The functional consequences of the biodiversity: Experimental studies with intertidal communities.

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ABSTRACT

Over the last decades, global lost of biodiversity has fuelled considerable research to investigate the functional consequences of declining diversity in communities. Rapid changes in the biological composition and richness of most of earth´s ecosystems as a result of human activities have brought new urgency into these questions. Biodiversity can affect traits of ecosystem function.

This study presents an experimental approach to examine the effects and interactions of species richness and evenness on the primary productivity of synthetic assemblages. We used a modification of the design proposed by Benedetti-Cechii (2004) which included explicitly diversity, evenness while controlling the effect of identity.

The experiment shows that Species identity was the most of the relevant effect on assemblages functioning the higher productivity and efficiency values were found for *Sargassum muticum*. Conversely, *Bifurcaria bifurcata* seems to be the species with lower overall performance

Our approach was unable to detect some of the effects of species richness and evenness suggested in literature. Probably only longer experiments would allow species to develop strong positive interactions and reduce the prevalence of large identity effects

Keywords: Biodiversity, Species Richness, Evenness, Productivity. Macroalgal assemblages

RESUMO

Nas últimas décadas, as perdas globais de biodiversidade tem incrementado a investigação científica sobre as consequências funcionais do declínio da diversidade nas comunidades. As rápidas mudanças na composição biológica e riqueza dos ecossistemas Terrestres como resultado das atividades humanas suscitou a resposta a este problema. A biodiversidade pode afetar as características e o funcionamento dos ecossistemas.

Este estudo apresenta uma abordagem experimental para aferir acerca dos efeitos e interações da riqueza específica e do Evenness na produtividade primária das comunidades artificias de macroalgas. Assim, foi utilizada uma modificação do desenho experimental proposto por Benedetti-Cechii (2004), que inclui a diversidade e o Evenness para o controlo do efeito da identidade de espécies.O trabalho realizado mostra que a identidade de espécies foi mais relevante no funcionamento das comunidades. Valores de produtividade e eficiência mais elevados foram encontrados para *Sargassum muticum*. Por outro lado, *Bifurcaria bifurcata* parece ser a espécie com um desempenho global inferior.

Neste trabalho não foram detetados os efeitos da riqueza especifica e do Evenness sugeridos na bibliografia. Provavelmente a aplicação de desenhos experimentais mais longos permitirão estabelecer interações positivas e reduzir a prevalência de grandes efeitos de identidade nas espécies..

Palavras chave: Biodiversidade, Riqueza Específica Evenness, Productividade, Comunidades macroalgas

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1 INTRODUCTION

Human activities during last century have severely altered Earth's ecosystems, eliminating genes, species and biological traits at unprecedented rates (Barnosky et al., 2011; Murphy & Romanuk, 2014). These alterations are leading to dramatic changes in the biotic composition and structure of ecological communities (Balmford, et al, 2003) with large and unanticipated impacts on ecosystem functioning (Hooper & Dukes, 2003).

The conceptual idea of a close relationship between diversity and ecosystem functioning is fairly recent but not new. In fact during the 1980s, concern about species extinction rates led to research showing that organisms can shape physically habitats (ecosystem engineering), modify biogeochemical cycles and the productivity of ecosystems. Such research suggested that loss of certain life forms could substantially alter the structure and functioning of whole ecosystems (Cardinale et al., 2012). Nowadays and despite the early intense debate on the nature of the diversity-ecosystem functioning relationship see Duffy et al., (2007) for a review and the real consequences of diversity loss (Thompson & Starzomski, 2006); the positive effects of diversity on ecosystem functioning is a well-established belief among ecologists. It is even considered by some as a new ecological paradigm (Hillebrand & Matthiessen, 2009; Naeem, 2002). For example, two comprehensive meta-analyses that examined the results of over 100 experiments and > 400 measures of biodiversity effects revealed evidences that, on average, mixtures of species produce more biomass and use more resources than do single species (Balvanera et al., 2006; Cardinale et al., 2006). Similarly, in a recent review of experiments (Hooper and colleagues (2012) found that intermediate levels of species loss (21–40%) reduced plant production by 5–10%. Biodiversity not only determine ecosystem processes but also ecosystem properties like stability (Loreau, et al 2002), including resistance to invasion of exotics (J. Stachowicz & Byrnes, 2006) or predictability (Mcgrady-steed, et al, 1997).

With all the empirical evidence accumulated in these three decades of research, the mechanisms to explain the influence of species diversity on ecosystem function are now clearly established. Three main mechanisms have been proposed: i) the complementary effect resulting from the complementary use of resources by different species (niche differentiation), which means that having more species results in a more efficient use of resources, ii) the sampling effect which hypothesizes that the higher productivity of a highly diverse assemblages results from the increase of the probability

of selecting some highly productive species (Aarssen, 1997; Huston, 1997) and iii) facilitation, here used as synonymous with positive interactions, represents the benefits provided by one species that can increase the effective niche of other species for example by habitat amelioration or predation refuge (Bruno et al 2003). The relative importance of those mechanisms in natural systems is very much unknown but in the studies done so far; descriptions of complementarity effects are less frequent than those of selection effects, but complementarity has been proposed to increase in importance over time(Reiss et al, 2009).

To date most of the research on the functional consequences of diversity derived from experimental systems and theoretical models in which the number and identity of species has been highly controlled at local scale. However biodiversity is a multifaceted concept and include not only number of species but other structural traits of the communities like the evenness and (i.e. the relative abundance of species in the assemblage).

In natural systems, biomass or individual density is not evenly distributed across species, and species on the way to extirpation is likely to go through a low-abundance stage before disappearing completely, thus evenness may decline long before species richness does (Mulder et al 2004). In fact, evenness and species richness are not always correlated and both positive and negative relationships have been described (Stirling & Wilsey, 2001).

In contrast to the research effort devoted to examine the richness productivity relationship, the functional consequences of evenness (or its complementary concept: dominance) have been largely overlooked (Hillebrand et al 2008). Changes in evenness alter the distribution of traits in communities, modifying the balance between intra and interspecific interactions. If species interactions drive biodiversity effects, increasing evenness should enhance complementarity and ecosystem processes (Kirwan et al., 2007), however if sampling effects is the prevailing diversity mechanisms, probably evenness has little functional influence. Empirical studies on the effects of relative abundance of species have found positive effects of evenness on the productivity of plant communities (Kirwan et al., 2007; Stevens & Carson, 2001; Wilsey, Brian J. Potvin, 2000), suggesting that complementary interactions may be relevant.

Although studies like those cited above have looked into the individual effects of species richness and evenness or dominance, we have very little understanding of their interactive effects on ecosystem functioning. This understanding is highly relevant to foresee the potential impacts of global change. In a recent meta-analysis, Walker et

al., (2006) revealed that experimental warming strongly altered species composition in tundra ecosystems across the entire circumpolar arctic region. Warmed communities had lower species richness and lower evenness than ambient controls, thus were more strongly dominated by few species. Also, the arrival of introduced species, often highly dominant in their host assemblages and with functional traits very different to native species may have strong effects on ecosystem functioning. Thus research on the functional interplay between species richness and evenness is required to fully understand the some of the consequences of the new diversity scenarios resulting from global change.

Research on the ecosystem functioning of the marine realm lags behind terrestrial research. In marine macroalgal communities, research on biodiversity ecosystem functioning have focused on the effects of species richness on primary production and on the stability of natural communities (Boyer et al 2009; Bracken & Williams, 2013; Bruno et al., 2003; Kraufvelin et al 2010; Stachowicz, 2008). Most of these studies have found significant effects of species richness on primary productivity and stability. To date only one study have examined simultaneously the effects of species richness and evenness on the primary productivity of natural macroalgal assemblages (Arenas, et al 2009). In this observational study, the relationship between primary productivity and several biodiversity related traits (namely identity, species richness, evenness and spatial arrangement) were examined simultaneously in macroalgal assemblages. However observational approaches have several limitations (i.e. impossibility to establish causality and to identify underlying mechanisms) and only experiments may help to understand how these structural diversity components interact to shape the ecosystem function of macroalgal communities (Arenas et al., 2009; Maestre et al 2012).

This Master Thesis present an experimental approach to examine the effects and interactions of species richness and evenness on the primary productivity of synthetic assemblages. We used a modification of the design proposed by Benedetti-Cecchi, 2004) which included explicitly diversity, evenness while controlling the effect of identity. While results from manipulative experimental results may be problematic to extrapolate to natural communities, they allow to establish causal connections between biodiversity and productivity or other measures of ecosystem functioning (Benedetti-Cecchi, 2004).

2 MATERIAL AND METHODS

2.1 Synthetic assemblages construction and experimental design

We conducted the experiment using synthetic assemblages built with eight different species of seaweeds collected in the coastal area of North Portugal. The fronds where collected in April and May 2013 at the shores of Praia Norte, Viana do Castelo (41°41′27″N, 8°50′57″W) and Praia de Moledo (41°50′22″ N, 8°52′30″ W). Both beaches are exposed rocky shores with large intertidal granite and slate platforms and with abundant rockpools. They have a semi-diurnal tidal regime, with the largest tidal range of 3.5–4 m during spring tides. At the collection dates, healthy fronds from the eight different target species (see below) were cut or scraped from the rock and transferred to the laboratory in plastic bags and cool boxes. Once in the laboratory, seaweed were sorted by species and rinsed in a bath of freshwater (about 30 seconds) to remove herbivores (Arenas et al., 2009). When present, epiphytes were removed by hand; however their complete elimination was not possible, yet only those fronds less epiphyte were used in the experiments. Fronds were them placed in separated aerated seawater tanks (80 liters).

As stated above we used eight different seaweed species. Those were four brown seaweeds: *Stypocaulon scoparium* (Linnaeus) Kützing, *Bifurcaria bifurcata* R. Ross, *Fucus vesiculosus* Linnaeus, *Sargassum muticum* (Yendo) Fensholt, and four red seaweeds: *Chondracanthus acicularis* (Roth) Fredericq, *Mastocarpus stellatus* (Stackhouse) Guiry, *Chondracanthus teedei* (Mertens ex Roth) Kützing, *Osmundea pinnatifida* (Hudson) Stackhouse. All these species are abundant perennial seaweeds at the two collection shores (Araújo et al., 2009) have different morphologies and are suitable for the manipulation procedures used to create the synthetic assemblages.

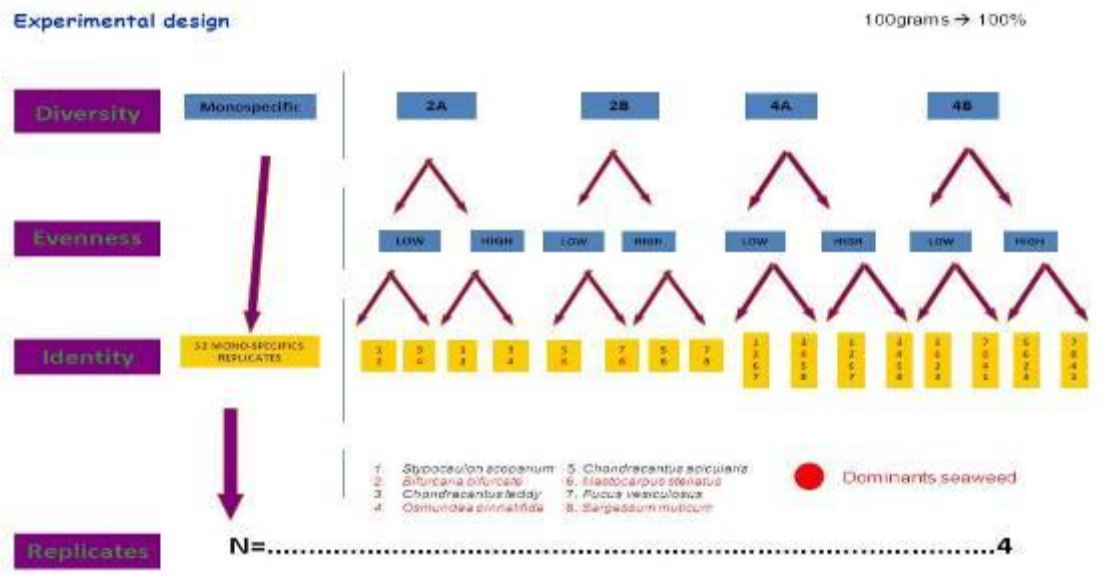


Figure 1 Diagram of the experiment designed to separate the effects of species richness, identity and evenness (distribution of biomass) of macroalgal in our synthetic assemblages. Species that occur at high richness level also occur in treatments with low richness treatments. Dominant species in low evenness treatment are in red.

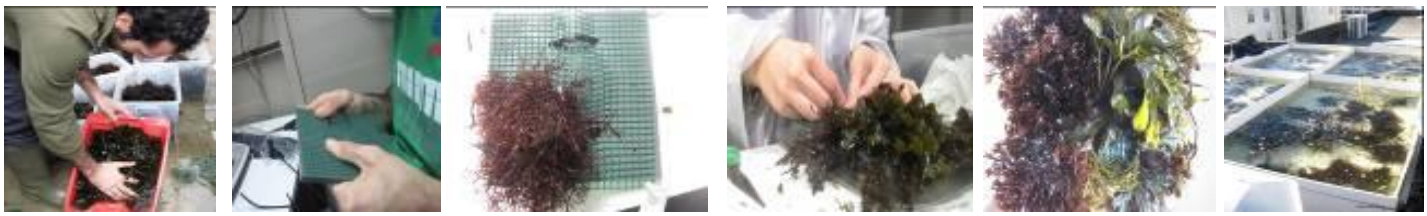


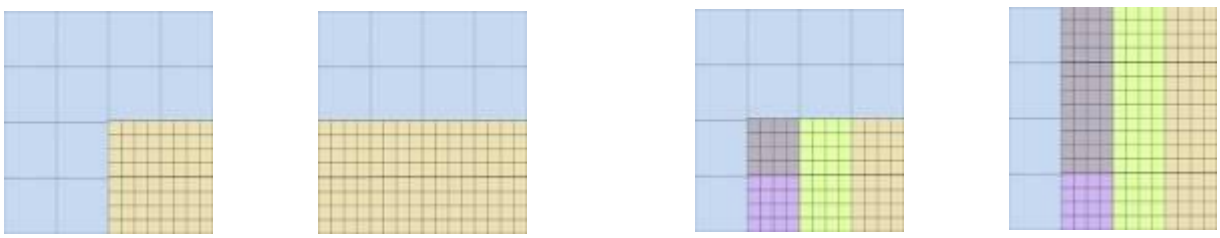
Figure 2.pictures taking during the process of building our synthetic assemblages

We built 96 synthetic communities in quadrates with an area of 196 cm^2. To assemble the communities, small groups of fronds were secured with small cable ties to a plastic 5 mm mesh in a way to keep fronds upright. Groups of fronds were regularly distributed throughout the mesh with a distance among frond groups of around 1 cm. Seaweeds and plastic mesh were them secured to a PVC plate to increase strength and ensure negative buoyancy. Once assembled, plates were submerged in a 300 l seawater outdoor sheltered tank set with aeration to create turbulence and temperature controlled at 16ºC using Aqua Medic® Titan 2000 and Teco TR 10-coolers units. To avoid nutrient limitation, seawater was enriched every two days by adding inorganic N (NaNO3) and P (NaH3PO4) to a final concentration of 50µM N and 5µM P. Salinity was regularly monitored and tanks were regularly refilled with freshwater to compensate for water evaporation.

Our experiment aimed to unravel the ecosystem functioning consequences of three different components of diversity, namely species richness, species identity and evenness. To do so, we used a similar design to the one proposed by Benedetti-Cecchi, (2004) considering in the experimental design the three components of diversity cited above: i) Species richness with two levels, 2 species and 4 species, ii) Evenness also with two levels (low and high) and iii) Identity, this factor have 4 levels for each species richness; i.e. we included four different combinations of 2 species and 4 species. Species were randomly selected to create the different Species richness and identities.

Species richness (SR) levels were built by creating assemblages with 2 species and with 4 species selected from the initial pool of eight species. Initially we created four different assemblages of 2 species assemblages randomly selecting species from the pool of 8 species. Them we created 4 species assemblages adding 2 new randomly selected species to the previous 2 species assemblages. In this way, we were able to include all the species present in the high species richness treatment also in the low species richness assemblages, preventing any effect confounding of strong identity effects with diversity ones (Bulling et al 2006; Stachowicz et al 2007). Thus, by selecting our assemblages randomly we were able to define an identity random factor which was nested in the factor species richness. We created 4 assemblages replicates per identity.

Evenness treatment was implemented by considering two different ways of distribution of biomass among species in the assemblages. Therefore, high evenness assemblages have equal amount of biomass per specie, while low evenness had a “dominant” species which presented a higher biomass than the other species in the assemblage. Dominant species were selected randomly and corresponded to *Bifurcaria bifurcata, Osmundea pinnatifida, Mastocarpus stellatus* and *Sargassum muticum*. Evenness was estimated using Shannon diversity index, following (Camargo, 1995) for the biomass data.



A 2 Species low evenness | B 2 Species high evenness | C 4 Species low evenness | D 4 Species high evenness

Figure 3 Evenness treatment

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Overall biomass in the plates was 100 g of FW seaweeds. This is within the range of FW biomass in rock-pools with erected seaweeds in the collecting area (personal observation). In plates with 2 species and high evenness, each species was represented by 50 g of FW biomass. In the case of low evenness the dominant species had 75 g FW and the other 25 g FW biomass. For the 4 species assemblages, in the case of high evenness each species included 25 g FW while in the low evenness the dominant species contributed with 62.5 g FW and the other three species with 12.5 g FW each. Wet weights were determined after removing excess water from the algae using a salad spinner (Bruno et al., 2005)

Additionally, we built single species assemblages to allow assessment of overyielding (D. U. Hooper & Dukes, 2003b). On overall, a total of 96 assemblages were created.

To gain further insight into the mechanisms underlying community attribute effects on ecosystem functioning, we calculated the overyielding for Gross primary productivity, Respiration and Net Primary Productivity. Overyielding allows to recognize the existence of increased biomass production in species mixtures relative to monoculture. Overyielding was estimated as ln(O/E), where O is observed value, and E is expected value (Orwin, et al 2014). Expected values were calculated based on monoculture responses and the relative proportion of each species within the mixed communities

Overyielding index(i+j):

Log (O(i+j)/E(i+j))

O(i+j) – Observed Primary Productivity (PP) for the assemblage i+j for the corresponding spatial aggregation

E(i+j) – Estimated Primary Productivity (PP) for the assemblage i+j for the corresponding spatial aggregation

2.2 Incubation procedures and Ecosystem functioning surrogates

Productivity-irradiance (P-I) curves were estimated by measuring oxygen fluxes inside a sealed incubation chamber at 7 irradiance levels. The incubation chamber consisted of a 12.5 l Plexiglas chamber partially submersed in a larger, thermo-statically controlled cooling chamber in white PVC, we used a temperature controlled chamber with 7 consecutive light irradiance levels, from 0 $\mu Em^{-2}s^{-1}$ (dark period) to 1578 $\mu Em^{-2}s^{-1}$. Mean ± SE temperature inside the incubation chamber was 16.5± 0.06°C (Aqua Medic® Titan 2000 and Teco TR10). Filtered sea water and the water movement inside the incubation chambers was maintained through a submersible pump (300 l h^{-1}) equipped with diffusers to reduce turbulence. Oxygen concentration variations were measured using a luminescent dissolved oxygen probe connected to a data-logger (Hach® HQ40) that registered a new measurement every 30 s and was continuously monitored. To reduce possible effects of circadian rhythms on algal productivity, incubations were always carried out during daylight hours (between 08:00 and 18:00h)

.

Figure 4 Incubation procedures

The irradiance levels facilities which allowed us to measure assemblage respiration and productivity rates at 7 successive and increasing light levels: 0 (dark), 24, 164, 262, 345, 417 and 1578 µE m^{-2} s^{-1}. Maximum irradiance levels in the chamber were lower than those recorded in the field at sea surface level where, during sunny days in winter, irradiance can reach >2000 µE m^{-2} s^{-1} (authors' pers. obs. using a scalar quantum sensor) (Arenas et al., 2009). The light source in the chamber was composed of sixty- four 30 W fluorescent tubes (Osram L® 965 Biolux). Irradiance inside the chamber was measured using a sensor (Walz® ULM500 Universal Light Meter). For each P-I incubation, the successive irradiance periods lasted between 20 min, the time necessary for the fluorescent tubes to warm up and the assemblages to reach linear rates of oxygen flux (Migné et al. 2002). All the timing of the light system was controlled

using Aqua Medic ® (AT Control System controllers, GmbH, Bissendorf, Germany). The entire set of incubations took around 2:30 h per assemblage.

Respiration and productivity were estimated through oxygen fluxes by regressing oxygen concentration (µmol) through time (s^{-1}) during dark and light periods of increasing intensities. Estimations were normalized by biomass and corrected by seawater volume inside the chamber to take into account the different volumes of the boulders.

The variables Respiration, Photosynthetic efficiency at low light irradiance (alpha, α) and Light compensation point were measured as surrogates of ecosystem functioning. Respiration of assemblages (mg O^2 h^{-1}); corresponded to the oxygen consumption rate during the dark period and we assessed net primary productivity (mg O^2 h^{-1}) as the pro- ductivity recorded at different irradiance intensities in order to calculate alpha. Both variables were calculated by plotting oxygen concentration over incubation time and fitting a linear regression line to calculate rates of oxygen change. Alpha (α) (mg O^2 $\mu Em^{-2}s^{-1}$), was estimated as the slope of P-I relationship at light-limited irradiances through linear regressions. Gross Primary Productivity where calculated as the sum of NPP and Respiration. Regressions were also used to estimate light compensation point of assemblages, the irradiance level at which respiration rate is equal to photosynthetic rate and net oxygen exchange is zero

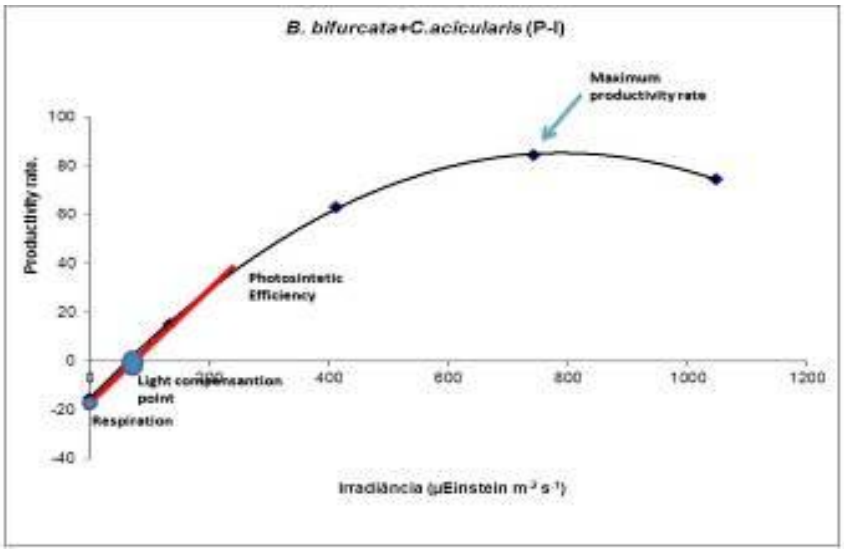

Figure 5 Example P-I curves and ecosystem functioning surrogates.

2.3. Statistical analyses

Analysis of variance (ANOVA) was used to test hypotheses involving productivity and all related surrogates. Analyses were done on data collected after 15 and 30 days. GMAV version 5 for Windows was used for computations (Underwood, & Chapman, 1998) Cochran's test was used to test for homogeneity of variance. Variances were not heterogeneous and data were not transformed. Student- Newman-Keuls procedure was used to make post hoc comparisons among levels of significant terms at alpha $p<0.05$. All nested factors are displayed in parentheses in the analysis tables.

Multiple linear regression models were used to examine simultaneously the influence of species identity, diversity and evenness on the functional performance of the assemblages (i.e. GPP, NPP, Respiration and Alpha).

We used linear models to analyze causality between predictors and functional responses. Therefore once each full multiple regression model was set, we proceeded to select those predictors with a truly significant effect on the response variable. Hierarchical partitioning, is particularly suitable for this task (Chevan, A., 1991). Hierarchical partitioning compares all possible models in a multiple regression setting and determines the independent capacities of the predictive variables to explain the patterns of variability in the corresponding response variable. For each predictor, its independent explanatory power on the dependent variable is characterized with an index ''I,'' which reflects the independent contribution of the predictor to the variance explained by the models. Variables that independently explained a larger proportion of variance than by chance were identified using randomization tests. For each predictor, the observed contribution to the explained variance (I) was compared to the distribution of a population of Is of 1000 randomizations of the data matrix. Significance was accepted at the upper 95% confidence limit. Hierarchical partitioning procedures were estimated using the hierarchical partitioning software for the public domain package R (Walsh & Nally, 2003)

3. RESULTS

3.1. Functional performance of single species assemblages

Assemblages with just one single species allows to characterize the functional performance of each species under the same density conditions occurring at multiple species assemblages. For all the four functional parameters estimated throughout incubations (GPP, Respiration, NPP and alpha) significant identity effects were detected (ANOVA, $p<0.05$ in all the cases). Consistently higher productivity and efficiency values were found for *Sargassum muticum*. Conversely, *Bifurcaria bifurcata* seemed to be the species with lower overall performance.

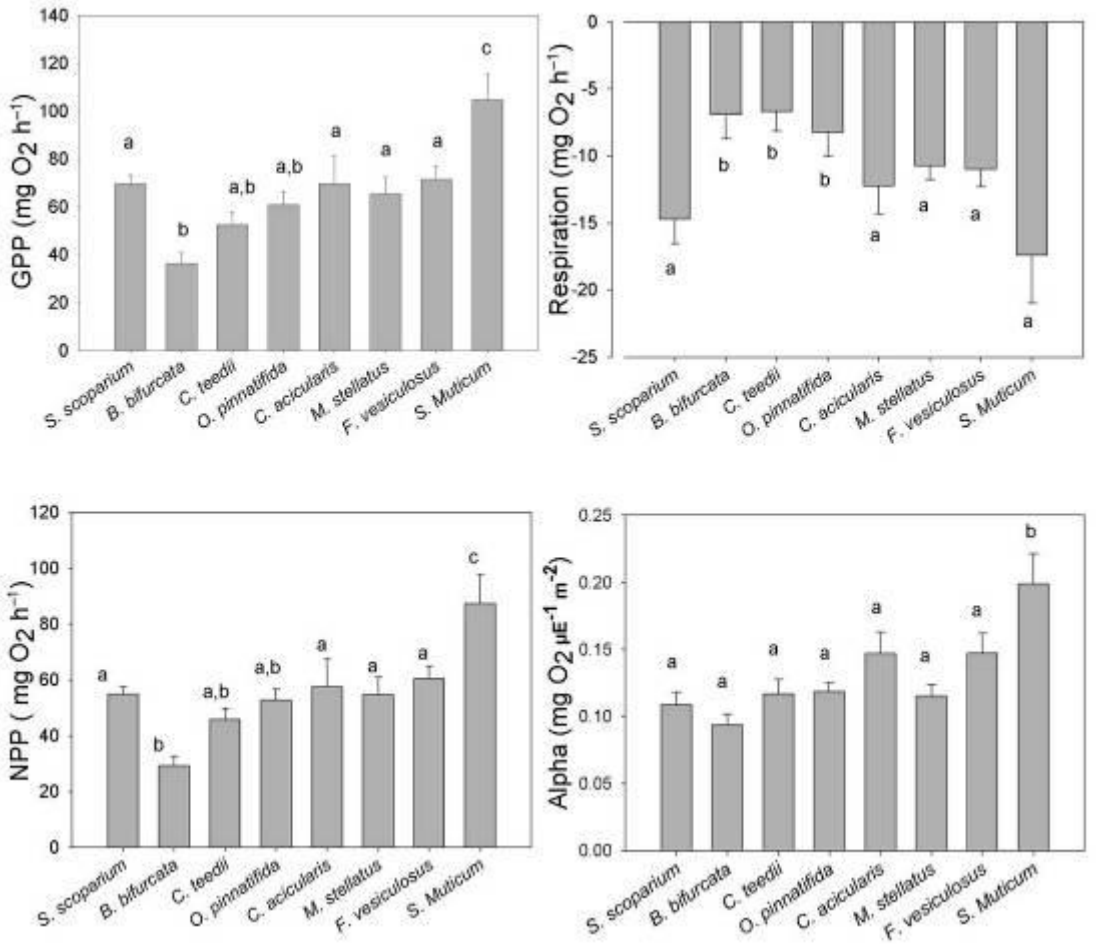

Figure 6 Mean (±SE, n=4) of the functional measures for the eight species utilized in our experiment. A) Gross primary productivity, GPP. B) Respiration rate (Resp). C) Net primary productivity (NPP) and D) Alpha, i..e assemblages efficiency under light restricting conditions. All measures refer to the whole assemblage. Bars sharing same letter did not differ in ANOVA test at p-level 0.05.

3.2. Functional performance of multiple species assemblages: Richness, Evenness and Identity effects

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When we measured the functional performance of our assemblages after the adjustment period, i.e. two weeks after the start of the experiment, we found that neither species richness, evenness nor their interactions drove functional responses in our synthetic assemblages. Species identity within the synthetic assemblages seemed to consistently be the most of the relevant effect on assemblages functioning (see table 1, Figure 2, a, b, c and d).

			a) GPP		**b) RESP**		**c) NPP**		**d) Alpha**	
	df effect	**df error**	**F**	**p-level**	**F**	**p-level**	**F**	**p-level**	**F**	**p**
Diversity	1	6	0.06	0.8088	0.19	0.6792	0.04	0.8461	0.03	0.8591
Evenness	6	48	0.88	0.3856	0.28	0.6161	0.82	0.3991	1.16	0.3237
Identity(Di)	1	6	7.29	**<0.001**	4.99	**0.0005**	6.40	**<0.001**	5.22	**0.0003**
DiXEv	1	6	0.06	0.8198	0.11	0.7541	0.12	0.7435	0.10	0.7675
EvXId(Di)	6	48	1.52	0.1929	1.25	0.2972	1.61	0.1643	1.97	0.0887

Table 1 Summary of ANOVA analyses of the effect of the experimental treatments for the four functional variables: A)Gross primary productivity, GPP. B) Respiration rate (RESP). C) Net primary productivity (NPP) and D) Alpha. Numbers in bold indicate significant effects.

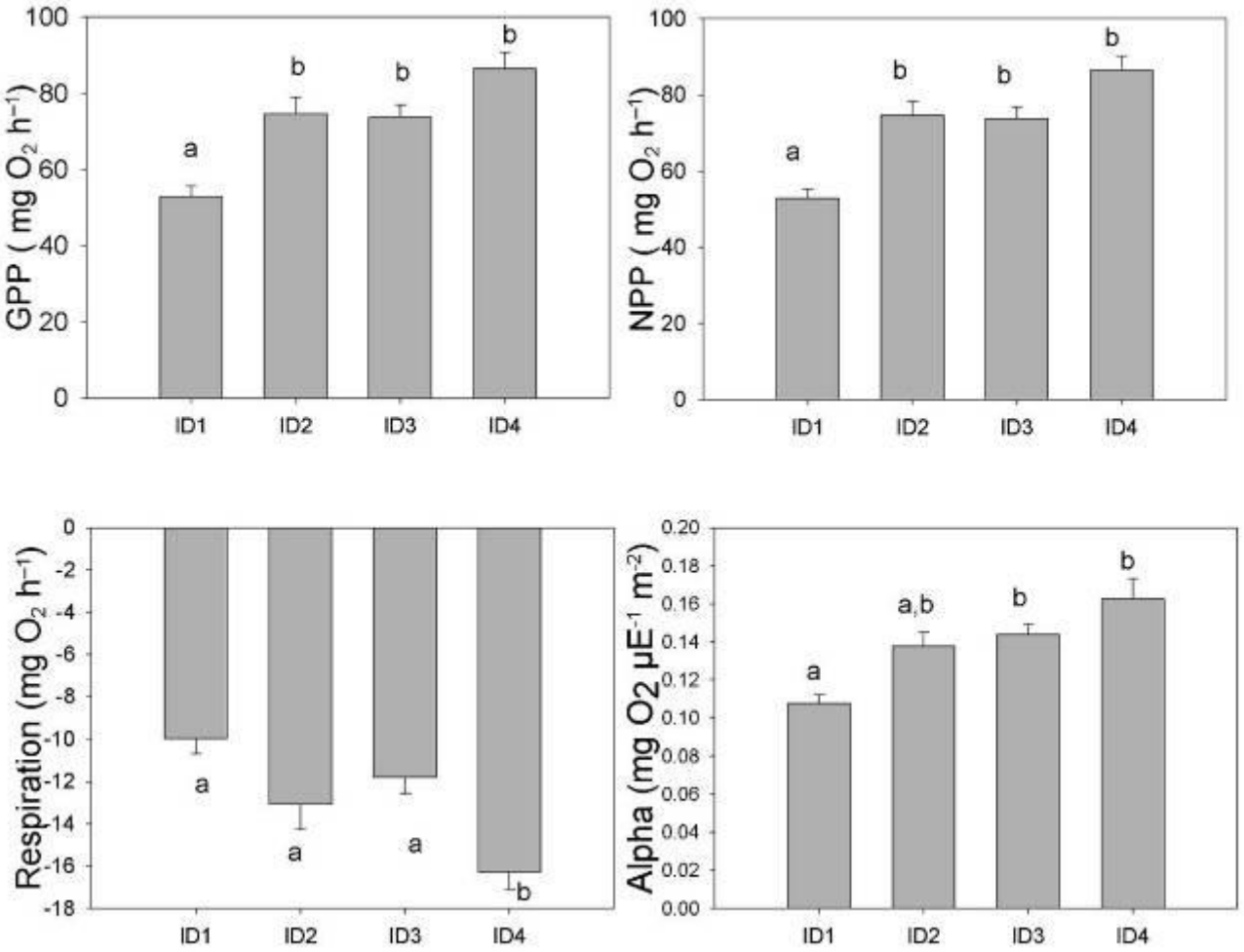

Figure 7 Mean (±SE, n=4) of the Identity source ID1 Bifurcaria. ID2 Osmundea ID3 Mastocarpus ID4 Sargasum. Different lowercase letters indicate differences between groups

3.3. Functional performance of multiple species assemblages: Overyielding

When we examined the effect of the different treatments on the overyielding in our assemblages no significant effects where observed in Gross Primary Productivity or Respiration but in the overyielding for Net Primary Productivity we observed significant differences in the Evenness x Identity interaction. These differences originated by the apparently low performance of the assemblages with ID 3 in low evenness assemblages. This identity corresponded to assemblages where the dominant species was *Mastocarpus stellatus* and included also C. teddi for the 2 spp assemblages plus B. bifurcata and *C. acicularis* in the 4 spp assemblages These assemblages produced less than expected showing a very negative value of overyielding, i.e. probably a significant underyielding (negative overyielding value).

			a) GPP		**b) RESP**		**c) NPP**	
	df effect	df error	F	p-level	F	p-level	F	p-level
Diversity	1	6	0.16	0.7036	4.21	0.0860	1.84	0.2242
Evenness	6	48	4.70	0.0732	3.28	0.1201	0.37	0.5660
Identity(Di)	1	6	1.50	0.1992	1.43	0.2219	4.38	**0.0013**
DiXEv	1	6	1.88	0.2195	1.81	0.2276	0.60	0.4667
EvXId(Di)	6	48	0.90	0.5038	1.14	0.3526	11.58	**<0.001**

Table 2 Summary of ANOVA analyses of the effect of the experimental treatments for overyielding effect in: a) gross primary productivity, GPP. b) Respiration rate (RESP) and c) Net primary productivity (NPP). Numbers in bold indicate significant effects.

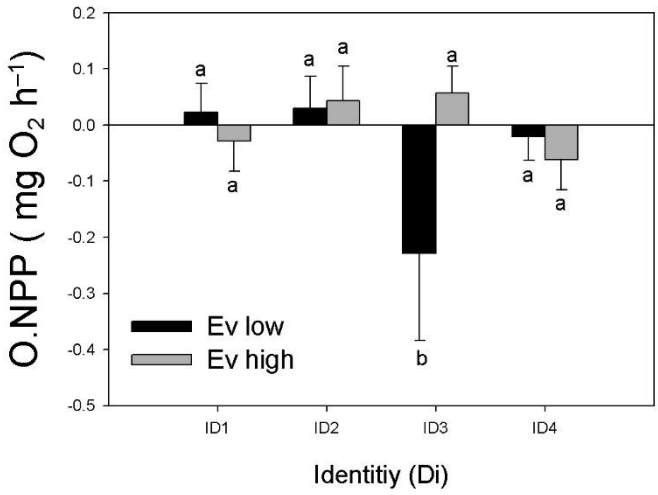

Figure 8 Mean (±SE, n=4) Interaction Evenness-Identity combination ID1 Bifurcaria. ID2 Osmundea ID3 Mastocarpus ID4 Sargasum. Different lowercase letters indicate differences between groups.

3.4. Functional performance of multiple species assemblages: Truly significant predictors

As expected from the previous analyses, hierarchical partitioning procedure to select si gnificant predictors did not find any relationship among assemblage's productivity and diversity or evenness (Table 3). However the presence of certain species had very stro ng effects on the performance of the assemblages. Particularly intense were the negati ve effects *Bifurcaria bifurcata* (Figure 8a, Table 3 and the very strong positive effects of Sargassum muticum (Figure 8b, Table 3).

a)	**I% (Independent contribution to the explained variance)**			
Predictors	**GPP**	**NPP**	**Respiration**	**Alpha**
Diversity	1.14 n.s.	0.91 n.s.	2.18 n.s.	0.92 n.s.
Evenness	3.4 n.s.	3.79 n.s.	2.25 n.s.	5.82 n.s.
S. scoparium	(-) 12.81**	(-) 14.72 **	5.73 n.s.	(-) 12.06 **
B. bifurcata (d)	(-) 30.18***	(-) 30.03***	(-) 26.04***	(-) 23.10 ***
C. tedii	2.07 n.s.	2.71 n.s.	4.97 n.s.	2.33 n.s.
O. pinnatifida (d)	3.31 n.s.	3.47 n.s.	3.22 n.s.	3.51 n.s.
C. acicularis	2.22 n.s.	2.38 n.s.	2.12 n.s.	3.33 n.s.
M. stellatus (d)	2.26 n.s.	1.93 n.s.	5.77 n.s.	2.49 n.s.
F. serratus	(-) 6.4*	5.56 n.s.	(-) 9.90**	(-) 8.07**
S. muticum (d)	(+) 36.10***	(+) 34.44 ***	(+)37.76***	(+) 37.32***
b)				
OLS linear model	**Adjusted R^2=0.44 P<0.001**	**Adjusted R^2=0.39 P<0.001**	**Adjusted R^2=0.33 P<0.001**	**Adjusted R^2=0.34 P<0.001**

Table 3 Relationships between Species richness, evenness and abundance of the different species with the different proxies of assemblages functioning used in our study (GPP, NPP, Respiration and Alpha). Notes: (a) Results of the hierarchical partitioning analysis, ''%I '' indicates the percentage of independent contribution to explained variance. Significant predictors are indicated by asterisks (see Methods for the significance determination procedure). Positive and negative symbols reflect the sign of the effect. (b) Linear model R^2 (ordinary least squares, OLS) and P values for the final model using the significant predictors. *P < 0.05; **P <0.01; ***P < 0.001. Significant predictors in bold. (d) indicates dominant species.

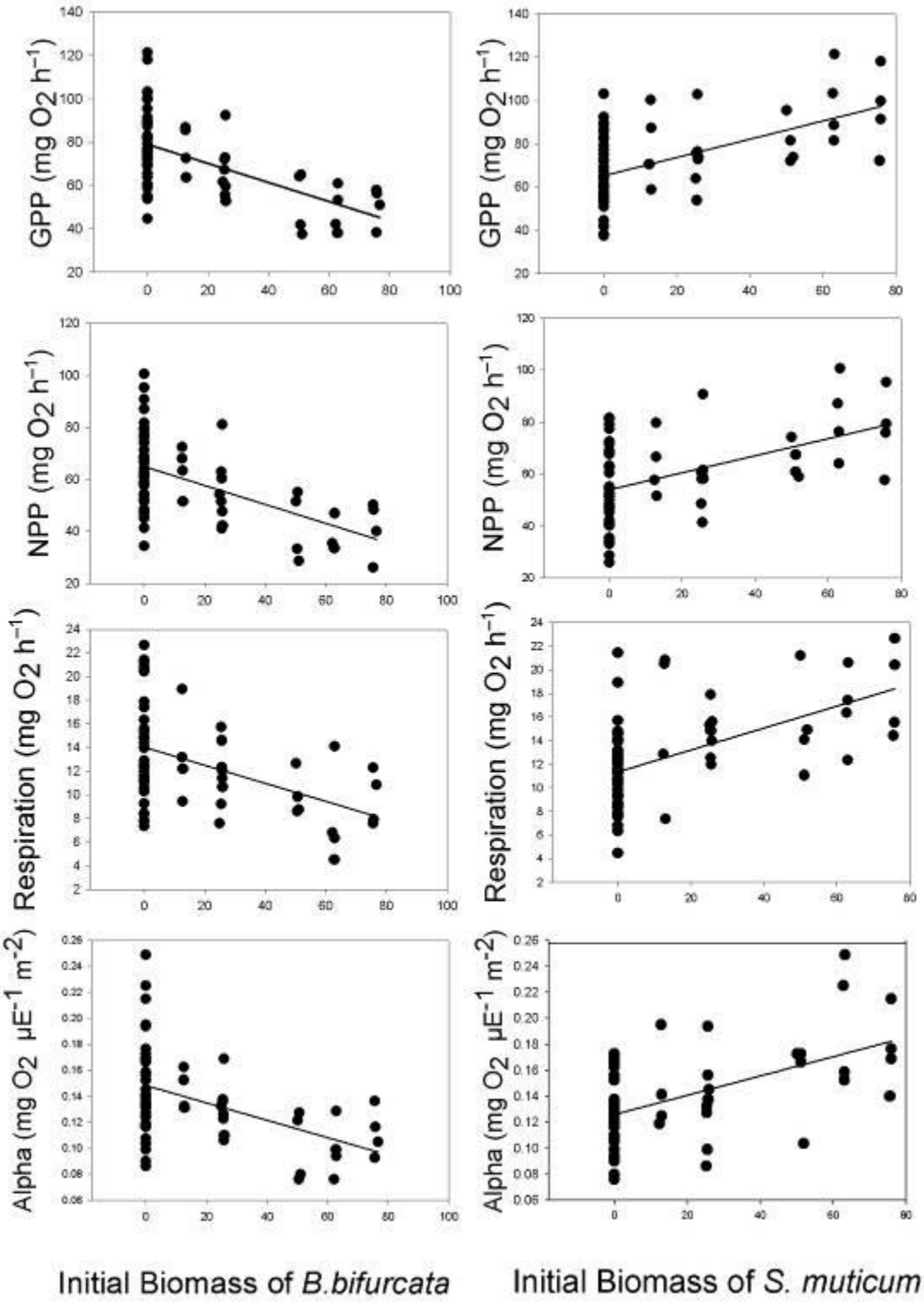

Figure 9 Linear relationships between the initial biomass of Bifurcaria bifurcata and Sargassum muticum with the GPP, NPP, Respiration rates and Alpha of the assemblages.

4. DISCUSSION AND CONCLUSSIONS

In our work on intertidal macroalgal communities we investigated the effects of species richness, evenness and identity on several primary production related processes as proxies for ecosystem function in marine algal communities system.

In our results, species richness effect were not significant for any of the functioning subrogated measured. On overall, multiplying by two the number of species in the assemblages, i.e. from 2 to 4 spp, only increased around 3 % GPP (average GGP 2spp= 70.7±3.4 mg O^2 h^{-1}, 4 spp= 73.2±3.2, n=32). This effect is far from the estimated effect of diversity found in literature. On terrestrial systems, (Hooper et al., 2012) estimated that a fifth percent species loss would mean a reduction of biomass production around 13 %. When we considered single species assemblages, average GPP in monospecific assemblages was 66.37±3.9 mg O^2 h^{-1}, i.e. a value almost 10 % lower than the higher diverse assemblages. Short term experiments frequently do not find any species richness effect (Cardinale et al., 2007; Ruijven & Berendse, 2005) Studies manipulating macroalgal richness have been often unsuccessful finding obvious richness effects. Bruno (2005) found that relative yields in multispecies assemblages of seaweeds where 13 % greater than monocultures but results were not significant. Some other experiments have also found positive effects of species richness on the performance of macroalgal assemblages, irrespective of the response measured. Middelboe & Binzer, (2004) and Bruno (2006) found positive effects of richness on macroalgal photosynthetic rates. Bruno et al (2005; 2006) reported higher biomass accumulation in richer assemblages, and (Bracken & Frielberg, Gonzalez-Dorantes, & Williams, 2008; Bracken & Stachowicz, 2006, Bracken & Nielsen, 2004), noticed positive richness effects on community nutrient uptake. However, whenever the experimental design allowed disentangling the mechanisms behind the positive relationship, species identity effects were identified as the dominant effect (Bruno et al., 2006)

It is also quite obvious from literature that species richness relationships with productivity are highly dependent on the environmental context (Cardinale, et al., 2000) and may have different shapes and directions depending in the response variable of interest, successional status and other community traits (Maestre et al., 2012; Mouillot & Mouquet, 2006). The overall lesson from many of these experiments is that in

primary producer biodiversity experiments richness effects are subtle and compositional effects are stronger (Bruno et al., 2005).

A similar situation was found in the case of evenness, where not significant differences among the two levels explored were found. In this case and looking to the overall trend, average gross primary productivity was slightly lower at high evenness treatment compared to low evenness (average GPP high evenness treatments: 69.8±3.9 mg O^2 h^{-1}, low evenness 4 spp= 74.1±3.9, n=32). Although not significant, this trend to reduce performance at high evenness is someway surprising. We are not aware of any other experimental study that have examined the effects of evenness on the productivity of macroalgal assemblages. Only Arenas et al., (2009) in an observational approach using communities inhabiting boulders from rock-pools found a positive but weak effect of evenness on NPP. In terrestrial ecosystems positive effects of evenness on productivity have been recorded in several experimental studies (Kirwan et al., 2007; Stevens & Carson, 2001; Wilsey & Potvin, 2000). Our complete absence of effect could result from the strong prevalence of identity effects in our assemblages. When species interactions drive biodiversity effects, increasing evenness should enhance complementary use of resources enhancing ecosystem performance however if identity effects is the prevailing diversity mechanisms, probably evenness has few functional influence (Kirwan et al., 2007).

The absence of strong effect of overyielding (just one interaction were observed) and the lack of effect by our experimental treatments (species richness or evenness) also confirm the strong prevalence of identity effects, since complementarity in resource use and facilitation are two mechanisms usually cited as the inductors of the phenomenon of "overyielding" (Fridley, Carolina, & Hill, 2001).

Identity effects shaped very strongly all the responses examined in the study. (Hooper,1998) and Hooper & Vitousek (1998) in some of the early experimental studies on biodiversity ecosystem functioning relationships found that composition, i.e. species identity, explain much more than richness in the effect on ecosystem processes. Identity effects are also have been also identified as very relevant when considering other community traits like resistance to invasion (Arenas,et al, 2006; Vaz-Pinto,et al. 2014). Our multiple regression approach allowed us to identity which species were more relevant in terms of their impact on the assemblage's productivity.

In fact assemblages with *Sargassum* generally showed higher rates of respiration, maximum net productivity and Gross primary productivity than others. The opposite trend was found on assemblages containing *Bifurcaria bifurcata.* In this case the more abundant was *B. bifurcata* the lower the rates of productivity. Interestingly *Sargassum*

muticum is a Japanese invasive species in the European Atlantic shores and in certain areas the species is displacing the very abundant native *Bifurcaria bifurcata* (Sánchez & Fernández, 2005). These changes in the identity are modifying severely primary productivity cycles in invaded areas as Vaz-Pinto et al., (2014) found recently in Portugal. Thus our assemblages mimicked patterns that are already described in other studies and in natural assemblages.

Our results regarding Overyielding in NPP were also surprising. Assemblages including *Mastocarpus* identity exhibit clearly "underyielding" when associated with the other 3 species, indicating a negative sampling effect which could result from inhibitory interactions among species; Usually treatments which have the highest diversity have a greater probability of being dominated by the most productive species of the entire species pool of selection effect (Wardle, 1999), in this case we observed the opposite behavior, because this plates also contained *Bifurcaria bifurcata*, and as we described above, it was the identity with lower rates in all the studied surrogates. This disparity in our results suggest that focusing on individual performances can be misleading, because single species behavior is not always correlated with their performance in multispecies assemblages. Hence, it is possible that the mixture performs worse than the best monoculture for each individual function (unifunctional underyielding), but still experiences multifunctional overyielding because the identity of the best monoculture species switches between functions (Gamfeldt, & Hillebrand, 2008). It is worthy to note that, the dominant species identity that resulted in some level of performance for one process or function did not necessarily also result in the highest level of functioning for other processes or functions.(Orwin et al., 2014)

The experimental approach used in this Master Thesis allowed to create all the experimental treatments required to disentangle direct and interaction effects among the ecological drivers of interest. Those direct and interactive effects are almost impossible to examine on natural assembles. Furthermore observational studies cannot establish causality among drivers and effects. Also synthetic assemblages like those used here are tractable systems and may also be a realistic resemblance of natural systems. Our approach was unable to detect some of the effects of species richness and evenness suggested in literature. If existing, the intensity of these effects were so subtle that most likely were overridden by the strong effects of identity displayed by some of the species included in our design. Probably only longer experiments, where species are able to adjust each other, and the use of a larger species pool would allow species to develop strong positive interactions and reduce the prevalence of large identity effects.

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