina*, *Potamogeton filiformis* and *P. perfoliatus*. Mean (±SE) shoot density, aboveground biomass, belowground biomass and total biomass in the presence and absence of each component species in the experimental communities. Asterisks indicate significant differences in community performance in the presence versus absence of a component species: * $p < 0.05$; ** $p < 0.005$. DW: dry weight

Species		Shoot density	Aboveground biomass (g DW)	Belowground biomass (g DW)	Total biomass (g DW)
Z. marina	Presence	115.6 ± 21.9	3.6 ± 0.4	4.8 ± 0.4 *	8.4 ± 0.6 *
	Absence	117.0 ± 14.5	2.9 ± 0.2	3.4 ± 0.1	6.3 ± 0.1
P. filiformis	Presence	166.9 ± 12.1 **	3.6 ± 0.3	4.0 ± 0.3	7.5 ± 0.7
	Absence	64.3 ± 2.8	2.9 ± 0.3	5.0 ± 0.5	7.9 ± 0.8
P. perfoliatus	Presence	115.5 ± 20.5	2.9 ± 0.2 **	4.2 ± 0.4	7.1 ± 0.5
	Absence	167.0 ± 21.8	4.3 ± 0.4	4.6 ± 0.6	8.9 ± 0.9

generally showed minor differences (Fig. 2). *Potamogeton filiformis* had the lowest production in monocultures, though this was non-significant, while *P. perfoliatus* performed significantly better in mixed cultures in terms of shoot density and aboveground biomass investment ($H = 9.7$, $p = 0.021$ and $H = 9.9$, $p = 0.019$, respectively). In particular, *P. perfoliatus* had a significantly higher ramet production in tricultures than in bicultures with *P. filiformis* ($U = 0.000$, $p = 0.029$, Fig. 2). Compared to its starting density, *P. filiformis* increased its shoot density by 1 order of magnitude (biculture with *P. perfoliatus*), while *P. perfoliatus* and *Zostera marina* reached, at maximum, ~0.5 and >3

times their initial densities, respectively (Fig. 2a). The relative increase in aboveground biomass followed the shoot density pattern (Fig. 2b), while the change in belowground biomass was surprisingly uniform between species and across treatments (Fig. 2c).

When measuring the relative (%) temporal change in terms of shoot density and aboveground biomass production at the plot level in mixed cultures, the greatest change was found in the biculture of *Zostera marina* and *Potamogeton filiformis* (Fig. 3a,b). In terms of belowground biomass, the greatest change was recorded in the biculture of *Z. marina* and *P. perfoliatus* (Fig. 3c), while in terms of total biomass the biculture
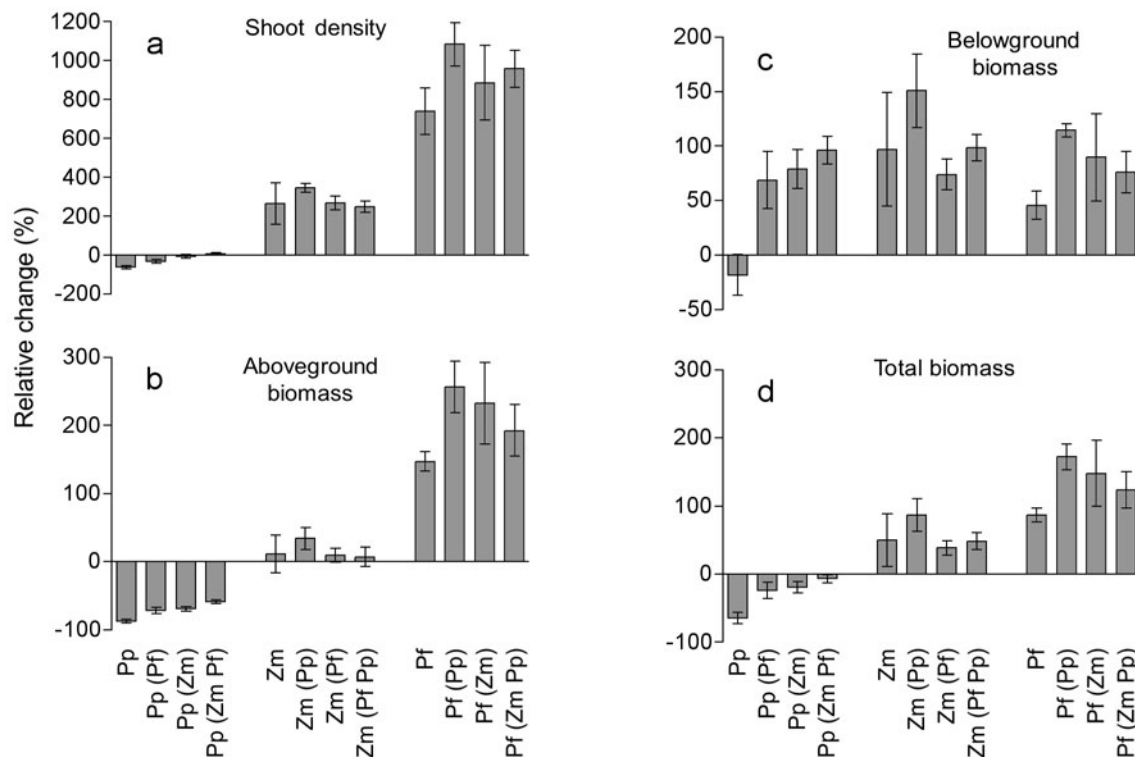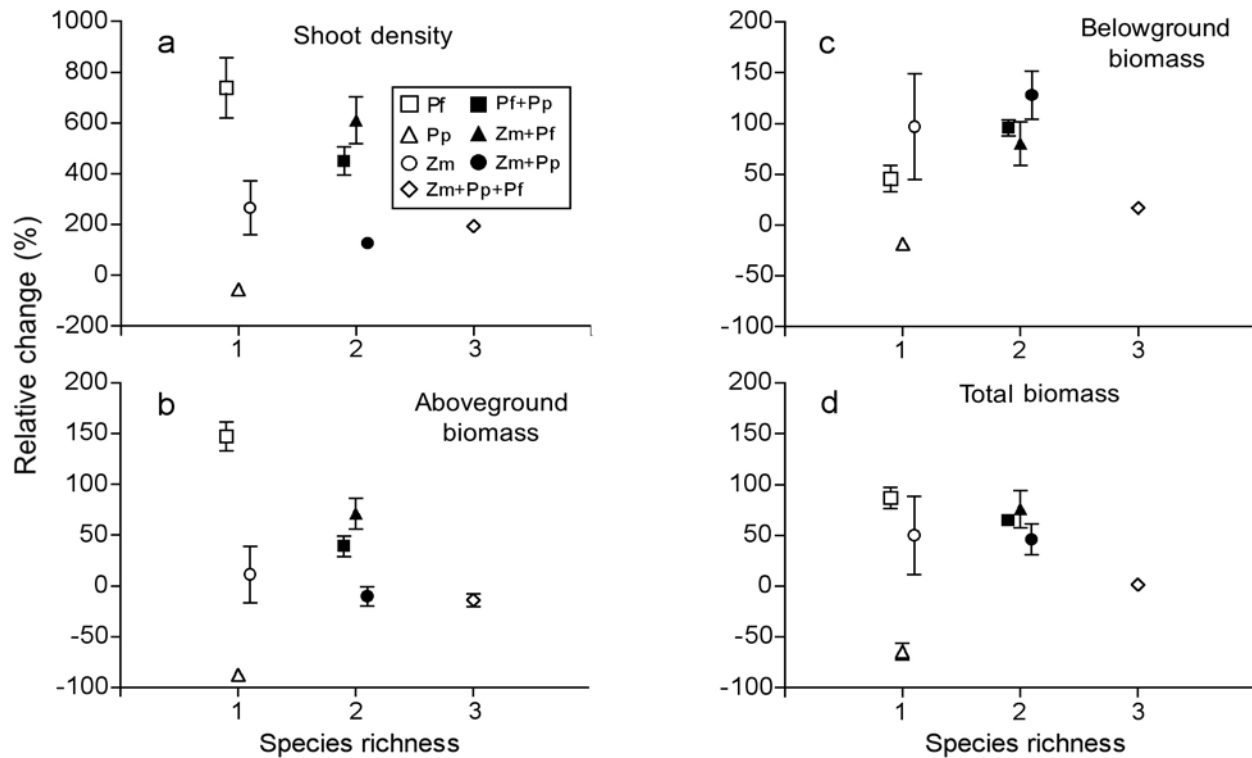

Fig. 2. *Zostera marina*, *Potamogeton filiformis* and *P. perfoliatus*. Relative (%) change in (a) shoot density, (b) aboveground biomass, (c) belowground biomass and (d) total biomass. Species in parentheses indicate other species present in the culture but not represented by the bars. Species abbreviations as in Fig. 1. Data are means ± SE

Fig. 3. *Zostera marina*, *Potamogeton filiformis* and *P. perfoliatus*. Relative (%) change in (a) shoot density, (b) aboveground biomass, (c) belowground biomass and (d) total biomass in relation to species richness. Species abbreviations as in Fig. 1. Data are means ± SE (not visible for all treatments due to scale)

of *Z. marina* and *P. filiformis* expressed the greatest relative change (Fig. 3d). The different treatments differed significantly from each other in terms of shoot density (H = 22.1, p = 0.001), aboveground biomass (H = 22.3, p = 0.001), belowground biomass (H = 16.0, p = 0.014) and total biomass (H = 15.6, p = 0.016).

For *Potamogeton perfoliatus*, species richness enhanced all biomass production variables, while *P. filiformis* and *Zostera marina* showed nonlinear relationships with increasing species number (Table 2, Fig. 4). The number of tubers and turions of *P. filiformis* and *P. perfoliatus*, respectively, correlated positively with species richness (tubers: $F_{1,14}$ = 8.882, p = 0.010, r^2 = 0.388; turions: $F_{1,13}$ = 5.631, p = 0.034, r^2 = 0.302) (Fig. 5).

Additive partitioning and D_i

The aboveground, belowground and total biomass production in biculture treatments showed similar, but non-significant, responses with positive CE and negative SE resulting in a positive NE. However, the biculture consisting of *Potamogeton filiformis* and *P. perfoliatus* showed a significant positive NE for total biomass production (*t*-test, p = 0.004). The triculture treatment also showed non-significant positive CE and negative SE for all the measured plant variables, resulting in a negative NE (Fig. 6). In terms of species richness, bicultures showed a significant positive CE for aboveground biomass (*t*-test, p = 0.016) (Fig. 6), while the other plant variables were non-significant and followed the same trend of positive CE and negative SE as described above.

Table 2. *Zostera marina*, *Potamogeton filiformis* and *P. perfoliatus*. Simple linear regression results for responses to increasing plant species richness in the field experiment

| Species	Plant variable	df	F	p	r^2
P. perfoliatus	Shoot density	1,13	20.7	0.001	0.610
	Aboveground biomass	1,13	12.88	0.003	0.500
	Belowground biomass	1,13	34.35	<0.001	0.730
	Total biomass	1,13	17.26	0.001	0.570
P. filiformis	Shoot density	1,14	0.41	0.843	0.003
	Aboveground biomass	1,14	0.03	0.867	0.002
	Belowground biomass	1,14	0.001	0.972	0.000
	Total biomass	1,14	0.002	0.964	0.000
Z. marina	Shoot density	1,14	1.3	0.274	0.085
	Aboveground biomass	1,14	0.56	0.469	0.038
	Belowground biomass	1,14	0.76	0.399	0.051
	Total biomass	1,14	0.68	0.425	0.046
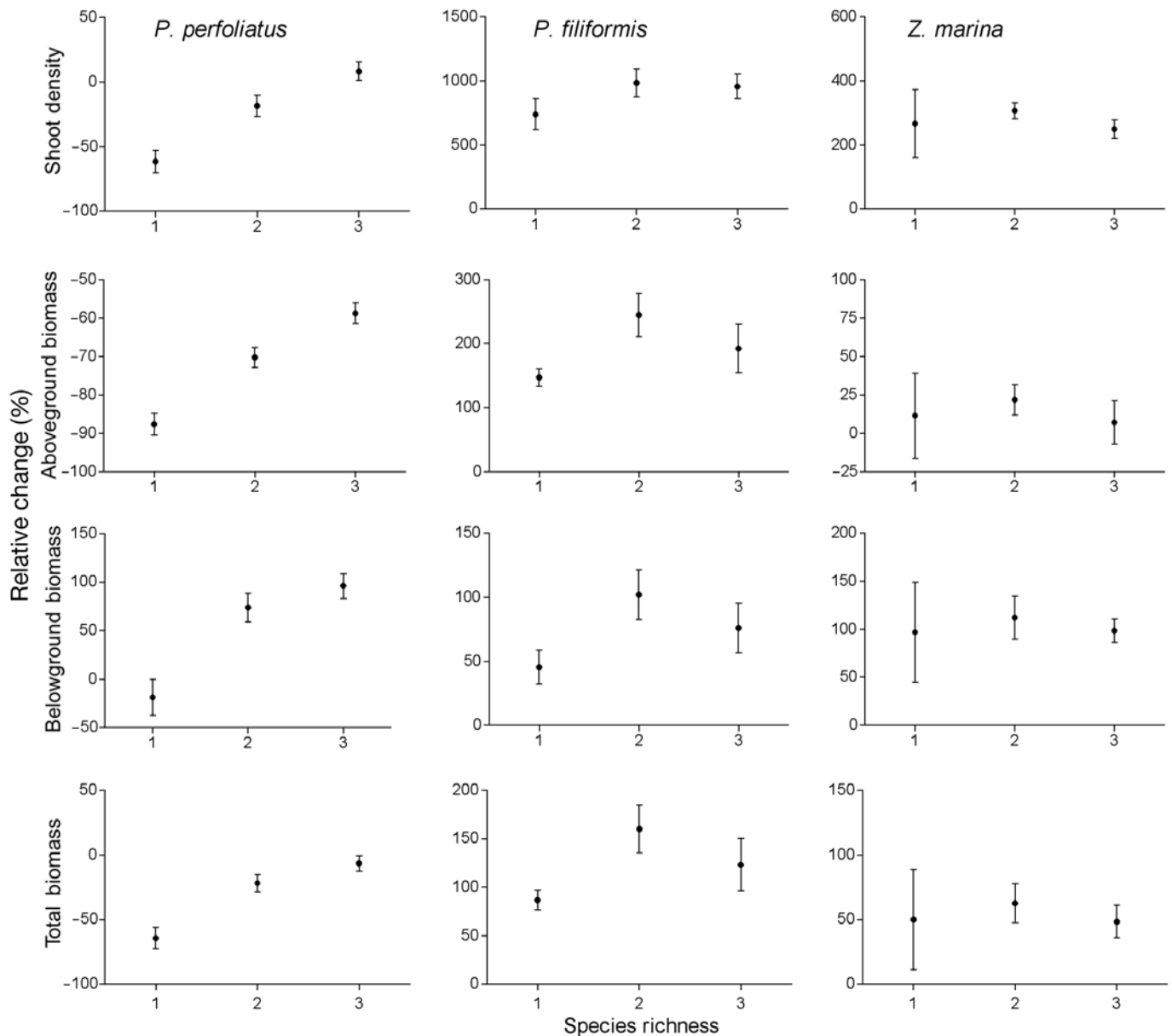
Fig. 4. *Zostera marina*, *Potamogeton filiformis* and *P. perfoliatus*. Relative (%) change in aboveground biomass, belowground biomass and total plant biomass and shoot density for each species in relation to species richness. Data are means ± SE

All treatments showed positive D_i values indicating that all species performed better in mixes than expected (Table 3). However, none of these D_i values were statistically significant.

Sediment and water column nutrients

The C:N ratio was low in all treatments including controls, and ranged from 3.9 to 4.5, with no richness effects ($F_{1, 29} = 4.0$, p = 0.055, $r^2 = 0.121$). The control plots revealed significantly higher porewater NH_4^+ concentra-

tions compared to other treatments ($F_{7, 22} = 6.5$, p < 0.001), while none of the individual plant treatments differed in terms of porewater nutrient concentrations. Among plant treatments, the NH_4^+ concentration ranged between 12.6 ± 4.1 (*Potamogeton filiformis* monoculture) and 22.3 ± 9.0 µM (triculture). In terms of richness, the NH_4^+ concentration was highest in the control treatments (67.2 ± 13.5 µM), lowest in monocultures (14.4 ± 2.5 µM) and increased somewhat with increasing species richness (quadratic regression: $F_{2, 27} = 15.95$, p < 0.001, $r^2 = 0.54$, Fig. 7a). The porewater PO_4^{3-} concentration ranged between 1.91 ± 0.78 (*Zostera marina* and *P. perfoliatus*
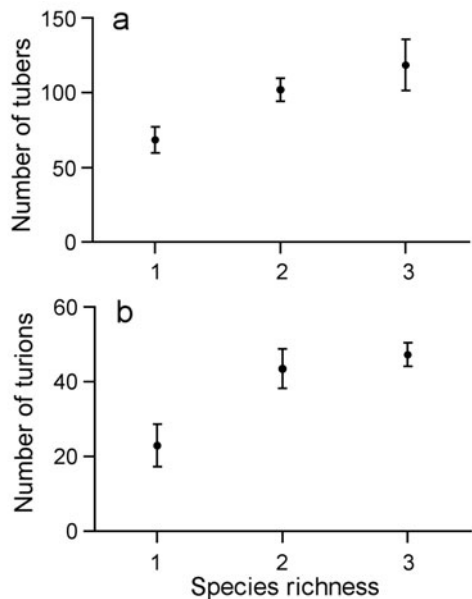
Fig. 5. *Potamogeton filiformis* and *P. perfoliatus*. Compensated number of (a) tubers (*P. filiformis*) and (b) turions (*P. perfoliatus*) in relation to species richness. Data are means ± SE

biculture) and 0.63 ± 0.19 µM (*P. perfoliatus* monoculture), but did not differ between treatments ($F_{7, 22} = 0.89$, p = 0.53). The PO_4^{3-} concentration in the controls was 1.45 ± 0.55 µM. No relationship between species richness and porewater PO_4^{3-} concentration was found ($F_{1, 28} = 0.05$, p = 0.82, $r^2 = 0.002$, Fig. 7b). Compared to the sediment nutrient pools, water column nutrient concentrations were substantially lower and ranged during the season from 0.17 to 0.38 µM (total N: 17.1 to 24.3 µM) and 0.05 to 0.17 µM (total P: 0.59 to 0.64 µM) for NH_4^+ and PO_4^{3-}, respectively.
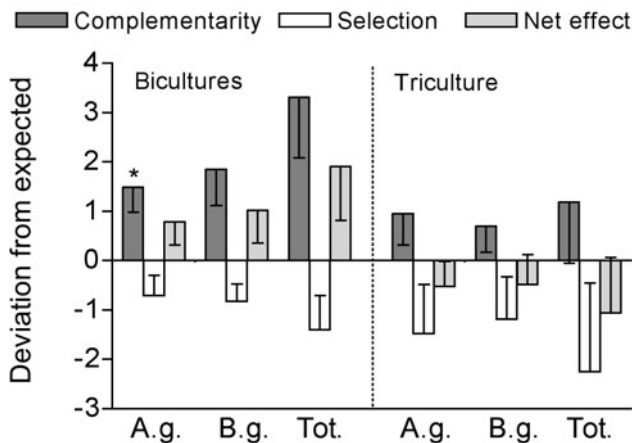

Fig. 6. *Zostera marina*, *Potamogeton filiformis* and *P. perfoliatus*. Additive partitioning of aboveground biomass (A.g.), belowground biomass (B.g.) and total biomass (Tot.) in the bicultures and the triculture. Asterisk denotes a significant (p = 0.016) effect. Data are means and SE

Table 3. *Zostera marina*, *Potamogeton filiformis* and *P. perfoliatus*. Species performance (D_i, mean ± SE) calculated for aboveground, belowground and total biomass production in mixed cultures. Asterisks in parentheses indicate significant differences from 0.0 (p < 0.05) that disappeared after correcting for multiple comparisons. Zm: *Zostera marina*; Pf: *Potamogeton filiformis*; Pp: *P. perfoliatus*

Plant culture	Aboveground biomass	Belowground biomass	Total biomass
Zm bicultures	0.82 ± 0.68	1.00 ± 0.82	0.92 ± 0.76
Zm triculture	0.23 ± 0.37	0.51 ± 0.65	0.39 ± 0.53
Pf bicultures	0.43 ± 0.19	0.39 ± 0.12(*)	0.40 ± 0.14(*)
Pf triculture	0.21 ± 0.21	0.26 ± 0.20	0.22 ± 0.19
Pp bicultures	1.56 ± 0.50(*)	1.27 ± 0.45(*)	1.34 ± 0.46(*)
Pp triculture	2.77 ± 1.31	1.72 ± 0.95	1.96 ± 1.03

DISCUSSION

Species interactions among rooted macrophytes

The results obtained in the present study indicate that ecosystem processes in macrophyte assemblages are influenced by species composition. Among the species tested, positive CE and negative SE were recorded, thus leading to both a positive and negative NE depending on the treatment. While complementarity did not lead to a significant increase in resource use, facilitation is suggested to occur in the communities. We found no increase in total primary production with increasing plant richness. The richness effects recorded were weak and species-specific, i.e. an enhanced biomass production of *Potamogeton perfoliatus* and tuber production of *P. filiformis*, while *Zostera marina* appeared unaffected by the presence of other species. To our knowledge, this is the first manipulative aquatic *in situ* BEF study on interactions and resource use among angiosperm species.

In marine ecosystems, the relationship between macrophyte richness and primary productivity is strongly influenced by species composition, while richness effects are generally non-existent or weak (Stachowicz et al. 2007). In the present study, richness effects were only found for *Potamogeton perfoliatus*, which increased its shoot density and biomass production with increasing species richness. In addition, the tuber production of *P. filiformis* correlated positively with species number. Richness effects may be dependent on the duration of the experiment. In accordance with the present study, short-term terrestrial grassland experiments have not found a relationship between species richness and primary production (Naeem et al. 1996, Weigelt et al. 2007), while positive correlations have emerged from long-term studies (Hector et al. 1999, Roscher et al. 2007). Since the production of previous years may alter
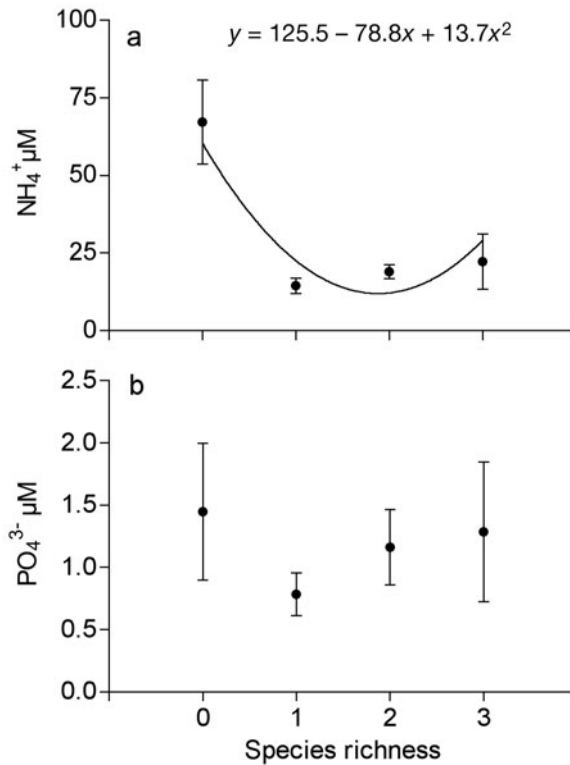
$$y = 125.5 - 78.8x + 13.7x^2$$

Fig. 7. *Zostera marina*, *Potamogeton filiformis* and *P. perfoliatus*. Relationships between sediment porewater nutrient concentration and plant species richness. (a) NH_4^+ (quadratic regression: $p < 0.001$, $r^2 = 0.54$). (b) PO_4^{3-} (no relationship was found). Data are means ± SE

long-term patterns in plant communities, short-term experiments may fail to detect species complementarity (Hooper & Dukes 2004). Alternatively, biodiversity effects in marine plant communities may, compared to terrestrial ecosystems, be subordinated in comparison to more influential limiting factors such as irradiance, waves and currents, nutrients, epiphytes and sediment characteristics. Thus, in order to fully capture potential biodiversity effects in aquatic plant communities, future manipulations should preferentially span several years and include different environmental settings (Cardinale et al. 2000, 2007).

In accordance with macroalgal BEF studies (Bruno et al. 2005, 2006), compositional effects had a strong effect on primary production. For example, *Zostera marina* enhanced both the total community and belowground production, while *Potamogeton filiformis* significantly contributed to increased ramet production. Similarly, in tropical seagrass systems, the community composition is thought to be of greater importance for the maintenance of ecosystem functions than the number of species per se (Duarte 2000).

The positive CE recorded in the present study indicates the presence of either facilitation or niche parti-

tioning (Bertness & Callaway 1994, Loreau & Hector 2001, Duffy 2006). Our findings are supported by the results of Kautsky (1991), who observed complementarity between *Potamogeton filiformis* and *P. perfoliatus* and suggested this was due to the differences in rooting depths. Surprisingly, the sediment porewater nutrient concentrations were higher in the biculture and triculture treatments compared to the monocultures. Thus, complementary nutrient use is unlikely to explain this positive effect. Other forms of positive interactions, e.g. enhanced oxygen release to the rhizosphere (Caffrey & Kemp 1991, Callaway & King 1996) or increased nutrient use efficiency (van Ruijven & Berendse 2005), may possibly explain this result.

The frequent occurrences of negative SE among treatments indicate that the communities were dominated by species with lower than average monoculture biomass. In the triculture, this effect was strong enough to cancel out the positive CE, resulting in a negative NE. While the NE was positive in the bicultures, thus suggesting a positive effect of biodiversity, the additive partitioning of the triculture indicated that the production decreased with increasing plant species richness (Loreau & Hector 2001).

Biomass allocation patterns

Biomass allocation patterns were most likely explained by exposure rather than interspecific interactions (Kautsky 1991, Boström et al. 2006). *Potamogeton perfoliatus* invested more into belowground parts than into shoots, while *P. filiformis* tended to show less variable R:S ratios (Kautsky 1988, 1991). The higher relative change in shoot density and aboveground biomass of *P. filiformis* compared to the other species suggests that exposed sandy bottoms represent optimal growing conditions for this species. In contrast, the performance of *P. perfoliatus* monocultures indicate that, despite the morphological plasticity of the species, sediment stability and shelter provided by other plants seem to be a prerequisite for successful population establishment and maintenance in harsh environments (Kautsky 1988, 1991). In a transplantation study in the Baltic Sea (Worm & Reusch 2000), a similar pattern was recorded for young *Zostera marina* plants.

In temperate macrophytes, tuber and turion production is initiated when carbohydrate production exceeds growth demands, and usually peaks towards the end of the season (Santamaría & Llano García 2004). In the present study, species richness correlated positively with tuber and turion abundance, implicating a coupling between interspecific competition and resource investment. As the number of propagules determines the initial density, and thus the ability of a species to

compete for space, light and nutrients early in the following growth season (Kautsky 1990), *Potamogeton filiformis* and *P. perfoliatus* may possess a competitive advantage over other species.

Ramet production and space occupation

In terrestrial plant studies, aboveground and belowground space partitioning has been recorded, e.g. increasing shoot density and cover in mixtures relative to monocultures (Lorentzen et al. 2008). While establishment and ramet production in freshwater plants depends on nutrient status and population density (Wolfer & Straile 2004), little is known about the importance of interspecific competition for space during plant succession in seagrass ecosystems (Williams 1990). Due to the differing repertoire of functional traits among the species studied here, space occupation patterns through clonal growth were species-specific, but not significantly influenced by species richness. However, the shoot density of *Potamogeton filiformis* tended to be higher in mixes compared to monocultures, while the linear increase in shoot density of *P. perfoliatus* only reflected an increase from a negative (–61.4%) to slightly positive (8.5%) level (Fig. 4, see 'Discussion' subsection 'Biomass allocation patterns'). In the study area, *Zostera marina* is a biomass storer with low productivity (Kautsky 1988, Boström et al. 2004) and space occupation appeared to be unaffected by the presence of other species. Based on a mesocosm study, the space occupation of *P. perfoliatus* was suggested to be negatively density-dependent (Wolfer & Straile 2004), but such a pattern was not observed in the present study. The spatial spread of *P. filiformis* was highest in monocultures (>60 cm) compared to mixed cultures. Decreasing space occupation capacity in mixed communities can be explained by interspecific competition (Grime 2001); alternatively, complementarity in mixed communities may result in shorter rhizome spacer lengths and thus increased concentration and foraging ability of ramets in nutrient-rich patches (Wolfer 2008). However, sediment porewater nutrient levels were not significantly lower in the mixed communities, suggesting that interspecific competiton is more important (than complementarity) for space occupation capacity.

Sediment porewater nutrients and resource use

Plant richness did not markedly affect the sediment organic deposition and mineralization, thus the C:N ratio did not differ between treatments. The low C:N ratios found in the present study indicate that the organic matter in the sediment is derived from benthic microalgae (as suggested by Hansen & Kristensen 1998), as seagrass detritus generally has a higher C:N ratio (Kristensen & Hansen 1995, Boschker et al. 2000). Because of differing biomass and penetration depth of rhizomes and roots (*Potamogeton filiformis*: slender roots, ~5 cm depth, *Zostera marina*: extensive rhizomes and roots, ~10 cm depth, *P. perfoliatus*: extensive rhizome, intermediate roots, ~15 cm depth; authors' pers. obs.), we expected to record complementary resource use. As water column nutrients were depleted throughout the productive season at the study site, sediment nutrients likely constituted the main source of nutrients for the plant communities (Hemminga 1998). When *Z. marina* grows on sandy sediments it is often considered N-limited (Short 1987). This is in accordance with the *Z. marina* treatments, with average NH_4^+ concentrations in porewater <100 µM (Fourqurean et al. 1992, present study). The highest porewater NH_4^+ concentration was recorded in unvegetated control plots, while surprisingly, the triculture had the highest NH_4^+ concentration among the plant treatments. Thus, in contrast to some terrestrial studies (e.g. Tilman et al. 1997), no resource use complementarity was recorded. This indicates weak richness effects on sediment porewater nutrient concentrations, i.e. plants in our mixed brackish water communities do not appear to use available nutrients more completely than in monocultures.

For some species, a more efficient nutrient use at high species richness has been recorded (van Ruijven & Berendse 2005), which may also explain the observations in the present study. However, in the present study, the leaf nutrient content was not analyzed, so the exact mechanism behind this observation requires further study. In contrast to NH_4^+, PO_4^{3-} levels did not differ between vegetated and unvegetated plots. Similar patterns have been recorded in the study area (Boström et al. 2004). Surprisingly, the PO_4^{3-} concentration was highest in the biculture of *Zostera marina* and *Potamogeton perfoliatus*, suggesting that despite the non-significant CE found for biomass production, these species did not utilize porewater nutrients as efficiently as other species combinations.

CONCLUSIONS

Our results show that the effects of plant richness per se on primary production and resource use in brackish water plant communities are weak or non-existent. However, species composition appears to be important for both aboveground and belowground production in angiosperm communities. In earlier work (Engelhardt & Ritchie 2002), richness responses of these plant compartments have not been analysed separately for indi-

vidual component species. The present study shows that a substantial part of angiosperm species interactions occurs in the sediment, and that these interactions might differ from aboveground dynamics. Understanding the interactions in multispecific plant communities is challenging, but crucial for determining the consequences of species loss for the functioning of these valuable coastal ecosystems.

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