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Colonization dynamics in trophic-functional patterns of biofilm-dwelling ciliates using two methods in coastal waters

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The colonization dynamics in trophic-functional structure of biofilm-dwelling ciliate fauna were studied using two methods based on an artificial substratum in Korean coastal waters of the Yellow Sea during April 2007. Polyurethane foam enveloped slide (PFES) and conventional slide (CS) systems were used to collect ciliate samples at a depth of 1 m. The ciliate fauna represented similar colonization dynamics in trophic-functional patterns that were driven mainly by the algivores, bacterivores and non-selectives in both systems. Simple trophic-functional patterns (e.g. algivores and non-selectives) occurred within the ciliate fauna at the initial stage (1–3 days), while complex patterns (e.g. algivores, non-selectives and bacterivores) were established at the transitional (5–7 days) and equilibrium (9–19 days) stages. However, the time in which ciliate fauna reached a stable trophic-functional pattern was shorter in the PFES than in the CS system. Among four trophic-functional types, the algivores and bacterivores significantly fitted the MacArthur-Wilson and logistic models in colonization and growth curves in both systems, respectively. Furthermore, the species richness and diversity of algivores and bacterivores were significantly higher in the PFES system than in the CS. These results suggest that the PFES system was more effective than the conventional slide method for a colonization survey on trophic-functional patterns of biofilm-dwelling ciliate fauna in marine ecosystems.

Keywords: artificial substratum, biofilm-dwelling ciliate, colonization dynamics, marine ecosystem, trophic-functional group

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INTRODUCTION

Biofilm-dwelling ciliates are a primary component of the periphyton microfauna and play an important role in the functioning of microbial food webs in aquatic ecosystems as a variety of consumers (Fischer *et al.*, 2002; Kathol *et al.*, 2009; Norf *et al.*, 2009; Zhang *et al.*, 2012, 2013; Xu *et al.*, 2014). Pratt & Cairns (1985) classified the ciliated protozoa into five trophic-functional (hereafter functional) groups based on their feeding strategies: bacterivores (B), algivores (A) saprotrophs (S), raptors (R) and non-selectives (N). Biofilm-dwelling ciliates can occur in high abundance in a microperiphyton fauna, where they can form complex communities of many different taxonomic and functional types in aquatic ecosystems (Norf *et al.*, 2009; Kathol *et al.*, 2011; Zhang *et al.*, 2012). As inhabitants of the sediment-water interface, these trophic-functional grazers can potentially feed on both surface-attached and suspended prey, and thus they can import the flux of both matter and energy from plankton (e.g. bacteria and microalgae) to benthos in most aquatic biotopes (Kathol *et al.*, 2009, 2011; Norf *et al.*, 2009; Xu *et al.*, 2011a, b). Bacterivores generally play an important role in maintaining and improving water quality by their grazing activities even in a niche with extreme environmental conditions for metazoa (Patterson *et al.*, 1989; Zhang *et al.*, 2013; Xu *et al.*, 2014).

The changes of trophic-functional (hereafter functional) patterns of the ciliate communities may significantly affect other components of the aquatic food web, and thus may influence the distribution and abundance of both lower and higher organisms (Finlay & Esteban, 1998). Some bacterivores can tolerate extreme environmental conditions and inhabit biotopes that are unfavourable to most metazoans (Patterson *et al.*, 1989). Furthermore, with their rapid growth and delicate external membranes, these microorganisms may react more quickly to environmental changes than most other eukaryotic organisms and thus serve as bioindicators of water pollution (Risse-Buhl & Küsel, 2009; Morin *et al.*, 2010; Xu *et al.*, 2014).

Biofilm-dwelling protozoan grazers feed on a variety of food particles, including bacteria, algae, flagellates, small ciliates and detritus (Geesey *et al.*, 1978; Parry, 2004; Scherwass *et al.*, 2005; Risse-Buhl & Küsel, 2009). Generally, the types of food acquisition in a biofilm are either the food particles from plankton origin by filter feeders, or the active search for food particles in the biofilm by gulper feeders (Norf *et al.*, 2009; Kathol *et al.*, 2011; Zhang *et al.*, 2012). However, the functional structure of the biofilm-dwelling ciliate communities may be subject to significant changes during the initial colonization of biotopes due to the food supply from the tidal current and circulation (Kiørboe *et al.*, 2004; Norf *et al.*, 2009). Thus, the conventional artificial substrate methods are problematic for biofilm-dwelling ciliate colonization in marine ecosystems (Xu *et al.*, 2009a,

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b). Xu *et al.* (2009a, b) has reported the effectiveness of a modified method, the polyurethane foam enveloped slide (PFES) system, for removing the influence of tidal waves on the colonization dynamics in taxonomic patterns of the ciliates in marine ecosystems. However, with regard to the colonization patterns in functional structure of the ciliate communities in this system, little information is known.

In this study, the colonization dynamics of the biofilm-dwelling ciliate communities in functional structures were investigated using the two methods based on the glass slide method in Korean coastal waters of the Yellow Sea during a 20-day period. Our aims of this study are focused on: (1) to document the functional composition of the biofilm-dwelling ciliate communities in both PFES and CS systems; (2) comparing the colonization dynamics in functional structure of the ciliate communities with the conventional slide method; and (3) to determine the effectiveness of the PFES system on collection of the ciliate communities with high diversity within a short time period for bioassessment of water quality in marine ecosystems.

MATERIALS AND METHODS

Study site and data collection

This survey was carried out during April 2007 in coastal waters, near Incheon Harbour, Korea (Xu *et al.*, 2009a, b) (Figure 1). This is a polluted area with a depth of ~8 m and a low transparency of ~1 m (mainly due to mixing of sediments from the muddy-sandy bottom) (Xu *et al.*, 2009a). Conventional (CS) and polyurethane foam enveloped slides (PFES) were deployed at 1 m. Sampling strategy followed that described by Xu *et al.* (2009a).

PFES and CS were designed, deployed, anchored and sampled as described by Xu *et al.* (2009a, b). A total of 80 glass slides (2.5×7.5 cm) were used as artificial substrates for collecting periphytic ciliates from a depth of 1 m below the water surface. For each method, a total of four PVC frames were used to hold 40 glass slides, 20 of which were used as an independent sampling replicate. For each replicate, two slides were randomly collected from each PVC frame at the time interval of 1, 3, 5,. . ., 19 days, during the study period. Samples were collected simultaneously from both

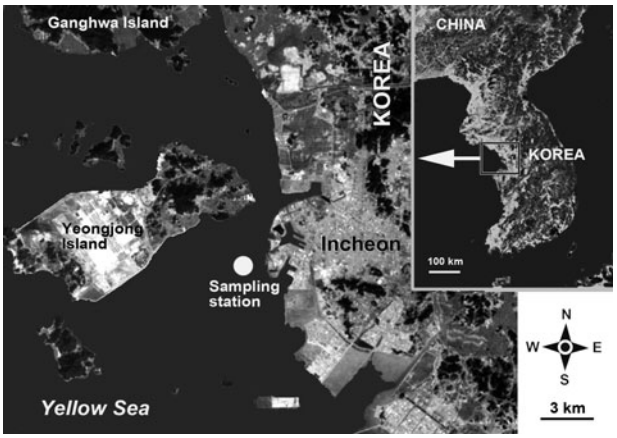

Fig. 1. Map showing the study area, which was located in the coastal waters of the Yellow Sea, near Incheon Harbor, Korea.

systems. Ciliate identification and enumeration followed methods outlined by Xu *et al.* (2009a).

Different periphytic ciliate species were assigned to the corresponding functional groups, which comprised bacterivores (B), algivores (A), raptors (R) and non-selectives (N), according to the literature (Pratt & Cairns, 1985; Fernandez-Leborans & Fernandez-Fernandez, 2002; Xu *et al.*, 2010; Zhang *et al.*, 2012). The different protist species recorded were assigned to the corresponding functional group taking into account the data from the literature (Pratt & Cairns, 1985; Fernandez-Leborans & Fernandez-Fernandez, 2002; Xu *et al.*, 2010; Zhang *et al.*, 2012) and from direct observation (e.g. non-selectives *Euplotes* spp. feeding on both bacteria and algae and algivores *Diophrys* spp. mainly feeding on algae) (Zhang *et al.*, 2012).

Data analysis

The colonization process in species number of each functional group of the ciliate communities was tested if it fitted the MacArthur & Wilson (1967) model:

$$S_t = S_{eq}(1 - e^{-GT})$$

where St is the species number at time T; S_{eq} is the estimated equilibrium species number of ciliate colonization; G is the colonization rate constant; T is the colonization time; and $T_{90\%}$ is the time taken to reach 90% S_{eq}. Three functional parameters (S_{eq}, G and $T_{90\%}$) were estimated using the program SIGMAPLOT v12.5. Fitness tests were carried out to determine whether the species numbers observed at each time interval fit the MacArthur–Wilson model at the 0.05 significance level (Zhang et al., 2012).

The increase of individual abundance of each functional group over the total study period was tested if it fitted the logistic model:

$$A_t = A_{max}/[1 + e^{(a-rt)}]$$

where A_t is the individual abundance at time t; A_{max} is the carrying capacity of individual abundance (maximum abundance); r is the grow rate constant; a is the coefficient constant of initial individual abundance; T is the colonization time; and $T_{50\%}$ is the time to 50% A_{max}. All growth curve parameters (e.g. A_{max}, r and $T_{50\%}$) were estimated using the SIGMAPLOT v12.5. Fitness tests were to determine whether the individual abundance recorded at each time interval fit the logistic model at the 0.05 significance level (Zhang *et al.*, 2012).

Species diversity (H'), evenness (J') and species richness (D) of five functional groups in samples were calculated as follows:

$$H' = -\sum_{i=1}^{s} Pi(\ln\ Pi)$$

$$J' = H'/\ln\ S$$

$$D = (S-1)/\ln\ N$$

where H' = observed diversity index; P_i = proportion of the total count arising from the ith species; S = total number of species; and N = total number of individuals.

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All multivariate analyses were performed using PRIMER v6.1 and the PERMANOVA+ for PRIMER (Clarke & Gorley, 2006; Anderson *et al.*, 2008). The colonization dynamics in functional pattern of the ciliate communities were summarized using the routine dbRDA (distance-based redundancy analysis) of PERMANOVA+ on Bray–Curtis similarity matrices from fourth root-transformed species-abundance data, and Euclidean distance matrices from log-transformed species-number data. The significance of correlations/differences in colonization patterns of the ciliate communities between two methods was tested using the routine RELATE/PERMANOVA (Clarke & Gorley, 2006).

The *t* test and non-parametric Kolmogorov–Smirnov test was used to evaluate the differences in the species number and colonization rates between two methods at the 0.05 level (Zhang *et al.*, 2012).

RESULTS

Taxonomic composition and functional structure

A total of 29 biofilm-dwelling ciliate species were recorded from both glass slide systems over the survey period, 27 taxa of which were identified in each of the PFES and CS systems. The functional composition of the ciliate communities is summarized in Table 1. The 29 ciliate species represented four functional groups: 14 algivores, 11 bacterivores, three non-selectives and one raptor (Table 1). The algivores and bacterivores were the most common functional forms, accounting for 51.9% (14 species) and 37.0% (10 species) in the PFES system, and for 48.1% (13 species) and 37.0% (10 species) in the CS system, respectively, compared with the other two groups (Table 1).

Colonization dynamics in species number and abundance

The colonization curves in species number of all four functional ciliate groups with colonization times of 1, 3, 5,..., 19 days in both systems during the study period are shown in Figure 2. In terms of species number, group A predominated the microbial communities in the initial stage (1–3 days), while groups A and B were the primary contributors to the transitional and mature stages (Figure 2A, B). Regression fitness test showed that groups A and B fitted well to the MacArthur–Wilson model (Table 2). Furthermore, the equilibrium species number (S_{eq}) of groups A and B were higher in the PFES system than in the CS ($P < 0.05$) (Table 2).

With regard to abundance, groups A and B showed higher values within the periods of more than ~9 days in the CS system than in the PFES ($P < 0.05$) (Figure 3A, B). Regression fitness test showed that groups A and B were

Table 1. List of biofilm-dwelling ciliate species, with trophic-functional type (func.) and abundance recorded in two artificial glass slide systems FPES and CS in Korean coastal waters of the Yellow Sea during three colonization stages (1–5 d, 7–9 d and 11–19 d).

Species	Func.	FPES			CS		
		1–5 d	7–9 d	11–19 d	1–5 d	7–9 d	11–19 d
Amphileptus litonotiformis	A	–	–	+	–	–	+
Amphileptus gui	A	–	–	+	–	–	+
Litonotus paracygnus	A	–	+	+	–	+	+
Loxophyllum rostratum	A	–	–	+	–	–	–
Dysteria monostyla Kahl	A	–	+	+	–	+	++
Vaginicola crystalline marina	B	–	–	+	–	+	–
Pseudovorticella sinensis	B	–	++	++	–	++	++++
Zoothamnium duplicatum	B	–	++	+++	+	++	+++++
Pleuronema coronatum	B	–	–	–	–	+	–
Metanophrys similis	B	–	+	+	–	+	++
Paranophrys magna	B	–	–	+	–	–	+
Uronema marinum	B	–	+	+	–	–	+
Condylostoma acuta	R	–	–	–	–	–	+
Gruberia lanceolata	A	–	–	+	–	–	+
Peritromus kahli	B	–	–	+	–	–	+
Aspidisca leptaspis	B	–	–	+	–	–	+
Aspidisca steini	B	–	–	++	–	+	+
Diophrys appendiculata	A	–	–	+	–	–	+
Diophrys scutum	A	+	–	+	–	+	–
Euplotes charon	N	–	+	+	–	+	+
Euplotes minuta	N	+	+	+	+	+	+
Euplotes vannus	N	–	+	–	–	+	–
Holosticha bradburyae	A	–	+	+	–	–	+
Holosticha diademata	A	+	–	+	–	+	+
Holosticha heterofoissneri	A	–	+	+	–	+	+
Stichotricha marina	B	–	+	–	–	–	–
Strombidium apolatum	A	+	–	–	+	–	–
Strombidium sulcatum	A	+	+	+	+	–	–
Tintinnopsis elongata	A	+	+	+	+	+	–

Functional type (func.): A, algivores; B, bacterivores; N, non-selectives; R, raptors; Abundance: +, 0–1; ++, 1–10; +++, 10–100; ++++, 100–400; +++++, over 400 ind. cm^{-2}.

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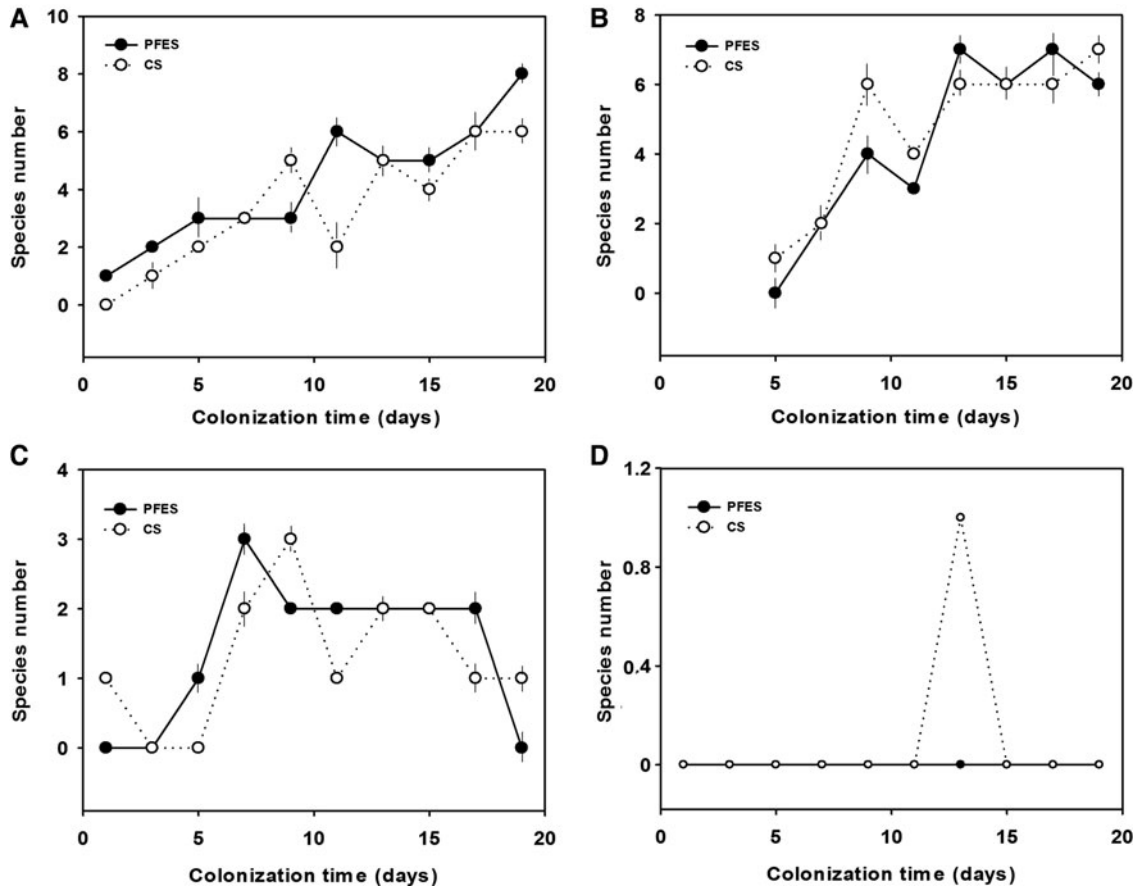

Fig. 2. Colonization curves in species number of algivores (A), bacterivores (B), non-selectives (C) and raptors (D) within biofilm-dwelling ciliate communities in the PFES and CS systems during the study period.

Table 2. Functional parameters of each trophic-functional assemblage (func.) of biofilm-dwelling ciliate fauna, based on datasets using the PFES and CS methods.

Method	Func.	S_{eq}	G	T_{90}	R^2
PFES	A	13.2	0.04	57.6	0.86*
	B	7.5	0.17	13.5	0.86*
	N	1.8	0.27	8.5	0.32
	R	–	–	–	–
CS	A	11.0	0.04	57.6	0.79*
	B	6.9	0.21	11.0	0.83*
	N	1.7	0.22	10.5	0.18
	R	–	–	–	–

S_{eq}, the estimated equilibrium species number; G, the constant value of colonization rate; T_{90}, the time taken for reaching 90% S_{eq} (day); R^2, determination coefficients. Functional type (func.): A, algivores; B, bacterivores; N, non-selectives; R, raptors. *Significant at $P < 0.05$.

well fitted to the logistic model (Table 3). It should be noted that the estimated maximum abundances (A_{max}) of bacterivores was significantly higher in the PFES system than in the CS ($P < 0.05$) (Table 3).

Temporal variations in functional structures

The colonization patterns of the ciliate communities in terms of relative species number and abundance in both systems are summarized in Figure 4. Simple functional patterns (e.g. algivores and non-selectives) occurred within the ciliate fauna at the initial stage (1–3 days), while complex patterns (e.g. algivores, non-selectives and bacterivores) were established at the transitional and equilibrium stages.

The matching correlation with temporality of the colonization dynamics in functional structure was similar in PFES and CS systems (correlation coefficient: 0.811 versus 0.812).

However, the colonization patterns of the ciliates were different in the dbRDA ordination plot. In the CS system, the dbRDA ordinations showed the colonization process of the ciliate communities was falling into three stages, respectively: the first axis (dbRDA 1) separated the initial stage (1–3 days) and the transitional stage (5–7 days) (left) from the equilibrium stage (9–19 days) (right), while the second axis (dbRDA 2) discriminated the samples at initial stage above (upper) from those at the transitional stage (lower) (Figure 5B, D). However, the time in which ciliate fauna reached a stable functional pattern was shorter in the PFES than in the CS system, i.e. the last sample (7-day) in the transitional stage was grouped with those in the equilibrium stage by the first axis (Figure 5A, C). PERMANOVA test showed that there were significant differences in community patterns between each pair of stages ($P < 0.05$). However, no significant difference was found in colonization patterns of the ciliate communities between the two methods ($P > 0.05$).

Vector overlay of Spearman correlations of species number of all five functional groups with the dbRDA axes is also shown

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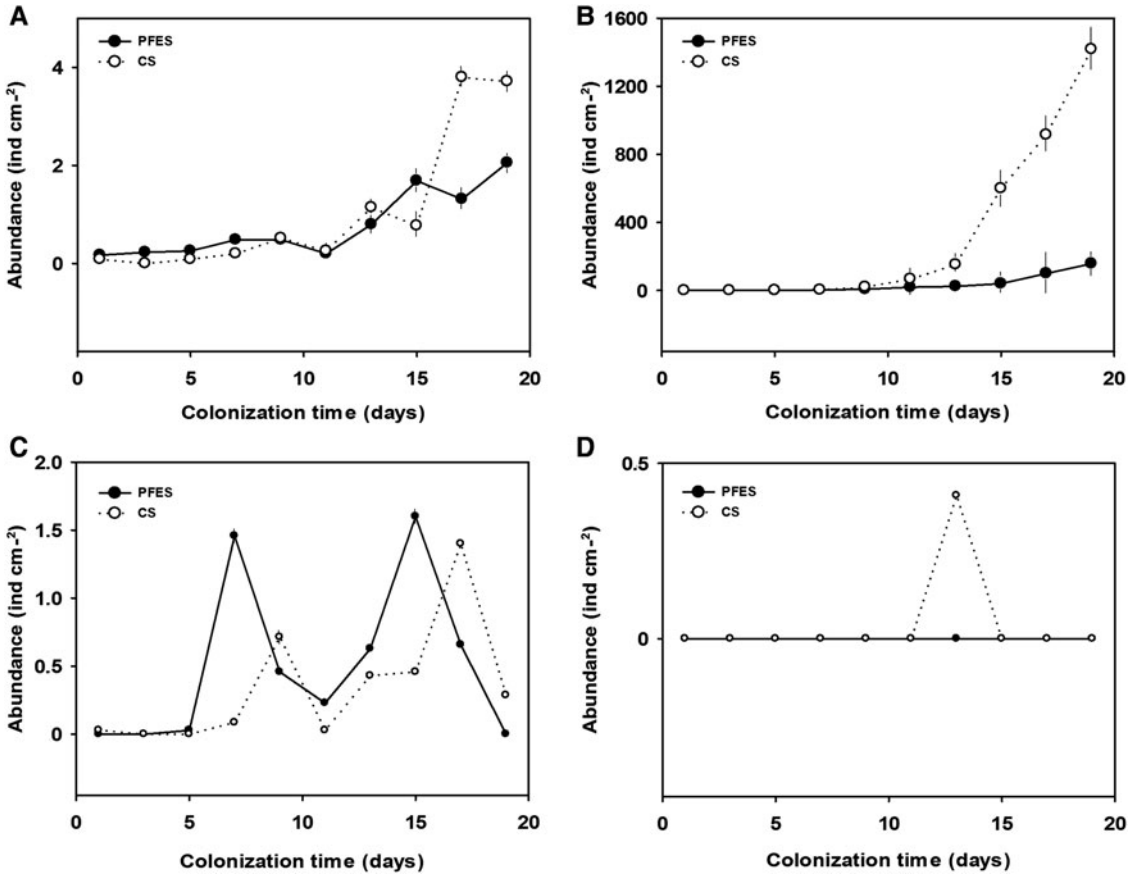

Fig. 3. Growth curves in abundance of algivores (A), bacterivores (B), non-selectives (C) and raptors (D) within biofilm-dwelling ciliate communities in the PFES and CS systems during the study period.

Table 3. Estimated parameters of logistic curves in abundance of each trophic-functional assemblage of the biofilm-dwelling ciliate fauna, based on datasets using the PFES and CS methods.

Method	Func.	A_{max}	r	T_{50}	R^2
PFES	A	6.8	0.18	23.9	0.87*
	B	351.4	0.40	19.5	0.99*
	N	–	–	–	–
	R	–	–	–	–
CS	A	3.8	2.6	15.5	0.91*
	B	1820.7	0.54	16.8	0.99*
	N	–	–	–	–
	R	–	–	–	–

A_{max}, the estimated equilibrium abundance; r, growth rate; T_{50}, the time taken to reach 50% A_{max} (day); R^2, determination coefficients. Functional type (func.): A, algivores; B, bacterivores; N, non-selectives; R, raptors. *Significant at $P < 0.05$.

in Figure 5. This shows that the bacterivores and non-selectives were the primary contributors driving the colonization dynamics in functional pattern in both systems (Figure 5).

Temporal variations in diversity patterns

Temporal variations in species richness, diversity and evenness of all four functional groups in both systems are summarized in Figure 6. In terms of species richness and diversity, groups A and B showed a similar trend in both systems, i.e. generally increasing in the initial and transitional stages and levelling off in the equilibrium stage (Figure 6A, D). In terms of species evenness, groups A and B showed a decrease trend in the equilibrium stage (Figure 6C, F). Non-parametric test revealed that the species richness and diversity of groups A and B were significantly higher in the PFES system than in the CS ($P < 0.05$).

DISCUSSION

Many investigations have demonstrated that grouping in functional assemblage is an effective approach to simplifying community structure, and allows investigation of ecosystem-level processes driven by the community members with similar function on different temporal scales (Fernandez-Leborans, 2001; Xu *et al.*, 2010; Zhang *et al.*, 2012). In the present study, the biofilm-dwelling ciliate communities in both sampling systems represented four functional groups: algivores, bacterivores, non-selectives and raptors. Groups A and B were the primary contributors to the ciliate communities. This result was consistent with the previous reports on benthic microfauna: even though a large number of species may be present, only a few commonly were the primary contributor to the communities (Azovsky, 1988; Fernandez-Leborans & Fernandez-Fernandez, 2002; Burkovskii *et al.*, 2011; Zhang *et al.*, 2012). It should be noted that the consumers (B and N) were the primary contributors driving the shift in functional pattern of the ciliate communities in both systems.

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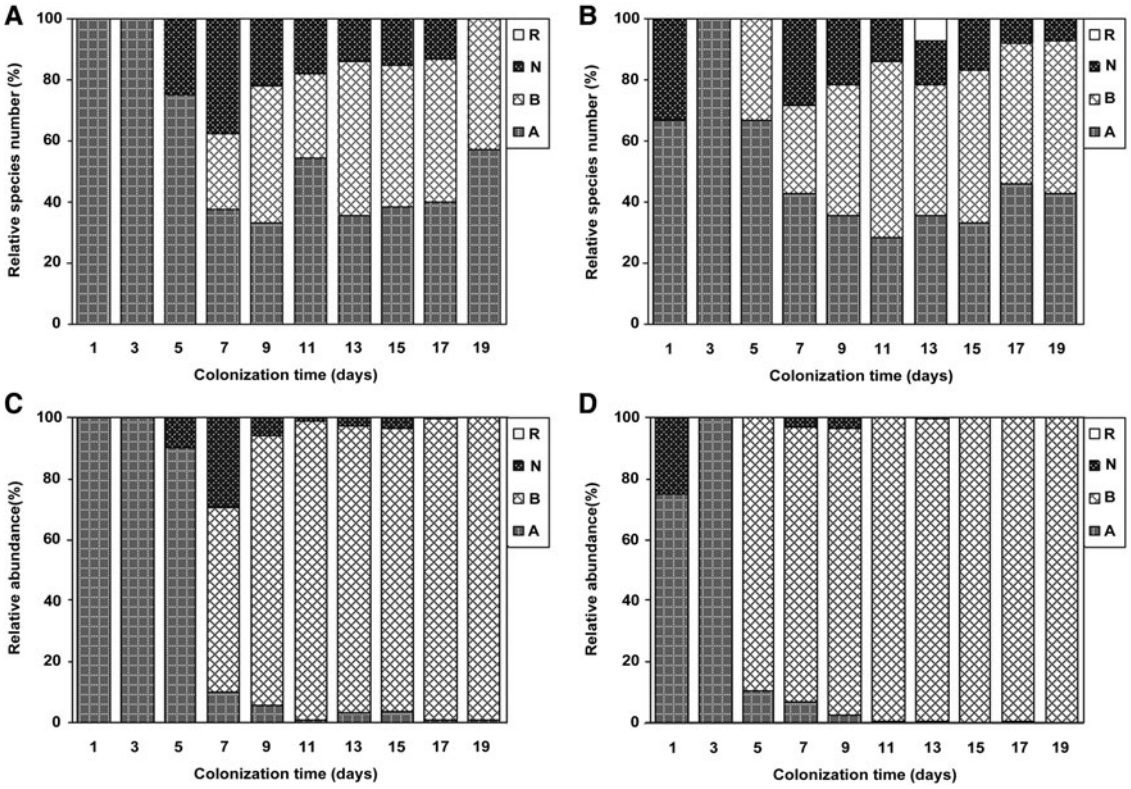

Fig. 4. Temporal variations of relative species number (A, B) and relative abundance (C, D) of four functional groups of biofilm-dwelling ciliate communities in the PFES (A, C) and CS (B, D) systems during the study period. A, algivores; B, bacterivores; N, non-selectives; and R, raptors.

Previous studies have demonstrated that during the colonization period, the number of species generally increases and then equilibrates, following the MacArthur–Wilson (1967) equilibrium model equation (Xu *et al.*, 2009a; Zhang *et al.*, 2012). However, it should be noted that the classic MacArthur–Wilson model also presupposes that number of species is determined by a dynamic balance between immigration and extinction. Based on our previous studies, the temporal patterns in community structure may be discriminated into three phases including the initial stage (e.g. 1–3 days), transitional stage (e.g. 5–7 days) and the equilibrium stage (e.g. 10–28 days) during the colonization period, among which there is a significant difference in community structure ($P < 0.05$) (Burkovskii & Mazei, 2001; Burkovskii *et al.*, 2011; Zhang *et al.*, 2012). In this study, the multivariate analysis revealed that the ciliate communities in functional structure might also be assigned into three phases including the initial stage (1–3 days), transitional stage (5–7 days) and the equilibrium stage (9–19 days).

In terms of both species number and abundance of each functional group, the colonization and growth curves of algivores and bacterivores significantly fitted the MacArthur–Wilson model in both systems, respectively. Otherwise, although the non-selective group (N) decreased in species number during the colonization period, they showed high contribution to the community compared with the raptor group (R). Thus, these three groups may be considered as main drivers in the shift of functional structure of the ciliate in both systems. It should be noted that these function groups exhibited different colonization curves, for example, bacterivore group (B) seemed to reach equilibrium stage, while the species number of non-selective group (C) decreased, but that of algivore group (A) increased slowly with a high value of $T_{90\%}$. This result was similar to our previous report on the ciliate colonization dynamics in coastal waters of the Yellow Sea, northern China (Zhang *et al.*, 2012). These findings suggest that during the colonization process in the taxonomic composition and the community structure, the functional groups have their own specific colonization dynamics.

Our previous studies have demonstrated that the tidal events influence both species diversity measures and colonization parameters of periphytic ciliate communities colonizing conventional slides (CS) (Xu *et al.*, 2009a, b). In this study, the matching correlation with temporality of the colonization dynamics in functional structure was similar in both systems. However, the dbRDA ordination revealed that the time at which ciliate fauna reached a stable functional pattern was shorter in the PFES than in the CS systems. Otherwise, the species richness and diversity of groups A and B were significantly higher in the PFES system than in the CS. This implies that the PFES system may collect the ciliate samples with high diversity within a shorter time period than the CS system, besides the removal of the tidal influence. Thus, we suggest that the PFES system was more effective than the conventional slide method for examining functional patterns of biofilm-dwelling ciliate fauna in marine ecosystems.

Our previous studies have demonstrated that the growth curves of the ciliates generally fitted the logistic model equation (Zhang *et al.*, 2012, 2013). The three functional parameters A_{max}, r and T_{50}, which were determined by this equation model, may reflect the environmental conditions, for example, the higher the A_{max} value, the higher the eutrophication level. However, in this study, the bacterivores were predominated by suspension feeding (planktivorous) consumers

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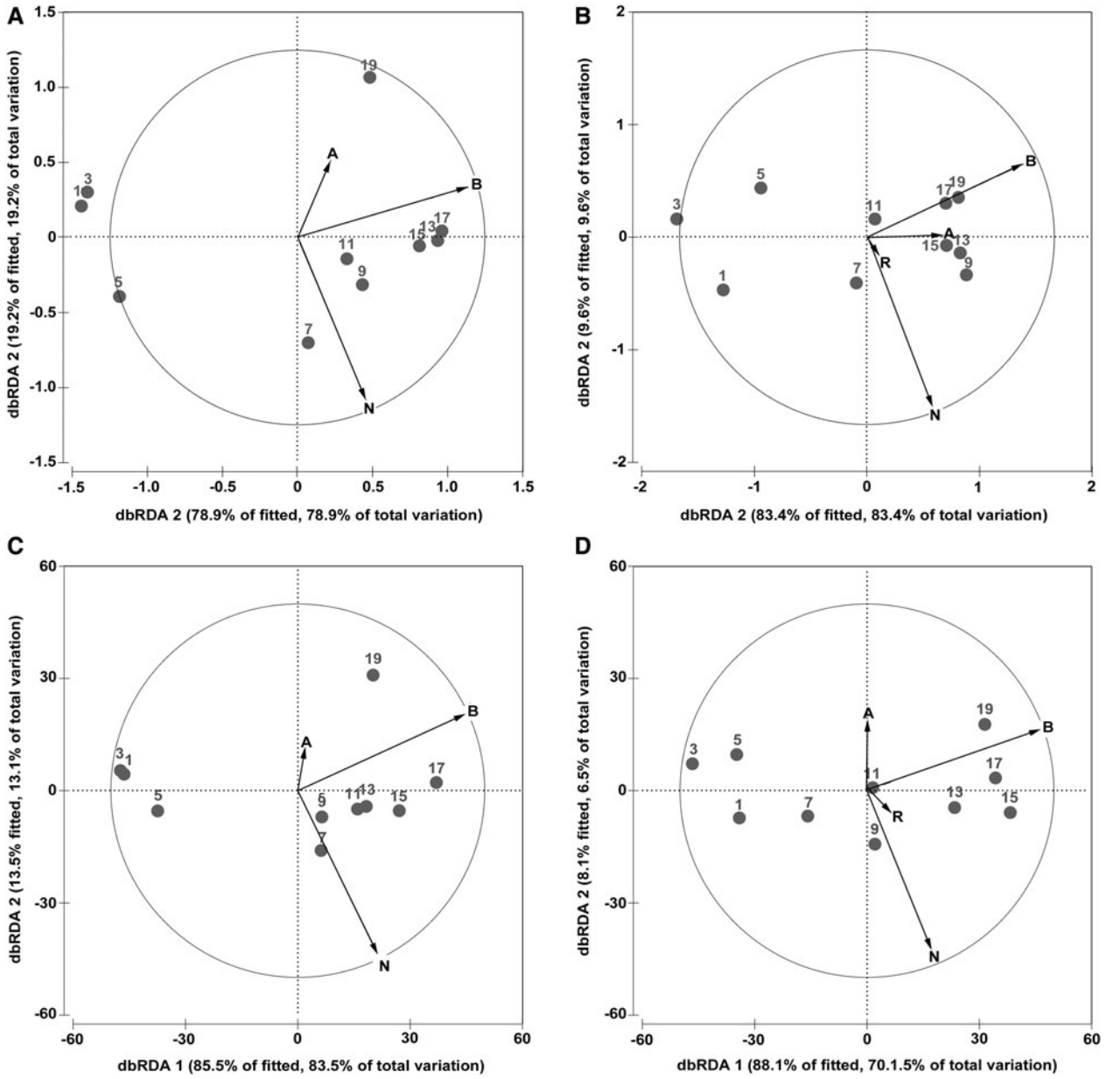

Fig. 5. Distance-based redundancy analysis (dbRDA) on Euclidean distance from the log-transformed species-number data (A, B) and on Bray–Curtis similarities for the species-abundance data (C, D) of the ciliate samples during the study period, respectively, and correlations of species numbers and individual abundance of four functional groups with the two dbRDA axes. See Fig. 4 for abbreviations. 1–19, colonization times.

(mainly peritrich ciliates). Their individual numbers and growth rate (*r*) were low due to less food supply from planktonic resources in the PFES system compared with the CS system. This may also be why the maximum abundance of bacterivores was significantly higher in the CS system than in the PFES system.

In summary, the ciliate fauna represented similar colonization dynamics in functional pattern that was driven mainly by the algivores, bacterivores and non-selectives in both systems. Simple trophic-functional patterns occurred within the ciliate fauna at the initial stage, while complex patterns were established at the transitional and equilibrium stages, respectively. However, the time at which ciliate fauna reached a stable functional pattern was shorter in the PFES system than the CS. Among four functional types, the algivores and bacterivores significantly fitted the MacArthur–Wilson and logistic models in colonization and growth curves in both systems. Furthermore, the species richness and diversity of groups A and B were significantly higher in the PFES system than in the CS. These results suggest that the PFES system was more effective than the conventional slide method for a colonization survey on functional patterns of biofilm-dwelling ciliate fauna in marine ecosystems.

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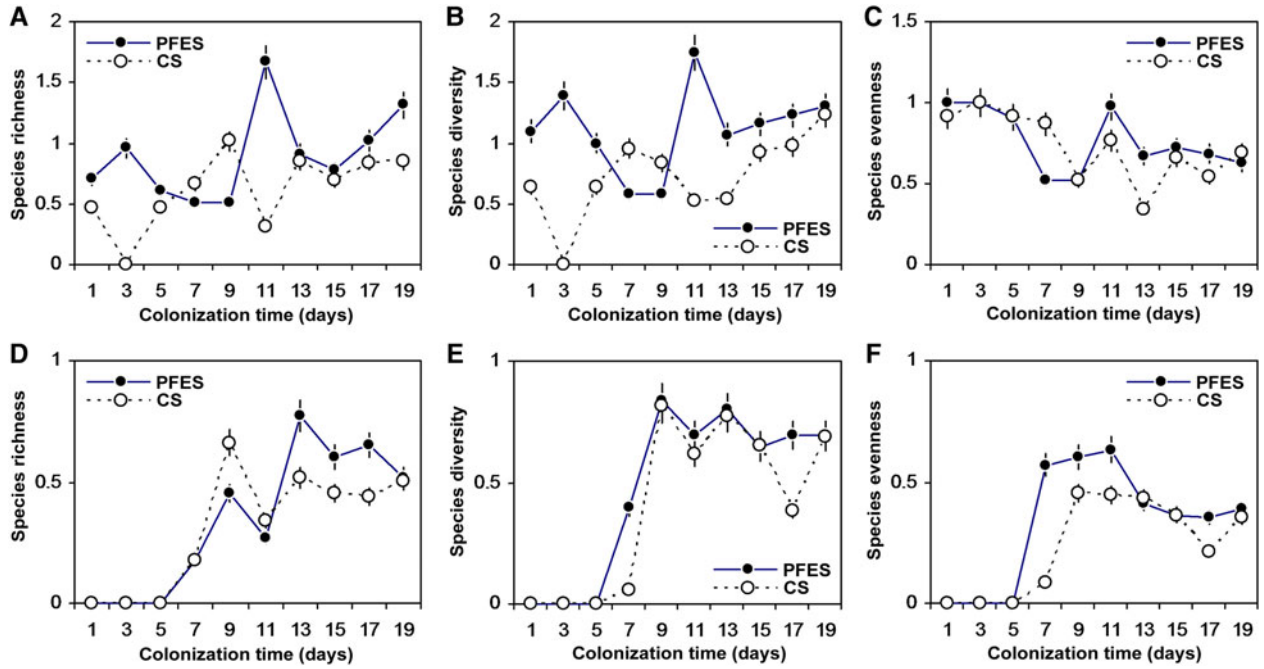

Fig. 6. Temporal variations in species richness (*D*) (A, D), diversity (*H′*) (B, E) and evenness (*J′*) (C, F) of functional groups A (A, B, C) and B (D, E, F) in the PFES and CS systems during the study period.

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