

# *Cycas micronesica* Trees Alter Local Soil Traits

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**Abstract:** Cycad plants possess uncommon morphological, chemical, and ontogenetic characteristics and they may introduce localized changes in soil traits that increase habitat heterogeneity. We used mature *Cycas micronesica* K.D. Hill trees growing in a range of soil types in Guam, Rota, and Yap to quantify differences between the soils beneath target trees and paired non-target soils away from cycad trees. The chronic presence of a *C. micronesica* tree introduced numerous localized changes in soil traits, increasing the heterogeneity of elemental stoichiometry in the community. Nitrogen, carbon:phosphorus, and nitrogen:phosphorus were increased in target soils among every soil type. Carbon increased and phosphorus decreased in most target soils. The habitats revealing the greatest number of elements with differences between target and non-target soils were the habitats with acid soils. The greatest number of metals exhibiting differences between the target and non-target soils occurred in the impoverished sandy habitat. This is the first report that indicates a cycad tree increases community spatial heterogeneity by localized changes in soil chemistry. Contemporary declines in cycad populations due to anthropogenic threats inadvertently decrease this spatial heterogeneity and its influences on primary producers in the landscape then cascading effects on the food web.

**Keywords:** cycad; home field advantage; fertile islands; plant-soil feedback; soil legacy effects

## 1. Introduction

*Cycas micronesica* K.D. Hill is an arborescent cycad species (Figure 1a) with a native range that includes Palau, Yap, Guam, and Rota Islands [1]. These Micronesian islands are positioned in the western belt of the Pacific Ring of Fire from ca. 7.5° to 14.1° N latitude. The 2003 invasion of Guam and 2007 invasion of Rota by the armored scale *Aulacaspis yasumatsui* Takagi caused widespread *C. micronesica* tree mortality [2–4]. This tree was the most numerous tree species on Guam at the time of the invasion [5], indicating that it served as a foundation species due to its abundance (Figure 1b). The density of the tree in Rota and Yap habitats was similar to or greater than in Guam habitats (unpublished data). The selective purging of this single species from in-situ and urban forests could lead to substantial ecological changes [4].

Cycad plants are unique in many respects, and more focused studies into cycad biology may inform contemporary research agendas [6,7]. *Cycas micronesica* is the only gymnosperm that is native to the Mariana Islands, produces long-lived leaves that senesce with high lignin/nitrogen and carbon/nitrogen traits [8], and like all cycads produce specialized coralloid root structures that house nitrogen-fixing cyanobacteria symbionts [6].

The international goals of threatened species recovery and ecological restoration are often hindered by the lack of benchmark information [4]. How does an attempt to return to some historical level of plant population health or ecosystem services occur if that historical benchmark is not quantified prior to the disturbances that generate the need for recovery or restoration? In that regard,

we set out to determine the mineral and metal content of soils near *C. micronesica* trees to more fully understand cycad ecology. The information may increase our understanding of how healthy *C. micronesica* trees contribute to ecosystem services and how those services may be lost when the trees are killed by invasive pests.



**Figure 1.** The arborescent *Cycas micronesica* tree exhibits a pachycaulous stem with radiating large compound leaves. (a) Isolated trees reveal the beauty and distinctiveness of this gymnosperm; and, (b) Extremely high densities illuminate the role as a foundation species in many of the habitats within its native range.

We were not interested in identifying mechanisms functioning in the means by which a healthy cycad tree directly interacts with its soils. Instead we set out to quantify soil traits within the leaf umbrella of large target cycad trees and compare those traits to the soils from nearby paired sites outside the zone of influence of the target plants. We tested the hypothesis that the soil traits beneath a mature cycad tree would differ from the soils that are outside the zone of influence, thereby increasing heterogeneity in community properties. When considering the importance of contemporary cycads to provide insights into the evolution of seed production and the ability to withstand mass extinction events that deleted countless other species from the biosphere [6], the fact that no cycad species has been included in the research on plant-soil feedback phenomena is surprising. This first look at the local contributions of a long-lived arborescent cycad species will fill this void.

## 2. Materials and Methods

We monitored the migration events of *A. yasumatsui* throughout Guam and Rota habitats during the years following the invasions, and for most habitats we recorded dates of initial *C. micronesica* plant infestations. For three habitats on Guam, we were able to collect soils to more fully understand the ecosystem contributions of the healthy *C. micronesica* trees prior to the *A. yasumatsui* infestations. These soils represent most of the soils throughout the native range of the tree species. Even though we were unable to collect soil samples from Guam's littoral sandy habitats prior to widespread plant mortality. We were able to collect soil samples on the island of Rota. The Yap *C. micronesica* populations do not have any biological threats to date. We augmented the Guam and Rota habitats with a Yap habitat to more fully understand how *C. micronesica* affects all the soils throughout its native range. This approach provided five habitats containing high density *C. micronesica* populations prior to the onset of non-native herbivore threats. Twelve paired micro-sites were selected in each habitat for sampling (methods below), and all the *C. micronesica* plants in excess of 1-m in height were counted within a linear 4-m × 100-m transect in order to estimate the adult population density.

An eastern Guam site was sampled 13–15 December 2004, and it contained 1159 trees per hectare. The karst soils formed in slope alluvium, loess, and residuum overlying limestone (Clayey-skeletal, gibbsitic, nonacid, isohyperthermic Lithic Ustorthents) [9]. The most common sympatric trees were *Cynometra ramiflora* L., *Eugenia reinwardtiana* (Blume) DC., *Ochrosia mariannensis* A. DC., *Ochrosia oppositifolia* (Lam.) K.Schum, and *Pandanus tectorius* Parkinson ex Zucc. *Aulacaspis yasumatsui* migrated into this habitat in February 2005. A western Guam habitat was sampled 4–7 March 2005, and it contained 1094 trees per hectare. The Guam cobbly clay soils were formed from sediment overlying porous limestone on uplifted plateaus (Clayey, gibbsitic, nonacid, isohyperthermic Lithic Ustorthents) [9]. The most common sympatric trees were *Aglaia mariannensis* Merrill, *Meiogyne cylindrocarpa* (Burck) Heusden, *Morinda citrifolia* L., *Ochrosia oppositifolia* (Lam.) K.Schum, and *Premna obtusifolia* R. Br. *Aulacaspis yasumatsui* migrated into this habitat in May 2005. A southern Guam site was sampled 15–18 June 2005, and it contained 889 trees per hectare. The volcanic soils formed in residuum derived from tuff (very fine, kaolinitic, isohyperthermic Oxic Haplustalfs) [9]. The most common sympatric trees were *Cocos nucifera* L., *Cynometra ramiflora* L., *Morinda citrifolia* L., *Pandanus dubius* Spreng., and *Pandanus tectorius* Parkinson ex Zucc. *Aulacaspis yasumatsui* migrated into this habitat in February 2006. A Rota coastal habitat was sampled 9–11 July 2009, and it contained 1328 trees per hectare. The sandy soils were formed in water-deposited coral sand derived from coral reef formations (Carbonatic, isohyperthermic Typic Ustipsamments) [10]. The most common sympatric trees were *Ficus prolixa* Forst. f., *Hernandia sonora* L., *Hibiscus tiliaceus* L., *Morinda citrifolia* L., and *Pandanus dubius* Spreng. *Aulacaspis yasumatsui* migrated into this habitat in Oct 2009. A Yap habitat was sampled 3–5 March 2018, and contained 1242 trees per hectare. The soils were formed in residuum derived from green, chlorite, and talc schist (Clayey-skeletal, mixed, isohyperthermic Lithic Tropudalfs) [11]. The most common sympatric trees were *Barringtonia racemosa* (L.) Spreng., *Calophyllum inophyllum* L., *Hibiscus tiliaceus* L., *Ficus tinctoria* Fort. f., and *Pandanus japensis* Mart.

General information about geology, soils, forest types, and climate of Guam and Rota is similar as they are separated by ca. 60 km [5,9,10,12]. The tropical marine climate is benign and it is characterized with daily minimum temperatures ranging from 23–25 °C and daily maximum temperatures ranging from 29–31 °C. Yearly rainfall is about 2500 mm, with about 55 percent occurring during the rainy season from Jul to Nov. The geological history and soils of Yap are dissimilar from that of Guam and Rota, but the tropical climate is similar [11,13]. Daily minimum temperatures ranging from 23–24 °C and daily maximum temperatures ranging from 30–31 °C. Yearly annual rainfall is 3050 mm, and even the driest month of Feb averages 146 mm of precipitation.

Guam (13.4° N) and Rota (14.1° N) are positioned at in the most active tropical cyclone basin worldwide [14], and these frequent violent storms deposit copious amounts of green plant tissues on the litter layer. Yap (9.5° N) is positioned at the southern edge of the tropical cyclone belt, so the frequency of these disturbances is minimal when compared to Guam and Rota.

## 2.1. Field Sampling

Within each habitat, 12 large *C. micronesica* trees greater than 3 m in stem height were selected as “target” sites. The height of each target tree was measured, then soil was harvested from the 0–15 cm zone while using a fixed pattern. The target soils were collected at the four cardinal points at distances of 1 m from the stem and at the leaf canopy drip line. Maximum *C. micronesica* leaf length is approximately 2 m, so this provided eight soil cores that were homogenized to represent soil status underneath the umbrella of influence of the *C. micronesica* trees. The “non-target” sites were defined by a ring of 5 m radius surrounding each target tree, and eight soil samples were obtained at the four cardinal points and the four intercardinal points. Due to the plant density, some of these prescribed sampling sites were in close proximity with other *C. micronesica* trees. In these cases, more distance from the target tree was added to ensure a distance of at least four meters from other *C. micronesica* trees. The eight non-target soil samples were homogenized into one sample.

## 2.2. Analyses

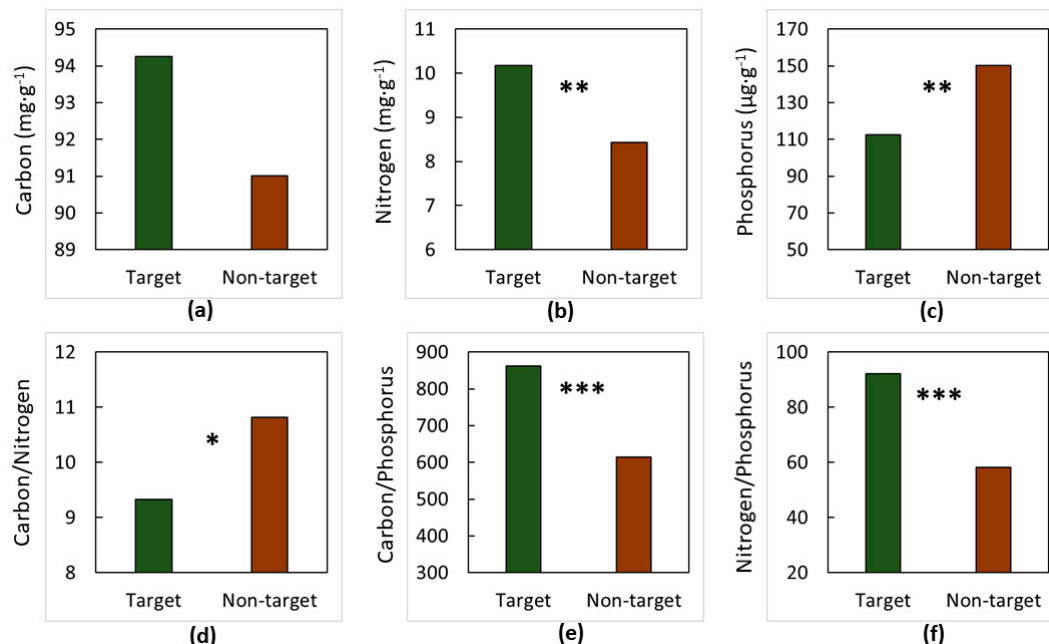
Total carbon (C) and nitrogen (N) contents were determined by dry combustion (FLASH EA1112 CHN analyzer; Thermo Fisher, Waltham, MA, USA). Extractable essential nutrients other than phosphorus (P) were quantified following digestion with diethylenetriaminepentaacetic acid, and total metals were quantified following digestion with nitric acid [15,16]. Analysis was by inductively coupled plasma optical emission spectrometry (Spectro Genesis; SPECTRO Analytical Instruments, Kleve, Germany). The available P was determined by the Olsen method [17] for alkaline soils and modified Truog method [18] for acid soils.

## 2.3. Statistics

Variables that met parametric requirements were subjected to paired *t* test to determine differences between target and non-target soils (SAS 9.3; SAS Institute, Cary, IN, USA). The stoichiometric variables C/N, C/P, and N/P were log-transformed prior to analysis. Some of the rare metals were undetected in some of the replications, and in these cases, no transformations were able to satisfy parametric requirements. For these metals, the Mann-Whitney *U* test [19] was used, as this test does not require any assumption about distribution of the data.

## 3. Results

The target soils beneath *C. micronesica* trees (height =  $367 \pm 22$  cm, mean  $\pm$  SE) differed from the meta-community non-target soils in most C:N:P traits in Guam cobbly clay soils (Figure 2. Target soils were 27% greater in N ( $t = 0.28$ ,  $p = 0.011$ ) and 25% less in P ( $t = 3.30$ ,  $p = 0.003$ ) than non-target soils. Target soils were 14% less in C/N ( $t = 2.12$ ,  $p = 0.046$ ), 29% greater in C/P ( $t = 3.80$ ,  $p = 0.001$ ), and 22% greater in N/P ( $t = 5.37$ ,  $p < 0.001$ ) than non-target soils.



**Figure 2.** The influence of proximity to *Cycas micronesica* on soil chemical traits in Guam cobbly clay soils on Guam. Target soils were collected underneath cycad trees. Non-target soils were collected 5-m from focal cycad trees. (a) Total carbon; (b) Total nitrogen; (c) Olsen phosphorus; (d) Carbon/nitrogen; (e) Carbon/phosphorus; and, (f) Nitrogen/phosphorus. \*, \*\*, \*\*\* = significant at 0.05, 0.01, or 0.001.

With the exception of Magnesium (Mg), which decreased 10% in target soils as compared with non-target soils, the remainder of minerals and metals were unaffected by the presence of *C. micronesica* trees in this Guam soil (Table 1). Arsenic (As) was not detected in any of the sites.

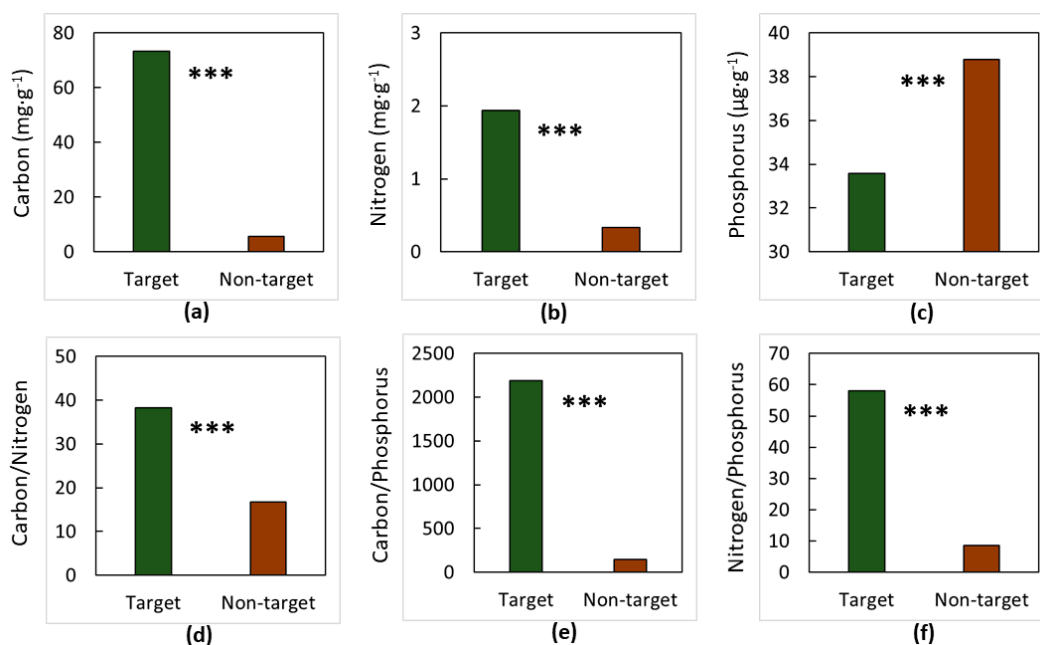


**Table 1.** The influence of proximity to *Cycas micronesica* trees on soil chemical traits in Guam cobbly clay soils on Guam. Target soils were collected underneath cycad trees. Non-target soils were collected 5-m from focal cycad trees. Means  $\pm$  SE,  $n = 12$ .

Soil trait	Target	Non-Target	Statistic <sup>1</sup>	Significance
pH	7.4 $\pm$ 0.1	7.3 $\pm$ 0.1	$t = 0.40$	0.697
Potassium <sup>2</sup>	342.7 $\pm$ 28.8	469.6 $\pm$ 58.3	$t = 1.95$	0.064
Calcium <sup>2</sup>	10,349.5 $\pm$ 625.7	10,133.3 $\pm$ 676.0	$t = 0.24$	0.817
Magnesium <sup>2</sup>	881.8 $\pm$ 27.2	980.8 $\pm$ 40.0	$t = 2.28$	0.033
Manganese <sup>2</sup>	55.1 $\pm$ 4.3	60.5 $\pm$ 5.9	$t = 0.60$	0.552
Iron <sup>2</sup>	36.1 $\pm$ 2.5	39.4 $\pm$ 2.1	$t = 0.99$	0.330
Cadmium <sup>3</sup>	6.2 $\pm$ 0.3	5.6 $\pm$ 0.2	$t = 0.95$	0.353
Cobalt <sup>3</sup>	20.3 $\pm$ 1.2	21.0 $\pm$ 1.7	$t = 0.35$	0.728
Chromium <sup>3</sup>	102.4 $\pm$ 9.0	96.9 $\pm$ 9.3	$t = 0.42$	0.682
Copper <sup>3</sup>	77.4 $\pm$ 3.2	82.6 $\pm$ 5.9	$t = 0.77$	0.448
Nickel <sup>3</sup>	17.5 $\pm$ 1.1	16.0 $\pm$ 1.2	$t = 0.91$	0.371
Lead <sup>3</sup>	3.1 $\pm$ 2.1	3.0 $\pm$ 2.0	$U = 72$	0.976
Selenium <sup>3</sup>	0.5 $\pm$ 0.2	0.3 $\pm$ 0.1	$U = 63$	0.603
Zinc <sup>3</sup>	105.8 $\pm$ 8.7	109.4 $\pm$ 8.6	$t = 0.29$	0.774

<sup>1</sup> Mann-Whitney  $U$  test, or standard  $t$  test. <sup>2</sup> diethylenetriaminepentaacetic acid extractable,  $\mu\text{g}\cdot\text{g}^{-1}$ . <sup>3</sup> nitric acid digestible,  $\mu\text{g}\cdot\text{g}^{-1}$ .

Guam's acid volcanic soils were changed to a greater degree by the presence of *C. micronesica* trees (height =  $343 \pm 19$  cm) than were the alkaline soils (Figure 3 Table 2). Target soils exhibited a 13-fold increase in C ( $t = 53.18$ ,  $p < 0.0001$ ), an 83% increase in N ( $t = 26.32$ ,  $p < 0.0001$ ), and a 13% decrease in P ( $t = 4.41$ ,  $p < 0.001$ ) when compared with the non-target soils. Target soils were 14% greater in C/N ( $t = 20.46$ ,  $p < 0.001$ ), 93% greater in C/P ( $t = 79.58$ ,  $p < 0.0001$ ), and 85% greater in N/P ( $t = 39.98$ ,  $p < 0.001$ ) than the non-target soils.



**Figure 3.** The influence of proximity to *Cycas micronesica* on soil chemical traits in Akina soils on Guam. Target soils were collected underneath cycad trees. Non-target soils were collected 5-m from focal cycad trees. (a) Total carbon; (b) Total nitrogen; (c) Modified Truog phosphorus; (d) Carbon/nitrogen; (e) Carbon/phosphorus; and, (f) Nitrogen/phosphorus. \*\*\* = significant at 0.001.

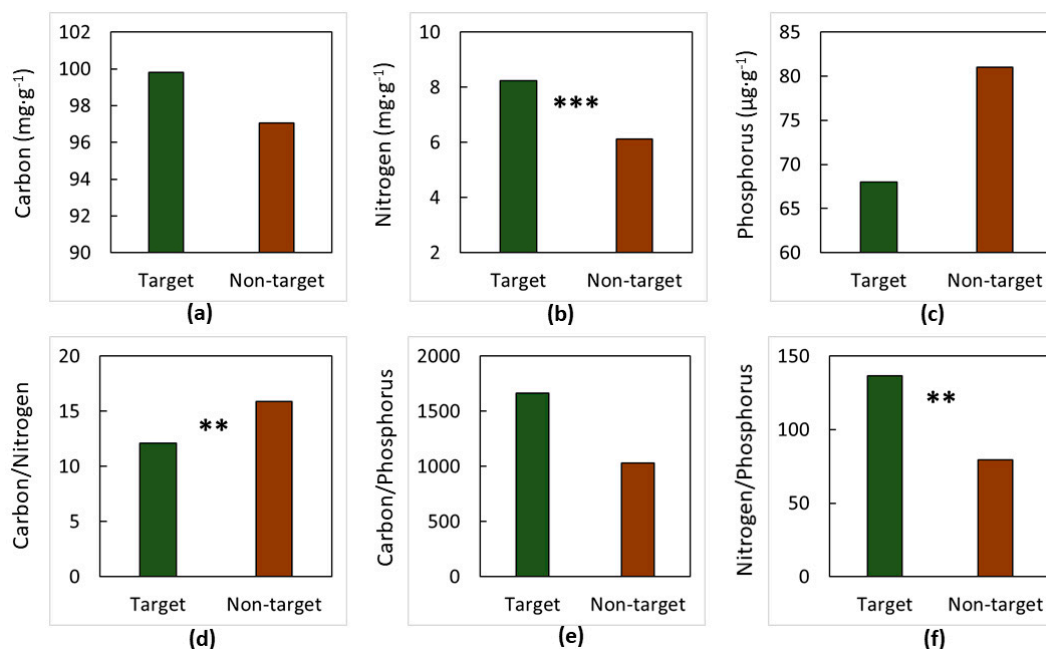
**Table 2.** The influence of proximity to *Cycas micronesica* trees on soil chemical traits in Akina soils on Guam. Target soils were collected underneath cycad trees. Non-target soils were collected 5-m from focal cycad trees. Means  $\pm$  SE,  $n = 12$ .

Soil Trait	Target	Non-Target	Statistic <sup>1</sup>	Significance
pH	6.8 $\pm$ 0.0	6.3 $\pm$ 0.1	$t = 5.54$	<0.001
Potassium <sup>2</sup>	1778.0 $\pm$ 21.1	1918.3 $\pm$ 36.4	$t = 3.34$	0.003
Calcium <sup>2</sup>	7957.7 $\pm$ 67.5	5846.5 $\pm$ 78.4	$t = 20.42$	<0.001
Magnesium <sup>2</sup>	2945.9 $\pm$ 15.3	3293.3 $\pm$ 115.2	$t = 2.99$	0.007
Manganese <sup>2</sup>	31.8 $\pm$ 1.8	55.1 $\pm$ 1.2	$t = 10.87$	<0.001
Iron <sup>2</sup>	49.9 $\pm$ 1.6	95.9 $\pm$ 2.1	$t = 17.37$	<0.001
Cobalt <sup>3</sup>	23.5 $\pm$ 1.0	17.0 $\pm$ 1.0	$t = 5.81$	<0.001
Chromium <sup>3</sup>	10.9 $\pm$ 0.8	12.0 $\pm$ 0.8	$t = 1.05$	0.304
Copper <sup>3</sup>	78.3 $\pm$ 1.8	80.0 $\pm$ 3.8	$t = 0.41$	0.344
Nickel <sup>3</sup>	9.3 $\pm$ 0.7	7.3 $\pm$ 0.9	$t = 1.74$	0.096
Selenium <sup>3</sup>	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0	$U = 29$	0.014
Zinc <sup>3</sup>	60.6 $\pm$ 0.9	98.2 $\pm$ 2.4	$t = 14.48$	<0.001

<sup>1</sup> Mann-Whitney  $U$  test, or standard  $t$  test. <sup>2</sup> diethylenetriaminepentaacetic acid extractable,  $\mu\text{g}\cdot\text{g}^{-1}$ . <sup>3</sup> nitric acid digestible,  $\mu\text{g}\cdot\text{g}^{-1}$ .

The acid target soils were decreased in potassium (K), Mg, manganese (Mn), iron (Fe), and zinc (Zn) (Table 2). The target soils were increased in pH, calcium (Ca), cobalt (Co), and selenium (Se) when compared with the non-target soils. Lead (Pb) and As were not detected in any of the soils.

Guam's karst soils were changed to a similar degree by the presence of *C. micronesica* trees (height =  $335 \pm 15$  cm), as were Guam's alkaline soils (Figure 4, Table 3). Target soils increased 26% in N ( $t = 5.17$ ,  $p < 0.0001$ ), decreased 24% in C/N ( $t = 2.77$ ,  $p = 0.011$ ), and increased 42% in N/P ( $t = 3.45$ ,  $p = 0.002$ ) when compared with the non-target soils. The remainder of the quantified minerals and metals were unaffected by the presence of *C. micronesica* trees. Cadmium (Cd), As, and Pb were not detected in any of the soils.



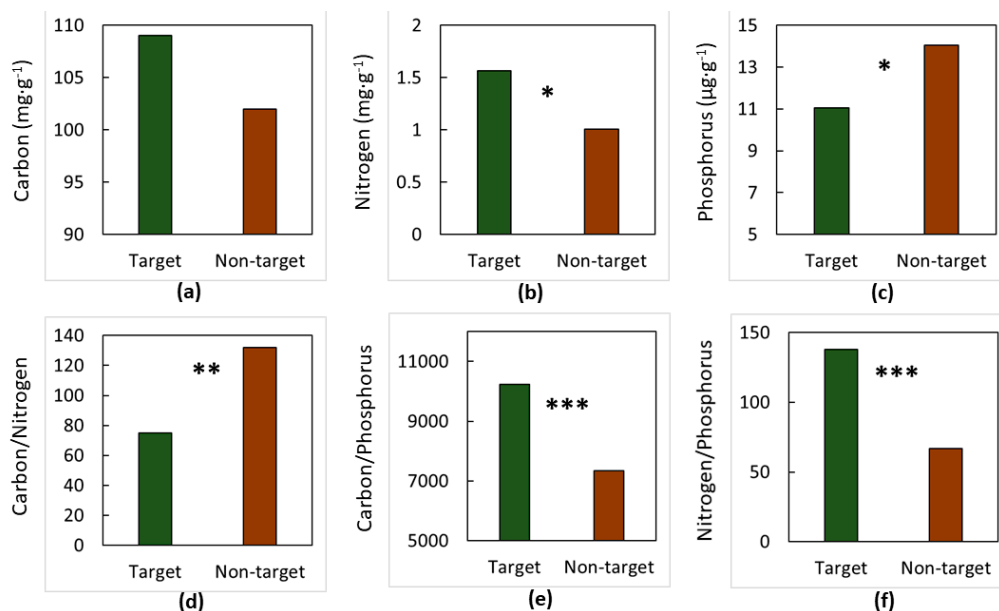
**Figure 4.** The influence of proximity to *Cycas micronesica* on soil chemical traits in Ritidian Rock Outcrop soils on Guam. Target soils were collected underneath cycad trees. Non-target soils were collected 5-m from focal cycad trees. (a) Total carbon; (b) Total nitrogen; (c) Olsen phosphorus; (d) Carbon/nitrogen; (e) Carbon/phosphorus; and, (f) Nitrogen/phosphorus. \*\*, \*\*\* = significant at 0.01 or 0.001.

**Table 3.** The influence of proximity to *Cycas micronesica* trees on soil chemical traits in Ritidian Rock Outcrop soils on Guam. Target soils were collected underneath cycad trees. Non-target soils were collected 5-m from focal cycad trees. Means  $\pm$  SE,  $n = 12$ .

Soil Trait	Target	Non-Target	Statistic <sup>1</sup>	Significance
pH	7.5 $\pm$ 0.1	7.4 $\pm$ 0.1	$t = 0.32$	0.752
Potassium <sup>2</sup>	298.3 $\pm$ 19.8	3551 $\pm$ 28.1	$t = 1.64$	0.114
Calcium <sup>2</sup>	8983.2 $\pm$ 666.3	8632.8 $\pm$ 638.3	$t = 0.38$	0.708
Magnesium <sup>2</sup>	758.7 $\pm$ 31.0	827.2 $\pm$ 43.3	$t = 1.28$	0.212
Manganese <sup>2</sup>	61.5 $\pm$ 6.0	79.7 $\pm$ 9.4	$t = 1.63$	0.118
Iron <sup>2</sup>	46.2 $\pm$ 3.1	54.0 $\pm$ 5.1	$t = 1.33$	0.197
Cadmium <sup>3</sup>	5.8 $\pm$ 0.4	5.7 $\pm$ 0.4	$t = 0.23$	0.821
Cobalt <sup>3</sup>	20.6 $\pm$ 1.5	21.4 $\pm$ 1.5	$t = 0.42$	0.676
Chromium <sup>3</sup>	105.0 $\pm$ 11.6	105.2 $\pm$ 10.7	$t = 0.01$	0.992
Copper <sup>3</sup>	7500 $\pm$ 2.4	81.2 $\pm$ 4.00	$t = 1.33$	0.196
Nickel <sup>3</sup>	19.3 $\pm$ 2.0	18.3 $\pm$ 1.5	$t = 0.41$	0.684
Lead <sup>3</sup>	3.4 $\pm$ 3.4	9.6 $\pm$ 7.6	$U = 72$	0.999
Selenium <sup>3</sup>	0.4 $\pm$ 0.2	0.3 $\pm$ 0.2	$U = 72$	0.976
Zinc <sup>3</sup>	88.9 $\pm$ 6.8	90.7 $\pm$ 5.9	$t = 0.19$	0.850

<sup>1</sup> Mann-Whitney  $U$  test, or standard  $t$  test. <sup>2</sup> diethylenetriaminepentaacetic acid extractable,  $\mu\text{g}\cdot\text{g}^{-1}$ . <sup>3</sup> nitric acid digestible,  $\mu\text{g}\cdot\text{g}^{-1}$ .

The sandy littoral soils from Rota were changed to a greater degree by the presence of *C. micronesica* trees (height =  $345 \pm 15$  cm) than were Guam's alkaline soils (Figure 5, Table 4). Target soils exhibited a 36% increase in N ( $t = 2.43$ ,  $p = 0.024$ ) and a 21% decrease in P ( $t = 2.57$ ,  $p = 0.017$ ) when compared with non-target soils. Target soils were 43% less in C/N ( $t = 3.14$ ,  $p = 0.005$ ), 28% greater in C/P ( $t = 7.12$ ,  $p < 0.0001$ ), and 52% greater in N/P ( $t = 6.30$ ,  $p < 0.001$ ) than the non-target soils. More of the soil metals were influenced by the long-term presence of *C. micronesica* trees than were the minerals. Target soils were greater in cobalt (Co), Cr, and selenium (Se), and lower in iron (Fe), Cd, and Pb than non-target soils. None of the sandy soils contained Copper (Cu) or nickel (Ni).



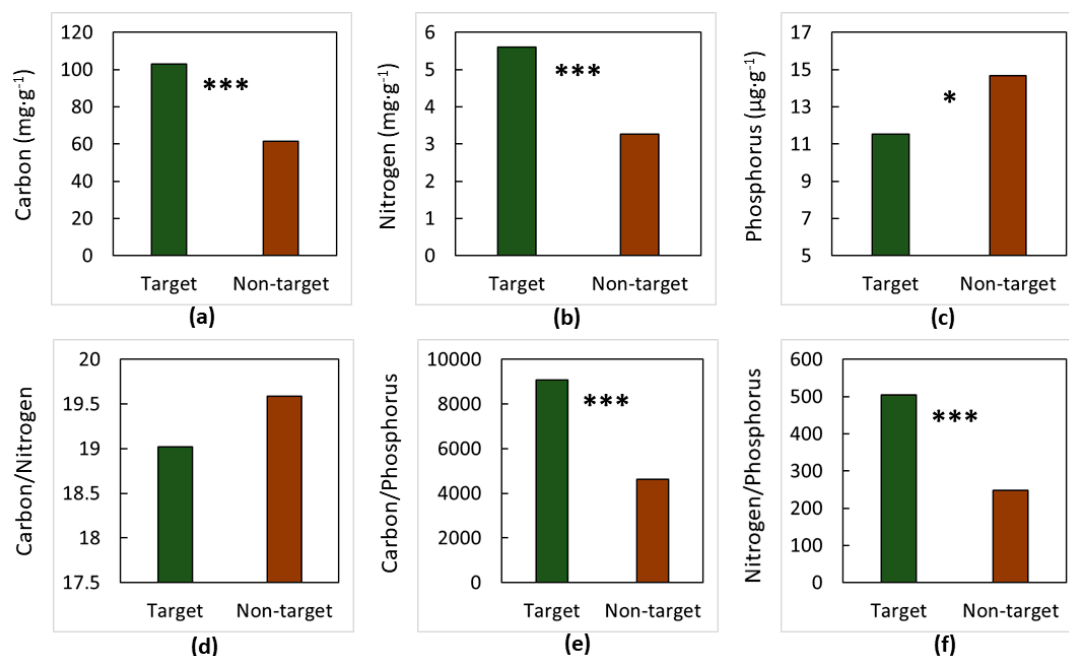
**Figure 5.** The influence of proximity to *Cycas micronesica* on soil chemical traits in Shioya sand soils on Rota. Target soils were collected underneath cycad trees. Non-target soils were collected 5-m from focal cycad trees. (a) Total carbon; (b) Total nitrogen; (c) Olsen phosphorus; (d) Carbon/nitrogen; (e) Carbon/phosphorus; and, (f) Nitrogen/phosphorus. \*, \*\*, \*\*\* = significant at 0.05, 0.01, or 0.001.

**Table 4.** The influence of proximity to *Cycas micronesica* trees on soil chemical traits in Shioya sand soils on Rota. Target soils were collected underneath cycad trees. Non-target soils were collected 5-m from focal cycad trees. Means  $\pm$  SE,  $n = 12$ .

Soil Trait	Target	Non-Target	Statistic <sup>1</sup>	Significance
pH	7.9 $\pm$ 0.0	8.0 $\pm$ 0.0	$t = 2.43$	0.024
Potassium <sup>2</sup>	21.1 $\pm$ 3.1	38.9 $\pm$ 6.0	$t = 2.64$	0.015
Calcium <sup>2</sup>	9525.5 $\pm$ 222.8	9044.4 $\pm$ 235.6	$t = 1.18$	0.253
Magnesium <sup>2</sup>	470.2 $\pm$ 30.9	404.8 $\pm$ 19.1	$t = 1.80$	0.086
Manganese <sup>2</sup>	18.5 $\pm$ 0.8	17.7 $\pm$ 1.3	$t = 1.54$	0.138
Iron <sup>2</sup>	5.7 $\pm$ 0.4	6.9 $\pm$ 0.3	$t = 2.22$	0.037
Arsenic <sup>3</sup>	0.3 $\pm$ 0.0	0.2 $\pm$ 0.1	$t = 1.09$	0.287
Cadmium <sup>3</sup>	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	$t = 4.01$	0.001
Cobalt <sup>3</sup>	0.2 $\pm$ 0.0	0.1 $\pm$ 0.0	$t = 5.60$	<0.001
Copper <sup>3</sup>	0.4 $\pm$ 0.0	0.3 $\pm$ 0.0	$t = 2.41$	0.025
Chromium <sup>3</sup>	3.9 $\pm$ 0.3	2.4 $\pm$ 0.1	$t = 4.73$	<0.001
Lead <sup>3</sup>	0.1 $\pm$ 0.0	0.4 $\pm$ 0.1	$t = 3.72$	0.001
Selenium <sup>3</sup>	3.5 $\pm$ 0.0	3.0 $\pm$ 0.0	$t = 13.82$	<0.001
Zinc <sup>3</sup>	10.6 $\pm$ 1.5	7.5 $\pm$ 1.1	$t = 1.73$	0.097

<sup>1</sup> Standard  $t$  test. <sup>2</sup> diethylenetriaminepentaacetic acid extractable,  $\mu\text{g}\cdot\text{g}^{-1}$ . <sup>3</sup> nitric acid digestible,  $\mu\text{g}\cdot\text{g}^{-1}$ .

Yap's schist soils were changed to a similar degree by the presence of *C. micronesica* trees (height =  $353 \pm 16$  cm) as were Guam's acid soils (Figure 6, Table 5). Target soils exhibited a 40% increase in C ( $t = 7.17$ ,  $p < 0.0001$ ), an 42% increase in N ( $t = 4.63$ ,  $p < 0.0001$ ), and a 21% decrease in P ( $t = 4.41$ ,  $p < 0.001$ ) when compared with the non-target soils. Target soils were 49% greater in C/P ( $t = 5.68$ ,  $p < 0.0001$ ), and 51% greater in N/P ( $t = 4.48$ ,  $p < 0.001$ ) than the non-target soils. Target soils were greater in Ca, Mg, Mn, and Cu; and, lower in K and Co non-target soils. None of the sandy soils contained As, Cd, or Pb.



**Figure 6.** The influence of proximity to *Cycas micronesica* on soil chemical traits in schist soils on Yap. Target soils were collected underneath cycad trees. Non-target soils were collected 5-m from focal cycad trees. (a) Total carbon; (b) Total nitrogen; (c) Modified Truog phosphorus; (d) Carbon/nitrogen; (e) Carbon/phosphorus; and, (f) Nitrogen/phosphorus. \*, \*\*, \*\*\* = significant at 0.05, 0.01, or 0.001.



**Table 5.** The influence of proximity to *Cycas micronesica* trees on soil chemical traits in schist soils on Yap. Target soils were collected underneath cycad trees. Non-target soils were collected 5-m from focal cycad trees. Means  $\pm$  SE,  $n = 12$ .

Soil Trait	Target	Non-Target	Statistic <sup>1</sup>	Significance
pH	5.9 $\pm$ 0.2	5.7 $\pm$ 0.1	$t = 2.03$	0.055
Potassium <sup>2</sup>	91.3 $\pm$ 10.0	120.5 $\pm$ 22.4	$t = 2.91$	0.043
Calcium <sup>2</sup>	2012.5 $\pm$ 417.5	1212.2 $\pm$ 174.2	$t = 4.33$	<0.001
Magnesium <sup>2</sup>	1031.0 $\pm$ 214.1	1367.1 $\pm$ 203.1	$t = 2.28$	0.033
Manganese <sup>2</sup>	11.7 $\pm$ 2.3	14.2 $\pm$ 2.8	$t = 2.79$	0.011
Iron <sup>2</sup>	329.2 $\pm$ 72.4	359.7 $\pm$ 41.8	$t = 0.89$	0.381
Cobalt <sup>3</sup>	31.5 $\pm$ 3.0	26.2 $\pm$ 2.2	$t = 3.41$	0.003
Chromium <sup>3</sup>	521.6 $\pm$ 28.3	545.8 $\pm$ 75.5	$t = 0.74$	0.469
Copper <sup>3</sup>	38.2 $\pm$ 4.5	49.8 $\pm$ 6.9	$t = 3.44$	0.002
Nickel <sup>3</sup>	231.1 $\pm$ 14.6	244.5 $\pm$ 28.9	$t = 1.02$	0.321
Selenium <sup>3</sup>	0.5 $\pm$ 0.3	0.5 $\pm$ 0.5	$U = 60$	0.509
Zinc <sup>3</sup>	43.8 $\pm$ 12.1	41.7 $\pm$ 6.2	$t = 0.37$	0.713

<sup>1</sup> Mann-Whitney  $U$  test, or standard  $t$  test. <sup>2</sup> diethylenetriaminepentaacetic acid extractable,  $\mu\text{g}\cdot\text{g}^{-1}$ . <sup>3</sup> nitric acid digestible,  $\mu\text{g}\cdot\text{g}^{-1}$ .

## 4. Discussion

### 4.1. The Soils

The variation in non-target soil chemistry among the five habitats was substantial. The ability of *C. micronesica* to establish high density populations in such a range of soil types is evidence of the adaptability of the species. The two acid soils behaved similarly with regard to the influence of *C. micronesica* trees, exhibiting significant increases of N, C, and Ca in target soils and significant decreases of P, K, and Mg in the target soils. The three alkaline soils were more heterogeneous with regard to the influence of *C. micronesica* trees on soil chemistry, as N was the only element that behaved similarly among the soils. Yap soils exhibited the lowest pH, the lowest macronutrient concentrations, and greatest metal concentrations. These conditions enabled the *C. micronesica* trees to increase N in target soils to a greater degree than in any of the other soils. Interestingly, the impoverished sandy soils with a mean total metal concentration of only 19  $\mu\text{g}\cdot\text{g}^{-1}$  exhibited differences between the target and non-target soils for more than half of the metals, but the other soils with total metal content as high as 1273  $\mu\text{g}\cdot\text{g}^{-1}$  exhibited less influence of *C. micronesica* tree on the concentration of individual metals. The influence of legume tree species on soil chemistry has also been shown to substantially vary among soil types [20].

### 4.2. The Elements

An increase in N occurred in all target soils, and N was the only element that exhibited a consistent significant change among all of the habitats. The direction of change caused by a *C. micronesica* tree was homogeneous among the soils for every other element, but the level of significance was inconsistent among the soils. For example, C increased in every target soil, but it was not significant in the three alkaline soils. Similarly, P decreased in every target soil, but it was not significant in the karst soils.

Carbon and N were the only elements that the *C. micronesica* trees entirely or partially derived from non-soil outside sources (the atmosphere), so root or leaf litter decomposition released C and N into the soil community that was not entirely recycled. The remainder of the minerals and metals were initially extracted from the local soil pools, so root or leaf litter decomposition re-released these elements as recycled soil contributions. The target soils were always higher in C and N, but were lower in P than the non-target soils. These traits generated similar changes in C/P and N/P among the five soils, but heterogeneous changes in C/N depending on which of the two elements was increased the most by the *C. micronesica* trees.

Carbon accumulations adjacent to the *C. micronesica* trees may have been due to recalcitrant senesced roots and leaves in comparison to decomposing organs from the metacommunity. The diversity and volume of production of bio-platform molecules is taxon-specific, and which of those molecules journey from within the plant body to released senesced tissues is also partially under genetic control. These plant-derived molecules are often complex and therefore recalcitrant towards deconstruction. Quantifying every fraction of the standing soil C pool would increase our understanding of how a cycad tree is able to increase C within close proximity to the unique trees.

The N relations of these soils may be more fully understood by calculating the relative net nitrification ( $RNN = (\text{net nitrification})/(\text{net total N mineralization}) \times 100$ ), where net total N mineralization is the sum of ammonification and nitrification. RNN was 95% for the target sites and 82% for the non-target sites. Clearly, the nitrifying genera *Nitrosomonas* and *Nitrobacter* [21] were more active in the target sites and less active in the non-target sites. As with the C relations, more quantifications of every fraction of the N pool, such as organic N, would increase our understanding of how the N heterogeneity that we have reported is sustained within cycad habitats.

#### 4.3. Informative Sub-Disciplines Literature

The underlying mechanisms that enabled the changes in soils following long-term direct contact with a *C. micronesica* tree were not determined. The literature contains abundant relevant informative examples within the following three areas of research. This literature may provide appropriate methods for future experimental approaches to tease apart the plant behaviors that lead to the soil change that we have documented.

##### 4.3.1. Fertile Islands

The literature on fertile islands has focused on arid and semi-arid environments where a woody plant becomes established in grassland or barren habitats. These shrubs then begin the process of creating “islands of fertility” directly beneath their canopies [22–24]. Drivers of this spatial change in soil fertility include microsite accumulation of high quality litter, nutrient inputs from enhanced animal visitations, nutrients concentrated in stemflow, unique vertical and horizontal root contributions, and increases in the mycorrhizal activities.

##### 4.3.2. Plant-Soil Feedback

Plant-soil feedback is one of the mechanisms that mediates ecological succession and biodiversity coexistence. The abiotic and biotic traits of soils are changed by plants, and those changes can influence subsequent plant growth [25,26]. Plant-soil feedback is usually studied in the context of priming the soils by first growing one plant species, and then monitoring conspecific or heterospecific subsequent growth in the same soils. The direction of the feedback can be negative, where nutrients are immobilized or depleted or pathogens accumulate; or, can be positive, where nutrients or mutualists accumulate [27]. “Soil legacy effects” are commonly studied in the context of invasive plant species that possess the power to engineer changes in ecosystem of the invaded habitats [28]. This subset of the plant-soil feedback agenda is often invoked for discussing allelopathic properties that remain in soils following invasive plant species growth. For example, soils that persist following the widespread mortality of *C. micronesica* due to long-term *A. yasumatsui* damage often remain barren due to unidentified residuals [29,30].

##### 4.3.3. Home-Field Advantage

The home-field advantage hypothesis predicts that plant litter decomposes more rapidly within the vicinity of the source plant than in some distant location, because decomposers that specialize in the source litter are in greater quantity close to the source plant [31]. This heterogeneity in specialist decomposer community can have cascading effects on the heterogeneity of biogeochemical traits in the soil layer.

#### 4.4. Synthesis

A large literature has accumulated on each of these sub-disciplines, and the direct influence of the plant's presence on the microsite soil is a common thread. Controlled experimental approaches are common, but in-situ observational studies in mixed forest stands are also represented [32]. Our objectives did not include approaches to identify the mechanisms that *C. micronesica* trees employ to exert local changes in the soil traits. However, these three subsets of the ecology and restoration literature provide ample examples of experimental approaches that could be used to more fully understand how cycad plants modify their habitats. Regardless of the causal mechanisms, our results were robust and some trait changes were consistent among the many habitat and soil types. Given the considerable changes in C:N:P and other soil traits, an in-situ *C. micronesica* tree creates the conditions to increase  $\alpha$ -diversity of soil microbiota [33]. The influence of *C. micronesica* trees on the heterogeneity of soil nitrogen is of particular interest, as nitrogen availability directly affects growth of primary producers in a landscape then exerts cascading effects on food web functioning among many trophic levels [34]. Moreover, we have shown that the selective removal of this foundation species from Guam and Rota habitats by the *A. yasumatsui* invasion has the potential to homogenize the degraded island communities and erode soil biodiversity.

Shortly after we conducted the field work in Guam and Rota, each habitat was invaded by *A. yasumatsui*. More than 90% of the resident *C. micronesica* population has been subsequently killed by the invasions (unpublished data), thereby removing this important biological genesis of community heterogeneity. The community responses will likely homogenize soil chemical traits and reduce soil biodiversity. This study provides crucial quantitative baseline information [35,36] that may be useful for future monitoring to determine localized biodiversity erosion in the medium term and ecological restoration attempts in the long term. Continued manipulative studies that are designed to more fully understand how *C. micronesica* trees behave in plant-soil feedbacks are no longer possible on Guam or Rota. However, the in-situ populations in Yap and Palau remain unthreatened, and these habitats remain available for continued research. Our information derived prior to the onset of the contemporary biological threats in Guam and Rota provides benchmark data that may enable quantifying ecological restoration goals in the future.

#### 4.5. Conservation

Few reports have included green leaf tissue nutrient levels of cycad species for the purpose of increasing our understanding of cycad biology and conservation. In order to be informative, the methods of studies with these objectives need to be unambiguous and repeatable. The ionome and resulting biochemistry of sessile perennial plants is under the control of the immediate environment. The absolute levels of essential and non-essential elements and the stoichiometry among these elements within soils can affect the corresponding plant tissue contents [37,38]. The reports that include green leaf element contents of cycad species have not adequately quantified these consequential local soil traits to carry out the methods appropriately. For example, most of the papers do not include any mention of the corresponding soil nutritional status [39–42]. Other papers include generic descriptions of the soil characteristics without collecting specific experimental soil samples to improve the accuracy of the methods [43]. Other papers include soil samples from the experimental sites, but they do not indicate proximity of the samples to the studied cycad plants [44]. We are aware of only two reports that included dedicated soil sampling with descriptions of proximity to the cycad plants that were being studied for green leaf nutrient content [45,46]. Continued research within this agenda must include descriptions of influential soil chemistry, and our results indicate the sampling sites must be constrained within close proximity to the studied cycad plants to enable the legitimacy of the methods.

## 5. Conclusions

The long-lived *C. micronesica* tree is able to modify the elemental profile of the soils within the drip line of its leaf canopy. The potential causal mechanisms that a *C. micronesica* tree employs to modify the soils in direct contact with it were not analyzed in this study. The results, however, do reveal that loss of these localized habitat modifications by the recent invasion of *A. yasumatsui*, which has killed most of the *C. micronesica* tree populations on Rota and Guam, will decrease soil chemical heterogeneity at the community level. The spatial changes in soil C:N:P and other chemical traits will then influence  $\alpha$ -diversity of soil microbiota and the ecological processes that these organisms modulate. This is the first report which indicates a healthy cycad tree increases soil community heterogeneity through modifications on localized soil mineral and metal concentrations. More case studies are needed to determine whether this is a common cycad trait or is variable among the other cycad species. Cycads are the most threatened plant group worldwide [47–49], and the consequences of continuing losses of cycads throughout the globe are magnified by the collateral loss of community heterogeneity. This case study also provides an example of how invasions of islands can damage ecological processes by removing the services of unique native species.

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