Science of the Total Environment 508 (2015) 402–410

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effects of nutrient enrichment on mangrove leaf litter decomposition

Joost A. Keuskamp [a,*], Mariet M. Hefting [a], Bas J.J. Dingemans [a], Jos T.A. Verhoeven [a], Ilka C. Feller [b]

[a] Ecology and Biodiversity, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
[b] Smithsonian Environmental Research Center, Edgewater, MD 21037, USA

HIGHLIGHTS

- Microbial decomposers are N-limited even in sites where tree growth is P-limited.
- Eutrophication increased mangrove litter decomposition exclusively via litter quality.
- Litter N and P dynamics were not modified by external amendments.
- Eutrophication effects on decomposition may depend on limitation of primary producers.
- Litter N and P dynamics were not modified by external amendments.

ARTICLE INFO

Article history:
Received 8 October 2013
Received in revised form 1 November 2014
Accepted 27 November 2014
Available online 11 December 2014

Editor: C.E.W. Steinberg

ABSTRACT

Nutrient enrichment of mangroves, a common phenomenon along densely populated coastlines, may negatively affect mangrove ecosystems by modifying internal carbon and nutrient cycling. The decomposition of litter exerts a strong influence on these processes and is potentially modified by eutrophication. This study describes effects of N and P enrichment on litter decomposition rate and mineralisation/immobilisation patterns.

By making use of reciprocal litter transplantation experiments among fertiliser treatments, it was tested if nutrient addition primarily acts on the primary producers (i.e. changes in litter quantity and quality) or on the microbial decomposers (i.e. changes in nutrient limitation for decomposition). Measurements were done in two mangrove forests where primary production was either limited by N or by P, which had been subject to at least 5 years of experimental N and P fertilisation.

Results of this study indicated that decomposers were always N-limited regardless of the limitation of the primary producers. This leads to a differential nutrient limitation between decomposers and primary producers in sites where mangrove production was P-limited. In these sites, fertilisation with P caused litter quality to change, resulting in a higher decomposition rate. This study shows that direct effects of fertilisation on decomposition through an effect on decomposer nutrient availability might be non-significant, while the indirect effects through modifying litter quality might be quite substantial in mangroves. Our results show no indication that eutrophication increases decomposition without stimulating primary production. Therefore we do not expect a decline in carbon sequestration as a result of eutrophication of mangrove ecosystems.

1. Introduction

Mangroves are among the most carbon-rich forests in the tropics (Donato et al., 2011), due to high primary production and slow decomposition (Cebrián, 1999). The latter process governs important ecosystem services such as soil carbon sequestration and nutrient exchange with through-flowing water. Microbial decomposers are a key functional group processing most of the carbon and nutrient fluxes in these coastal systems (Holguin et al., 2001). Leaf litter is a primary resource for decomposers, and its quality and quantity have strong effects on decomposer activity (Couteaux et al., 1995; Strickland et al., 2009; Mooshammer et al., 2012). Decomposers have a major impact on nutrient availability for the vegetation through conversion of organically bound nutrients to mineral forms (mineralisation) and vice-versa (immobilisation) (Swift et al., 1979; Berg and Laskowski, 2005; Cherif and Loreau, 2009) As a change in nutrient availability in turn affects both litter composition and litter production rate, changes in decomposition dynamics have a strong potential feedback on the decomposer community composition, nutrient availability, and primary production (Holguin et al., 2001; Hessen et al., 2004; Norris et al., 2012).

The rate of decomposition generally correlates positively with nutrient availability in terrestrial and wetland ecosystems (Cebrián et al., 1998). Anthropogenic nutrient enrichment might therefore have a

* Corresponding author.
E-mail addresses: j.a.keuskamp@uu.nl (J.A. Keuskamp), m.m.hefting@uu.nl (M.M. Hefting), b.j.j.dingemans@uu.nl (B.J.J. Dingemans), j.t.a.verhoeven@uu.nl (J.T.A. Verhoeven), feller@serc.si.edu (I.C. Feller).

http://dx.doi.org/10.1016/j.scitotenv.2014.11.092
0048-9697/

negative effect on carbon storage by stimulating decomposition more strongly than primary production (Hessen et al., 2004). Nutrient enrichment may change litter decomposition in two different ways, i.e. directly, via increased availability of mineral nutrients for decomposers (Norris et al., 2012), and indirectly, via fertiliser-induced changes in litter composition (Bryant et al., 1983; Prescott, 2010; Hobbie et al., 2012). Indirect effects of nutrient enrichment may include an increase in litter nutrient content (Hobbie et al., 2012) and a decrease in carbon-rich secondary compounds such as lignin (Bryant et al., 1983), both resulting in a higher decomposability.

Direct effects of nutrient enrichment on decomposition are quite complex, as both retarding and stimulating effects of nutrient amendment have been reported. While decomposition of labile litter compounds is mostly stimulated by nutrient addition, the effect on more recalcitrant litter compounds is element specific, as nitrogen generally retards, (O'Connell, 1993; Knorr et al., 2005; Craine et al., 2007), while phosphorus stimulates breakdown of these litter compounds.

Nutrient enrichment thus affects decomposer activity, litter quality, and their interaction. Nutrient availability and litter quality influence patterns of nutrient immobilisation and mineralisation during decomposition, (O'Connell, 1993; Osono and Takeda, 2004). As these processes are tightly coupled to nutrient uptake by mangrove trees (Holguin et al., 2001), changes in nutrient dynamics of decomposing leaves may have important consequences for primary production. Nutrient enrichment therefore could result in complex interactions culminating in net accumulation or net loss of litter and soil organic matter in the soil. For example, enrichment in one nutrient may decrease availability of another nutrient through plant uptake or immobilisation. This, in turn, may slow down decomposition, primary production, or both.

In the present study, we investigated the effects of long-term nitrogen (N) and phosphorus (P) enrichment on patterns of litter decomposition as well as nutrient mineralisation and immobilisation in two mangrove forests on Twin Cays in Belize and on North Hutchinson Island in Florida. These mangroves have a contrasting soil carbon content, but a similar vegetation structure with trees fringing the coastline being much taller than trees growing further inland. In this very nutrient-limited inner zone, mangrove trees are stunted and growth is limited by P on Twin Cays and by N on North Hutchinson Island.

Manipulation of nutrient limitations by the long-term fertilisation treatments applied in these locations allows for testing effects of nutrient availability on decomposition without the confounding effects of location and plant species composition. By comparing decomposition dynamics of litter in the different locations and treatments we can assess i) whether nutrient enrichment stimulates decomposition directly, through increasing soil nutrient availability, or indirectly, through changing leaf litter quality, ii) Whether N- or P-limited systems respond differently to enrichment with either of these elements, and iii) Whether nutrient retention during the decomposition process is diminished by external nutrient enrichment.

2. Materials and methods

This study was conducted at two mangrove-dominated locations: Twin Cays, Belize, and an impounded mangrove (Mosquito Impoundment 23) on North Hutchinson Island, Indian River Lagoon, Florida, USA (Fig. 1a). Twin Cays is a relatively pristine, peat-based, 92-ha archipelago, 12 offshore (16;50;N, 88;06;W) where it receives no terrigenous freshwater or sediment inputs (Ruetzler and Feller, 1996). Mosquito impoundment 23 is a 122-ha abandoned impoundment, located on the lagoon side of North Hutchinson Island, Indian River County, Florida, USA (27;33;N, 80;20;W). Both locations have been the focus of long-term research (e.g. (Feller et al., 2003, 2007, 2009)) with respect to eutrophication effects.

Twin Cays is underlain by deep deposits of mangrove peat, 8–12 thick (Macintyre et al., 2004). On North Hutchinson Island, in contrast, the soil is highly disturbed with little structure, composed primarily of sand and shell fragments that were deposited when the impoundment was built (Feller et al., 2002, 2003).

In spite of their contrasting edaphic properties, vegetation structure is similar at both locations with a clear zonation perpendicular to the

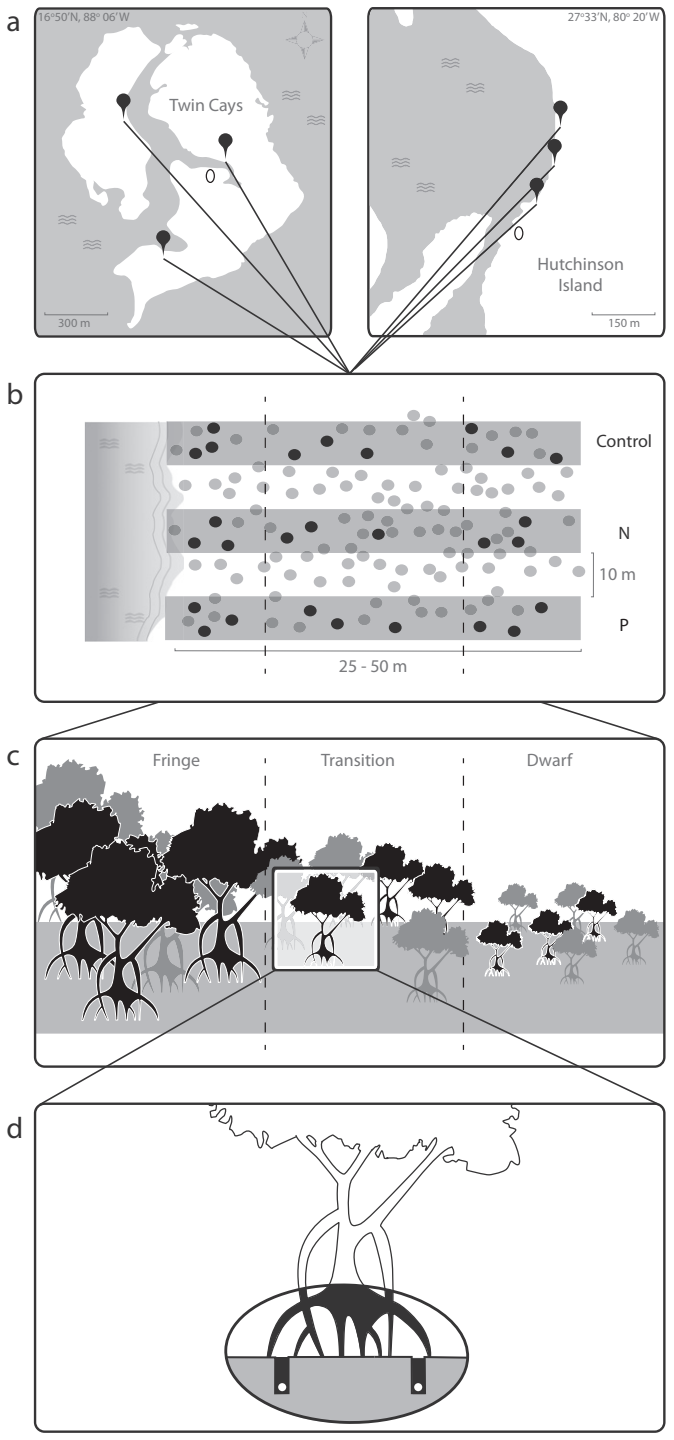

Fig. 1. a) The setup of the mangrove fertilisation experiment in Twin Cays, Belize and North Hutchinson Island, Florida, USA. Per location, three transect blocks, 2550 long, were oriented perpendicular to the shoreline. In addition, a reference plot was established at each location (open circles). b) Each transect block consisted of three parallel transects separated by 10 buffer zones against possible lateral migration of fertilisers. All transects randomly received one of three fertilisation treatments: Control, nitrogen (N) and phosphorus (P). c) Transects were subdivided into fringe, transition, and dwarf zones based on tree height. In each transect, three trees per zone were selected for treatment. d) The fertilisation was applied to specific trees by burying fertiliser-filled dialysis tubing near the roots and plugging the hole with soil substrate.

shore line. Both primarily dominated by red mangrove (*Rhizophora mangle*) and black mangrove (*Avicennia germinans*) intermingled with white mangrove (*Laguncularia racemosa*) (Feller et al., 2009). Both locations showed a clear zonation perpendicular to the shoreline. The outer zone of 5–20 consists of a narrow stand of uniformly tall (5–6) *R. mangle* trees. Further from the shoreline, trees rapidly decrease in height (2–4) and form a transition zone that varies in width from 5–30. This transition zone is still dominated by *R. mangle* on Twin Cays and by *A. germinans* on North Hutchinson Island. On the landward side of this transition zone, both locations are dominated by stunted trees of low stature (≤1.5). This so-called dwarf zone is dominated by *R. mangle* on Twin Cays and by *A. germinans* on North Hutchinson Island. Fertilisation studies have shown that mangrove trees at North Hutchinson Island are strongly N-limited (Feller et al., 2002), while trees on Twin Cays are strongly P-limited in the dwarf zone, while the fringe zone is somewhat N limited (Feller et al., 2003).

2.1. Experimental design

Trees for experimental manipulation were chosen at Twin Cays in 1995 and at North Hutchinson Island in 1997 as described in Feller et al. (2002, 2003). Per location, three transect blocks, 25–50 long, were oriented perpendicular to the shoreline (Fig. 1). Each transect block consisted of three parallel transects separated by 10 buffer zones to exclude effects of possible lateral migration of fertilisers. Transects were subdivided into fringe, transition, and dwarf zones based on tree height as described above. In each zone, 3 trees per transect were selected to be treated and were fertilised with N, P or were left unfertilised so that in total 81 trees per location, 9 per transect, were selected to accommodate the experimental treatments. In addition to the above, reference plots were established in the fringe of both locations, outside the influence of the nutrient enrichment. These plots served as a location-specific control for transplantation effects and were used for incubation of litter from other sites and as a source of reference litter. The reference plot on Twin Cays was established in a *Rhizophora*-dominated fringe, while on North Hutchinson Island it was located in an *Avicennia*-dominated fringe site.

The fertilisation treatments (N, P, Control) were randomly assigned to the three transects within each transect block (Feller et al., 2002, 2003). Fertilisation consisted of the semiannual burial of dialysis tubing filled with 300 urea ($CO(NH_2)_2$) or triple superphosphate ($Ca(H_2PO_4)_2 \cdot H_2O$), leading to the application of 276 of N and 165 of PO_4 per plot respectively. To deliver the fertiliser to the soil matrix, two 30 deep holes, 4 in diameter, were made at opposing sides of each tree, beneath the outermost margin of the canopy and approximately 30 from a root of the target tree (Fig. 1). Twice a year, these holes were filled with the fertiliser contained in dialysis tubing (Spectrapor Membrane Tubing, 50 diameter, 6000–8000 MWCO). Each hole was sealed with soil substrate. For control trees, holes were cored and sealed but no fertiliser was added. This method was based on previous studies (Feller, 1995), in which plant growth dramatically increased in response to addition of the limiting nutrient and small patches of fertilised trees immediately around the target tree were created.

Table 1
Experimental setup of litter incubation in mangrove stands at Twin Cays, Belize and North Hutchinson Island, Florida. Treatment consisted of at least five years of semiannual nitrogen (N) or phosphorus (P) fertilisation with a non-fertilised control treatment as a reference. The setup is repeated for each of the three zones, in each of three transect blocks, at both locations. Within a location, all zones and all transects share a single reference plot.

Experiment	Origin		Incubation	Treatment effect
Native litter (NL)	C	→	C	Soil nutrients, litter quality
	N	→	N	
	P	→	P	
Common site (CS)	C	→		Litter quality
	N	→	Reference	
	P	→		
Common litter (CL)		→	C	Soil nutrients
	Reference	→	N	
		→	P	

2.2. Litter collection and incubation

Litter was collected by picking fully senescent leaves directly from the target trees at the experimental sites. Within each zone, litter from each treatment was combined and thoroughly mixed, so that nine different litter types were collected per location. In addition, litter was collected from the reference plot at each location.

Approximately 8–10 of dry senescent leaf litter was included in 1 mesh bags of 10 · 10. Replicate sets of seven bags were deployed randomly among the three replicates per zone at each location where they were collected. To amass enough leaf litter to set up these experiments, senescent leaves were collected over a 6-month period in 2000 and the experiments were deployed in October 2000. These bags were retrieved after 2, 4, 8, 24 and 36 weeks of incubation to determine remaining mass, carbon (C), N, and P. In addition, bags were brought to the field and immediately retrieved as a control for transportation effects. In addition to measuring C, N and P concentrations, these zero-day bags were used in to determine lignin content.

To quantify the effects of direct, indirect, and interactive effects of soil nutrients and litter quality, three incubation experiments were conducted: i) Native litter (NL), where litter was incubated at its site of origin; ii) Common site (CS), where litter harvested from different treatments was incubated at a reference plot; iii) Common litter (CL), where litter harvested at the reference plot was incubated in all treatments plots. (See also Table 1). These experiments were conducted in parallel on Twin Cays and on North Hutchinson Island.

2.3. Chemical analysis and mass loss determination

After incubation, litter bags were carefully rinsed and dried at 70 for 48 before further processing. Initial weights were corrected for handling losses and initial moisture content to determine initial litter mass. These correction factors were determined on litter from bags that were taken into the field, but not incubated (0 weeks). Litter mass loss was determined by weighing litter after incubation and subtracting the corrected initial weight.

Total C, N, P and lignin concentrations were measured after grinding the litter in a Wiley Mill to pass through a 0.38 screen. Concentrations of C and N were determined with a Model 440 CHN Elemental Analyser (Exeter Analytical, North Chelmsford, US-MA) at the Smithsonian Environmental Research Center, Edgewater, MD. Concentrations of P were determined using an inductively coupled plasma spectrophotometer (ICP) by Analytical Services, Pennsylvania State University, US-PA. Lignin analysis was done at Northern Arizona University, based on methods outlined by Iiyama and Wallis (1990) as modified by Chapman et al. (2003). Briefly, 10 of deionised water was added to 10–15 of dry, ground litter samples in test tubes. The tubes were then placed in a dry block, heated at 60 for 1, and stirred at 10 intervals. Samples were then filtered through GF/A glass fibre filters and rinsed three times with each of the following sequence of solutions: water, ethanol, acetone and diethyl ether. After drying overnight at 70, samples were digested in 25 acetyl bromide in acetic acid and perchloric acid for 40 at 70, cooled, diluted with 10 of 2 NaOH, then brought to 50 volume with glacial acetic acid. Samples were filtered and absorbances were read on a spectrophotometer at 280 (Spectromax Plus 184 Molecular Devices, Sunnyvale, US-CA). National Institute of Standards and Technology pine standard (NIST no. 1575) was used for calibration.

2.4. Statistical analysis

Results were analysed using R (R Core Team, 2012) and the R packages *nlme* (Pinheiro et al., 2012) for linear-mixed effect modelling, *car* (Fox and Weisberg, 2011) for type II sum of squares and *mgcv* (Wood, 2011) for generalised additive mixed modelling (GAMM). Hypothesis testing for linear models was done using Wald's χ^2 test with type II sum of squares. Homoscedasticity was confirmed by Levene's test and normality was checked using Shapiro's test.

Treatment effects for GAMM models were tested using a Wald's F test for general additive models. Lignin, N, and P concentrations of fresh litter were analysed using a linear model with treatment, zone and their interactions as fixed effects and transect as a random effect. Changes in absolute N and P content during decomposition were tested separately at the two locations using a GAMM with a cubic regression splined smooth for N and P content, factorial splines and parametric terms for each fertilisation and zone within transect as random effect.

3. Results

3.1. Litter quality

Overall, the litter quality on Twin Cays is lower as compared to North Hutchinson Island, with lower N and P concentrations and higher lignin contents (Fig. 2, Table 2). This is true for all three zones, including the fringe zone which is dominated by *R. mangle* in both Twin Cays and North Hutchinson Island.

At both locations, the composition of leaf litter is significantly related to zone and nutrient treatments (Table 2). While fertilisation had a larger effect on litter quality on Twin Cays as compared to North Hutchinson Island, the zonation effect is relatively stronger at North Hutchinson Island.

At Twin Cays, the N concentration of fresh litter is highly variable with multiple interactions between zone and fertilisation treatment. Nevertheless, fresh leaf litter N concentration is clearly elevated in the N-fertilised treatments (Fig. 2). In the P treatments, the N concentration of fresh litter is lower, particularly in the dwarf zone. On North Hutchinson Island, fresh leaf litter N concentration is also higher in the N-fertilised plots, but at this location, the effect of zone is stronger. On Twin Cays, P-fertilised trees produced litter with a higher P concentration as compared to other treatments, while on North Hutchinson Island there is no effect of P-fertilisation on litter composition. In this location, there is only an effect of zone, with senescent leaves in the transition zone having a higher P concentration.

Table 2
Summary of the results of Wald's χ^2 test on linear mixed-effect models examining the concentration of nitrogen ([N]), phosphorus ([P]) and lignin of fresh leaf litter in Twin Cays, Belize (TC) and North Hutchinson Island, Florida, USA (HI). Effects of fertilisation with N and P between and within locations are examined. Between locations, only fringe trees were compared, as they were the same species. Within locations, the effect of zonation and its interaction with N or P fertilisation is also examined. (***p < 0.001, **p < 0.01, *p < 0.05, ·p < 0.1).

			[N]		[P]		Lignin	
		Df	χ^2	*p*	χ^2	*p*	χ^2	*p*
Between	Location	1	4.12	*	87.91	***	4.49	*
	N:location	2	11.69	**	7.80	*	0.19	
	P:location	2	0.38		17.00	***	2.69	
Within								
TC	N	1	19.62	***	0.81		1.79	
	P	1	23.00	***	77.53	***	0.20	
	Zone	2	78.68	***	3.93		5.72	·
	N:zone	2	8.68	*	0.92		6.52	*
	P:zone	2	13.90	***	2.41		3.67	
HI	N	1	13.03	***	0.01		1.65	
	P	1	1.71		0.24		2.54	
	Zone	2	8.06	*	33.38	***	21.24	***
	N:zone	2	1.37		1.65		0.23	
	P:zone	2	0.01		1.72		1.39	

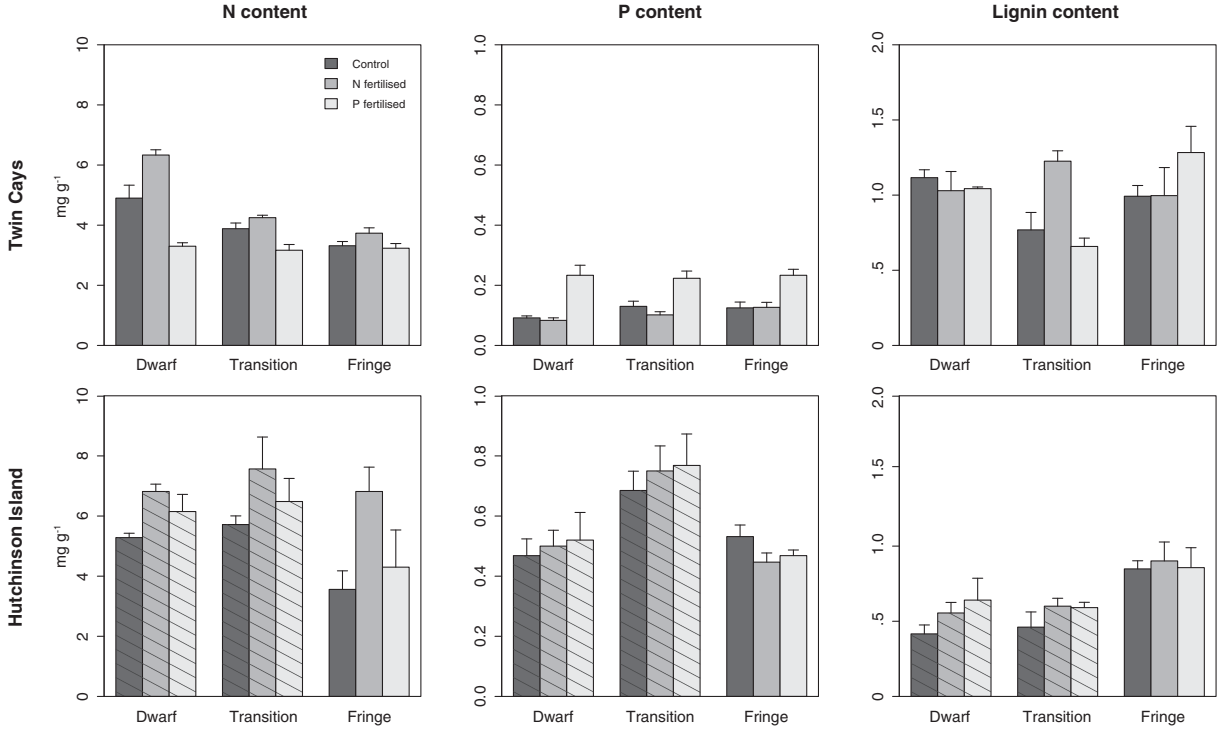

Fig. 2. Nitrogen (N), phosphorus (P), and lignin concentration of fresh leaf litter from *Avicennia germinans* (hatched bars) and *Rhizophora mangle* (solid bars) mangrove trees at Twin Cays, Belize and North Hutchinson Island, Florida, USA. Values were measured in control plots and in plots that received semiannual nitrogen- (N) or phosphorus-fertilisation (P) for at least five years.

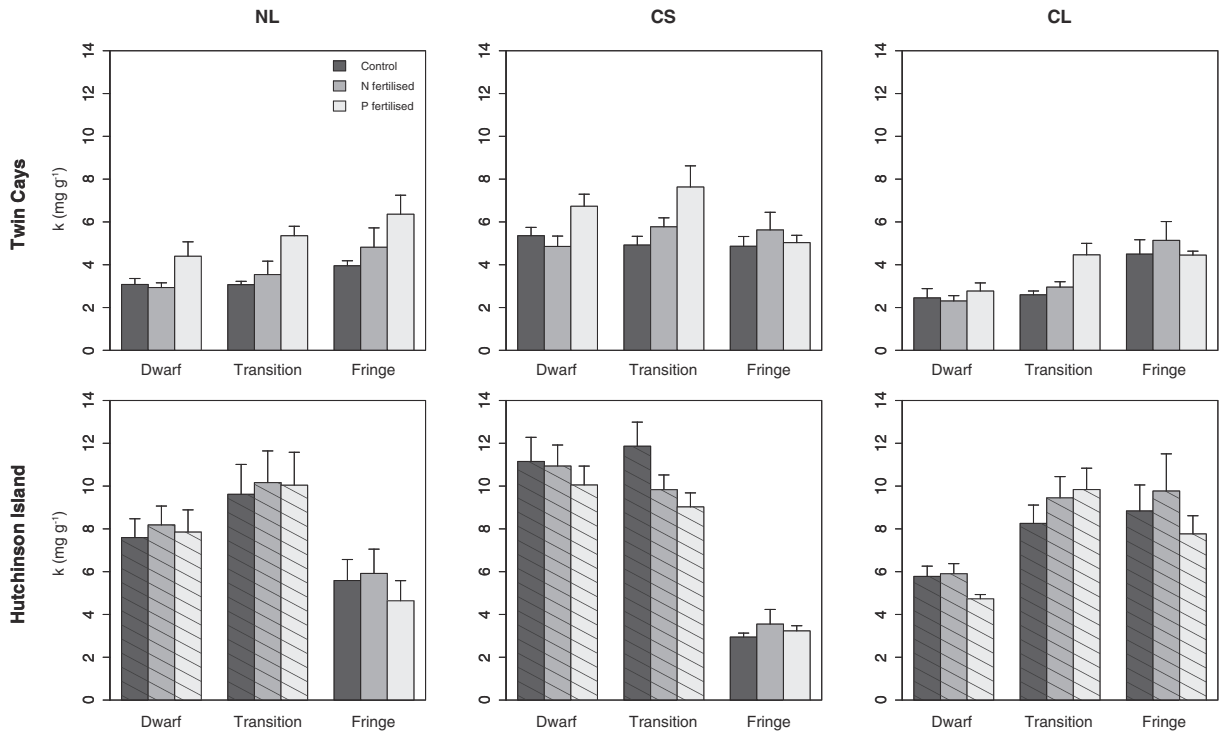

Fig. 3. Decomposition rate constants (k) of leaf litter produced by *Avicennia germinans* (hatched bars) and *Rhizophora mangle* (solid bars) at Twin Cays, Belize and North Hutchinson Island, Florida, USA. k Values were calculated from mass loss at four sampling moments between 64 and 400 days, using a single pool exponential decay model $W_t = W_0 \cdot e^{-kt}$. Both stands showed a distinct zonation perpendicular to the waterfront, differing in hydrology and primary production: fringe (F), transition (T) and dwarf (D) zones. In each zone, plots were fertilised with nitrogen (N), with phosphorus (P) or not fertilised (Control). In each location, litter was incubated in its own treatment (Native Litter — NL), in a reference location (Common Site — CS), or litter from the reference location was incubated in the treatment sites (Common Litter — CL); see also Table 1.

3.2. Decomposition rates

Leaf litter decomposition rates are generally lower on Twin Cays than on North Hutchinson Island (Fig. 3). However, in the Native Litter experiment, decomposition rates of fringe *R. mangle* litter do not differ between the two locations, while in the Common Site experiment, *R. mangle* litter decomposes even more rapidly on Twin Cays as compared to North Hutchinson Island (Fig. 3, Table 3).

Table 3
Summary of the results of Wald's χ^2 test on a linear mixed-effect model examining the relationship of decomposition rate constant k with N and P enrichment in Twin Cays, Belize (TC) and North Hutchinson Island, Florida, USA (HI). Effects for the three incubation experiments are examined separately between and within locations. Between locations, only fringe trees were compared, as they were of the same species. Within locations, the effect of zonation and its interaction with N or P fertilisation is also examined. Symbols denote significance levels (***p < 0.001, **p < 0.01, *p < 0.05).

			Native litter		Common site		Common litter	
		Df	χ^2	*p*	χ^2	*p*	χ^2	*p*
Between	Location	1	0.00		25.43	***	6.62	*
	N:location	2	0.71		0.70		0.35	
	P:location	2	7.08	*	0.45		1.09	
Within								
TC	N	1	0.05		0.58		0.30	
	P	1	22.43	***	8.87	**	5.71	*
	Zone	2	16.01	***	4.08		41.62	***
	N:zone	2	0.10		2.55		0.77	
	P:zone	2	0.86		3.49		2.71	
HI	N	1	0.83		0.30		1.34	
	P	1	0.71		1.95		0.09	
	Zone	2	109.92	***	398.09	***	53.24	***
	N:zone	2	0.06		4.28		0.48	
	P:zone	2	2.85		1.85		4.44	

In each of the three litter incubation experiments, i.e. with Native Litter, Common Site and Common Litter, decomposition rates significantly differ between fertilisation treatments, zones, or both. On Twin Cays, P-fertilisation is the most important modifier in all three experiments, while on North Hutchinson Island differences in decomposition rate are only explained by zone. Nitrogen does not have any significant effects on decomposition rates in any of the experiments (Table 3).

Results from the Native Litter (NL) experiments incorporate both litter quality and environmental influences on decomposition rate. On Twin Cays, decomposition rate is significantly higher in the P-fertilised sites than in the other treatments, and also in the fringe zone decomposition rates are significantly higher in comparison to the dwarf and transition zone (Table 3, Fig. 3). On North Hutchinson Island, however, fertilisation did not have any significant effect on decomposition rate but the rate of this process was significantly lower in the *R. mangle*-dominated fringe, than in the *A. germinans*-dominated transition and dwarf zones.

The Common Site (CS) experiments investigate the effects of litter quality differences on decomposition rates regardless of effects of soil conditions, as in both locations litter from all treatments was incubated in the same reference plot. On Twin Cays, the effects of fertilisation are similar to those in the NL experiment, i.e. a significant stimulation of decomposition by the P treatment, except for the fringe zone (Fig. 3). On North Hutchinson Island, fertilisation did not have a significant effect on decomposition rates (Table 3), while the decomposition rate constant of fringe-derived (*R. mangle*) litter is significantly lower than that of the other zones (Fig. 3).

The Common Litter (CL) experiments test for environmental effects on decomposition rates only, as the incubated litter in all treatment plots is derived from the same reference plot. On Twin Cays, there is a significant zone effect, with faster decomposition in the fringe as compared to the other zones. Also, decomposition of this reference litter was significantly faster in the P-fertilised plots, most notably in the

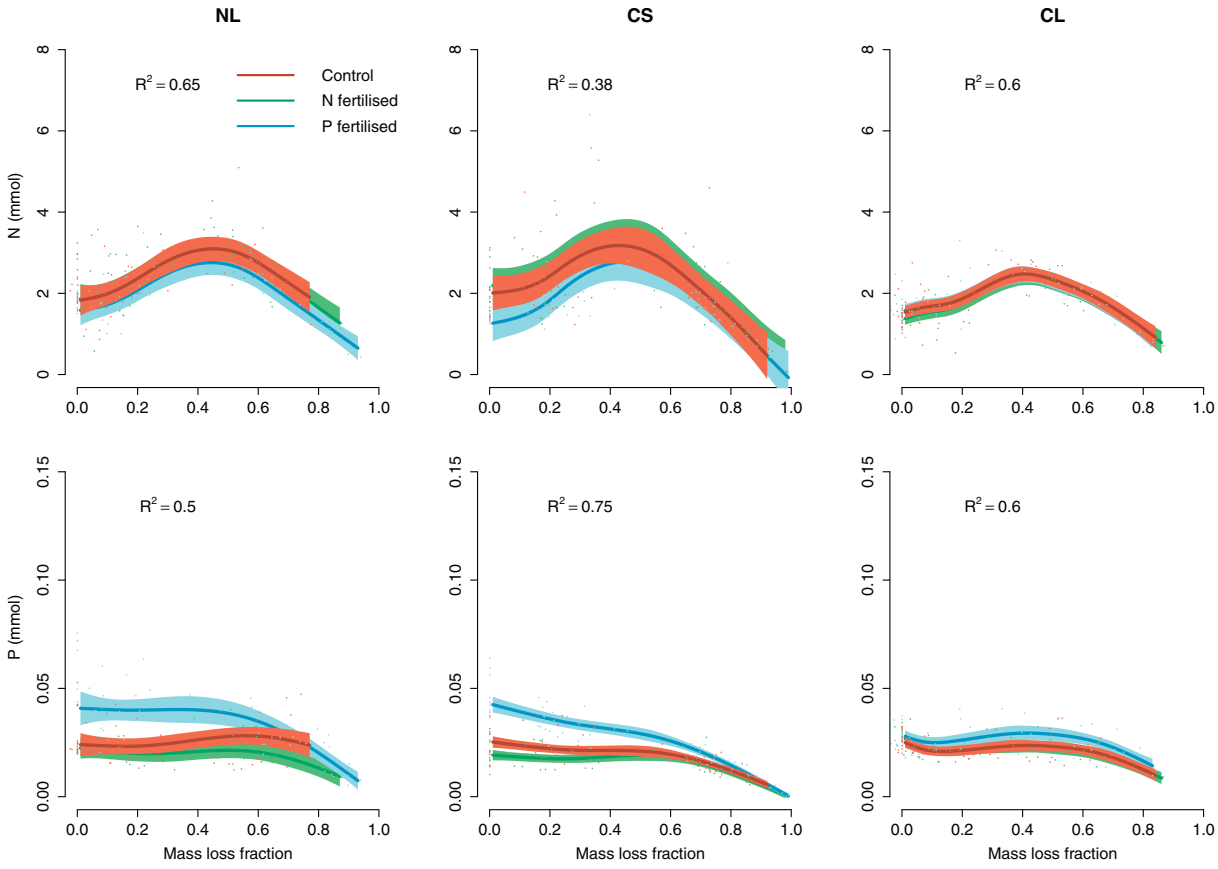

Fig. 4. Absolute amounts of nitrogen (N) and phosphorus (P) (mmol) against relative mass loss of mangrove leaf litter during decomposition in litter bags on Twin Cays, Belize. Litter was incubated in its own treatment (Native Litter — NL), in a reference location (Common Site — CS), or litter from the reference location was incubated in the treatment sites (Common Litter — CL); see Table 1. Lines and shaded areas are splined fits with 95 confidence intervals of the non-parametric term in a model with linear effects of nutrient treatments and zonation. R^2 indicates the goodness of fit.

transition zone. On North Hutchinson Island there is again only an effect of zone with the decomposition rate constant of reference litter incubated in the dwarf zone being notably lower as compared to the other zones.

3.3. Nutrient dynamics during decomposition

The absolute amounts of N and P in the decomposing material (i.e. plant litter plus attached microorganisms) in the course of the decomposition process are shown in Figs. 4 and 5 for Twin Cays and North Hutchinson Island, respectively. Microbial growth and attachment of soil particles led in some cases to an initial increase in weight so that some data points show negative weight loss. Fertilisation treatments are plotted separately, and the significance of those effects is given in Table 4. Note that the fertilisation effects depicted in Figs. 4 and 5 do not include the effects of zonation so that a considerable amount of datapoints fall outside the 95% confidence interval. Between the NL, CS, and CL experiments, overall patterns of N and P dynamics during decomposition are quite similar, whereas the observed patterns are clearly distinct among both locations. On Twin Cays, the absolute amount of N increases during the initial phase of decomposition until almost half of the initial litter weight is lost (Fig. 4). At this point, 25 to 50% of the N in the litter results from net immobilisation (i.e. the binding of external N in microbial biomass). After this immobilisation phase, the N amounts start to decrease. Phosphorus amounts do not increase, but stay more or less constant except for a slight decline after the start of N decrease.

On North Hutchinson Island, there is generally a much less pronounced increase in N content during the initial decomposition phase, but its decline starts around the same stage of proportional weight loss as on Twin Cays. Phosphorus is initially mineralised rapidly on North Hutchinson Island, but this is followed by a net immobilisation phase relatively late in the decomposition process after about one-third of the initial mass is lost. When about half the initial litter mass is lost, both P and N amounts start to decrease (Fig. 5).

Within each location, there is little difference in the pattern of immobilisation and mineralisation between fertilisation treatments (Figs. 3 and 4, Table 4). In fact, there was only one instance where nutrient dynamics during decomposition significantly deviated between fertilisation treatments: In the CL experiment on North Hutchinson Island both fertilisation treatments showed smaller initial loss of P as compared to the control treatment.

4. Discussion

While the two study area (Twin Cays in Belize and North Hutchinson Island in Florida) differed fundamentally in soil type, climatic conditions, and hydrology, we still found comparable effects of within-stand zonation on decomposition rates at both locations. We expected that these differences in decomposition rate among zones were driven by nutrient availability, based on results from a belowground decomposition experiment (Feller et al., 2002). This was not confirmed by our results, however. The effects of zonation were not modified by nutrient treatments at either Twin Cays or at North Hutchinson. This clearly shows that decomposition rates across within-stand zonation are

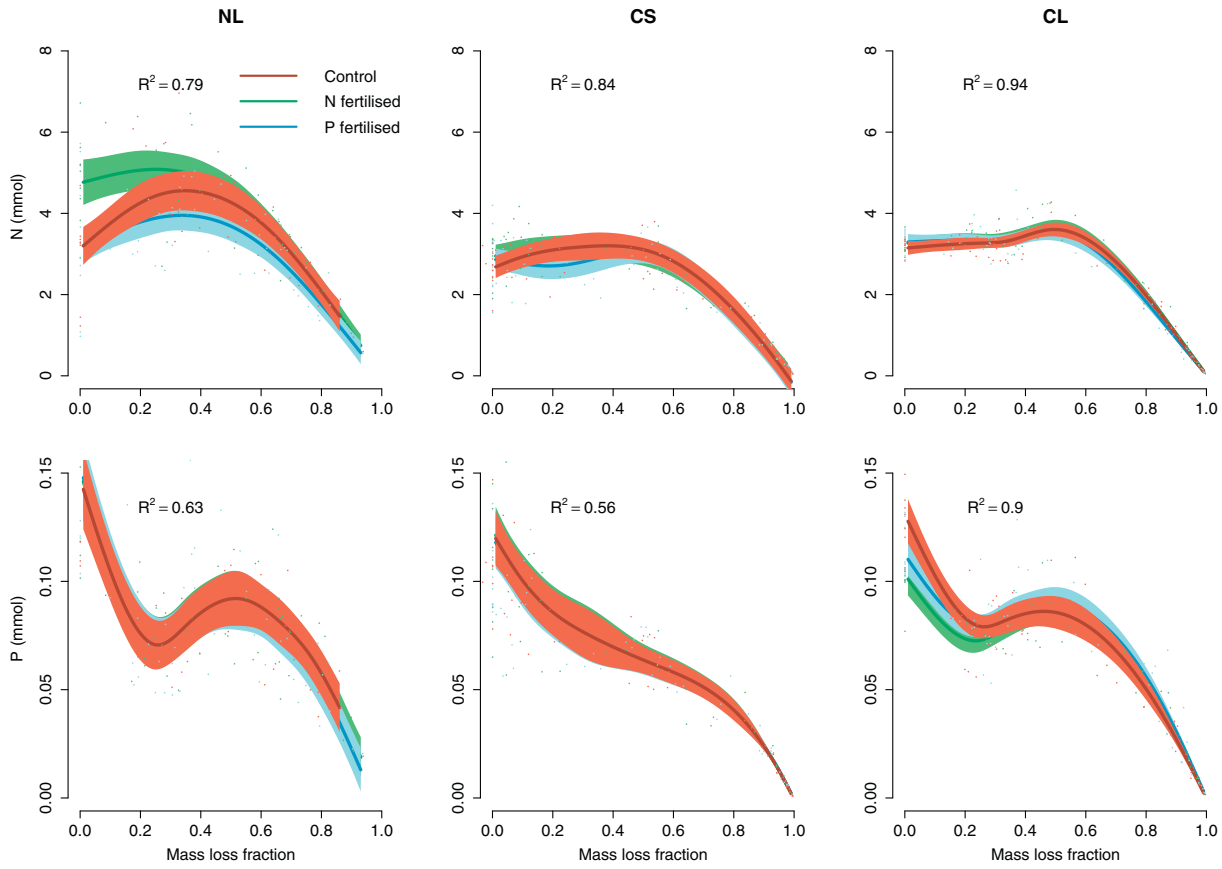

Fig. 5. Absolute amount of nitrogen (N) and phosphorus (P) (mmol) against relative mass loss of mangrove leaf litter during decomposition in litter bags on North Hutchinson Island, Florida, USA. Litter from the treatment sites was incubated in its original location (Native Litter — NL), in a reference location (Common Site — CS), or litter from a reference location was incubated in the treatment sites (Common Litter — CL); see Table 1. Lines and shaded areas are splined fits with 95 confidence intervals of the non-parametric term in a model with linear effects of nutrient treatments and zonation. R^2 indicates the goodness of fit.

Table 4
Spline fitting results of a GAMM model describing the amounts of nitrogen and phosphorus in mangrove leaf litter during the decomposition process. The fitted model consists of parametric terms for fertilisation treatment, and non-parametric cubic splined smoothing terms, describing the change of absolute nitrogen and phosphorus amounts during the decomposition process. Within the smoothing terms, an overall relation, and deviations for each treatment were fitted: Control, N and P-fertilised. For each line the estimated degrees of freedom, the F-value and p-level are shown. Separate models were fitted for each combination of experimental setup (Native Litter, Common Site and Common Litter) and location (Twin Cays, Belize (TC) and Hutchinson, Florida, USA (HI)). Symbols denote significance levels (***p < 0.001, **p < 0.01, *p < 0.05).

		Term	Native litter			Common site			Common litter		
			Df	*F*	*p*	*Df*	*F*	*p*	*Df*	*F*	*p*
TC	Nitrogen	N	1.00	0.12		1.00	1.90		1.00	2.20	
		P	1.00	12.33	***	1.00	9.22	*	1.00	0.00	
		Mass loss	3.32	39.85	***	3.30	29.41	***	3.69	45.37	***
		C:mass loss	1.00	0.00		1.00	0.05		1.00	0.00	
		N:mass loss	1.00	0.02		1.00	0.05		1.00	0.00	
		P:mass loss	1.00	0.02		1.00	0.02		1.20	0.01	
	Phosphate	N	1.00	17.41	***	1.00	20.84	***	1.00	0.34	
		P	1.00	41.73	***	1.00	97.56	***	1.00	19.25	***
		Mass loss	2.39	8.81	***	3.11	14.17	***	3.65	9.93	***
		C:mass loss	1.00	0.00		1.00	0.35		1.01	0.02	
		N:mass loss	1.00	0.07		2.1	0.21		1.69	0.30	
		P:mass loss	2.19	2.00		1.00	1.33		1.73	0.55	
HI	Nitrogen	N	1.00	7.71	**	1.00	1.34		1.00	1.88	
		P	1.00	6.66	*	1.00	0.59		1.00	0.01	
		Mass loss	2.79	18.26	***	3.41	66.95	***	3.54	120.45	***
		C:mass loss	2.08	1.39		2.07	2.16		1.75	0.31	
		N:mass loss	1.00	0.48		1.00	0.55		1.00	0.32	
		P:mass loss	1.00	0.22		3.66	1.44		2.71	1.18	
	Phosphate	N	1.00	0.76		1.00	0.05		1.00	7.37	**
		P	1.00	0.86		1.00	0.04		1.00	0.14	
		Mass loss	4.25	22.76	***	4.06	4.22	*	4.46	38.41	***
		C:mass loss	1.00	0.05		1.07	0.26		3.03	2.75	*
		N:mass loss	1.00	0.06		1.06	0.28		1.00	0.17	
		P:mass loss	1.00	0.07		1.08	0.32		1.00	0.21	

mainly determined by factors other than nutrient availability, e.g. environmental factors such as hydrology, soil texture and temperature and species identity (with *R. mangle* litter decomposing slower than *A. germinans* litter).

The direct effects of nutrient amendments on decomposer nutrient limitations can be deduced from the nutrient content of the litter–decomposer complex during the decomposition process. These changes are brought about by microbial growth and the associated requirements for nutrient and/or energy: A nutrient in excess is promptly leached from this complex (mineralisation), whereas nutrients limiting decomposers are kept in the complex, or even absorbed from the environment (immobilisation) (Manzoni et al., 2008). These patterns generally show a shift from nutrient limitation, where nutrients are immobilised, to a phase of energy limitation, where nutrients are mineralised (Manzoni et al., 2008). Hence, a higher immobilisation rate and an earlier onset of mineralisation is expected if more nutrients are available. To facilitate comparability between nutrient dynamics of litter with different decomposition rates, we have expressed the N and P content of the litter–decomposer complex as a function of mass loss, rather than as a function of time.

To our surprise, the location (Twin Cays or North Hutchinson Island) where decomposition took place was a much better predictor of the observed mineralisation and immobilisation patterns than fertilisation treatment. On Twin Cays, decomposition started with a clear immobilisation phase of N where N content doubled as a result of N uptake from the environment. Although such a buildup of N is fairly common, the increase in N was rather extreme as compared to other ecosystems (Parton et al., 2007), presumably because of the N-binding capacity of mangrove derived tannins (Kraus et al., 2003), rendering part of the litter-bound N unavailable to microorganisms. The consistent initial build-up of N (and not P) on Twin Cays clearly shows that here N is limiting microbial decomposition, despite the P limitation for primary production on large parts of the island. This is consistent with the N-limitation of the soil microbial community on this location (Keuskamp et al., 2015).

On North Hutchinson Island, N was initially retained in the litter while P was immobilised from the environment somewhat later in the decomposition process. Again, this pattern was rather consistent between fertilisation treatments and even mangrove species. The contrasting immobilisation/mineralisation patterns between locations and the insensitivity to fertilisation treatments must have been due to between-location differences in key environmental variables such as soil texture and hydrology. The P buildup in North Hutchinson Island for example was accompanied by an increase in litter iron (Fe) content (data not shown). This suggests that a chemical rather than a biological process has driven this late immobilisation of P. The sites at North Hutchinson Island are irregularly flooded, resulting in dissolution of Fe–P complexes under release of soluble P (Krom and Berner, 1980). The released P may have been absorbed to hydrous ferric oxide particles on the litter (Lucotte and D'Anglejan, 1983) with lowering water tables.

Despite its strong influence on initial litter N concentration, N enrichment did not affect litter decomposition rates at either of the locations. This result is striking, as litter-mediated effects of N enrichment have commonly been found to increase decomposition rates in mangroves (Mfilinge et al., 2002; Kristensen et al., 2008). Litter initial N concentration and lignin:N ratios have often been proposed as major factors controlling decomposability of litter (Melillo et al., 1982; Prescott, 2010). In our study, we did not find these relations. P fertilisation of trees which had been P-limited, however, led to a high increase in decomposition rate of leaf litter produced by these trees. This litter, incidentally, had the lowest nitrogen concentrations. P is generally reported to have little effect on decomposition, although similar increases of decomposition by P-enrichment have been reported in a P-limited freshwater wetland (Rejmánková and Houdková, 2006). Based on this and our own result we hypothesise that the effects of nutrient enrichment depend on whether the amended nutrient limits primary production. The predominance of N limitation in terrestrial ecosystems then would lead to a bias towards observing strong N effects on decomposition rates.

The increased decomposition rates of leaf litter from P-fertilised trees which had been P-limited either results from a higher P content or from a lower fraction of recalcitrant compounds in response to alleviation of a limiting nutrient (Waring et al., 1985; Entry et al., 1998; Kraus et al., 2004). The fact that P enrichment of P-limited sites did not increase decomposition rates of reference litter, while litter originating from these sites decomposed faster in otherwise N-limited reference locations points to the latter. Because P was not limiting decomposition and the N-content of the leaf litter was lower than in the control treatments, the increase in decomposition rate must have resulted from a change in litter recalcitrance, induced by alleviation of the P limitation of the tree that the litter originated from. A similar effect on litter recalcitrance has been described in *Sphagnum* were increased N availability has been shown to lead to a reduced polyphenol content (Bragazza and Freeman, 2007), which then accelerated decay. In case of our mangroves, increased availability of a nutrient limiting mangrove growth could have decreased the litter tannin content, which then stimulated decay.

Summarising, we can conclude that the effects of nutrient enrichment on mangrove litter decomposition were primarily mediated by changes in litter quality, whereas direct effects of N and P enrichment on decomposition are rather small. Nutrient limitations for primary production and litter decomposition are not necessarily the same, as decomposers were always N-limited, even in our study area where mangrove growth was P-limited. In such cases of differential nutrient limitation, N amendment did, however, not lead to a massive increase in decomposition upon amendment of N. Instead, P amendment did stimulate growth as well as decomposition. Our results show no indications that anthropogenic nutrient inputs significantly decrease carbon sequestration from mangrove litter.

Acknowledgements

We thank Anne H. Chamberlain, Smithsonian Environmental Research Centre, who put great effort in collecting, processing and analysing the leaf material used in this study. We also thank Dr. Samantha Chapman, Villanova University, who analysed lignin content of our samples and Annick Spoelstra who designed Fig. 1 of this paper.

References

Berg B, Laskowski R, editors. Litter Decomposition: A Guide to Carbon and Nutrient Turnover. Advances in Ecological ResearchNew York: Academic Press; 2005.
Bragazza L, Freeman C. High nitrogen availability reduces polyphenol content in *Sphagnum* peat. Sci. Total Environ. 2007;377:439–43.
Bryant JP, Chapin III FS, Klein DR. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 1983;40:357.
Cebrián J. Patterns in the fate of production in plant communities. Am. Nat. 1999;154:449–68.
Cebrián J, Williams M, McClelland J, Valiela I. The dependence of heterotrophic consumption and C accumulation on autotrophic nutrient content in ecosystems. Ecol. Lett. 1998;1:165–70.
Chapman SK, Hart SC, Cobb NS, Whitham TG, Koch GW. Insect herbivory increases litter quality and decomposition: an extension of the acceleration hypothesis. Ecology 2003;84:2867–76.
Cherif M, Loreau M. When microbes and consumers determine the limiting nutrient of autotrophs: a theoretical analysis. Proc. Biol. Sci. 2009;276:487–97.
Couteaux MM, Bottner P, Berg B. Litter decomposition climate and litter quality. Trends Ecol. Evol. 1995;10:63–6.
Craine JM, Morrow C, Fierer N. Microbial nitrogen limitation increases decomposition. Ecology 2007;88:2105–13.
Donato DC, Kauffman JB, Murdiyarso D, Kurnianto S, Stidham M, Kanninen M. Mangroves among the most carbon-rich forests in the tropics. Nat. Geosci. 2011;4:293–7.
Entry JA, Runion GB, Prior SA, Mitchell RJ. Influence of CO_2 enrichment and nitrogen fertilization on tissue chemistry and carbon allocation in longleaf pine seedlings. Plant Soil 1998:3–11.
Feller IC. Effects of nutrient enrichment on growth and herbivory of dwarf red mangrove (*Rhizophora mangle*). Ecol. Monogr. 1995;65:477–505.

Feller IC, McKee KL, Whigham DF, O'Neill JP. Nitrogen vs. phosphorus limitation across an ecotonal gradient in a mangrove forest. Biogeochemistry 2002;62:145–75.
Feller IC, Whigham DF, McKee KL, Lovelock CE. Nitrogen limitation of growth and nutrient dynamics in a disturbed mangrove forest, Indian River Lagoon, Florida. Oecologia 2003;134:404–14.
Feller IC, Lovelock CE, McKee KL. Nutrient addition differentially affects ecological processes of *Avicennia germinans* in nitrogen versus phosphorus limited mangrove ecosystems. Ecosystems 2007;10:347–59.
Feller IC, Lovelock CE, Piou C. Growth and nutrient conservation in *Rhizophora mangle* in response to fertilization along latitudinal and tidal gradients. Smithson. Contrib. Mar. Sci. 2009;38:345–58.
Fox J, Weisberg S. An R Companion to Applied Regression. Second ed. Thousand Oaks CA: Sage; 2011.
Hessen DO, Ågren GI, Anderson TR, Elser JJ, De Ruiter PC. Carbon sequestration in ecosystems: the role of stoichiometry. Ecology 2004;85:1179–92.
Hobbie SE, Eddy WC, Buyarski CR, Carol Adair E, Ogdahl ML, Weisenhorn P. Response of decomposing litter and its microbial community to multiple forms of nitrogen enrichment. Ecol. Monogr. 2012;82:389–405.
Holguin G, Vazquez P, Bashan Y. The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview. Biol. Fertil. Soils 2001;33:265–78.
Iiyama K, Wallis AFA. Determination of lignin in herbaceous plants by an improved acetyl bromide procedure. J. Sci. Food Agric. 1990;51:145–61.
Keuskamp JA, Feller IC, Laanbroek HJ, Verhoeven JTA, Hefting MM. Short- and long-term effects of nutrient enrichment on microbial exoenzyme activity in mangrove peat. Soil Biol Biochem 2015;81:38–47.
Knorr M, Frey SD, Curtis PS. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 2005;86:3252–7.
Kraus TEC, Dahlgren RA, Zasoski RJ. Tannins in nutrient dynamics of forest ecosystems — a review. Plant Soil 2003;256:41–66.
Kraus TEC, Zasoski R, Dahlgren R. Fertility and pH effects on polyphenol and condensed tannin concentrations in foliage and roots. Plant Soil 2004;262:95–109.
Kristensen E, Bouillon S, Dittmar T, Marchand C. Organic carbon dynamics in mangrove ecosystems: a review. Aquat. Bot. 2008;89:201–19.
Krom MD, Berner RA. Adsorption of phosphate in anoxic marine sediments. Limnol. Oceanogr. 1980;25:797–806.
Lucotte M, D'Anglejan B. Forms of phosphorus and phosphorus–iron relationships in the suspended matter of the St. Lawrence Estuary. Can. J. Earth Sci. 1983;20:1880–90.
Macintyre IG, Toscano MA, Lighty RG, Bond GB. Holocene history of the mangrove islands of Twin Cays, Belize, Central America. Atoll Res. Bull. 2004;510:1–16.
Manzoni S, Jackson RB, Trofymow JA, Porporato A. The global stoichiometry of litter nitrogen mineralization. Science 2008;321:684–6.
Melillo JM, Aber JD, Muratore JF. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 1982;63:621–6.
Mfilinge PL, Atta N, Tsuchiya M. Nutrient dynamics and leaf litter decomposition in a subtropical mangrove forest at Oura Bay, Okinawa, Japan. Trees 2002;16:172–80.
Mooshammer M, Wanek W, Schnecker J, Wild B, Leitner S, Hofhansl F, Blöchl A, Hämmerle I, Frank AH, Fuchslueger L, Keiblinger KM, Zechmeister-Boltenstern S, Richter A. Stoichiometric controls of nitrogen and phosphorus cycling in decomposing beech leaf litter. Ecology 2012;93:770–82.
Norris MD, Avis PG, Reich PB, Hobbie SE. Positive feedbacks between decomposition and soil nitrogen availability along fertility gradients. Plant Soil 2012;367:347–61.
O'Connell AM. Decomposition and nutrient content of litter in a fertilized eucalypt forest. Biol. Fertil. Soils 1993;17:159–66.
Osono T, Takeda H. Accumulation and release of nitrogen and phosphorus in relation to lignin decomposition in leaf litter of 14 tree species. Ecol. Res. 2004;19:593–602.
Parton W, Silver WL, Burke IC, Grassens L, Harmon ME, Currie WS, King JY, Adair EC, Brandt LA, Hart SC, Fasth B. Global-scale similarities in nitrogen release patterns during long-term decomposition. Science 2007;315:361–4.
Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. nlme: Linear and Nonlinear Mixed Effects Models. 3.1–105 Ed; 2012.
Prescott CE. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry 2010;101:133–49.
R Core Team. R: A Language and Environment for Statistical Computing; 2012.
Rejmánková E, Houdková K. Wetland plant decomposition under different nutrient conditions: what is more important, litter quality or site quality? Biogeochemistry 2006;80:245–62.
Ruetzler K, Feller I. Caribbean Mangrove Swamps. Scientific American; 1996.
Strickland MS, Osburn E, Lauber C, Fierer N, Bradford MA. Litter quality is in the eye of the beholder: initial decomposition rates as a function of inoculum characteristics. Funct. Ecol. 2009;23:627–36.
Swift MJ, Heal OW, Anderson JM. Decomposition in terrestrial ecosystems. Volume Studies in Ecology 5. Berkeley, California, USA: University of California Press; 1979.
Waring RH, McDonald A, Larsson S, Ericsson T. Differences in chemical composition of plants grown at constant relative growth rates with stable mineral nutrition. Oecologia 1985;66:157–60.
Wood SN. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. J. R. Stat. Soc. Ser. B Stat. Methodol. 2011;73:3–36.