

Article

Leaf Litter Mixtures in Guam: Decomposition Synergism and Antagonism of Two Endangered Tree Species

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Abstract

Leaf litter traits among tree species exert a direct influence on spatiotemporal nutrient turnover and an indirect influence by shifting the decomposition dynamics of leaf litter mixtures including other sympatric species. *Cycas micronesica* and *Serianthes nelsonii* are two Mariana Island tree species that are endangered, and developing a greater understanding of the influence of these trees on biogeochemistry may improve information-based conservation decisions. The objectives of this study were to quantify the influence of mixing the leaf litter of these species with 12 sympatric forest plants to determine the additive and nonadditive influences on decomposition. The *C. micronesica* litter was collectively antagonistic when litter mixtures were incubated in a mesocosm study and a field litterbag study, and the response was similar among the included species. The *S. nelsonii* litter was collectively synergistic among the same mixed species, and the response was dissimilar among the included species. The contributions of these two threatened tree species to spatiotemporal diversity in biogeochemistry are dissimilar and considerable. These findings indicate that species recovery efforts for these two species are of paramount importance for maintaining Mariana Island ecological integrity and native biodiversity by sustaining their contributions to ecosystem services.

Keywords: antagonistic effects; *Cycas micronesica*; litter quality; litter mixture; nonadditive effects; *Serianthes nelsonii*; synergistic effects



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1. Introduction

The decomposition of leaf litter in forest communities plays a central role in numerous crucial processes including regional nutrient cycling, carbon sequestration dynamics, sustaining food webs, and soil microbiology [1–3]. Among the drivers which control the decomposition processes is the quality of litter, indicating that the spatial and temporal aspects of litterfall need to be more fully understood [4–6]. Single-species litters with greater concentrations of nutrients and lower concentrations of complex molecules are expected to decompose more rapidly. These phenomena are critical to the global biodiversity crisis and for endangered species conservation science. The individual contributions of threatened tree species to ecosystem processes need to be more fully understood to develop more knowledge-based decisions for species recovery and to more fully acknowledge the contributions before they are potentially lost.

All ecological processes should be viewed with contextual integrity, and litter decomposition research must acknowledge the mixing of various plant taxa. Single-species litter decomposition studies are important for understanding species rankings in decomposition lability and recalcitrance. However, in natural forest settings, the litter from one

species is never decomposed in the absence of litter from other sympatric species. Therefore, research to address the decomposition of multi-species litter mixtures provides more context-dependent outcomes [7,8]. When multi-species decomposition speed is increased or decreased relatively to the arithmetic averages of single-species incubation, the response is referred to as nonadditive. Some of the factors that lead to nonadditive litter mixture decomposition are transfers of nutrients from high-quality litters to low-quality litters during incubations, changes in water retention in the litter layer, the transfer of toxic or recalcitrant molecules from recalcitrant litters to labile litters, and changes in the microbial community due to the enhanced chemical diversity [9–11]. One of the issues that need to be more fully understood in relation to biodiversity loss is that the direct impact of each species represented in litter mixtures alters litter decomposition dynamics [12].

The Micronesia region of the western Pacific Ocean has not received adequate research to fully understand ecosystem function, so contributions from the region to global issues e.g., [13], remains minimal. Two endangered tree species from the Micronesian Mariana Islands associate with nitrogen-fixing root endosymbionts, and therefore are important contributors to island biogeochemical relations. The cycad *Cycas micronesica* K.D. Hill is the region's only gymnosperm, and has been listed under the International Union for Conservation of Nature's Red List since 2006 [14] and under the United States Endangered Species Act (ESA) since 2015 [15]. The legume *Serianthes nelsonii* Merr. is the most threatened tree species in the region, and has been listed under the Red List since 1978 [16] and under the ESA since 1987 [17]. These two tree species are valued as horticultural specimens. A greater understanding of how these important trees contribute to ecosystem function is urgently needed. Towards that goal, leaf litter nutrient relations have been determined [18] and single-species litter decomposition has been reported, indicating that low-quality *C. micronesica* litter is relatively recalcitrant and high-quality *S. nelsonii* litter is relatively labile [19,20].

No reports from Micronesia have adequately addressed multi-species litter decomposition, so knowledge is lacking to understand nutrient cycling in these oceanic islands. Therefore, the objective of this study was to determine the litter mass loss of dual-species mixtures involving these two endangered tree species. In addition to the *C. micronesica* and *S. nelsonii* litter mixture incubations, the study included 12 other sympatric species, each mixed with *C. micronesica* or *S. nelsonii*. For any nonadditive decomposition outcomes, the low-quality *C. micronesica* litter was predicated to exhibit antagonistic changes and the high-quality *S. nelsonii* litter was predicted to exhibit synergistic changes to the mixed litter decompositions.

2. Materials and Methods

Four litter decomposition experiments were conducted on the island of Guam using leaf litter from 14 forest species. The experimental approach was created to determine the influence of *C. micronesica* and *S. nelsonii* leaf litter on decomposition dynamics of the remaining 12 species in two-species mixtures. Two experiments were conducted under closed canopy forest conditions to ensure ecological relevance under natural weather conditions. In addition, two experiments were conducted in a constructed mesocosm to provide more controlled conditions that are more easily repeatable. Mesocosm research is useful for bridging the gap between laboratory experimental conditions and in situ field conditions [21]. Mesocosms provide more realism than laboratory studies, yet variables can be controlled to a greater degree than field studies.

Litter collection methods, storage conditions, and litterbag preparation protocols were previously described [18,22]. The collection locality was a biodiverse karst forest habitat in northeast Guam which developed on an uplifted coralline soil series (clayey-skeletal,

gibbsitic, nonacid, isohyperthermic Lithic Ustorthents). The 14 species were sympatric taxa in this habitat, and included monocot, eudicot, and gymnosperm species (Table A1). A major canopy defoliation event during Typhoon Dolphin in May 2015 was exploited to collect the litter samples from freshly dislodged stems containing intact leaves. The litter was collected within 48 h of the defoliation event. The 2015 litter samples were stored in air-conditioned laboratory conditions until the incubations were conducted. The litterbag protocol [23] was employed in the four experiments. Litterbags for the determination of single-species incubations were 20×20 cm and the nylon screen contained 1.5 mm diameter holes. Single-species litterbags were constructed with 3 g of litter, and therefore contained $75 \text{ g} \cdot \text{m}^{-2}$. Litterbags for the determination of two-species incubations were constructed with 0.5 g of each species for a total of 1 g, and litterbags were 10×10 cm. These litterbags contained $100 \text{ g} \cdot \text{m}^{-2}$. These mixture litterbags contained 0.5 g of *C. micronesica* or *S. nelsonii* litter, and 0.5 g of each of the remaining species. To ensure that the pre-incubation litter was not altered by oven drying, air-dried litter weights were used initially. Five samples of each of the 14 species were weighed as 2 g air-dried samples, dried at 75°C for two days, and then reweighed to obtain oven dry weight. The ratio of air-dried/oven-dried weight was used to determine the weight of the air-dried litter for each species that would provide the equivalent of 3 g or 0.5 g oven-dried litter.

2.1. Mesocosm Methods

The mesocosm experiments were initiated 6 January 2018 using the University of Guam laboratory site and methods that were previously described [20]. The mesocosm exposed $0.6 \text{ m} \times 8.8 \text{ m}$ of medium surface, and the substrate was created with a 10 cm layer of perlite as the base, then a surface 10 cm layer of field soil collected in a native forest community in Dededo, Guam. Care was taken to ensure that none of the 12 experimental species were in the vicinity of the soil collection sites in order to avoid any home field advantage complications among the taxa [24,25]. The litter layer was not included, but the humus layer was harvested with the soil and homogenized. The soil series was clayey, gibbsitic, nonacid, isohyperthermic Lithic Ustorthents [26]. The litterbags were installed on the same day as the mesocosm construction to ensure that the soil microorganism community did not change prior to litterbag deployment. Nutrient contents were typical for northern Guam soils (Table A2). The scattered indirect light to which the litterbags were exposed was $30\text{--}35 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Skye SKP200 Quantum Sensor, Skye Instruments, Llandrindod Wells, Powys, UK). The plastic mesocosm cover was removed one time per week and overhead sprinklers were employed to apply 5 cm of irrigation water. This was approximately the average of historical weekly rainfall for Guam. The mean daily maximum temperature was $29\text{--}30^\circ\text{C}$, and the mean daily minimum temperature was $27\text{--}28^\circ\text{C}$. Environmental factors such as temperature, light, and water relations influence decomposition [27], and therefore, the previously mentioned factors are needed to compare to similar studies.

Eight replications were constructed for each single-species litterbag category. The litterbags were deployed on the surface of the mesocosm substrate in eight blocks, and each block contained a single litterbag for each 3 g replication. Four of the blocks were randomly selected for harvest on 7–8 April 2018 (three months of incubation), and the remaining four blocks were harvested 10–11 July 2018 (six months of incubation). Eight litterbags were constructed for each two-species combination, and deployed as a second mesocosm experiment. There were 10 co-occurring species for the mesocosm experiment (Table A1). This dual-species mixture experiment also included 1 g litterbags for each species. The deployment date, three-month harvest date, and six-month harvest dates were as described for the single-species experiment.

Following harvest, adhering soil and humus particles were gently washed from the surface of each litterbag, and the remaining litter was carefully removed and the litterbags were discarded. The litter was allowed to dry in ambient conditions, then adhering soil was gently brushed from the litter. The litter was placed on a filter paper, then gently washed with reverse osmosis water. The litter was dried at 75 °C for two days and then weighed.

2.2. Field Methods

The field experiments were initiated 15 December 2022 on a site of the University of Guam campus, and the soil series was the same as for the mesocosm study. The litterbags were constructed according to the methods described for the mesocosm experiments, except that there were 12 co-occurring species included (Table A1). A fragment forest comprised *Leucaena leucocephala* (Lam.) de Wit was selected because of the homogeneity of emergent canopy species that reduced spatial heterogeneity in litterfall history. Soil nutrients were similar to those of the mesocosm study, but nitrogen concentrations were elevated due to the *L. leucocephala* tree cover (Table A3). The small plants that contributed to the subcanopy were removed to enable the deployment of the litterbags. All stems that directly contributed to the emergent canopy were left intact to avoid creating large canopy gaps. Each block was 0.8 m × 1.0 m, and the litterbags for each block were installed in a contiguous manner. The blocks were not contiguous. The entire footprint of this field site was 6.4 m × 9.2 m.

The amount of incident light at the soil surface was directly quantified with a 0.75 m line quantum sensor (EMS-7, PP Systems, Amesbury, MA, USA) and was 105–140 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Rainfall was allowed to impact the litterbags. A mean of 5.1 cm rainfall per week occurred during the first three months, and a mean of 7.4 cm rainfall per week occurred during the second three-month period. The mean daily maximum temperature was 28–29 °C, and the mean daily minimum temperature was 26–28 °C.

Four of the blocks were randomly selected and all of the litterbags within each were harvested 14–15 March 2023 to provide remaining litter weight after three months of incubation. The four remaining blocks were harvested 20–21 June 2023 to yield the six-month samples. Oven dry weight was determined as described above.

2.3. Statistical Methods

Several one-way and two-way analysis of variance (ANOVA) approaches were employed to determine the levels of significance in the response variables. The same tests were used for the mesocosm and field experiments. Parametric prerequisites were confirmed with Levene's test and the Shapiro–Wilk test.

The differences in the remaining litter dry weight among the species were determined with a one-way ANOVA for 12 species in the mesocosm study and 14 species in the field study. This ANOVA included only the 3 g litterbag replications. The three- and six-month harvests were analyzed separately.

The influence of *C. micronesica* litter on the decomposition speed of the co-occurring species was analyzed as a two-factor ANOVA. The treatment factor contained two levels with the calculated additive weight and actual weight of each replication considered as the treatment factor and the species were treated as a second factor in a 2 × 10 factorial for the mesocosm study and a 2 × 12 factorial for the field study.

The influence of the *S. nelsonii* litter on the decomposition speed of the co-occurring species was analyzed with the same two-factor ANOVA approach. These two-way ANOVAs did not include the 3 g litterbag replications, but included the 1 g litterbags containing only single-species and dual-species litter.

The *C. micronesica* and *S. nelsonii* litterbag treatments were analyzed with a *t*-test comparing the calculated additive weight with the actual weight. The three-month and six-month data were analyzed separately.

In order to standardize each dual-species dataset with a single-response variable, the relative deviation from additivity was calculated for each replication as $((\text{absolute weight} - \text{additive weight}) / \text{additive weight}) \times 100$. A positive outcome indicated nonadditive antagonism with less decomposition than predicted. A negative outcome indicated nonadditive synergism with greater decomposition than predicted.

In order to more fully understand some of the underlying mechanisms of the experimental outcomes, a Pearson correlation analysis was conducted using litter chemical trait dissimilarity. Total N and C were determined by dry combustion (FLASH EA1112 CHN Analyzer, Thermo Fisher, Waltham, MA, USA). Samples were also digested by a microwave system with nitric acid and peroxide, and then Ca, K, and P were quantified by inductively coupled plasma optical emission spectroscopy (Spectro Genesis; SPECTRO Analytical Instruments, Kleve, Germany). Litter tissue lignin concentration was determined according to the acetyl-bromide method. The chemical concentrations were described in detail from previous reports [18,28]. The differences between each species pair for concentrations of lignin (ΔLig), carbon (ΔC), nitrogen (ΔN), phosphorus (ΔP), potassium (ΔK), and calcium (ΔCa) were sorted then correlated with the relative deviation from the additivity metric. Additionally, the stoichiometric variables $\Delta\text{C:N}$, $\Delta\text{C:P}$, and $\Delta\text{C:K}$ were correlated with the same metric.

The statistical procedures were executed with R [29]. For variables which were significant, means separation was accomplished with the Tukey–Kramer post hoc test of each pairwise comparison.

3. Results

3.1. Single-Species Incubations

The biomass loss during the incubations in the mesocosm differed among the 12 species during the first three months ($f_{11,55} = 438.278$, $p < 0.001$) and after six months ($f_{11,55} = 608.378$, $p < 0.001$). The least biomass loss occurred for *B. tetrandra*, *C. nucifera*, *C. micronesica*, and *H. longipetiolata*. More than 80% of the original litter remained after 3 months of incubation for these species (Table 1). Biomass loss was greater during the 3- to 6-month period for these recalcitrant species, and more than 40% of the original biomass remained in the litterbags at 6 months. The labile species experienced the greatest biomass loss and included *M. citrifolia*, *P. grandis*, *P. serratifolia*, and *S. nelsonii*. Less than 20% of the original biomass remained after 3 months of incubation for these species (Table 1). The speed of biomass loss during the 3- to 6-month incubation period also increased for these species such that less than 10% of the original biomass remained in the litterbags at 6 months. The remaining species in the study exhibited intermediate decomposition speed in the mesocosm (Table 1).

The mass loss during the incubations in the field study differed among the 14 species during the first three months ($f_{13,55} = 89.811$, $p < 0.001$) and after six months ($f_{13,55} = 104.055$, $p < 0.001$). The same four species (*B. tetrandra*, *C. nucifera*, *C. micronesica*, *H. longipetiolata*) exhibited recalcitrant decomposition, but the biomass loss was greater than in the mesocosm study for both litterbag harvest periods (Table 2). The same four species (*M. citrifolia*, *P. grandis*, *P. serratifolia*, *S. nelsonii*) exhibited labile decomposition (Table 2). The remaining species including the added *F. prolixa* and *O. mariannensis* were intermediate compared to the four recalcitrant and four labile species (Table 2).

Table 1. Remaining litter biomass of 12 plant species from 3 g litterbags after 3 or 6 months of incubation in a mesocosm in Guam. Mean $\pm$ SE, $n = 4$.

Species	3 Months	6 Months
<i>Artocarpus mariannensis</i>	0.81 $\pm$ 0.02 f ¹	0.40 $\pm$ 0.01 f
<i>Bikkia tetrandra</i>	2.14 $\pm$ 0.06 b	1.19 $\pm$ 0.03 b
<i>Cocos nucifera</i>	2.13 $\pm$ 0.06 b	1.20 $\pm$ 0.03 b
<i>Cycas micronesica</i>	2.60 $\pm$ 0.06 a	1.35 $\pm$ 0.05 a
<i>Heritiera longipetiolata</i>	2.48 $\pm$ 0.06 a	1.26 $\pm$ 0.05 b
<i>Hibiscus tiliaceus</i>	1.19 $\pm$ 0.02 e	0.59 $\pm$ 0.01 e
<i>Morinda citrifolia</i>	0.35 $\pm$ 0.01 h	0.03 $\pm$ 0.01 g
<i>Pandanus tectorius</i>	1.59 $\pm$ 0.04 c	0.79 $\pm$ 0.02 c
<i>Pisonia grandis</i>	0.61 $\pm$ 0.02 g	0.03 $\pm$ 0.01 g
<i>Premna serratifolia</i>	0.34 $\pm$ 0.01 h	0.03 $\pm$ 0.01 g
<i>Serianthes nelsonii</i>	0.66 $\pm$ 0.02 g	0.02 $\pm$ 0.01 g
<i>Tabernaemontana rotensis</i>	1.39 $\pm$ 0.03 d	0.55 $\pm$ 0.01 d

¹ Means separation by Tukey–Kramer post-hoc test. Means with same letters within each column are not significantly different.

Table 2. The remaining litter biomass of 14 plant species from 3 g litterbags after 3 or 6 months of incubation in a field litterbag study in Guam. Mean $\pm$ SE, $n = 4$.

Species	3 Months	6 Months
<i>Artocarpus mariannensis</i>	0.68 $\pm$ 0.04 e ¹	0.34 $\pm$ 0.02 e
<i>Bikkia tetrandra</i>	2.07 $\pm$ 0.09 b	1.03 $\pm$ 0.05 b
<i>Cocos nucifera</i>	2.01 $\pm$ 0.09 b	1.01 $\pm$ 0.05 b
<i>Cycas micronesica</i>	2.48 $\pm$ 0.11 a	1.26 $\pm$ 0.05 a
<i>Ficus prolixa</i>	1.98 $\pm$ 0.10 b	0.94 $\pm$ 0.04 b
<i>Heritiera longipetiolata</i>	2.23 $\pm$ 0.11 a	1.20 $\pm$ 0.05 a
<i>Hibiscus tiliaceus</i>	1.11 $\pm$ 0.07 d	0.56 $\pm$ 0.02 d
<i>Morinda citrifolia</i>	0.35 $\pm$ 0.01 g	0.09 $\pm$ 0.01 f
<i>Ochrosia mariannensis</i>	2.01 $\pm$ 0.10 b	0.97 $\pm$ 0.05 b
<i>Pandanus tectorius</i>	1.46 $\pm$ 0.05 c	0.69 $\pm$ 0.03 c
<i>Pisonia grandis</i>	0.71 $\pm$ 0.04 e	0.34 $\pm$ 0.02 e
<i>Premna serratifolia</i>	0.34 $\pm$ 0.02 f	0.11 $\pm$ 0.01 f
<i>Serianthes nelsonii</i>	0.77 $\pm$ 0.04 e	0.32 $\pm$ 0.02 e
<i>Tabernaemontana rotensis</i>	0.96 $\pm$ 0.05 d	0.53 $\pm$ 0.03 d

¹ Means separation by a Tukey–Kramer post-hoc test. Means with the same letters within each column are not significantly different.

In summary, there was a 11.5-fold difference among the 6-month biomass means for the field study and a 67.5-fold difference among the 6-month biomass means for the mesocosm study. In general, the recalcitrant species exhibited greater mass loss and the labile species exhibited less mass loss in the field study when compared with the mesocosm study.

3.2. Mixing Litter with *Cycas micronesica*

The biomass remaining after 3 months of incubation in the mesocosm with *C. micronesica* litter mixtures was influenced by the main treatment factor ($f_{1,60} = 22.386$; $p < 0.001$) and the main species factor ($f_{9,60} = 105.662$; $p < 0.001$). In contrast, the interaction of treatment $\times$ species was not significant ($f_{9,60} = 0.952$; $p = 0.488$), indicating that the extent and direction of the nonadditive effects of the *C. micronesica* litter was similar among the species. The actual biomass of litter remaining after 3 months of incubation was 7% greater than the calculated additive mean (Figure 1a), and after 6 months of incubation, the actual biomass was 6% greater than the additive means (Figure 1b). These results indi-

cated that the *C. micronesica* litter rendered an antagonistic influence on the decomposition of litter from the 10 forest species.

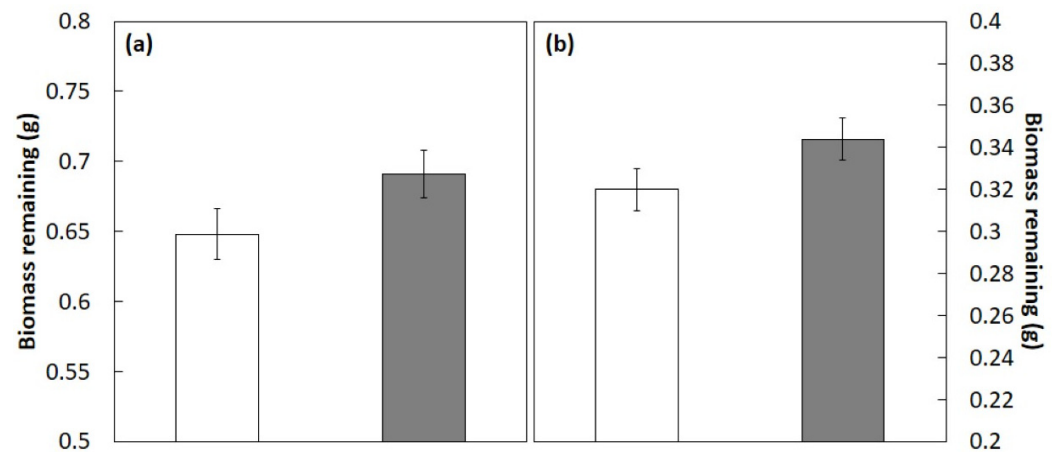


Figure 1. Litter biomass remaining after 3 months (a) or 6 months (b) of incubation in a Guam mesocosm. The original 1 g of litter tissue was derived from 10 plant species from Guam's northern karst forests that were mixed 50:50 with *Cycas micronesica* litter. Open bars represent the additive mean, and shaded bars represent the actual biomass. Mean $\pm$ SE, $n = 40$. Differences are significant at $p < 0.001$.

The leaf litter mixtures contained 50% *C. micronesica* litter separated into six non-overlapping means after 3 months of incubation (Table 3). The *H. longipetiolata* litter exhibited the least biomass loss, and the *M. citrifolia* and *P. serratifolia* litters exhibited the greatest level of biomass loss. Biomass loss was about 33% for the combined 3-month data set. These litter mixtures lost about 67% for the combined 6-month dataset, and the differences among the species mixtures were diminished (Table 3). The mixtures containing *B. tetrandra* and *C. nucifera* merged with the *H. longipetiolata* litter as the most recalcitrant mixtures, and the mixtures containing *P. grandis* merged with the *M. citrifolia* and *P. serratifolia* as the most labile mixtures.

Table 3. Remaining litter biomass of 10 plant species from the 1 g litterbags after 3 or 6 months of incubation in a mesocosm in Guam. The overall means were a combination of the predicted additive mean from single-species incubations and the actual remaining biomass of 50:50 mixtures with *Cycas micronesica* litter. Mean $\pm$ SE, $n = 8$.

Species	3 Months	6 Months
<i>Artocarpus mariannensis</i>	0.59 $\pm$ 0.02 e ¹	0.30 $\pm$ 0.01 d
<i>Bikkia tetrandra</i>	0.80 $\pm$ 0.01 b	0.42 $\pm$ 0.01 a
<i>Cocos nucifera</i>	0.79 $\pm$ 0.01 b	0.43 $\pm$ 0.01 a
<i>Heritiera longipetiolata</i>	0.84 $\pm$ 0.02 a	0.44 $\pm$ 0.02 a
<i>Hibiscus tiliaceus</i>	0.66 $\pm$ 0.01 d	0.33 $\pm$ 0.01 c
<i>Morinda citrifolia</i>	0.51 $\pm$ 0.01 f	0.25 $\pm$ 0.01 e
<i>Pandanus tectorius</i>	0.73 $\pm$ 0.02 c	0.36 $\pm$ 0.01 b
<i>Pisonia grandis</i>	0.56 $\pm$ 0.01 e	0.24 $\pm$ 0.01 e
<i>Premna serratifolia</i>	0.50 $\pm$ 0.01 f	0.25 $\pm$ 0.01 e
<i>Tabernaemontana rotensis</i>	0.68 $\pm$ 0.01 d	0.32 $\pm$ 0.01 cd

¹ Means separation by Tukey–Kramer post hoc test. Means with same letters within each column are not significantly different.

The biomass remaining after 3 months of incubation in the field study with *C. micronesica* litter mixtures was influenced by the main treatment factor ($f_{1,95} = 15.659$; $p < 0.001$) and the main species factor ($f_{11,95} = 18.678$; $p < 0.001$). In contrast, the interac-

tion of treatment $\times$ species was not significant ($f_{11,95} = 0.652$; $p = 0.778$), indicating that the extent and direction of the nonadditive effects of the *C. micronesica* litter was similar among the species, as for the mesocosm study. The actual biomass of litter remaining after 3 months of incubation was 9% greater than the calculated additive mean (Figure 2a), and after 6 months of incubation, the actual biomass was 8% greater than the additive means (Figure 2b). These results indicated that the *C. micronesica* litter rendered an antagonistic influence on the decomposition of litter from the 12 forest species.

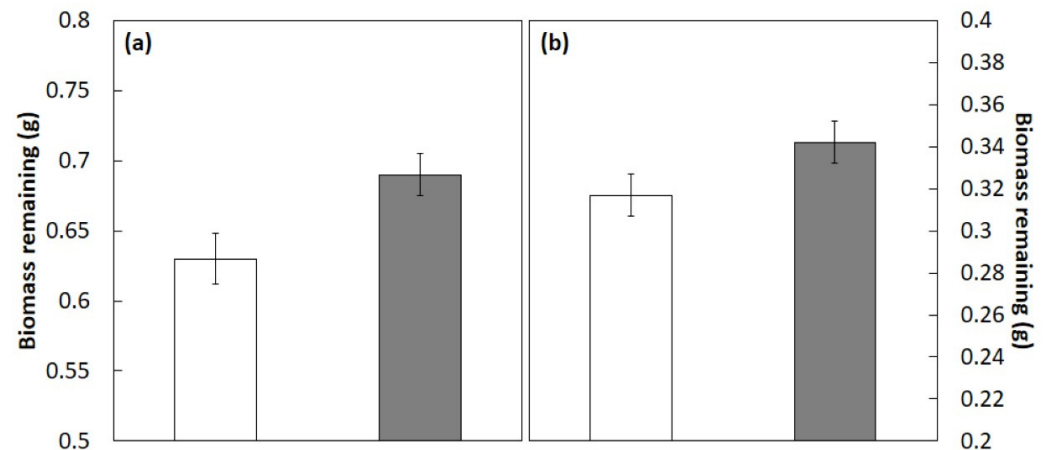


Figure 2. Litter biomass remaining after 3 months (a) or 6 months (b) of incubation in a Guam field study. Original 1 g of litter tissue was derived from 12 plant species from Guam’s northern karst forests that were mixed 50:50 with *Cycas micronesica* litter. Open bars represent the additive mean, shaded bars represent the actual biomass. Mean $\pm$ SE, $n = 48$. Differences significant at $p < 0.001$.

The 12 species with litter that was mixed with *C. micronesica* litter exhibited a 1.6-fold difference among the 3-month biomass means and a 1.6-fold difference among the 6-month biomass means for the field study (Table 4). There were five overlapping groups of means in the 3-month dataset and four groups of non-overlapping means in the 6-month groups of means. The relatively diminished differences among the species indicated that mixing the leaf litter with the *C. micronesica* leaf litter reduced the differences among the individual species to a greater extent in the field study than in the mesocosm study. The litter mixtures with the *M. citrifolia* and *P. serratifolia* litter remained the most labile litter mixtures, with about half of the litter lost by 3 months of incubation and about three-fourths of the litter lost by 6 months.

In summary, the influence of mixing the leaf litter of various forest species with *C. micronesica* leaf litter on changes in decomposition was similar among the 12 different species, as indicated by the lack of significant treatment $\times$ species interactions in the 3- and 6-month factorial ANOVAs. Therefore, the magnitude and direction of non-additivity was not influenced by the litter identity when mixed with *C. micronesica* litter. The species that exhibited the least amount of decomposition as non-mixed litter also exhibited the least amount of decomposition when mixed with *C. micronesica*. The species that exhibited the greatest levels of decomposition as non-mixed litter also exhibited the greatest levels of decomposition when mixed with *C. micronesica*. However, the decomposition was collectively non-additive, and as a group, the *C. micronesica* litter caused an antagonism that slowed down the litter decomposition of these 12 forest species. The overall means were similar for the mesocosm versus the field study for these litter treatments that were mixed with *C. micronesica* litter. Mixtures with *C. micronesica* litter exhibited a collective 7% antagonism in mesocosm and 9% antagonism in field incubations.

Table 4. Remaining litter biomass of 12 plant species from 1 g litterbags after 3 or 6 months of incubation in a field study in Guam. Overall means were a combination of the predicted additive mean from single-species incubations and the actual remaining biomass of 50:50 mixtures with *Cycas micronesica* litter. Mean $\pm$ SE, $n = 8$.

Species	3 Months	6 Months
<i>Artocarpus mariannensis</i>	0.57 $\pm$ 0.03 d ¹	0.29 $\pm$ 0.01 c
<i>Bikkia tetrandra</i>	0.77 $\pm$ 0.02 a	0.38 $\pm$ 0.02 a
<i>Cocos nucifera</i>	0.76 $\pm$ 0.02 a	0.38 $\pm$ 0.02 a
<i>Ficus prolixa</i>	0.75 $\pm$ 0.03 a	0.37 $\pm$ 0.01 a
<i>Heritiera longipetiolata</i>	0.79 $\pm$ 0.03 a	0.41 $\pm$ 0.02 a
<i>Hibiscus tiliaceus</i>	0.64 $\pm$ 0.03 bc	0.32 $\pm$ 0.01 b
<i>Morinda citrifolia</i>	0.49 $\pm$ 0.02 e	0.25 $\pm$ 0.01 d
<i>Ochrosia mariannensis</i>	0.76 $\pm$ 0.03 a	0.38 $\pm$ 0.01 a
<i>Pandanus tectorius</i>	0.69 $\pm$ 0.02 b	0.32 $\pm$ 0.01 b
<i>Pisonia grandis</i>	0.58 $\pm$ 0.02 d	0.29 $\pm$ 0.01 c
<i>Premna serratifolia</i>	0.51 $\pm$ 0.02 e	0.26 $\pm$ 0.01 d
<i>Tabernaemontana rotensis</i>	0.62 $\pm$ 0.03 cd	0.32 $\pm$ 0.01 b

¹ Means separation by Tukey–Kramer post hoc test. Means within each column with same letters are not significantly different.

3.3. Mixing Litter with *Serianthes nelsonii*

The biomass remaining after 3 months of incubation in the mesocosm with *S. nelsonii* litter mixtures was influenced by the treatment main factor ($f_{1,60} = 63.316$; $p < 0.001$), the species main factor ($f_{9,60} = 334.874$; $p < 0.001$), and the interaction of treatment $\times$ species ($f_{9,60} = 9.176$; $p < 0.001$). Therefore, the extent and direction of non-additive effects of the *S. nelsonii* litter was heterogeneous among the 10 species (Table 5). The means separated into nine non-overlapping groups. Some species exhibited an additive effect as shown by a lack of significant difference between the additive mean and the actual mean. These included *A. mariannensis*, *M. citrifolia*, *P. grandis*, *P. serratifolia*, and *T. rotensis*. The remainder of the species exhibited a nonadditive effect of litter mixing, whereby the 50:50 mixture with *S. nelsonii* accelerated litter decomposition in a synergistic manner. The species with the greatest level of synergistic decomposition were the most recalcitrant species. For example, the actual biomass mean for *B. tetrandra* was 18% less than the predicted additive mean.

The biomass remaining after 6 months of incubation in the mesocosm with *S. nelsonii* litter mixtures was influenced by the treatment main factor ($f_{1,60} = 46.189$; $p < 0.001$), the main species factor ($f_{9,60} = 815.342$; $p < 0.001$), and the interaction of treatment $\times$ species ($f_{9,60} = 3.97$; $p < 0.001$). Therefore, the extent and direction of non-additive effects of the *S. nelsonii* litter was heterogeneous among the 10 species as with the 3-month incubations (Table 5). There was more overlap among the 6-month means than the 3-month means, indicating the means converged as the leaf litter decomposed. The same five species exhibited an additive effect as shown by a lack of significant difference between the additive mean and the actual mean. The 50:50 mixture with *S. nelsonii* accelerated litter decomposition for the five remaining species. The three most recalcitrant species were *B. tetrandra*, *C. nucifera*, and *H. longipetiolata*, and the actual remaining mixed litter was 14% less for these three species than what was predicted from the additive means.

The biomass remaining after 3 months of incubation in the field study with *S. nelsonii* litter mixtures was influenced by the main treatment factor ($f_{1,95} = 69.140$; $p < 0.001$), the species main factor ($f_{11,95} = 90.123$; $p < 0.001$), and the interaction of treatment $\times$ species ($f_{11,95} = 2.785$; $p = 0.005$). The significant interaction indicated that the extent and direction of the non-additive effects of the *S. nelsonii* litter was not similar among the 12 species (Table 6). The number of species exhibiting an additive response was less than it was for the mesocosm study, in that only the labile *M. citrifolia*, *P. grandis*, and *P. serratifolia* litter

means revealed an additive decomposition response. The recalcitrant species exhibited the greatest level of synergy when mixed with *S. nelsonii*, with actual biomass means that were about 19% less than the predicted additive means. About 71% of the litter had decomposed by 3 months in this field study, and about 91% of the litter had decomposed by 6 months.

Table 5. Remaining litter biomass of 10 plant species from 1 g litterbags after 3 or 6 months of incubation in a mesocosm in Guam. Means are the predicted additive mean from single-species incubations and the actual remaining biomass of 50:50 mixtures with *Serianthes nelsonii* litter. Mean $\pm$ SE, $n = 4$.

Species	Treatment	3 Months	6 Months
<i>Artocarpus mariannensis</i>	Predicted	0.247 $\pm$ 0.008 g ¹	0.071 $\pm$ 0.006 h
<i>Bikkia tetrandra</i>	Additive	0.467 $\pm$ 0.012 b	0.204 $\pm$ 0.007 a
<i>Cocos nucifera</i>	Mean	0.466 $\pm$ 0.012 b	0.205 $\pm$ 0.007 a
<i>Heritiera longipetiolata</i>		0.523 $\pm$ 0.013 a	0.216 $\pm$ 0.007 a
<i>Hibiscus tiliaceus</i>		0.310 $\pm$ 0.010 e	0.110 $\pm$ 0.006 f
<i>Morinda citrifolia</i>		0.171 $\pm$ 0.008 i	0.013 $\pm$ 0.005 i
<i>Pandanus tectorius</i>		0.378 $\pm$ 0.010 c	0.137 $\pm$ 0.005 d
<i>Pisonia grandis</i>		0.213 $\pm$ 0.008 h	0.012 $\pm$ 0.005 i
<i>Premna serratifolia</i>		0.169 $\pm$ 0.006 i	0.012 $\pm$ 0.005 i
<i>Tabernaemontana rotensis</i>		0.344 $\pm$ 0.009 d	0.097 $\pm$ 0.006 fg
<i>Artocarpus mariannensis</i>	Actual	0.245 $\pm$ 0.007 g	0.067 $\pm$ 0.006 h
<i>Bikkia tetrandra</i>	Biomass	0.384 $\pm$ 0.011 c	0.167 $\pm$ 0.007 c
<i>Cocos nucifera</i>	Remaining	0.386 $\pm$ 0.010 c	0.187 $\pm$ 0.007 b
<i>Heritiera longipetiolata</i>		0.442 $\pm$ 0.009 b	0.188 $\pm$ 0.006 b
<i>Hibiscus tiliaceus</i>		0.279 $\pm$ 0.008 f	0.096 $\pm$ 0.005 fg
<i>Morinda citrifolia</i>		0.172 $\pm$ 0.008 i	0.012 $\pm$ 0.005 i
<i>Pandanus tectorius</i>		0.346 $\pm$ 0.011 d	0.122 $\pm$ 0.006 e
<i>Pisonia grandis</i>		0.215 $\pm$ 0.007 h	0.012 $\pm$ 0.005 i
<i>Premna serratifolia</i>		0.167 $\pm$ 0.007 i	0.011 $\pm$ 0.005 i
<i>Tabernaemontana rotensis</i>		0.342 $\pm$ 0.009 d	0.087 $\pm$ 0.006 g

¹ Means separation by Tukey Kramer post hoc test. Means with same letters within each column are not significantly different.

The biomass remaining after 6 months of incubation in the field study with *S. nelsonii* litter mixtures was influenced by the treatment main factor ($f_{1,95} = 38.954$; $p < 0.001$), the species main factor ($f_{11,95} = 76.499$; $p < 0.001$), and the interaction of treatment $\times$ species ($f_{11,95} = 2.205$; $p = 0.023$). The extent and direction of nonadditive effects of the *S. nelsonii* litter was heterogeneous among the 12 species (Table 6). Seven of the 12 species exhibited additive litter decomposition when mixed with *S. nelsonii* after 6 months of incubation in the field study. The recalcitrant species like *B. tetrandra* and *H. longipetiolata* exhibited the greatest nonadditive response with actual litter weight of 18% to 19% less than that predicted by the additive mean.

In summary, the influence of mixing the leaf litter of various forest species with *S. nelsonii* leaf litter on changes in decomposition was not consistent among 12 different species, as indicated by the significant treatment $\times$ species interactions in the 3- and 6-month factorial ANOVAs. Three to seven species exhibited additive decomposition in this mixture, depending on the study and incubation duration. For the species which demonstrated nonadditive litter decomposition, the *S. nelsonii* litter caused a universal synergism that increased the litter mass loss. About 70% of the mixed litter had decomposed by 3 months in the field study, and about 86% of the litter had decomposed by 6 months. Mixtures with labile *S. nelsonii* litter exhibited 7% synergism in the mesocosm and 12% synergism in the field incubations.

Table 6. Remaining litter biomass of 12 plant species from 1 g litterbags after 3 or 6 months of incubation in a field study in Guam. Means are the predicted additive mean from single-species incubations and the actual remaining biomass of 50:50 mixtures with *Serianthes nelsonii* litter. Mean $\pm$ SE, $n = 4$.

Species	Treatment	3 Months	6 Months
<i>Artocarpus mariannensis</i>	Predicted	0.244 $\pm$ 0.012 e ¹	0.112 $\pm$ 0.006 f
<i>Bikkia tetrandra</i>	Additive	0.476 $\pm$ 0.023 ab	0.226 $\pm$ 0.009 ab
<i>Cocos nucifera</i>	Mean	0.463 $\pm$ 0.021 b	0.222 $\pm$ 0.011 ab
<i>Ficus prolixa</i>		0.469 $\pm$ 0.023 b	0.213 $\pm$ 0.012 bc
<i>Heritiera longipetiolata</i>		0.515 $\pm$ 0.022 a	0.255 $\pm$ 0.014 a
<i>Hibiscus tiliaceus</i>		0.317 $\pm$ 0.018 d	0.148 $\pm$ 0.010 e
<i>Morinda citrifolia</i>		0.174 $\pm$ 0.009 f	0.075 $\pm$ 0.009 g
<i>Ochrosia mariannensis</i>		0.486 $\pm$ 0.015 ab	0.218 $\pm$ 0.017 bc
<i>Pandanus tectorius</i>		0.384 $\pm$ 0.015 c	0.171 $\pm$ 0.012 d
<i>Pisonia grandis</i>		0.254 $\pm$ 0.011 e	0.115 $\pm$ 0.009 f
<i>Premna serratifolia</i>		0.187 $\pm$ 0.010 f	0.082 $\pm$ 0.008 g
<i>Tabernaemontana rotensis</i>		0.291 $\pm$ 0.014 d	0.139 $\pm$ 0.009 e
<i>Artocarpus mariannensis</i>	Actual	0.241 $\pm$ 0.012 e	0.111 $\pm$ 0.006 f
<i>Bikkia tetrandra</i>	Biomass	0.387 $\pm$ 0.023 c	0.188 $\pm$ 0.009 d
<i>Cocos nucifera</i>	Remaining	0.376 $\pm$ 0.015 c	0.191 $\pm$ 0.012 cd
<i>Ficus prolixa</i>		0.383 $\pm$ 0.021 c	0.175 $\pm$ 0.009 d
<i>Heritiera longipetiolata</i>		0.416 $\pm$ 0.021 c	0.208 $\pm$ 0.011 bc
<i>Hibiscus tiliaceus</i>		0.261 $\pm$ 0.014 e	0.128 $\pm$ 0.010 e
<i>Morinda citrifolia</i>		0.170 $\pm$ 0.009 f	0.076 $\pm$ 0.006 g
<i>Ochrosia mariannensis</i>		0.395 $\pm$ 0.012 c	0.177 $\pm$ 0.011 d
<i>Pandanus tectorius</i>		0.321 $\pm$ 0.012 d	0.170 $\pm$ 0.010 d
<i>Pisonia grandis</i>		0.241 $\pm$ 0.011 e	0.113 $\pm$ 0.009 f
<i>Premna serratifolia</i>		0.177 $\pm$ 0.010 f	0.083 $\pm$ 0.008 g
<i>Tabernaemontana rotensis</i>		0.244 $\pm$ 0.013 e	0.115 $\pm$ 0.006 f

¹ Means separation by Tukey–Kramer post hoc test. Means with same letters within each column are not significantly different.

3.4. General Trends

The mixture of *C. micronesica* and *S. nelsonii* litter exhibited a significant antagonistic effect after 3 months (Figure 3a) or 6 months (Figure 3b) of incubation in the mesocosm. The recalcitrant *C. micronesica* litter significantly antagonized the *S. nelsonii* litter, with an actual decomposition that was about 8% greater than the additive mean after 3 months and about 11% greater than the additive mean after 6 months.

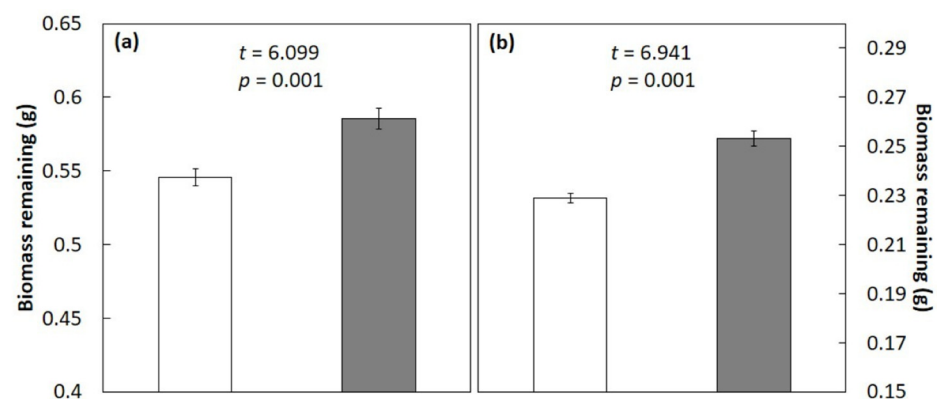


Figure 3. Litter biomass remaining after 3 months (a) or 6 months (b) of incubation in a Guam mesocosm study. Original 1 g of litter tissue was derived from a 50:50 mixture of *Cycas micronesica* litter and *Serianthes nelsonii* litter. Open bars represent additive mean, and shaded bars represent actual biomass. Mean $\pm$ SE, $n = 4$.

The mixture of *C. micronesica* and *S. nelsonii* litter exhibited additive decomposition effects after 3 months (Figure 4) or 6 months (Figure 4b) of incubation in the field study. Under these field conditions, the relative influences of these two contrasting litter types on the bulk litter sample clearly cancelled the effects of each other during incubation.

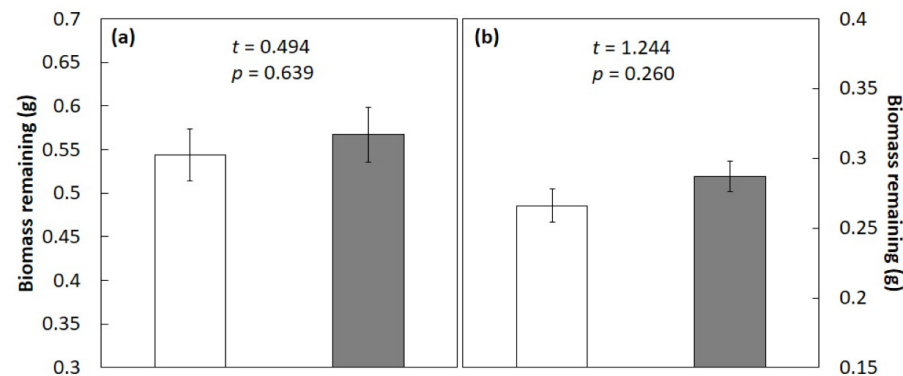


Figure 4. Litter biomass remaining after 3 months (a) or 6 months (b) of incubation in a Guam field study. Original 1 g of litter tissue was derived from a 50:50 mixture of *Cycas micronesica* litter and *Serianthes nelsonii* litter. Open bars represent additive mean, and shaded bars represent actual biomass. Mean $\pm$ SE, $n = 4$.

The relative decomposition of each species combination may best be revealed graphically by plotting the relative increase or decrease in decomposition as compared with additivity. For the 3 month mesocosm data, the overall means were about 6% antagonized by *C. micronesica* and 7% synergized by *S. nelsonii* (Figure 5a). Most species that were antagonized by *C. micronesica* exhibited near additivity for *S. nelsonii*, and most species that were synergized by *S. nelsonii* exhibited near additivity for *C. micronesica*. However, *H. tiliaceus* and *P. tectorius* appeared to be influenced in opposite directions by the two species.

The deviation from additivity was greater after 6 months of incubation than after 3 months of incubation in the mesocosm study (Figure 5b). The litter mixed with *C. micronesica* decomposed about 8% slower and the litter mixed with *S. nelsonii* decomposed about 7% faster than the additive means. Variation among the replications also increased, as shown by the relatively greater SE bars.

The data from the field litterbag study exhibited greater deviations from additivity than the mesocosm study (Figure 6a). After 3 months of decomposition, the litter mixed with *C. micronesica* decomposed about 10% slower and the litter mixed with *S. nelsonii* decomposed about 13% faster than the additivity.

More of the species mixtures exhibited additivity after 6 months of incubations in the Guam field litterbag study than in the mesocosm study (Figure 6b). Overall, the mixtures with *C. micronesica* litter decomposed about 8% slower and the mixtures with *S. nelsonii* litter decomposed 10% faster than additivity.

In summary, the litterbags containing mixed *C. micronesica* and *S. nelsonii* litter revealed a significant antagonism in the mesocosm study but not in the field study. The *C. micronesica* litter antagonized the mixed species in litterbags and the *S. nelsonii* litter synergized the mixed species in litterbags. The level of antagonism caused by *C. micronesica* litter was similar to the level of synergism caused by the *S. nelsonii* litter in the mesocosm study. In contrast, the synergistic effects of the *S. nelsonii* litter were relatively greater than the antagonistic effects of *C. micronesica* litter in the field study. These dissimilarities in some of the response variables illuminated the importance of using various research protocols to improve the context dependency to more fully understand complex litter decomposition dynamics.

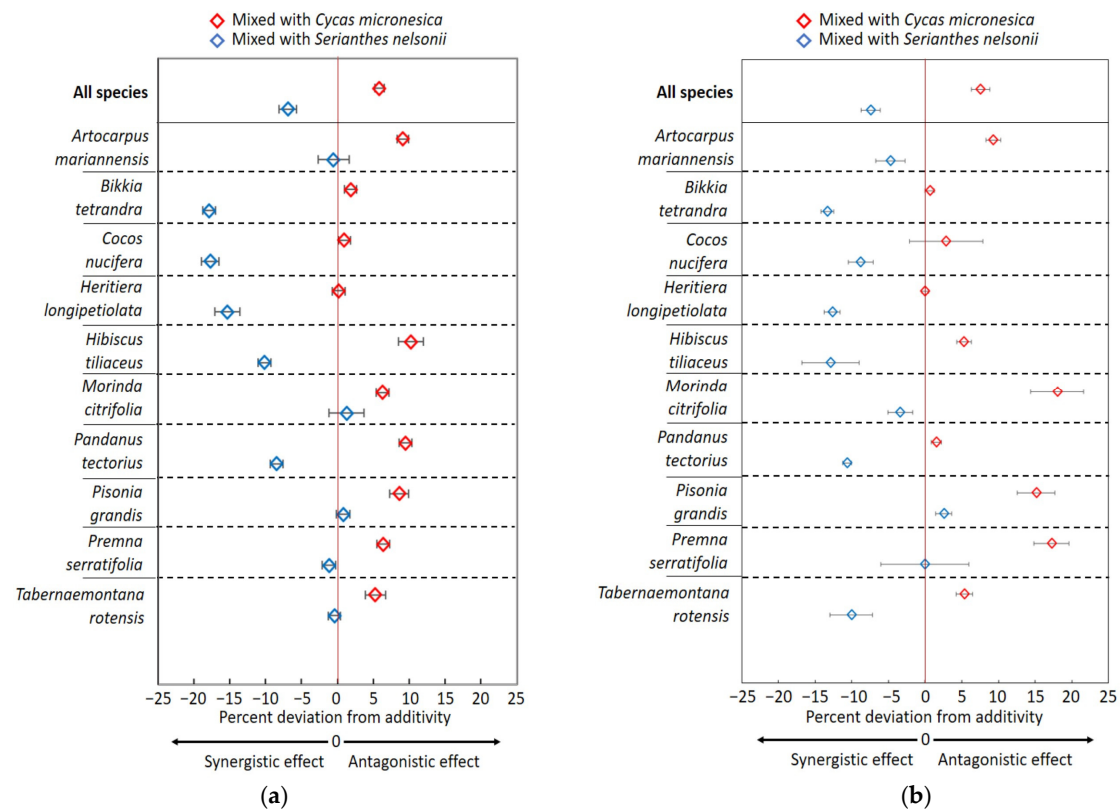


Figure 5. Graphic comparison of mixing the litter of 10 Guam forest species with *Cycas micronesica* litter or *Serianthes nelsonii* litter after (a) 3 months of incubation and (b) 6 months in a mesocosm. Original 1 g of litter tissue was derived from a 50:50 mixture. Vertical red line is additivity.

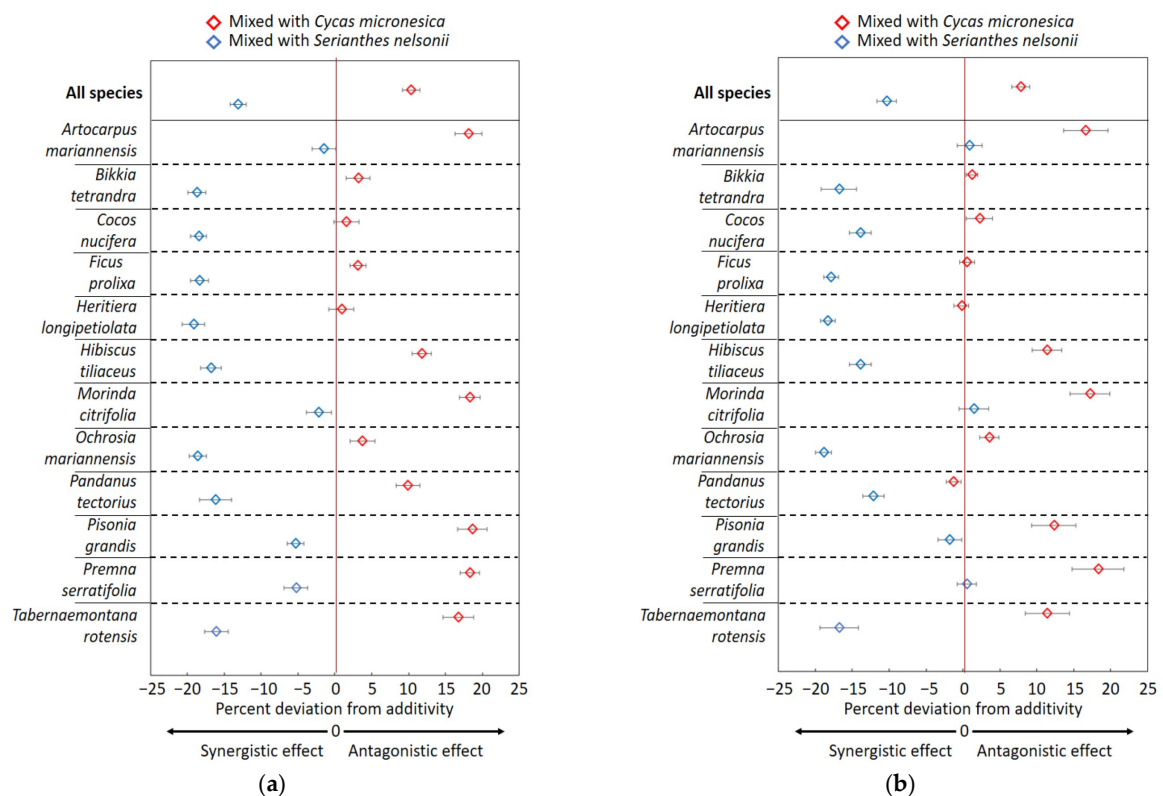


Figure 6. Graphic comparison of the mixing litter of 12 Guam forest species with *Cycas micronesica* litter or *Serianthes nelsonii* litter after (a) 3 months of incubation or (b) 6 months in a field litterbag study. Original 1 g of litter tissue was derived from a 50:50 mixture. Vertical red line is additivity.

3.5. Pearson Correlations

The differences in various leaf litter chemicals between the pairwise comparisons of species and *C. micronesica* exhibited correlations with the deviation from decomposition additivity after 3 months and 6 months of incubation in the mesocosm study (Figure 7). After 3 months of incubation, the variables with the greatest correlations were $\Delta C:N > \Delta C:K > \Delta N$. After 6 months of incubation, the variables with the greatest correlations were $\Delta N > \Delta C:N > \Delta \text{lig}$. For these mesocosm incubations, the 3-month data were not correlated with the 6-month data.

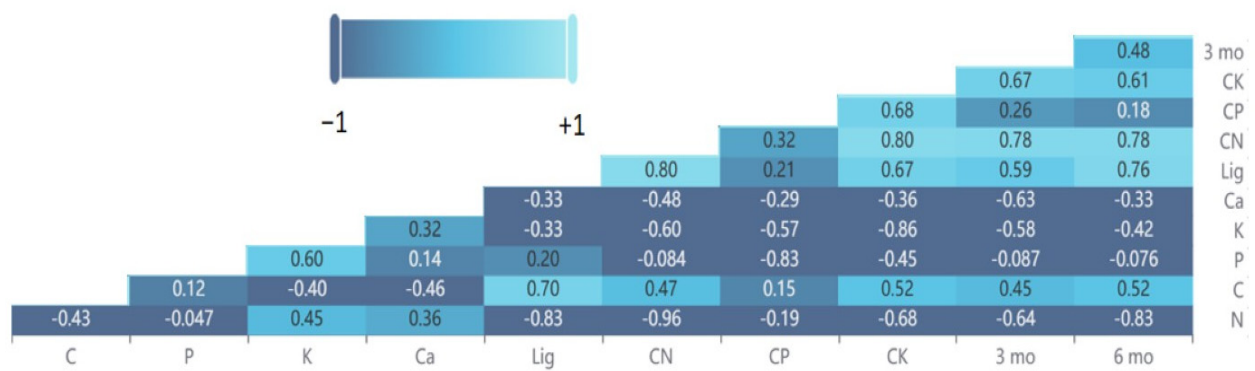


Figure 7. Heat map and coefficients for Pearson correlations among the leaf litter of various litter species mixed with *Cycas micronesica* litter after 3 and 6 months of incubation in a mesocosm litterbag study. Lig = lignin.

The differences in various leaf litter chemicals between the pairwise comparisons of species and *S. nelsonii* exhibited correlations with the deviation from decomposition additivity after 3 months and 6 months of incubation in the mesocosm study (Figure 8). After 3 months of incubation, the variables with the greatest correlations were $\Delta N > \Delta C:N > \Delta C:K$. After 6 months of incubation, none of the litter chemical traits exhibited a substantial correlation with the litter mass loss. The 3-month data were again not correlated with the 6-month data for the *S. nelsonii* mesocosm mixtures.

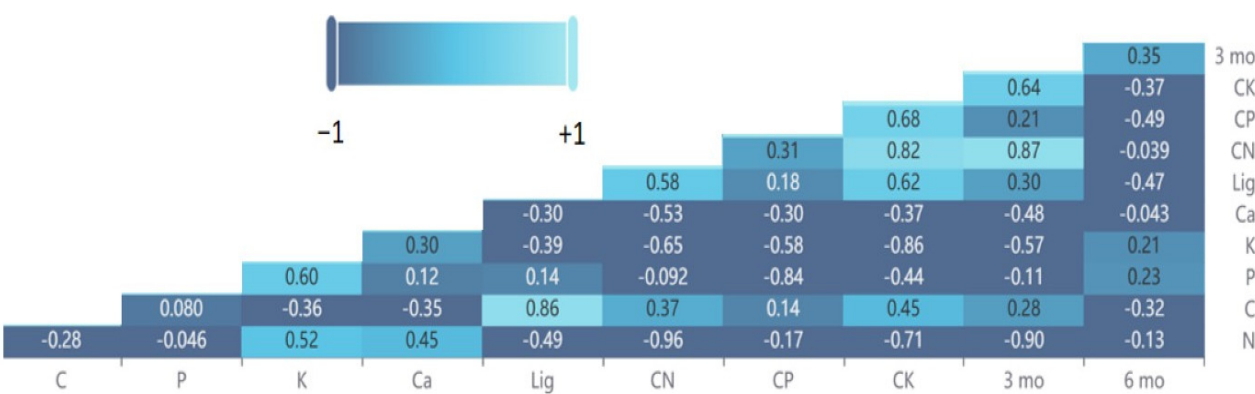


Figure 8. Heat map and coefficients for Pearson correlations among leaf litter of various litter species mixed with *Serianthes nelsonii* litter after 3 and 6 months of incubation in a mesocosm litterbag study. Lig = lignin.

The differences in various leaf litter chemicals between the pairwise comparisons of species and *C. micronesica* exhibited correlations with the deviation from decomposition additivity after 3 months and 6 months of incubation in the field study (Figure 9). After 3 months of incubation, the variables with the greatest correlations were $\Delta C:N > \Delta N$. After 6 months of incubation, the variables with the greatest correlations were $\Delta N > \Delta C:N > \Delta \text{lig}$.

In contrast to the mesocosm study, the 3-month data exhibited substantial correlation with the 6-month data.

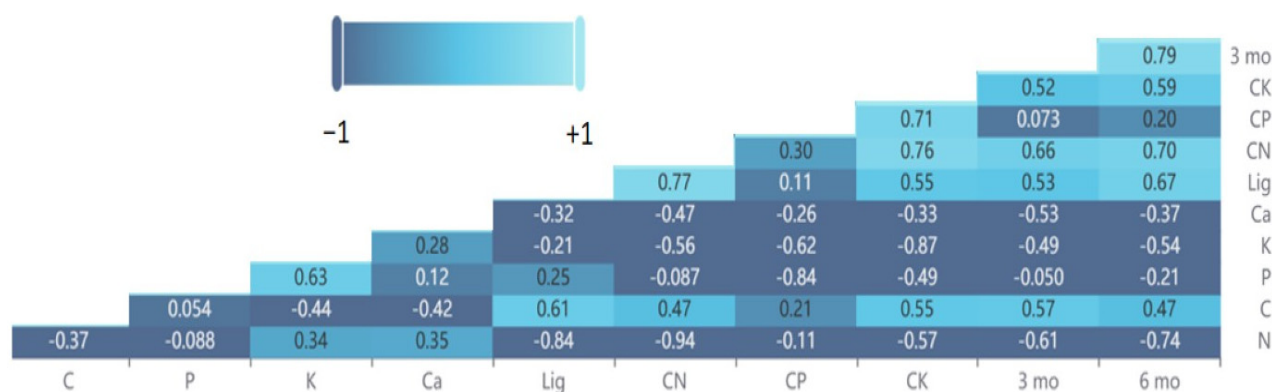


Figure 9. Heat map and coefficients for Pearson correlations among the leaf litter of various litter species mixed with *Cycas micronesica* litter after 3 and 6 months of incubation in a field litterbag study. Lig = lignin.

The differences in various leaf litter chemicals between the pairwise comparisons of species and *S. nelsonii* exhibited correlations with the deviation from decomposition additivity after 3 months and 6 months of incubation in the field study (Figure 10). After 3 months and 6 months of incubation, the variables with the greatest correlations were $\Delta N > \Delta C:N$. As with the *C. micronesica* field study mixtures, the 3-month data were correlated with the 6-month data.

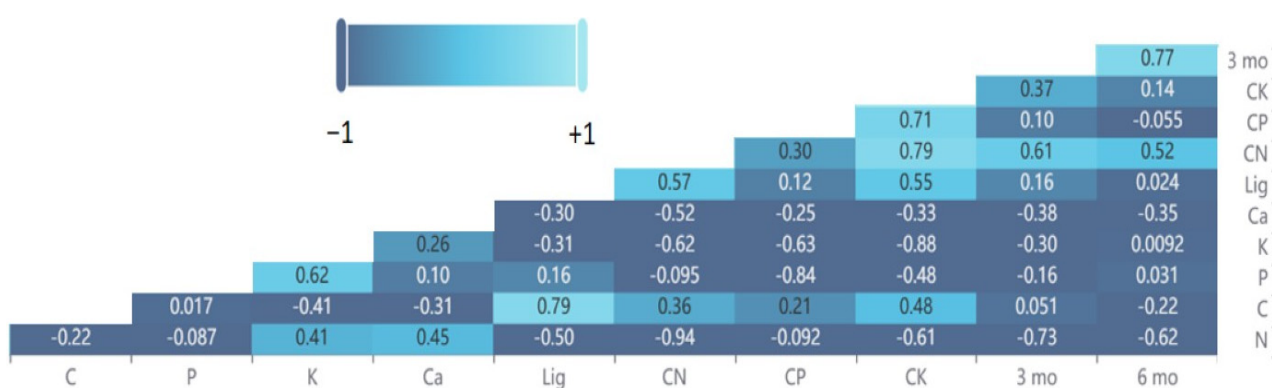


Figure 10. Heat map and coefficients for Pearson correlations among the leaf litters of various litter species mixed with *Serianthes nelsonii* litter after 3 and 6 months of incubation in a field litterbag study. Lig = lignin.

4. Discussion

Litterbags incubated in two studies in Guam confirmed the prediction of the non-additive effects of *C. micronesica* and *S. nelsonii* litter on mixtures with 12 Guam forest species. Mixtures with the recalcitrant *C. micronesica* litter exhibited a collective antagonism, and the mixtures with labile *S. nelsonii* litter exhibited a collective synergism. Literature reviews reveal that the nonadditive effects of litter mixtures occur in a majority of these studies [10,30,31], and these Guam data corroborate those reports.

4.1. Recalcitrance Versus Lability

A considerable body of literature exists on the direct influence of litter quality on single-species decomposition [4–6]. The role of litter quality may also explain much of the extent and direction of nonadditive trends in mixed litters in relation to recalcitrance and

the lability of the constituents of the bulk litter matrix [32–35]. Indeed, the low quality *C. micronesica* litter exhibited recalcitrant decomposition traits when decomposed alone but also antagonized the mixed litter incubations. In contrast, the high quality *S. nelsonii* litter exhibited labile decomposition traits when decomposed alone but also synergized mixed litter incubations.

Several mechanisms of nonadditive litter mixture decomposition have been proposed [9–11]. For the synergism caused by high-quality litter types, the direct transfer of nutrients from high-to-low quality litters within the mixture has been verified [36]. For antagonism caused by low-quality litter types, the role of recalcitrant components like lignin and cellulose and their transfer among the mixed leaf materials may be partly causal [37,38]. In addition to the transfer of these labile and recalcitrant chemical components among the mixed litter materials, their transfer to the soil substrate can facilitate or impede changes in the microbial community due to the enhanced chemical diversity. The litterfall zone beneath a *S. nelsonii* tree may therefore generate a mini-ecocline whereby the collective chemical environment facilitates litter decomposition within the ecocline in relation to the zones outside of the litterfall zone. Contrarily, the litterfall zone beneath a *C. micronesica* tree may generate a mini-ecocline whereby the collective chemical environment impedes litter decomposition in relation to the zones outside of the litterfall zone. These heterogeneous creations of mini-ecoclines may subsequently lead to mini-ecotones that benefit the community by improving biodiversity heterogeneity at the local level.

Species recovery plans are crucial for identifying approaches for conserving threatened plant species [39]. These plans typically explicate the need for expanded research to improve knowledge about the contributions of endangered species to ecosystem function. This Guam case study illuminates how two endangered tree species contribute to biogeochemical relations and add to the global literature on how biodiversity losses may influence litter decomposition [12]. If ongoing conservation efforts are ultimately unsuccessful, their loss will exert a direct negative impact on ecosystem function.

4.2. Mesocosm Versus Field Incubations

The overall litter mass loss for single-species litterbags was similar for the mesocosm versus field study incubations, but individual litter types behaved differently. The 6-month litter mass loss was greater in the field venue for recalcitrant species but greater in the mesocosm venue for the labile species. Moreover, nonadditive effects were greater in the field incubations than the mesocosm incubations for both *C. micronesica* and *S. nelsonii* litter mixing. The 3-month data were not correlated with the 6-month data for the mesocosm study, but were highly correlated for the field study.

The quality of the litter material is consistently considered the major driver of heterogeneous decomposition processes, but site factors also play a role. Among these are the temperature, humidity and rainfall patterns, soil substrate nutrient supply, incident light, slope aspect, altitude, and local decomposer community identities [27,40–47]. The temperature and soil nutrient regimes of the two studies were similar, so these factors were not likely causal of the differences between the mesocosm and field studies. The plastic cover of the mesocosm provided 100% humidity for the duration of the study, but diel fluctuations of ambient humidity characterized the field study. Additionally, the weekly application of a consistent amount of irrigation in the mesocosm created a homogeneous temporal pattern in relation to water inputs, but heterogeneous inputs of water occurred for the open field litterbags. For example, the field litterbags received more than 32 cm of rainfall during one week in May 2023, and these stochastic water relations may have influenced decomposer activities or leached more nutrients from the litterbag contents. Rainfall pulses per se have been identified as consequential drivers of litter decomposition [48]. Moreover, incident

light was less than 2% of sunlight and homogeneous throughout every photoperiod in the mesocosm study, but was 5% to 7% of sunlight and heterogeneous in the field study. The mesocosm litter was never exposed to sunflecks, but the field litter was impacted with bursts of full sun during midday sunflecks. Specific short wavelengths of the incident light, as occurs during sunflecks, have been identified as causal of the photodegradation influences on leaf litter decomposition [49–51].

These various drivers may impart a portion of their influence indirectly by modifying the soil microbial community function [52]. Recalcitrant litter components such as lignin and cellulose may deter the activity of the decomposer community, thereby altering the decomposer community. The role of the decomposer community identity on decomposition may be more important in the initial decomposition of fresh litters when the bacterial community dominates the processes, and become more muted in later stages of decomposition when the fungal decomposer community becomes more influential [9]. Decomposition during these two short-term litterbag studies may have been partly influenced by an inconsistent initial decomposer community. The mesocosm substrate was obtained from a native tree forest which had a decomposer community that had developed under mixed litters, and the field study was conducted under the canopy of the non-native *L. leucocephala* which produced a labile leaf litter [53–56]. This legume tree increases soil fertility partly through its beneficial influence on the soil microorganism community [57].

4.3. Litter Quality Drivers

Several of the initial litter quality metrics were correlated with the differences in litter mixture effects, but ΔN and $\Delta C:N$ emerged as the most prevalent drivers for every correlation. These two metrics were highly correlated with the results of 3- and 6-month incubations for both *C. micronesica* and *S. nelsonii* mixtures. The recalcitrant lignin fraction emerged as influential in defining the *C. micronesica* mixtures for the 3- to 6-month incubation period, but not the initial 3-month period. Lignin differences among these litter types may be important during the later stages of decomposition when *C. micronesica* litter is part of the mixture.

4.4. Future Directions

The loss of global biodiversity is raising alarm bells among many research priorities, and the influence on litter decomposition is among those priorities [12]. The forest ecological communities in the oceanic islands of Micronesia have largely been ignored in this research agenda. The ongoing loss of biodiversity is increasing threats to ecosystem function in numerous ways, and this study augments recent papers [18–20,22] that indicate species recovery efforts for *C. micronesica* and *S. nelsonii* are of paramount importance for maintaining ecosystem function and biogeochemical spatiotemporal diversity in Guam.

Design of successful research on litter decomposition in Guam and other Micronesian islands will require more context dependency in the protocols. The direct influence of the home field advantage [24,25,58] on litter decomposition was purposefully disallowed in both of these pioneer studies by design. This design assured a level substrate playing field among all of the single- and dual-species litterbags. The home field advantage of litters that fall on soil substrates that have historically been influenced by conspecific litters predicts a universal positive influence on litter mass loss speed. The extent of this advantage is modified by how dissimilar the chemical constituents of the fresh litter are from the bulk litter matrix [59]. More litterbag incubations using home and away microsites as the experimental substrates within in situ forests settings will be required to tease apart the many context-dependent issues in this *C. micronesica* and *S. nelsonii* research agenda.

Copious resources have been spent in recent years for *C. micronesica* and *S. nelsonii* conservation and recovery [60–62]. Most heavily funded projects have focused on expensive endeavors that do not address the primary threats and resulted in no adaptive management research that could have added horticultural and ecological knowledge. Therefore, the history of failed terrestrial resource conservation in Guam is partly a social science issue in which the policy- and decision-makers have suppressed science to rely on anecdotes and gray literature to inform decisions. This established culture has caused international species experts to be marginalized from having a seat at the table for *C. micronesica* and *S. nelsonii* conservation decisions [60,61]. The greatest need for continued research on the role of biodiversity and specifically threatened taxa on ecosystem function is a change in culture of the decision-makers who continue to invest millions of U.S. dollars of public funds into ineffective conservation programs. These ineffective conservation expenditures have led to a federal lawsuit [61].

Human activities continue to drive global change factors which collectively threaten biodiversity, demanding greater levels of coordination among various disciplines and requiring a change in direction [63]. Global climate change includes elevated carbon dioxide concentrations, and these atmospheric changes may decrease leaf litter mass loss through feedback loops [64]. Invasive species have emerged as another major driver of global change [65], and invasive insect herbivores comprise the primary threat to *C. micronesica* survival [62]. These invasive species may exert direct changes to litter decomposition dynamics [20], and this phenomenon deserves further study as the interplay between climate change and invasive species is more fully researched. Climate change and invasive species will continue to be major drivers that should be studied in linkages rather than in isolation as biodiversity management policy continues to evolve [66].

5. Conclusions

Cycas micronesica and *Serianthes nelsonii* leaf litters influenced the decomposition of dual species litter mixtures in mesocosm and field conditions on the island of Guam. The *C. micronesica* litter was collectively antagonistic, whereas the *S. nelsonii* litter was collectively synergistic. These two tree species are endangered and these findings enable a greater understanding of how losing them from the palette of native trees may influence the biogeochemistry of the forest communities. The contributions of these two threatened tree species are dissimilar and considerable. Species recovery efforts for these two species are of paramount importance for maintaining Mariana Island ecological integrity and native biodiversity by sustaining their contributions to ecosystem services

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Conflicts of Interest: Author Marler represented the company Philippine Native Plants Conservation Society. The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A

Table A1. List of plant species selected to decompose in northern Guam. M = mesocosm study; F = field study.

Species	Family	Study	Notes
<i>Artocarpus mariannensis</i> Trécul	Moraceae	M,F	Eudicot tree
<i>Bikkia tetrandra</i> (L.f.) A. Rich.	Rubiaceae	M,F	Eudicot shrub
<i>Cocos nucifera</i> L.	Arecaceae	M,F	Monocot tree
<i>Cycas micronesica</i> K.D. Hill ^{1,2}	Cycadaceae	M,F	Gymnosperm tree
<i>Ficus prolixa</i> G. Forst.	Moraceae	F	Eudicot tree
<i>Heritiera longipetiolata</i> Kaneh. ²	Malvaceae	M,F	Eudicot tree
<i>Hibiscus tiliaceus</i> L.	Malvaceae	M,F	Eudicot tree
<i>Morinda citrifolia</i> L.	Rubiaceae	M,F	Eudicot tree
<i>Ochrosia mariannensis</i> A.DC.	Apocynaceae	F	Eudicot tree
<i>Pandanus tectorius</i> Parkinson ex Du Roi	Pandanaceae	M,F	Monocot tree
<i>Pisonia grandis</i> R. Br.	Nyctaginaceae	M,F	Eudicot tree
<i>Premna serratifolia</i> L.	Lamiaceae	M,F	Eudicot tree
<i>Serianthes nelsonii</i> Merr. ^{1,2}	Fabaceae	M,F	Eudicot tree
<i>Tabernaemontana rotensis</i> (Kaneh.) P.T. Li ²	Apocynaceae	M,F	Eudicot tree

¹ Associated with nitrogen-fixing root endosymbionts. ² Listed under the United States Endangered Species Act.

Table A2. The concentration of plant nutrients in mineral soils in a Guam mesocosm. Total carbon and nitrogen content was determined by dry combustion. Available phosphorus was determined by the Olsen method [67]. Other macro- and micro-nutrients were quantified following digestion with diethylenetriaminepentaacetic acid [68]. Analysis was performed using inductively coupled plasma optical emission spectrometry. Mean $\pm$ standard error, $n = 4$.

Variable	Soil Trait
pH	7.82 $\pm$ 0.13
Calcium	7.91 $\pm$ 0.17 mg·g ⁻¹
Carbon	90.44 $\pm$ 5.62 mg·g ⁻¹
Copper	6.09 $\pm$ 0.61 µg·g ⁻¹
Iron	34.72 $\pm$ 2.14 µg·g ⁻¹
Magnesium	880.27 $\pm$ 31.29 µg·g ⁻¹
Manganese	64.19 $\pm$ 5.11 µg·g ⁻¹
Nitrogen	8.05 $\pm$ 0.72 mg·g ⁻¹
Phosphorus	80.22 $\pm$ 7.27 µg·g ⁻¹
Potassium	577.64 $\pm$ 19.54 µg·g ⁻¹
Zinc	17.17 $\pm$ 3.29 µg·g ⁻¹

Table A3. The concentration of plant nutrients in mineral soils in a Guam field study. Chemical analyses were as described in Table A2. Mean $\pm$ standard error, $n = 4$.

Variable	Soil Trait
pH	7.85 $\pm$ 0.17
Calcium	7.88 $\pm$ 0.15 mg·g ⁻¹
Carbon	91.44 $\pm$ 4.98 mg·g ⁻¹
Copper	5.39 $\pm$ 0.55 µg·g ⁻¹
Iron	32.75 $\pm$ 2.10 µg·g ⁻¹
Magnesium	870.22 $\pm$ 29.22 µg·g ⁻¹
Manganese	68.12 $\pm$ 5.15 µg·g ⁻¹
Nitrogen	8.92 $\pm$ 0.78 mg·g ⁻¹
Phosphorus	77.82 $\pm$ 6.32 µg·g ⁻¹
Potassium	562.52 $\pm$ 17.55 µg·g ⁻¹
Zinc	18.38 $\pm$ 2.97 µg·g ⁻¹

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