Variable nutrient stoichiometry (carbon:nitrogen:phosphorus) across trophic levels determines community and ecosystem properties in an oligotrophic mangrove system
Variable nutrient stoichiometry (carbon:nitrogen:phosphorus) across trophic levels determines community and ecosystem properties in an oligotrophic mangrove system

U. M. Scharler1 · R. E. Ulanowicz2,3 · M. L. Fogel4,5 · M. J. Wooller6,7 · M. E. Jacobson-Meyers8 · C. E. Lovelock9 · I. C. Feller10 · M. Frischer11 · R. Lee12 · K. McKee13 · I. C. Romero14 · J. P. Schmit15 · C. Shearer16

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Abstract Our study investigated the carbon:nitrogen:phosphorus (C:N:P) stoichiometry of mangrove island of the Mesoamerican Barrier Reef (Twin Cays, Belize). The C:N:P of abiotic and biotic components of this oligotrophic ecosystem was measured and served to build networks of nutrient flows for three distinct mangrove forest zones (tall seaward fringing forest, inland dwarf forests and a transitional zone). Between forest zones, the stoichiometry of primary producers, heterotrophs and abiotic components did not change significantly, but there was a significant difference in C:N:P, and C, N, and P biomass, between the functional groups mangrove trees, other primary producers, heterotrophs, and abiotic components. C:N:P decreased with increasing trophic level. Nutrient recycling in the food webs was highest for P, and high transfer efficiencies between trophic levels of P and N also indicated an overall shortage of these nutrients when compared to C. Heterotrophs were sometimes, but not always, limited by the same nutrient as the primary producers. Mangrove trees and the primary tree consumers were P limited, whereas the invertebrates consuming leaf litter and detritus were N limited. Most compartments were limited by P or N (not by C), and the relative depletion rate of food sources was fastest for P. P transfers thus constituted a bottleneck of nutrient transfer on Twin Cays. This is the first comprehensive ecosystem...
study of nutrient transfers in a mangrove ecosystem, illustrating some mechanisms (e.g. recycling rates, transfer efficiencies) which oligotrophic systems use in order to build up biomass and food webs spanning various trophic levels.

**Keywords** Oligotrophic environment · Recycling · Nutrient limitation · Mangrove food web · Transfer efficiency

### Introduction

Nutrient dynamics in aquatic ecosystems have been studied in recent decades mainly due to concerns over eutrophication, resulting algal blooms and their impacts on other biotic communities. As such, eutrophic systems have gained much attention, especially with regards to the nutrient-algae link (e.g. Smith et al. 1999; Anderson et al. 2014). However, there are two areas regarding nutrient dynamics that have received less consideration. Higher trophic levels are rarely included in studies, and oligotrophic systems are relatively unrepresented in the literature. Nutrient dynamics in oligotrophic systems differ from those in eutrophic systems by having considerably lower availability of nutrients in their dissolved and non-living particulate form (e.g. Zhang et al. 2007). This lower nutrient availability implies that in order to satisfy nutrient demands of higher trophic levels, recycling rates should be high and transfers of nutrients efficient across trophic levels.

Studies of elemental stoichiometry in ecosystems, especially that of macronutrients [i.e. carbon (C), nitrogen (N) and phosphorus (P)], have become important to explain a wide range of community and ecosystem properties. Such properties include taxa-specific variability and ontogenetic shifts, effects of consumer-driven nutrient cycling, and the integration of stoichiometric and metabolic theory to explain ecosystem functioning and stability (e.g. Sterner and Elser 2002; Vanni et al. 2002; Cross et al. 2005; Allen and Gillooly 2009; Hillebrand et al. 2009; Persson et al. 2010). In this regard, ecological stoichiometry is an integral part of community and food web ecology. In this paper we use C:N:P stoichiometry to infer both ecosystem and community-level properties of recycling, transfer efficiency and nutrient limitation of a mangrove forest.

In the past, aquatic ecosystems have generally been divided into those that are limited, sensu Liebig, by N (marine) or P (freshwater). These generalisations were mostly derived from nutrient limitations investigated in growth experiments, or nutrient concentrations of algae which were assumed to reflect their nutrient environment (Redfield 1934). However, nutrient limitations are not confined to primary producers, and can be exacerbated and sometimes switched at higher trophic levels. For example, algae may be N limited in a particular ecosystem, whereas fish in the same ecosystem may be P limited due to their higher requirement of P for bone synthesis (Ulanowicz and Baird 1999; Sterner and George 2000; Vanni et al. 2002). Limiting nutrients may vary between ecosystem components and it is difficult, if not impossible, to label an entire ecosystem as limited by one particular nutrient.

The overall aim of our study was to investigate the nutrient dynamics in an oligotrophic mangrove ecosystem. We established the stoichiometry (C:N:P) of biotic and abiotic ecosystem components and then used these data to calculate nutrient transfer efficiencies between trophic levels, stoichiometry at the various trophic levels and extent of recycling. We hypothesised that the nutrient stoichiometry changes between trophic levels and that recycling rates are higher for the limiting nutrients. Lastly, we investigated the changes of nutrient limitations of ecosystem components.

### Materials and methods

We established the stoichiometry (C:N:P) of the biotic and abiotic ecosystem components, and nutrient flows between them for three different forest zones on the mangrove islands Twin Cays, Belize. Data were gathered during a 5-year USA-National Science Foundation BioComplexity programme, which included ten research groups focussing on biochemistry, physiology and ecology of various ecosystem components (Table 1). Data emanating from this research served to establish the C:N:P stoichiometry of various abiotic and biotic components, and to build trophic-flow networks for three tree growth zones by quantifying standing stocks and trophic links.

### Study site

This study was conducted on Twin Cays (16°50′N, 88°06′W), Belize, a 92-ha pair of mangrove islands situated about 12 km offshore (MacIntyre et al. 2004) that is part of a system of mangrove islands between the Belizean mainland and the Mesoamerican barrier reef. In the 1990s, fertilisation experiments examined nutrient limitations of the mangrove trees in each of three forest zones (fringe, transition, dwarf) (Feller et al. 2002). These studies demonstrated that mangrove trees were N limited in the fringe zone, N+P limited in the adjacent transition zone, and P limited in the interior dwarf zone (Feller et al. 2002). The fringe zone on Twin Cays is composed primarily of *Rhizophora mangle* L. (red mangrove), with lesser amounts of
Table 1 Data sources for stocks and flows used to build the carbon (C), nitrogen (N) and phosphorus (P) networks for the fringe, transition and dwarf mangrove forest zones

<table>
<thead>
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<th>Compartment</th>
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<td>1</td>
<td><em>Rhizophora mangle</em></td>
<td>Leaf biomass, production, respiration, wood biomass, wood production, C content, prop-root biomass, proportion of belowground root biomass, belowground root production, prop-root production P:B, C:N, nutrient sources, N:P</td>
<td>This study; CARICOMP data; Cintrón and Schaeffer Novelli (1984); Jin-Eong et al. (1995); Feller and Mathis (1997); Koltes et al. (1998); McKee et al. (2007); McKee (2011)</td>
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<td><em>Avicennia marina</em></td>
<td>Wood/leaf biomass, P:B, P:R, wood biomass, C content, wood litterfall, root biomass, C:N, nutrient sources, N:P</td>
<td>This study; CARICOMP data; Koltes et al. (1998); Cintrón and Schaeffer Novelli (1984); McKee et al. (2007); McKee (2011)</td>
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<td><em>Laguncularia racemosa</em></td>
<td>Wood/leaf biomass, P:B, P:R, wood litterfall, root biomass, C:N, nutrient sources, N:P</td>
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<td>89</td>
<td>DIC, NH₄⁺, NO₂⁻, NO₃⁻, PO₄⁻</td>
<td>Standing stock</td>
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</table>

*Production, B biomass, R respiration, E egestion, POC particulate organic C, DOC dissolved organic C, DON dissolved organic N, DOP dissolved organic P, DIC dissolved inorganic C, C consumption, CARICOMP Caribbean Coastal Marine Productivity Program, ATLSS across trophic levels system simulation*
Avicennia germinans (L.) Stearn. (black mangrove) and Laguncularia racemosa (L.) C.F. Gaertn. (white mangrove). The transition zone was populated by a mixture of the three tree species, and the interior dwarf zone by monospecific stands of R. mangle.

Field data

Representative species of various functional groups (see Table 1) were sampled in each of the three forest zones and their dry weight per area was determined as described below.

Primary producers

Leaf, wood and root biomass (grams per square metre) was calculated for the mangrove tree species R. mangle, L. racemosa and A. germinans. Leaf biomass of R. mangle was estimated from the leaf area index assessed using hemispherical photography analysed with the Hemiview software package (Delta T Devices, UK) and wood and prop-root biomass from allometric relationships based on tree height and diameter (Cintrón and Schaeffer Novelli 1984) measured on nine replicate trees in each forest zone. Wood biomass of A. germinans and L. racemosa was obtained from Caribbean Coastal Marine Productivity Program (CARICOMP) data (CARICOMP—UNESCO; see also Koltes et al. 1998). To determine belowground root biomass, 50-cm deep cores were taken with a Russian peat corer (cross-sectional area 5 cm²) in the transition zone, which contained primarily A. germinans and L. racemosa. Live and dead roots were separated from the background material, washed, dried at 80 °C, and weighed. Leaf production was measured from litterfall (CARICOMP data). Belowground accumulation rates of mangrove roots were determined using an implanted mass technique (McKee et al. 2007; McKee 2011).

Total lichen cover on the bark of trees was estimated for each tree species in the three different zones from percent cover estimations and an estimate of tree surface area, based on main stem height and diameter. Macroalgae were divided into two groups including submerged macroalgae and the most conspicuous intertidal macroalgae, Bostrychia sp., Bostrychia sp. biomass was measured in nine replicate 25 × 25-cm plots in each vegetation zone, whereas data on submerged macroalgae were taken from the literature (Table 1). Microbial mats were abundant on Twin Cays, especially in the dwarf zone, often building thick layers of floc (unconsolidated microbial layers). Coverage, biomass, productivity, respiration, N fixation, denitrification and P content of microbial mats were assessed (Joye and Lee 2004; Lee and Joye 2006; Lee et al. 2008). These mats were dominated by cyanobacteria and exhibited high rates of primary production, respiration, and N fixation. Microbial mats exhibited unique stable isotopic signatures (Lee 2006) and contributed to ecosystem productivity, especially in the dwarf forest zone.

Heterotrophic compartments

The heterotrophic compartments of the habitats on Twin Cays consisted of several crab species, molluscs, fauna inhabiting leaf litter and detritus (micro-, meio- and macrofauna), arthropods utilising the mangrove trees as a food source or for shelter, reptiles, birds, bacteria and fungi, and in the fringe forest zone root-fouling organisms on submerged R. mangle roots. Mammals were not part of the Twin Cays food web due to a lack of land-based mammals. Fish were excluded from the network due to a lack of quantitative data.

Densities of the largest and most conspicuous gastropods that were not part of the smaller litter fauna, namely Cerithidea sp., Littorina angulifera and Melampus coffeeus, were sampled in three transects across all three zones at the CARICOMP site where leaf litter was taken. Snails were dried at 60 °C, weighed, and stored for stable isotope analysis. Leaf litter fauna was sampled at eight transects in April and November 2001. Three replicates were taken in the fringe, transition and dwarf zones. An area of 25 × 25 cm of leaf litter and underlying detritus (soft mud on peat surface) was sampled, and detritus and leaves were rinsed above a fine-mesh sieve (63 µM). The organisms were grouped into Harpacticoidea, Copepoda, Amphipoda, Isopoda, Tanaidacea, Ostracoda, Nematoda, Oligochaeta, Polychaeta, Gastropoda, Turbellaria and Acari. An initial examination on invertebrate infauna in mangrove peat revealed extremely low numbers. As extraction of fauna from mangrove peat is highly time consuming, the fauna in mangrove peat was not included in this study.

Information on arthropods utilising the mangrove trees was collated from the literature (Feller and Mathis 1997; Feller 2002) and data generated during the present study. The density of leaf miners and stem miners on R. mangle was measured according to methods in Feller and Chamberlain (2007). Tree density measurements obtained from CARICOMP (Koltes et al. 1998) were used to estimate arboreal arthropod density in each forest zone.

Biomass of sediment bacteria from peat samples was estimated by epifluorescent counting and biovolume estimation after staining with 4',6-diamidino-2-phenylindole (Alongi 1988; see Online Resource 1). Bacteria associated with decaying leaf litter and fresh leaves were extracted similarly to peat samples, except that ten hole punches (0.625 cm diameter) from each leaf were formalin fixed.
Biomass of fungi was estimated by determining the activity of chitinase in the peat, following Miller et al. (1998). Estimation was a two-part process. First, enzyme activity in peat was measured in the field. Secondly, fungi from peat samples were cultivated, their enzyme activity measured, standard curves were developed, and fungal biomass was calculated from enzyme activity (see Online Resource 1).

Abiotic components

Dead root biomass for A. germinans and L. racemosa was measured from cores taken in the transition zone as described above. Leaf litter sampled during the leaf litter invertebrate sampling was separated into R. mangle, A. germinans and L. racemosa trees and Thalassia sp., rinsed, dried at 60 °C and weighed. To measure dead wood biomass, dead wood on the ground and in the canopy was collected in three replicate 4 × 4-m plots in the three forest zones. The coarse woody debris was sorted by species, fresh and dry weight measured, and nutrient content determined. Plots were resampled after 2 years in order to determine input rate into each forest zone.

A thin layer termed ‘detritus’ sometimes overlaid the mangrove peat. The mass of this detrital layer was determined by measuring the thickness of this layer, which ranged from 0 to 2 cm, and dry weight from a known volume. The mangrove peat below the thin detrital layer was termed ‘particulate organic matter’, and its dry mass was determined by drying a known volume of peat.

Dissolved organic nutrients were measured using a high temperature catalytic oxidation system [for dissolved organic C (DOC), and dissolved organic N] and ash hydrolysis-spectrophotometry for dissolved organic P (Lee et al. 2008). DOC leaching from leaves was determined from two decomposition experiments starting in April and November 2001, respectively (see Online Resource 1). Dissolved inorganic N [nitrate (NO$_3^-$), nitrite (NO$_2^-$), ammonia and phosphate (PO$_4^{3-}$)] were measured using the phenol hypochlorite method for ammonium, the cadmium reduction method for NO$_3^-$ + NO$_2^-$, spectrophotometry for NO$_2^-$, and PO$_4^{3-}$ was analysed colorimetrically using the molybdate blue method (see Lee et al. 2008 for methodological details).

**Determination of C, N, and P contents**

Samples for total C, N and P analysis were dried at the Carrie Bow Cay field station laboratory at 50–80 °C in air, brought back to the Carnegie Institution of Washington, where they were completely dried at 50 °C under N$_2$. Samples were weighed (0.2–2 mg) into tin boats for further analysis (Wooller et al. 2003). The C and N contents of primary producers, heterotrophs, and detrital components were measured using an elemental analyser (Carlo Erba 2500) attached to a Thermo Finnigan Delta Plus XL isotope ratio mass spectrometer (Fogel et al. 2008) (see Table 1 for exceptions). P content was analysed using methods modified from Aspila et al. (1976) for the various ecosystem components by comparing digestion of weighed, dried and combusted samples which were subsequently measured spectrophotometrically for total P concentration normalized for dry weight (see Table 1 for exceptions). Organic PO$_4^{3-}$ was determined by subtraction.

The C, N and P contents (as % of dry weight) were used to express the stoichiometry of individual compartments by weight, and to calculate C, N and P standing stock per area for each compartment in each forest zone.

**Network building**

We calculated biomass in C, N and P and quantified trophic and respiratory flows for all identified compartments (see Table 1). With these, balanced C, N and P budgets for all compartments and for all three forest zones were produced as described below.

Species abundance and biomass were measured for the components included in the networks. Productivity (P) and/or respiration (R) for certain species were measured during this project and metabolic parameters not measured were estimated using P:biomass (P:B), P:R, and consumption (C):B ratios from the literature. Where C, N or P contents were not available for a certain compartment, that of a compartment similar in feeding guild, taxonomic group and size was used. All biomass was expressed as grams of C or N or P per square metre and all flows as grams of C or N or P/square metre per year. Sources for data, ratios, and equations used to calculate flows are listed in Table 1. In addition to the trophic and respiratory flows, boundary flows were included as imports and/or exports to and from the systems (Online Resource 1).

Using this information, quantities were assigned to feeding links among and between biotic and abiotic compartments following the method of Ulanowicz and Scharler (2008) (see Online Resource 1). This resulted in nine networks, i.e. a C, N and P network for each of the three mangrove forest zones. Networks were mass balanced so that the nutrient balance for autotrophic nodes becomes—gross primary production (GPP) = net primary production (NPP) + R; and for all heterotrophic nodes—consumption (C) = P + R + E. R is included only for the C networks; N networks feature gaseous exchange only for microbial mats (denitrification); and there is no gaseous exchange for P.
Calculation of nutrient limitations and limiting sources

To identify the element limiting a given recipient compartment, the criterion of Liebig (1840) is usually invoked. In the network context, this translates into finding the element with the longest residence, or turnover time, in the given compartment. Furthermore, the limiting flow originating from a particular source compartment was calculated. The Liebig procedure normally cannot be applied to identify which particular source of that limiting nutrient is most crucial to the given compartment. Ulanowicz and Abarca-Arenas (1997), however, generalised the Liebig procedure by showing that both limiting elements and limiting flows are those to which the overall biomass inclusive system ascendency is most sensitive (see Online Resource 1).

Ascendency is a system-level index that quantifies jointly the degree of trophic-flow organisation inherent in the network and its total system throughput (TST) (Hirata and Ulanowicz 1984). The theory of the ascendency index and associated calculations are outlined in Online Resource 1, whereas the specific equations (1, 2) used for the sensitivity calculations are presented here to demonstrate which changes in biomass and flows impact the ascendency value. Applying the ascendency calculations to identify (1) limiting nutrients and (2) limiting flows, the activities on the compartmental level are put into context of the ecosystem level. For each forest zone, the turnover time for each nutrient in each recipient compartment was calculated to identify the limiting nutrient (1). In the ascendency calculation, an increased biomass of an element \( k \), and a slower turnover time of \( k \) in the node \( p \) compared to the turnover time of the entire system, contribute to a higher value of ascendency (Eq. 1; Online resource 1):

\[
\frac{\partial A_B}{\partial B_{pk}} = 2 \left( \frac{T_{..}}{B_{..}} - \frac{1}{2} \frac{T_{pk}}{B_{pk}} + \frac{T_{pk}}{B_{pk}} \right),
\]

where \( A_B \) is the biomass inclusive ascendency, \( B_{pk} \) the biomass of node \( p \) in terms of element \( k \), \( T \) the total throughput and \( B \) the total biomass.

Nutrients with a faster turnover time compared to that of the system contribute negatively to the system’s ascendency, and nutrients with an equal turnover time in a compartment to that of a system contribute a very small amount to the system’s ascendency. The system is therefore most sensitive to the slowest compartmental turnover times in relation to the system’s turnover time for the same element.

As the ascendency calculations in the identification of limiting nutrients have not yet been widely applied, we also calculated the turnover time for each compartment for comparison.

Then, the limiting flows (2) were calculated from the rate of depletion of a specific nutrient in a source node relative to its standing stock, where the nutrient flow with the highest relative depletion rate constitutes the limiting flow (Ulanowicz and Abarca-Arenas 1997). This limiting flow results in a high ascendency value (Eq. 2; Online Resource 1).

\[
\frac{\partial A_B}{\partial T_{ip}} = \log \left( \frac{T_{ip}B_{..}^2}{T_{..}B_{..}B_{ip}} \right)
\]

The highest sensitivity value from the above equation is calculated for the limiting flow that depletes its source at the fastest rate compared to its availability.

The limiting nutrient, and the limiting flow are not necessarily the same, since Eq. 1 is calculated for recipient nodes, and Eq. 2 is calculated for source nodes (see details in Online Resource 1).

To investigate the stoichiometry of and transfer efficiencies between trophic levels, the individual compartments were then apportioned over the various trophic levels according to their feeding activity (Ulanowicz 1986; Ulanowicz and Kay 1991). Investigations on the elements C, N and P per trophic level, their transfer efficiencies and recycling rates were conducted by analysing all networks using the software WAND (Allesina and Bondavalli 2004).

Results

C:N:P stoichiometry of biota and trophic levels

General biotic and abiotic groups

The C:N:P stoichiometry differed strongly between living and non-living compartments in all three mangrove forest zones (Fig. 1). High SDs of mean C:N, C:P and N:P resulted from considerable variability of C:N:P within groups (as in Fig. 1). Therefore, results of a two-way ANOVA (on log-transformed data) showed statistically significant differences only between the three major groups including primary producers, heterotrophic compartments and abiotic compartments (C:N, \( F = 346.7, p < 0.001; \) C:P, \( F = 309.47, p < 0.001; \) N:P, \( F = 20.64, p < 0.001)\), but not between zones (\( p > 0.05)\) nor for a compartment groups \( \times \) zone interaction (\( p > 0.05)\). Heterotrophs showed lower C:N and C:P and had less variable ratios than primary producers or abiotic compartments (Fig. 1).

Functional groups and compartments

\( R. mangle \) green and senescent leaves and microbial mats are two of the most important primary producer groups in terms of biomass and occur in all three forest zones (Table 2). The C:N for \( R. mangle \) green leaves was similar from one zone to another, whereas the C:N for microbial
Mats was halved in the dwarf zone compared to the fringe and transition zones. The C:P and N:P increased from the fringe to the dwarf zone for *R. mangle* green leaves, illustrating the relative decrease of P in the dwarf zone.

Microbial mats, however, showed a decreasing C:P and an increasing N:P from the fringe to the dwarf zone (Table 2), which is due to the greater presence of N and P relative to C. *R. mangle* leaf litter had higher C:N and C:P compared to those of green leaves in all three zones. Primary tree consumers (Fig. 1) had, in general, a higher C:N ratio, but a lower C:P and N:P ratio compared to organisms feeding primarily on leaf litter (Fig. 1) in all three zones. These ratios may reflect the lower P content in source material such as leaf litter compared to e.g. *R. mangle* green leaves (Table 2). The higher C:P and N:P of leaf litter in the dwarf zone compared to the fringe and transition zone support the assertion that there was a lower amount of available P in this zone. However, overall differences between C, N and P biomass (in grams per square metre; Online Resource 2) of all compartments were not statistically different between the three forest zones (*p* > 0.05), but there were significant differences (for C, *F* = 81.38, *p* < 0.001; for N, *F* = 47.79, *p* < 0.001; for P, *F* = 39.88, *p* < 0.001) between functional groups (trees, other primary producers, heterotrophs, abiotic compartments). Across trophic levels, both N and P were incorporated in higher relative proportions compared to C in organisms feeding on trophic levels ≥II (Fig. 2). In addition, P is accumulated at a higher rate relative to N in heterotrophs in comparison to trophic level I (Fig. 2).

**Nutrient limitations**

*Limiting nutrient of recipient compartment: ascendency calculations*

The nutrient limitation of individual compartments was calculated by the system ascendency’s sensitivity to changes in turnover rates of a particular nutrient in a particular compartment, and also by calculating compartmental turnover rates for each nutrient and compartment as a reference. From the ascendency analysis it was apparent that in all zones mangrove trees were primarily P limited. Sensitivity values for N were in most cases slightly lower, in contrast to those for C, which diverged considerably more, indicating a possible co-limitation by N (Online Resource 2). Similarly, microbial mats were P limited, and their co-limitation by N was less pronounced compared to that of mangrove trees. Sensitivity values for N and P were more similar at higher trophic levels.

### Table 2  
C:N:P (by weight) of two primary producer groups (*R. mangle* leaves and microbial mats) and *R. mangle* leaf litter in the three mangrove forest zones on Twin Cays, Belize.

<table>
<thead>
<tr>
<th>Mangrove forest zones</th>
<th><em>R. mangle</em> green leaves</th>
<th><em>R. mangle</em> leaf litter</th>
<th>Microbial mats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fringe</td>
<td>46.7</td>
<td>1570.2</td>
<td>33.6</td>
</tr>
<tr>
<td>Transition</td>
<td>45.2</td>
<td>1537.6</td>
<td>34.0</td>
</tr>
<tr>
<td>Dwarf</td>
<td>45.4</td>
<td>2136.5</td>
<td>47.1</td>
</tr>
</tbody>
</table>

Fig. 1  
Mean (±SD) of C:N (a), C:P (b) and N:P (c), by weight, for major abiotic and biotic groups in the fringe, transition and dwarf zone. MT Mangrove trees, OPP other primary producers, CR crabs, GA gastropods, LF litter fauna, SMF sessile macrofauna, MMF mobile macrofauna, IN insects, OA other arthropods, RE reptiles, BI birds, BF bacteria and fungi, AB abiotic.

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and were most similar at the highest trophic levels (reptiles, selected compartments of birds, crabs, leaf litter fauna, insects; Fig. 3). Bacteria and fungi (compartments 68–73) were P limited throughout all zones, as were the first-order consumers of trees (insects consuming green leaves, twigs and wood, compartments 37–51). Crabs, larger gastropods and leaf litter fauna (compartments 9–28) were limited by N. More than 50 % of reptile and bird compartments (compartments 54–67) were limited by N, followed by C and P. The fauna associated with submerged *R. mangle* prop roots in the fringe zone (compartments 29–36) were limited by P.

**Limiting nutrient of recipient compartment: turnover/biomass calculations**

There was nearly a 100 % overlap in the compartmental nutrient limitations derived from ascendency analysis and those calculated by individually comparing supply (total input stoichiometry) to requirement (biomass stoichiometry) for comparison (Online Resource 2). Only one of 174 compartmental nutrient limitations of all three forest zones differed and showed C limitation from the sensitivity value, and P limitation from the calculation of turnover rates. The latter were very similar for the two elements, differing by only 0.3 %. In summary, both P and N showed slower turnover rates in individual compartments in relation to the system from the ascendency analysis, causing nutrient limitation. Nutrient limitations apparent from the stoichiometry calculation of supply and requirement (the check to the sensitivity values) arise when the stoichiometry of the flow entering a compartment is dissimilar to the stoichiometry of the node.

**Limiting flows emanating from source compartment: ascendency calculations**

The limiting flows in the systems, which convey the highest depletion rate of a nutrient from a source compartment in relation to depletion of other sources (Ulanowicz and Abarca-Arenas 1997), were almost exclusively those for P transport (P, 97 %; N, 2 %; C, 0 %). The proportionally
highest depletion rate, and therefore possible bottlenecks in P transport in all three zones predominantly originated from the sediment bacteria and fungi compartments (68–73), followed by non-living compartments (74–89) and insect compartments (37–52). Overall, of the three nutrients investigated, P was depleted at the fastest rate from the various source compartments, highlighting P transfers between compartments as bottlenecks in the Twin Cays ecosystem.

**Ecosystem-level characteristics**

The material exchange of internal flows (excluding flows across system boundary) between trophic levels in general decreased from trophic level I to trophic level IV for C and N flows resembling an exponential decline, whereas that of P flows was more akin to a linear decrease (Fig. 4). The largest differences between C, N and P flows were apparent especially for the first two trophic levels. The networks from the dwarf zone showed the smallest C flows, and from the transition zone they were lowest for N and P flows over all trophic levels. The relatively high N and P flows in the dwarf zone resulted from the extensive microbial mats that were relatively enriched in N and P compared to the mangrove trees.

Mean transfer efficiencies (the proportion of flow into one trophic level that is passed on to the next) for the first four trophic levels (I–IV) were in general highest for P, followed by N and C (Fig. 5; Table 3). Transfer efficiencies for N were twice as high compared to those for C for the fringe and transition zones, and about three times as high in the dwarf zone. Transfer efficiencies for P were four times that of C in the fringe and transition zones, and ca. nine times in the dwarf zone (Fig. 5; Table 3). The highest transfer efficiencies were apparent at trophic level I, where they ranged from ca. 21 to 27 % for C, 38–48 % for N and 30–81 % for P, and the lowest at trophic level IV (Fig. 5). Although transfer efficiencies were generally higher for P and N compared to C, especially at trophic level I, they were similar to each other at trophic level IV. The largest difference between trophic levels for C, N and P was apparent in the dwarf zone (Fig. 5). Overall, these results reflect the biomass dominance of proportionally C-rich mangrove trees (lower C transfer efficiency) and rates of recycling of the three nutrients. The percentage of the TST recycled, which was calculated as Finn’s cycling index (FCI) (Finn 1980), was highest for P overall, and highest in the dwarf zone at 84.3 % (fringe, 51.0 %; transition, 15.4 %; Table 3). Recycling rates were fairly similar between the zones for N and decreased slightly from the dwarf (16.8 %) to the fringe zone (12.4 %). Recycling of C was highest in the fringe forest zone (10.6 %) and decreased to 6.5 % in the dwarf (Table 3).

**Discussion**

Although the concept of ecological stoichiometry has its beginnings in marine ecosystems through the Redfield ratio (Redfield 1934), most subsequent stoichiometric analyses, especially those of consumers, have been conducted in freshwater lakes or river ecosystems. Wide ranges of nutrient ratios are reported in the literature for invertebrates, comprising the largest number of heterotrophs in this and other studies, both on a molar and percent weight basis (e.g. Cross et al. 2003, 2005; Evans-White et al. 2005; Zhang et al. 2013). Comparisons to literature values are not exceptionally informative due to the ranges reported being generally large; still, our values for invertebrates fell within those from the literature (e.g. Cross et al. 2003; Evans-White et al. 2005). To further elucidate the stoichiometry within the invertebrate group on Twin Cays, we looked at feeding guilds, especially at herbivores (mangrove tree consumers) and detritus feeders (on abiotic sources). Heterotrophs on Twin Cays incorporated proportionally
more N and P into their biomass relative to their diets and showed stoichiometric homeostasis in their nutrient ratios (i.e. lower variability) when compared to primary producers. Organisms utilising mangrove trees or their litter as a primary food source, such as leaf, wood and twig borers, stem girdlers as well as crabs and larger gastropod species, achieved the highest degree of homeostasis in the system. These groups may supplement their herbivorous diet through nutrient richer microbial cells attached to sediment or leaf litter, or employ an omnivorous strategy when needed to supplement a herbivorous diet [e.g. the tree crab *Aratus pisonii* (Diaz and Conde 1998)]. This strategy and the presence of nutrient-rich primary producers (e.g. microbial mats), as well as bacteria and fungi, may alleviate N and P shortage in the food web as a whole and contribute to a biomagnification of N and P at higher trophic levels, as described e.g. for the Baltic Sea ecosystem by Bradshaw et al. (2012). Mangrove trees on Twin Cays also showed some degree of compensation for the different availability of nutrients through reabsorption of nutrients before leaf abscission (Feller et al. 2002).

The mangrove trees had the highest C:N and C:P of all primary producers and this may make this food source less desirable as a large gap to consumer stoichiometry must be bridged. Nevertheless all parts of the trees were not only highly important food sources to the invertebrates consuming trees, but also featured prominently in the quantity consumed. However, in mangrove systems such as Twin Cays, the fraction of the nutrient-poor primary production consumed may largely depend on the specialisation of feeding guilds present in the ecosystem rather than on stoichiometry. Previous studies showed that on Twin Cays, xylem- and phloem-feeding wood borers remove over 50 % of the *R. mangle* canopy, and leaf-feeding herbivores about 6 % (Feller 2002), which constitutes a significant percentage of primary production removed from the system. In addition, wood borers may supplement their diets with e.g. nutrient richer fungi, but we lack specific information on this feeding link in the Twin Cays forest.

In nutrient-poor systems such as mangroves, the recycling of nutrients is especially important to sustain suitable stoichiometric requirements of organisms. Speculations that small-sized organisms are highly important to allow efficient consumer-driven nutrient recycling (e.g. Vanni 2002) can, for mangrove systems, be extended to include larger organisms that can efficiently utilise the abundant mangrove litter. Here, consumers of different sizes are necessary to break down leaf litter into a series of progressively smaller fragments to allow further digestibility and a larger surface area to be colonised by fungi and bacteria (Scharler 2012). The high degree of nutrient cycling of the limiting nutrient corroborates the notion that both the larger (crabs, gastropods) and smaller (leaf litter fauna) invertebrate communities are of utmost importance in the recycling process along with the microbial organisms.

Few whole ecosystem studies have been reported in the literature that include the three macronutrients, and we compare indices pertinent to our work to those calculated in one such study of a temperate shallow coastal ecosystem.
of the Sylt-Rømø Bight, Germany (Baird et al. 2008). We calculated a higher TST for C (from two to ten times), highest contributors to which were the mangrove trees and throughputs through the dissolved organic and inorganic nutrient compartments. On the contrary, the TST of N and P were considerably lower on Twin Cays (0.1–0.5 times for N and 0.01–0.2 times for P) compared to the Sylt-Rømø Bight. Recycling of nutrients (as FCI) was lower on Twin Cays for C and N as compared to the Sylt-Rømø Bight, and recycling of P was comparable only for the Twin Cays dwarf zone (ca. 80%), but lower in the fringe and transition zones. Mangrove trees, which contribute a considerably large zone (ca. 80%), but lower in the fringe and transition zones. Mangrove trees, which contribute a considerable amount to throughputs, have overall lower recycling rates compared to the prominent primary producers in the Twin Cays networks, is the lack of species-specific information, applicable to the Sylt-Rømø Bight. Recycling of nutrients (as FCI) was lower on Twin Cays for C and N as compared to the Sylt-Rømø Bight, and recycling of P was comparable only for the Twin Cays dwarf zone (ca. 80%), but lower in the fringe and transition zones. Mangrove trees, which contribute a considerable amount to throughputs, have overall lower recycling rates compared to the prominent primary producers in the Twin Cays model. Only 3% of compartments were C limited in the former, whereas 12% were C limited on Twin Cays. The limiting flows from the source compartments revealed further differences. Where 98% of flows from source compartments were P limited on Twin Cays, this amounted to 52% for P, and 43% for N in Chesapeake Bay (Ulanowicz and Baird 1999). Although the two studies used the same algorithms to calculate nutrient limitations, there are two major differences due to network construction which may influence this comparison. Firstly, the network structure is different, which is mainly due to the nature of the ecosystems (e.g. mangrove trees and associated insects, lack of water column on Twin Cays). Secondly, whereas for the Twin Cays networks, the integrity of the measured stoichiometry of compartments was carried through network building and the layered balancing procedure (Online Resource 1), the Chesapeake C, N and P networks were constructed and balanced separately (Ulanowicz, personal communication).

Recycling was an important mechanism on Twin Cays to provide adequate amounts of nutrients to the food web, aided by the relatively high transfer efficiencies of especially N and P between trophic levels, resulting in a substantial decrease of C:N and C:P at trophic levels ≥ II. Calculations of the compartmental nutrient limitations have revealed the dominance of P limitation, followed by N, implying that both P and N demands are sometimes unmet. Efficiencies of transfer were of similar magnitude at all trophic levels, but higher for P in the dwarf forest zone compared to the fringe or transition forest zones. In a previous study, the severe P limitation of mangrove trees in the dwarf forest zone has been highlighted through fertilisation experiments and growth responses, and a N or N+P limitation in the fringe and transition zones (Feller et al. 2002).

Although our study calculated P limitations for all trees in all forest zones, the sensitivity coefficients for N were very close to those of P. The analysis of limiting flows on the other hand showed that P was depleted proportionally the fastest from most compartments, revealing an overall shortage of P on Twin Cays. The microbial mats on the other hand may have contributed to the enrichment in N relative to P across the food web supporting various trophic levels. Overall, the P limitation of the primary producers was repeated in some, but not all compartments at higher trophic levels. Although the first-order tree consumers were similarly limited by P, the leaf litter consumers (crabs, larger gastropods, leaf litter fauna) were limited by N. This pattern was repeated in all forest zones.

In conclusion, we found high recycling rates for the nutrient that showed higher transfer efficiencies and was the limiting nutrient. The compartmental nutrient limitation of the primary producers was repeated for some, but not for all higher trophic level groups. Significant differences in C:N:P were found between primary producers, heterotrophs and abiotic compartments, however not between forest zones. C:N:P decreased with trophic level, where the largest differences between trophic levels were apparent for C:P. The analysis of flows revealed a comparatively faster source node depletion rate for P. P was thus the limiting flow constituting a bottleneck for nutrient transfers in the Twin Cays ecosystem which possibly influences food web structure (e.g. Sterner and Elser 2002), and population (Andersen et al. 2004) and system growth.

The results and conclusions from this study are based on networks that were partly constructed from system-specific data, and partly from data originating from the literature. As it is not possible to measure each flow in a network, supplements from the literature are in general necessary in ecological network analysis; however, these may introduce errors in model structure. In addition, another common drawback in network construction, applicable to the Twin Cays networks, is the lack of species-specific information for each single species and changes thereof over time, resulting in a temporal snapshot. The interpretations from our study arose from networks that were constructed
as best as possible within the framework of this study. To increase the level of confidence more system-specific data are needed, especially increased temporal resolution would assist in interpreting variability.

Author contribution statement  U. M. S. collated data, built and analysed networks, and wrote the manuscript. R. E. U. developed the network analysis methodology. All authors provided input data to the network analysis and to the manuscript.

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