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Usefulness of tissue nitrogen content and macroalgal community structure as indicators of water eutrophication

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Abstract We tested the hypothesis that the community structure and biochemical composition of macroalgae reflect the degree of nutrient concentrations in the water column. Benthic community structure and tissue nitrogen (N) content of macroalgae on intertidal rocky shores at three sites were investigated in relation to sewage effluents on Mireuk Island, Tongyeong city, on the southern coast of Korea. *Ulva australis* clearly dominated at site 1, which was close to a sewage treatment plant, where higher dissolved inorganic N and dissolved inorganic phosphate concentrations were observed. *U. australis*-dominated communities also appeared at site 2 (intermediate levels of nutrient enrichment). The macroalgal assemblage at site 3 (unimpacted site) was significantly different from those at sites 1 and 2. Five species (*U. australis*, *Sargassum fusiforme*, *Grateloupia elliptica*, *Gelidium amansii*, and *Sargassum horneri*) were dominant at site 3, representing 87 % of the total coverage throughout the study period. Species richness (d), evenness (J'), and diversity index (H') were highest at site 3, intermediate at site 2, and lowest at site 1, showing a negative relationship with nutrient levels. These results indicate that macroalgal community structure can be used as a bioindicator in water quality assessment. The tissue N content of green and red algae was responsive to nutrient availability, while the tissue N content of brown algae was relatively unchanged among the sites. This suggests that tissue N content as a bioindicator for detecting the influence of sewage effluent should be considered to reflect the N storage capacity of macroalgae.

Keywords Community structure · Macroalgae · Nutrient enrichment · Sewage effluent · Tissue nitrogen content

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Introduction

Eutrophication caused by the inflow of excess nutrients (e.g., nitrogen (N) and phosphorus (P)) from human-derived sources is one of the most serious problems in estuarine and coastal areas. Nutrient over-enrichment stimulates the growth of macro- or microalgae, which leads to algal blooms, such as "green tides" or "red tides" (Fletcher 1996; Anderson et al. 2002). Algal blooms reduce the light available to seagrass and subtidal macroalgae and promote the development of anoxia, which in turn leads to massive mortalities among marine organisms. This facilitates considerable alterations in species diversity and food web dynamics in estuarine and coastal ecosystems (Raffaelli et al. 1989; Rabalais et al. 1996; Lee et al. 2007). Therefore, early detection of eutrophication is vital to prevent or minimize the negative effects of algal blooms and to properly manage and conserve estuarine and coastal ecosystems.

Macroalgal benthic community structure has been considered an effective tool for assessing the impacts of natural and anthropogenic disturbances (Borowitzka 1972;

Eriksson et al. 1998; Díaz et al. 2002; Martins et al. 2012). In particular, several studies have investigated the effects of marine pollution such as nutrient enrichment on macroalgal assemblages (Díez et al. 1999; Soltan et al. 2001; Arévalo et al. 2007; Pinedo et al. 2007). Unlike mobile marine organisms, the sedentary nature of macroalgal assemblages on the intertidal rocky shore means that they reflect present as well as previous environmental conditions (Reish 1987; Gorostiaga and Díez 1996). As the industrial or urban effluent with nutrient enrichment flows near the sea surface, benthic intertidal communities are more sensitive to this factor than other communities (Soltan et al. 2001). In general, the effects of sewage and pollution are dependent on the algal group. For example, reductions in perennial brown algae, simplification of community structure, and increases in opportunistic algae were observed under conditions of nutrient over-enrichment, and the combination of these factors led to dramatic decreases in species diversity (Díez et al. 1999; Middelboe and Sand-Jensen 2000; Benedetti-Cecchi et al. 2001). Thus, macroalgal communities on intertidal rocky shores can be useful indicators to estimate the degree of eutrophication in estuarine and coastal areas (Perez et al. 2000).

Traditionally, water quality sampling techniques have been used to detect and monitor nutrient enrichment in the water column. However, it is difficult to estimate water column nutrient enrichment in estuarine and coastal ecosystems, as the techniques used are conspicuously affected by tides, waves, currents, and nutrient uptake rates of marine plants. Macroalgal indicators have been developed as a complimentary method of detecting water nutrient enrichment (Fong et al. 1998; Costanzo et al. 2000; Jones et al. 2001; Lin and Fong 2008). Macroalgae continuously respond to enhanced nutrients, which change nutrient uptake rates, growth rates, and storage rates (Fujita 1985; Fong et al. 1994). Macroalgal tissue N content increases with increasing water N concentration until it reaches its saturation point (Cohen and Neori 1991). The N/P ratio of macroalgae was significantly lower under low N conditions than under high N conditions (Björnsäter and Wheeler 1990). Thus, the tissue N content of macroalgae reflects in situ nutrient availability over a period of time (Wheeler and Björnsäter 1992; Fong et al. 1994). However, biochemical responses of macroalgae to N enrichment vary among algal groups because different algal groups have different N storage capacities. In particular, brown algae, which have a high storage capacity, cannot respond immediately to high N levels (Thomsen and McGlathery 2007). This implies that the use of tissue N content as a bioindicator of N enrichment may depend on the nutrient storage capacity of the particular algal group.

The sewage treatment plant located in Tongyeong Bay on the southern coast of the Korean peninsula was built in 1994. An average sewage outfall of 54,000 m^3 day^{-1} has been discharged into the sea since 2001, consisting primarily of untreated anthropogenic wastes. The wastewater is treated by a conventional activated sludge process. Average biological oxygen demand and chemical oxygen demand in the effluent were 10.29 and 6.75 mg L^{-1}. Average T-N and T-P in the effluent in 2002 was 12.38 and 1.25 mg L^{-1}. This discharge, including high nutrient concentrations, spreads along the coast by tidal, wave, and current actions, and high N concentrations in the water column have been observed in this area. However, few studies have examined the effects of sewage treatment plant discharge on the macroalgal benthic community in Korea. The objective of this study was to assess the effectiveness of using the benthic community structure and biochemical composition of macroalgae as biological indicators to evaluate nutrient enrichment in the water column. In the present study, we hypothesized that (1) macroalgal benthic community structure in the vicinity of a sewage treatment plant would be simplified via decreased species diversity and increased opportunistic species compared with that at other sites, and (2) the use of algal tissue N content as an indicator for the early detection of eutrophication would depend on macroalgal species. To test these hypotheses, the benthic community structures and tissue N content of macroalgae were investigated on intertidal rocky shores at three study sites along a gradient of nutrient concentrations.

Materials and methods

The study sites were located on rocky intertidal shores in Tongyeong Bay on the southern coast of Korea (Fig. 1). Three accessible study sites were established at different distances from a sewage treatment plant. Site 1 (34°49′ N, 128°23′ E) was located approximately 0.3 km from the outfall of the sewage treatment plant. Site 2 (34°48′ N, 128°26′ E) was positioned approximately 5.5 km from the outfall site and was weakly affected by the effluents via tidal currents and wave action. Site 3 (34°45′ N, 128°24′ E) was considered as a reference site due to its lack of exposure to high nutrients. All sites were semi-exposed to waves around small islands and land. The tidal regime is semidiurnal, and the system is classified as mesotidal with a maximum tidal range of about 3.0 m during spring tides (Tide Tables for the Coast of Korea, National Oceanographic Research Institute of Korea).

Physical and chemical factors

Daily incident irradiance data (moles of photons per square meter per day mol photons m^{-2} day^{-1}), monthly precipitation (mm), and air and water temperatures (ºC) were obtained from the Tongyeong Meterological Observatory (http://www.kma.go.kr/sfc/sfc_02_03_jsp), about 3.5 km north of site 2. To determine dissolved inorganic N (dissolved inorganic nitrogen (DIN)=NH_4^++NO_3^-+NO_2^-) and phosphate

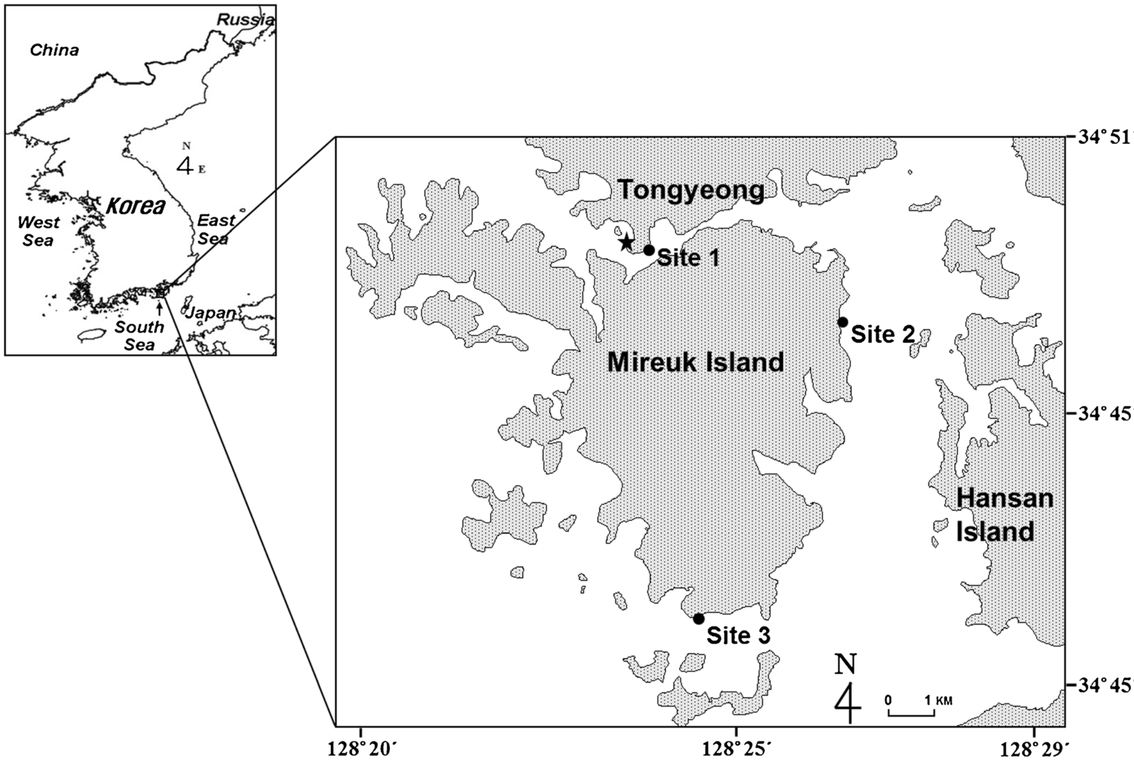

Fig. 1 Map showing the study area and sampling sites on the southern coast of Korea. *Star* indicates the Tongyeong city sewage treatment plant

(dissolved inorganic phosphate (DIP)=PO_4^{3-}) concentrations in the water column, four replicate surface water samples were collected monthly from January to December 2002 at the three sites. Water samples were placed on ice in the field and frozen until analyzed. Samples were filtered through Whatman GF/C filters. Before determining the $NO_3^-+NO_2^-$ concentration, samples were passed through a column containing copper-coated cadmium to reduce $NO_3^-+NO_2^-$. Water column nutrient concentrations were determined using standard colorimetric techniques following the methods of Parsons et al. (1984).

Experimental design and sampling

Observations were made on gently sloping rocky intertidal shores from January to December 2002. Three transect lines were located perpendicular to the shoreline at each site: the zone ranged from 0.4 to 2.0 m below mean sea level. Three replicates (50×50 cm) per transect line were randomly located on the rocky substratum. The distance between quadrats at each site depended on the size and was approximately 5–7 m. The percentage cover of each species was measured monthly in the field using a 0.25-m^2 stainless frame with 100 subplots. Recorded algal cover included only those thalli whose holdfasts were in the plot. The recorded algal percentage was sometimes more than 100 % because both the canopy and understory coverages were recorded. The percentage cover of each species was estimated using an efficient and accurate visual estimation method (Dethier et al. 1993). To identify macroalgal species accurately, samples of each species were collected, preserved with 4 % formalin in seawater, and observed under a microscope in the laboratory. The relative cover (RC) of each species was determined as follows: RC=(% cover of one species/% cover of total species in quadrat)×100.

Tissue N content analysis

To determine tissue N content, samples of the seaweed species commonly observed at the three sites on each sampling date were collected. The collected samples were rinsed to remove nutrients, epiphytes, and sediment from the thallus surface and were dried at 60 °C for 48 h. The dried samples were ground using a mortar and pestle, and approximately 2–3 mg of ground tissue were placed in a tin boat for the determination of N content using a CHNS/O elemental analyzer (Perkin Elmer 2400 Series II; USA).

Data analysis

To investigate changes in macroalgal species diversity and compare macroalgal communities among sites, Margalef's Richness Index (d), Pielou's Evenness Index (J'), Shannon's Diversity Index (H'), and K-dominance curves were calculated using PRIMER software (version 6.0). Similarity in species composition was analyzed using the Bray–Curtis similarity coefficient. Data were log($x+1$) transformed prior to analysis of the Bray–Curtis similarity coefficient. To compare macroalgal assemblages of different monthly samples among sites, cluster analysis was carried out using a hierarchical method with group-average linking, and non-metric multidimensional scaling (nMDS) was performed. Significant differences were assessed among seaweed composition from monthly samples using an analysis of similarity (ANOSIM). The cluster analysis, nMDS and ANOSIM were analyzed using PRIMER (version 6.0).

The significance of differences in water column nutrient concentrations (dissolved inorganic N and dissolved inorganic phosphate) and diversity indices (d, J', and H') were tested using one-way ANOVA with time as a block. Differences in tissue N content of each species among sites were tested for significance by one-way ANOVA. Data were tested for normality and homogeneity of variance to meet the assumptions of parametric statistics prior to ANOVA analysis. As these assumptions were not satisfied, data were log-transformed. When significant differences among treatments were observed, a Student–Newman–Keuls (SNK) post hoc test was performed. Statistical significance was set at alpha< 0.05. All ANOVA analyses were conducted using SPSS (version 15.0).

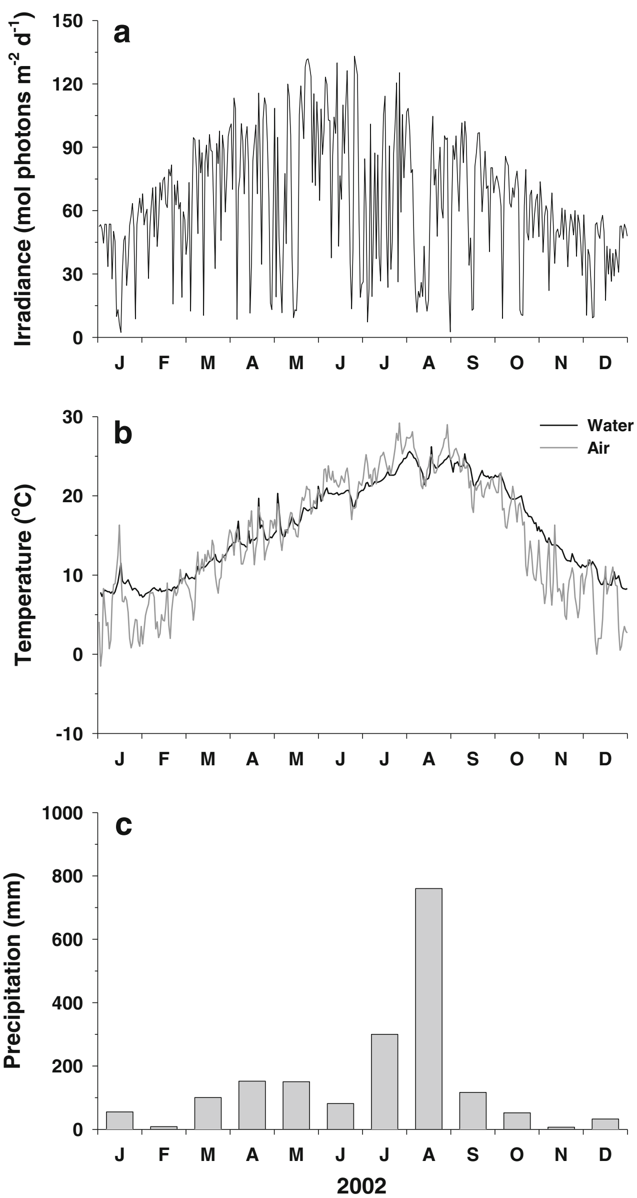

Fig. 2 Surface irradiance (**a**), air and water temperatures (**b**), and monthly precipitation at Tongyeong on the southern coast of Korea

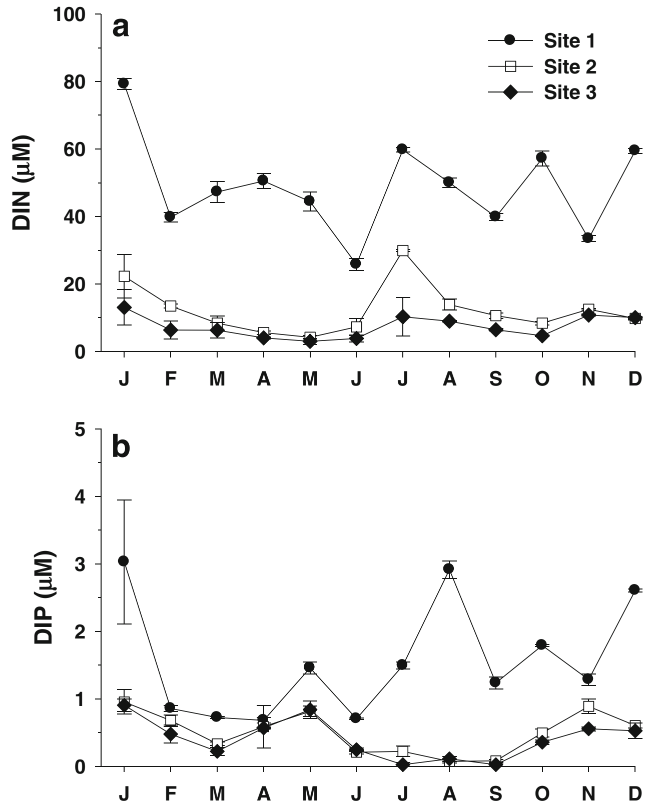

Fig. 3 Seasonal variations in water column DIN (**a**) and DIP (**b**) concentrations at three sites in Tongyeong Bay on the southern coast of Korea. Values are means±SE (n=4)

Results

Physical and chemical factors

Daily surface irradiance fluctuated greatly with sampling time, with increases during spring-summer and decreases during fall-winter (Fig. 2a). In particular, daily surface irradiance showed relatively low levels during July-August when the rainy season or typhoon season occurred. Air and water temperatures also exhibited strong seasonal variation (Fig. 2b). Air temperature was highest in July and August (29 °C) and lowest in January

Table 1 List of taxa with the monthly life form (LF), coverage (C), and relative coverage (RC) at three sites

Species	LF	Site 1		Site 2		Site 3	
		C	RC	C	RC	C	RC
Chlorophyta							
Ulva australis	A	63.5	73.8	36.0	44.4	22.2	18.9
Ulva compressa	A	1.8	2.1	3.0	3.7	+	+
Ulva intestinalis	A					1.3	1.1
Cladophora sakaii	A			+	+		
Codium fragile	A			+	+	+	+
Phaeophyceae							
Dictyota dichotoma	A			1.1	1.4	+	+
Dictyota divaricata	A			+	+	+	+
Rugulopteryx okamurae	A					+	+
Colpomenia sinuosa	A					1.0	+
Scytosiphon lomentaria	A	1.3	1.5			+	+
Undaria pinnatifida	A	6.4	7.4	6.9	8.5	5.8	4.9
Saccharina japonica	P			+	+		
Sargassum fusiforme	P			2.3	2.8	22.1	18.9
Sargassum horneri	P	1.7	2.0	12.1	14.9	9.8	8.4
Sargassum piluliferum	P			1.8	2.2		
Sargassum siliquastrum	P					+	+
Sargassum thunbergii	P			1.8	2.2	6.1	5.2
Rhodophyta							
Pyropia yezoensis	A	+	+	+	+	+	+
Amphiroa ephedraea	P					+	+
Corallina pilulifera	P			+	+	+	+
Lithophyllum okamurae	P			1.4	1.7	+	+
Gelidium amansii	P	+	+	+	1.0	14.7	12.5
Gelidium divaricatum	P					+	+
Pterocladiella capillacea	A					+	+
Gloiopeltis furcata	P			+	+		
Chondracanthus intermedius	P					+	+
Caulacanthus ustulatus	A	+	+	2.0	2.5	+	+
Chondrus ocellatus	P	+	+	1.8	2.2	2.7	2.3
Hypnea charoides	A			1.2	1.5		
Callophyllis rhynchocarpa	P			+	+	+	+
Solieria tenuis	A			+	+		
Polyopes affinis	P			+	+	+	+
Polyopes prolifera	P			+	+		
Grateloupia divaricata	A	+	+			4.5	3.8
Grateloupia elliptica	P	5.2	6.0	4.0	5.0	17.8	15.2
Gracilaria textorii	P					+	+
Gracilariopsis verrucosa	A	+	+	+	+	+	+
Plocamium telfairiae	A					+	+
Lomentaria catenata	A	3.8	4.4	+	+	3.2	2.7
Ceramium boydenii	A			1.0	1.2	+	+
Chondria crassicaulis	A			+	+	1.6	1.4
Total coverage (%)		86.0		81.0		117.2	
No. of *Chlorophyta* species		2		4		4	
No. of *Phaeophyceae* species		3		8		10	
No. of *Rhodophyta* species		8		17		20	
Total number of species		13		29		34	

A annual, *P* perennial, "+" cover is less than 1 %

(−1.5 °C). Water temperature ranged from 26.0 °C in July and August to 7.2 °C in January. Monthly precipitation increased notably during July and August, in part due to heavy rains during the rainy season and typhoon "RUSA" (Fig. 2c). The monthly average and annual precipitation amounts were 151 and 1,817 mm, respectively.

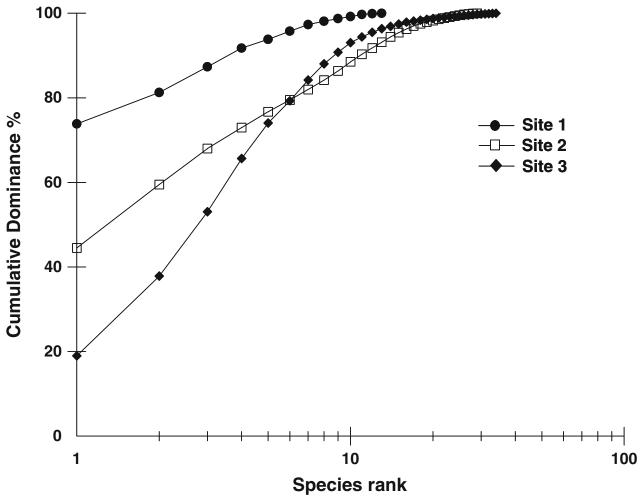

Fig. 4 *K*-dominance curves for macroalgal coverage at three sites in Tongyeong Bay on the southern coast of Korea

The DIN and DIP concentrations at site 1 was affected by the effluent from the sewage treatment plant, while site 3 remained the DIN and DIP concentrations in natural seawater. The DIN concentration in the water column was significantly higher ($P<0.001$) at site 1 than at sites 2 and 3 (Fig. 3a). Post hoc testing showed that the order of significant differences was site 1>site 2>site 3. The average DIN concentrations at sites 1, 2, and 3 were 48.8, 11.0, and 8.3 μM, respectively. In contrast, the DIP concentration in the water column showed results similar to those of the DIN concentration (Fig. 3b). The average water column DIP concentration at site 1 was 3.2–3.9 times those at sites 2 and 3.

Algal community structure

The total number of macroalgal species observed at each site increased with increasing distance from the sewage treatment plant (Table 1). At site 1, closest to the treatment plant, algal community structure was extremely simple over the experimental period. *Ulva australis* was the dominant species at site 1 (Table 1). The relative coverage of *U. australis* was very high. Only two other species (*Undaria pinnatifida* and *Grateloupia elliptica*) were recorded at more than 5 % coverage and relative coverage. Although the total number of species at site 2 was twice that at site 1, the algal community assemblage showed similar patterns at both sites. The algal community at site 2 was dominated by *U. australis* followed by *Sargassum horneri* (Table 1). Twelve macroalgal species were observed at more than 1 % coverage. At site 3, a total of 34 macroalgal species were observed during the experimental period, including four Chlorophyta species, 10 Phaeophyta species, and 20 Rhodophyta species (Table 1). The total algal percentage cover exceeded 100 % because macroalgae with different morphological forms usually coexisted. *U. australis* and *Sargassum fusiforme* showed the highest cover. The cover of *Gelidium amansii* and *G. elliptica* was more than 10 %. The main species other than these four were *U. pinnatifida*, *S. horneri, Sargassum thunbergii, Chondrus ocellatus, Grateloupia divaricata*, and *Lomentaria catenata*.

Dominance curves and diversity indices

The shape of the *K*-dominance curve differed among communities (Fig. 4). For site 1, dominated by *U. australis*,

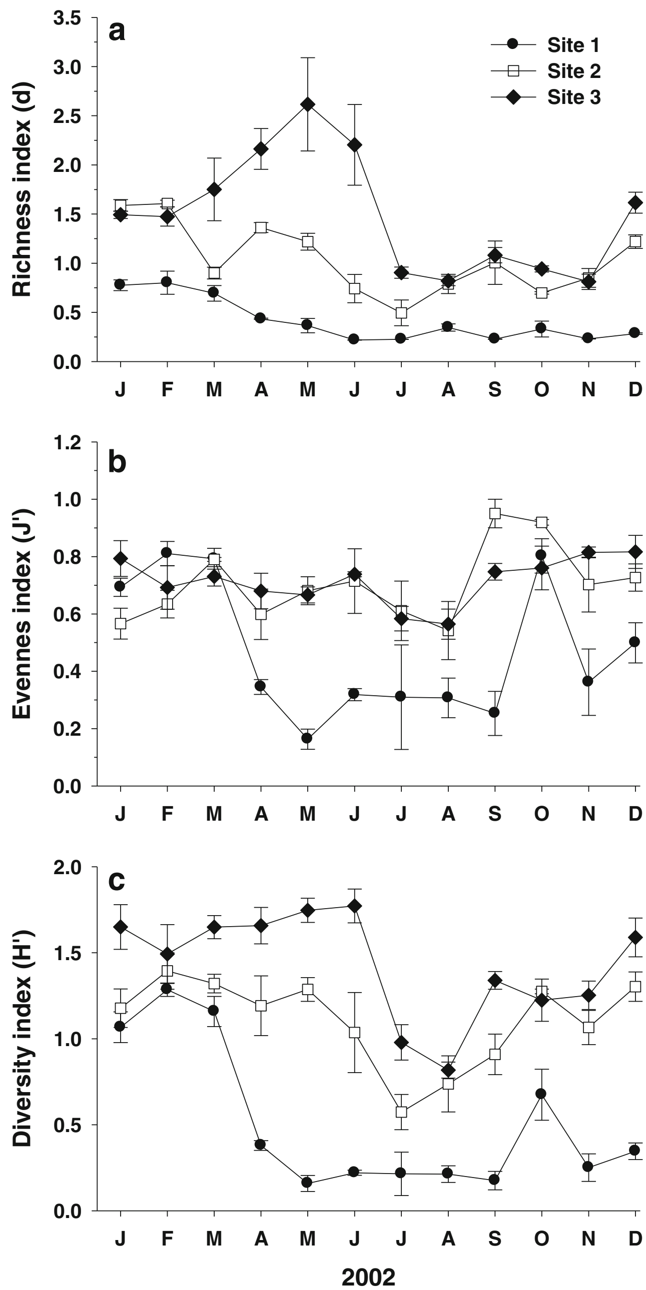

Fig. 5 Margalef's Richness Index (d; **a**), Pielou's Evenness Index (J'; **b**), and Shannon's Diversity Index (H'; **c**) of macroalgae at three sites in Tongyeong Bay on the southern coast of Korea. Values are means±SE ($n=9$)

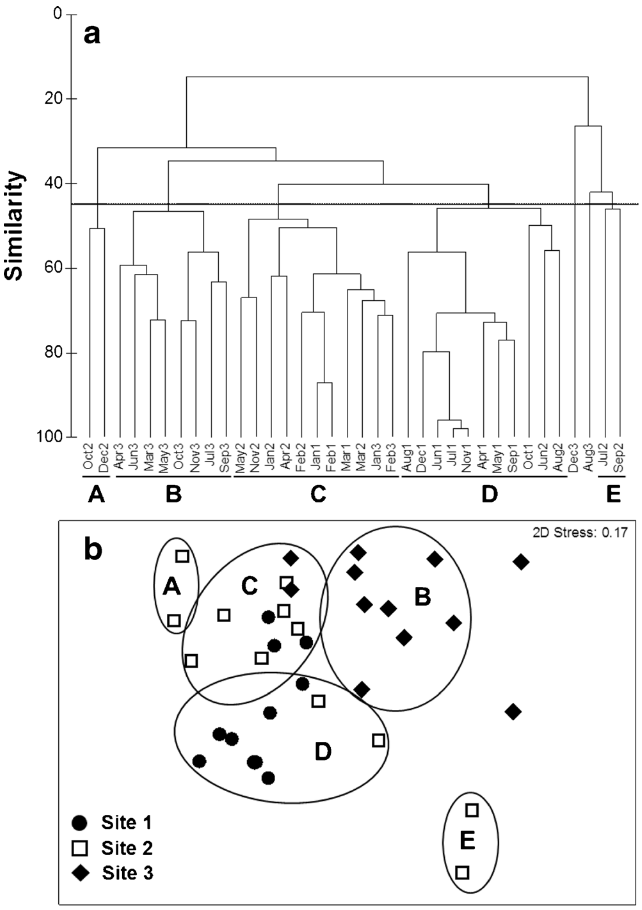

Fig. 6 Dendrogram of the hierarchical clustering of macroalgal communities (similarity threshold, 45 %) based on Bray–Curtis similarities (**a**) and a nMDS plot (**b**) based on log(x+1) transformed data. The *1*, *2*, and *3* after the date indicate sites 1, 2, and 3, respectively

the curve was gently sloped, while the curves for sites 2 and 3, dominated by several species, had diagonal and S-shaped slopes, respectively. Species richness (d) was significantly higher (P<0.001) at site 3 than at sites 1 and 2 over the experimental period (Fig. 5a). Species richness at site 3 was highest in May (2.62±0.47) and lowest during summer-fall (0.82±0.05). By contrast, species richness at site 1 was constant over the sampling time. Site 3 also showed the highest mean evenness (J'; 0.72±0.02), and the lowest mean evenness was found at site 1 (0.47±0.26), with site 2 exhibiting an intermediate value (0.70±0.03) (Fig. 5b). Site 3 had significantly higher (P<0.001) species diversity (H'; 1.43±0.06) compared with sites 1 (0.51±0.07) and 2 (1.11±0.05) (Fig. 5c).

Cluster analysis and nMDS

The results of the cluster analysis and MDS ordination of species coverage showed the existence of five groups (A to E, with a stress value of 0.17) and two isolated samples (August and December at site 3) at a similarity of 45 % (Fig. 6a, b). Group B was composed of the samples from most seasons at site 3, while group C included the samples from January and February, when algal diversity was highest, at the three sites. Group D was composed mainly of most samples from site 1, where green algae dominated during the experimental period. These results were supported by the ANOSIM test for differences between sites (global test, R=0.383, P=0.001) or among groups (global test, R=0.787, P=0.001).

Tissue N content

The tissue N content of macroalgal species varied significantly among the sites (Table 2). The tissue N content of *U. australis* at site 1 was significantly higher (SNK test, P<0.05) than those at sites 2 and 3 (Table 2). Additionally, the average tissue N content of each red algae species (*G. elliptica*, *Pyropia yezoensis*, *L. catenata*, *C. ocellatus*, and *Caulacanthus ustulatus*) was significantly higher (SNK test, P<0.05 in all cases) at site 1 than at sites 2 and 3 (Table 2). However, the average tissue N content of *S. horneri* or *U. pinnatifida* did not differ significantly (one-way ANOVA, P=0.281 and 0.135, respectively) among the sites (Table 2).

Discussion

In the present study, the observed differences in water column nutrient concentrations among sites were related with variations in macroalgal benthic community structure and in macroalgal species diversity on the intertidal rocky shore. To

Table 2 Mean tissue nitrogen content of dominant species at the three study sites

Species	Site 1	Site 2	Site 3
Chlorophyta			
Ulva australis	4.34±0.30 [a]	3.30±0.29 [b]	2.80±0.18 [b]
Phaeophyceae			
Sargassum horneri	3.17±0.71	2.95±0.26	2.29±0.05
Undaria pinnatifida	3.88±0.28	3.83±0.36	2.89±0.43
Sargassum fusiforme	-	2.30±0.54	1.67±0.01
Sargassum thunbergii	-	3.38±0.55	2.56±0.23
Rhodophyta			
Grateloupia elliptica	3.48±0.22 [a]	2.79±0.22 [b]	2.19±0.23 [b]
Pyropia yezoensis	5.18±0.19 [a]	4.01±0.06 [b]	3.78±0.21 [b]
Lomentaria catenata	3.77±0.29 [a]	2.87±0.21 [b]	2.71±0.33 [b]
Chondrus ocellatus	4.06±0.34 [a]	2.53±0.36 [b]	2.56±0.28 [b]
Caulacanthus ustulatus	5.91±0.22 [a]	3.41±0.08 [b]	2.50±0.05 [c]

Values with the same letter are not significantly different among sites (P<0.05) our knowledge, this is the first study investigating the responses of the macroalgal benthic community to effluents from a sewage treatment plant on the intertidal rocky shore in Korea. The *K*-dominance curves and diversity index (d, J', and H') revealed that site 1 was less diverse compared with the other two sites. This can be explained by the blooming of green algae (dominance of one or two species), especially *U. australis*, at site 1. Green algal blooms inhibit the settlement of other algae and consequently lead to declines in species number and diversity (Park et al. 2011).

A few studies have reported that inorganic nutrients enrichment by wastewater disposal significantly increased macroalgal density and species richness or did not affect total cover and species richness of macroalgae (Terlizzi et al. 2002; Reopanichkul et al. 2009). However, most studies were similar to these results which showed the change of benthic community structure such as the decline of species diversity and increase of opportunistic and pollution tolerant species by the sewage discharge (Littler and Murray 1975; Rodríguez-Prieto and Polo 1996; Díez et al. 2003; Liu et al. 2007). Arévalo et al. (2007) reported that due to the blooming of *U. rigida*, the species richness and diversity of the macroalgal community near a sewage outfall were decreased by more than 70 % compared with those at a control site. High nutrient loading allowed an increase of opportunistic algae such as *Ulva*, *Cladophora*, and *Chaetomorpha*, which resulted in the decline of species richness and algal coverage (Middelboe and Sand-Jensen 2000). These results strongly support our first hypothesis, that the macroalgal community close to a sewage treatment plant would be simplified by decreased species diversity and increased opportunistic and pollution tolerant species.

A further conspicuous sign of alterations in the macroalgal benthic community under high N environmental conditions was the decline in large brown or perennial algae (Littler and Murray 1975; Munda 1993; Díez et al. 2003; Liu et al. 2007; Connell et al. 2008). Doblin and Clayton (1995) demonstrated declines in zygote germination, delays in embryo growth, and increases in embryo mortality in brown algae owing to sewage effluents. When exposed to urban water for 26 days, the photosynthetic efficiency of brown algae decreased by more than 40 % compared with the initial level (Scherner et al. 2012). Discharge from a sewage plant facilitated the replacement of large brown or perennial algae by nutrient-stress-tolerant species such as opportunistic or turf-forming species because of differences in their nutrient uptake rates (Middelboe and Sand-Jensen 2000; Connell et al. 2008).

In the present study, two large brown algae (*U. pinnatifida* and *S. horneri*) were recorded at site 1 at less than 10 % cover, and only two perennial species, *S. horneri* and *G. elliptica*, exhibited more than 1 % cover. However, more than five large brown algae were observed at both sites 2 and 3 throughout the experimental period. At site 3, *U. pinnatifida* and three *Sargassum* species (*S. fusiforme*, *S. horneri*, and *S. thunbergii*) were recorded at over 5 % monthly cover, and their total cover was more than 40 %. The number of perennial species at sites 2 and 3 was four times that at site 1. These results indicate that large brown or perennial algae are seriously negatively affected by the effluent from a sewage plant, leading to declines in both brown and perennial algae and in species diversity. Thus, understanding the response of the macroalgal benthic community to discharge from a sewage treatment plant is essential for establishing the management policy of marine ecosystem in vicinity of the plant.

Macroalgae tissue N content is a potential indicator for estimating N availability in the water column (Wheeler and Björnsäter 1992; Cohen and Fong 2006), as macroalgae have the ability to store large reserves of "luxury" N (Horrocks et al. 1995). The tissue N content of macroalgae was higher at a site in the proximity of the discharge from a shrimp farm and decreased down the creek away from the discharge site (Lin and Fong 2008). Jones et al. (2001) demonstrated that the tissue N content of marine plants such as seagrass and macroalgae can be used as a biological indicator to assess the ecological impacts of discharges from shrimp farms and sewage treatment plants. In the present study, the tissue N content of macroalgae varied significantly according to distance from a sewage treatment plant. Among green and red algae, tissue N contents were highest at site 1 and lowest at site 3. Algae from site 2 showed intermediate tissue N content, although the difference in tissue N content between samples from sites 2 and 3 was not significant. This would indicate that the tissue N content of green and red algae reflects nutrient availability in the water column and can be a useful tool for monitoring water quality parameters.

However, the use of tissue N content as a bioindicator for detecting nutrient enrichment was dependent on the algal group. In the present study, the tissue N content of the brown algae, *S. horneri* and *U. pinnatifida*, did not differ significantly among sites. In cage experiments, nutrient addition did not appear to increase significantly the biomass or tissue N content of brown algae (Thomsen and McGlathery 2007). Pfister and Van Alstyne (2003) showed that the growth and tissue N content of the kelp species *Hedophyllum sessile* in the intertidal zone was positively affected by nutrient enrichment. The absence of a nutrient effect on the kelp species may be attributable to their high nutrient storage capacity (Fujita et al. 1989). The tissue N content of macroalgae with higher nutrient storage did not closely reflect ambient nutrient conditions because of the confounding effects of previous supplies of N (Germann et al. 1987). This implies that brown algae species with high nutrient storage capacities are not appropriate as tools for monitoring nutrient over-enrichment in the water column. Thus, we suggest that the application of physiological characteristics of macroalgae as bioindicators for evaluating sewage effluent should be undertaken with caution and with consideration of algal nutrient storage capacities.

In conclusion, the species composition and biodiversity indices of macroalgal communities strongly reflected environmental N exposure. Discharge from a sewage treatment plant facilitated the dominance of green algae and the decline of large brown or perennial algae, consequently leading to decreased biodiversity. The response of macroalgal tissue N content to nutrient enrichment was dependent on the algal group. The tissue N contents of green and red algae were positively related to the DIN concentration, whereas brown algae, due to their high N storage capacity, did not respond to the DIN concentration. This suggests that the N storage capacities of macroalgae are an important factor to consider when using tissue N content as a water-monitoring tool.

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