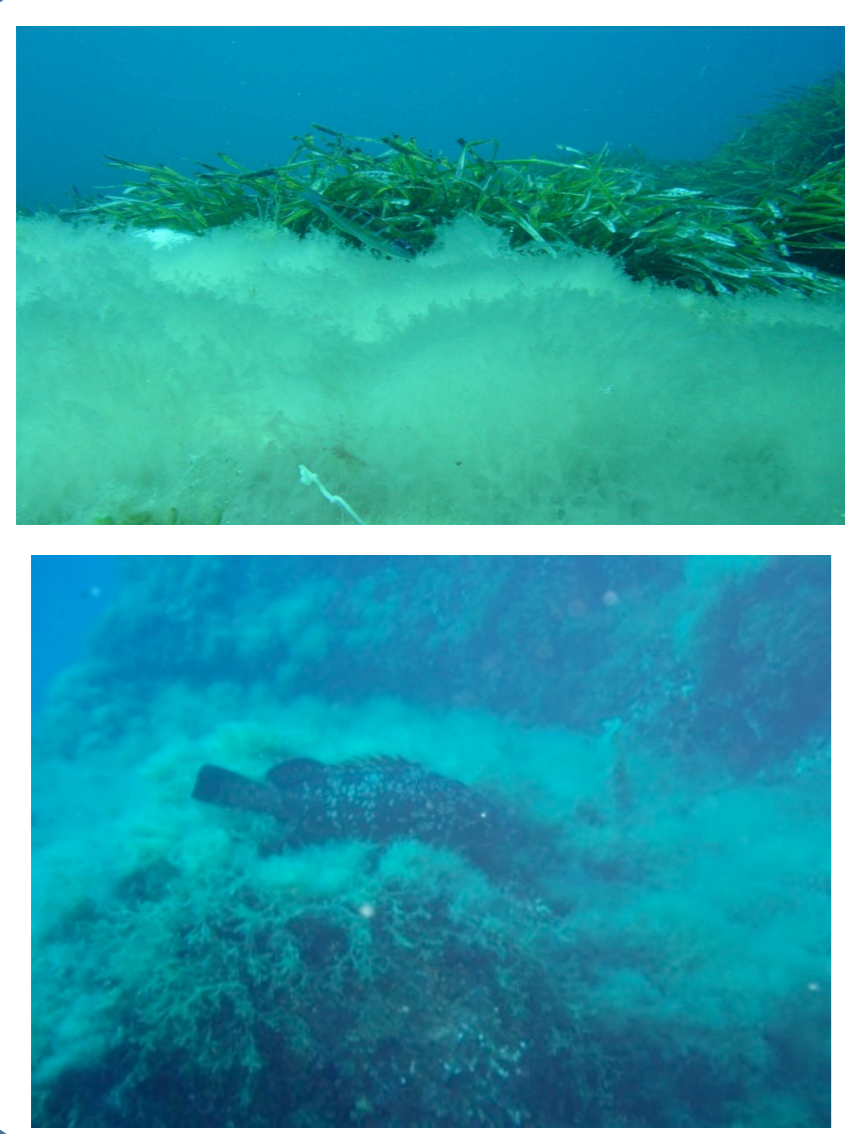


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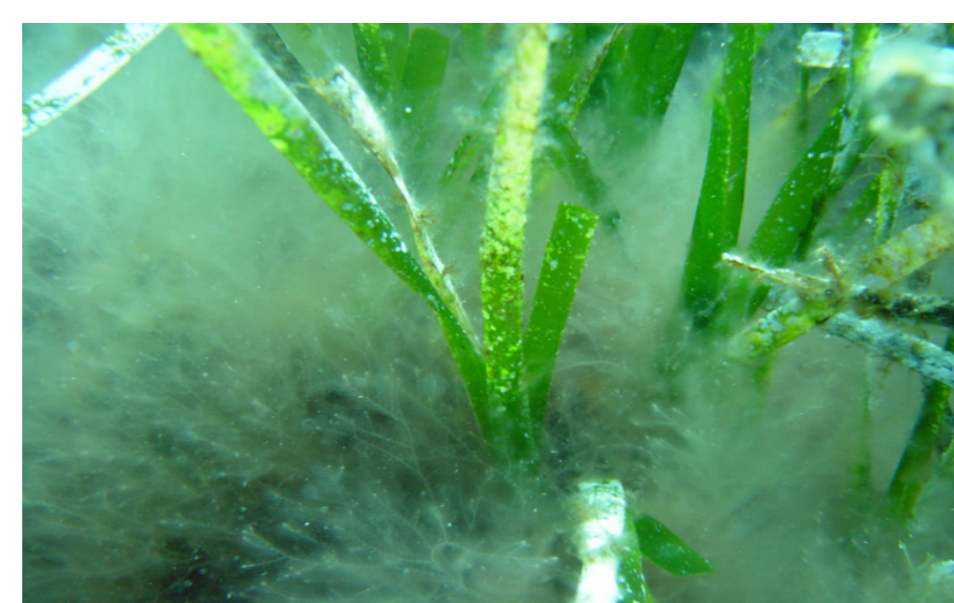
INTRODUCTION- AIMS

Species invasions are increasing worldwide producing ecological effects on native communities by inducing changes in biodiversity and ecosystem functioning that result in economical costs. Most studies deal with biodiversity changes on few species or assemblages. MPAs are not protected against species invasions, and worldwide fragmented knowledge on ecosystems responses is provided. In this study a new approach integrating changes at community level and functional biodiversity after macroalgal invasions over seagrass beds have been applied. Changes in functional biodiversity of invaded systems have been revealed after application of stable isotope as tracers of matter and energy fluxes on key species. Modifications of trophic strategies at benthic species have been encountered as an ecological adaptation to invasion. Several case studies at algae, invertebrates and fishes applying biomarkers of oxidative stress have evidenced physiological responses of different taxa at invaded mats. Activation of defense mechanisms of enzymes after production of reactive oxygen species ROS is found in most studied species. Combination of different techniques has been proven to be providing better holistic comprehension of mechanisms underlying species invasions. An array of indicators of change at temperate mediterranean ecosystems is presented with different approaches to assess coastal species responses.

RESULTS & DISCUSSION- INDIVIDUAL RESPONSES

Ecological markers: Reactive Oxygen Species ROS (oxidative stress biomarkers) and Stable Isotopes

Invasive *Lophocladia lallemandii* epiphytes *Posidonia oceanica*



Oxidative damage markers and antioxidant system in *Posidonia oceanica*

	<i>P. oceanica</i>	<i>P. oceanica</i> + <i>L. lallemandii</i>
MDA (nmol/mg prot)	5.75 ± 0.27	8.28 ± 0.80*
Protein carbonyl derivatives (nmol/mg prot)	4.57 ± 0.47	9.47 ± 0.89*
GSH (nmol/mg prot)	76.7 ± 4.7	101 ± 6*
Catalase (mK/mg prot)	71.2 ± 6.3	91.3 ± 6.9*
GPX (nKat/mg prot)	0.90 ± 0.09	1.71 ± 0.17*
SOD (pKat/mg prot)	7.67 ± 0.36	9.73 ± 0.45*
H ₂ O ₂ (pmol/min-mg prot)	3.73 ± 0.36	4.62 ± 0.42*

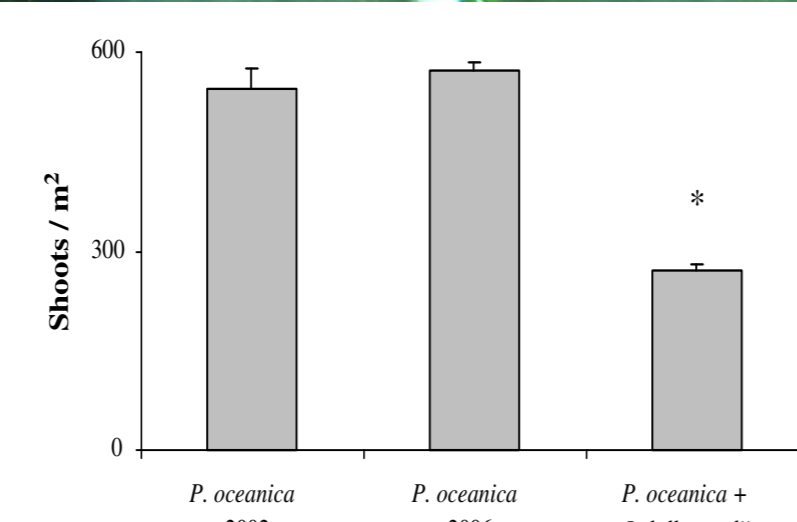


Fig. 1. Mean shoot densities in *Posidonia oceanica* meadows determined in October 2002 and 2006 in the control station and in 2006 in the epiphytized station. Values are expressed as mean shoot densities/m² ± s.e.m. N = 10 samples in each location. (*) Indicates significant differences respect control station in 2006. One-way ANOVA, p < 0.05.

Markers of oxidative damage (MDA concentration protein carbonyl derivatives), GSH concentration, antioxidant enzyme activities (Catalase, GPX and SOD) and H₂O₂ generation in the leaves of *Posidonia oceanica* not epiphytized and epiphytized by *Lophocladia lallemandii*. Values are expressed as means ± s.e.m., n = 8 samples in each location. (*) Indicates significant differences. One-way ANOVA, p < 0.05.

Bryozoan *Reteporella grimaldii*

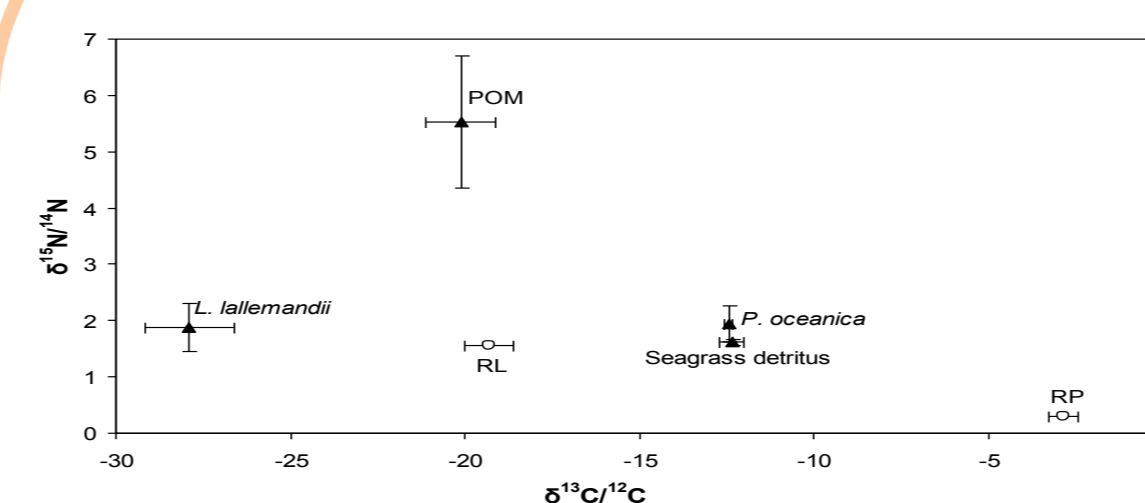
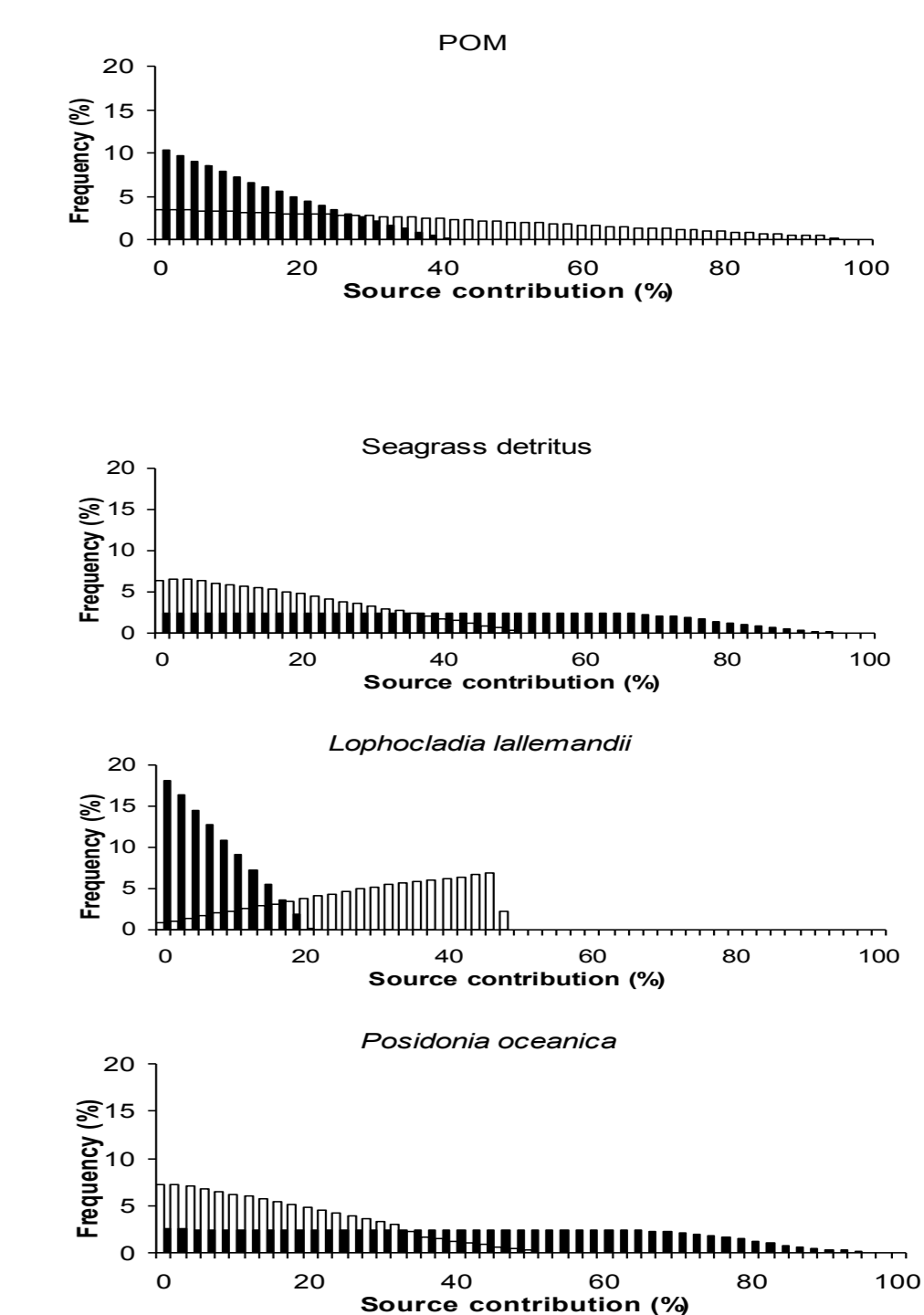
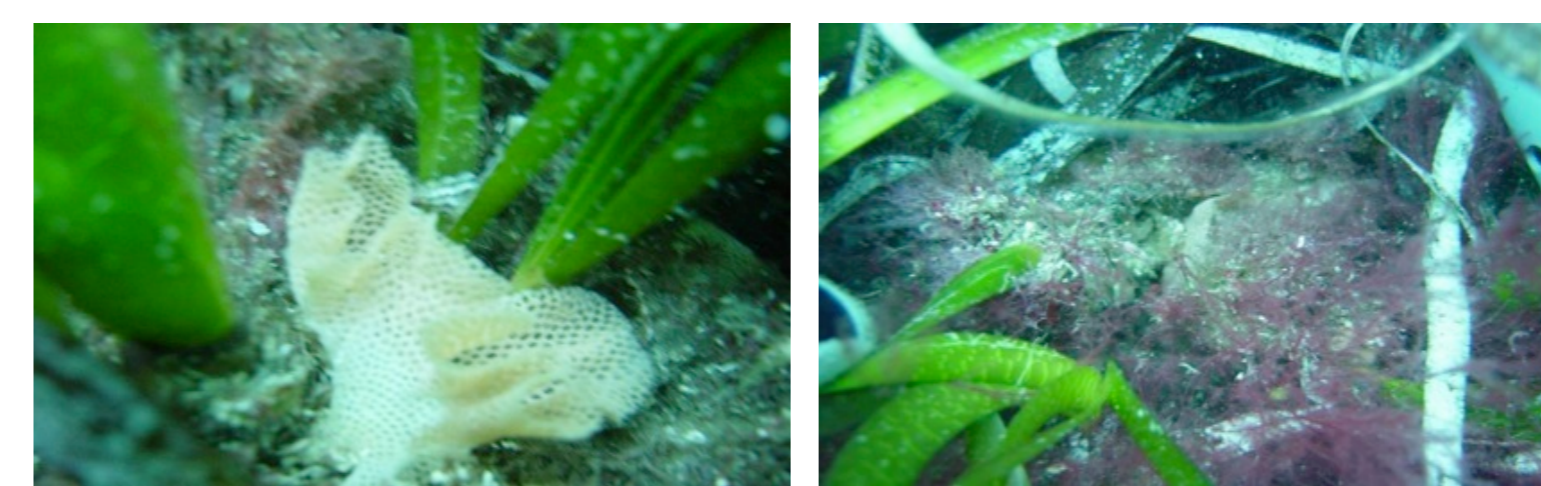


Table 2. Antioxidant enzyme activities and MDA concentration in *Reteporella grimaldii* (n = 8 colonies)

	<i>Reteporella</i> noninvaded	<i>Reteporella</i> + <i>Lophocladia</i>
Catalase	65.5 ± 7.3	113 ± 9*
SOD	3.61 ± 0.34	4.61 ± 0.51*
GPX	4.31 ± 0.15	5.2 ± 0.30*
MDA	0.80 ± 0.06	1.08 ± 0.03*

Catalase (mK/mg prot), SOD (pKat/mg prot), GPX (nKat/mg prot) activities, and MDA concentration (nmol/mg prot) in *Reteporella grimaldii* invaded and noninvaded by *Lophocladia lallemandii*. (*) Significant differences between plots (one-way ANOVA, P < 0.05). Mean ± SEM



Assessing effects of macroalgae invasions

Echinoderm *Paracentrotus lividus*

Antioxidant enzyme activities.

	Control	<i>P. oceanica</i>	<i>C. racemosa</i>	<i>L. lallemandii</i>
Catalase (K (s ⁻¹)/mg protein)	101 ± 25	104 ± 32	123 ± 29	124 ± 35
Superoxide dismutase (pmol/min/mg protein)	0.95 ± 0.08	0.97 ± 0.10	1.08 ± 0.24	1.24 ± 0.30*
Glutathione peroxidase (nmol/min/mg protein)	1.11 ± 0.11	1.19 ± 0.11	1.38 ± 0.18*	1.54 ± 0.21*
Glutathione reductase (nmol/min/mg protein)	2.85 ± 0.84	2.78 ± 0.78	5.25 ± 1.34*	7.15 ± 1.67*#

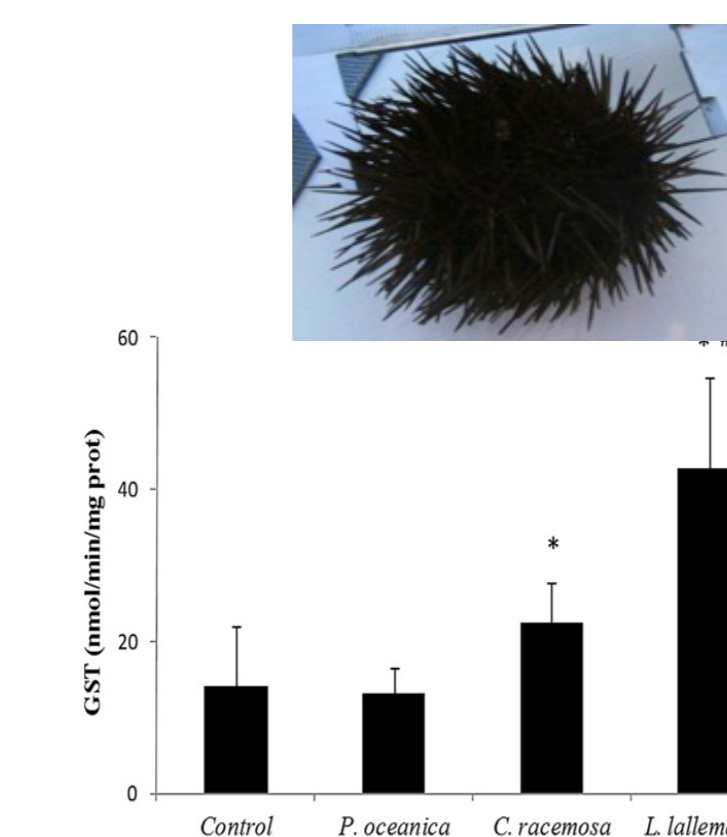


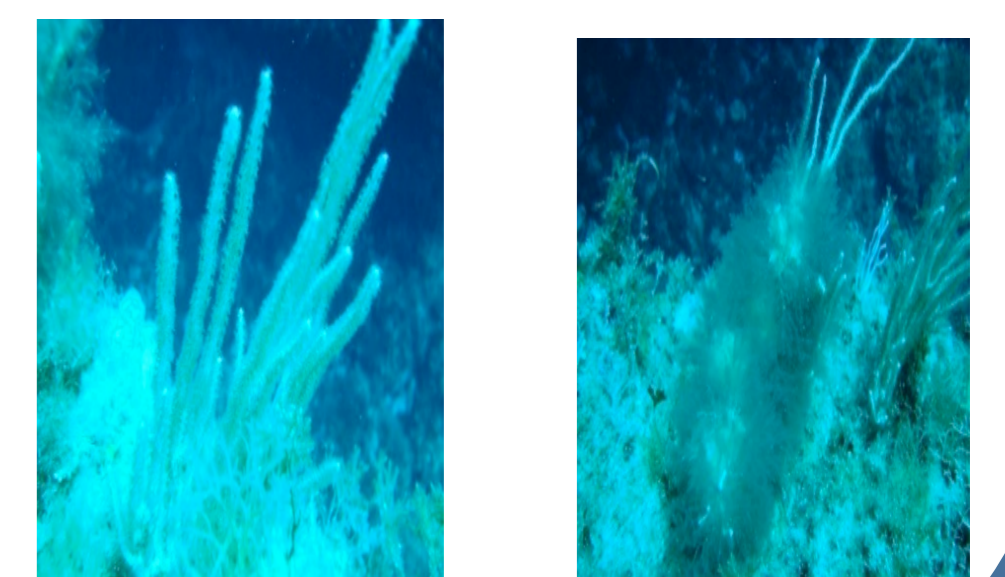
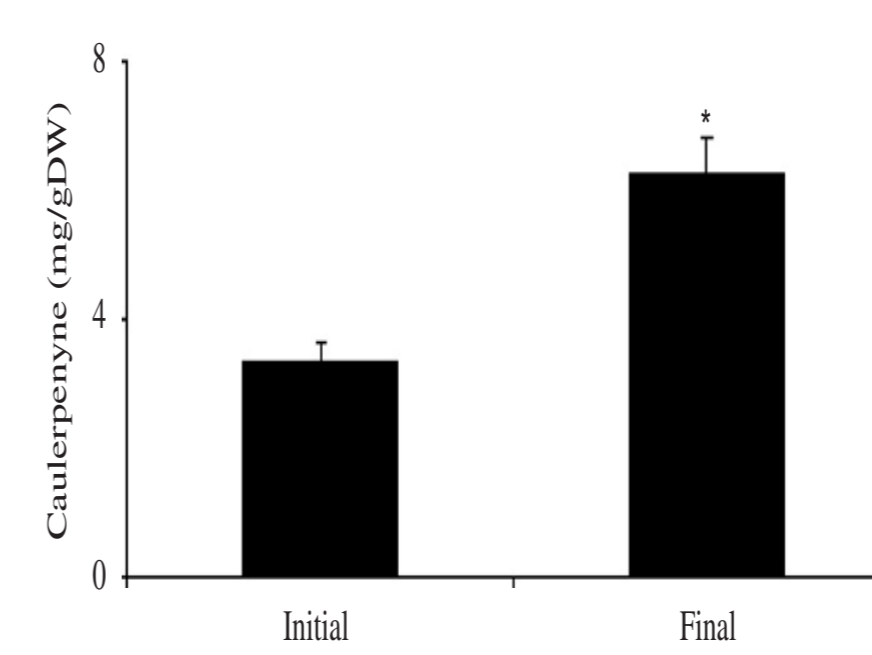
Fig. 1. Glutathione S-transferase (GST) activity in gonads of *Paracentrotus lividus*. Values were computed as means ± S.D. One-way ANOVA. Significant differences respect control and *Posidonia oceanica* groups. # Significant differences between *C. racemosa* and *L. lallemandii* groups. p < 0.05.

Enzymatic activities determined in the gonad homogenates of the sea urchin *Paracentrotus lividus*. *p < 0.05 when compared with control and *P. oceanica* (one-way ANOVA analysis). #p < 0.05 when compared with *C. racemosa*. Values are expressed as mean ± S.D.

Gastropod *Bittium reticulatum*

Antioxidant enzyme activities and MDA levels in *Bittium reticulatum*

	<i>P. oceanica</i>	<i>C. taxifolia</i>
CAT (K/mg prot)	43.1 ± 5.1	44.9 ± 4.5
GPx (nKat/mg prot)	10.8 ± 1.0	13.9 ± 0.8*
GR (nKat/mg prot)	15.1 ± 2.1	23.7 ± 2.3*
GST (nKat/mg prot)	211 ± 20	278 ± 24*
MDA (nmol/mg prot)	5.23 ± 0.62	6.05 ± 0.7*



RESULTS AND DISCUSSION- ECOSYSTEM RESPONSES

Invasive macroalgae generate trophic web changes

Spondyliosoma cantharus



Nested multifactorial analysis of variance of antioxidant enzyme activities and MDA concentration in *Spondyliosoma cantharus*.

df	CAT (MS)	SOD (MS)	EROD (MS)	GST (MS)	MDA (MS)
Invaded	1 95.284***	0.1989*	98.918***	312767***	0.01606a
Site (invaded)	2 9388	0.0073	1660	29076	0.00013
Error	44 135.677	0.0473	5657	19597	0.00402

MS means square, ***p < 0.001 **p < 0.01 *p < 0.05. a means p = 0.052.

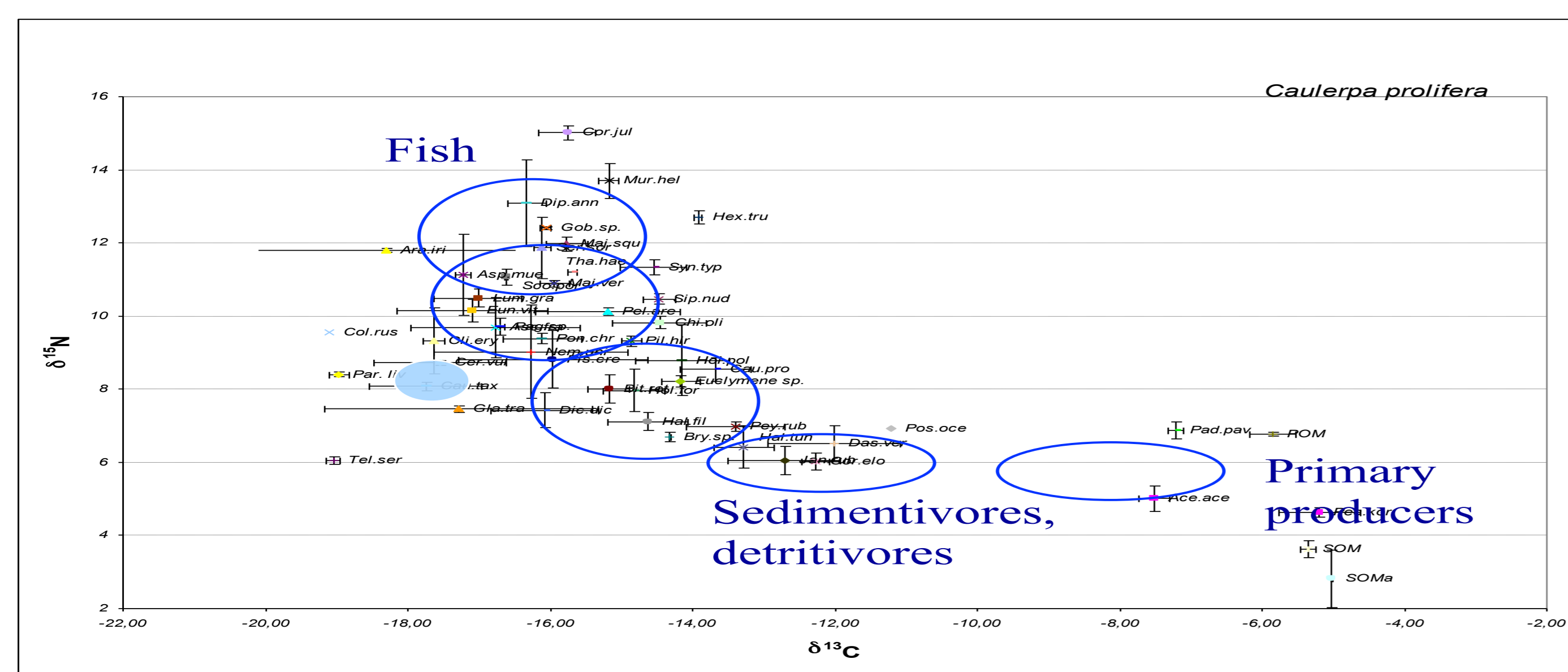
Nested multifactorial analysis of variance of isotopic composition (δ¹³C, δ¹⁵N and C:N ratio).

df	δ ¹³ C	δ ¹⁵ N	C:N
Invaded	1 14.55***	11.91***	0.045***
Site (invaded)	2 0.86**	2.32	0.012**
Error	48 0.12	0.89	0.001

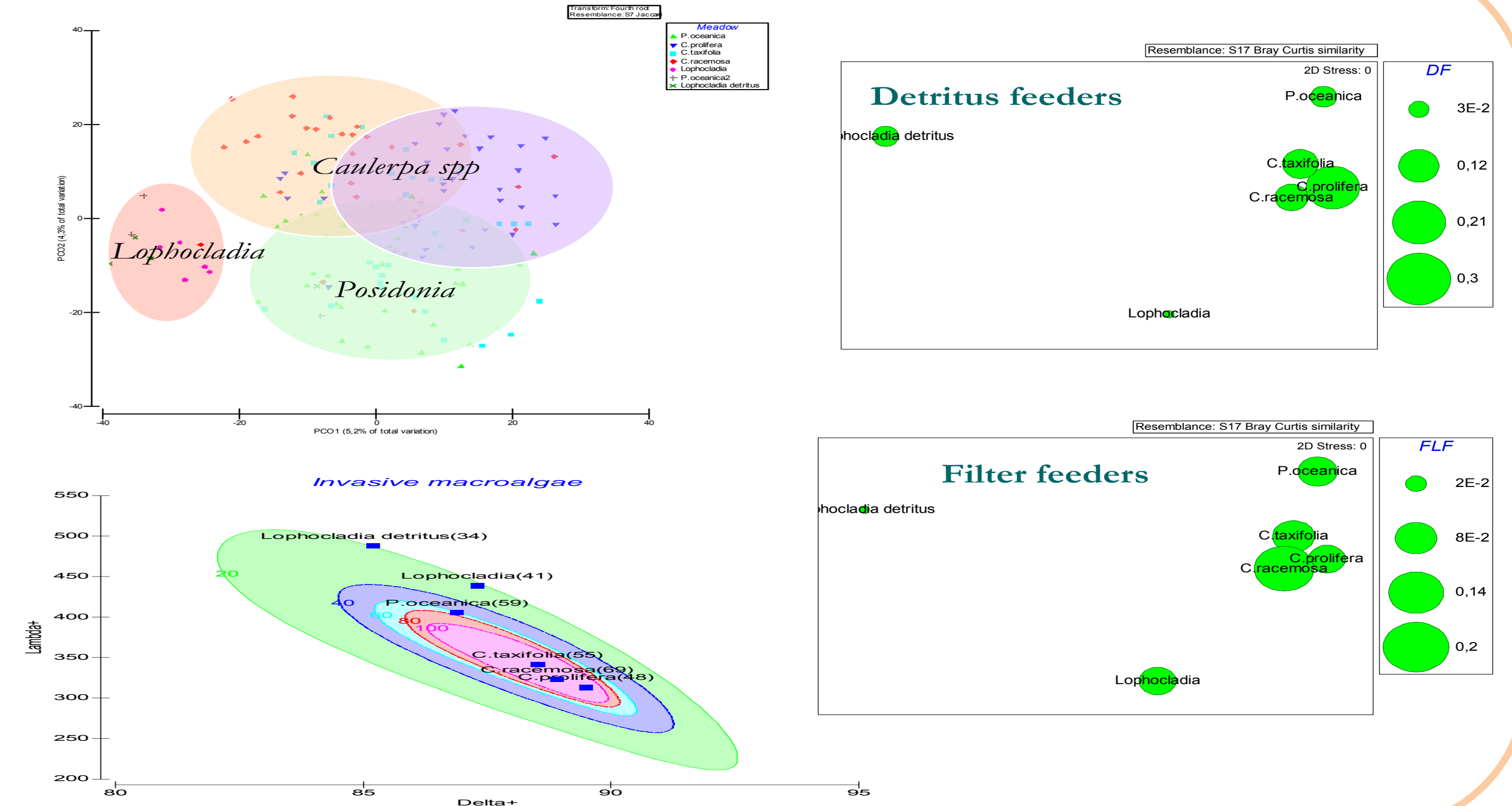
MS means square, ***p < 0.001 **p < 0.01 *p < 0.05.

Combination of biomarkers and stable isotope signals corroborate individual effects at physiological levels of various taxa. Ecosystems responses are generated through food web alterations and changes in community composition

Energy fluxes- Trophic web changes



Stable isotopes demonstrate functional changes after macroalgal invasions at MPAs



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