Succession in an intertidal benthic community affected by untreated sewage effluent: A case of study in the SW Atlantic shore

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ABSTRACT

A study of benthic succession related with sewage pollution was conducted in a warm-temperate coastal area of Mar del Plata city, Argentina. The effect of nutrient enrichment and starting period of the succession was tested after cleared space in the intertidal rocky shore benthic community. The time of recovery after a disturbance in enriched sites was considerably lower (20–29 weeks) than in non-enriched sites based on the diatom *Berkeleya* sp. and the polychaete *Boccardia proboscidea* developed in the early succession stage, and *Brachidontes rodriguezii* or *B. proboscidea* occurring with *Polysiphonia* sp., *Petalonia fascia* and *Ulva* spp. developed in the late succession stage of the enriched sites. The very low time at which the community recovered in the enriched environments turned out to be one of the assets of the present study and this parameter can be used as a quick indicator of sewage pollution in the area.

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1. Introduction

The nutrient enrichment of coastal marine environments is a phenomenon of global concern. One prominent mode in which the human populations increase the mobilization of nutrient elements to coastal areas is through the discharge of sewage outfall (Nixon, 1995; Cloern, 2001). Thus, contamination with sewage outfall is one of the main anthropogenic stressors in intertidal rocky shore benthic communities, and it had been intensely studied in temperate coastal waters (López Gappa et al., 1990, 1993; Soltan et al., 2001; Díaz et al., 2002; Arévalo et al., 2007; Díez et al., 1999; Elías et al., 2006; Muniz et al., 2011). The concentration of dissolved nitrogen and phosphorus in the water column regulate the growth rates of epilithic algal communities (Kraufvelin, 2007; Littler et al., 2010), promoting the development of early successional stages macrophytes in the surrounding communities (Littler and Murray, 1975; Soltan et al., 2001; Bokn et al., 2003; O'Shanahan Roca et al., 2003; Martinetto et al., 2010) owed to their high nutrients requirements (Karez et al., 2004). The most extreme environmental response to a nutrient enrichment is the intensive algal blooms registered in lagoons, bays or even open coastal systems (Menesguen, 1992; Sfriso et al., 1993; Valiela et al., 1997; Teichberg et al., 2010). However, other response to nutrient enrichment could be, for example, a less dramatic change of the abundance and the species composition of the algal community (Orfanidis et al., 2001; Ballesteros et al., 2007; Arévalo et al., 2007; Pinedo et al., 2007; Dongyan et al., 2007; Pinedo et al., 2013). Moreover, the organic enrichment produced by sewage outfall may modify the faunal composition in near-shore benthic environments, predicting a peak in opportunists' organisms, mainly certain polychaetes species (Pearson and Rosenberg, 1978; Borja et al., 2006; Jaubet et al., 2011; Elías et al., 2015).

Studies of disturbed communities with nutrient enrichment have generally focused on the ecological analysis of communities, field descriptions of populations, or laboratory analyses of physiological responses of individual organisms (López Gappa et al., 1990, 1993; Díaz et al., 2002; Karez et al., 2004; Elías et al., 2006; Ballesteros et al., 2007; Arévalo et al., 2007; Pinedo et al., 2007; Dongyan et al., 2007; Torres and Caille, 2009; Littler et al., 2010). However, studies of benthic succession related to nutrient enrichment have not been developed in extension (Murray and Littler, 1978), especially in South America (Fricke et al., 2015). Mar del Plata, the largest coastal city of Argentina, holds a pre-treatment sewage plant which had been discharging directly in the intertidal zone since 1989 until December of 2014, when the City Council inaugurated a submerged sewage outfall which extended the point of discharge a distance of 4 km from the coastline along the seafloor. Previous to this modification, the area showed an increasing nutrient enrichment condition, turning into a sewage-polluted habitat (Vallarino et al., 2002; Elías et al., 2006; Elías et al., 2009; Jaubet et al., 2011; Sánchez et al., 2013). The benthic community near the sewage discharge area has being studied for over the past 15 years, chiefly assessing the effect of organic enrichment over the epilithic mussels bed-forming *Brachidontes rodriguezii* (family Mytilidae) (Vallarino et al.,

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2002; Elías et al., 2006; Sánchez et al., 2013) and the invasive polychaete *Boccardia proboscidea* (family Spionidae) (Jaubet et al., 2011; Garaffo et al., 2012; Jaubet et al., 2013; Elías et al., 2015). The macrobenthos community affected by the sewage outfall shows populations of *B. rodriguezii* in a lower density and with bigger individuals than those inhabiting non-impacted areas (Vallarino et al., 2014), and with eventually high density of the tube-building reef *Boccardia proboscidea* (Jaubet et al., 2011, 2013). Regarding to the algal assemblage, a recent study assessed the variability of the species coverage at different distances of the outfall, being the tube dwelling diatom *Berkeleya* sp. more abundant near the sewage outfall whereas *Ulva* spp. was distributed with similar abundances in both impacted and non-impacted areas (Becherucci et al., 2016).

The area of the present study also undergoes natural disturbance events, because severe wind storms (from the S-SE sector) (Manolidis and Alvarez, 1994) produce patches of opening space in littoral areas which may suffer different patterns of species recovery. Given that the benthic community of the study area responds to sewage outfall discharge varying its functioning and structure (Garaffo et al., 2012; Jaubet et al., 2013) the present study was designed to determinate: 1) if the macrobenthos characteristics of impacted areas with nutrient enrichment show lower time of recovery during succession, and 2) whether a primary effect of environmental enrichment and starting period of the succession alters the diversity and composition of macrobenthos communities.

2. Methods

2.1. Study area

The study area was located on the north coast of Mar del Plata city, Buenos Aires, Argentina (38° S, 57° 33′ W) (Fig. 1). Along the north city shoreline sandy open beaches alternate with extended abrasion platforms composed of compact sedimentary rock, sometimes cemented by crystalline calcium carbonate (Amor et al., 1991). The area is affected by a littoral current (flowing predominantly from South to North). The tidal regime is semidiurnal with tidal amplitude ranging from approximately 0.8 m to 1.6 m during exceptional tides. Sea surface temperature shows a great seasonal variation (9.3 °C in winter and 20 °C in summer) (Guerrero and Piola, 1997), while sea pH remains between 7 and 8.5 (Isla et al., 1998). Sánchez et al. (2013) observed that both sediment organic matter and water turbidity in sewage impacted areas of Mar del Plata were 1% and 50% higher than in non-impacted areas respectively.

2.2. Experimental design

Four sampling sites distributed along the coastline at increasing distances from the local sewage outfall were selected. One sampling site was located at 800 m (hereinafter site IS) south of the point of discharge, whereas the other sampling sites were located at 3.7 km (IN), 8 km (R1) and 9 km (R2) to the north of the outfall (Fig. 1). According to previous studies sites IS and IN were considered impacted sites whereas sites R1 and R2 were treated as reference non-impacted sites (Elías et al., 2006; Sánchez et al., 2013; Jaubet et al., 2015; Becherucci et al., 2016). In each sampling site, eight experimental plots (square areas of 0.40 m^2) were randomly distributed in independent rocks on the mid height level of the eulittoral shore (Raffaelli and Hawkins, 1999). At the beginning of the experiment, each experimental plot was scraped aimed by a spatula, thus removing all organisms in the plots and then washed with a solution of sodium hypochlorite. In order to avoid an "edge effect", the plots were scraped leaving a thick 10-cm perimeter which was maintained in
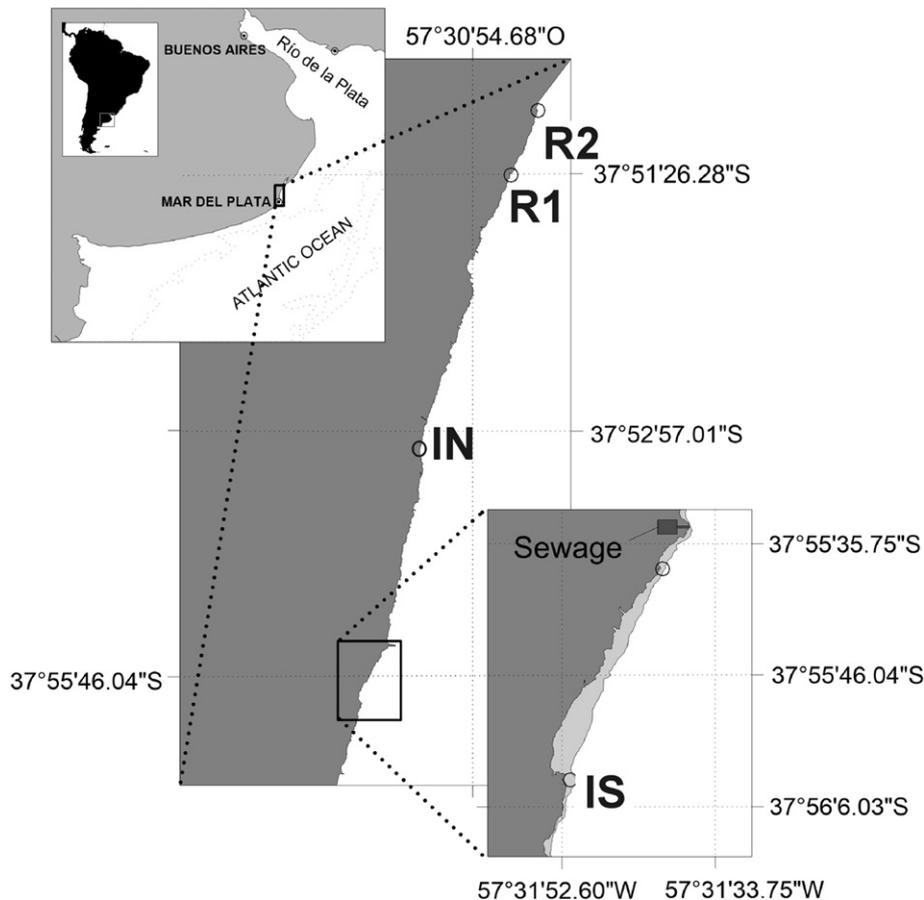

Fig. 1. Distribution of sampling sites (IS, IN, R1 and R2) and intertidal sewage outfall location in the study area. Site IS was located at 800 m to the south of the outfall, site IN at 3700 m north, R1 at 8000 m north and R2 9000 m to the north of the outfall.

every monitoring visit. Corresponding unaltered control plots were located neighboring each experimental plot (hence a total of 42 control plots were used). The later consisted of a square area of the same dimension. The experiments were started in two different seasons: spring (November 2013) and autumn (May 2014). The experiments were conducted until there were no significant differences in the taxa composition (according to taxa percentage coverage) among the assemblages of the experimental and control plots in at least one sampling site (regardless of the season).

2.3. Data collection and analysis

The sites were visited every two weeks approximately (successional times) during low tides. Photographs of all plots (experimental and control) were taken during each monitoring visit. Considering the total area of the plot as the 100%, the coverage (%) of individual benthic taxa was determinate using a software image package (Klonk light v. 13.1.3.8) and at least two layers of organisms were recorded in some plots. When layering occurred, the underlying taxon coverage was calculated by maintaining the relative coverage that it had in the non-layering sector. Total coverage, computed as the sum of the covers of all taxa, could thus exceed 100% (Foster et al., 1991).

The resemblance among both experimental and control assemblages in each sampling site of each starting period were tested using a PERMANOVA analysis (permutational multivariate analysis of variance). Taxon richness (S), Shannon-Wiener diversity (H′) (Shannon and Wiener, 1963) and evenness (J′) (Pielou, 1969) indexes were calculated for each sampling unit in all successional times. The effect of sampling site and starting period of the sampling over the S, H′ and J′ indexes of the communities during the succession process was analyzed by using a repeated measures ANOVA, being "successional time" the repeated measurement. The assumption of Mauchley Sphericity was previously tested and according to a p value > 0.01 or < 0.01, a univariate or Wilks multivariate test was performed respectively. The relation of taxa composition according to successional times and sampling sites were analyzed using a correspondence analysis (Kenkel et al., 2002). In order to achieve a better view of the correspondence plots, the median of the taxa data for each sampling site in each successional time were calculated. The effect of sampling site and starting period over composition of the late stage community (week 20 to spring and week 29 to autumn) was analyzed by using a PERMANOVA analysis. The taxa contribution to the assemblage differentiation was measured by applying a SIMPER analysis (similarity percentage analysis). The multivariate analyses were performed on a Bray-Curtis similarity matrix with a fourth-root transformation.

3. Results

A total of 16 taxa were recorded during the succession experiments (Table 1A and B), being *Ulva* spp., *Ralfsia* sp. and *Berkeleya* sp. the most common taxa amongst the algae and *B. proboscidea* and *B. rodriguezii* between the zoobenthos species. A biofilm, mainly represented by several species of cyanophyta, diatoms and green spores, covered the bare substratum in the early succession. The taxa composition of this microphytobenthos was not measured in this study given that the experimental design was not suitable for this fraction. Thus, the cover of those taxa was recorded as biofilm. The tube-dwelling diatom *Berkeleya* sp. was the only species considered in the data analysis as it was present with very high abundance near the sewage outfall, forming evident macroscopic mats attaining several centimeter in length. The spionidae *B. proboscidea* was found in two forms: endolithic form, when it inhabits inside the rock and epilithic form, when the species *B. proboscidea* built soft sand tubes

Table 1
Patterns of development of macrobenthos colonizing experimental plots in each sampling sites (IS, IN, R1 and R2) during weeks (W) of succession. Values represent mean cover (%) for 8 replicate of 0.40 m² plots cleared in spring (A) and autumn (B).

A

Taxa	Site	W02	W07	W12	W20
Biofilm	IS	21.15	8.24		
	IN	1.66			
	R1	2.33			
	R2	4.37	12.86	–	–
Ulva spp.	IS		21.00	29.69	19.10
	IN	0.33	4.87	12.89	25.39
	R1	0.03	11.36	22.37	28.74
	R2	2.30	27.53	8.45	11.37
Ulva lactuca	IS				
	IN				
	R1				
	R2	–	–	13.20	–
Bryopsis plumosa	IS		6.63		
	IN	1.38			
	R1		0.48		
	R2	–	–	–	–
Porphyra sp.	IS		0.08		
	IN				
	R1				
	R2	–	–	0.08	–
Polysiphonia sp.	IS		2.34	0.19	0.35
	IN		3.48	1.94	4.29
	R1		2.01		
	R2	–	–	–	–
Ceramium uruguayense	IS				
	IN				
	R1				
	R2	–	8.32	–	–
Gelidium crinale	IS				
	IN				
	R1				
	R2		0.75	0.02	0.27
Ralfsia sp.	IS			0.39	
	IN	3.67	0.51	2.78	0.50
	R1	6.93	1.61	8.11	2.34
	R2	–	–	0.08	–
Berkeleya sp.	IS		10.49		
	IN	0.66	15.73		
	R1		15.00		
	R2	–	9.48	–	0.37
Brachidontes rodriguezii	IS	0.74	3.35	18.92	73.39
	IN	0.24	3.90	5.10	44.89
	R1	0.29	2.63	0.88	2.41
	R2	0.87	2.86	6.51	29.96
Boccardia proboscidea	IS	6.22	64.17	76.64	22.76
	IN	39.47	69.16	62.02	21.75
	R1	2.88	51.96	15.21	5.71
	R2	13.97	14.00	1.99	2.18
Balanus glandula	IS				
	IN	0.03	0.03		
	R1	0.13	0.04		0.01
	R2	0.02	–	–	0.01
Siphonaria lessoni	IS				
	IN	0.01	0.41	0.69	1.46
	R1	0.03	0.15	0.15	0.79
	R2	0.07	1.06	2.15	5.82
Macrobenthos total cover	IS	6.96	97.48	125.83	115.60
	IN	45.12	82.45	85.42	98.27
	R1	10.29	70.24	46.72	40.00
	R2	17.23	54.52	32.48	49.61

B

Taxa	Site	W02	W07	W12	W20	W29
Biofilm	IS	68.79	9.13	12.31		
	IN	28.53	58.53	63.03	31.61	
	R1	14.57	39.22	81.96	13.58	
	R2	60.58	92.05	89.40	70.46	–
Cladophora sp.	IS					
	IN			0.39	2.06	0.90

Table 1 (continued)

B

Taxa	Site	W02	W07	W12	W20	W29
	R1			0.39	1.97	1.53
	R2	–	–	–	0.43	0.03
Ulva spp.	IS		36.63	43.88	22.67	22.54
	IN		0.55	0.69	3.22	
	R1	3.40	2.73	2.88	5.06	4.93
	R2	0.01	–	–	–	–
Ulva lactuca	IS					
	IN			1.89	19.75	26.33
	R1					0.88
	R2	–	0.83	5.93	54.69	30.44
Bryopsis plumosa	IS		0.87	0.12	0.09	0.88
	IN					
	R1		0.02			
	R2	–	–			
Porphyra sp.	IS			0.13	0.02	
	IN					
	R1				0.07	4.52
	R2	–	–	0.58	2.15	1.22
Polysiphonia sp.	IS		0.81	1.91	1.32	0.25
	IN		0.03			
	R1		1.25	0.62	1.27	9.86
	R2	–	0.00	–	–	–
Ceramium uruguayense	IS					
	IN				0.10	
	R1					0.30
	R2	–	–	–	–	–
Gelidium crinale	IS					
	IN		0.16	0.52	0.53	2.11
	R1	0.02	1.23	0.76	0.80	0.71
	R2		1.88	1.90	0.25	
Ralfsia sp.	IS	0.95	1.99	2.07	3.01	2.37
	IN	0.28	1.05	0.70	0.92	1.07
	R1	0.21	0.13	0.35		
	R2		0.33	0.19	1.96	0.37
Petalonia fascia	IS		1.23	2.18		
	IN					
	R1		25.24		3.09	1.08
	R2	–	–	–	–	–
Berkeleya sp.	IS		22.69	4.20	10.84	7.84
	IN					
Brachidontes rodriguezii	IS	0.54	0.48	0.36	2.26	4.13
	IN	0.78	1.15	1.38	2.29	3.88
	R1	0.20	1.05	0.73	1.28	3.15
	R2	1.42	1.30	0.58	5.83	8.34
Boccardia proboscidea	IS	36.01	48.80	43.92	94.85	33.84
	IN	13.71	35.75	28.46	59.38	14.07
	R1	18.41	52.35	10.69	78.35	30.08
	R2	13.02	1.10	0.27	10.24	0.11
Balanus glandula	IS					0.03
	IN					0.01
	R1				0.26	0.72
	R2	0.10	0.03	0.02	4.01	3.62
Siphonaria lessoni	IS	0.06	0.49	0.34	1.37	0.62
	IN	1.06	1.85	1.56	3.59	2.28
	R1	0.46	1.89	1.65	2.68	0.77
	R2	0.40	3.14	2.26	6.83	0.35
Macrobenthos total cover	IS	36.61	91.18	94.75	122.83	62.31
	IN	16.50	41.48	36.97	93.94	51.94
	R1	22.95	60.46	18.04	91.85	57.79
	R2	14.97	7.95	10.60	86.94	45.18

over the rock (Jaubet et al., 2013). During this study, the two forms of *B. proboscidea* were recorded.

3.1. Successional time

The experimental plots cleared in spring were monitored in 4 successional times: weeks 02, 07, 12 and 20. The experimental plots cleared in autumn were monitored at weeks 02, 07, 12, 20 and 29 from the beginning of the experiment. The benthic community developed in experimental plots within impacted sites (IS and IN) and starting in spring was similar to that obtained for control plots at week 20 (Pseudo F = 0.43, $p = 0.662$ and Pseudo F = 0.24, $p = 0.841$; IS and IN respectively). At that time only c. 40% of the substratum in non-impacted sites (R1 and R2) was covered and the community developed was different to that of control plots (Pseudo F = 5.50, $p = 0.022$ and Pseudo F = 3.82, $p = 0.031$; R1 and R2 respectively). In plots started in autumn, the assemblages of experimental plots resembled to that of control plots at week 29 in the impacted site IS (Pseudo F = 1.61, $p = 0.199$) while it varied in sites IN (Pseudo F = 5.43, $p = 0.007$), R1 (Pseudo F = 7.56, $p = 0.011$) and R2 (Pseudo F = 11.66, $p = 0.008$), covering more than 50% of the plots.

3.2. Benthic assemblages

The first two axes of the correspondence analysis between taxa and the experimental plots started in spring represented 62.14% of the total variance. Overall, in the ordination plot, a spatial gradient was observed in the horizontal axis and a temporal gradient in the vertical axis. The impacted sites (IS and IN) were grouped separately from non-impacted sites (R1 and R2) in the horizontal axis. However, some exceptions of this general pattern were observed. One IS and IN sites corresponded to week 02 were placed within the group of non-impacted sites, and one R1 site that corresponded to week 07 was placed within the group of impacted sites. In the vertical axis, the successional times were placed from weeks 02 and 07 to weeks 12 and 20 (from down to top). According to the gradients, biofilm, *Ralfsia* sp., *Ceramium uruguayense*, *Balanus glandula* and *Porphyra* sp. were associated with the early successional times (02 and 07 weeks) in non-impacted sites and with week 02 in IS and IN sites, while *Berkeleya* sp., *Bryopsis plumosa*, *Polysiphonia* sp. and *B. proboscidea* (both endolithic and epilithic forms) were associated with the early successional times (07 week) in impacted sites. *Ulva lactuca*, *Gelidium crinale*, *Siphonaria lessoni* and bare substratum were associated with the late successional times in non-impacted sites, while *B. rodriguezii* and *Ulva* spp. was associated with late successional times in impacted sites (Fig. 2A).

The first two axes of the correspondence analysis between taxa and the experimental plots started in autumn represented 63.59% of the total variance. The ordination plot reflected a spatial gradient in axis one where IS site form a solo group separated from IN, R1 and R2 sites, being one IS site corresponding to week 02 an exception grouped with the latter group. In axis 2 a temporal variation was reflected, however, a mix of successional times was observed. A quite similar association between taxa and sites during the spring period was observed; however, there was a clear segregation between the two forms of *B. proboscidea*, being the epilithic form associated with the impacted site (IS) and the endolithic form with other sites. Moreover, *B. rodriguezii* appeared associated with non-impacted sites and *Petalonia fascia* (seasonal species) was associated with an impacted IS site (Fig. 2B).

The sampling sites (Pseudo F = 10.23, $p = 0.001$) and starting period of the experiments (Pseudo F = 27.85, $p = 0.001$) as well as their interaction (Pseudo F = 5.89, $p = 0.001$) had an effect over the assemblage composition in the late succession. When plots were cleared in spring, site R2 differed from the rest of the sites where *B. rodriguezii* and *S. lessoni* were more abundant; site R1 differed from site IS but resembled to site IN, mainly due to the abundance of *Ulva* spp. and *Ralfsia* sp.; and site IS differed from sites R1 and R2 but resembled to site IN due to the high abundance of *B. rodriguezii*, *Ulva* spp. and *B. proboscidea* (epilithic) (Table 2). When plots were cleared in autumn, site R2 and site IS differed between them and with the rest of the sites mainly due to the high abundance of *B. glandula*, *Ralfsia* sp., *U. lactuca* and biofilm in site R2 and the high abundance of *B. proboscidea*, *Ulva* spp. and *Berkeleya* sp. in site IS. Sites R1 and IN resembled between
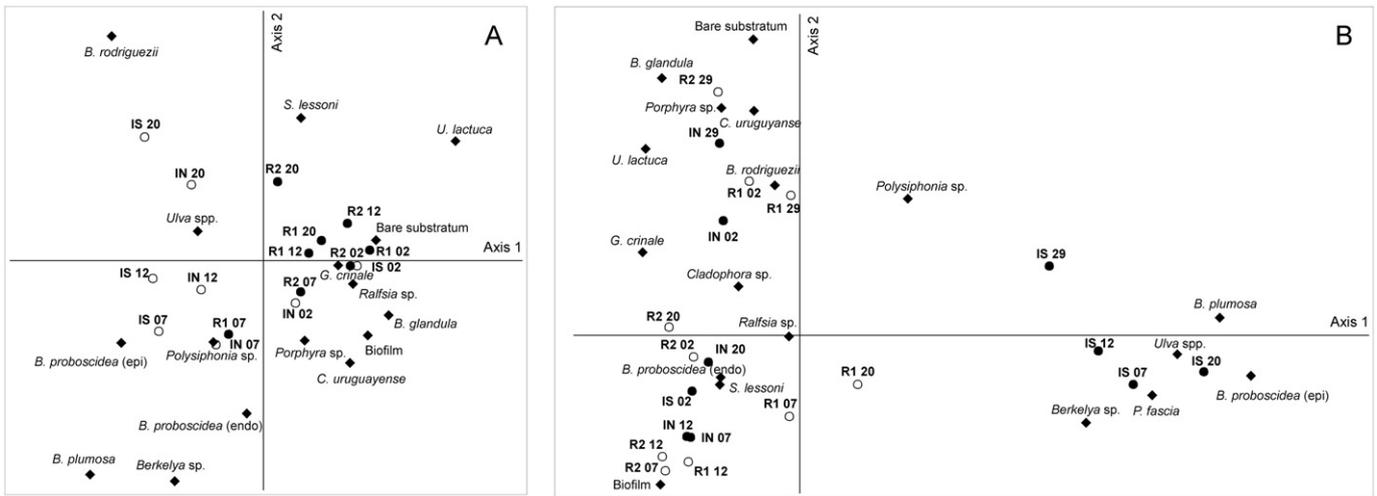
Fig. 2. Ordination plots of correspondence analysis between taxa and sampling sites (IS, IN, R1 and R2) in each week (02, 07, 12, 20 and 29) of the succession started in spring (A) and autumn (B). White circle represent impacted sites (IS and IN) and black circle references sites (R1 and R2).

them and differed from the rest, being *B. proboscidea* (endolithic) more abundant in those sites (Table 2).

3.3. Community parameters

The taxa richness (S), Shannon-Wiener diversity (H′) and evenness (J′) indexes increased after the second week and then all parameters remained relatively constant in the later weeks. The interaction of the successional time and sampling sites had a significant effects over S (F = 2.13, p = 0.031) and H′ (F = 2.29, p = 0.020), being in week 02 of site IS lower than the rest of the sites (Fig. 3A and B). The interaction of the successional time and starting period had a significant effects over S (F = 13.58, p ≪ 0.001) and H′ (F = 5.80, p ≪ 0.001). A decreased of species richness (S) was observed in weeks 12 and 20 in plots cleared in spring (Fig. 3C). The diversity index (H′) increased in week 20 in plots cleared in autumn (Fig. 3D). Overall, the J′ was higher during the succession in plots cleared in autumn than in spring (F = 4.48, p = 0.005).

4. Discussion

4.1. Successional time

The time of succession of the overall community was markedly lower in impacted sites than in non-impacted sites. This is line with a previous study conducted in other impacted area. Murray and Littler (1978), near Wilson Cove (San Clemente Island, California), found short recovery times of algal population in denuded plots in sewage-polluted habitat (1 month) compared to unpolluted habitat (30 months). Those experiments suggested that algal population development in sewage-polluted habitat encompass opportunistic species which show rapid recruitment and growth parameters, and thus represent resilient subclimax associations. Our findings agreed with this hypothesis, showing that the time of species recovery decrease significantly near to the point of the nutrient discharge. Regarding the plots commenced in autumn, only the site IS completed the succession at 29 weeks, while the assemblage composition of the experimental plots of the site IN at that time was still different to the control plots. This pattern may be the result of the impact generated by the sewage outfall, which is known to vary spatially according to season. As suggested by Elías et al. (2009) during the summer months the prevailing winds from the northern sector disperse the sewage flume to the southern shore of the sewage outfall, resulting in a northern area holding less organic enrichment. Thus, the site IN, located to the north of the sewage

outfall, was probably less influenced by the flume nearby the week 29 coinciding which the proximity of the summer months. Nevertheless, the time of recovery of the communities in both experiments (starting in spring and autumn) in the site nearest to the sewage outfall was significantly lower when compared to reference sites denoting a clear relation with the sewage discharge. In natural conditions, the succession of the community may take up to three years until it returns to its initial condition in intertidal rocky shores of Mar del Plata dominated by *B. rodriguezii* (Penchazsadeh, 1973). The very low time at which the community recovered in the enriched environments turned out to be one of the assets of the present study and this parameter can be used as a quick indicator of sewage pollution in the area.

4.2. Benthic assemblages

Both biofilm and *Ralfsia* sp. were the more relevant taxa associated with the early successional (02 and 07 weeks) times in non-impacted sites and with week 02 in IS and IN sites, while *Berkeleya* sp. and *B. proboscidea* (both endolithic and epilithic forms) were associated with the week 07 in impacted sites. The biofilm represent the first stage of the succession in rocky marine habitats (Begon et al., 1994). Although the biofilm was composed by several unidentified taxa including diatoms, the tube-dwelling diatom *Berkeleya* sp. was evidently visible in the biofilm developed in the impacted sites chiefly in spring. Moreover, as the coverage of *Berkeleya* sp. became apparent from the biofilm group in early succession (week 07), the ordination of sites IS and IN shifts towards the impacted group. These tube dwelling diatoms were previously proposed as an indicator of sewage pollution in the study area (Becherucci et al., 2016). Other *Berkeleya* diatom, were registered in association with artificial nitrogen (N) and phosphorus (P) enrichment in the North Sea (Hillebrand et al., 2000). A recent experimental study conducted in San Antonio coastal Bay (Rio Negro, Argentina) concluded that the nutrient enrichment linked with human activities in an early succession stages (with occurrence of tube-dwelling diatoms) modify the succession trajectory and thus the final benthic community assemblage (Fricke et al., 2015). In our study, the lack of the assemblage composition data of the biofilm prevented relating enrichment impact with the first steps of succession, and that disserves to be analyzed.

The presence of *B. proboscidea* was expected in the early succession due to its opportunistic behavior, however an epilithic development of *B. proboscidea* in impacted sites was observed. The epilithic form of the species was previously identified as an enriched pollution indicator (Sánchez et al., 2013) and in the area has an explosive development (reaching 1.6 million individual per square meter) forming large reefs

Table 2
Pair-wise test and SIMPER result showing the taxa contribution to sampling sites dissimilarities according starting period (spring and autumn). Cumulative percentages that exceed 70% are not show.

Pair-wise test			SIMPER				
	t	p	Taxon	Av. abundance	Av. abundance	Contribution (%)	Cum (%)
Spring							
C2 ≠ C1	2.3045	0.01		C2	C1		
			B. rodriguezii	2.13	0.9	21.46	21.46
			Ulva spp.	1.31	2.25	18.17	39.63
			S. lessoni	1.21	0.47	16.51	56.14
			Ralfsia sp.	0	0.74	12.18	68.32
C2 ≠ IN	1.8288	0.036		C2	IN		
			B. proboscidea (epi)	0.22	1.43	21.56	21.56
			B. rodriguezii	2.13	1.96	18.22	39.77
			Ulva spp.	1.31	2.11	16.69	56.46
C2 ≠ IS	2.3295	0.01		C2	IS		
			B. proboscidea (epi)	0.22	1.54	23.08	23.08
			S. lessoni	1.21	0	21.17	44.25
			Ulva spp.	1.31	1.73	16.8	61.05
C1 = IN	1.4255	0.117					
C ≠ IS	2.914	0.001		C1	IS		
			B. rodriguezii	0.9	2.88	32.03	32.03
			B. proboscidea (epi)	0.54	1.54	20.5	52.53
			Ulva spp.	2.25	1.73	13.52	66.05
IN = IS	1.2933	0.219					
Autumn							
C2 ≠ C1	2.9473	0.002		C2	C1		
			Ulva lactuca	2.61	0	19.75	19.75
			B. proboscidea (endo)	0.78	2.19	13.79	33.53
			Biofilm	2.88	1.23	12.93	46.46
			Ulva spp.	0	1.14	8.65	55.11
			B. glandula	1.08	0.3	7.04	62.15
			B. proboscidea (epi)	0	0.77	5.81	67.95
C2 ≠ IN	2.2621	0.012		C2	IN		
			B. proboscidea (endo)	0.78	2.33	19.19	19.19
			Biofilm	2.88	1.36	16.94	36.13
			Ulva lactuca	2.61	1.51	12.63	48.76
			B. glandula	1.08	0.06	10.67	59.43
			Ralfsia sp.	1.14	0.69	8.34	67.77
C2 ≠ IS	8.1469	0.002		C2	IS		
			B. proboscidea (epi)	0	3.11	17.41	17.41
			Biofilm	2.88	0	16.14	33.55
			Ulva lactuca	2.61	0	14.53	48.08
			Ulva spp.	0	2.04	11.36	59.43
			Berkeleya sp.	0	1.32	7.08	66.51
C1 ≠ IS	3.1755	0.004		C1	IS		
			B. proboscidea (epi)	0.77	3.11	21.25	21.25
			B. proboscidea (endo)	2.19	0.23	18.79	40.04
			Biofilm	1.23	0	10.68	50.72
			Berkeleya sp.	0.46	1.32	10.37	61.08
IN ≠ IS	4.8646	0.003		IN	IS		
			B. proboscidea (epi)	0	3.11	21.45	21.45
			B. proboscidea (endo)	2.33	0.23	15.27	36.72
			Ulva spp.	0.56	2.04	10.67	47.39
			Ulva lactuca	1.51	0	10.61	58
			Biofilm	1.36	0	8.93	66.93
C1 = IN	1.5363	0.052					

over the intertidal rocks (Jaubet et al., 2011, 2013). The presence of *Ralfsia* sp. on the non-impacted area was related to the availability of bare substratum rather with the pollution condition. Other factor affecting the *Ralfsia* sp. distribution may be related with the absence of the epilithic form of *B. proboscidea* in non-impacted sites. This is also evident in plots cleared in autumn where *Ralfsia* sp. developed in site IN where the abundance of the epilithic form of *B. proboscidea* decreased comparing to those plots cleared in spring.

According to the succession started in spring, *B. rodriguezii* was related to impacted sites in the late successional stage given that the time of recovery of the species was relatively lower in these sites, and not because the species did not inhabit in non-impacted sites. In fact, *B. rodriguezii* is known as a bed-forming mussel species which dominates on intertidal rocky substrata forming monocultures that exclude other

epilithic species in warm-temperate shores of Argentina (Adami et al., 2004). The effect of sewage pollution over the population of *B. rodriguezii* and its associated community was previously documented by other authors (López Gappa et al., 1990, 1993; Vallarino et al., 2002; Vallarino et al., 2014). Moreover, a storm occurred after the week 12 produced high levels of sand deposition on the intertidal rocky shore in site IS. This mussel was the sole species that prevailed when the intertidal freed of sand. Thus, *B. rodriguezii* dominated the experimental plots accompanied by a variable coverage of *B. proboscidea* and *Ulva* spp. A similar pattern to the above described observation (i.e. before and after storms, causing sand deposition) was previously discussed by Jaubet et al. (2013, 2015).

Several species including *C. uruguayense*, *Ulva lactuca*, *Siphonaria lessoni* and bare substratum were associated with the late successional
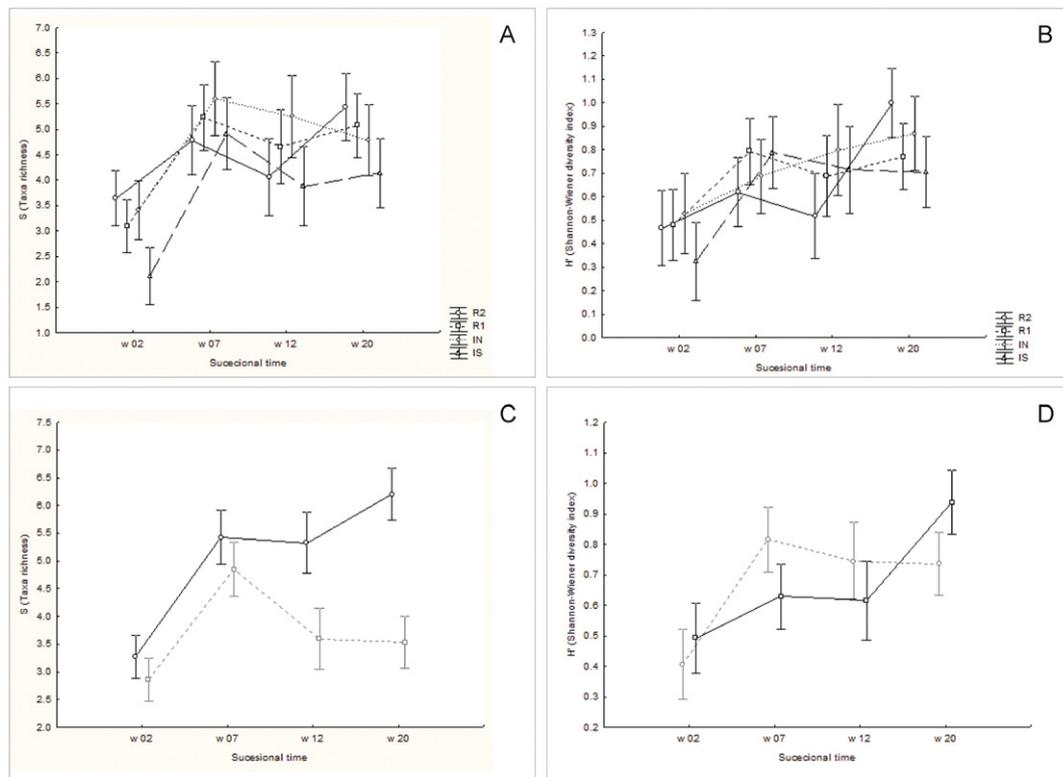
Fig. 3. Taxa richness (A) and Shannon-Wiener diversity (B) of sampling sites (IS, IN, R1 and R2) during weeks of the succession (02, 07, 12 and 20). Taxa richness (C) and Shannon-Wiener diversity (D) of experimental plots cleared in spring (dotted line) and autumn (full line). Vertical bars denote 0.95 confidence intervals.

times in non-impacted sites. *C. uruguayense*, although observed in a low coverage, was recognized as an indicator species for the non-impacted areas in the study area (Becherucci et al., 2016). In a previous study conducted in northern Argentine Patagonia, *U. lactuca* showed a high growth velocity (20–25% day^{-1}) in an enriched channel of San Antonio Bay, being directly related to nutrient input (Teichberg et al., 2010). Moreover, large biomass accumulations of free-floating macroalgae *U. lactuca* were reported in the absence of grazing by invertebrates in a eutrophic Danish estuary (Geertz-Hansen et al., 1993). However, *U. lactuca* was related to site R2 in a previous study conducted in Mar del Plata (Becherucci et al., 2016). The association of bare substratum with reference sites indicated a long time of recovery for the substrata in these sites. The limpet *S. lessoni* was potentially associated with the availability of the bare substrata, where it can graze on diatoms and algal propagules, although, the behavior of this motile limpet depends on other factors as well (e.g. arid or humid climate, food availability) (Nuñez et al., 2014).

According to the succession commenced in autumn, several differences to that started in spring were observed. For example, there was a clear development of the epilithic form of *B. proboscidea* in the late successional stage in the impacted site (IS). Jaubet et al. (2011) described for the first time large reefs produced by the tube-dwelling *B. proboscidea* which consisted in a compact sand reef with a high density of polychaete tubes built over the intertidal rocks. The authors found a temporal variation on its abundance, which increased at the final spring or early summer; this being in line with our results. Other species such as *Polysiphonia* sp., *P. fascia* and *Ulva* spp. were associated with impacted sites in the late succession stage; these are all opportunistic species which were well developed in association with the epilithic form of *B. proboscidea*. Any relation between these opportunistic algae and the sand tubes formed by *B. proboscidea* deserves to be studied. *Ulva* spp. is widely reported as an opportunistic species related with enriched habitats (López Gappa et al., 1990, 1993; Díez et al., 1999; O'Shanahan Roca et al., 2003; Kraufvelin, 2007; Torres and Caille, 2009; Martinetto et al.,

2010). However, in the study area no clear relation between *Ulva* spp. and the sewage outfall was previously observed (Becherucci et al., 2016).

Regarding to the taxa and sites association in plots cleared in autumn, both R1 and IN sites seemed to be an intermediate location between impacted (IS) and non-impacted (R2) sites. It appears that sites R1 and IN represented a transition between sites IS and R2 with an intermediate-impacted condition. According to this hypothesis of intermediate-impacted condition, *B. rodriguezii* and *Ralfsia* sp. were associated with sites (R1 and IN) in the late succession stage of the experiment started in autumn probably because these species did not coexist with the epilithic form of *B. proboscidea* (in site IS) or neither was not the appropriate succession stage for both species to develop (in site R2). Considering the PERMANOVA and SIMPER results, the assemblages of both impacted sites (IS and IN) were similar in the plots started in spring, however, in those that started in autumn, were different because the site IS presented higher coverage of the epilithic form of *B. proboscidea* and *Ulva* spp. than the site IN. Moreover, a higher coverage of *B. proboscidea* (endo), *U. lactuca* and biofilm (indicating bare substrata) were observed in the site IN, reaffirming the hypothesis of intermediate-impacted condition.

The temporal gradient of the plots cleared in autumn was not clear because the varying occurrence of opportunistic species (such as *B. proboscidea* and *Ulva* ssp.) in the late succession stage of the impacted site IS. The time at which a patch is formatted may affect the course of succession, chiefly due to the seasonality of species reproduction and growth parameters (Benedetti-Cecchi and Cinelli, 1993). In our study, *B. proboscidea* showed a seasonally pattern, being spring (coinciding with the late succession stage of the plot cleared in autumn) the season in which high coverage of the epilithic form of *B. proboscidea* was found.

4.3. Community parameters

It is well established that the diversity of a community (referring as S, J′ and H′ parameters) increase according to the successional times

(Begon et al., 1994). A significant lower S and H′ were registered in week 02 in the impacted site (IS), however, any interpretation deserves of a microscopic essay including the taxa identification of the assemblages composition. Over the later succession stages of the experiment started in spring, the S decreased after week 12 possible due to the sand deposition on the intertidal rocky shore in the site IS in which *B. rodriguezii* was the sole species that prevailed when the intertidal freed of sand. Moreover, the evenness parameter in the community was overall lower in spring, possible due to the dominance of *B. rodriguezii* registered in the site IS. The benthic community of plots cleared in autumn exhibited a higher H′ over the late stage of the succession beside a higher evenness.

To summarize, although this coastal system has not shown an evident response of elevated ephemeral macroalgae coverage, a shift in the macrozoobenthos fraction was observed, particularly in the coverage of the non-indigenous *Boccardia proboscidea*. This shift appears to be a response of two main events: an increasing of a nutrient input to the system and the seasonal pattern of *Boccardia proboscidea*. As Cloern (2001) discussed, the global translocation of organisms and inoculation of coastal ecosystems with non-indigenous species, sometimes cause a profound ecological disturbance, and this disturbance interacts with nutrient enrichment. Moreover, climatic factors may change the responds of the system to nutrient enrichment. In our case, the dominance of a non-indigenous species like *B. proboscidea* was facilitated by an enrichment condition, indicating a clearly polluted environment though this pattern were occasionally modified by storms causing intertidal sand deposition (Jaubet et al., 2011, 2013).

5. Conclusions

The time of recovery after a disturbance (measured as the time spanned for a benthic community to reach its non-cleared composition) in enriched sites was lower than in non-enriched sites based on the observed shift of the communities' composition. In enriched sites the diatom *Berkeleya* sp. and the spionid *B. proboscidea* developed in the early succession stages, while *B. rodriguezii* or *B. proboscidea* occurring with *Polysiphonia* sp., *P. fascia* and *Ulva* spp. developed in the late succession. Environmental variables as storms causing sand deposition also alter the succession pattern in impacted sites, removing species such as the epilithic form of *B. proboscidea* and promoting the dominance of other epilithic species such as *B. rodriguezii*.

Acknowledgments

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