Research article

Effects of an experimental resource pulse on the macrofaunal assemblage inhabiting seagrass macrophytodetritus

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ABSTRACT. Physical disturbances and resource pulses are major structuring drivers of terrestrial and aquatic ecosystems. The accumulations of exported dead leaves from the Neptune grass, *Posidonia oceanica* (L.) Delile are ephemeral and highly dynamic detrital habitats offering food sources and shelter for vagile macrofauna community. These habitats are frequently subject to wind and storms which can add "new" detrital material to previous accumulations; these can be defined as resource pulses and could potentially impact the associated macrofauna. This study assesses the impact of an experimental resource pulse on the macrofauna associated with exported *P. oceanica* litter accumulations. The experimental design consisted of two pulse treatments (the addition of dead leaves with and without the associated fauna), and two controls (one procedural, and one total control), where the added material was left underwater for 14 days. Invertebrates then present in the sampled detritus were all identified and counted. Our data suggest that the responses of these invertebrates to resource pulses present intermediate characteristics between aquatic and terrestrial ecosystems responses. Inputting a moderate amount of dead *P. oceanica* leaves into experimental mesocosms had a non-negligible impact and rapidly affected the macrofauna community. Specialist detritivores species were boosted while herbivore/detritivore species dramatically decreased. Predators also showed a modest but significant density increase, demonstrating the fast propagation of the pulse response throughout the entire community and through several trophic levels. Strict hypoxia-tolerant species were also only observed in the treated mesocosms, indicating the strong influence of resource pulses on physico-chemical conditions occurring inside litter accumulations.

KEYWORDS. Resource pulses, *Posidonia oceanica*, macrofauna, experimental ecology, macrophytodetritus.

Introduction

In terrestrial, estuarine and marine ecology, disturbances are regarded as playing a central structuring role in ecosystems (GILLER 1996; LAKE 2000). Resource pulses have recently been defined as "rare, brief and intense episodes of increased resource availability in space and time" (OSTFELD & KEESING 2000; YANG *et al.* 2008). They can take place in many different ecosystems (e.g., massive floods in arid ecosystems or floodplains, dead leaf litter input in mangroves or forests, massive emergence of insects, mass seeding events or storm driven nutrient runoffs). Some pulses are recurrent in time (e.g., terrestrial

leaf litter input in autumn in decidual temperate forests) and others are purely stochastic in occurrence, duration, and amplitude (HOLT 2008; NOWLIN *et al.* 2008; LEVI *et al.* 2015). Pulses can be caused by different factors: (1) climatic or environmental causes, (2) temporal accumulation and release, (3) spatial accumulation and release, (4) outbreak population dynamics (YANG *et al.* 2008; LEROUX & LOREAU 2012). Studying these potentially structuring events is an important topic in the perspective of building a better collective understanding of the controversial climate change impact on extreme stormy events in the Southern European seas (MÖLTER *et al.* 2016).

The endemic and highly productive Neptune grass, *Posidonia oceanica* L. Delile, 1813, covers between 25.10³ km² and 45.10³ km² of the Mediterranean coastal area, which represents 1–2 % of the Mediterranean Sea (PASQUALINI *et al.* 1998). Detrital pathway is considered as a very important pathway for the incorporation of *P. oceanica* organic matter into the coastal food webs as a large amount of the foliar primary production (up to 90 %) can end up in the detrital compartment (MATEO & ROMERO 1997; ROMERO *et al.* 1992; VALENTINE & HECK 1999). These dead leaves, once exported out of the meadow to unvegetated places (e.g., bare sand patches) form accumulations associated with bacteria, fungi, microalgae, macroalgae, living leaves, uprooted rhizomes, dead organisms and fine sediment ("exported macrophytodetritus accumulations", hereafter EMAs) (ANESIO *et al.* 2003; BOUDOURESQUE *et al.* 2006; LEPOINT *et al.* 2006; LEMKE *et al.* 2007; MASCART *et al.* 2015a).

These EMAs can be considered as constantly subject to the influence of pulsed perturbation (storm, wind events) and resource pulses ("new litter" input) affecting both the resource availability and habitat physico-chemical conditions in the litter. EMAs are known to serve as shelter and food source for a variety (80–115 species) of vagile macroinvertebrates (size > 500 µm) (GALLMETZER *et al.* 2005; DIMECH *et al.* 2006; LEPOINT *et al.* 2006; STURARO *et al.* 2010; REMY 2016) and are comparable to macrophytodetritus habitats found in Mediterranean estuaries, brackish lakes or lagoons (*Phragmites* accumulation, for example) (MANCINELLI *et al.* 2005).

Since these invertebrates live, reproduce and feed in EMAs all year long, resource pulses might potentially play a strong structuring role for this vagile macrofauna community.

To our knowledge, this is the first study that experimentally explores the impact of resource pulses on *P. oceanica* exported macrophytodetritus accumulations and specifically on the associated macrofauna. We aimed to answer the following questions: (1) Can a clear response of the macrofauna to litter pulses be identified in terms of density and/or diversity? (2) Does the presence/absence of the animal fraction of the litter influence the macrofauna's response to a resource pulse? (3) Do pulsed events maintain biodiversity inside EMAs?

Material and methods

Sampling site

The *in situ* experiment was carried out in 2014, from October 7th to October 21st near the STARESO (STAtion de REcherches Sous-marines et Océanographiques) (University of Liège) research station in Calvi Bay (42°35′ N; 8°43′ E) in Corsica. The sampling site was situated at 10 m depth on a 200 m² bare coarse sediment patch regularly covered with dead *P. oceanica* litter, just next to the northwestern part of Punta Oscelluccia (Figure 1). During the whole experimentation period, a constant salinity of 38 and temperatures between 22.8 and 23.1 °C were observed.

Experimental setup

Since resource pulses occurring in EMAs consist mainly of dead leaf input, two controls and two treatments (N=5) were designed to assess the impact of "fresh" litter supply on this community. Each mesocosm (21 L) consisted of a transparent PVC box (20 x 30 x 35 cm) with a 38 µm nylon mesh to

exclude predation by fishes, but allowing free water exchange. The first procedural control, hereafter referred as **C**, (N=5) and comprised PVC mesocosms placed on the EMA without addition of dead leaves. To assess the impact of mesocosms on the macrofauna community, a total control, hereafter referred as T_{final}, (N=6) was taken at the end of the experiment. This T_{final} was a sample of the "natural" community present in the EMA outside of the mesocosms, on a 20 x 30 cm surface, to compare it to the procedural control. The first treatment (N=5), hereafter referred as "**T-defaun**", was composed of PVC mesocosms containing a given additional supply of "defaunated litter", to assess the potential impact of purely vegetal detrital supply. The second treatment (N=5), hereafter referred as "**T-fauna**", was composed of PVC mesocosms each containing a given additional supply of "natural litter", comprising the vegetal detrital material and the associated fauna present inside (fauna was not characterised in this "additional litter", but assumed to be similar to what was identified in the T_{final} samples). The litter intended for being added to the two treatments was sampled 24 h before the beginning of the experiment; half of it was defaunated and the other half was kept in 750 L storage. Defaunation was achieved by rinsing the
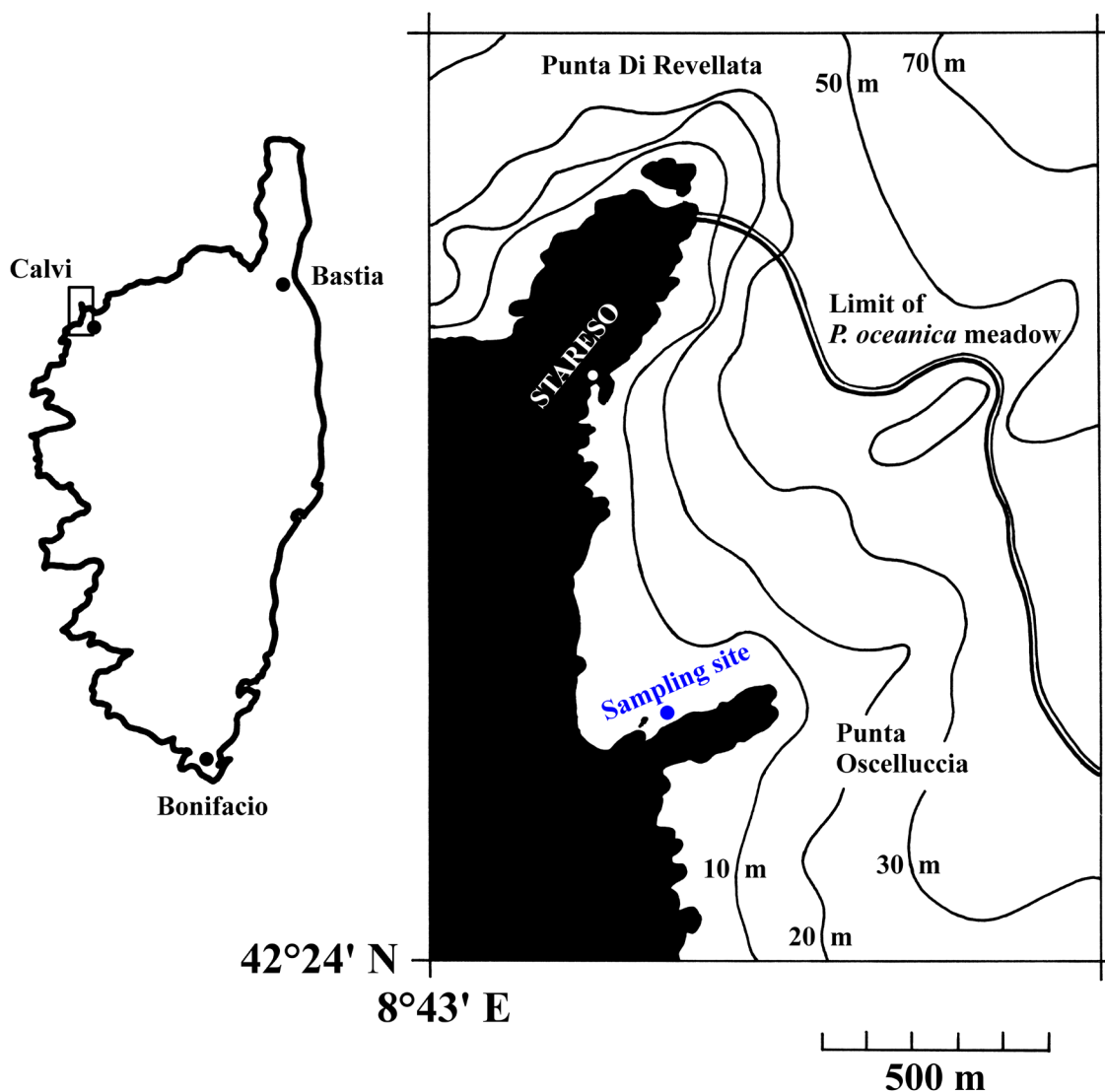

Figure 1 – Location of the STARESO research station (University of Liège) and the sampling site in the Bay of Calvi indicating the lower limit of the *P. oceanica* meadow (modified after GOBERT 2002).

sampled litter on a 10 mm sieve stacked on a 500 µm nylon-mesh sieve in order to separate the detrital material from the macrofauna.

The 15 mesocosms were placed at a depth of 9 m at the sampling site (Figure 1) and on an adjacent EMA situated directly north-east. Treatments and controls were placed randomly to prevent any bias from a potential position effect. Just before starting the experiment, 205 ± 3 g (wet mass) of litter were placed inside each mesocosm for both treatments. This amount of litter represented visually a doubling (+100 %) of the amount of litter naturally present at the sampling site at the beginning of the experiment. This was considered as corresponding to a "moderate" resource pulse in terms of litter input (see Remy 2016). Mesocosms were anchored on the sediment with marine steel poles and weights to prevent any movement of the setup and avoid dead leaf movements in or out of the mesocosm-sediment interface. This anchoring did not prevent the possibility of colonisation/escaping by macrofauna inhabiting the surrounding litter. After 14 days, all mesocosms were sealed underwater and brought back to the lab for further processing.

Sample processing

In the laboratory, macrofauna was separated from the dead *P. oceanica* leaves using freshwater on successive 10 mm and 500 µm sieves for optimal and handy separation. The 500 µm fraction was preserved in a 4 % formaldehyde seawater solution and kept for further analysis. After 48 h of formaldehyde fixation, the 4 % formaldehyde seawater solution was replaced by distilled water for final sorting. Specific identification and counting of all individuals was effected using a stereomicroscope (Zeiss Stemi 2000-C). Animals were then stored in 99.8 % ethanol. The remaining defaunated detrital fraction was dried at 60 °C for 5 days and then weighed. Density data are, therefore, expressed per gram of dried litter present in the mesocosm at the end of the experiment. All presented data are presented in the form of mean ± standard deviation.

Data analysis

A non-metric multidimensional scaling (n-MDS) coupled with an ANOSIM analysis was performed on the square root-transformed density data of all the sampled species. The n-MDS analysis is based on an iterative procedure. For a given number of dimensions, many iterations are computer-generated. Each iteration corresponds to a possible ordination. A "stress" value is calculated for each attempt, which is in fact a way to express the error associated with the ordination procedure, i.e., the mismatch between theoretical inter-sample similarities and real similarities between those samples, measured on the ordination. The iteration that shows the lowest stress value is then considered as being the best way to map the samples. In this study, we performed a 2D n-MDS using the "MDS" routine of PRIMER v6.1.13 for Windows. The resemblance matrix was built by calculating Bray-Curtis similarity. The number of iterations was set to 99, and the minimum stress level at 0.01. ANOSIM analysis is widely used in ecology and has some analogies with ANOVA-like analysis; however, it is used to evaluate directly a dissimilarity matrix rather than raw data. Together with the complementary n-MDS, this analysis is adapted to differentiate two or more groups for multivariate data.

A SIMPER analysis was also performed. The purpose of one-way SIMPER (SIMilarity PERcentage) analyses is to highlight the variables (here, species) best explaining the similarity between samples forming a single group and dissimilarity between two groups. The program calculates Bray-Curtis similarity between all pairs of items of a group. In parallel to this similarity breakdown procedure, the program also performs an inter-group dissimilarity breakdown. This procedure is very similar to the similarity breakdown described above but is based on the breakdown of the total inter-group dissimilarity, giving the relative contribution (expressed in %) of each variable (here, species) to the dissimilarity between groups.

The widely used Shannon-Wiener index (hereafter, H') and Simpson index (hereafter, 1-λ') were also calculated for each sample.

Classical statistical analysis (ANOVA and MANOVA) were performed using R. nMDS, ANOSIM and SIMPER analyses were performed using PRIMER 6.1.13 (CLARKE & GORLEY 2006) with PERMANOVA additional software (ANDERSON *et al.* 2008b). A significance level of $p < 0.01$ was used in all tests to limit "Type I error".

Graphs were built with PRIMER 6.1.13 and GraphPad PRISM 6.01 software for Windows (GraphPad Software, San Diego, USA).

Results

At the end of the experiment, there was no significant difference in litter biomass sampled inside the mesocosms composing the four treatments (1-way ANOVA, $p = 0.5024$).

A total of 3503 individuals from 36 species were sampled at the end of the 14 days in situ experiment, representing a mean global density of 4.4 ± 1.4 ind. gDM^{-1}. A total of 25 species were sampled in the total control (T_{final}), 26 species in the control (hereafter referred as "**C**"), 35 species in the defaunated litter treatment (**T-defaun.**) and 36 species in the litter + fauna treatment (**T-fauna**).

Arthropods (24 species) were extremely dominant, representing on average 85.6 ± 2.8 % (3.54 ± 1.2 ind. gDM^{-1}) of the whole sampled community (Table 1). Within arthropods, amphipods were the most dominant taxa, representing 78.2 ± 12.5 % of arthropod abundance, followed by decapods representing 9.6 ± 6.1 %, isopods representing 7.3 ± 3.3 % and leptostraceans representing 4.7 ± 3.8 % (Table 1). The amphipod *Gammarella fucicola* (Leach, 1814) was typically the most abundant species, representing by itself 62.9 ± 17.6 % of the amphipods and 44.0 ± 20.2 % (1.9 ± 1.3 ind. gDM^{-1}) of the total macrofauna. Arthropods were followed by annelids (6 species) representing 8.2 ± 2.2 % (0.4 ± 0.2 ind. gDM^{-1}), mollusks (4 species) representing 4.2 ± 1.7 % (0.2 ± 0.1 ind. gDM^{-1}) and echinoderms (2 species) representing 2.03 ± 1.33 % (0.1 ± 0.04 ind. gDM^{-1}) of the community.

A preliminary MANOVA (1-way MANOVA, $p > 0.95$) demonstrated that the total control (T_{final}) was not different from the procedural control (C) for either global density, H', 1-λ' or for diversity at high level taxa and at specific level. Since the "mesocosm effect" was negligible, and in order to simplify future analyses, T_{final} was not included any further in the next MANOVAs. Another MANOVA analysis showed that global density and high taxonomic level data presented no significant differences (1-way MANOVA, $p > 0.06$) according to the treatment. On the other hand, density data of amphipods, decapods and leptostraceans presented highly significant differences according to the treatment (1-way MANOVA, $p < 0.0005$ and ANOVA, $p < 0.0002$).

The n-MDS analysis based on the squared density data of the 36 species showed clearly this pattern, forming two main significant (ANOSIM, $p < 0.008$) groups; one of them being the "Control group" composed of C replicates and T_{final} replicates, and the significantly different other group being the "Pulse cluster" composed of T-defaun. and T-fauna replicates (Figure 2).

The Shannon-Wiener diversity index (**H'**) and Simpson evenness index (**1-λ'**) also showed important variations from one treatment to the other. H' index showed a minimum value of 2.42 ± 0.17 in C, and values of 2.94 ± 0.16 and 3.01 ± 0.15 in T-defaun. and T-fauna respectively, indicating a lower diversity in C. 1-λ' index showed a minimum value of 0.86 ± 0.03 in C, and a maximum value of 0.94 ± 0.01 in both T-defaun. and T-fauna, indicating that a few species were highly dominant in all treatments and that their dominance increased in T-defaun. and T-fauna.

Multivariate analysis (1-way MANOVA, $p < 0.0001$ and ANOVA, $p < 0.0001$) showed that both H' and 1-λ' were significantly influenced by the treatment.

TABLE 1

Density of macrofauna found in experimental treatment. Results are mean ± standard deviation.

	T_{final}			Control			T-defaun			T-fauna		
	Avg.	±	SD	Avg.	±	SD	Avg.	±	SD	Avg.	±	SD
Gammarella fucicola	3.28	±	0.51	3.5	±	1	1.14	±	0.19	1.08	±	0.19
Gammarus aequicauda	0.13	±	0.04	0.11	±	0.06	0.7	±	0.18	0.72	±	0.12
Melita hergensis	0.17	±	0.12	0.19	±	0.16	0.13	±	0.08	0.11	±	0.04
Apherusa chiereghinii	0.03	±	0.04	0.01	±	0.02	0.01	±	0.01	0.03	±	0.02
Leptocheirus guttatus	0.01	±	0.01	0.01	±	0.01	0.01	±	0.02	0.02	±	0.03
Microdeutopus chelifer	0.21	±	0.11	0.25	±	0.2	0.11	±	0.06	0.04	±	0.03
Stenothoe monoculoides	0.01	±	0.02	0.02	±	0.03	0.01	±	0.01	0.01	±	0.01
Lysianassa costae							0.02	±	0.03	0.02	±	0.03
Nototropis guttatus	0.05	±	0.03	0.06	±	0.04	0.03	±	0.02	0.09	±	0.04
Maera grossimana	0.01	±	0.01	0.01	±	0.01	0.01	±	0.02	0.01	±	0.01
Dexamine spinosa	0.03	±	0.03	0.02	±	0.02	0.01	±	0.02	0.02	±	0.03
Athanas nitescens	0.02	±	0.03	0.03	±	0.04	0.13	±	0.02	0.15	±	0.06
Palaemon xiphias	0.04	±	0.02	0.05	±	0.04	0.08	±	0.01	0.18	±	0.07
Alpheus glaber	0.01	±	0.01	0	±	0.01	0.02	±	0.05	0.03	±	0.02
Galathea intermedia	0.02	±	0.03	0.01	±	0.02	0.01	±	0.02	0.07	±	0.03
Anapagurus chiroacanthus							0.07	±	0.06	0.07	±	0.03
Pisa tetraodon	0.01	±	0.01	0.01	±	0.01				0.01	±	0.01
Liocarcinus navigator	0.02	±	0.03	0.02	±	0.04	0.01	±	0.01	0.01	±	0.02
Jaera nordmanni	0.13	±	0.09	0.15	±	0.12	0.2	±	0.05	0.24	±	0.04
Idotea balthica							0.02	±	0.02	0.02	±	0.03
Stenosoma lancifer	0.02	±	0.03	0.02	±	0.04	0.03	±	0.04	0.01	±	0.02
Cymodoce truncata							0.02	±	0.01	0.02	±	0.02
Nebalia strausi							0.19	±	0.08	0.24	±	0.06
Achelia echinata				0.01	±	0.01	0.01	±	0.02	0.01	±	0.01
Platynereis dumerilii	0.24	±	0.13	0.3	±	0.21	0.08	±	0.04	0.15	±	0.03
Polyophthalmus pictus	0.16	±	0.04	0.15	±	0.04	0.08	±	0.05	0.08	±	0.05
Hesiospina autantiaca							0.03	±	0.02	0.04	±	0.03
Chrysopetalum debile							0.03	±	0.02	0.05	±	0.04
Protodorvillea kefersteini							0.02	±	0.02	0.02	±	0.02
Hesione panthernia							0.01	±	0.01	0.01	±	0.01
Bittium reticulatum	0.15	±	0.1	0.17	±	0.14	0.11	±	0.05	0.12	±	0.06
Tricolia tenuis	0.02	±	0.03	0.03	±	0.04	0.03	±	0.03	0.03	±	0.04
Rissoa violacea	0.01	±	0.02	0.01	±	0.02	0.01	±	0.02	0.01	±	0.01
Chiton olivaceus							0.01	±	0.01	0.01	±	0.01
Ampipholis squamata	0.03	±	0.03	0.02	±	0.02	0.08	±	0.05	0.09	±	0.03
Holothuria sp.	0.01	±	0.02	0.01	±	0.02	0.01	±	0.02	0.01	±	0.01

Fifteen species made up to 90% of all sampled individuals and only those 15 species will be discussed in detail. However, although representing a relatively limited contribution, the shrimp *Palaemon xiphias* (Risso, 1816), the only big predator found in EMAs was added to the analysis to monitor the potential impact of a pulsed event on a predator species. Multivariate analysis based on the density of these 16 species showed a significant effect of the treatment on population densities for only 7 of them (1-way MANOVA, $p < 0.001$). Two amphipod species (*G. fucicola* and *G. aequicauda*) and the leptostracean species (*Nebalia strausi* (Risso, 1826)) showed very highly significant effects of the treatment on population densities (ANOVA, $p < 0.00001$). The three decapod species (*Athanas nitescens* (Leach, 1813), *P. xiphias* and *Galathea intermedia* (Lilljeborg, 1851)) showed significant effects of the treatment on population densities (ANOVA, $p < 0.01$). The remaining amphipod species (*Nototropis guttatus* (Costa, 1853)) showed no significant effect on population density. For these 7 species, densities show drastic variations between C, on the one hand, and the pulse treatments on the other (Figure 3). *Gammarella fucicola* showed a much higher density in C than in T-defaun. or T-fauna. The 6 other species showed an opposite pattern with lower densities in C. *Gammarus aequicauda* experienced by far the most drastic differences, going from a density of 0.11 ± 0.06 ind. gDM^{-1} in C, to 0.71 ± 0.13 ind. gDM^{-1} in the two pulsed treatments. Decapods and the leptostracean *N. strausii* showed similar trends as in *G. aequicauda*.

SIMPER similarity analysis for treatment factor based on density data for the 36 species showed that *G. fucicola* was always the strongest contributor to similarity (Table 2). Similarity was high for all
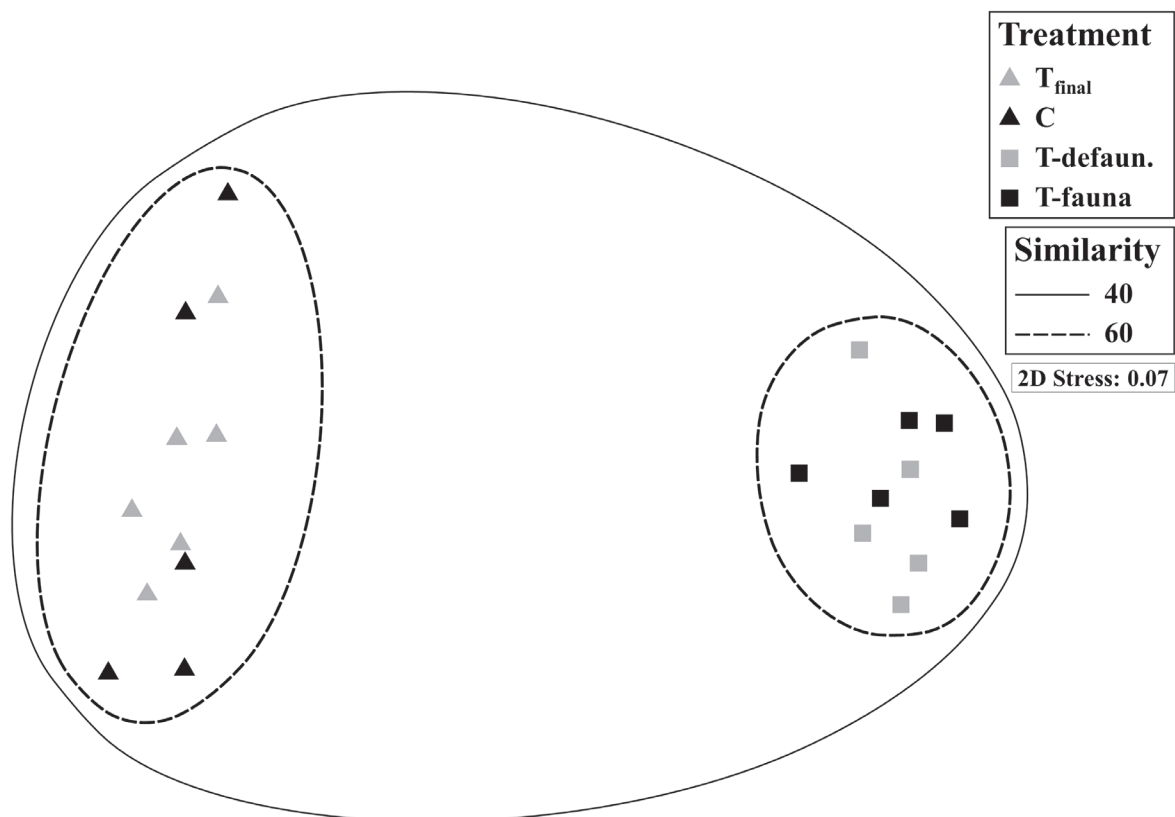

Figure 2 – 2D n-MDS ordination of the 21 samples and superposed Bray-Curtis similarity curves based on the square root-transformed density data of the 36 sampled species. Similarity curves represent 40% (solid black) and 60% (dotted black) of similarity between samples. Black and grey triangles represent C and T$_{final}$, respectively; and black and grey squares represent T-fauna and T-defaun., respectively.

TABLE 2

Summary table of the SIMPER analysis with factor "treatment" for macrofauna species contribution to intra-group similarity, and inter-group dissimilarity.

Treatment factor

Similarity

T_{final} (76.91% similarity)

Species	%	% Cum.
Gammarella fucicola	37.07	37.07
Platynereis dumerilii	8.53	45.60
Polyophthalmus pictus	7.70	53.30
Microdeutopus chelifer	7.15	60.45
Melita hergensis	6.35	66.80
Bittium reticulatum	6.19	72.99
Jaera nordmanni	6.17	79.16
Gammarus aequicauda	5.90	85.06
Nototropis guttatus	3.53	88.59
Palaemon xiphias	3.51	92.09

Control (71.35% similarity)

Species	%	% Cum.
Gammarella fucicola	38.54	38.54
Platynereis dumerilii	9.25	47.79
Polyophthalmus pictus	8.09	55.88
Jaera nordmanni	7.07	62.95
Bittium reticulatum	6.44	69.39
Microdeutopus chelifer	6.39	75.77
Gammarus aequicauda	6.21	81.98
Melita hergensis	5.46	87.44
Nototropis guttatus	3.78	91.23

Dissimilarity

T_{final} & Control (22.39% dissimilarity)

Species	%	% Cum.
Gammarella fucicola	8.14	8.14
Microdeutopus chelifer	7.14	15.27
Melita hergensis	6.12	21.39
Platynereis dumerilii	5.50	26.89
Bittium reticulatum	4.76	31.65
Tricolia tenuis	4.63	36.28
Athanas nitescens	4.61	40.89
Apherusa chiereghinii	4.40	45.29
Liocarcinus navigator	4.28	49.57
Jaera nordmanni	4.14	53.71
Stenosoma lancifer	4.14	57.84
Ampipholis squamata	4.01	61.85

T_{final} & T-defaun. (41.32% dissimilarity)

Species	%	% Cum.
Gammarella fucicola	14.11	14.11
Gammarus aequicauda	9.16	23.27
Nebalia strausi	7.69	30.96
Athanas nitescens	4.62	35.57
Anapagurus chiroacanthus	4.35	39.92
Platynereis dumerilii	3.56	43.48
Ampipholis squamata	3.19	46.67
Microdeutopus chelifer	3.08	49.75
Hesiospina autantiaca	2.92	52.67
Chrysopetalum debile	2.69	55.36
Melita hergensis	2.43	57.80
Jaera nordmanni	2.40	60.20

T-defaun. (75.99% similarity)

Species	%	% Cum.
Gammarella fucicola	18.19	18.19
Gammarus aequicauda	14.02	32.21
Jaera nordmanni	7.50	39.71
Nebalia strausi	6.71	46.42
Athanas nitescens	6.12	52.54
Bittium reticulatum	4.99	57.53
Melita hergensis	4.95	62.48
Palaemon xiphias	4.91	67.39
Microdeutopus chelifer	4.60	71.99
Platynereis dumerilii	4.12	76.11
Polyophthalmus pictus	3.70	79.81
Ampipholis squamata	3.36	83.17
Anapagurus chiroacanthus	3.22	86.39
Hesiospina autantiaca	2.05	88.44
Nototropis guttatus	1.79	90.23

T-fauna (78.56% similarity)

Species	%	% Cum.
Gammarella fucicola	15.54	15.54
Gammarus aequicauda	12.66	28.20
Jaera nordmanni	7.34	35.54
Nebalia strausi	7.20	42.73
Palaemon xiphias	5.71	48.44
Platynereis dumerilii	5.64	54.08
Athanas nitescens	5.24	59.32
Melita hergensis	4.64	63.96
Bittium reticulatum	4.47	68.43
Ampipholis squamata	4.17	72.60
Nototropis guttatus	4.07	76.67
Anapagurus chiroacanthus	3.64	80.31
Galathea intermedia	3.40	83.71
Polyophthalmus pictus	3.25	86.96
Hesiospina autantiaca	2.56	89.52
Chrysopetalum debile	1.92	91.44

Control & T-defaun. (42.17% dissimilarity)

Species	%	% Cum.
Gammarella fucicola	13.63	13.63
Gammarus aequicauda	9.32	22.95
Nebalia strausi	7.60	30.55
Anapagurus chiroacanthus	4.29	34.84
Athanas nitescens	4.16	39.00
Platynereis dumerilii	4.11	43.11
Microdeutopus chelifer	3.52	46.63
Ampipholis squamata	3.42	50.05
Hesiospina autantiaca	2.88	52.93
Melita hergensis	2.88	55.81
Jaera nordmanni	2.69	58.50
Chrysopetalum debile	2.66	61.16

T_{final} & T-fauna (41.31% dissimilarity)

Species	%	% Cum.
Gammarella fucicola	13.83	13.83
Gammarus aequicauda	8.89	22.72
Nebalia strausi	8.34	31.06
Athanas nitescens	4.75	35.82
Microdeutopus chelifer	4.53	40.35
Anapagurus chiroacanthus	4.41	44.75
Palaemon xiphias	3.62	48.37
Hesiospina autantiaca	3.41	51.79
Chrysopetalum debile	3.26	55.05
Galathea intermedia	2.80	57.85
Ampipholis squamata	2.78	60.63

Control & T-fauna (42.68% dissimilarity)

Species	%	% Cum.
Gammarella fucicola	13.2	13.2
Gammarus aequicauda	8.93	22.13
Nebalia strausi	8.13	30.27
Microdeutopus chelifer	4.66	34.92
Anapagurus chiroacanthus	4.30	39.22
Athanas nitescens	4.28	43.5
Palaemon xiphias	3.73	47.23
Hesiospina autantiaca	3.33	50.56
Chrysopetalum debile	3.18	53.74
Galathea intermedia	3.09	56.83
Ampipholis squamata	3.08	59.9
Jaera nordmanni	2.93	62.83

T-defaun. & T-fauna (22.93% dissimilarity)

Species	%	% Cum.
Galathea intermedia	5.28	5.28
Microdeutopus chelifer	4.40	9.68
Nototropis guttatus	4.25	13.92
Alpheus glaber	4.20	18.13
Palaemon xiphias	3.78	21.91
Tricolia tenuis	3.52	25.43
Apherusa chiereghinii	3.50	28.94
Lysianassa costae	3.41	32.34
Platynereis dumerilii	3.24	35.58
Stenosoma lancifer	3.21	38.79
Chrysopetalum debile	3.18	41.96
Cymodoce truncata	3.11	45.08
Idotea baltica basteri	3.03	48.11
Polyophthalmus pictus	2.93	51.04
Gammarus aequicauda	2.83	53.87
Gammarella fucicola	2.79	56.66
Leptocheirus guttatus	2.77	59.44
Dexamine spinosa	2.73	62.17

treatments ranging from 71.4 to 78.6 %. Specific contributions showed a similar pattern in C and in T_{final} and this pattern was very different from the one observed in T-defaun. or T-fauna treatments. An important result was that in C or T_{final}, *G. fucicola* was by far and away the species contributing most to the similarity, while in T-defaun. and T-fauna, *G. fucicola* was followed closely by *G. aequicauda*. SIMPER dissimilarity analysis showed that dissimilarity between both treatments and C or T_{final} was much higher (Table 2) than any dissimilarity between the treatments. It also showed that the species explaining most of the dissimilarity between the treatments and the controls were *G. fucicola*, *G. aequicauda* and *N. strausi*, which is consistent with what was observed in terms of density variations.

Discussion

This *in situ* experiment demonstrated the potential impact of resource pulses on the macrofauna associated to exported dead litter from *P. oceanica*, an ephemeral habitat characteristic of the Mediterranean coastal zone.

The global density, diversity and dominance patterns observed in the control during this experiment correspond to previous studies of this coastal detrital community (GALLMETZER *et al.* 2005; DIMECH *et al.* 2006; REMY 2016), showing lower diversity but higher abundance than in the adjacent seagrass meadow (STURARO *et al.* 2014; MICHEL *et al.* 2015) and higher diversity and higher abundance than in reed macrophytodetritus from Mediterranean brackish water (MANCINELLI *et al.* 2007) or seagrass (*Cymodocea nodosa/Zostera* spp.) detritus from the Po estuary (MANCINELLI & ROSSI 2002).

The two controls showed no significant difference between each other either, demonstrating that the effect of the experimental design was quite negligible compared to the effect of the treatments (i.e., addition of litter).
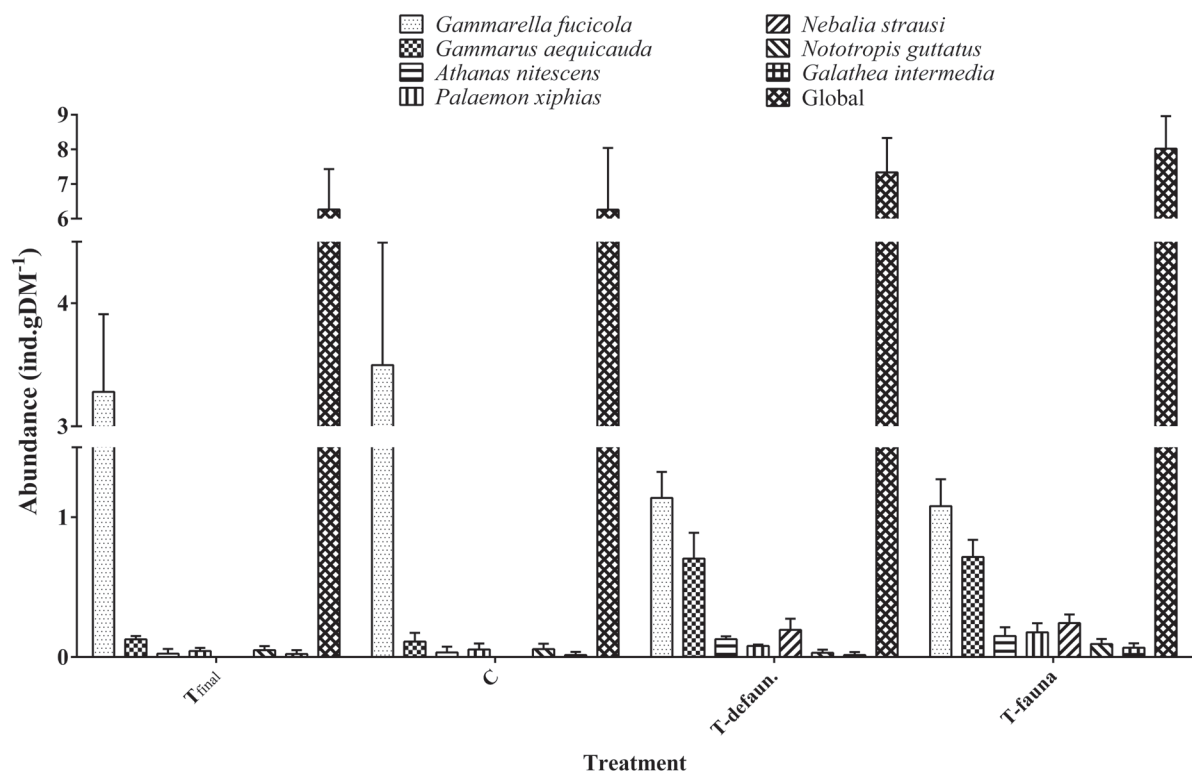

Figure 3 – Mean densities of most abundant species in relation to treatment. Error bars are standard deviations.

This experiment showed that diversity (H') and dominance (1-λ') in pulsed litter were influenced by both pulse treatments, and that diversity increased significantly with the addition of dead leaves, with or without the associated macrofauna (diversity: increase of 9-11 species between the control and the treatments). The dominance of some species also significantly increased, indicating that while the number of species encountered increased, a limited number of species seemed literally boosted by the addition of the dead leaves. Such important density variations observed in the relatively short duration of the experiment (14 days) leads to the conclusion that active colonisation and (e)migration of macro-invertebrates have more than likely occurred during the experiment (see MASCART *et al.* 2015b for meiofauna). Such short term migration/emigration is also observed in reed macrophytodetritus accumulations, occurring over even shorter time scales (i.e., within days) and explaining the non-random variability of macrofauna in these accumulations (MANCINELLI *et al.* 2005). In our experiment, the mesocosm design allows fauna movement inside the litter or via the sediment. Such behavioural aggregation of consumers is a common fast response to resource pulses (ANDERSON *et al.* 2008a; HOLT 2008; YANG *et al.* 2008; YANG *et al.* 2010; MASCART *et al.* 2015b) and the linked density variations observed in both treatments could be considered as a community-level 'first response' to a dead leaf pulse.

Another major result was the absence of effect of the addition of the animal fraction on the global abundance of the macrofauna community. Moreover, there were no differences in global abundance between T-fauna and C or T_{final}, which is also surprising since the amount of added "higher quality" animal organic matter was high in this treatment (YEE & JULIANO 2012). Different hypotheses could explain this result: first, the experiment was carried out for 14 days, which could be too long to witness such short-term effects of an animal pulse. Indeed, during these two weeks, the added organisms' density might have been levelled by predation and/or by behavioural migration resulting from the potentially increased competition for space and resources inside the mesocosms. Secondly, since it was very difficult to really estimate the invertebrates' density inside the added litter without over disturbing the community, it could be that the density of added macrofauna was too low, inducing damped responses within the first days of the experiment.

Except for *G. fucicola*, all the other species experienced a significant increase in density in both T-fauna and T-defaun. The most striking observation is the seven-fold increase in density of *G. aequicauda*. It must be noted that the SIMPER analysis coupled to the n-MDS ordination confirmed that the increased / reduced densities of these 7 species allow the strong differentiation of the controls and the two treatments into two very distinct groups, the first being what could be expected from a "natural" community at that moment of the year, and the second potentially reacting to a resource pulse. The significant density increase of detritivores is potentially a mixed result of the active colonisation of the treatments' mesocosms by new *G. aequicauda* and of increased juvenile recruitment. While *G. aequicauda* is a very active swimmer (swimming speed up to 20 cm.s⁻¹ in laboratory conditions) with a moving capacity considered relatively high for a benthic amphipod (VERSCHUT *et al.* 2015; LONGO *et al.* 2016), amphipod juveniles are a major component of litter meiofauna (MASCART *et al.* 2015a) and the experiment duration (14 days) would have allowed juveniles to reach the 500 µm size needed to be retained by sieving.

The drastically increased density of a purely detritivore species such as *G. aequicauda* could be a credible explanation for the absence of difference in litter biomass between the controls and the treatments. Even if loss of material is never excluded, the presence of these important litter fragmenters and consumers associated with potentially lower oxygen concentrations and increased microbial activity could explain the increased degradation of litter in the treatments.

Gammarus aequicauda is known to be a detritivore, ingesting and assimilating *P. oceanica* dead leaves (LEPOINT *et al.* 2006; MICHEL *et al.* 2015), biting but also shredding dead seagrass leaves (MANCINELLI 2012). It is a species found in various macrophytodetritic accumulations such as *Phragmites* accumulations (MANCINELLI *et al.* 2009) or *Cymodocea/Zostera* accumulations (MANCINELLI & ROSSI 2002). The density increase of the most important detritivore species found in the accumulation of

dead litter exported *P. oceanica* constitutes to one of the main observations of this study. Indeed, such detritivore-based responses to resource pulses are mostly found in terrestrial ecosystems (NOWLIN *et al.* 2008; YANG *et al.* 2008), but are also found in other aquatic phanerogam detritus accumulations such as reed (MANCINELLI *et al.* 2005). The exported dead *P. oceanica* litter accumulations present similarities with terrestrial forest ecosystems from the pulse point of view. Like most terrestrial plants, *P. oceanica* is a flower plant which sheds its leaves in autumn. Like most terrestrial macrophyte-driven ecosystems, these leaves form an important detrital pool with associated well-developed "detrital" food webs. These characteristics lead to a bottom-up response to pulses, typical of some forest ecosystems: detritivore organisms are able to take much more advantage of resource pulses (YANG 2006) than herbivores, which is what was observed during this experimental study. Indeed, *G. fucicola*, the most dominant species in natural accumulations, is considered as a detritivore/herbivore species, probably relying more on epiphytes than *G. aequicauda* (LEPOINT *et al.*, 2006 MICHEL *et al.* 2015). Moreover, some observations indicate that *G. fucicola* could need more degraded litter (more colonised by microbes) than *G. aequicauda* (Compère & Trevisan, unpublished data). In our control litter, *G. fucicola* dominated the assemblage as it usually does the natural litter, but litter added for our treatment was "new" litter (senescing leaves) coming directly from the meadow, probably in the first stage of the degradation process. Therefore, the two gammarids do potentially not share the same detritivore niche: *G. aequicauda* could be regarded as a pioneer detritivore and *G. fucicola* as a secondary coloniser.

Such primary bottom-up responses are not the only responses to pulses. Top-down responses of predators always follow after a certain lag time (secondary response) (OSTFELD & KEESING 2000; CHESSON *et al.* 2004; YANG *et al.* 2010), depending on the generation time of the organisms comprising the impacted community. This study was not primarily designed to document such longer time-sensitive outcomes and secondary responses but the observed significant abundance increase of carnivore species such as *Palaemon xiphias* and *Athanas nitescens* could be linked to the fresh litter input. This secondary response was relatively fast and occurred in less than 14 days, which probably also implies an active colonisation of the treatment mesocosms by these two species. This response could be congruent with HOLT (2008), who stated that a very intense but very brief resource pulse could result in a drastic primary consumer density increase within the first day after the event, and a moderate but fast predator density increase within the first 10 days after the event. This is also in accordance with NOWLIN *et al.* (2008), who stated that invertebrate communities composed of organisms with short generation times could respond very quickly to resource pulses. The increase of *P. xiphias* and *A. nitescens* densities could thus be a result of the rapid increase of one of their potential prey, *G. aequicauda*. This predatory response could also induce cascading effects on other prey, like the very abundant *G. fucicola*. The important decrease of *G. fucicola* density could be the result of the potential reduced fitness of the species, of the increased competitiveness of *G. aequicauda* (emigration effect) but also of increased predatory stress due to *P. xiphias* or *A. nitescens* (predation effect).

Another result was the non-negligible presence of *Nebalia strausi* in both treatments, and its absence from the control and T$_{final}$. Considering the very small size of *N. strausi*, active migration inside T-defaun. and T-fauna is more than probable. The *Nebalia* genus (OKEY 2003) and specifically the species *N. strausi* is known to be highly tolerant to hypoxia (GALLMETZER *et al.* 2005; REMY 2016), indicating that the addition of dead leaves to the treatment mesocosms potentially induced a certain level of hypoxia.

Conclusions

In conclusion, this experimental study provides insights into the impact of a resource pulse on the vagile macrofauna associated with an exported dead *P. oceanica* litter accumulation. We demonstrated that several major species of invertebrates showed drastic density variations in response to the pulse treatments, and that the driving parameter was the input of "fresh" litter and not the presence of the associated animal fraction. More precisely, the marked increase in *G. aequicauda* density, a pioneer detritivore species, was of major importance in our comprehension of the pulse response of the litter

macrofauna community and how it could increase litter degradation and assimilation in the coastal macrofauna food web. Indeed, this response of a purely detritivore species is found in other terrestrial ecosystems but also in brackish areas and is a sign that exported *P. oceanica* litter accumulations and terrestrial macrophyte-driven ecosystems potentially share more common characteristics than we previously thought.

Because macrophytodetritic habitats (e.g., seagrass or reed for example) may be found everywhere in the shallow coastal areas of the Mediterranean Sea (MATEO & ROMERO 1997; MANCINELLI *et al.* 2007; LEPOINT *et al.* 2006), they probably play an important role in local trophic webs (MOORE *et al.* 2004) and in the seagrass-based C cycle. However, in comparison with other detrital ecosystems, the impact of the natural dynamics (e.g., resource pulses) affecting these accumulations and their functioning (i.e., C cycling, nutrients cycling, biodiversity driver) remain largely understudied and our understanding of them would benefit from them receiving particular attention in future research.

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