Estuarine, Coastal and Shelf Science 214 (2018) 141–148

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

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

Differential responses of litter decomposition to regional excessive nitrogen input and global warming between two mangrove species

Ziyao Yang[a,b], Weimin Song[a], Yan Zhao[a,b], Jian Zhou[a,b], Zhonglei Wang[b], Yiqi Luo[a], Yuhong Li[c,**], Guanghui Lin[a,b,*]

[a] Ministry of Education Key Laboratory for Earth System Modeling, Department of Earth System Science, Tsinghua University, Beijing, 100084, China
[b] Division of Ocean Science and Technology, Graduate School at Shenzhen, Tsinghua University, Shenzhen, Guangdong, 518000, China
[c] Department of Environmental Science and Engineering, Huaqiao University, Xiamen, Fujian, 361021, China

ARTICLE INFO

Keywords:
Mangroves
Litter decomposition
Nitrogen input
Warming effect

ABSTRACT

Excessive nitrogen (N) input and warming affect litter decomposition and nutrient dynamics, especially in coastal areas. Understanding how mangrove litter decomposition responds to both excessive N input and warming is essential for estimating coastal carbon (C) and N cycling in the context of global change. In this study, we measured litter quality before and during the decomposition process of two mangrove species—*Avicennia marina*, a pioneer species, and *Bruguiera gymnorrhiza*, a late successional species—in a controlled experiment under treatments of N addition and warming. These two species dominate mangroves in southern China. Mass loss, C and N contents, and isotope values were measured over time as litter decomposed. N addition significantly increased the dry mass loss rates of *B. gymnorrhiza* litter by 52%, and warming significantly enhanced the dry mass loss of both *A. marina* and *B. gymnorrhiza* litter by 43% and 112%, respectively. N loss rate was not affected by the treatments but differed between the two mangrove species. There were no discernible changes in litter $\delta^{13}C$ or $\delta^{15}N$ throughout the decomposition process under N addition or warming, but the litter $\delta^{15}N$ content was higher for *A. marina* than for *B. gymnorrhiza,* which indicated feedback between decomposers and litter chemistry. These results highlight that the litter decomposition rates of both mangrove species respond positively to regional N loading or global warming. The litter decomposition rate differed significantly between mangrove species because of differences in litter N content, C/N ratio and associated microorganisms.

1. Introduction

Mangrove litter decomposition is a key process that regulates energy conversion and nutrient cycling in the ecosystems, thereby influencing the net ecosystem carbon (C) storage and blue carbon sinks of tropical and subtropical coastal areas (Alongi, 2014; Sukardjo et al., 2013; Wafar et al., 1997). These areas are being affected by regional excessive nitrogen (N) loading due to aquatic eutrophication (Jordan et al., 2011) and by global warming due to the accumulation of greenhouse gases in the atmosphere. Many experiments have examined the independent effects of these regional and global environmental changes on litter decomposition (Alongi, 2015; Knorr et al., 2005), but surprisingly, the combined effects of these changes on mangrove litter decomposition dynamics have not been examined. Thus, it is important to explore the combined effects of regional and global environmental changes on mangrove litter decomposition processes.

In general, litter decomposition can be affected by many factors including environment (e.g. temperature, moisture, and soil pH), nutrients availability and activities of decomposers in the soil (Chapin et al., 2002). On the other hand, litter quantity such as initial lignin content, initial N content and the ratios of initial lignin/N and C/N can also has impact on litter decomposition dynamics (Aber and Melillo, 1982; Melillo et al., 1982; Parton et al., 1987; Taylor et al., 1989). Initial C content (e.g., cellulose) and lignin content mainly control the litter decomposition rate. Cellulose, which contains 20–30% of the initial C content, is the most abundant biopolymer in plants and determines the early phase of decomposition (Berg and Laskowski, 2006). Lignin is one of the most difficult components of plant tissue to

* Corresponding author. Ministry of Education Key Laboratory for Earth System Modeling, Department of Earth System Science, Tsinghua University, Beijing, 100084, China.
** Corresponding author.
E-mail addresses: liyuh@hqu.edu.cn (Y. Li), lingh@tsinghua.edu.cn (G. Lin).

https://doi.org/10.1016/j.ecss.2018.09.018
Received 18 May 2018; Received in revised form 13 September 2018; Accepted 19 September 2018
Available online 20 September 2018

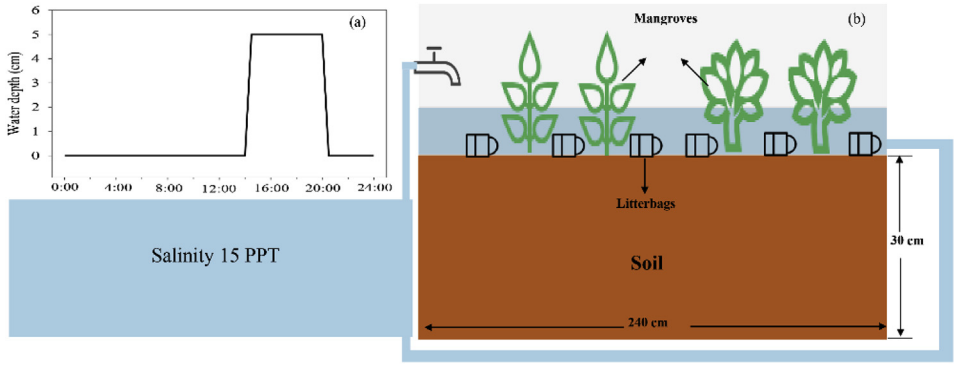

Fig. 1. Changes in water depth in the experimental mesocosms over time (a) and a cross-sectional view of the mesocosms (b) used in this study.

decompose and higher temperatures can lead to higher lignin contents in litters (Liu et al., 2017; Melillo et al., 1982).

The long-term effects of global warming due to rising atmospheric greenhouse gas concentrations will change the C/N ratios in leaves because of the CO_2 fertilization effect (Tu et al., 2014), subsequently complicating the decomposition processes (Fang et al., 2007). Moreover, N inputs to estuaries and coastal wetlands are much higher than those to terrestrial forests because of water eutrophication (Wu et al., 2014), which may have significant influences on the litter quality and decomposition rate of mangrove litters. Many experiments have demonstrated that the relationship between exogenous N addition and litter decomposition varies greatly (Aber et al., 1998; Hobbie, 2005), with studies reporting positive effects (Downs et al., 1996; Hobbie and Vitousek, 2000), negative effects (Carreiro et al., 2000; Hobbie, 2008; Hobbie et al., 2012; Magill and Aber, 1998), and no effects (Hobbie and Vitousek, 2000; Prescott, 1995; Vitousek, 1998). In addition to having impact on litter quality, long-term warming and N addition will greatly change the soil microbial community structure (Hu et al., 2001) and soil microbial activities (Chauvet and Suberkropp, 1998; Dang et al., 2009; Martinez et al., 2014), thereby changing the priming effect during the decomposition process and the decomposition rate of litter leaves (German et al., 2011).

Plant functional traits that are tightly coupled to litter quality (e.g., initial C and N contents, the C/N ratio, and lignin content) vary greatly among different species (Chen et al., 2014; Szefer et al., 2017), so they determine the decomposability of organic matter and the availability of nutrients to decomposers (Berg and McClaugherty, 2014). Although previous decomposition experiments have investigated the differential responses of litter to environmental factors among terrestrial plant species (Chen et al., 2014; Szefer et al., 2017), no decomposition study has evaluated the influence of plant species variation on litter decomposition dynamics in mangrove forests. Unlike other types of terrestrial ecosystems, mangrove forests are often submerged and exposed under periodic tides. Frequent drying and wetting cycles induced by tides in mangrove forests could have a profound impact on litter decomposition processes, with the potential to influence nutrient cycling and blue carbon sinks in mangrove forests (Friesen et al., 2018; Holmer and Olsen, 2002; Reef et al., 2010). Moreover, plant species in different succession stages usually tend to have different growth rate and leaf renewal rate (Bazzaz, 1979; Kazakou et al., 2006; Navas et al., 2003), which means that the litter quality of different species will differ strongly and should be estimated for different species in order to exactly understand the litter decomposition processes of mangrove forests. Thus, variation in litter quality associated with species succession may determine the rate of mangrove litter decomposition and its responses to environmental changes at regional and global scales.

We conducted a mesocosm experiments that manipulated atmosphere warming (+3 °C) and N loading (25 mg N L^{-1}) in a constructed ecosystem planted with the seedlings of *Avicennia marina*, a pioneering mangrove species and *Bruguiera gymnorrhiza*, a late succession mangrove species in the subtropical coastal regions of China. The goals of this study were to examine the combined effects of simulated N addition and warming on the litter decomposition of the two mangrove species and to test whether the responses of litter decomposition to these treatments differ between the two successional species.

2. Materials and methods

2.1. Experimental design

Our decomposition experiment was implemented in a mesocosm-scale ecosystem in a greenhouse at the Graduate School at Shenzhen, Tsinghua University, China (22°59′N, 113°97′E). The mesocosm experiment was designed to evaluate the effects of excessive nitrogen (N) input (229 g NH_4Cl and 91 g $NaNO_3$ were added to one reservoir with 3000 L seawater to make a concentration of 20 mg L^{-1} NH_4^+ and 5 mg L^{-1} NO_3^-, respectively, which represents the highest N levels in mangrove water influenced by aquaculture waste water discharge in this region, see Wu et al., 2014) and warming (3 °C warming, expected the median temperature rise by 2100 over 186–2005 due to anthropogenic CO_2 emission, see IPCC, 2014) on mangrove ecosystems. Sixteen cement pools (2.4 × 1.1 × 0.5 m) were planted with one-year-old seedlings of two mangrove species: *Avicennia marina*, a pioneer species, and *Bruguiera gymnorrhiza*, a late successional species.

The seedlings were divided into four treatment groups (four replicates for each treatment): (1) ambient control (CK, no warming and no N addition), (2) N addition (+N), (3) warming (+W) and (4) N addition plus warming (WN). The tidal circulation of the simulated mangrove ecosystem was controlled by a water supply system on a timer that was connected to two seawater reservoirs (7.5 × 1.0 × 0.7 m each, one with N addition and the other without). The tide was set to occur at 14:00 and lasted until 20:00 (local time) each day (Fig. 1).

Approximately 18 months after establishing the above treatments (March 2015 to September 2016), fresh leaf litters of *A. marina* and *B. gymnorrhiza* were collected from each treatment pool, washed with deionized water and oven-dried at 60 °C before being placed into nylon litterbags. Though the oven-dried method may change litter structure and lead to metamorphic changes in chemical composition such as protein. However, the not very high temperature (60 °C) used here may reduce this effect and the change in litter structure can be negligible. Approximately 1–2 g of *A. marina* litter and 3 g of *B. gymnorrhiza* litter were placed into separate nylon litterbags (10 × 20 cm, with a mesh size of 1 mm^2), and 6 nylon litterbags per species were placed in each pool. The opening of each litterbag was sealed with 6 Monel staples (Genuine Arrow T50 staples made of rust-resistant Monel; designed and assembled in the USA), and the litterbags were then fixed to the soil surface with bamboo sticks at each corner to prevent them from being washed away by the artificial tides. On days 0, 7, 14, 28, 56, 84 and 168 during the decomposition process, we collected 1 litterbag per species

from each treatment pool at each time point. Prior to analysis, the samples were rinsed with distilled water to remove adhered soil and oven-dried at 60 °C.

2.2. Measurements of litter quality

The dry mass weight, total C and N contents (TC and TN), C and N isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and lignin content of the litter samples were measured. The $\delta^{13}C$ and $\delta^{15}N$ values were expressed using the delta notation, i.e., $\delta^{13}C$ and $\delta^{15}N$: δ (‰) = $(R_{samples} - R_{standard})/R_{standard} \times 1000$, where $R_{samples}$ and $R_{standard}$ represent the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of the samples and the standard, respectively. The $\delta^{13}C$ values were determined in parts per thousand (‰) relative to the international standards of Pee Dee Belemnite (PDB), and the $\delta^{15}N$ values were presented relative to atmospheric N (N_2-atm). After the dry mass weight measurements, all of the samples were ground with a ball mill to pass through a #80-mesh screen prior to analysis. TC and TN were measured using a Macro cube Elemental Analyzer (Elementar, Inc., Italy). $\delta^{13}C$ and $\delta^{15}N$ were measured with a Delta V Advantage isotope ratio mass spectrometer coupled with a Flash HT 2000 Elemental Analyzer (Thermo Fisher Scientific Inc., USA). The lignin content was determined using the acetyl bromide method (Chang et al., 2008). The key properties of the litter collected after approximately 18 months of treatment and then used in the decomposition experiment are described in Table 1.

2.3. Statistical analyses

The decomposition rates of the leaf litters were determined using the negative exponential function (Olson, 1963) given by $M = a\ e^{-kt}$, where M represents the fraction of the remaining dry mass at time t (%), k represents the decomposition constant, t represents the time since the beginning of the experiment (days), and a is the constant for the function fitting. This model relies on two assumptions: k is constant and characterizes the dry mass loss during decomposition, and the relative rate of decomposition is constant.

Litter quality were calculated and presented as the mean ± standard error (SE) of the four replicates. The differences in both leaf litter quality and decomposition rate between both species and treatment were assessed by analysis of variance (ANOVA). When significance was observed at $P < 0.05$, the least significant difference (Duncan) test was used. All statistical analyses were conducted using R version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Initial A. marina and B. gymnorrhiza litter quality

Except that the WN treatment significantly decreased the N content and thus increased the C/N ratio of the litter of *B. gymnorrhiza*, the N addition or warming treatments did not have significant effects on the litter quality of either mangrove species after 18 months of treatments. The N content of the pioneer mangrove species *A. marina* was significantly higher than that of the late successional species *B. gymnorrhiza* ($P < 0.001$, Table 1), and the C/N ratio of *A. marina* litter was significantly lower than that of *B. gymnorrhiza* litter ($P < 0.01$). Lignin content in the litter did not differ between before and after the N addition and warming treatments ($P > 0.05$).

3.2. Mass loss and dynamics of C and N

As reported in other litter decomposition studies, mass loss during mangrove litter decomposition could be divided into two phases (Fig. 2): an early phase involving a rapid loss of easily degradable compounds and a late phase involving a slow loss of recalcitrant materials (Berg and McClaugherty, 2014). The dry mass and C loss patterns, which were expressed as percentages of the initial total amounts, were similar for both species treated with warming and N addition. The litter in all treatments rapidly degraded during the first 28 days and then exhibited slower and steadier decreases over the remaining period of the decomposition experiment. The litter decomposition patterns of the two mangrove species were well described using a simple negative exponential equation and fitted parameters (Olson, 1963) plus other related parameters as shown in Table 2. The litter decomposition of each mangrove species showed different responses among the N addition and warming treatments (Fig. 2a and b; Table 2). According to the decay constant (Table 2), the litter of *A. marina* seedlings decomposed faster under the warming treatment than under the CK treatment ($P = 0.02$), but this was not the case for the litter of *A. marina* seedlings under the N addition treatment in comparison with the CK treatment ($P = 0.07$). Regarding the litter of *B. gymnorrhiza*, N addition and warming each significantly enhanced the mass loss rate during decomposition ($P < 0.001$ and $P = 0.02$, respectively). At the end of the study period, warming had increased the decomposition rate of *A. marina* and *B. gymnorrhiza* by 43% and 112%, respectively, whereas N addition had increased the decomposition rate of *B. gymnorrhiza* by 52%.

Similar results were observed for the litter C loss rate during the decomposition process (Fig. 2c and d; Table 2), which was rapidly reduced in the initial stage of decomposition. The litter C content of each species did not significantly differ among the four treatments (Table 1). After 168 days of decomposition of *A. marina*, the TC in the control and treatment groups had decreased to 32% (CK), 42% (+N), 38% (+W), and 40% (WN) of the initial values. After 168 days of decomposition of *B. gymnorrhiza*, the TC in the control and treatment groups had decreased to 43% (CK), 40% (+N), 43% (+W), and 46% (WN) of the initial values. Although the TC of *A. marina* was higher than that of *B. gymnorrhiza*, its loss rate during decomposition was higher than that of *B. gymnorrhiza* ($P < 0.01$). The N loss patterns during litter decomposition exhibited similar trends among the four treatments (all $P > 0.05$), but differences were observed between *A. marina* and *B. gymnorrhiza* (Fig. 2e and f). The TN in the litter of *A. marina* decreased rapidly (by more than 60%) during the first 3 weeks of decomposition and remained stable and low (approximately 40% of the initial level)

Table 1
The effects of N addition and warming treatments on select parameters of leaf litter from the seedlings of two mangrove species growing for more than 18 months under four different treatments. Different superscript letters indicate significant differences between two treatments at P < 0.05 level.

Species	Treatment	TC (g kg^{-1})	TN (g kg^{-1})	C/N	Lignin (%)
A. marina	CK	470.04 ± 23.17^{a}	23.34 ± 1.81^{a}	20.56 ± 2.19^{a}	41.76 ± 0.73^{a}
	+N	499.22 ± 17.13^{a}	26.10 ± 0.90^{a}	19.22 ± 1.05^{a}	40.87 ± 1.03^{a}
	+W	481.69 ± 30.79^{a}	25.20 ± 0.98^{a}	19.17 ± 1.23^{a}	41.11 ± 0.92^{a}
	WN	445.32 ± 7.36^{a}	26.32 ± 1.79^{a}	17.16 ± 1.21^{a}	41.32 ± 1.18^{a}
B. gymnorrhiza	CK	328.45 ± 49.01^{a}	14.59 ± 0.66^{a}	22.65 ± 3.65^{a}	38.38 ± 0.40^{a}
	+N	405.52 ± 21.14^{a}	15.62 ± 1.24^{a}	26.20 ± 1.28^{a}	38.79 ± 0.85^{a}
	+W	408.27 ± 12.19^{a}	12.25 ± 1.38^{ab}	34.86 ± 4.71^{ab}	40.19 ± 1.99^{a}
	WN	421.86 ± 5.40^{a}	9.89 ± 0.87^{b}	43.80 ± 4.46^{b}	40.20 ± 0.65^{a}

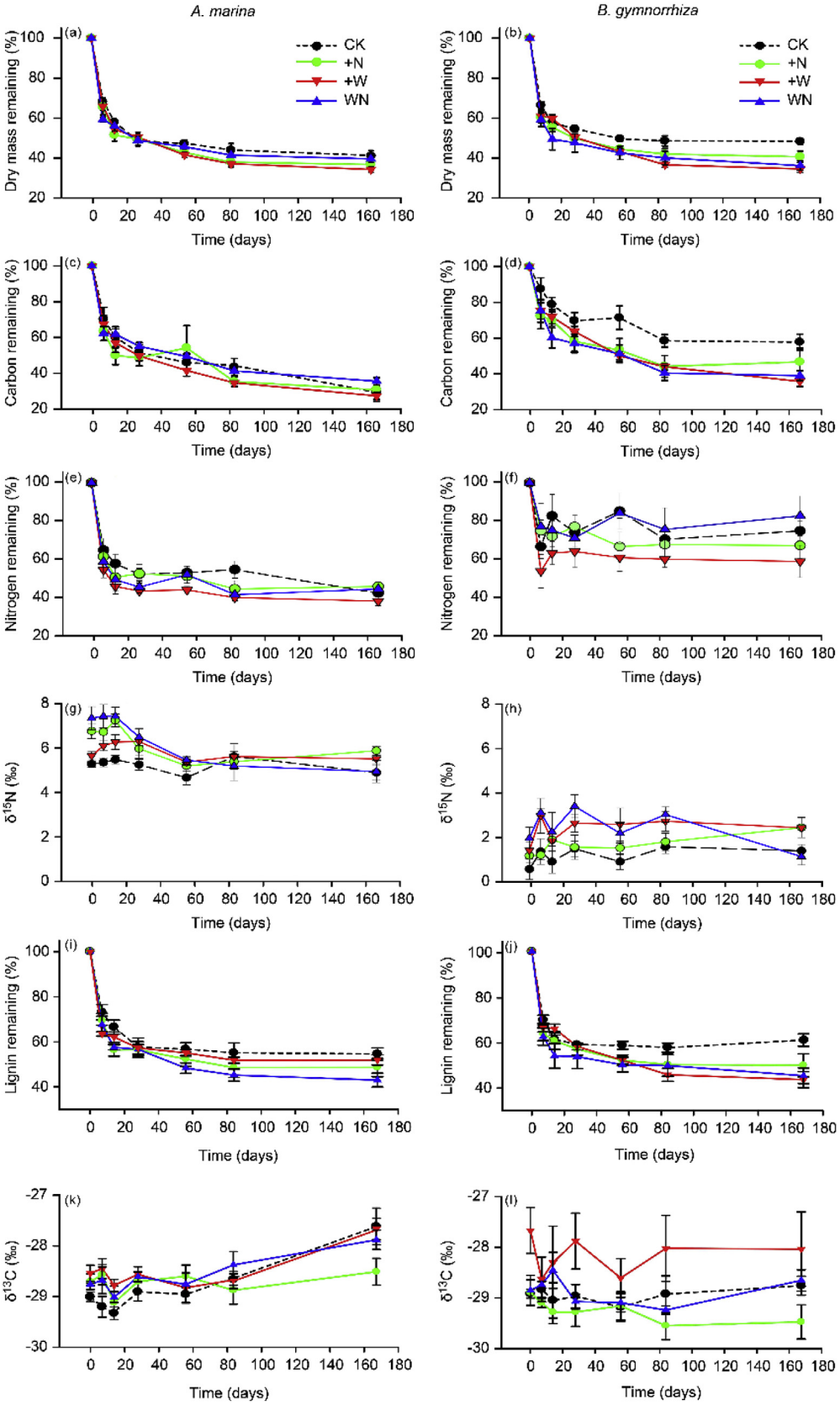

Fig. 2. The remaining percentages of dry mass; C, N and lignin contents and the C and N isotope ratios of litter from *A. marina* and *B. gymnorrhiza* under four different N addition and warming treatments.

Table 2
The effects of N loading and warming on select parameters related to the litter decomposition processes of *A. marina* and *B. gymnorrhiza*. Different superscript letters indicate significant differences between two treatments at $P < 0.05$ level.

Species	Treatment	Dry mass loss	C loss	N loss rate	Lignin loss	$\Delta\delta^{13}C$	$\Delta\delta^{15}N$
		decay constant	decay constant	1st to 3rd week	decay constant	7th to 24th week	in 24 weeks
		(per day)	(per day)	(%)	(per day)	(‰)	(‰)
A. marina	CK	0.0035 ± 0.0006[a]	0.0063 ± 0.0009[a]	47.92 ± 3.16[a]	0.0025 ± 0.0003[a]	−1.34 ± 0.46[a]	0.38 ± 0.23[a]
	+N	0.0048 ± 0.0010[ab]	0.0053 ± 0.0001[a]	47.60 ± 4.89[a]	0.0030 ± 0.0007[a]	−0.45 ± 0.15[a]	0.88 ± 0.22[a]
	+W	0.0050 ± 0.0001[b]	0.0065 ± 0.0003[a]	56.69 ± 1.64[a]	0.0035 ± 0.0003[a]	−1.16 ± 0.22[a]	0.14 ± 0.37[a]
	WN	0.0038 ± 0.0005[ab]	0.0048 ± 0.0003[a]	54.64 ± 3.12[a]	0.0023 ± 0.0003[a]	−0.88 ± 0.18[a]	**2.38 ± 0.81**[b]
B. gymnorrhiza	CK	0.0025 ± 0.0006[a]	0.0027 ± 0.0002[a]	26.01 ± 5.69[a]	0.0025 ± 0.0004[a]	−0.49 ± 0.19[a]	−0.82 ± 0.71[a]
	+N	0.0038 ± 0.0005[b]	0.0050 ± 0.0005[b]	22.97 ± 6.13[a]	0.0033 ± 0.0006[a]	−0.24 ± 0.26[a]	−1.27 ± 0.68[a]
	+W	0.0053 ± 0.0010[b]	0.0055 ± 0.0005[b]	36.05 ± 8.45[a]	0.0038 ± 0.0005[a]	−0.67 ± 0.41[a]	−1.36 ± 0.89[a]
	WN	0.0043 ± 0.0005[b]	0.0043 ± 0.0003[ab]	28.95 ± 4.41[a]	0.0030 ± 0.0004[a]	−0.51 ± 0.26[a]	0.82 ± 0.61[a]

thereafter. However, the TN in the litter of *B. gymnorrhiza* significantly decreased (to approximately 20–40%) only during the first week of decomposition and remained at a high level thereafter (60–80% of the initial level).

3.3. Dynamics of $\delta^{15}N$ and $\delta^{13}C$

The $\delta^{15}N$ values of *A. marina* exhibited depletion characteristics prior to 28 days and were approximately 1‰ more negative than the values in the initial phase. After day 28, the change patterns of *A. marina* $\delta^{15}N$ varied among the four groups: The $\delta^{15}N$ value in the CK group first increased and then decreased, the $\delta^{15}N$ value in the +N group increased slightly, and the $\delta^{15}N$ values in the +W and WN groups did not change significantly. The $\delta^{15}N$ values of *B. gymnorrhiza* were alternately enriched and depleted, although the fluctuation pattern under N addition was opposite that of the other three groups. When considering the entire decomposition process, the changes in $\delta^{15}N$ values were not significant (except under WN treatment in the litter of *A. marina*) (Table 2). We found that the $\delta^{15}N$ values of the *A. marina* litter were significantly higher than those of the *B. gymnorrhiza* litter (Fig. 2g and h). The $\delta^{13}C$ values ranged from −29.66 to −26.89‰ for *A. marina* and from −30.26 to −26.43‰ for *B. gymnorrhiza* in the control and treatment groups. However, neither N addition nor warming altered the litter $\delta^{13}C$ patterns (Fig. 2k and l).

3.4. Dynamics of lignin content

The lignin content increased with time and showed a similar pattern of change across treatments, with significant increases for both species observed in the first 28 days ($P < 0.01$) and the content approaching largely stable values in the following three months (Fig. 2i and j). Warming led to higher lignin content in *A. marina* but lower lignin content in *B. gymnorrhiza*. No significant differences were observed in the litter lignin decay constant among the four treatments or between the two mangrove species (Table 2).

4. Discussion

Our study found that the initial litter N content and C/N ratio of both species were not significantly changed under continuous warming and N addition (Table 1). These findings are consistent with previous studies (Breeuwer et al., 2008; Siegenthaler et al., 2010) in which plants were found to respond to N addition by improving biomass growth while showing no pronounced changes in C or N content. The initial N content of leaves grown in an environment with high N availability may be diluted by additional biomass growth. We found significant changes in the N content and C/N ratio of *B. gymnorrhiza* in the WN group, possibly due to enhancement of the dilution effect caused by N addition by the increase of air temperature.

The lack of clear responses in the litter decomposition rate to N addition in *A. marina* was likely due to the high N content of the litter (Table 2). *A. marina* typically has higher contents of leaf nutrient than does *B. gymnorrhiza*; accordingly, we observed a much higher N content and a lower C/N ratio in *A. marina* than in *B. gymnorrhiza* under all of the N addition and warming treatments. These findings can be attributed to differences in plant physiology and chemical composition between the two successional species. Compared with the late species *B. gymnorrhiza*, the pioneer species *A. marina* tends to have lower tannin contents that are poor in N because they grow in lower tidal zones with longer inundation period (Wang et al., 2013). In addition, litter with a higher N content not only decomposes faster than that with a lower content but also exhibits a higher abundance of leaf-colonizing microbes (Nordhaus et al., 2017). Leaf-colonizing microbes tend to preferentially decompose *A. marina* litter because of its higher N content and lower C/N ratio. Thus, the litter decomposition of *A. marina* is not sensitive to N addition. However, compared with that of *A. marina*, the litter of *B. gymnorrhiza* contains less N and has a higher C/N ratio, leading to a higher responsiveness to N addition.

Previous studies have reported that there was a tight link between litter decomposition rate and initial litter quality parameters, with patterns of more rapid decomposition associated with high N content, low initial lignin content and low initial lignin/initial N ratios (Melillo et al., 1984; Robertson et al., 1992; Twilley, 1986). However, our study showed a negative relationship between initial lignin content and the final remaining dry mass of *A. marina*, which indicates that a higher initial lignin content in *A. marina* litter corresponds to a faster decomposition rate (Fig. 3). Recent studies have shown that the decomposition rate of lignin in soil can be equal to or faster than that of other organic compounds. This pattern is observed because lignin content is usually determined by the acid-unhydrolyzable residue, also called Klason lignin, which contains aliphatic structures that are difficult to decompose (Hilli et al., 2012; Preston et al., 2009). The acetyl bromide method used in this study can fully exploit the lignin absorption effect of the spectrum to eliminate acid deficiency, which can decrease the loss of lignin and the inclusion of aliphatic structures, thereby improving the quality and quantity of lignin used in the following analysis. In our study, warming enhanced the decomposition of the lignin-derived compounds of *A. marina* but not *B. gymnorrhiza* (Fig. 2i and j). Notably, lignin can only be degraded by a few fungi groups (Neff et al., 2002). This positive effect of warming might have been due to the stimulation of fungal activity by the increased temperature (Feng et al., 2008). In addition, at high temperatures, fungi groups tend to preferentially target litters with high N contents. Such a significant association was only observed in *A. marina.* Another negative relationship was observed between initial C/N ratio and final remaining dry mass of *B. gymnorrhiza,* which indicated that high C/N ratios led to high decomposition rates. A previous study analyzed a data set of 2800 observations and found that the C-use efficiency of decomposers tended to

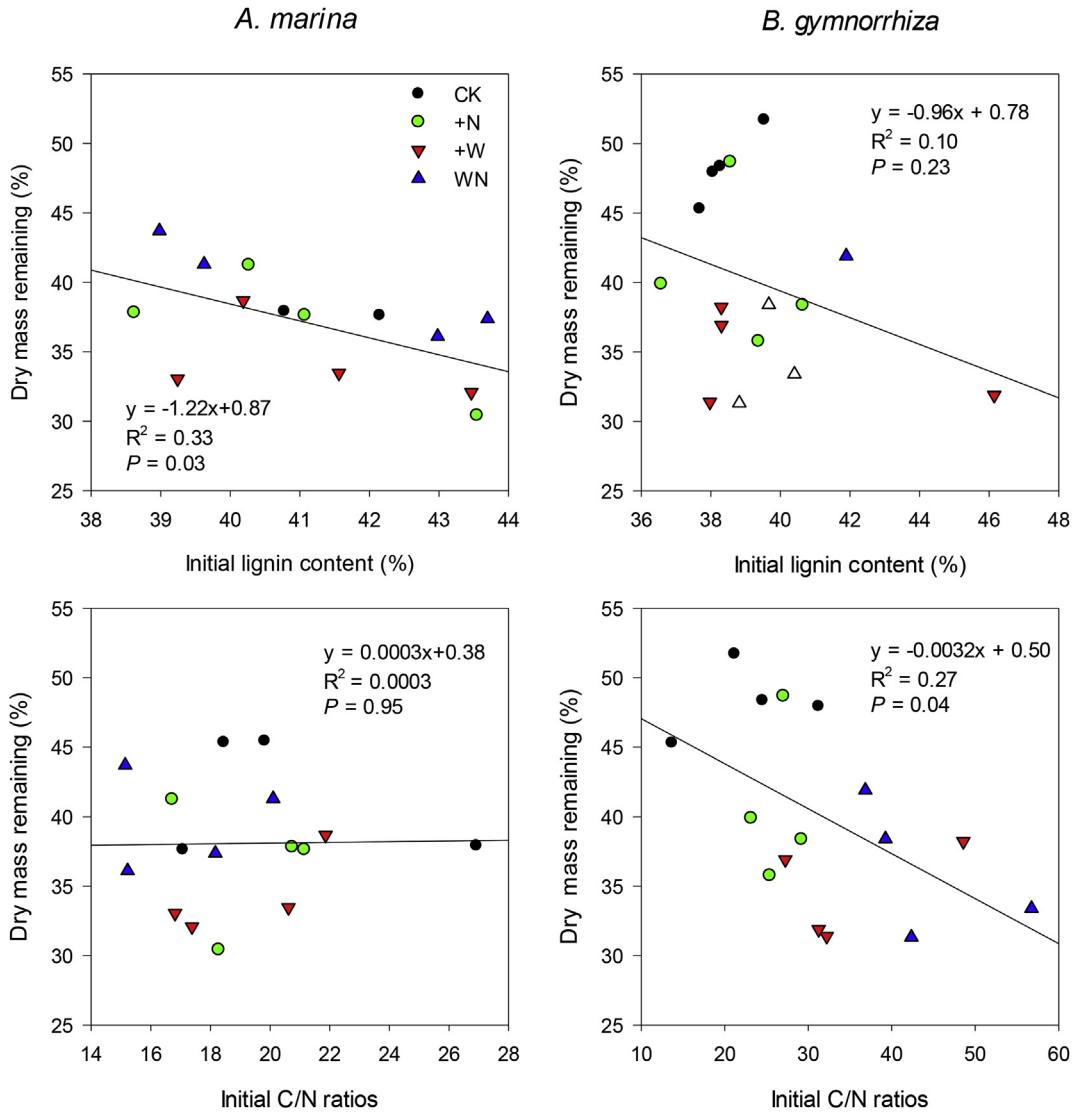

Fig. 3. Relationships between the initial leaf quality and their remaining dry mass: (1) black filled circles indicate ambient control (CK), (2) green filled circles indicate N addition (+N), (3) red downward triangles indicate warming (+W), and (4) blue upward triangles indicate N addition and warming (WN). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

decrease with increasing substrate C/N ratio. Low efficiency leads to a high heterotrophic respiration rate per unit mass of litter, which yields high decomposition rates (Manzoni et al., 2008). The initial C/N ratio of *B. gymnorrhiza* was much higher than that of *A. marina*, suggesting the potential existence of these relationships in *B. gymnorrhiza* but not *A. marina*. Litter that contains more N (or has a lower C/N ratio), such as that of *A. marina*, tends to exhibit net mineralization, whereas litter that contains less N (or has a higher C/N ratio), such as that of *B. gymnorrhiza*, demonstrates net immobilization (Alongi et al., 2013). Thus, *A. marina* exhibited a net nitrogen loss, possibly because N was mineralized and released into the sediment, which resulted in a faster decrease in the remaining N content, whereas *B. gymnorrhiza* showed mineralization and immobilization, leading to slower N loss.

No clear relationship was observed between litter quality and $\delta^{13}C$ or $\delta^{15}N$ value in our study (Table 3). In *A. marina*, the C content accounted for 43.0% of the $\delta^{13}C$ value in the CK group but only 29.6% of this value in the +W group. In *B. gymnorrhiza*, the remaining C accounted for 24.4% of the $\delta^{13}C$ value in the CK group, and the C/N ratio accounted for 23.4% of the $\delta^{13}C$ value in the +W group. The C content accounted for 20.1% of the $\delta^{15}N$ value in the CK group and 32.7% of the $\delta^{15}N$ value in the +N group in *A. marina*. The C/N ratio accounted for 31.1% and 40.3% of the $\delta^{15}N$ value in the +W and WN groups of *A. marina*, respectively. The remaining N content accounted for 20.1% and 23.6% of the $\delta^{15}N_{litter}$ value in the WN group of *B. gymnorrhiza*. No other variables were significant ($P > 0.05$). Studies have indicated that changes in $\delta^{15}N$ values are determined by the loss or incorporation of exogenous biomass during N immobilization and mineralization. Increases in the remaining N lead to net N accumulation via immobilization, and decreases in the remaining N lead net N losses via N mineralization (Aber and Melillo, 1980; Benner, 1991).

The magnitude and direction of $\delta^{15}N$ value change during decomposition are based on a range of factors (Bouillon et al., 2008; Bragazza and Iacumin, 2009; Fourqurean and Schrlau, 2003). In the present study, the similar change patterns of C/N and $\delta^{15}N$ suggested that the N in the remaining litter either belonged to the same pool as that at the beginning of decomposition or was not altered by the mineralization and immobilization processes. The $\delta^{15}N$ values of the *A. marina* litter were higher than those of the *B. gymnorrhiza* litter, which indicated that the preferential uptake of ^{15}N enriched the NH_4^+−N of this pioneer mangrove species in this mesocosm system (unpublished data). Moreover, the relationship between $\delta^{15}N$ value and remaining N was the opposite between *A. marina* and *B. gymnorrhiza*. Species and substrate

Table 3
Results of a stepwise regression of the relationships of C and N isotope values of the litter with the remaining C, N and lignin contents; C/N ratios; and C and N contents. Variables were retained in the regression model at $P < 0.05$.

Isotope value	Species	Treatment	Litter quality	Slope	Partial R^2	*P*-value
$\delta^{13}C$	*A. marina*	CK	C content (%)	−0.060	0.430	< 0.001
		+W	C content (%)	−0.057	0.296	0.002
		WN	CN ratio	−0.064	0.288	0.024
			Lignin (%)	4.889	0.188	0.004
	B. gymnorrhiza	CK	C remaining (%)	0.923	0.244	0.008
			Lignin (%)	4.955	0.182	0.026
		+W	N content (%)	−1.326	0.165	0.036
			CN ratio	−0.051	0.234	0.011
$\delta^{15}N$	*A. marina*	CK	C content (%)	0.040	0.201	0.017
		+N	C content (%)	−0.109	0.327	0.002
			CN ratio	0.268	0.319	0.002
		+W	CN ratio	0.090	0.311	0.001
		WN	N remaining (%)	2.423	0.230	0.010
			CN ratio	0.258	0.403	< 0.001
	B. gymnorrhiza	WN	N remaining (%)	−2.832	0.201	0.019
			N content (%)	−1.293	0.236	0.010

diversity also result in complicated isotope change patterns during decomposition (Bragazza and Iacumin, 2009).

The clear decrease in the $\delta^{13}C$ values of the litter in the early phase occurred because of the preferential retention of the recalcitrant lignin that is depleted in ^{13}C. However, in the later phase of decomposition (from the 7th to the 24th week), the $\delta^{13}C$ values of the remaining litter increased by ~1‰ in most treatments for both species (Table 2); this increase can be attributed to fungal ^{13}C enrichment during the late decomposition period (Potapov et al., 2013). We also observed that microbial respiration released ^{13}C-depleted CO_2 because of C isotope fractionation during the late phase of litter decomposition (unpublished data), resulting in significant increases in $\delta^{13}C$ values. However, further studies are needed to reveal the associated mechanisms. The lack of regular $\delta^{13}C$ and $\delta^{15}N$ changes in the decomposition process reflects the complexity of C isotope fractionations during mangrove litter decomposition process and the feedback process between the decomposer activity and the litter quality.

One limitation of our study was the small number of different test conditions and the small number of replicates. Due to the conditions of our greenhouse, there were only 3 mesocosms per test condition and only two levels of each treatment for both temperature and nitrogen conditions. In addition, we did not include elevated CO_2 treatment, another important aspect of global change, due to our space limitation. The interpretations of our results and the implications of our new findings should be cautious, and future studies with more replications, CO_2 treatment and treatment levels are needed to verify the key findings from this study.

5. Conclusions

In conclusion, our results imply that regional excessive N input and global warming will accelerate the C and N cycles of mangroves in subtropical China through faster litter decomposition. The observed $\delta^{13}C$ enrichment in the late stage of decomposition is more likely related to the isotope fractionation during the respiration process of the fungal community, which indicates the feedback processes between decomposer activity and litter substrate quality and makes it possible to describe and predict the decomposition process by the C stable isotopes. However, more studies are needed to confirm whether these patterns exist in other mangrove species and to determine how the differences between mangrove species relate to C and N cycling processes under changing N and temperature regimes at regional and global scales.

Declarations of interest

None.

Acknowledgments

We thank Jianzhang Chen for operating the greenhouse experiments and assisting in stable isotope and elemental analyses. We also thank other team members (especially Xiawan Zheng, Xiaofang Huang, etc.) for help in running the Shenzhen greenhouse experiment. This work was financially supported by the National Basic Research Program of China or the “973” Program (2013CB956601), the Shenzhen Basic Research Discipline Layout Project (JCYJ20150529164918736), the Ocean Public Fund Research Projects (201305021) and Science and Technology Project of Quanzhou City (2014Z120).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2018.09.018.

References

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., Fernandez, I., 1998. Nitrogen saturation in temperate forest ecosystems. Bioscience 48, 921–934.
Aber, J.D., Melillo, J.M., 1980. Litter decomposition: measuring relative contributions of organic matter and nitrogen to forest soils. Can. J. Bot. 58, 416–421.
Aber, J.D., Melillo, J.M., 1982. Nitrogen immobilization in decaying hardwood leaf litter as a function of initial nitrogen and lignin content. Can. J. Bot. 60, 2263–2269.
Alongi, D., Boto, K., Robertson, A., 2013. Nitrogen and phosphorus cycles. In: Alongi, D., Boto, K., Robertson, A. (Eds.), Tropical Mangrove Ecosystems. John Wiley & Sons, NJ, pp. 251–292.
Alongi, D.M., 2014. Carbon cycling and storage in mangrove forests. In: Carlson, C.A., Giovannoni, S.J. (Eds.), Annual Review of Marine Science. vol. 6. pp. 195–219.
Alongi, D.M., 2015. The impact of climate change on mangrove forests. Curr. Clim. Change Rep. 1, 30–39.
Bazzaz, F.A., 1979. The physiological ecology of plant succession. Annu. Rev. Ecol. Systemat. 10, 351–371.
Benner, R., 1991. Diagenesis of belowground biomass of Spartina alterniflora in salt-marsh sediments. Limnol. Oceanogr. 36, 1358–1374.
Berg, B., Laskowski, R., 2006. Litter decomposition: a guide to carbon and nutrient turnover. Adv. Ecol. Res. 38, 1–421.
Berg, B., McClaugherty, C., 2014. Decomposition as a process: some main features. In: Berg, B., Mcclaugherty, C. (Eds.), Plant Litter. Decomposition, Humus Formation, Carbon Sequestration, third ed. Springer, Berlin, Heidelberg, pp. 11–31.
Bouillon, S., Borges, A.V., Castañeda-Moya, E., Diele, K., Dittmar, T., Duke, N.C., Kristensen, E., Lee, S.Y., Marchand, C., Middelburg, J.J., 2008. Mangrove production and carbon sinks: a revision of global budget estimates. Global Biogeochem. Cycles 22, 1–12.
Bragazza, L., Iacumin, P., 2009. Seasonal variation in carbon isotopic composition of bog plant litter during 3 years of field decomposition. Biol. Fertil. Soils 46, 73–77.

Breeuwer, A., Heijmans, M., Robroek, B.J., Limpens, J., Berendse, F., 2008. The effect of increased temperature and nitrogen deposition on decomposition in bogs. Oikos 117, 1258–1268.
Carreiro, M., Sinsabaugh, R., Repert, D., Parkhurst, D., 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81, 2359–2365.
Chang, X.F., Chandra, R., Berleth, T., Beatson, R.P., 2008. Rapid, microscale, acetyl bromide-based method for high-throughput determination of lignin content in Arabidopsis thaliana. J. Agric. Food Chem. 56, 6825–6834.
Chapin, F., Matson, P., Mooney, H., 2002. Principles of Terrestrial Ecosystem Ecology. Springer, New York.
Chauvet, E., Suberkropp, K., 1998. Temperature and sporulation of aquatic hyphomycetes. Appl. Environ. Microbiol. 64, 1522–1525.
Chen, H., Gurmesa, G.A., Liu, L., Zhang, T., Fu, S., Liu, Z., Dong, S., Ma, C., Mo, J., 2014. Effects of litter manipulation on litter decomposition in a successional gradients of tropical forests in southern China. PLoS One 9, e99018.
Dang, C.K., Schindler, M., Chauvet, E., Gessner, M.O., 2009. Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition. Ecology 90, 122–131.
Downs, M.R., Nadelhoffer, K.J., Melillo, J.M., Aber, J.D., 1996. Immobilization of a 15 N-labeled nitrate addition by decomposing forest litter. Oecologia 105, 141–150.
Fang, H., Mo, J., Peng, S., Li, Z., Wang, H., 2007. Cumulative effects of nitrogen additions on litter decomposition in three tropical forests in southern China. Plant Soil 297, 233–242.
Feng, X., Simpson, A.J., Wilson, K.P., Williams, D.D., Simpson, M.J., 2008. Increased cuticular carbon sequestration and lignin oxidation in response to soil warming. Nat. Geosci. 1, 836–839.
Fourqurean, J.W., Schrlau, J.E., 2003. Changes in nutrient content and stable isotope ratios of C and N during decomposition of seagrasses and mangrove leaves along a nutrient availability gradient in Florida Bay, USA. J. Chem. Ecol. 19, 373–390.
Friesen, S.D., Dunn, C., Freeman, C., 2018. Decomposition as a regulator of carbon accretion in mangroves: a review. Ecol. Eng. 114, 173–178.
German, D.P., Chacon, S.S., Allison, S.D., 2011. Substrate concentration and enzyme allocation can affect rates of microbial decomposition. Ecology 92, 1471–1480.
Hilli, S., Stark, S., Willför, S., Smeds, A., Reunanen, M., Hautajärvi, R., 2012. What is the composition of AIR? Pyrolysis-GC–MS characterization of acid-insoluble residue from fresh litter and organic horizons under boreal forests in southern Finland. Geoderma 179, 63–72.
Hobbie, S.E., 2005. Contrasting effects of substrate and fertilizer nitrogen on the early stages of litter decomposition. Ecosystems 8, 644–656.
Hobbie, S.E., 2008. Nitrogen effects on decomposition: a five-year experiment in eight temperate sites. Ecology 89, 2633–2644.
Hobbie, S.E., Eddy, W.C., Buyarski, C.R., Adair, E.C., Ogdahl, M.L., Weisenhorn, P., 2012. Response of decomposing litter and its microbial community to multiple forms of nitrogen enrichment. Ecol. Monogr. 82, 389–405.
Hobbie, S.E., Vitousek, P.M., 2000. Nutrient limitation of decomposition in Hawaiian forests. Ecology 81, 1867–1877.
Holmer, M., Olsen, A.B., 2002. Role of decomposition of mangrove and seagrass detritus in sediment carbon and nitrogen cycling in a tropical mangrove forest. Mar. Ecol. Prog. Ser. 230, 87–101.
Hu, S., Chapin, F.S., Firestone, M., Field, C., Chiariello, N., 2001. Nitrogen limitation of microbial decomposition in a grassland under elevated CO2. Nature 409, 188–191.
IPCC, 2014. Climate change 2014: synthesis report. In: Core Writing Team, Pachauri, R.K., Meyer, L.A. (Eds.), Contribution of working groups I, II and III to the Fifth Assessment Report of the the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland, pp. 151.
Jordan, S.J., Stoffer, J., Nestlerode, J.A., 2011. Wetlands as sinks for reactive nitrogen at continental and global scales: a meta-analysis. Ecosystems 14, 144–155.
Kazakou, E., Vile, D., Shipley, B., Gallet, C., Garnier, E., 2006. Co-variations in litter decomposition, leaf traits and plant growth in species from a Mediterranean old-field succession. Funct. Ecol. 20, 21–30.
Knorr, M., Frey, S., Curtis, P., 2005. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86, 3252–3257.
Liu, G., Sun, J., Tian, K., Xiao, D., Yuan, X., 2017. Long-term responses of leaf litter decomposition to temperature, litter quality and litter mixing in plateau wetlands. Freshw. Biol. 62, 178–190.
Magill, A.H., Aber, J.D., 1998. Long-term effects of experimental nitrogen additions on foliar litter decay and humus formation in forest ecosystems. Plant Soil 203, 301–311.
Manzoni, S., Jackson, R.B., Trofymow, J.A., Porporato, A., 2008. The global stoichiometry of litter nitrogen mineralization. Science 321, 684–686.
Martinez, A., Larranaga, A., Perez, J., Descals, E., Pozo, J., 2014. Temperature affects leaf litter decomposition in low-order forest streams: field and microcosm approaches. FEMS Microbiol. Ecol. 87, 257–267.
Melillo, J., Naiman, R., Aber, J., Linkins, A., 1984. Factors controlling mass loss and nitrogen dynamics of plant litter decaying in northern streams. Bull. Mar. Sci. 35, 341–356.
Melillo, J.M., Aber, J.D., Muratore, J.F., 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63, 621–626.
Navas, M.L., Ducout, B., Roumet, C.J., Garnier, J., Garnier, E., 2003. Leaf life span, dynamics and construction cost of species from Mediterranean old-fields differing in successional status. New Phytol. 159, 213–228.
Neff, J.C., Townsend, A.R., Gleixner, G., Lehman, S.J., Turnbull, J., Bowman, W.D., 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature 419, 915–917.
Nordhaus, I., Salewski, T., Jennerjahn, T.C., 2017. Interspecific variations in mangrove leaf litter decomposition are related to labile nitrogenous compounds. Estuar. Coast Shelf Sci. 192, 137–148.
Olson, J.S., 1963. Energy storage and the balance of producers and decomposers in ecological systems. Ecology 44, 322–331.
Parton, W., Schimel, D.S., Cole, C., Ojima, D., 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. Soil Sci. Soc. Am. J. 51, 1173–1179.
Potapov, A.M., Semenina, E.E., Kurakov, A.V., Tiunov, A.V., 2013. Large 13C/12C and small 15N/14N isotope fractionation in an experimental detrital foodweb (litter–fungi–collembolans). Ecol. Res. 28, 1069–1079.
Prescott, C., 1995. Does nitrogen availability control rates of litter decomposition in forests? Plant Soil 168, 83–88.
Preston, C.M., Nault, J.R., Trofymow, J., 2009. Chemical changes during 6 years of decomposition of 11 litters in some Canadian forest sites. Part 2. 13 C abundance, solid-state 13 C NMR spectroscopy and the meaning of “lignin”. Ecosystems 12, 1078–1102.
Reef, R., Feller, I.C., Lovelock, C.E., 2010. Nutrition of mangroves. Tree Physiol. 30, 1148–1160.
Robertson, A.I., Alongi, D.M., Boto, K.G., 1992. Food chains and carbon fluxes. Aust. Inst. Mar. Sci. 41, 293–326.
Siegenthaler, A., Buttler, A., Bragazza, L., Van der Heijden, E., Grosvernier, P., Gobat, J.-M., Mitchell, E.A., 2010. Litter-and ecosystem-driven decomposition under elevated CO 2 and enhanced N deposition in a Sphagnum peatland. Soil Biol. Biochem. 42, 968–977.
Sukardjo, S., Alongi, D.M., Kusmana, C., 2013. Rapid litter production and accumulation in Bornean mangrove forests. Ecosphere 4.
Szefer, P., Carmona, C.P., Chmel, K., Konečná, M., Libra, M., Molem, K., Novotný, V., Segar, S.T., Švamberková, E., Topliceanu, T.S., 2017. Determinants of litter decomposition rates in a tropical forest: functional traits, phylogeny and ecological succession. Oikos 126, 1101–1111.
Taylor, B.R., Parkinson, D., Parsons, W.F., 1989. Nitrogen and lignin content as predictors of litter decay rates: a microcosm test. Ecology 70, 97–104.
Tu, L.-h., Hu, H.-l., Chen, G., Peng, Y., Xiao, Y.-l., Hu, T.-x., Zhang, J., Li, X.-w., Liu, L., Tang, Y., 2014. Nitrogen addition significantly affects forest litter decomposition under high levels of ambient nitrogen deposition. PLoS One 9, e88752.
Twilley, R.W., 1986. Litter production and turnover in basin mangrove forests in Southwest Florida. Ecology 67, 670–683.
Vitousek, P.M., 1998. Foliar and litter nutrients, nutrient resorption, and decomposition in Hawaiian Me t rosideros polymorpha. Ecosystems 1, 401–407.
Wafar, S., Untawale, A.G., Wafar, M., 1997. Litter fall and energy flux in a mangrove ecosystem. Estuar. Coast Shelf Sci. 44, 111–124.
Wang, Y., Zhu, H., Tam, N.F.Y., 2013. Polyphenols, tannins and antioxidant activities of eight true mangrove plant species in South China. Plant Soil 374, 549–563.
Wu, H., Peng, R., Yang, Y., He, L., Wang, W., Zheng, T., Lin, G., 2014. Mariculture pond influence on mangrove areas in south China: significantly larger nitrogen and phosphorus loadings from sediment wash-out than from tidal water exchange. Aquaculture 426, 204–212.