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Combining stable isotope enrichment, compartmental modelling and ecological network analysis for quantitative measurement of food web dynamics

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Summary

1. Quantitative food web analysis is made difficult by the need for simultaneous measurement of multiple carbon (C) flows. C flow rates can potentially be quantified indirectly using conventional methods such as gut contents analysis, but the involvement of many conversion factors and assumptions results in significant errors in the final estimation. Such difficulties have limited quantitative data on food web dynamics, restricting community-level hypothesis testing.

2. We conducted laboratory microcosm and field experiments, using combined enriched stable isotope (^{13}C), compartmental modelling and ecological network analysis (ENA) as a novel approach to quantifying food web dynamics. Feasibility of this approach was demonstrated via the quantification of C flow, backflow and cycling in a subtropical estuarine sandflat food web comprising three trophic levels: microphytobenthos (MPB), three dominant meiofaunal groups (juvenile polychaetes, harpacticoid copepods and nematodes) and soldier crabs (*Mictyris longicarpus*).

3. Enriched sodium bicarbonate ($NaH^{13}CO_3$) was used to trace C flow. The compartmental modelling software WinSAAM quantified C flow by comparing the temporal patterns of producer and consumer ^{13}C enrichment. Network analysis software EcoNet was used to assess food web dynamics by providing system indices that quantified the flows and food web interactions.

4. This approach allows concurrent quantitative assessment of food web structure and ecosystem functioning. WinSAAM results showed that C return rate from nematodes to MPB was greater than the other two meiofaunal groups and soldier crabs assimilated more C from nematodes than from the other two meiofaunal groups. System indices from EcoNet suggested that indirect flow contributed more than direct flow in this system. Unique capabilities of this approach in food web studies and assumptions underlying the proposed and conventional food web analytical approaches are discussed.

5. Another significant advance of this approach is that it allows sensitive quantification of trophic linkages in a food web without requiring equilibrium to be achieved, thus enabling instantaneous assessment of the relative importance of various C flow pathways. Specific hypotheses about food web dynamics, such as the impact of eutrophication and urbanization, can then be tested quantitatively through manipulative experiments by comparing indices reflecting system behaviour.

Key-words: carbon assimilation, ecosystem function, trophodynamics

Introduction

Food webs have high heuristic value for ecological theory and have been the subject of considerable interest in ecology (Berlow *et al.* 2004). External drivers, whether anthropogenic or environmental, can profoundly impact communities, causing a re-arrangement of their structure and a deviation from the original successional pattern, ultimately resulting in a breakdown in homeostasis of the ecosystem (Pimm 2002). It is important to understand food web structure because this knowledge improves our understanding of the fundamental processes responsible for the structure of all ecological systems (Martinez 1994). Although a challenging task often compromised by errors inherent in the methods used, the characterization of food webs represents an essential step in understanding ecosystem function (Pasquaud, Lobry & Elie 2007).

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The quantification of food webs encounters many practical problems, and methodological and logistical limitations often impede simultaneous measurement of all flows necessary for quantifying carbon (C) flow (van Oevelen *et al*. 2006). Further, empirical food web analytical tools, such as gut content analysis, are biased and time-consuming, and can only provide snapshot quantifications of dietary composition (Hyslop 1980; Créach *et al*. 1997). Assemblage-level hypothesis testing is still not possible unless overall trophic linkages are all quantified. Some tools, such as stable isotopic tracers (Peterson & Fry 1987) and modelling programs (Ulanowicz 1983) have been developed in recent decades as alternatives for improving our understanding of food web linkages, but their use remains limited to providing quantitative data for hypothesis testing.

Stable isotope analysis can identify the relative contributions of potential food sources as animals are on average only slightly δ^{13}C enriched (by $\sim 1‰$) relative to their diet (McCutchan *et al*. 2003). In the enrichment approach, the abundance of the rare isotope is greatly increased so that the movement of differentially labelled organic matter can be traced in the food web of interest (e.g. Winning *et al*. 1999). This allows more precise and sensitive identification of the diet and the transfer of organic matter in the food web than using isotopes at natural abundance levels (Rossi 2007). Enriched stable isotope experiments have been used for identifying feeding relationships (e.g. Galván, Fleeger & Fry 2008) and analysing C and nitrogen pathways (e.g. Mulholland *et al*. 2000).

Compartmental modelling allows dynamic assessment of trophodynamics and description of the interaction and energy flow between organisms (Pimm 2002). Determination of C transfer rates in metabolism at the organismal level is made possible by the Windows version of Simulation Analysis and Modelling (WinSAAM 3.0.7). WinSAAM is a nonlinear mathematical computer modelling program that incorporates model compilation, model solution and model fitting functions designed for analysing experimental data in compartmental modelling (Stefanovski, Moate & Boston 2003). WinSAAM has been reviewed extensively for studying non-ecological systems (Boston *et al*. 2003). It has been widely used to simulate and fit data, and to model the transfer rate of tracers in a system, without requiring the system to have reached steady state (Hamilton *et al*. 2004).

At the whole-system level, ecological network analysis (ENA) describes and assesses ecosystem function in terms of its biological productivity, interaction between species, interaction between the system and its surroundings, and other functional processes (Ings *et al*. 2009). In particular, ENA incorporates a number of indices to quantify the flows and interactions between compartments. These indices can be used to compare functional variability among ecosystems (Scharler & Baird 2005). However, most ENA studies have used data collected from empirical studies (e.g. Lobry *et al*. 2008). The lack of purposely collected data for quantitative determination of food web dynamics and linkages is a major obstacle in assessing and comparing the functional responses of biotic assemblages to disturbances (Berlow *et al*. 2004). This deficiency is in stark contrast to the large range of metrics available for comparing assemblage structure, such as species diversity indices. It is therefore not surprising that previous assessments were almost exclusively conducted on the structure rather than function of biotic assemblages.

We demonstrate an alternative and more efficient approach to empirical food web analysis, through combined application of *in situ* pulse-chase ^{13}C labelling, compartmental modelling techniques and ENA. We quantified C flow among compartments and C cycling in an estuarine sandflat food web with this approach. C transfer rates among compartments determined using WinSAAM were entered to EcoNet. EcoNet then iterates the model and calculate system indices that provide an assessment of ecosystem functioning. Both WinSAAM and EcoNet are freely available to researchers.

Materials and methods

CONSTRUCTING THE EXPERIMENTAL FOOD WEB

All organisms for the microcosm experiment were collected from the intertidal sandflat at The Spit, south-east Queensland, Australia (27°26′37″ S, 153°25′19″ E). A preliminary survey conducted to ascertain the major components of the food web for experimentation showed that the most abundant meiofaunal groups of the estuarine sandflat were nematodes, harpacticoid copepods and juvenile polychaetes (abundance of nematodes: 9895 ± 1803 individuals per m^2; harpacticoid copepods: 1138 ± 228 individuals per m^2; juvenile polychaetes: 126 ± 27 individuals per m^2). In order to provide background information on the trophic linkages, level of enrichment and the timing required to detect significant changes in ^{13}C label of the organisms, a laboratory microcosm experiment was conducted before application of this approach to the field.

QUANTIFYING C FLOW BETWEEN MICROPHYTOBENTHOS, MEIOFAUNA AND SOLDIER CRABS IN MICROCOSMS

Experimental design

Twelve sediment samples ($0{\cdot}3 \times 0{\cdot}4 \times 0{\cdot}1$ m^3) were collected from The Spit during low tide and washed *in situ* through a 1-mm sieve to remove any macrofauna, before being transported to a constant temperature room (25·5 ± 0·5 °C). Each microcosm container was connected to a recycling automatic semi-diurnal tidal system, subjected to a 11-h : 13-h light : dark cycle. Light was provided using eight fluorescent light tubes (Crompton lighting 36 W, 4000 K tri-phosphor cool-white) 25 cm above the sediment surface. Field density of soldier crabs was measured by counting the number of burrows in 20 quadrats ($0{\cdot}25 \times 0{\cdot}25$ m^2). Four soldier crabs were kept in each container to simulate field density at The Spit, estimated to be 5·9 ± 0·9 individuals per 0·0625 m^2. Density of crabs in the microcosms should not affect their feeding ability as the density of soldier crabs in the microcosms was less than that in the field. Crabs were allowed to acclimatize in the microcosms for 2 weeks prior to the commencement of the experiment. Observations of the crabs' behaviour were made for about 6 h day^{-1}

throughout the experiment. Chl *a* concentration and organic content of the microcosm samples at the commencement of the experiment were measured and compared with those collected from the field site (see below for details). A 1 g L^{-1} $NaH^{13}CO_3$ (Novachem) enrichment solution was sprayed evenly over the sediment surface at the beginning of the experiment at low tide (as per Middelburg *et al*. 2000), and again at days 7 and 14 in order to maintain ^{13}C enrichment of the microphytobenthos (MPB). MPB, meiofauna and one crab in each container were harvested 0, 7, 14 and 21 days after the first enrichment. MPB was scraped from the sand surface (0–5 mm) and meiofauna was collected from the top 5 cm of sand using two 10-cm-diameter corers. Samples were stored at −20 °C until processing.

QUANTIFYING *IN SITU* C FLOW FROM MICROPHYTOBENTHOS TO MEIOFAUNA AND SOLDIER CRABS IN A FIELD EXPERIMENT

A manipulative experiment was conducted on an intertidal estuarine sandflat located 2 km upstream from the mouth of Tallebudgera Creek, SE Queensland, Australia (28°06′29″ S, 153°26′57″ E). The sandflat is about 220 m wide at ∼1 m above mean low water.

The C transfer rates between MPB, the meiofauna and soldier crabs were studied by enclosing crabs in cages made with polyethylene mesh. Each cage was 0·2 m in diameter and 0·4 m high, and had bottom and lid covers made of the same material, the latter protecting the crab from seabirds and other predators. It had minimal disturbance on the crabs, as the crabs were observed to behave normally and feed inside the cages.

Within the aggregation zone of soldier crabs on the sandflat, 25 cages were randomly placed 3–5 m apart at around 2 m from extreme low tide, 2 weeks prior to the commencement of the experiment. Soldier crabs generally burrow down to 15–20 cm of sand (Warwick, Clarke & Gee 1990); the cages were therefore buried 30 cm into the sand, i.e. the top of the cage stood 10 cm above the sand surface. Prior to the experiment, the enclosed sand was sieved through a 1-mm mesh to remove macrofauna and then returned into the cage. After a week to allow settlement of the sand and its meiofaunal inhabitants, adult soldier crabs with similar carapace lengths and widths were collected locally. One individual was put into each cage at natural density at Tallebudgera Creek (1·5 ± 0·2 individuals per 0·0625 m^2). Crabs were allowed to acclimatize for 1 week before the commencement of the enrichment experiment. Chl *a* concentration and organic content of the sediment inside and outside cages were measured and compared as described below.

A 1 g L^{-1} $NaH^{13}CO_3$ enrichment solution was sprayed evenly over the sediment surface in the cage, at the application rate of 2 g m^{-2} surface area on day 0 of the experiment. The application rate of enriched $NaH^{13}CO_3$ was higher and the sampling time shorter than those in the laboratory microcosm experiment to compensate for tidal dilution of the ^{13}C label (Supporting information, Table S1). Each cage was randomly allocated for destructive sampling 0 (before), 1, 2, 4 and 6 days after enrichment, with five independent replicates in each treatment. On each sampling date, the soldier crab was collected and put in ice slurry, while sediment samples were collected from the top 10 cm of sand using a 10-cm diameter corer and stored at −20 °C. MPB and the dominant meiofaunal groups were extracted from the sediment and soldier crab hepatopancreas tissues dissected for stable isotopic analysis, from both microcosms and field experiment, as described below.

SPECIMEN PROCESSING AND ISOTOPIC ANALYSIS

Chl *a* concentration was analysed according to Lorenzen (1967) and organic content was measured using the loss-on-ignition technique after acid treatment to remove carbonates. MPB was extracted based on density gradient centrifugation in silica gel (de Jonge 1979). The green band formed upon centrifugation consisted predominantly of microalgae, mainly diatoms (*Amphora robusta*, *Cymbella affinis*, *Entomoneis* sp. and *Pleurosigma* sp.), with rare occasional contamination (< 5% by abundance) by fine detritus. Meiofauna were handpicked from the sediment, identified to the lowest practical taxonomic level and counted. Meiofaunal species with carbonate shells were acid washed using 5% HCl prior to stable isotope analysis. All samples (MPB, meiofauna, crab hepatopancreas tissues) were dried to constant weight at 60 °C. The C stable isotope ratio is presented using the standard notation:

$$\delta^{13}C = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000‰$$

where R represents the ratio $^{13}C : {}^{12}C$. The standard for C was Pee–Dee Belemnite equivalent ($R_{standard}$ is 0·0112372). $\delta^{13}C$ was measured using a continuous flow isotope ratio mass spectrometer (GV Isoprime, Manchester, UK), with precision among subsamples being 0·5‰ (1 SD).

MODEL CONSTRUCTION

Assumptions of the proposed compartmental modelling approach were as follows: (i) a significant food source is included and labelled successfully (assured through *a priori* examination of dietary composition of consumers and the temporal profile of isotopic signatures of the food source and consumers); and (ii) each compartment is a single well-mixed pool. In reality, organisms are composed of tissues with different C assimilation rates, but those included in this study were small enough for this assumption to broadly hold true. The proposed approach does not require a closed system assumption. The ability of the proposed approach to quantify food web dynamics is achieved through: (i) a known amount of tracer administrated to the food source; (ii) temporal profiles of tracer in the compartments were obtained; and (iii) biomass of the compartments was measured.

WinSAAM

The modelling software WinSAAM (available free at http://www.winsaam.org) has been used to examine physiology-based nutrient transport problems using stable isotope data (Sian *et al.* 2002). WinSAAM was developed to analyse experimental data in the context of biological systems and can adjust the input parameters iteratively to find the best-fit flow rates among all compartments in the model (Wastney *et al.* 1999). Although WinSAAM is capable of dealing with dynamic data at higher levels of organization, such as communities, its application has to date been confined to organismal-level studies. We used WinSAAM to determine the C transfer rate among compartments using temporal changes in the amount of tracer in the compartments in this experiment, taking advantage of the program's ability to iterate the input parameters to find the best-fit transfer rates among all compartments in the model without the need for the levels to reach equilibrium (Stefanovski, Moate & Boston 2003).

C flow among compartments was estimated by comparing the temporal patterns of producer and consumer ^{13}C enrichment.

Compartments within the model were: (i) MPB C source; (ii) juvenile polychaetes; (iii) harpacticoid copepods; (iv) nematodes; and (v) soldier crabs. The calculated fluxes were constrained by: (i) the temporal variation in the amount of ^{13}C in the compartments: there must be at least two time points of changes in the amount of ^{13}C in the compartments for WinSAAM to estimate the fluxes; and (ii) the extent of ^{13}C enrichment between food source and consumers: the food source has a higher level of enrichment than those of the consumers.

The amount of ^{13}C uptake of each compartment was back calculated using the stable isotope signature standard formula. $\delta^{13}C$ signature, proportion of ^{13}C and F ratio

$$\left(\frac{^{13}C}{^{13}C + ^{12}C}\right) \times 100\%$$

of the samples were measured and reported using IRMS. Transfer of ^{13}C label to each compartment was described in terms of a differential equation:

$$\int \mathrm{d}(^{13}\mathrm{C\ label\ in\ compartment})\mathrm{d}t = \int \mathrm{d}(\mathrm{inputs} - \mathrm{outputs})\mathrm{d}t$$

i.e. the net change in label ^{13}C is equal to the time-weighted sum of the ^{13}C label fluxes in and out.

The fractional transfer rate, $L_{i,j}$, is a nonlinear parameter representing the fractional rate of flow of C from compartment j to i in unit time. $L_{i,j}$ was estimated from the initial fractional slope of each compartment over time, derived from the temporal changes of the $\delta^{13}C$ signature of each compartment. By specifying $L_{i,j}$, the program assembles the corresponding set of differential equations. Backflow of ^{13}C from one compartment to another was estimated from the fractional slope of the decreasing amount of ^{13}C in the consumers after reaching the peak value. C backflow to MPB C pool occurs when the semi-log plot of the amount of ^{13}C in MPB against time resulted in an exponential curve instead of a straight line (R. Boston, personal communication). In ecological sense, C backflow is interpreted as the amount of C released from consumer, mainly mediated by respiration, excretion and mortality, to the C pool, which is then assimilated by its food source again. Temporal data on the amount of ^{13}C of an individual sample in each compartment provide the input for the calculation of transfer rates. The upper and lower input boundaries of the amount of ^{13}C flow are 5·00E + 02 and 9·00E−08 μg day^{-1} respectively.

Model results can also be easily tracked in WinSAAM using the event logging window. WinSAAM provides the adjustment details of the parameters altered during the iteration process. The final best-fit combination of model parameters is achieved when there is no further improvement of fit (Stefanovski, Moate & Boston 2003). In addition to adjusting parameters by least squares minimization to fit observed data, the program provides statistical measures of fit and parameter estimates. Data were weighted using fractional standard deviation (FSD, standard deviation and/or mean) of estimated parameters generated using WinSAAM. FSD is one of the data sensitivity parameters in WinSAAM for estimating how well the model fits the observed values (Wastney *et al.* 1999). The data are considered well described by the model if FSD is $< 0{\cdot}5$ (Stefanovski, Moate & Boston 2003). Further, sensitivity analysis was conducted to assess the robustness of ^{13}C transfer rates determined using WinSAAM. The sensitivity of calculated flows to variation in C input was determined by calculating new flow rates resulting from varying MPB C input by ±10%, while keeping the other input values constant (Niquil *et al.* 1998; Donali *et al.* 1999).

MODEL ANALYSIS

EcoNet

The food web in focus was expressed as a set of compartments, and flows among and between these compartments (Pimm 2002). C transfer rates determined using WinSAAM were entered into EcoNet (available free at http://eco.engr.uga.edu/index.html) to determine the energy cycling pattern and to quantify the relative contribution of direct and indirect flows to the system. EcoNet is a simulation and network analysis software that helps assess the food web structure of an ecosystem (Kazanci 2007). We used this software because it integrates dynamic simulation with ENA. EcoNet is one of the few software programmes that include both deterministic and stochastic algorithms. Stochastic solvers such as the Langevin equation (Gillespie 2000) provide information on how the system evolves from the given initial condition. The time–course plot resulted from stochastic solvers is different at each simulation run. Alternatively, deterministic solvers (fourth-order Runge–Kutta and Runge–Kutta–Fehlberg) are distinct from stochastic solvers by fitting data in a set of fixed rate equations and produce smooth time–course plots. The user can choose the most appropriate numerical method based on the nature of the targeted system. EcoNet uses network environ analysis to quantify the actual relation between compartments, environmental inputs and outputs (Tollner & Kazanci 2007). Computation of EcoNet and definition of matrices are described in Kazanci (2007). Its capability in effectively analysing complex ecological systems has been demonstrated (Tollner & Kazanci 2007). The selected method in the present study was the deterministic approach (fourth-order Runge–Kutta), with sensitivity set at 0·01 and iterated until the system reached steady state. The fourth-order Runge–Kutta solver was chosen because it provides the optimal balance between complexity of the model and duration of the iteration process (C. Kazanci, personal communication).

Five quantitative indices on system function: Finn cycling index (FCI), indirect effect index, total system throughput (TST), throughflow (N) and storage (S) analyses were used in this study. The amount of energy cycling in the system or FCI reflects the degree of maturity and resilience of a system (Odum 1969). 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 (Finn 1976), i.e.

$$\sum_{i=1}^{n} \frac{T_i}{\mathrm{TST}} \frac{N_{ii} - 1}{N_{ii}},$$

where N_{ii} is the ith coefficient along the diagonal of the Leontief matrix and T_i is the inflow to compartment i. When FCI is high and there are many cycles of long length, the high FCI is indicative of maturity according to the definition given by Odum (1969). However, a high FCI with most cycled flow along a few, short cycles, is indicative of stressed systems (R. Ulanowicz, personal communication). Indirect effect index measures the amount of flow that occurs through indirect connections instead of direct connections, i.e.

$$\frac{\sum_{i=1}^{n}\sum_{j=1}^{n}(N_{ij} - i_{ij} - g_{ij})}{\sum_{i=1}^{n}\sum_{j=1}^{n} g_{ij}}$$

where $g_{ij} = f_{ij}/T_j$, i_{ij} is the initial input from component i to component j. TST reflects the size of the system in terms of the sum of flows through all the individual compartments. Throughflow (N) represents how much environmental input to one compartment

is received by another. $N_{jj} > 1$ represents a unit of C cycling through the food web and re-entering the compartment j, whereas $N_{jj} = 1$ represents a unit of C input to compartment j from an environmental source all retained in compartment j. Storage represents the relationship between input flow rates and compartment storage values. Storage $S = c^{-1}$ where $c_{ij} = f_{ij}/x_j$; f_{ij} represents the actual flow rate from compartment j to i at steady state; x_j represents the input received in the originating component j at a discrete time interval (Patten 1978; Fath & Patten 1999). The above indices were calculated for the microcosm and field experiments. Stability analysis was performed by calculating new solutions of the model after changing the input value of the MPB by ±10%.

Results

THE EXPERIMENTAL FOOD WEB

The starting conditions in the microcosm resembled that at The Spit (Chl *a*: field: 907·0 ± 39·5 mg m^{-3}, microcosm: 786·3 ± 76·4 mg m^{-3}; one-way ANOVA, $n = 24$, $P = 0{\cdot}245$; organic content: field: 1·57 ± 0·09%, microcosm: 1·65 ± 0·08%; one-way ANOVA, d.f. = 23, $P = 0{\cdot}452$). No significant caging effect was observed in the field experiment (Chl *a*: inside cage: 460·7 ± 30·8 mg m^{-3}, outside cage: 391·4 ± 31·4 mg m^{-3}, one-way ANOVA, d.f. = 49, $P = 0{\cdot}221$; organic content: inside cage: 0·85 ± 0·03%, outside cage: 0·87 ± 0·08%, one-way ANOVA, d.f. = 49, $P = 0{\cdot}833$). Soldier crabs collected from the microcosms were of similar sizes (non-enriched sediment: CL = 14·0 ± 0·4 mm; enriched sediment: CL = 14·5 ± 0·2 mm; one-way ANOVA, d.f. = 47, $P = 0{\cdot}690$). No mortality of crabs was observed throughout the experiment and all crabs were moving constantly and feeding in the microcosms during 'day' time. Crabs collected from Tallebudgera Creek were also of a similar size (CL, non-enriched sediment: 21·2 ± 0·8 mm; CL, enriched sediment: 20·6 ± 0·40 mm; one-way ANOVA, d.f. = 24, $P = 0{\cdot}476$) and had the same dietary composition between enriched and non-enriched sediments (Fig. S1).

The meiofaunal assemblage was dominated by nematodes in abundance (microcosm: 49·5 ± 6%; field experiment: 67·8 ± 8%) followed by juvenile polychaetes (microcosm: 34·8 ± 5%; field experiment: 7·1 ± 2%) and harpacticoid copepods (microcosm: 10·3 ± 3%; field experiment: 15·9 ± 3%) at the commencement of the experiments. There was a clear difference in ^{13}C labelling between MPB, meiofauna and soldier crabs (Fig. 1). The $\delta^{13}C$ signatures and enrichment levels of MPB and the meiofaunal groups were more depleted in the field samples, which may be due to dilution of the label in the field (Fig. 1b). The enrichment levels of the three meiofaunal groups were greater in the microcosms (Fig. 1a), probably due to accumulation of the ^{13}C label in the organisms from the multiple ^{13}C spikes.

WINSAAM COMPARTMENTAL MODELLING

The best-fit ^{13}C transfer rates among all compartments in both microcosm and field experiments were determined using

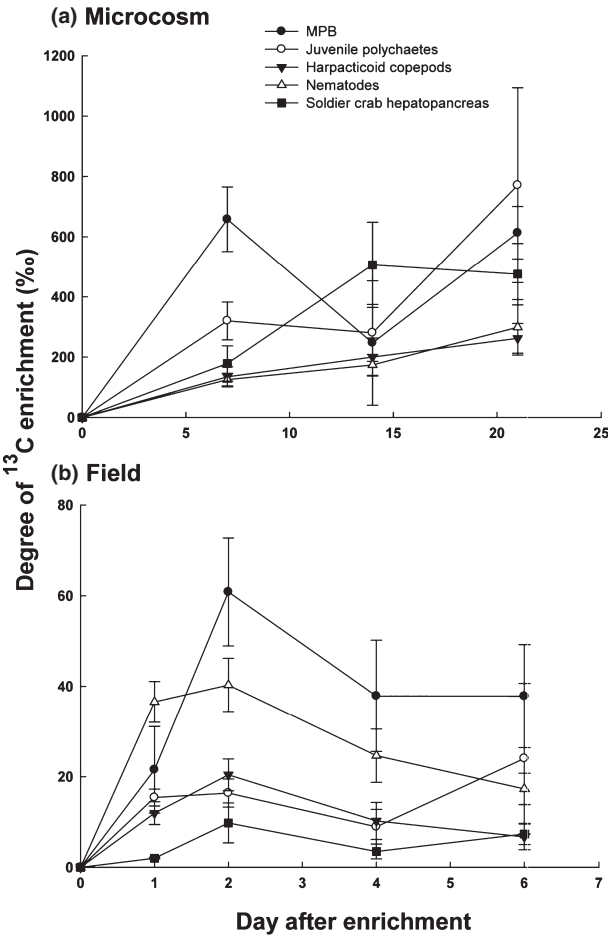

Fig. 1. Degree of enrichment over the course of the ^{13}C addition experiments, with $\delta^{13}C$ values corrected for non-enriched MPB, juvenile polychaetes, harpacticoid copepods, nematodes and soldier crabs, in (a) microcosm; (b) Tallebudgera Creek. Data are mean ± 1 SE.

WinSAAM (Fig. 2). All adjusted parameters had FSD values < 0·5 (Table S2) and no adjustable parameter had an estimated value equal to a boundary condition value. This suggested that the data pattern was well defined by the model. When the ^{13}C input rate was varied by ±10%, WinSAAM was sufficiently robust to indicate similar ^{13}C flow rates among compartments under the changed MPB ^{13}C uptake rates and, again, all FSDs were < 0·5, indicating very good fitting of models (Table S3). The order of ^{13}C transfer rates among the compartments remained the same in the microcosms and field experiments, regardless of the ±10% changes of MPB ^{13}C uptake rates (Figs S2 and S3). In general, the higher the MPB ^{13}C uptake rate, the more was the C available to the meiofauna and hence, the more was the meiofaunal C uptake by the crabs. Therefore, the resulting models were robust with respect to variation of the MPB C input data.

The transfer rates between compartments and the C return rates determined are shown beside the solid and dashed arrows, respectively, in Fig. 2. Assimilation of MPB C was the greatest by the nematodes among the dominant meiofaunal groups (Fig. 2). The MPB C assimilation rates (total C

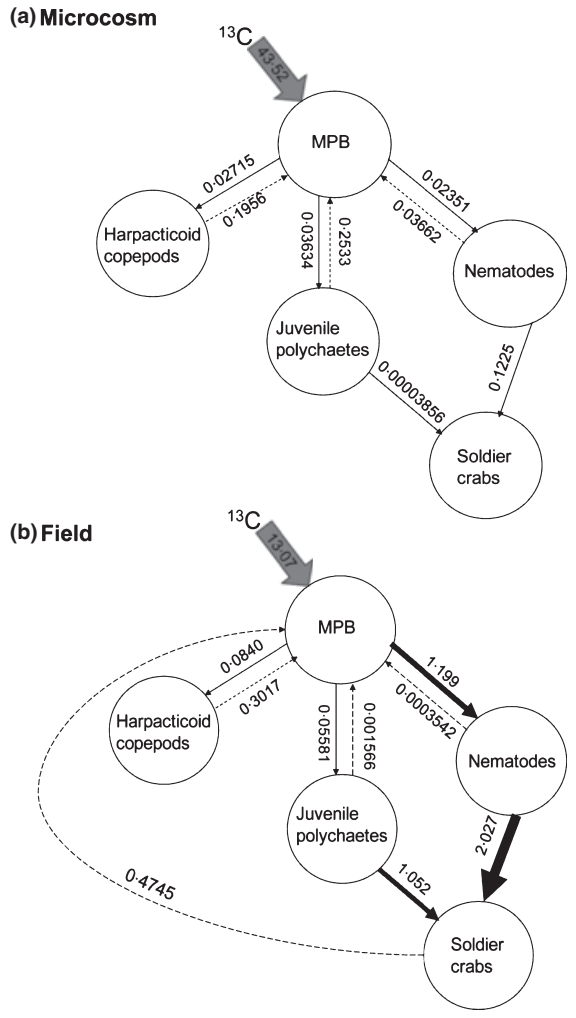

Fig. 2. The carbon transfer rates (μg ^{13}C day^{-1}) as estimated using WinSAAM modelling between MPB, juvenile polychaetes, harpacticoid copepods, nematodes and soldier crabs, in (a) microcosm; (b) Tallebudgera Creek. Solid lines represent the direction of C flow from lower to higher trophic levels, whereas dash lines represent the return pathway. Line thickness is scaled to the magnitude of C flow. Number in the arrow represents the amount of ^{13}C label (in μg) assimilated using MPB. Insignificant transfer rates ($\leq 9{\cdot}00 \times 10^{-8}$ μg) are not included.

calculated from the ^{13}C transfer rate) of the three meiofaunal groups in the microcosm and field experiments were 0·15 and 2·27 μg C cm^{-2} day^{-1} respectively. Soldier crabs in the field experiment assimilated 25 times more C from the meiofauna than those in the microcosm (microcosm: 0·21 μg C cm^{-2} day^{-1}; field: 5·22 μg C cm^{-2} day^{-1}). The amount of nematode C assimilated by soldier crab was 0·21 and 3·44 μg C cm^{-2} day^{-1} in microcosm and field experiment respectively.

ECONET ANALYSIS

The system indices FCI, indirect effect index and TST were higher in the field than in the microcosm (FCI : microcosm = 0·07, field = 0·61; indirect effect index : microcosm = 0·21, field = 5·9; TST : microcosm = 53·4 μg C cm^{-2} day^{-1}, field = 81·3 μg C cm^{-2} day^{-1}). The high FCI and indirect effect index reflected strong connection strength between compartments and indicated that indirect flow was more important in the field. The same total amount of ^{13}C was applied to the sediment in both microcosm and field experiments. The N matrix traces the energy generated by inputs into various compartments. $N_{\text{nematode,nematode}}$ is >1, which means that a unit of C in MPB was more likely to cycle through the sandflat food web and re-enter the nematode compartment before being dissipated from the system. By contrast, a unit of C derived from MPB indirectly would be stored in the crab compartment ($N_{\text{soldiercrabs,soldiercrabs}} = 1$). The non-zero entries in the N matrix indicated that a unit of C input into MPB generated subsequent flow through every compartment except the crabs in microcosm. There was no direct environmental input to soldier crabs in microcosms since $S_{\text{MPB,soldiercrabs}} = S_{\text{juvenilepolychaetes,crabs}} = S_{\text{harpacticoids,soldier crabs}} = S_{\text{nematode,soldiercrabs}} = 0$; therefore, an environmental input to soldier crabs would never affect the storage value of other compartments in microcosm ($S_{\text{soldiercrabs,soldiercrabs}} = 364$). The absence of C backflow from the soldier crabs to MPB or other compartments and the high storage value in the crab compartment suggest that bottom-up control was more important than top-down effect in the microcosm. Because of recycling in the food web, even more energy from the environmental input was stored in the crab compartment ($S_{\text{soldiercrabs,MPB}} = 7$ in microcosm and $S_{\text{soldiercrabs,MPB}} = 3$ in the field). Smaller amounts of energy were stored in all of the other compartments available from MPB. A robust steady-state value of both experiments was reached after 10 000 iterations of EcoNet. Time–course figures of each simulation with changes of MPB stocks by $\pm 10\%$ resulted in overlapping of the solutions for each compartment. Hence, the models were considered to be stable in the present study.

Discussion

The proposed approach combines the capabilities of stable isotope enrichment technique, compartmental modelling and ENA to allow concurrent assessment of sandflat food web structure and functioning. Hamilton *et al.* (2004) demonstrated the application of compartment modelling to assess the contribution of a ^{15}N-enriched producer to consumers in a three-compartment freshwater stream food web. We extended this technique to a five-compartment food web of an estuarine sandflat system and applied the data of C transfer rates obtained from WinSAAM into ENA to provide further functional assessment at the system level.

UNIQUE THEORETICAL CAPABILITY OF THE PROPOSED APPROACH

C flow among compartments was quantified using ^{13}C labelling: real-time C transfer and backflow rates among compartments can be estimated using compartmental modelling (WinSAAM). The utility of compartmental modelling with ^{13}C isotopic tracer offers important insights into C uptake and release among organisms. By quantifying C flow in the system,

other system indices can be evaluated using EcoNet which enhance our understanding on food web dynamics.

The proposed approach can also be applied to testing of hypotheses on changes in ecosystem functioning driven by external forces. This can be done by designing manipulative experiments that compare the amount and pattern of material flow as well as system indices across treatments. This approach would thus allow monitoring of changes in ecosystems function more efficiently and at lower analytical costs, with fewer conversions and assumptions, compared with conventional approaches (e.g. gut contents analysis) in collecting data for network analysis. Similarly, long-term monitoring of functional variability of ecosystems can be conducted using the proposed approach. Changes in the amount of material flow among compartments and system indices can be compared within the same system over time, e.g. seasonal or inter-annual, as indicators of trends in ecosystem health (Table 1).

ECOLOGICAL REALISM OF THE WINSAAM RESULTS

The modelled transfer rates should reflect changes in availability of C associated with particular compartments. Although the physical conditions of microcosms were well controlled and resembled those in the field, the abundance of meiofauna in microcosms decreased throughout the experiment (total meiofauna abundance decreased by 53%). The increased mortality of meiofauna, especially juvenile polychaetes and nematodes (nematode abundance decreased by 18% and polychaetes by 77%), as expected, caused an increase in C

Table 1. Theoretical advantages and disadvantages of conventional and the proposed approaches for food web analysis

Method	Advantages	Disadvantages
Gut content analysis	Qualitative and quantitative descriptions of diet	Favours identification of relatively large food items with hard parts and long residence in stomach
	Suitable for macro-organisms, such as fish and crabs	Only provides a snapshot of feeding activity and results in a bias in prey analysis
	Low analytical cost	Conversion to C transfer rates is error prone No indication on time-integrated assimilation by organisms
		No indication of ecosystem functioning Time consuming
Stable isotopes (natural abundance and enrichment) and mixing models	Suitable for micro- and macro-organisms	Assumption on system at equilibrium state can be violated because of the difficulty to determine whether isotopic equilibrium has been reached in enrichment experiments, especially when the degree of enrichment varies over time
	Qualitative and quantitative descriptions of trophic relationships	No indication of material release and/or backflow rate among organisms
	Medium analytical cost	No indication of ecosystem functioning
Biochemical markers, e.g. fatty acids	Qualitative information on food source utilization by consumers	No indication of material release rate among organisms, non-quantitative
	Suitable for micro- and macro-organisms	No indication of ecosystem functioning
		High analytical cost
This study	Quantitative assessment of food web structure and ecosystem functioning	More sampling effort: requires at least two time-points samples, i.e. the initial and final amount of tracer in the compartments for WinSAAM to estimate fluxes.
	High flexibility and accuracy to estimate fluxes because it does not require system to reach equilibrium state	Knowledge of ENA is required
	Suitable for micro- and macro-organisms	
	Can estimate time-integrated material assimilation fluxes among compartments	
	Allows hypothesis testing to compare functional variability, by comparison of flow rates and system indices in manipulative experiments	
	Medium analytical costs	

backflow from these meiofaunal groups to MPB in microcosms. MPB C uptake rate of nematodes was higher than those of harpacticoid copepods and juvenile polychaetes in the field. This was probably because of the large abundance of nematodes compared with the other two meiofaunal groups. The high abundance of nematodes has also been found in empirical ecological studies (Coull & Chandler 1992).

COMPARISON OF ASSUMPTIONS BETWEEN THE PROPOSED AND CONVENTIONAL APPROACHES

Conventionally, quantification of C flow in ecosystems is conducted mainly by: (i) measuring the productivity and consumption rates of the majority of individual species (Gerlach 1971) and/or using conversion factors based on the data retrieved from previous studies (Chardy & Dauvin 1992; Kennedy 1994); (ii) isotopic analysis followed by application of numerical mixing models; and/or (iii) utility of trophic biomarker analysis. However, time-integrated C assimilation rates among organisms are not quantified and no indication of ecosystem functioning is achievable using the conventional approaches (Table 1).

The first conventional approach, i.e. measurements of the productivity and consumption rates of organisms, suffers from the use of many conversion factors and the inability to measure instantaneous flow rates between compartments due to the laborious data collection process. For example, C assimilation by fiddler crabs, a common species on intertidal sandflats (Reinsel 2004), could be estimated from its dietary composition (e.g. through gut content analysis) and the C content of each food item in its diet. As gut content analysis of fiddler crabs often showed no trace of meiofauna, the difference in abundance of meiofauna between feeding pellets and undisturbed sediment is assumed to represent all meiofauna assimilated by the fiddler crabs (Dye & Lasiak 1986). Estimates of nematode C assimilated by fiddler crabs could then be calculated by using the following pieces of information gathered from, usually, prior to other studies: (i) the abundance of meiofauna on the surface sediment (e.g. 41 individuals per cm^2) (Dye & Lasiak 1986), (ii) the proportion of nematodes in the surface sediment (e.g. 98%) (Dye & Lasiak 1986), (iii) wet weight of nematodes ($\sim$0·93 μg per individual), e.g. retrieved from Gerlach (1971) and Heip, Vincx & Vranken (1985) and (iv) C content of nematodes retrieved from the literature (12·4%) (Jensen 1984). This would yield, by multiplying the above numbers, $\sim$4·64 μg C cm^{-2} day^{-1} of nematode C being assimilated by fiddler crabs.

The use of these 'static' conversion factors means that the estimates are only applicable to particular defined situations that are similar to those generating the conversion factors in the first place. Further, differences in meiofaunal abundance between disturbed and undisturbed sediments might not be solely attributable to crab diets. Any difference may be due to other confounding factors, e.g. the burrowing behaviour of crabs could lower the amount of food (i.e. MPB) available to the meiofauna (Reinsel 2004) and indirectly cause a decline in meiofaunal abundance.

Mixing models assume that the consumer must be close to isotopic steady-state or equilibrium with respect to its food sources before the isotopic signatures (with or without enrichment) of a consumer can be compared with its potential food sources at a particular point in time. Isotopic equilibrium between consumers and their food sources is generally assumed to exist in studies of natural abundances of stable isotopes. However, changes in ecological conditions may result in temporal fluctuations in isotopic composition of the consumers (O'Reilly *et al.* 2002). Thus, it is difficult to determine whether isotopic equilibrium has been reached in isotope addition experiments involving macrofauna, especially when the degree of isotopic enrichment varies over time.

Biochemical trophic markers, such as fatty acid (FA) analysis, could effectively discriminate food sources (Hall, Lee & Meziane 2006) and show temporal diet shifts in consumers (Wai *et al.* 2008). However, this method relies on the use of unique biomarkers that are found in a producer for tracing a food source. This may lead to confounding results if food sources share the same FA markers (see review of Dalsgaard *et al.* 2003). Further, high analytical cost of FA may limit the sample size and this could be problematic in manipulative experiments for hypothesis testing. Similarly, biochemical markers provide only qualitative information and do not measure fluxes from consumers to food sources or give indication on ecosystem functioning.

Combining stable isotope enrichment and compartmental modelling techniques provides an alternative approach to analysing food webs both qualitatively and quantitatively. Our proposed approach gives almost instantaneous measurement of C flow among compartments and provides sensitive indication on ecosystem functioning, e.g. C cycling and relative contribution of direct and indirect flows in the food web. The unique capability of this approach offers a great advantage in using this method for quantitative hypothesis testing of food web responses to manipulative experimentation.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Gut content analysis of soldier crab collected from Tallebudgera Creek before (day 0) and 1, 2, 4 and 6 days after the application of ^{13}C tracer. MPB, microphytobenthos; Seagrass, seagrass fragment; Poly, polychaetes; Harpact, harpacticoid copepods; Nemat, nematodes; Amph, amphipods. Data are mean + 1 SE.

Fig. S2. The carbon transfer rates (μg ^{13}C day^{-1}) as estimated using WinSAAM modelling between MPB, juvenile polychaetes, harpacticoid copepods, nematodes and soldier crabs, in the microcosms. (a)

the original MBP ^{13}C assimilation rate; (b) initial MPB C assimilation rate of ^{13}C is −10% of the original rate; (c) initial MPB C assimilation rate of ^{13}C is +10% of the original rate. Solid lines represent the direction of carbon flow from lower to higher trophic levels, whereas dash lines represent backflow. Line thickness represents the relative magnitude of carbon flow. Number in the arrow represents the amount of ^{13}C label (in μg) assimilated using MPB. Insignificant transfer rates ($\leq 9{\cdot}00 \times 10^{-8}$ μg) are not included.

Fig. S3. The carbon transfer rates (μg ^{13}C day^{-1}) as estimated using WinSAAM modelling between MPB, juvenile polychaetes, harpacticoid copepods, nematodes and soldier crabs, in the field. (a) in the original MBP ^{13}C assimilation rate; (b) initial MPB C assimilation rate of ^{13}C is −10% of the original rate; (c) initial MPB C assimilation rate of ^{13}C is +10% of the original rate. Solid lines represent the direction of carbon flow from lower to higher trophic levels, whereas dash lines represent backflow. Line thickness represents the relative magnitude of carbon flow. Number in the arrow represents the amount of ^{13}C label (in μg) assimilated using MPB. Insignificant transfer rates ($\leq 9{\cdot}00 \times 10^{-8}$ μg) are not included.

Table S1. $\delta^{13}C$ signatures (‰) of MPB, juvenile polychaetes, harpacticoid copepods, nematodes and soldier crabs before and after 7-day enrichment at an application rate of 1 g $NaH^{13}CO_3$ per m^{-2}.

Table S2. FSD of adjusted ^{13}C transfer rates (L_{ij}) from compartment *j* to *i*. Poly, juvenile polychaetes; Harpact, harpacticoid copepods; Nemat, nematodes; Crab, soldier crabs. Insignificant transfer rates ($\leq 9{\cdot}00 \times 10^{-8}$) are not included. All values are $< 0{\cdot}5$.

Table S3. FSD of adjusted ^{13}C transfer rates (L_{ij}) from compartment *j* to *i*. Poly, juvenile polychaetes; Harpact, harpacticoid copepods; Nemat, nematodes; Crab, soldier crabs, in (a) microcosms; and (b) field experiment. Insignificant transfer rates ($\leq 9{\cdot}00 \times 10^{-8}$) are not included.

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