Vol. 17(1), pp. 13-27, January-March 2025
DOI: 10.5897/JENE2024.0974
Article Number: A52D1C972972
ISSN 2006-9847

http://www.academicjournals.org/JENE

Journal of Ecology and The Natural Environment

Full Length Research Paper

Woody plants richness in the tropical dry forest at the RAMSAR site Playa Tortuguera El Verde Camacho, Sinaloa, México

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Received 17 August, 2024; Accepted 27 January, 2025

The species richness of tropical dry forests is heterogeneous. To prevent species exploitation, a 3 km barrier was constructed within the tropical dry forest of the RAMSAR site in 1991. Theoretically, the area inside the fence should be the best preserved, with a greater number of species expected. To determine whether the fence influenced woody species richness, 20 transects (0.01 ha each) were established on both sides of the fence. Woody species with a diameter at breast height (DBH) ≥ 1 cm were recorded. Non-parametric estimators, such as sampling effort, bias, precision, accuracy, and the U Mann-Whitney test, were used for data analysis. A total of 77 and 80 species were quantified inside and outside the barrier, respectively. The most species-rich plant families were Fabaceae, Euphorbiaceae, Cactaceae, Rubiaceae, and Burseraceae. Chao 1 and ACE estimators most closely approximated the actual observed species value, with a sampling effort exceeding 92%. Jackknife 1, Chao 1, Chao 2, and ICE showed the least bias, with higher precision and accuracy. The U Mann-Whitney test revealed no significant differences in species richness between the sampled sites inside and outside the fence.

Key words: Bias, precision and accuracy, conservation fencing, natural protected areas, non-parametric estimators, sampling effort.

INTRODUCTION

The number of species is a primary characteristic of biotic communities, heavily influenced by natural and/or anthropogenic processes and interactions, and is the most fundamental data on diversity in different spaces (Schluter and Ricklefs, 1993). As a key indicator, it quantifies biodiversity (Shimadzu, 2018) and informs conservation priorities (Hellmann and Fowler, 1999). Therefore, measuring species number is essential to

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understanding assemblies, structure, and biodiversity conservation.In Tropical Dry Forests (TDFs), species quantity is heterogeneous and varies over time. Richness increases with the expansion of the sampled area, encompassing more heterogeneous environments (Veiskarami et al., 2021). Additionally, species composition changes over time, representing different successional stages, such as pioneer, climax, and transitional forest species. TDFs are complex and fragile ecosystems with unique richness (Fernández-Méndez et al., 2014). In Mexico, for example, an average of 74.2 species of woody plants with a Diameter at Breast Height (DBH) over 1 cm has been recorded in a 0.1 ha area (Trejo and Dirzo, 2002).

Non-parametric estimators are commonly employed to quantify species richness (Palmer, 1990; Chao and Chiu, 2016; Ulrich et al., 2020). However, determining the total richness of taxonomic inventories and species listings of assemblages is practically impossible (Jiménez-Valverde and Hortal, 2003; Dos Santos, 2012; Revermann et al., 2018). Therefore, techniques based on incomplete samples of biological communities are used (Walther and Moore, 2005).

Measuring species richness is subject to bias, as many rare taxa (scarce in number and spatially uncommon) remain undetected. This underestimates the total species richness and poses a statistical challenge when comparing richness between sites or samples, as the observed quantity is sensitive to the number of individuals counted and/or the size of the sampled area (Colwell et al., 2012).

There are various estimators of richness, including Chao 1 and 2, Jackknife 1 and 2, Bootstrap, ICE, and ACE (Chazdon et al., 1998; Villarreal et al., 2006; Gotelli and Chao, 2013), which predict the true richness based on the species registered *in-situ*. These coefficients calculate the potential number of species present in an area by summing the observed species and the "missing" ones in the samples (Palmer, 1991). TDF is the predominant vegetation type at the RAMSAR site of Playa Tortuguera, El Verde Camacho (SRPTVC), which is surrounded by irrigation agriculture and cultivated pastureland. This natural protected area (ANP) includes a coastal nesting site for the olive ridley sea turtle (*Lepidochelys olivacea*). However, the site is threatened by land-use changes and species exploitation. In 1991, conservationists and landowners constructed a 3 km long wire barb wire fence and cyclonic mesh at the north of the RAMSAR site to regulate human presence, vehicle access, and prevent livestock intrusion. This study aims to quantify the number of woody plant species to estimate richness and compare richness measurements between the areas inside and outside the fence in the TDF of the SRPTVC. Due to the isolating effect of the barrier, a higher number of woody species is expected to be recorded inside the boundary compared to the area outside the fence. Significant differences in species richness is anticipated between the two areas.

MATERIALS AND METHODS

Description of the study site

Northern geographic coordinates are: 23° 29' 28.86" N; 106° 35' 33.31" W and 23° 28' 38.07" N; 106° 37' 20.68" W, and southern 23° 17' 53.82" N; 106° 29' 9.97" W and 23° 19' 48.96" N; 106° 26' 44.42" W. The listing performed in Playa Tortuguera El Verde Camacho, resulted in 375 species, being the *Fabaceae* and *Poaceae* families with the highest richness measures (Briseño-Dueñas, 2003). The types of vegetation and land use are: tropical deciduous forest, mangrove, hydrophilic vegetation, annual irrigation agriculture, cultivated grassland and human settlement (Figure 1).

Located in the lowlands of the Pacific Coastal Plains, north of the municipality of Mazatlán and to the south of San Ignacio, Sinaloa, Mexico, and its altitude oscillates between 0 and 50 m. The ANP is irrigated by the Quelite River, which have formed soils of lacustrine and alluvial origin with great sand content and more recent conglomerates. It is characterized by its semi-arid climate BS1(h')w, with a mean annual temperature of 22ºC and a coldest month holds a temperature above 18ºC. Summer precipitations and a percentage of winter rain between 5 and 10.2% of the total annual amount (INEGI, 2000).

Vegetation sampling

Sampling sites were selected using random numbers, and a grid mesh was created, comprising 100 grids over the northernmost polygon of the tropical dry forest. Forty 50 x 2 m (100 m^2) transects were established, covering a total area of 4000 m^2 (Gentry, 1988). To ensure equal sampling effort and minimize statistical and ecological biases when comparing richness as a measure of biodiversity (Gardner, 2014; Schluter and Ricklefs, 1993), 20 transects were placed inside the protected area and 20 outside the fence. Transect orientation consisted of a combination of north-south and east-west heading lines. Within each transect, all woody plant species with a stem diameter ≥ 1 cm at 1.30 m DBH were recorded (Ibarra-Manriquez et al., 2023).

Species registration

The species were identified *in-situ* and the unrecognized ones were collected and photographed. For the identification of these unrecognized specimens collected on field, the nomenclature of the taxonomic classification APG IV was employed (Angiosperm Phylogeny Group, 2016). The validation of the scientific names was carried out consulting the web pages: The Word Flora Online (WFO), TROPICOS, POWO (Plants of the World Online) and the geographic distribution through the Global Biodiversity Information Facility (GBIF 2024). Families were sorted from most to least taxa richness, the generic and specific epithets were arranged alphabetically. The transects and the inventory of species were carried out during the rainy season, in the months of July, August and September of 2021 and 2022.

Plants with extinction risk categories

The singletons (species with just one individual registered), doubletons (two individuals in total), uniques (those species present in a single sampling site) and duplicates (species present in two

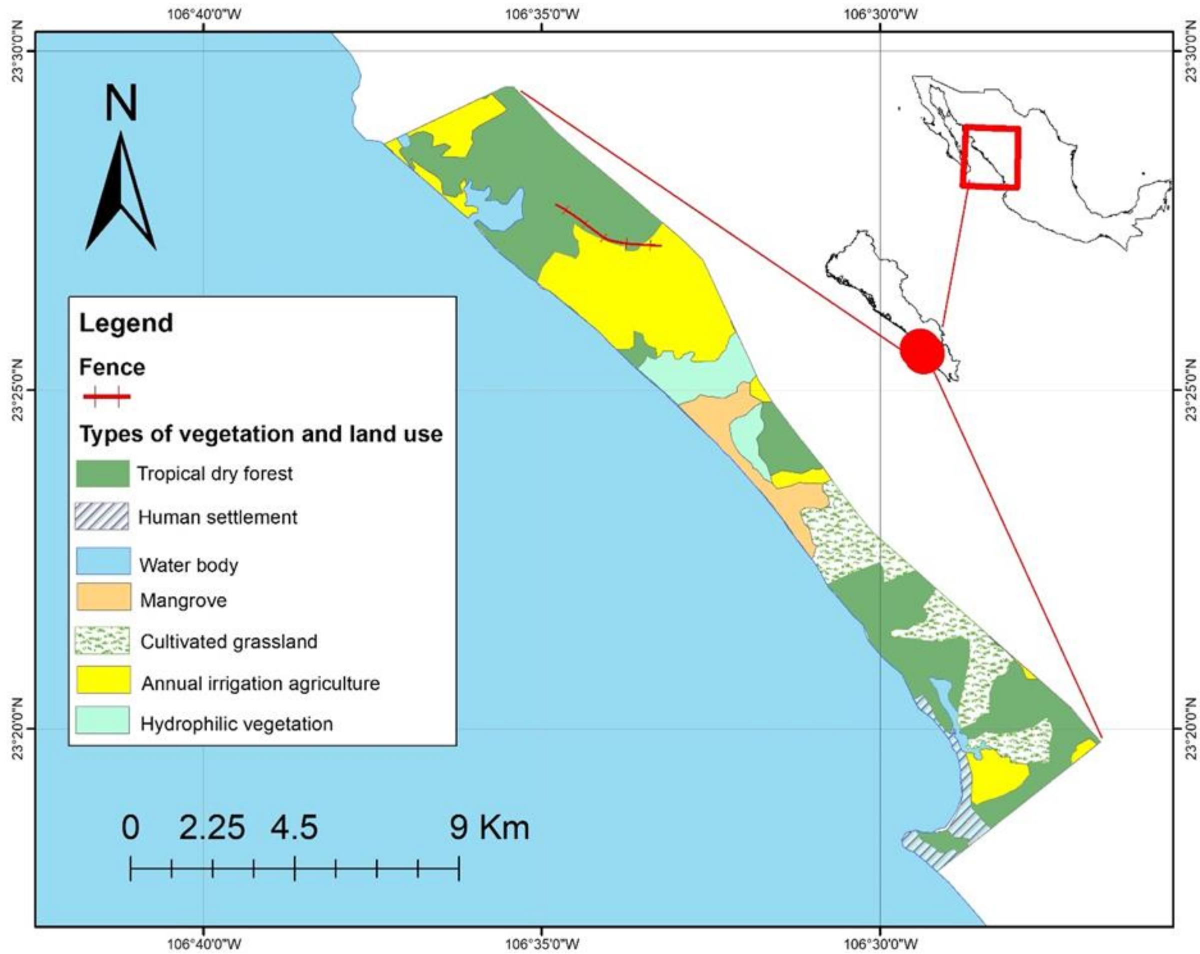

Figure 1. Location of the study site, types of vegetation and soil use of the RAMSAR site Playa Tortuguera El Verde Camacho, Sinaloa, México.

sampling locations) recorded were collated with the lists of endangered plants in Mexico, included in the NOM-059-SEMARNAT-2010 (SEMARNAT, 2019), internationally included in the red list IUCN (IUCN, 2022) and finally the catalogue of native vascular plants of Mexico (Villaseñor, 2016), as main sources to assess the conservation status and taxa distribution in Mexico.

Data analysis, non-parametric estimators of species richness

Seven non-parametric estimators were utilized: Chao 1, Chao 2, Jackknife 1, Jackknife 2, Bootstrap, ACE and ICE (Chao, 1984; Chao, 1987; Burnham and Overton, 1978, 1979; Smith and van Belle, 1984; Chao and Lee, 1992; Chao et al., 1993; Lee and Chao, 1994). They function under a simple logic, once several rare taxa are registered, it is an indicator of the existence of a large number of non-registered invisible species in the samples (Magurran, 2017) and the highly estimated values are produced when the samples contain large proportions of rare species (Melo, 2004).

Estimates application

Two matrices were constructed for processing species richness information, where rows represented species and columns represented sampling sites. Using Excel, the matrices were converted into text files delimited by tabulations, facilitating analysis with the EstimateS software (Colwell, 2013). This software is a useful and straightforward tool for obtaining information on species aggregation and various richness estimators.

Sampling efficiency

A contrast between the observed richness values versus the estimated was performed, using data obtained from the non-parametric techniques, which can proportionate an estimation of the suitable sample size (Hao et al., 2002). The expected values generated by the estimators can be utilized to determine the sampling efficiency (Villarreal et al., 2006).

If the results were 80% or higher, the achieved sampling would be considered good, very great or excellent. Efficiency is a simple arithmetic operation determined by dividing the number of observed species by the estimated ones and the quotient multiplied by 100.

Scaled measurements for bias, precision and accuracy

To evaluate the performance of species richness estimators, scaled measurements of bias, precision, and accuracy were employed to obtain comparable results (Walther and Moore, 2005). This approach helped avoid inconsistent results and errors in decisionmaking or choosing an unsuitable estimator (Hellmann and Fowler, 1999). Data from aggregated species were utilized from each recorded sample in the field, along with the results of seven non-parametric estimators from inside and outside the fence. Bias, the difference between the expected values of the estimator and the real richness values (Hellmann and Fowler, 1999), was used as a neutral or balanced value between high and low quantities (Brower et al., 1998). A global bias of zero was considered ideal (Kotz et al., 2006). At the lecture scale, zero is the predetermined value for bias. A negative value indicates underestimation of richness, while a positive value indicates overestimation (Walther and Moore, 2005). The bias was calculated using the formula for the mean scaled error (Walther and Moore, 2005). Ideally, the best richness estimator should have the lowest bias (Archaux, 2009).

Precision refers to the difference between one species richness estimator based on sample data and the average of all possible richness estimations based on all possible samples of the same size from the sampled community (Hellmann and Fowler, 1999). It measures the degree of repeatability in replicated samples using a determinate sampling procedure (Ludwig and Reynolds, 1988). A precise estimator should show slight variation (Kotz et al., 2006). A variation and standard deviation algorithm coefficient were employed (Walther and Moore, 2005), with results expressed as percentages (0-100) for easy interpretation. Accuracy represents the difference between the species richness estimation based on sampled data and the real richness of the sampled community (Hellmann and Fowler, 1999). It is the closeness of a measured value to its real value (Krebs, 1999). The scaled mean square error was used to calculate accuracy (Walther and Moore, 2005).

Hypothesis testing between the richness of sites outside and inside the fence

To compare the woody plants richness between the sites, a non-parametric *U* Mann-Whitney test was run through SPSS (IBM Corp., 2022), applied to data which do not follow a normal distribution (Hodgson and Thompson, 1993); where the difference of medians was contrasted, employing a critic value of $\alpha < 0.05$, to conclude if the woody species richness from two sites of the same community, would differentiate significantly (Pielou, 1975). The technique contrasts the null hypothesis (*H0*) and the alternative (*H1*). *H0* assumes the median richness in tree, shrubs and climbing plants species are the same, while *H1* claims the opposite, which are all different from one other.

RESULTS

Floristic richness of the TDF from the SRPTVC

A total of 31 taxa families of woody plants were registered at the site. 28 families were registered outside the fence and 29 inside. The number of counted species added to a total of 94 taxa. The shared species from both sides were 63 and the absent ones 31. Outside the fence 80 species were recorded. The Fabaceae family contributed with 19 species (23.8%), Euphorbiaceae 12 (15%), Cactaceae 6 (7.5%), Rubiaceae 5 (6.3%), Burseraceae 4 (5%) and Apocynaceae 4 (5%), which totaled 62.5%. Inside the fence 77 species were counted. Here, of the Fabaceae, 15 species were registered (19.5%), Euphorbiaceae 10 (13%), Cactaceae 7 (9.1%), Rubiaceae 5 (6.5%), Burseraceae 4 (5.2%) and Capparaceae 4 (5.2%), which added 58.4% (Table 1).

Plants in extinction risk categories

Four registered species appear as Threatened (A) in the NOM-059-SEMARNAT-2010 (SEMARNAT 2019). *Handroanthus impetiginosus* and *Laguncularia racemosa* both recorded inside the fence, although *Hesperalbizia occidentalis* was registered outside. *Guaiacum coulteri* remains present in both areas. 74 taxa are included in the IUCN red list. 67 classified as low risk (LC), three under Near Threatened (NT): *Manihot chlorosticta*, *Bursera laxiflora*, and *H. impetiginosus* and four inside the Vulnerable category (VU): *Stenocereus alamosensis*, *Esenbeckia hartmanii*, *Eugenia sinaloae* and *G. coulteri*.
The total species linked with the following categories: under extinction risk, native to Mexico, with low abundance and limited spatial location, added to 26 and those included in the red list under the Low risk (LC) category, added 24, only one species under Near Threatened (NT) and another one Vulnerable (VU). Meanwhile, only three species were present in the NOM-059-SEMARNAT-2010 (SEMARNAT 2019), the three under the threatened categories, plus showing up in both legislations.

The singletons (S) totaled eight taxa, the doubletons (DO) added to six, the recorded uniques (U) were 23 species and the duplicates (Du) under a risk class added six species (Table 2).

Non-parametric estimators of species richnesss

Inside the fencing (A), 77 species were observed. Non-parametric estimators yielded the following richness estimates: Jackknife 2, 106.33; Jackknife 1, 96; Chao 2, 95.05; Bootstrap, 85.63; ACE, 79.23; and Chao 1, 79.99.
Outside the fencing (B), 80 species were observed, with estimated richness values exceeding 100 for Jackknife 2 (113.88), Chao 2 (104.38), and Jackknife 1 (100.9). The lowest estimates were obtained for Chao 1 (84.5), ACE (86.25), and Bootstrap (89.11) (Figure 2).

Sampling effort

The best estimators with quantities near the absolute number of observed species were Chao 1, ACE, Bootstrap, and ICE, respectively. Chao 2 and Jackknife 1 inside the fence showed high efficacy, however their results came under 80 % outside. Jackknife 2 showed low results on both sites. The efficiency values were higher inside than outside of the fence (Table 3).

Bias, precision and accuracy of the non-parametric estimators

The bias values (SME) of the observed species richness and Jackknife 2 oscillated negatively and positively both inside the fencing (- 0.2422 to 0.0879) and outside

Table 1. Floristic woody plants richness from the TDF at the RAMSAR site Playa Tortuguera El Verde Camacho, Sinaloa, México. Presence (1), absence (0). Risk category from NOM-059-SEMARNAT 2010. Threatened (A) and from IUCN Red list: Low risk (LC), Near Threatened (NT) and Vulnerable (VU).

Families / specie	Presence – Abscence		Risk category
	Inside	Outside	
FABACEAE			
Bauhinia pauletia Pers.	0	1	LC
Calliandra houstoniana (Mill.) Standl.	0	1	LC
Cenostigma eriostachys (Benth.) Gagnon & G. P. Lewis	1	1	LC
Chloroleucon mangense (Jacq.) Britton & Rose.	1	1	LC
Coulteria platyloba (S. Watson) N. Zamora	1	1	LC
Entada polystachya (L.) DC.	1	1	LC
Erythrina flabelliformis Kearney	1	1	LC
Erythrina lanata Rose	1	0	LC
Erythrostemon palmeri (S.Watson) Gagnon & G. P. Lewis	1	1	LC
Haematoxylum brasiletto Karst.	1	1	LC
Hesperalbizia occidentalis (Brandegee) Barneby & J.W.Grimes	0	1	A. LC
Libidibia sclerocarpa (Standl.) Britton & Rose	1	1	LC
Lonchocarpus lanceolatus Benth.	1	1	LC
Lysiloma divaricatum (Jacq.) J. F. Macbr.	1	1	LC
Microlobius foetidus (Jacq.) M. Sousa & G. Andrade	0	1	LC
Pithecellobium unguis-cati (L.) Benth.	1	1	LC
Pityrocarpa obliqua (Pers.) Brenan	1	1	LC
Senna pallida (Vahl) H. S. Irwin & Barneby	0	1	LC
Senna quinquangulata (Rich.) H. S. Irwin & Barneby	1	1	LC
Vachellia macracantha (Humb. & Bonpl. ex Willd.) Seigler & Ebinger	1	1	LC
EUPHORBIACEAE			
Adelia vaseyi (J. M. Coult.) Pax & K. Hoffm.	0	1	
Croton morifolius Willd.	1	1	LC
Croton niveus Jacq.	1	1	LC
Croton reflexifolius Kunth	1	1	LC
Enriquebeltrania disjuncta De-Nova & Sosa	1	1	
Euphorbia bracteata Jacq.	1	1	
Euphorbia californica Benth.	1	1	
Euphorbia schlechtendalii Boiss.	1	1	
Jatropha cordata (Ortega) Müll. Arg.	1	1	LC
Jatropha curcas L.	1	1	LC
Jatropha marquezii Pío-León, Millán-Otero & B. Salomón	0	1	
Manihot chlorosticta Standl. & Goldman	1	1	NT
CACTACEAE			
Acanthocereus tetragonus (L.) Hummelinck	1	1	LC
Opuntia feroacantha Britton & Rose	1	1	
Opuntia rileyi J. G. Ortega	1	0	
Opuntia spraguei J. G. Ortega	1	1	
Pachycereus pecten-aboriginum (Engelm. ex S.Watson) Britton & Rose	1	1	LC
Pereskiopsis porteri (Brandegee ex F. A. C.Weber) Britton & Rose	0	1	LC
Selenicereus vagans (K. Brandegee) Britton & Rose	1	0	LC
Stenocereus alamosensis (J. M. Coult.) A. C. Gibson & K.E. Horak	1	1	VU
RUBIACEAE			
Chiococca alba (L.) Hitchc.	1	1	LC

Table 1. Cont'd

Hintonia latiflora (Sessé & Moc. ex DC.) Bullock	0	1	LC
Randia aculeata L.	1	1	LC
Randia armata (Sw.) DC.	1	0	LC
Randia capitata DC.	1	1	LC
Randia thurberi S. Watson	1	1	LC
APOCYNACEAE			
Cascabela ovata (Cav.) Lippold	0	1	LC
Plumeria rubra L.	0	1	LC
Rauvolfia tetraphylla L.	0	1	LC
Ruehssia edulis (S. Watson) L. O. Alvarado	0	1	
BURSERACEAE			
Bursera excelsa (Kunth) Engl	1	1	LC
Bursera fagaroides (Kunth) Engl.	1	1	LC
Bursera laxiflora S. Watson	1	1	NT
Bursera simaruba (L.) Sarg.	1	1	LC
CAPPARACEAE			
Crateva palmeri Rose	1	0	LC
Morisonia americana L.	1	1	LC
Morisonia flexuosa L.	1	1	LC
Morisonia indica (L.) Ined.	1	1	
MALVACEAE			
Ayenia aculeata (Jacq.) Christenh. & Byng	0	1	
Ceiba aesculifolia (Kunth) Britten & Baker f.	1	0	LC
Helicteres baruensis Jacq.	1	1	LC
RHAMNACEAE			
Colubrina heteroneura (Griseb.) Standl.	1	1	LC
Gouania rosei Wiggins	1	1	
Sarcomphalus amole (Sessé & Moc.) Hauenschild	1	1	LC
RUTACEAE			
Esenbeckia hartmanii B. L. Rob. & Fernald	1	1	VU
Zanthoxylum fagara (L.) Sarg.	1	1	LC
Zanthoxylum schreberi (J. F. Gmel.) Reynel ex C. Nelson	1	0	LC
BIGNONIACEAE			
Dolichandra unguis-cati (L.) L. G. Lohmann	0	1	
Handroanthus impetiginosus (Mart. ex DC.) Mattos	1	0	A. NT
COMBRETACEAE			
Combretum fruticosum (Loefl.) Stuntz	1	0	
Laguncularia racemosa (L.) C. F. Gaertn.	1	0	A. LC
CONVOLVULACEAE			
Ipomoea arborescens (Humb. & Bonpl. ex Willd.) G. Don	1	1	LC
Ipomoea bracteata Cav.	1	0	
MALPIGHIACEAE			

Table 1. Cont'd

Heteropterys laurifolia (L.) A. Juss.	1	1	LC
Malpighia emarginata DC.	1	1	
MYRTACEAE			
Eugenia sinaloae Standl.	1	1	VU
Psidium oligospermum Mart. ex DC.	1	1	LC
VITACEAE			
Cissus trifoliata L.	0	1	
Cissus verticillata (L.) Nicolson & C. E. Jarvis	1	0	LC
ACHATOCARPACEAE			
Phaulothamnus spinescens A. Gray.	1	1	LC
BORAGINACEAE			
Cordia alliodora (Ruiz & Pav.) Oken	1	0	LC
EBENACEAE			
Diospyros aequoris Standl.	1	0	LC
ERYTHROXYLACEAE			
Erythroxylum mexicanum Kunth	1	1	LC
MELIACEAE			
Trichilia trifolia L.	1	1	LC
MORACEAE			
Ficus cotinifolia Kunth	1	1	LC
NYCTAGINACEAE			
Neea psychotrioides Donn. Sm.	1	1	LC
OPILIACEAE			
Agonandra racemosa (DC.) Standl.	1	1	LC
PICRAMNIACEAE			
Alvaradoa amorphoides Liebm.	0	1	LC
POLYGONACEAE			
Ruprechtia fusca Fernald	1	1	LC
PRIMULACEAE			
Bonellia macrocarpa (Cav.) B. Ståhl & Källersjö	0	1	LC
SALICACEAE			
Casearia nitida (L.) Jacq.	1	1	
SAPINDACEAE			
Paullinia fuscescens Kunth	1	1	
SAPOTACEAE			
Sideroxylon celastrinum (Kunth) T. D. Penn.	1	1	LC

Table 1. Cont'd

ZYGOPHYLLACEAE			
Guaiacum coulteri A. Gray	1	1	A. VU

Table 2. Risk category and native woody plants with low abundance (singletons and doubletons) and scarce spatial dispersion (uniques and duplicates) S: singletons; DO: doubletons; U: uniques; Du: duplicates.

Specie	Inside the fence				Outside the fence			
	S	Do	U	Du	S	Do	U	Du
Acanthocereus tetragonus				*				
Agonandra racemosa					*		*	
Bauhinia pauletia						*	*	
Bonellia macrocarpa					*		*	
Cascabela ovata					*		*	
Ceiba aesculifolia			*					
Chiococca alba			*					
Chloroleucon mangense				*				
Cissus verticillata	*		*					
Colubrina heteroneura		*			*		*	
Cordia alliodora				*				
Erythrostemon palmeri			*					
Erythrina flabelliformis			*				*	
Ficus cotinifolia						*	*	
Guaiacum coulteri			*					*
Helicteres baruensis			*					
Hesperalbizia occidentalis						*	*	
Laguncularia racemosa	*		*					
Manihot chlorosticta		*		*	*		*	
Microlobius foetidus						*	*	
Morisonia americana					*		*	
Morisonia flexuosa			*					
Pereskiopsis porteri								*
Rauvolfia tetraphylla							*	
Selenicereus vagans			*					
Sideroxylon celastrinum							*	

(- 0.2539 and 0.0900). Jackknife 1 was the best bias estimator of species richness in both sites because it showed the nearest quantities to zero. The precision percentage data (CV) varied inside, between 22.14 (Chao 1) and 29.54 (Jackknife 2); outside, 21.27 (ICE) and 29.86 (Jackknife 2). The accuracy quantities (SMSE) inside fluctuated between 0.0562 (Chao 2) and 0.1105 (Jackknife 2); outside with 0.0530 (ICE) and 0.1139 (Jackknife 2) (Table 4).

Results of the Mann-Whitney test

The median number of species (± MAD) inside the fencing reached 20, with a superior value of 23 and outside of 22, plus a maximum value of 25. The value of error probability was $P < 0.158$. Both diagrams show a difference of two species (Figure 3).

DISCUSSION

Floristic richness of the TDF from the SRPTVC

The *Fabaceae, Euphorbiaceae, Cactaceae*, and *Rubiaceae* families exhibited the highest species richness, contributing 48 and 52.5% of the species inside and outside the fence, respectively. This finding

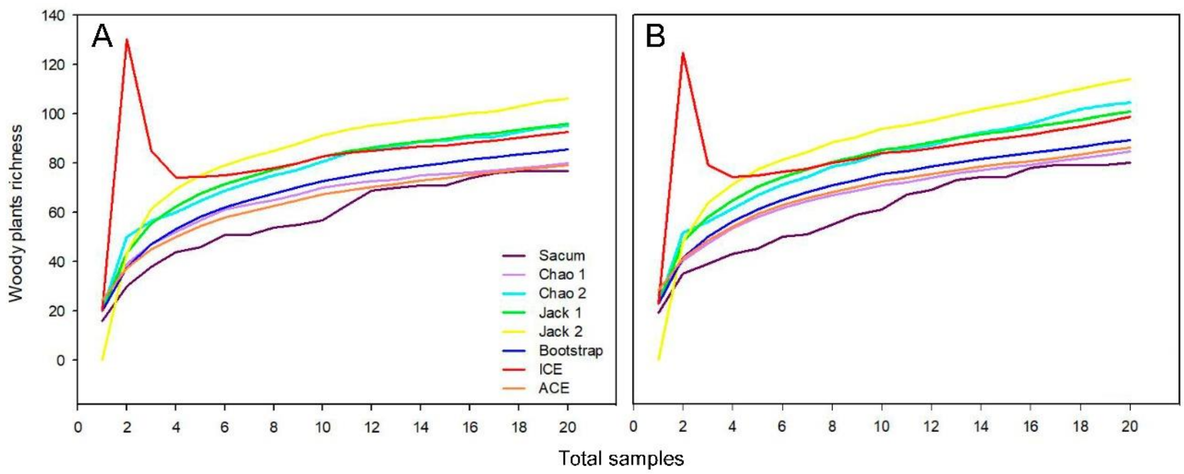

Figure 2. The results of the non-parametric estimators obtained from inside the fencing (A) and outside the fencing (B) at the tropical dry forest at the RAMSAR site Playa Tortuguera El Verde Camacho, Sinaloa, México.

Table 3. Sampling efficiency of the richness estimators of the stational dry forest at the RAMSAR site El Verde Camacho, Sinaloa, México.

Estimator	Inside		Outside	
	Estimator's result	Sampling efficiency (%)	Estimator's result	Sampling efficiency (%)
Chao 1	80.0	96.3	84.5	94.7
Chao 2	95.1	81.0	104.4	76.6
Jackknife 1	96.0	80.2	100.9	79.3
Jackknife 2	106.3	72.4	113.9	70.3
Bootstrap	85.6	89.9	89.1	89.8
ICE	92.6	83.1	98.6	81.2
ACE	79.2	97.2	86.2	92.8

Table 4. Bias, precision and accuracy of the non-parametric estimators of species richness (ICE, ACE, Chao 1, Chao 2, Jackknife 1, Jackknife 2 and Bootstrap) with measuring scale.

Estimator	Inside the fencing			Outside the fencing		
	(Bias) SME	(Precision) CV	(Accuracy) SME	(Bias) SME	(Precision) CV	(Accuracy) SMSE
Observed species richness	- 0.2422	28.93	0.1063	- 0.2539	28.57	0.1061
ICE	0.0774	22.28	0.0634	0.0535	21.27	0.0530
ACE	- 0.1757	22.77	0.0658	- 0.1428	22.24	0.0567
Chao 1	- 0.1519	22.14	0.0581	- 0.1609	22.57	0.0617
Chao 2	- 0.0101	23.98	0.0562	- 0.0006	25.08	0.0627
Jackknife 1	0.0010	24.53	0.0601	0.00003	23.86	0.0569
Jackknife 2	0.0879	29.54	0.1105	0.0900	29.86	0.1139
Bootstrap	- 0.1168	24.85	0.0616	- 0.1158	23.79	0.0576

corroborates previous studies, which identified Fabaceae as the family with the highest species richness at the site (Briseño-Dueñas, 2003; Pennington et al., 2006; Roncal-Rabanal et al., 2023). When the sampling sites were reduced by half (0.1 ha), 57 and 61 species were quantified inside and outside the fence, respectively. Conversely, doubling the study area to 0.2 ha resulted in an increase of 20 and 19 species inside and outside the

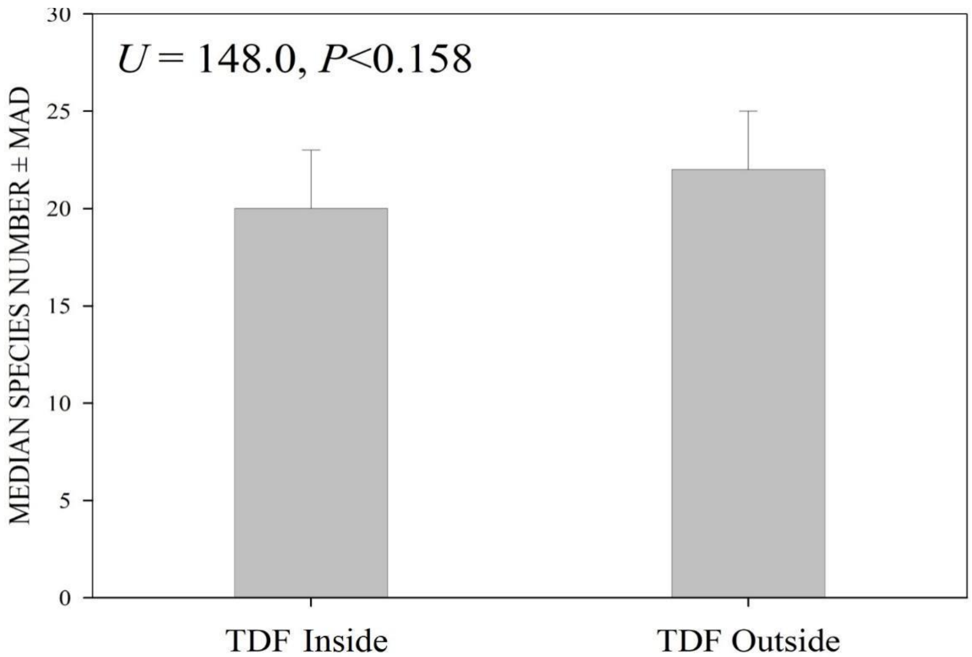

Figure 3. Bar chart comparing the median number of species between the sites (inside and outside) the fence built in 1991. The *U* and *P* values of the non-parametric test of Mann-Whitney.

fence, respectively, but did not show a proportional increase in taxa with the expansion of the sampling area. Instead, the increase in species richness decreased as it approached the maximum number of recorded species. These findings are comparable to other studies in the region, with some reporting higher richness values, such as Mendoza (1999) in Colombia (78 species), Carrillo-Fajardo et al. (2007) in Colombia (79 species), and Trejo and Dirzo (2002) in 20 TDF sites in Mexico (average of 74.2 species). The recorded values were higher than those found in Alamos, Sonora (46 species) and the central region of Sinaloa (51 species), similar to those in La Burrera, Baja California Sur (56 species), but lower than those in Cosalá, Sinaloa (80 species) and Jesús María, Nayarit (63 species). These results align with the patterns of taxonomic richness distribution, which exhibit an inverse relationship with latitude and an increase with altitude. Alamos, with a higher latitude, recorded lower species richness, whereas Jesús María, located southward with fewer degrees of latitude, showed a higher number of species, similar to the quantity recorded outside the fencing. La Burrera, at a similar latitude but separated longitudinally by the Gulf of California, recorded an equivalent quantity of species to that obtained within the fence. Cosalá, geographically close but at a higher altitude, registered higher richness, which can be attributed to its location near El Mineral de Nuestra Señora, a natural protected area managed by the Universidad Autónoma de Sinaloa, with policies focused on species preservation and plant community conservation. The low richness values in our study area can be partially explained by the flat surface and low altitudes (5 to 20 m), characteristic of the Pacific Coastal Plains, which contrasts with the nearby low hills that provide greater abiotic variability and higher rates of beta biodiversity. These results are consistent with those of Linares-Palomino and Ponce-Álvarez (2005), who found that Tropical Dry Forests with lower richness are typically located in lowlands and near coastal areas along an altitude gradient.

Endangered plants

In TDF species assemblages of the SRPTVC, singletons, doubletons, uniques, and duplicates are particularly significant, as they often appear in endangered species catalogues, listed as Amenazada (A) on the NOM-059-SEMARNAT-2010 or as Low Risk (LC), Near Threatened (NT), and Vulnerable (VU) on the IUCN list. Combining singletons and uniques, the following species were registered: *Cissus verticillata* and *Laguncularia racemosa* inside the fencing, and *Agonandra racemosa, Bonellia macrocarpa, Cascabela ovata, Colubrina heteroneura, Manihot chlorosticta*, and *Morisonia americana* outside the perimeter. Most of these species are classified as Low Risk (LC), except for *M. chlorosticta*, which is listed *racemosa*, which appears on both listings. Notably, these species are native to Mexico, with their northernmost distribution located among the northwest states of Mexico. Specifically, *C. ovata* and *C. heteroneura* have their northern limit in the states of Durango and Sinaloa, and *M. americana* in Sinaloa.

Among doubletons and duplicates, only *M. chlorosticta* was found inside the fence. In the mixture of species with low abundance and limited distribution, *Bauhinia pauletia, Ficus cotinifolia, Hesperalbizia occidentalis*, and *Microlobius foetidus* were present outside the fence.

These species are native to Mexico, with their northern border of geographical distribution limited to the northwest states of Mexico. Notably, the distribution of all individuals of one species was concentrated in a single location site.

Inside the fence, the unique species were: *Casearia nitida, Ceiba aesculifolia, Chiococca alba, Morisonia flexuosa, Erythrostemon palmeri, Erythrina flabelliformis, Guaiacum coulteri, Helicteres baruensis*, and *Selenicereus vagans.*

Outside the fencing, *Jatropha marquezii* and *Sideroxylon celastrinum* were found. These species are native to Mexico, with their geographic frontier limited to the northwest states of Mexico. Specifically, *M. flexuosa, E. palmeri, H. baruensis,* and *S. vagans* have their northern borders in Sonora and Sinaloa, while *C. nitida* and *J. marquezii* have their northernmost borders in Sinaloa.

Non-parametric estimators and sampling effort

In both sites, Chao 1 and ACE resulted in lower estimated values, close to the number of observed species. Inside the fence, ACE increased in two with twenty-two species not included in samples, which boosted to 79.22. Adding to the 40 abundant species (S_{common}), 39.22 the product of the quotients between rare species (S_{rare}), singletons frecuency (F_1) and the sample abundance coverage estimator (C_{ace}), multiplied by the coefficient of variation of F_1 for rare taxa (γ_{ace}^2). In Chao 1 the value of the product between singletons and doubletons averaged by EstimateS increased in 2.19 the observed species value and rose to 80.0 the existing woody species. Outside the fence by Chao 1, the radius between those species which registered one or two individuals were averaged and resulted in 4.5, reaching 84.5 the sums of observed species plus the estimated ones. ACE added 6.2 to the 80 observed species. Adding to the 40 common taxa, the product of the quotients yielded 46.2. Notably, the Jackknife 2 estimator produced the highest values, with an overestimation of 106.32 species within the fenced area.

The Jackknife 2 estimator added 29.3 species to the observed 77 inside the fence, resulting from the products of the uniques radius and duplicates between the 20 samples and the samples minus the unit. Outside the fence, Jackknife 2 overestimated by 113.9 species, adding 33.9 to the observed 80. Intermediate estimates were provided by Bootstrap and ICE. The non-parametric richness estimators showed that ICE and Chao 1 were closest to the observed species values, with estimates of 79.2 and 80.0 inside the perimeter, and 86.2 and 84.5 outside, compared to the actual values of 77 and 80. ACE and Chao 1 estimated 2.2 and 3.0 missing species inside the fencing, and 6.2 and 4.5 outside, with percentage sampling effort values exceeding 90% for both estimators and registration sites.

Bootstrap and ICE showed quantities over 80% in both sites, while Chao 2 and Jackknife 1 recorded amounts above 80% solely within the fence. Jackknife 2 overestimated the observed species value in both places, predicting over 100 species. Jackknife 1 and Chao 2 outside the fencing accounted for a similar number.

Comparative studies, such as Fernández-Méndez et al. (2014), reported a range of 8-34 species of trees in 12 TDF fragments, with Chao 1 estimates between 8.75 and 39.70 species. Dzib-Castillo et al. (2014) accounted for 51 species of trees and estimated 60 using Chao 1, with a sampling effort of 85%. Revermann et al. (2018) reported extreme values of 215 (high) and 111 (low) woody species.

Employing four non-parametric estimators of richness (Chao 2, ICE, Jackknife 1, and Jackknife 2), Jackknife 1 and ICE were found to be the most accurate, closely aligning with the observed species values. In the site with the highest richness, these estimators predicted similar quantities of 282 and 283 species, respectively, with 67 and 68 missing species. In the site with the lowest richness, they estimated 148 and 162 species, respectively, predicting 37 and 51 non-registered species. The high number of estimated and missing species resulted in a sampling efficiency below 80%. This finding is consistent with the results of Jackknife 2, which provided the highest estimation values for the number of species.

Similar results have been reported in other studies. David-Higuita and Álvarez-Dávila (2018) recorded 318 species and, using Chao 2, estimated a total of 413 species, representing an efficacy of less than 80% in their sampling. This result is comparable to the estimator outside the fencing. Ortega-Baranda et al. (2020) reported 33 and 47 species of trees in two contrasting dry forests and, applying Chao 2, estimated 42 and 63 species, respectively, with sampling efforts below 80%, similar to Chao 2 outside the fencing.

Houngnon et al. (2021) recorded 185 vascular plants and employed non-parametric estimators, including Bootstrap, Chao, Jackknife 1, and Jackknife 2. The estimated values revealed that Jackknife 2 yielded the highest estimate with 243 species, including 58 non-observed species, followed by Bootstrap with 201 species and 16 non-detected taxa. In contrast, Gharnit et al. (2025) found Chao 2 and Jackknife 1 to be the most accurate richness estimators. The results showed that Jackknife 1, Chao 1, and Bootstrap achieved a sampling effort exceeding 80%. Notably, Bootstrap exhibited a 92.5% similarity with Chao 1 and ACE. Conversely, Jackknife 2 presented the highest estimation value but a low sampling effort.

The quantification of sampling effort demonstrated an inverse relationship with the number of estimated species. The variability in observed species across different sources can be attributed to the inherent nature of dry forests, as well as differences in plot sizes, registration plots, and total sampling surface. The findings suggest that low estimations, closer to the actual observed species value, result in high percentages of sampling effort. In contrast, highly estimated numbers of species generate low sampling efforts.

Bias, precision and accuracy

The estimators with the least bias (SME < 0.1) within and outside the fencing were Jackknife 1, ICE, and Jackknife 2, all exhibiting a positive bias. In contrast, Chao 2 displayed the lowest negative bias. Bootstrap, Chao 1, ACE, and observed richness showed the highest negative biased figures, exceeding 0.1. In terms of accuracy (CV), Chao 1, ICE, and ACE from both sides of the fencing yielded the lowest values, around 20%. Observed richness and Jackknife 2 achieved the highest numbers, nearing 30%. The percentage quantities of observed and estimated species richness showed minimal variation. Precision results (SMSE) differed inside and outside the fence. Chao 2 and 1, Jackknife 1, Bootstrap, and ICE presented the lowest values (< 0.1), varying only in order of relevance. Jackknife 2 and observed richness showed the highest quantities (> 0.1). These findings align with Palmer (1990, 1991), who identified Jackknife 1 and 2 as the least biased estimators, and Colwell and Coddington (1994), who noted that the Jackknife procedure reduces bias in estimations.

The findings of this study align with those of Brose (2002), who reported Chao 2 as the most precise estimator, albeit with negative bias. Similarly, the results show that the estimators significantly reduced bias compared to observed richness. The study also concurs with Chiarucci et al. (2003), who found Jackknife 2 to be the least biased estimator, although in this study, Jackknife 1 resulted in the least bias, with both being positively biased. In contrast, Chao 2 and Bootstrap exhibited negative bias. The conclusion that estimators perform better than observed richness (Sobs) is reinforced, sharing similarities with Archaux (2009), who found Jackknife 1 to be the least biased. However, this study diverges from Archaux's conclusion that Jackknife 1 is the most accurate estimator. A link between bias and accuracy was found, where the least biased estimator was also the most accurate.

The results differ from Chun-Huo (2023), who reported Chao 2 as an estimator that tends to overestimate true species richness. Regarding Wei et al. (2010), this study concurs that Jackknife 2 is the most precise but disagrees on its accuracy, as it showed the highest values. Similarities were found with Chiu et al. (2014), who noted that Jackknife 1 and 2's positive results are related to sample size, leading to overestimation in large samples. No clear pattern of small or large differences exists between estimated or observed values of richness in field samples, regarding bias, precision, and accuracy. However, this study differs from Houngnon et al. (2021), who found Bootstrap to be the least biased estimator, as it showed non-low bias values in this study.

U Mann-Whitney test results

The U Mann-Whitney test yielded a probability value of $P < 0.15$, exceeding the significance level of $\alpha < 0.05$. This suggests a small variation in woody plant richness between the two sites, with no significant statistical differences. Consequently, the results fail to confirm the alternative hypothesis, which posits that the fence would lead to a significant difference in the number of tree, shrub, or climbing species between the two sites. These findings align with those of Gillespie et al. (2000), who found no significant differences in tree, shrub, and vine richness between dry forests in Central America and similar ones in the Neotropics. Similarly, Gutiérrez-Flores and Canales-Gutiérrez (2012) did not detect significant differences in wild flora richness between different altitudes. Lanuza et al. (2022) also found no differences in tree richness across a fragmented landscape in Nicaragua.

In contrast, the results differ from those of Gentry (1995), who compared TDF sites in the Caribbean islands and the American mainland, finding a less pronounced tendency for West Indian dry forests to be less diverse than continental ones. Trujillo and Henao-Cárdenas (2018) also reported significant differences in richness across an altitudinal gradient.

The recorded species in 0.1 ha ranged from 57 to 61 woody plants inside and outside the fence, respectively, which is lower than the average richness reported for Mexico by Trejo and Dirzo (2002) of 74.2 species. In 0.2 ha, 77 species were recorded within the fence, with non-parametric models estimating an increase to 79.2-106.3 species. Outside the fence, 80 species were recorded, with models estimating an increase to 84.5-113.9 species. The sampling effort assessment revealed that Chao 1 and ICE had values above 92% in both areas. Jackknife 1 was the least biased estimator on both sides, while Chao 1 and ICE were the most precise, and Chao 2 and ICE were the most accurate within and outside the fence, respectively.

The total species in 0.4 ha reached 94, with 64 taxa shared between both sides of the fence, showing a similarity of 67%. Notably, 59 registered species were included on the IUCN and NOM-059-SEMARNAT red lists, with 14 taxa standing out due to their low abundance and reduced spatial dispersion, making them the most endangered. Contrary to the hypothesis, the results showed a slight superiority in the number of species outside (80) versus inside (77) the fence. This finding does not concur with Teketay et al. (2018), who concluded that species richness increases within fenced areas.

Conclusion

The conservation role of a 3 km long fence in a tropical dry forest in northwest Mexico was assessed by examining the total number of woody plant species. Built in 1991, the fence restricted access to vehicles, people, and domesticated fauna. It was assumed that the fence would provide a protective advantage, resulting in a greater number of taxa inside the barrier compared to the outside. To investigate this, 40 transects were established, with 20 on each side of the fence. Seven richness estimators and the U Mann-Whitney test were employed for data analysis. The results revealed a total of 77 and 80 species inside and outside the fence, respectively, with the highest number of species found outside.

The Chao 1 and ACE estimators showed the highest values of efficiency in sampling. However, when comparing the number of species between both sides of the fence, no significant differences were found, indicating that the fence did not perform its conjectured function. This may be attributed to the slow growth and development of woody plants in dry seasonal forests, as well as the lack of research on the effects of anthropic impact on tree, shrub, and vine richness outside the fence. The 33-year period since the fence's construction may not have been sufficient to discern changes in woody plant richness.

To expand knowledge on species richness, further studies on non-woody plants and faunal groups are necessary, both at the Playa Tortuguera El Verde Camacho RAMSAR site and in other Natural Protected Areas in Sinaloa and northwestern Mexico.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors extend their gratitude to the Comisión Nacional de Áreas Naturales Protegidas for facilitating the investigation. They also appreciate the valuable suggestions on content and structure provided by Vladimir Márquez Stone, Jorge G. Sánchez Zazueta, Roberto C. Cárcamo Aréchiga, and Juan F. Pio León, which significantly contributed to the manuscript.

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