Therefore, the observations in Tinian reveal a need to persistently pursue new evidence-based outcomes. An adaptive management approach calls for action in the absence of relevant publications to inform decision. Total carbon, total nitrogen, calcium, and net ammonification were greater in native forest cover than in L. leucocephala patches. Net nitrification and net mineralization were greater under L. leucocephala cover. Trace metals also differed between the 2 forest cover types, with chromium, cobalt, and nickel accumulating to greater concentration under L. leucocephala cover and zinc accumulating to greater concentration under native forest cover. The results indicated that L. leucocephala cover generated substantial changes in soil chemical properties when compared with native forest tree cover, illuminating one means by which understory vegetation may be affected by changes in invasive tree cover.

**ABSTRACT**

An ex situ germplasm collection of the endangered *Cycas micronesica* was established in a transition zone between biodiverse native forest and mature stands of the invasive species *Leucaena leucocephala*. Soil chemical properties were determined for the 2 tree cover types to inform management decisions. Total carbon, total nitrogen, calcium, and net ammonification were greater in native forest cover than in *L. leucocephala* patches. Net nitrification and net mineralization were greater under *L. leucocephala* cover. Trace metals also differed between the 2 forest cover types, with chromium, cobalt, and nickel accumulating to greater concentration under *L. leucocephala* cover and zinc accumulating to greater concentration under native forest cover. The results indicated that *L. leucocephala* cover generated substantial changes in soil chemical properties when compared with native forest tree cover, illuminating one means by which understory vegetation may be affected by changes in invasive tree cover.

**Introduction**

*Cycas micronesica* transitioned from the most abundant tree on the island of Guam in 2002 to Endangered status under IUCN and Threatened status under the United States Endangered Species Act mainly as a result of exotic phytophagous insects that invaded the island. Plant mortality following the invasions was epidemic, and population size and structure dramatically changed. Ex situ and in situ conservation programs were subsequently initiated, including a living collection of Guam germplasm established on the island of Tinian and funded by the United States Department of the Navy. The germplasm collection was situated in a transition zone with a portion of the plants established beneath a diverse native forest canopy, and a portion established beneath adjacent mature stands of non-native *Leucaena leucocephala*. Growth and health of the *C. micronesica* plants have been highly variable (see Fig. 2 in ref. 8), with larger plants under the *L. leucocephala* cover than under native tree cover.

Conservation and restoration projects are often initiated in the absence of relevant publications to inform decisions. An adaptive management approach calls for persistent pursuit of new evidence-based outcomes. Therefore, the observations in Tinian reveal a need to identify which factors may be mediating the disparity in *C. micronesica* plant performance. Invasive plant species often exert profound changes in the habitats they invade. The literature on this subject reveals a bias toward several well-known invasive plant species, which limits a comprehensive evaluation of how invasive plants fit into global change issues. *Leucaena leucocephala* has been extensively exploited in agroforestry settings as a source of nitrogen-rich green mulch to improve soil quality for cash crops. Nitrogen inputs, soil nitrification, and soil ammonification are among the components of the nitrogen cycle that may be influenced by *L. leucocephala*. Therefore, one potential factor that could influence understory *C. micronesica* plant growth is the difference in nutrient relations of the soils beneath the native forest cover versus the *L. leucocephala* patches.

Long-standing patches of vegetation contribute to system spatial heterogeneity through chronic influences on biogeochemical cycling and through interactions with microbial communities associated with the vegetation. These plant-soil feedbacks are orchestrated by complex integrated relationships among many biotic and abiotic factors. Influences of tree genotype on these processes include prolonged extraction and sequestration of soil elements in plant organs, extent of element resorption...
prior to leaf senescence then abscission, litter quality effects on organic matter lability, and local amplification of root-associated microorganisms and specialist saprophytic microorganisms. These phenomena are one means by which invasive plant species can affect native plants through changes in soil properties.\textsuperscript{16,17}

Our objective was to use paired sampling sites throughout the \textit{C. micronesica} germplasm to determine how forest tree cover type influenced soil chemical traits and nitrogen mineralization dynamics. The results may improve management decisions in our ex situ conservation program. Moreover, the information will increase knowledge of broader topics of tropical invasive species management, rare plant conservation, and integrative biological influences on soil chemical properties.

**Results**

Total carbon concentration (Table 1) and total nitrogen concentration (Fig. 1) were less in soils from \textit{L. leucocephala} sites than soils from native forest sites. The quotients carbon:nitrogen did not differ between the 2 tree cover types. The pH of soils beneath \textit{L. leucocephala} tree cover was slightly less than that of soils from native tree cover (Table 1). From highest to lowest, the concentrations of extractable macronutrients in the soils from both tree cover types were in the following order: calcium > magnesium > potassium > phosphorus (Table 1). Calcium concentration was greater under diverse native tree cover than under \textit{L. leucocephala} cover, but the other nutrients were not influenced by tree cover type (Table 1).

The differences in various components of nitrogen cycling were highly contrasting between the 2 forest tree cover types. Net nitrification rate of soils beneath \textit{L. leucocephala} cover was 4.25-fold greater than soils beneath native tree cover (Fig. 1). Net ammonification of soils was positive beneath native tree cover, but negative beneath \textit{L. leucocephala} tree cover (Fig. 1). These results caused the soils from \textit{L. leucocephala} microsites to exhibit net mineralization that was 156% greater than that from the diverse native forest microsites (Fig. 1). Available nitrate, available ammonium, and total available nitrogen did not differ between the 2 forest tree cover types (Table 1, Fig. 1).

From highest to lowest, the concentrations of measured metals in the soils were in the following order: zinc > copper > chromium > cobalt > nickel > cadmium > selenium > lead (Table 2). Chromium, cobalt, and nickel were greater in the \textit{L. leucocephala} soils than in the native tree soils. Selenium and zinc were greater in the native tree soils than in the \textit{L. leucocephala} soils. Cadmium, copper, and lead were not influenced by tree cover type.

**Discussion**

Insular habitats including islands may be more susceptible to plant invasions than continental habitats.\textsuperscript{18} Yet the Pacific islands have been under-represented in publications on invasive plant species.\textsuperscript{11} We have addressed this bias with a look at a widespread invasive woody tree species in a small oceanic island habitat.

This diagnosis of the integrative changes in soil chemical properties caused by persistent \textit{L. leucocephala} tree cover is the first empirical look at how this invasive tree affects ecosystems in the Mariana Islands. We have shown that a high proportion of soil nutrients and metals were substantially changed by the \textit{L. leucocephala} tree cover. Various components of the nitrogen cycle were among the soil properties that were most affected by tree cover type. That total nitrogen was greater under native tree cover may seem counterintuitive, as \textit{L. leucocephala} is a well-known legume, its \textit{Rhizobium} endosymbionts are prevalent in the calcareous soils of the Mariana Islands,\textsuperscript{19} and none of the prevalent native tree species at our site associate with nitrogen-fixing symbionts. Indeed, habitats dominated by legume species tend to exhibit greater soil nitrogen.\textsuperscript{20} These interesting relationships were reconciled by the acute differences in nitrification and ammonification. Net mineralization rates in the \textit{L. leucocephala} microsites were 256% of those of native forest microsites. Moreover, net nitrification of the \textit{L. leucocephala} soils exceeded net mineralization, but net nitrification of the native forest soils was only 87% of net mineralization. Clearly, nitrogen-fixing bacteria genera \textit{Nitrosomonas} and \textit{Nitrobacter} were highly active under \textit{L. leucocephala} cover and less active under native tree cover.

We propose that the prodigious nitrification in soils beneath \textit{L. leucocephala} patches causes excessive nitrogen losses from the system through leaching since nitrate

\begin{table}[h]
\centering
\caption{Soil properties as influenced by \textit{Leucaena leucocephala} tree cover vs. native forest cover on Tinian Island. Mean $\pm$ SE, $n = 10$. $D = \text{Kolmogorov-Smirnov statistic}$.}
\begin{tabular}{lccc}
\hline
Trait & Native tree cover & \textit{Leucaena} tree cover & $D$ & $P$ \\
\hline
pH & 7.44 $\pm$ 0.06 & 7.15 $\pm$ 0.07 & 0.600 & 0.031 \\
Total Carbon (mg$\cdot$g$^{-1}$) & 130.4 $\pm$ 3.5 & 73.7 $\pm$ 7.4 & 0.900 & 0.001 \\
Carbon/Nitrogen & 11.1 $\pm$ 0.3 & 10.5 $\pm$ 0.3 & 0.300 & 0.675 \\
Phosphorus (mg$\cdot$g$^{-1}$) & 37.7 $\pm$ 6.3 & 49.7 $\pm$ 4.7 & 0.400 & 0.313 \\
Potassium (mg$\cdot$g$^{-1}$) & 130.1 $\pm$ 19.8 & 100.0 $\pm$ 8.4 & 0.400 & 0.313 \\
Magnesium (mg$\cdot$g$^{-1}$) & 218.1 $\pm$ 25.2 & 237.6 $\pm$ 15.2 & 0.300 & 0.675 \\
Calcium (mg$\cdot$g$^{-1}$) & 4957 $\pm$ 374 & 3316 $\pm$ 281 & 0.700 & 0.007 \\
Available nitrogen (mg$\cdot$g$^{-1}$) & 102.2 $\pm$ 14.9 & 107.6 $\pm$ 15.2 & 0.300 & 0.675 \\
\hline
\end{tabular}
\end{table}
is highly mobile\textsuperscript{21} In contrast, the limited nitrification of the native forest microsites protects those soils from similar losses. Annual precipitation in Tinian is 204 cm (www.wunderground.com), evincing considerable leaching potential. As a result, the soil nitrogen pool is labile in the \textit{L. leucocephala} patches and recalcitrant in the native forest patches.

Biodiversity is critical for sustaining many components of ecosystem services and maintaining forest productivity.\textsuperscript{22} Indeed, species mixtures and high plant biodiversity may increase nitrogen retention, reduce nitrogen losses, and decrease the potential for groundwater contamination due to nitrogen leaching.\textsuperscript{23,24} Therefore, our native forest may have exhibited greater

\textbf{Figure 1.} Available nitrate, available ammonium, net nitrification rate, net ammonification rate, net mineralization rate, and total nitrogen of soils as influenced by \textit{Leucaena leucocephala} tree cover versus biodiverse native tree cover. \(D = \) Kolmogorov-Smirnov statistic. Mean + SE, \(n = 10\).
retention of nitrogen simply because it had greater biodiversity than the *Leucaena leucocephala* microsites, which were monospecific.

Two issues from our study are relevant for climate change research. First, climate change is predicted to increase nitrate leaching as extreme events increase in frequency. Therefore, in a climate change scenario nitrate leaching in *L. leucocephala* sites could be aggrovated. Second, soil organic matter is the largest terrestrial carbon pool, and soil carbon and nitrogen mineralization are closely coupled. Our results indicate that monospecific *L. leucocephala* sites act as less effective carbon sinks than native forest sites.

Ecosystem changes caused by invasive species are some of the complex consequences of anthropogenic changes to the global environment. A full understanding of how to manage invasive species cannot develop in the absence of an all-inclusive viewpoint founded in empirical studies. Meta-analyses have shown that net primary production, litter decomposition, and altered nitrogen cycle are some of the most common ways that successful invasive plants modify nutrient cycling. Bardon et al. recently reported that root exudates of a successful invasive plant reduced metabolic activity of 2 denitrifying bacteria species, adding a previously unknown means by which invasive plants may directly influence the nitrogen cycle.

The differences in soil chemical components we have reported from *L. leucocephala* vs. diverse native tree forest cover may influence understory vegetation growth and health. In our experimental site, the main understory species of interest was the Guam-sourced *C. micronesica* germplasm that we introduced and managed. *Cycas micronesica* is one of more than 350 species of extant cycads. This plant group is among the most threatened groups of plants worldwide. Developing successful management strategies for cycad conservation is an urgent agenda. An ongoing refinement of our *C. micronesica* conservation program based on national threatened and international endangered listings fits into that international agenda. Although we have shown the soils in *L. leucocephala* microsites exhibited substantial differences from soils in biodiverse native forest microsites, the magnitude and direction of differences in macronutrients (Table 1) and metals (Table 2) were not likely to explain why *C. micronesica* plants have grown better as understory plants in the *L. leucocephala* microsites. Furthermore, the increased net nitrification and net mineralization of soils in *L. leucocephala* microsites are not likely to substantially benefit the understory *C. micronesica* plants, as available nitrogen did not differ between the soils from the 2 forest cover types. Additionally, all cycad plants associate with nitrogen-fixing cyanobacteria endosymbionts, therefore cycads may not be affected by ecologically relevant differences in nitrogen among various soils.

Several critical research needs are illuminated by this study. A greater understanding of litterfall quantity, seasonality, and quality may reveal some of the factors that mediate the differences in soil traits between the native forest cover and *L. leucocephala* cover. Reciprocal litter incubation studies would tease apart the influences of litter quality and soil microbes that influence decomposition speed. Which soil microbes are influential players in the changes that *L. leucocephala* imposes on soils may be identified by DNA sequencing of bulk soils or rhizosphere, and would greatly improve mechanistic insight. *Cycas micronesica* plant growth has been greater under *L. leucocephala* cover than under native tree cover in our ex situ germplasm, yet soil nutrition does not appear to mediate this response. Site differences in incident light, relative humidity, and temperature may be more important factors than the soil properties for explaining the disparity in growth and health of the *C. micronesica* germplasm.

Many Guam and Tinian habitats that have experienced past disturbance are characterized by *L. leucocephala* cover. Once established, this species effectively monopolizes emergent canopy cover. Future large-scale restoration plans for Guam and Tinian include landscape-scale efforts to remove invasive, non-native plant species. Our results indicate that forest restoration goals may require many years to achieve following the invasive tree removal, considering the transformed soil properties that need to be restored.

### Materials and methods

The experimental site was located on the island of Tinian in karstic outcrop soils (Loamy-skeletal, carbonatic, iso-hyperthermic Lithic Haplustolls). A robust *C. micronesica* planting is being maintained along a north-south oriented ecotone between diverse native tree cover and an adjacent belt of *L. leucocephala* cover. The soil samples were obtained within this ex situ germplasm. The *L. leucocephala* cover. The soil samples were obtained within this ex situ germplasm. The *L.

### Table 2. Metal content of soils in Tinian Island as influenced by *Leucaena leucocephala* tree cover versus native forest cover. Mean ± SE, n = 10. D = Kolmogorov-Smirnov statistic.

| Property (µg g⁻¹) | Native cover | Leucaena cover | D     | P      |
|-------------------|--------------|----------------|-------|--------|
| Cadmium           | 2.97 ± 0.21  | 3.14 ± 0.22    | 0.200 | 0.975  |
| Chromium          | 32.32 ± 1.83 | 53.21 ± 1.21   | 1.000 | 0.001  |
| Cobalt            | 9.39 ± 0.64  | 21.61 ± 2.39   | 1.000 | 0.001  |
| Copper            | 49.20 ± 7.08 | 50.81 ± 11.68  | 0.500 | 0.111  |
| Lead              | 0.012 ± 0.004| 0.015 ± 0.002  | 0.300 | 0.675  |
| Nickel            | 3.52 ± 0.28  | 12.91 ± 0.88   | 1.000 | 0.001  |
| Selenium          | 1.43 ± 0.04  | 1.27 ± 0.03    | 0.600 | 0.031  |
| Zinc              | 95.73 ± 3.85 | 77.33 ± 2.78   | 1.000 | 0.001  |
leucocephala sites were primarily mono-specific for the emergent canopy cover, but contained a diverse understory vegetation palette. The native forest sites were highly diverse, and dominant tree species were Pisonia grandis, Psychotria mariana, Aglaia mariannensis, Cynometra ramiflora, and Eugenia palumbis.

We collected paired soil samples from 10 locations along the ecotone on 10 Sept 2014. Each sample from the L. leucocephala cover was located 25-35 m east of its paired sample from the native forest cover. The soil cores were collected from the A horizon from 0-10 cm. Rainfall for the 2 weeks prior to the soil harvests averaged 9.54 mm d⁻¹ (www.wunderground.com).

Analyses
A portion of each sample was dried at 50°C then total carbon and nitrogen were determined by dry combustion (Nelson and Sommers); extractable P, K, Mg, and Ca were determined by Mehlich-3 digestion (Mehlich 1984); and total metals were determined by nitric acid digestion. Nitrate and ammonium were determined colorimetrically from fresh moist soil samples following 2M KCl extraction. Soil was incubated using the buried bag method in a homogeneous site at 28°C (range 25°C – 31°C) soil temperature for 32 d. Nitrate and ammonium were determined at the end of the incubation period. Net nitrification rate was calculated by subtracting initial from final nitrate concentration and dividing by the incubation period. Net ammonification rate was calculated by subtracting initial from final ammonium concentration and dividing by the incubation period. Net mineralization was calculated as the sum of nitrification and ammonification.

The data did not meet requirements for parametric analysis, primarily because of unequal variances. We used the non-parametric and distribution-free Kolmogorov-Smirnov 2-sample test to test the null hypothesis that the 2 groups of soil samples were not different in each of the chemical properties that were quantified. This test does not require any assumption about the distribution of data. Levels of significance of at least P < 0.05 were considered significant.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

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