

SHORT COMMUNICATION

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## *Leucaena leucocephala* and adjacent native limestone forest habitats contrast in soil properties on Tinian Island

Thomas E. Marler, Nirmala Dongol, and Gil N. Cruz

Western Pacific Tropical Research Center, University of Guam, UOG Station, Mangilao, Guam, USA

### 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.

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### Introduction

*Cycas micronesica* transitioned from the most abundant tree on the island of Guam in 2002<sup>1</sup> to Endangered status under IUCN<sup>2</sup> and Threatened status under the United States Endangered Species Act<sup>3</sup> mainly as a result of exotic phytophagous insects that invaded the island.<sup>4,5</sup> Plant mortality following the invasions was epidemic, and population size and structure dramatically changed.<sup>6</sup> 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.<sup>7</sup> 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.<sup>9,10</sup> 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.<sup>11</sup> *Leucaena leucocephala* has been extensively exploited in agroforestry settings as a source of nitrogen-rich green mulch to improve soil quality for cash crops.<sup>12</sup> Nitrogen inputs, soil nitrification, and soil ammonification are among the components of the nitrogen cycle<sup>13</sup> 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.<sup>14</sup> These plant-soil feedbacks are orchestrated by complex integrated relationships among many biotic and abiotic factors.<sup>15</sup> 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.<sup>16,17</sup>

Our objective was to use paired sampling sites throughout the *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 *L. leucocephala* sites than soils from native forest sites. The quotient carbon:nitrogen did not differ between the 2 tree cover types. The pH of soils beneath *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 *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 *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 *L. leucocephala* tree cover (Fig. 1). These results

**Table 1.** Soil properties as influenced by *Leucaena leucocephala* tree cover vs. native forest cover on Tinian Island. Mean + SE, n = 10. D = Kolmogorov-Smirnov statistic.

Trait	Native tree cover	<i>Leucaena</i> tree cover	D	P
pH	7.44 ± 0.06	7.15 ± 0.07	0.600	0.031
Total Carbon (mg·g <sup>-1</sup> )	130.4 ± 3.5	73.7 ± 7.4	0.900	0.001
Carbon/Nitrogen	11.1 ± 0.3	10.5 ± 0.3	0.300	0.675
Phosphorus (μg·g <sup>-1</sup> )	37.7 ± 6.3	49.7 ± 4.7	0.400	0.313
Potassium (μg·g <sup>-1</sup> )	130.1 ± 19.8	100.0 ± 8.4	0.400	0.313
Magnesium (μg·g <sup>-1</sup> )	218.1 ± 25.2	237.6 ± 15.2	0.300	0.675
Calcium (μg·g <sup>-1</sup> )	4957 ± 374	3316 ± 281	0.700	0.007
Available nitrogen (μg·g <sup>-1</sup> )	102.2 ± 14.9	107.6 ± 15.2	0.300	0.675

caused the soils from *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).

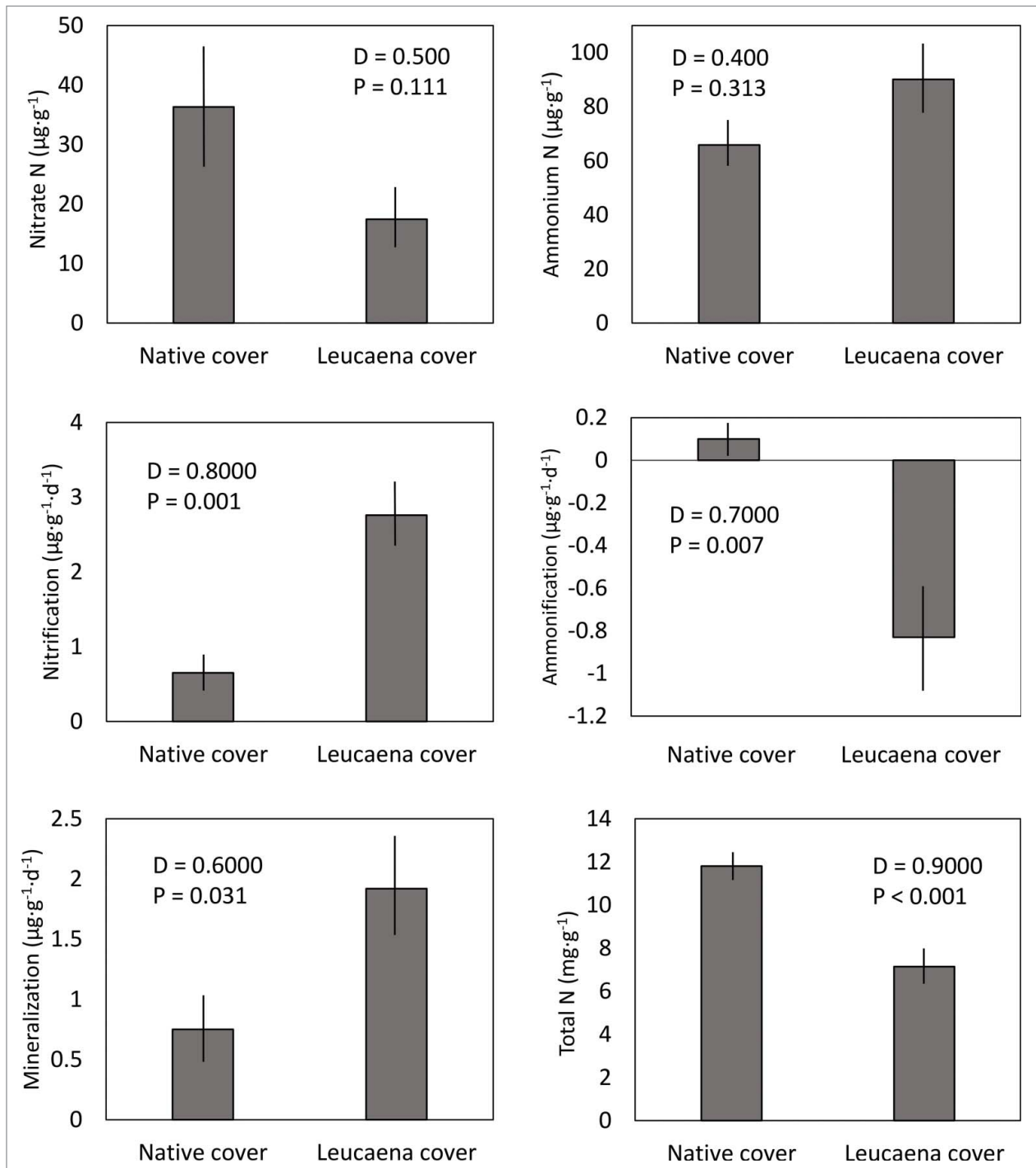
From highest to lowest, the concentrations of measured metals in the soils were in the following order: zinc > copper > chromium > cobalt > nickel > cadmium > selenium > lead (Table 2). Chromium, cobalt, and nickel were greater in the *L. leucocephala* soils than in the native tree soils. Selenium and zinc were greater in the native tree soils than in the *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.<sup>18</sup> Yet the Pacific islands have been under-represented in publications on invasive plant species.<sup>11</sup> 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 *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 *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 *L. leucocephala* is a well-known legume, its *Rhizobium* endosymbionts are prevalent in the calcareous soils of the Mariana Islands,<sup>19</sup> 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.<sup>20</sup> These interesting relationships were reconciled by the acute differences in nitrification and ammonification. Net mineralization rates in the *L. leucocephala* microsites were 256% of those of native forest microsites. Moreover, net nitrification of the *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 *Nitrosomonas* and *Nitrobacter* were highly active under *L. leucocephala* cover and less active under native tree cover.

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



**Figure 1.** Available nitrate, available ammonium, net nitrification rate, net ammonification rate, net mineralization rate, and total nitrogen of soils as influenced by *Leucaena leucocephala* tree cover versus biodiverse native tree cover. D = Kolmogorov-Smirnov statistic. Mean + SE, n = 10.

is highly mobile.<sup>21</sup> 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](http://www.wunderground.com)), evincing considerable leaching potential. As a result, the soil nitrogen pool is labile in the *L. leucocephala* patches and recalcitrant in the native forest patches.

Biodiversity is critical for sustaining many components of ecosystem services and maintaining forest productivity.<sup>22</sup> 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.<sup>23,24</sup> Therefore, our native forest may have exhibited greater

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

Property ( $\mu\text{g}\cdot\text{g}^{-1}$ )	Native cover	<i>Leucaena</i> cover	D	P
Cadmium	2.97 $\pm$ 0.21	3.14 $\pm$ 0.22	0.200	0.975
Chromium	32.32 $\pm$ 1.83	53.21 $\pm$ 1.21	1.000	0.001
Cobalt	9.39 $\pm$ 0.64	21.61 $\pm$ 2.39	1.000	0.001
Copper	49.20 $\pm$ 7.08	50.81 $\pm$ 11.68	0.500	0.111
Lead	0.012 $\pm$ 0.004	0.015 $\pm$ 0.002	0.300	0.675
Nickel	3.52 $\pm$ 0.28	12.91 $\pm$ 0.88	1.000	0.001
Selenium	1.43 $\pm$ 0.04	1.27 $\pm$ 0.03	0.600	0.031
Zinc	95.73 $\pm$ 3.85	77.33 $\pm$ 2.78	1.000	0.001

retention of nitrogen simply because it had greater biodiversity than the *L. 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.<sup>25</sup> Therefore, in a climate change scenario nitrate leaching in *L. leucocephala* sites could be aggravated. Second, soil organic matter is the largest terrestrial carbon pool,<sup>26</sup> and soil carbon and nitrogen mineralization are closely coupled.<sup>27,28</sup> 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.<sup>29</sup> 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.<sup>30</sup> Bardon et al.<sup>31</sup> 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.<sup>32</sup> This plant group is among the most threatened groups of plants worldwide.<sup>33,34</sup> 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<sup>2,3</sup> 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.<sup>35</sup> 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 karsty outcrop soils (Loamy-skeletal, carbonatic, isohyperthermic Lithic Haplustolls).<sup>36</sup> 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* 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  $9.54 \text{ mm} \cdot \text{d}^{-1}$  ([www.wunderground.com](http://www.wunderground.com)).

### Analyses

A portion of each sample was dried at  $50^{\circ}\text{C}$  then total carbon and nitrogen were determined by dry combustion (Nelson and Sommers);<sup>37</sup> extractable P, K, Mg, and Ca were determined by Mehlich-3 digestion (Mehlich 1984);<sup>38</sup> and total metals were determined by nitric acid digestion.<sup>39</sup> Nitrate and ammonium were determined colorimetrically from fresh moist soil samples following 2M KCl extraction. Soil was incubated using the buried bag method<sup>40</sup> in a homogeneous site at  $28^{\circ}\text{C}$  (range  $25^{\circ}\text{C} - 31^{\circ}\text{C}$ ) 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<sup>41</sup> 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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### References

- [1] Donnegan JA, Butler SL, Grabowiecki W, Hiserote BA, Limtiaco D. Guam's forest resources, 2002. Resource Bulletin PNW-RB-243. Portland: US. Department of Agriculture, Forest Service, Pacific Northwest Research Station; 2004.
- [2] Marler T, Haynes J, Lindström A. *Cycas micronesica*. The IUCN Red List of Threatened Species 2010; e. T61316A12462113; <http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T61316A12462113.en>
- [3] USFWS [US. Fish and Wildlife Service]. Endangered and threatened wildlife and plants; endangered status for 16 species and threatened status for 7 species in Micronesia. Federal Register 2015; 80(190):59424-97.
- [4] Marler TE. *Cycad aulacaspis* scale invades the Mariana Islands. *Memoirs New York Botanical Garden* 2012; 106:20-35.
- [5] Marler TE. Temporal variations in leaf miner, butterfly, and stem borer infestations of *Cycas micronesica* in relation to *Aulacaspis yasumatsui* incidence. *HortScience* 2013; 48:1334-8.
- [6] Marler TE, Lawrence JH. Demography of *Cycas micronesica* on Guam following introduction of the armoured scale *Aulacaspis yasumatsui*. *J Trop Ecol* 2012; 28:233-42; <http://dx.doi.org/10.1017/S0266467412000119>
- [7] Anonymous. Conserving our nation's only native cycad species. *Currents* 2014; Fall, p. 28-31. Available at: [http://greenfleet.dodlive.mil/files/2014/10/Fall14\\_Conserving\\_Cycad\\_Species.pdf](http://greenfleet.dodlive.mil/files/2014/10/Fall14_Conserving_Cycad_Species.pdf)
- [8] Marler TE, Dongol N, Cruz GN. Plastic responses mediated by identity recognition in below-ground competition in *Cycas micronesica* K.D. Hill. *Trop Cons Science* 2016; 9:648-57.
- [9] Pyšek P, Jarošík V, Hulme PE, Pergl J, Hejda M, Schaffner U, Vilà M. A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. *Glob Change Biol* 2012; 18:1725-37; <http://dx.doi.org/10.1111/j.1365-2486.2011.02636.x>
- [10] Vilà M, Espinar JL, Hejda M, Hulme PE, Jarošík V, Maron JL, Pergl J, Schaffner U, Sun Y, Pyšek P. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecol Letters* 2011; 14:702-8; <http://dx.doi.org/10.1111/j.1461-0248.2011.01628.x>
- [11] Hulme PE, Pyšek P, Jarošík V, Pergl J, Schaffner U, Vilà M. Bias and error in understanding plant invasion impacts. *Trends Ecol & Evol* 2013; 28:212-8; <http://dx.doi.org/10.1016/j.tree.2012.10.010>
- [12] Read MD, Kang BT, Wilson GF. Use of *Leucaena leucocephala* (Lain.de Wit) leaves as nitrogen source for crop production. *Fert Res* 1985; 8:107-16; <http://dx.doi.org/10.1007/BF01048894>
- [13] Smil V. *Cycles of Life*. Scientific American Library. New York: 2000.

- [14] Van Der Heijden MG, Bardgett RD, Van Straalen NM. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* 2008; 11:296-310; PMID:18047587; <http://dx.doi.org/10.1111/j.1461-0248.2007.01139.x>
- [15] Van der Putten WH, Bardgett RD, Bever JD, et al. Plant-soil feedback: the past, present and future challenges. *J Ecol* 2013; 101:265-76; <http://dx.doi.org/10.1111/1365-2745.12054>
- [16] Haubensak KA, Parker IM. Soil changes accompanying invasion of the exotic shrub *Cytisus scoparius* in glacial outwash prairies of western Washington [USA]. *Plant Ecology* 2004; 175:71-9. <http://dx.doi.org/10.1023/B:VEGE.0000048088.32708.58>; <http://dx.doi.org/10.1023/B:VEGE.0000048088.32708.58>
- [17] Niu H, Liu W, Wan F, Liu B. An invasive aster (*Ageratina adenophora*) invades and dominates forest understories in China: altered soil microbial communities facilitate the invader and inhibit natives. *Plant Soil* 2007; 294:73-85; <http://dx.doi.org/10.1007/s11104-007-9230-8>
- [18] Gimeno I, Vilà M, Hulme PE. Are islands more susceptible to plant invasion than continents? A test using *Oxalis pes-caprae* L. in the western Mediterranean. *J Biogeography* 2006; 33:1559-65; <http://dx.doi.org/10.1111/j.1365-2699.2006.01525.x>
- [19] Marutani M, Manalastas E. Evaluation of two methods to estimate the populations of indigenous N-fixers associated with tropical legumes. *Micronesica* 2000; 33:153-60.
- [20] Chimphango SBM, Potgieter G, Cramer MD. Differentiation of the biogeochemical niches of legumes and non-legumes in the Cape Floristic Region of South Africa. *Plant Ecology* 2015; 216:1583-95; <http://dx.doi.org/10.1007/s11258-015-0542-0>
- [21] Cameron KC, Di HJ, Moir JL. Nitrogen losses from the soil/plant system: a review. *Ann Appl Biol* 2013; 162:145-73; <http://dx.doi.org/10.1111/aab.12014>
- [22] Scherer-Lorenzen M. The functional role of biodiversity in the context of global change. In *Forests and global change*, Eds Burslem D, Coomes D, Simonson W, Cambridge Univ Press, Cambridge, MA: 2014; 195-238.
- [23] Lang AC, von Oheimb G, Scherer-Lorenzen M, et al. Mixed afforestation of young subtropical trees promotes nitrogen acquisition and retention. *J Applied Ecol* 2014; 51:224-33; <http://dx.doi.org/10.1111/1365-2664.12157>
- [24] Scherer-Lorenzen M, Palmborg C, Prinz A, Schulze E-D. The role of plant diversity and composition for nitrate leaching in grasslands. *Ecology* 2003; 84:1539-52; [http://dx.doi.org/10.1890/0012-9658\(2003\)084%5b1539:TROPDA%5d2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(2003)084%5b1539:TROPDA%5d2.0.CO;2)
- [25] Dirnböck T, Kobler J, Kraus D, Grote R, Kiese R. Impacts of management and climate change on nitrate leaching in a forested karst area. *J Envir Management* 2016; 165:243-52; <http://dx.doi.org/10.1016/j.jenvman.2015.09.039>
- [26] Schlesinger WH, Bernhardt ES. *Biogeochemistry: An analysis of global change*. 3rd ed. Academic Press; 2013.
- [27] Gutiérrez-Girón A, Díaz-Pinés E, Agustín Rubio A, Gavilán RG. Both altitude and vegetation affect temperature sensitivity of soil organic matter decomposition in Mediterranean high mountain soils. *Geoderma* 2015; 237:1-8; <http://dx.doi.org/10.1016/j.geoderma.2014.08.005>
- [28] Quan Q, Wang CH, He NP, et al. Forest type affects the coupled relationships of soil C and N mineralization in the temperate forests of northern China. *Sci Rep* 2014; 4:1-8.
- [29] Barney JN. Invasive plant management must be driven by a holistic understanding of invader impacts. *Applied Vegetation Science* 2016; 19:183-4; <http://dx.doi.org/10.1111/avsc.12239>
- [30] Liao C, Peng R, Luo Y et al. Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. *New Phytol* 2008; 177:706-14; PMID:18042198; <http://dx.doi.org/10.1111/j.1469-8137.2007.02290.x>
- [31] Bardon C, Piola F, Bellvert F, Zhou X, Wu X, Fang C, Chen J, Li B. Evidence for biological denitrification inhibition (BDI) by plant secondary metabolites. *New Phytol* 2014; 204:621-31; <http://dx.doi.org/10.1111/nph.12944>
- [32] Osborne R, Calonje M, Hill K, Stanberg L, Stevenson DW. The world list of cycads. *Memoirs New York Botanical Garden* 2012; 106:480-508.
- [33] Brummitt NA, Bachman SP, Griffiths-Lee J, Lutz M, Moat JF, Farjon A, Donaldson JS, Hilton-Taylor C, Meagher TR, Albuquerque S, et al. Green plants in the red: A baseline global assessment for the IUCN sampled Red List Index for plants. *PloS one* 2015; 10:e0135152; PMID:26252495; <http://dx.doi.org/10.1371/journal.pone.0135152>
- [34] Fragniere Y, Bétrisey S, Cardinaux L, Stoffel M, Kozłowski G. Fighting their last stand? A global analysis of the distribution and conservation status of gymnosperms. *J Biogeography* 2015; 42:809-20; <http://dx.doi.org/10.1111/jbi.12480>
- [35] USFWS [US. Fish and Wildlife Service]. Biological opinion for the joint Guam program office relocation of the US. Marine Corps from Okinawa to Guam and associated activities on Guam and Tinian. 2010; United States: Department of the Interior.
- [36] Young FJ. Soil survey of the islands of Aguijan, Rota, Saipan, and Tinian, Commonwealth of the Northern Mariana Islands. United States Department of Agriculture, Soil Conservation Service; 1989.
- [37] Nelson DW, Sommers LE. Total carbon, organic carbon, and organic matter. p. 961-1010. In: Sparks DL (ed.) *Methods of soil analysis. Part 3. Chemical methods*. SSSA Book Series No. Five. SSSA and ASA, Madison, WI: 1996.
- [38] Mehlich A. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Commun Soil Sci Plant Anal* 1984; 15:1409-16; <http://dx.doi.org/10.1080/00103628409367568>
- [39] E.P.A. [United States Environmental Protection Agency]. EPA Method 3005. Cincinnati, Ohio: USEPA; 1992.
- [40] Eno CF. Nitrate production in the field by incubating the soil in polyethylene bags. *Proc Soil Sci Soc Am* 1960; 24:277-9; <http://dx.doi.org/10.2136/sssaj1960.03615995002400040019x>
- [41] Sokal RR, Rohlf FJ. *Biometry: The principles and practice of statistics in biological research*. 4th Edition. New York: WH Freeman; 2011.