Combining process indices from network analysis with structural population measures to indicate response of estuarine trophodynamics to pulse organic enrichment

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ABSTRACT

Indicators of ecosystem structures and processes are important in estuarine health studies but are rarely assessed simultaneously. Organic enrichment affects the abundance and diversity of meio- and macro-fauna, but its impact on food web dynamics is less well known. We used a manipulative field experiment combining stable isotope enrichment with compartmental modelling (WinSAAM) and ecological network analysis (EcoNet) to investigate the impacts of organic enrichment on the food web structure and dynamics of an estuarine sandflat, and to evaluate the combined use of structural and process indicators in estuaries. Organic enrichment was achieved by addition of powdered algae to increase organic content (OC) from 0.8% (background) to 3, 5 and 10%. Six dominant compartments including microphytobenthos, meiofauna and soldier crabs (*Mictyris longicarpus*) were sampled. Increasing organic content decreased the total abundance of meiofauna and number of meiofaunal taxa. C flow rates and indices reflecting system functioning, however, showed non-linear responses. Carbon flow rates between compartments and the proportion of carbon cycling within the system both decreased from background to enrichment at 3 and 5% OC, but then increased at 10% OC. On these measures the highest OC treatment was most similar to background controls, which could be misinterpreted as a sign of health of highly enriched sites. Additional interpretation of indicators for the 10% treatment showed, however, that the high proportion of internal cycling was a result of a much reduced number of compartments (i.e. some taxa had been lost). The combination of trophodynamic process indices and structural population measures together provided the more comprehensive and robust assessment of the effects of organic loading on estuaries.

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1. Introduction

Water quality in coastal areas is deteriorating globally as a result of rapid urban and agricultural development (Smith et al., 2000). In many inshore areas, particularly estuaries, anthropogenic organic enrichment from treated and untreated sewage effluent (Oliveira and Soares-Gomes, 2003), oil spillage (Slocum and Mendelssohn, 2008) and aquaculture (Sutherland et al., 2007) is a major concern. Sediments in such systems are a sink for organic contaminants (Nilsson et al., 1991).

Organic enrichment, either natural (Rossi and Underwood, 2002) or anthropogenic (Pinto and Bemvenuti, 2004), induces changes in species abundance and diversity in benthic sediments (Veríssimo et al., 2011). The response of macrofauna to organic enrichment has been widely studied and a general pattern has been proposed (Pearson and Rosenberg, 1978), as a gradual loss of benthic macrofaunal species along spatial/temporal gradients of organic enrichment. Macrofaunal response to organic pollution may also be determined by exposure duration, spatial distribution, and seasonal fluctuation of pollutant concentration (Saunders et al., 2007). Meiofauna have been used as an indicator of organic enrichment in benthic sediments due to their high abundance, fast turnover, the lack of larval dispersal, and sensitivity to changes in environmental conditions (Coull and Chandler, 1992). For instance, nematode community structure was related to sediment organic content and has been suggested as an indicator of organic enrichment (Sutherland et al., 2007).

Although the response of meio- and macrofaunal assemblage structure to organic enrichment has been well studied, field demonstrations of the effects on system function are rare (Lee et al., 2006). Early work by Odum (1985) and more recently by Rapport et al. (1998) proposed a suite of functional changes expected of ecosystems under stress, underpinning the use of process indicators in estuarine condition. Several tools are available for generating process indicators based on empirical measurements. Ecological network analysis (ENA) has been used to assess interactions among

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system components and functional processes (e.g. Jørgensen and Fath, 2006). The effects of anthropogenic activities on ecosystem structure, however, and particularly on ecological processes and system resilience, are still poorly quantified in marine and estuarine environments (Elliott and Quintino, 2007).

Simultaneous assessment of ecosystem structure and function is rare in estuarine ecosystem health assessment. We applied ecological indicators and network modelling to investigate the effects of organic enrichment on estuarine sandflat structure and trophodynamics, an indicator of ecosystem health and resilience (sensu Holling, 1973). We hypothesised that meiofaunal abundance and diversity and C flow at lower trophic levels would all decrease at high enrichment levels. We conducted a field experiment on the impact of pulse organic enrichment on sandflat trophodynamics, employing a novel approach integrating stable isotope (^{13}C) tracers, compartmental modelling and ENA (Lee et al., 2011). Since microphytobenthos (MPB) is a major C source in sandflats (Middelburg et al., 2000) and soldier crabs have been shown to consume meiofauna by flotation feeding (Dittmann, 1993), we focussed on how the utilisation of MPB by meiofauna and hence soldier crabs might change in response to organic enrichment. The use of ENA and process indices in conjunction with structural ecological measures potentially offers a more comprehensive assessment of system status than either method alone.

2. Materials and methods

2.1. Field site

The experiment was conducted on an intertidal sandflat 2 km upstream from the mouth of Tallebudgera Creek, southeast Queensland, Australia (28°06′S, 153°26′E). The sandflat is about 220 m long parallel to the shore at around 1 m above mean low water. The meiofaunal assemblage is dominated by harpacticoid copepods, amphipods (mainly *Maera masteri*), juvenile polychaetes and nematodes. This site was chosen because it is inside a Fish Habitat Area and is therefore protected from harvesting activities, and because it receives little organic enrichment (EHMP, 2007), providing sufficient scope for experimental manipulation of organic enrichment.

2.2. Experimental design

The C transfer rates between MPB, the meiofauna and soldier crabs were studied by enclosing crabs in cages (0.2 m diam., 0.4 m depth) made from polyethylene mesh (8 mm × 8 mm, refer to Lee et al. (2011) for cage design and deployment). Within the aggregation zone of soldier crabs on the sandflat, 80 cages were randomly placed 3–5 m apart at around 2 m from extreme low tide in an overall belt of ~4 m × 200 m two weeks prior to the commencement of the experiment. The sand enclosed was sieved through a 1 mm mesh to remove soldier crabs before being returned to the cage. Sediment was enriched with powdered dried alga *Ascophyllum nodosum* (Algae Products A/S, Norway; max. particle diam. 120 μm) at the commencement of the experiment. The algal powder contained 31.5% C and 0.9% N. Powder may have contained some Chl *a* but was unlikely to be influencing the Chl *a* concentration of the sediment measured in the field because Chl *a* associated with dead algal material, especially in powder form, is rapidly degraded to phaeopigments. Addition of algal powder in the present study simulated an important aspect of eutrophication in that the sediment concentration of simple, non-toxic, organic matter was increased. There were three treatments, each with 20 replicates, dosed to nominal levels equivalent to 3, 5 and 10% of organic content (OC) in the sediment (148, 345 and 738 g of algal powder incorporated,

respectively, into the top 10 cm of sediment). Preliminary studies showed that the background OC of sediment was ~0.8%. Desired levels of organic enrichment were determined from a review of studies on organic enrichment effects on meio- and macrofauna (e.g. Pinto and Bemvenuti, 2004; Austen and Widdicombe, 2006). Twenty cages were left un-enriched ('background' samples) as controls; and 20 sediment samples in the same experimental area (without cages and without addition of algal powder – 'in-between' samples) were treated as procedural controls.

Chl *a* concentration and organic content inside and outside cages were monitored before (day 0), day 4, 8, 14, and 18 after addition of algae powder and until the sediment was finally harvested, to ensure that the designated organic content of the sediment was maintained throughout the experiment. Adult soldier crabs (*Mictyris longicarpus*) with similar carapace lengths (CL) were collected locally by hand. Two weeks after cage establishment (to allow settlement of the sand and algal powder), one crab was put into each cage to simulate natural field density, estimated to be 1.5 ± 0.2 individuals per 0.0625 m^2. Crabs were allowed to acclimatise in the cage for one week before the commencement of the isotope tracer experiment.

Enriched sodium bicarbonate, NaH^{13}CO$_3$, was used as a tracer to track MPB C flow among compartments. Only a small amount of NaH^{13}CO$_3$ was added in order to avoid significant additional C input to the system. A 1 g L^{-1} NaH^{13}CO$_3$ tracer solution was sprayed evenly over the sediment surface in the cage, at the application rate of 2 g NaH^{13}CO$_3$ m^{-2} surface area (=0.306 g ^{13}C m^{-2}) on day 0 of the experiment. Each cage was haphazardly allocated for destructive sampling 0 (before), 1, 2, 4 and 6 days after the addition of ^{13}C tracer, with four replicates of each treatment. On each sampling date, 20 sediment samples (16 from the cages and four haphazard 'in-between' samples), crabs inside the allocated cages and four crabs freely wandering on the shore near the cages were collected. Sediment was collected from the cage using a 10 cm diam. corer. In order to determine the structure of the food web, meiofauna were extracted using elutriation, handpicked and counted under a dissecting microscope (Zeiss, 10×) and identified to the lowest practical taxonomic level using available identification keys. Individuals of the same groups were placed on the same glass petri-dish and washed with distilled water while counting. Refer to Lee et al. (2011) for details of the processing procedures for MPB and meiofaunal taxa and dissection of crab hepatopancreas tissue for stable isotopic analysis.

2.3. Process indices

2.3.1. Modelling of carbon flow using WinSAAM

WinSAAM (Novotny et al., 2003) was used to determine the carbon transfer rate between organisms using temporal changes in the amount of tracer in the organisms throughout the experiment. Compartments within the model were: (1) MPB (carbon source); (2) juvenile polychaetes; (3) harpacticoid copepods; (4) nematodes; (5) amphipods; and (6) soldier crabs; items 2–5 are the main taxa found in crab guts at this site (Lee et al., 2011). Transfer of ^{13}C label to each compartment was described in terms of a differential equation:

$$\int (^{13}C \text{ in compartment})dt = \int (^{13}C \text{ input} - {}^{13}C \text{ output})dt$$

i.e. the net change in ^{13}C label in a compartment is equal to the time-weighted sum of the ^{13}C label flow rates in and out of the same compartment (see Lee et al., 2011 for further details). The upper and lower input boundaries of the amount of ^{13}C flow are 5.00E+02 and 9.00E−08 μg d^{-1}, respectively. Data were weighted using fractional standard deviation (FSD) to estimate the error of the data

input in the WinSAAM model. The errors associated with the estimated C fluxes were represented by the FSD, which is generated by WinSAAM after solving and fitting the model. The calculated FSD is one of the data sensitivity parameters in WinSAAM, to estimate how well the model fits observed values. The data are considered well described by the model if FSD is <0.5 (Wastney et al., 1999).

2.3.2. Process indices from EcoNet

Food web structure and energy cycling were analysed using EcoNet (eco.engr.uga.edu/index.html), a simulation and network analysis package for studying food web structure. This software was chosen because it can handle non-linear models and features fast stochastic simulation algorithms based on Langevin equations and Gillespie's Stochastic Algorithm (Kazanci, 2007). EcoNet uses Network Environ Analysis to quantify the actual relation among compartments, environmental inputs and outputs (Kazanci, 2007; Tollner and Kazanci, 2007). Six quantitative indices on system function, namely, Finn Cycling Index (FCI), total system throughput (TST), indirect effect index, throughflow analysis matrix (N), storage analysis matrix (S) and utility analysis matrix (U), were calculated for each treatment. These are used as an indication of the differences in organisation and system functioning between treatments and controls. The amount of energy cycling or FCI has been used to determine the degree of resilience of a system (e.g. Christensen, 1995). FCI is derived from the fraction of total flow that is devoted to cycling, and is defined as the ratio of the amount recycled to the sum of all flows in the ecosystem. Total system throughput (TST) reflects the size of the system in terms of the sum of flows through all the individual compartments (Baird and Ulanowicz, 1993). Indirect effect index measures the amount of flow that occurs over indirect connections versus direct connections (Fath and Borrett, 2006). Throughflow analysis matrix (N) represents how much environmental input to one compartment is received by another. Storage analysis matrix (S) represents the relationship between input flow rates and compartment storage values. Utility analysis matrix (U) indicates the relationship, and indirect interaction strengths between compartments in a food web. A negative value of U between compartments indicates that an increase in abundance of one compartment will decrease the C flow to the other compartments.

The C transfer rates between compartments determined from WinSAAM were entered into EcoNet. Stability analysis of the model was performed by calculating new solutions after changing the input C value of the MPB by ±10% for each treatment. The time-course data generated by EcoNet of each compartment in each simulation were grouped and plotted on a new time-course figure. The model was defined as stable when all simulations of the same compartment within a given parameter range overlap in one figure (C. Kazanci, pers. commun.). The relative distance among time-course figures was compared by calculating the relative difference in the time-course data of each compartment after the first iteration generated by EcoNet (Supplementary Information Table 1). Details of the use of EcoNet are available in Lee et al. (2011).

2.4. Statistical analysis

Differences in the total abundance of meiofauna among treatments were tested using one-way ANOVA, with enrichment level as a fixed factor. CL of the crabs for isotopic analysis were tested separately using one-way ANOVA with level of enrichment as a fixed factor, to ensure that crabs had similar body size and thus could be expected to show similar dietary composition and consistent response to ^{13}C labelling. Differences in Chl a concentration of sediment throughout the experiment was assessed using a two-way ANOVA, with Treatment and Time as fixed factors. As these two factors were found to interact significantly, we used planned
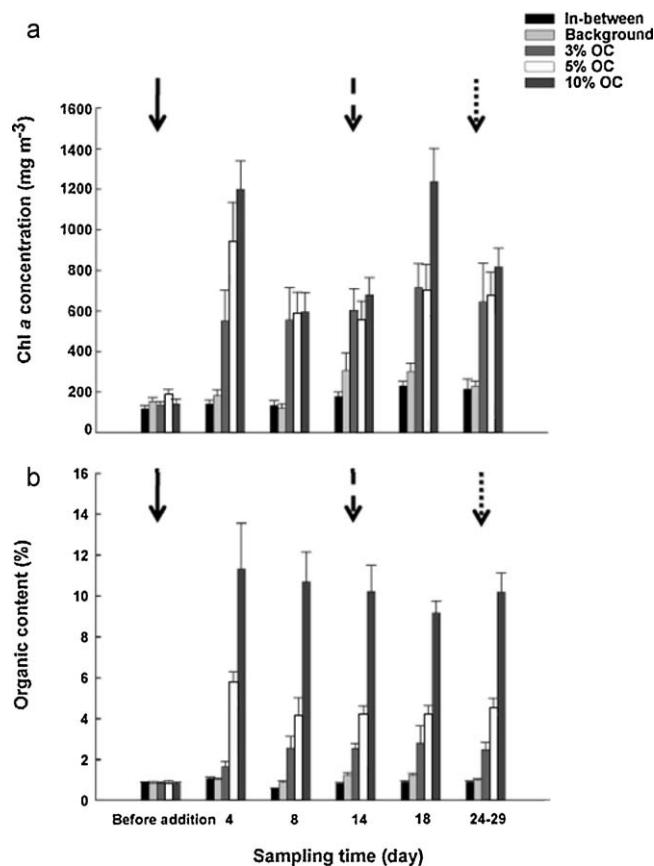
Fig. 1. (a) Chlorophyll a concentration and (b) organic content of sediment across treatments. Error bar represents +1 SE. Solid arrow represents the time cages were buried and algal powder was mixed with sediment inside the cages; dashed arrow represents the time crabs were placed in the cages; dotted arrow represents the time ^{13}C label was sprayed on sediment. Spiking occurred on day 24 and samples were collected between day 24 and day 29 inclusive.

contrasts separately for each time period to compare: (1) 'in-between' versus 'background' means, and (2) enrichment treatment means versus the pooled mean for 'in-between' and 'background'. Plots of residuals versus means showed that data satisfied assumptions of homogeneity of variance. Statistical comparison of the similarity of meiofaunal assemblages between treatments and controls was performed using one-way analysis of similarities (ANOSIM, in PRIMER v.6), with enrichment level as the treatment factor.

3. Results

3.1. Physical conditions and assemblage structure

The measured organic content of sediment was close to intended values, indicating that organic enrichment was broadly maintained at the intended treatment level throughout the experiment (Fig. 1). Addition of algal powder triggered growth of MPB and hence Chl a concentration increased with enrichment level (Fig. 1a; df = 4, 570; ANOVA interaction time × treatment $p < 0.001$; planned contrasts between enriched and pooled mean for 'in-between' and 'background' were significant for all time periods). Chl a concentration was similar between 'in-between' and 'background' sediment at all times (all planned contrasts non-significant), suggesting that deployment of cages did not create any artefact on MPB or carbon abundance (e.g. by limiting light penetration). The organic content of sediment was similar between 'background' (cage with addition of NaH^{13}CO$_3$) and 'in-between' (no cage, without the addition
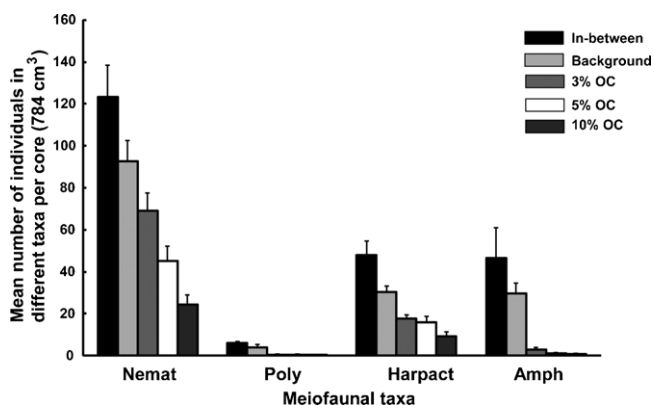
Fig. 2. Mean number of animals in dominant taxa of the meiofauna assemblage composition across the organic enrichment gradient in the harvesting period (6 days). Nemat, nematodes; Poly, juvenile polychaetes; Harpact, harpacticoid copepods; Amph, amphipods. Error bar represents +1 SE.

of $NaH^{13}CO_3$) during the harvesting period (df = 1, 39; $p = 0.169$). This suggested that the addition of $NaH^{13}CO_3$ label did not have a significant effect on the C content of sediment.

Total abundance of meiofauna decreased along the enrichment gradient ($p < 0.001$). Nematodes and harpacticoid copepods were the major taxa in the sediment in all treatments. However, there was a significant difference in the meiofaunal assemblage composition across enrichment levels (ANOSIM, $p = 0.001$), due to the low abundance (and probably high mortality) of some meiofaunal groups at elevated enrichment levels; e.g. at 3, 5 and 10% OC, respectively, juvenile polychaetes were 85, 88 and 99% lower than those at 'background', and amphipods were 89, 97 and 97% lower (Fig. 2).

Crabs had similar body sizes across treatments (ANOVA on CL, df = 4, 67; $p = 0.801$). No crabs died in 'background' cages, so any mortality of crabs in the treatments was due to enrichment. Mortality of crabs was highest at 10% OC (65%), followed by 3% OC (45%) and 5% OC (35%).

3.2. Process indices - C flow rates among compartments and C cycling

There was a clear difference in ^{13}C labelling between MPB, meiofauna and soldier crabs (Supplementary information Fig. 1). The best fit of the compartmental model was achieved using the transfer rates between compartments shown beside the arrows in Fig. 3 (all adjusted parameters in WinSAAM had FSD values <0.5, demonstrating that the pattern was well defined by the model). The high mortality of juvenile polychaetes and amphipods in response to organic enrichment caused the loss of these compartments in the corresponding food webs (Fig. 3). A decrease in carbon assimilation rates between compartments with increasing enrichment was observed. Most of the MPB C was assimilated by nematodes, followed by juvenile polychaetes, amphipods and harpacticoid copepods. In enriched sediment, harpacticoid copepods assimilated two times more MPB carbon at 10% OC than at 3% and 5% OC (Fig. 3b–d). Soldier crabs assimilated most of their carbon from amphipods in the absence of organic enrichment, but much less at 3% OC and none at 5% and 10% OC (Fig. 3). The rapid turnover rate of abundant nematodes in non-enriched sediment may result in large amount of C backflow from nematodes to MPB C pool. Carbon flow rates between amphipods and juvenile polychaetes were below the lower boundary condition value in WinSAAM ($9.00E-08\ \mu g\ ^{13}C\ d^{-1}$) in 5 and 10% OC treatments, suggesting that MPB C assimilation by these two compartments were negligible at 5 and 10% OC. In the 10% OC treatment, MPB

and/or meiofaunal C assimilation by soldier crabs were beyond the lower iteration boundary in WinSAAM, which indicated that the assimilation of MPB and/or meiofaunal C by soldier crabs was negligible.

The total amount of C flow through all the individual compartments was greatest in the 'background', followed by 10%, 5% and 3% OC (Table 1). C cycling and indirect effect index were greatest in the 'background', followed by 10%, 3% and 5% OC (Table 1). FCI was higher at 10% OC compared to other treatments and hence, more C was retained in that system. $N_{MPB,\ MPB}$ was >1 in all treatments, representing that MPB C recycled through the food web and re-entered the MPB compartment before this C left the system. The diagonal values of meiofauna in through-flow matrix analysis were >1 in all treatments, suggesting that the above compartments had the potential to cycle energy within them (Table 2). Soldier crabs received the greatest amount of MPB C indirectly at 3% OC ($N_{soldier\ crabs,\ MPB} = 0.30$), followed by background ($N_{soldier\ crabs,\ MPB} = 0.28$) and 5% OC ($N_{soldier\ crabs,\ MPB} = 0.05$), but there was no direct flow from MPB to soldier crabs in all treatments and control ($N_{MPB,\ soldier\ crabs} = 0$).

The storage analysis matrix S traces the amount of C storage in each compartment. More C from the enrichment was stored in the soldier crab compartment ($S_{soldier\ crabs,\ MPB} = 102, 108$ and 19 in background, 3% and 5% OC treatment respectively) whereas less C was stored in other compartments ($S < 1$). Juvenile polychaetes and harpacticoid copepods gained more C when the abundance of soldier crabs increased. The beneficial effect for juvenile polychaetes and harpacticoid copepods was greatest at background and 5% OC, respectively. However, nematodes and amphipods lost more C when the abundance of soldier crabs increased and the detrimental effect was greater at higher enrichment levels (Table 2).

4. Discussion

Energy cycling is considered an important aspect of estuarine function and interest in the comparison of ecosystem attributes appears to be growing (Vassallo et al., 2005). Most ecosystem health assessments are based on measurements of ecological structural changes at specific sites and times (Yoccoz et al., 2001), such as diversity measures or assemblage composition changes to indicate potential changes in ecosystem function (Elliott and Quintino, 2007), without measuring holistic changes of ecosystem functioning in response to stressors. We demonstrated the approach of using structural and functional measures of sandflat responses to pulse organic enrichment in a manipulative field experiment. The efficiency with which carbon was assimilated, transferred and dissipated provides significant insight into the fundamental structure and function of the system.

4.1. Interpreting responses using process indices and structural measures

This study reflects what might happen in estuaries due to large-scale pulse increases in organic load. The strongly non-linear response of C flow patterns to organic loading was unexpected. While population responses at 3, 5 and 10% OC were predictable, with very high OC resulting in loss of faunal elements, most C flow measures showed a V-shaped response. C throughput, flow between compartments, and cycling, all showed a pattern of decrease from background controls to 3 and 5% OC, but were then higher at 10% OC.

Cycling indices themselves give some indication that at 10% OC the system was vastly different from background cycling. The high FCI with additional characteristics of few, short-cycled flows is
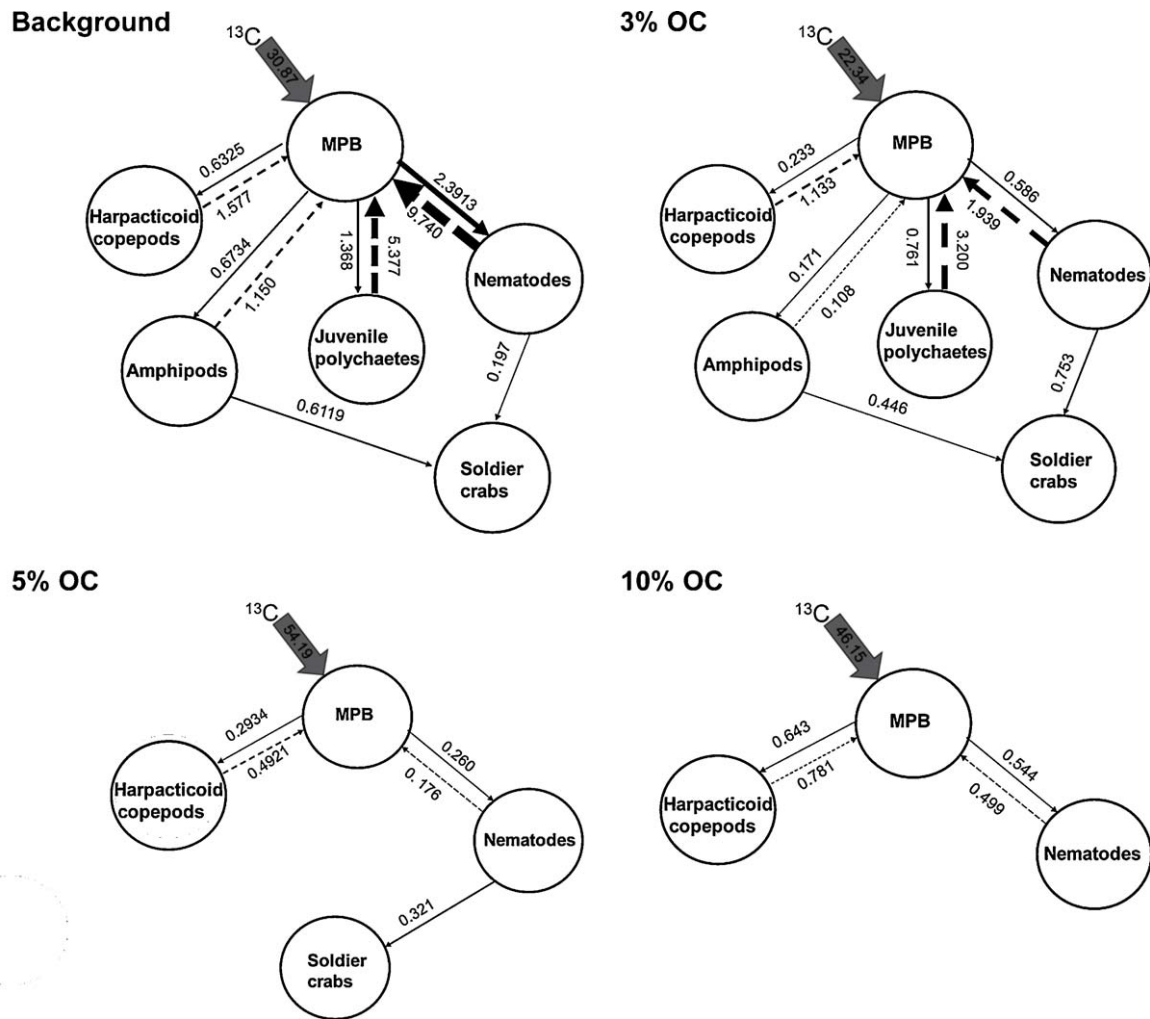
Fig. 3. The carbon transfer rate (μg ^{13}C d^{-1}) as estimated by WinSAAM modelling between MPB, juvenile polychaetes, harpacticoid copepods, nematodes, amphipods and soldier crabs. (a) Without the addition of algal powder; (b) 3%; (c) 5%; and (d) 10% organic content, respectively. Solid lines represent the direction of carbon flow from lower to higher trophic levels, whereas dashed lines represent the return pathway. Line thickness represents the relative magnitude of carbon flow. Number in the arrow represents the amount of ^{13}C label (μg) assimilated by MPB.

Table 1
Summary of system indices included in this study.

System indices	Background	3% OC	5% OC	10% OC
Finn Cycling Index (FCI)	0.69	0.44	0.34	0.52
Indirect Effect Index (IEI)	9.37	2.70	1.49	3.17
Total system throughput (TST, μg C cm^{-2} d^{-1})	342	99	132	184

Table 2
Diagonal values of throughflow analysis matrix (N) and utility analysis matrix (U) of MPB, juvenile polychaetes, harpacticoid copepods, nematodes and amphipods across enrichment levels. N/A, this interaction is not applicable due to the absence of the compartments in the corresponding treatment.

	Background	3% OC	5% OC	10% OC
Diagonal values of throughflow analysis matrix (N)				
$N_{\text{MPB, MPB}}$	5.74	2.41	1.65	2.44
$N_{\text{juvenile polychaetes, juvenile polychaetes}}$	2.36	1.75	N/A	N/A
$N_{\text{harpacticoid copepods, harpacticoid copepods}}$	1.62	1.22	1.36	1.80
$N_{\text{nematodes, nematodes}}$	3.32	1.41	1.28	1.64
$N_{\text{amphipods, amphipods}}$	1.44	1.03	N/A	N/A
Diagonal values of utility matrix analysis (U)				
$U_{\text{juvenile polychaetes, soldier crabs}}$	0.0000203	0.000127	N/A	
$U_{\text{harpacticoid copepods, soldier crabs}}$	0.000225	0.00115	0.000297	
$U_{\text{nematodes, soldier crabs}}$	−0.015	−0.17	−0.13	
$U_{\text{amphipods, soldier crabs}}$	−0.266	−0.51	N/A	

Note. Interaction between the meiofauna and soldier crabs is not applicable at 10% OC because of the loss of the soldier crab compartment.

indicative of a stressed system (R. Ulanowicz, pers. commun.). This response was also demonstrated by Scharler and Baird (2005), who found that highly impacted estuaries in South Africa had high FCI but shorter and fewer cycles. It is the additional information from community structure, however, that reinforces our interpretation. Very high OC (10%) results in C flows superficially most similar to background controls. This is a mere artefact, and in fact the system indices at 10% OC reflect a newly stabilised system with a much reduced number of compartments.

The V-shaped pattern of responses to organic loading support the Le Chatelier–Braun principle as it has been applied in ecology (Bondavalli et al., 2006). The number of compartments in 5% and particularly 10% OC food webs decreased after the addition of algal powder, probably because of the inability of some meiofauna groups and soldier crabs to survive at high enrichment levels. The number of individuals in remaining compartments in each treatment did not change significantly through sampling time. C fluxes among the remaining meiofauna components and from meiofauna to soldier crabs at 10% OC were thus higher. The higher C backflow from meiofauna to MPB compared to the MPB C uptake by the meiofauna suggested that there might be additional sources of C for the meiofauna.

4.2. Response of meiofauna and soldier crabs to organic enrichment

Increased supply of nutrients and organic matter may not necessarily result in increased biomass and/or abundance of macrofauna (e.g. Lee, 1999), while low levels of enrichment often produce a biostimulatory effect in oligotrophic habitats. Our results showed that the abundance of meiofauna and species diversity decreased with increased enrichment level following the same trend as demonstrated in previous studies (see review by Coull and Chandler, 1992). Since soldier crabs do not have fixed home-range, the only way to track C flows from meiofauna to the crabs is to confine the crabs in the sampling area. The mesh size of cages was chosen carefully in order to minimise caging effect, e.g. significant change in physical conditions other than the treatment applied. Any potential artefact of caging on the abundance of meiofauna should be equal in all treatments while comparing the C flow rates with the control. Although the abundance of harpacticoid copepods and nematodes decreased to similar extents at increased enrichment levels, recovery of harpacticoid copepods, in terms of the MPB C assimilation rates, was greater than that of nematodes. It is generally believed that higher diversity of species would enhance ecosystem functioning (Cardinale et al., 2002). A decrease in meiofaunal diversity and C flow between meiofauna and other compartments in organically enriched sediment would therefore change the food web structure and ultimately reduce resilience at the base of the sandflat food web.

Soldier crabs were sensitive to organic enrichment. Increased mortality was observed in the enriched treatments. Although the crabs would have died in the cages at high OC before we harvested the samples, findings indicated how C flow rates among the compartments were modified in organically enriched sediment. Furthermore, the macrofauna are suggested to be less capable of re-establishing as rapidly as the meiofauna in disturbed sediments (Austen and Widdicombe, 2006). This is supported by previous findings such as in Chesapeake Bay, where organic enrichment caused a decline in benthic macro-infauna and blue crabs (*Callinectes sapidus*) whereas copepods were relatively unaffected (Kemp et al., 2005).

4.3. Integration of structural measures and process indices in estuarine ecosystem health assessment

Previous ecological studies investigating the impacts of urbanisation on ecosystem health were mainly based on structural measures, e.g. changes in faunal abundance and diversity along pollution gradients (e.g. Morris and Keough, 2003), without measuring holistic changes of ecosystem functioning in response to stressors. Although measuring certain biotic and abiotic factors, e.g. assemblage composition and nutrient flows among organisms, can improve our understanding of fundamental ecological processes and how ecosystems might respond to stress, there are no standardised ways to combine biotic and abiotic metrics into a single or multi-metric score.

By incorporating compartmental and simulation modelling with a field manipulative experiment, we were able to show how a vital ecological process, namely, C dynamics, changed among compartments in organically enriched sediment whereas such information cannot be provided by standard structural measures based on taxa abundance and diversity. However, caution is needed while interpreting the information gained by the system indices. For example, the loss of trophic linkages at 10% OC treatments resulted in more C being retained in the system and shift in equilibrium state of the system. This might provide misleading information that 10% OC treatment was more stable than the other treatments. In contrast, additional characteristics provided by the structural measures showed that the system was sensitive to organic enrichment and 10% OC treatment was in poorer ecological status. Further, the loss of certain functional taxa, also directly indicated by the structural measures, in 10% OC treatment might result in lower quality of ecosystem service provided by the sandflats. Results showed that combined use of structural measures and process indices provides complementary information when assessing ecosystem health.

Field tracer experiments works effectively on small-scale food webs, nevertheless, be misinterpreted if migration in and out of experimental plots is significant. We minimised the issue of migration of animals by running the tracer experiment for a relatively short (6 days) period, starting three weeks after initially setting up cages. Minor rates of migration are a necessary shortcoming of field tracer experiments (e.g. Galvan et al., 2008). Experiments in the laboratory using closed systems, while allowing a high degree of control (e.g. Lee et al., 2011), often suffer from a lack of realism.

Addition of algal powder may have indirect effects on the organisms, including reduction of oxygen concentration (see review by Pearson and Rosenberg, 1978), decrease in water clarity (Cloern, 2001), and changes in biogeochemistry of the sediment (Hargrave et al., 2008). The sediment was well-flushed in this site by natural tidal cycles. Although we did not investigate the mechanism for the reduction, oxygen depletion caused by the addition of algal powder may be one possibility. Bioturbation of soldier crabs was suggested to increase the oxygen concentration of sediment (Webb and Eyre, 2004). However, darker colour of sediment was observed at organically enriched sediment regardless of the presence or absence of soldier crabs. This may lead to changes in abundance, composition and biogeochemical functioning of microbial communities along the oxic to anoxic gradient of sediment surface. Ultimately, organic enrichment may result in changes of abundance of organisms and assemblage composition (e.g. Rossi and Underwood, 2002).

5. Conclusions

The findings demonstrated the complementary value of the use of process indices and ecological indicators for assessing resilience of ecosystems. Synthesis of the structural measures and process indices approaches allows the inclusion of trophodynamics in

ecosystem health assessment and the adaptive sustainable management of natural resources. This research demonstrated a new approach to measuring ecological resilience and similar manipulative experiments could be done on other coastal areas, e.g. mangroves and saltmarshes. Such information can provide an early warning to decision makers before a major threshold of an ecosystem is crossed due to threats such as urbanisation.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ecolind.2012.01.015.

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