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Spatial and Temporal Diversity Variation in the *Anopheles* Communities in Malaria-Endemic Regions of Colombia

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Abstract. This study aimed to evaluate at a temporospatial scale, the influence of anthropogenic land cover changes in the *Anopheles* species community composition and diversity in two Colombian malaria-endemic regions, Bajo Cauca and Pacific. To determine variations over time, mosquitoes were collected in two time periods; land cover types were characterized on orthorectified aerial photographs, and landscape metrics were estimated for each locality and period. A temporal dissimilarity analysis to evaluated species replacement and the nestedness species loss/gain showed the influence of the species loss or gain component on *Anopheles* species assemblage (23%). The relationship between land cover variation and *Anopheles* beta diversity, evaluated by regression analysis, showed the effect of forest variation in the *Anopheles* community (β_{sim} and forest $r^2 = 0.9323$; β_{sne} and forest $r^2 = 0.9425$). Furthermore, a canonical correspondence analysis showed that the land cover types associated with *Anopheles* species presence were bare soil, shrub, wet areas, and forest. Results demonstrated the impact of land cover changes attributed to human activities on *Anopheles* population dynamics, over time; this was evidenced as species loss or gain, which was specific to each locality. Notably, the main malaria vectors were dominant in most localities over time, suggesting their tolerance to anthropogenic transformations; alternatively, the environmental changes are providing adequate ecological conditions for their persistence. Finally, the data generated are relevant for understanding the impact that environmental change may have on the dynamics of the neotropical malaria vectors. Thus, this research has potential implications for vector control interventions.

INTRODUCTION

Landscape structure and composition play an important role in mosquito ecology.[1–3] Hence, land cover changes have a direct impact on mosquito species presence and diversity.[4–6] Moreover, anthropogenic changes of the landscape cause environmental modifications that affect species dynamics, affecting their abundances, biodiversity, and geographic distributions.[7–9] Environmental alterations may cause habitat loss by deterioration of appropriate habitats or the establishment of suitable areas for species colonization, resulting in either a decrease in biodiversity or selection with species adaptation to unsuitable areas.[10] Furthermore, human dynamics are also affected because the environmental modifications have effects at the social and economic levels[11]; in public health, they have an impact on the transmission of diseases such as malaria, causing increased risk and occurrence.[12]

Land use and landscape modifications such as deforestation indirectly affect vector capacity because the resultant shifts in weather patterns and temperature rise increase the mosquito reproduction rate.[13] Specifically, for vector-borne diseases like malaria, mosquito vector proliferation affects disease incidence,[11] and species displacement produces changes in malaria distribution.[14–16] Landscape modifications such as open-pit mining may also originate human migration to rural areas,[17] with individuals becoming a suitable mosquito blood source, thus increasing human–mosquito vector contact rate and malaria risk.[18–20] In Latin America, several studies have explored the relationship between land use and land cover changes with malaria.[11,21,22] It has been shown that mining activities have a high impact on the landscape and are related to malaria transmission.[23,24] Likewise, deforestation can increase both the density of larval habitats in the environment and the human–mosquito vector contact rate.[15,16] In particular, deforested areas and forest edges provided adequate habitats for the vector *Anopheles darlingi* in the Brazilian Amazon region.[19] Moreover, deforestation processes in this region increased the number of malaria cases, a phenomenon associated with a forest patch size < 5 km^2.[25] Other human activities that cause landscape modification and deforestation are open-pit mining, fish-farming, and cattle raising; these also favor the presence and abundance of *Anopheles* by promoting larval habitat establishment and proliferation.[26,27] A couple of studies conducted in northwestern Colombia demonstrated that these activities propitiated the formation of suitable larval habitats for the important vectors *An. darlingi* and *Anopheles nuneztovari*.[20,28]

Malaria is a public health problem in Colombia, a country that ranks third in the number of cases in Latin America,[29] with 72,022 cases registered in 2021.[30] The important Colombian malaria-endemic regions where this study was carried out, Bajo Cauca (BC) and Pacific (PAC), together report more than 50% of the total malaria cases in the country.[30] In recent decades, these regions have experienced severe landscape alterations.[31] A few studies conducted in these regions evaluated the relationship between land use and land cover types with *Anopheles* species composition and diversity at specific times.[20,28,32] Considering that for various organisms, a temporospatial effect of anthropogenic land cover changes has had an impact on community biodiversity—with species loss, replacement, or colonization events[13,33,34]—and also that no previous study has estimated the temporal effects of land cover variation in the *Anopheles* communities assemblage in the neotropical region, this study aimed to evaluate, at a temporospatial scale, the impact of anthropogenic land cover changes in the composition and diversity of the *Anopheles* species communities in two malaria-endemic regions of Colombia.

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MATERIALS AND METHODS

Collection sites. *Anopheles* mosquitoes were collected in localities of the Colombian most malaria-endemic regions, BC in the northwest and PAC in the west of the country. The localities in BC were Puerto Astilla in Nechi municipality and Villa Grande in El Bagre. The localities in PAC were Córdoba in Buenaventura municipality and La Playa in Francisco Pizarro (Figure 1). For this work, we took as baseline the data obtained for two localities in BC during 2013 and performed the analyses and generated new data for them in a more recent period (2019). The two localities in PAC had not been evaluated before; therefore, the analyses were performed to generate the data for the two periods to conduct the temporospatial analysis. The main economic activities in BC include cattle raising, open-pit mining, and small-scale agriculture. For many years, this region has also been the largest gold producer in the country.[35] In particular, open-pit mining, mostly exploited with a nonsystematic approach and low government control, has produced significant environmental disruption.[35,36] In PAC, wood extraction and mining are important economic activities and the cause of deforestation and severe environmental disturbance; in recent decades, open-sky and alluvial mining in particular have expanded, resulting in large modifications to the landscape.[37]

Mosquito collection. Mosquitoes were collected in each locality during two periods (2013–2015 and 2019–2021). A similar sampling effort was applied in all collections and periods. Collections were performed over 4 consecutive nights, from 6 PM to 12 AM, each night in two houses up to 1 km apart. Two methodologies were used to maximize collections and reduce sampling bias owed to a particular methodology; two protected human-landing catches (HLC) were conducted under an informed consent agreement and protocol reviewed and approved by Comité de Bioética SIU-UdeA (code 18-35-810); and two barrier screens (70% darkness, 2 m high and 10 m long) located within 10 m from the house being sampled, surrounding its entrance and open spaces, were used to collect resting mosquitoes.[38,39] Taxonomic species assignment was carried out using a morphological key.[40] The polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) of the ITS2 region was used to confirm species assignment.[41–43]

Landscape dataset analysis. Coordinates corresponding to the location of collection sites were registered using a global positioning system (Garmin MAP76 CSX1). A 1.5-km radius landscape area from the collection site was evaluated, which corresponds to the average dispersion range estimated for *Anopheles*.[44] At each sample site and for each collection time, land cover patches were characterized on orthorectified aerial photographs SPOT-6-7. The satellite images were selected from the portfolio Image Hunter of Apollo Mapping®. The images had a resolution of 1.5 m and a minimum area of 100 km^2; before acquisition, each image was checked to verify that an area with a radius of 1.5 km from the sample site had no cloud coverage. Images used were taken within maximum 12 months with respect to mosquito collection. Land cover categories were defined according to the Corine Land Cover methodology adapted for the

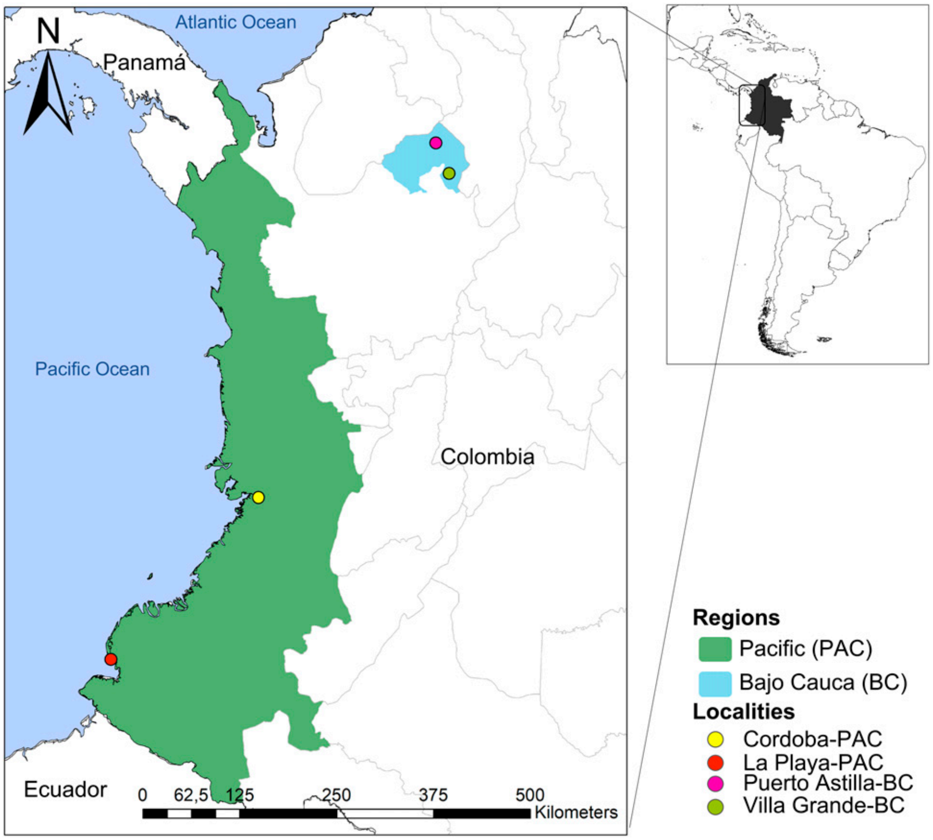

FIGURE 1. Localities where *Anopheles* collections were carried out in the Bajo Cauca and Pacific regions.

Colombian territory, specified in the National Land Cover Legends defined by the Colombian Instituto de Hidrología, Meteorología y Estudios Ambientales.[45] The land cover categories are described as follows: 1) forest—areas with arboreal presence and defined treetops; 2) water bodies—permanent, intermittent, and seasonal water bodies, such as lakes, lagoons, water tanks, natural or artificial freshwater ponds, dams, flowing waters (e.g., rivers and waterways); 3) crops—terrains mainly dedicated to food, fiber, and raw material production; 4) Grass—land occupied by neat grass covering 70% or more of its extension; 5) shrub—mainly shrubby vegetation with irregular dossal and presence of shrubs, palms, and low vegetation; 6) bare soil—territory with scarce or no vegetation composed by burned and bare soils or sandy covers and rocky outcrop; and 7) wet areas—waterlogging zones and swamps in which the phreatic level is at ground level.

Land cover characterization was carried out in the aerial photographs by visual inspection using ArcGIS v. 10.2[46] and corroborated by ground truthing. The landscape indices estimated were as follows: total landscape area, number of classes or covers, patch area, total cover area, percentage cover area, and mean patch size. Measures of landscape fragmentation were also conducted and included, number of patches, number of fragments per unit area or patch density, splitting index, and the effective mesh size. Landscape diversity was estimated using Shannon's diversity index.[47] All landscape indices were obtained in V-LATE 2.0 software.[48] In addition, deforestation rate was estimated as change rate between two periods; the rate was calculated for all land cover types[49] as shown in Equation 1, where A_{t1} is the area in the recent collection period, A_{t0} is the area in the past collection period, t_1 is the year in the recent collection, and t_0 is year in the past collection.

$$\text{Change rate} = \frac{Ln(A_{t1}) - Ln(A_{t0})}{t_1 - t_0} \times 100 \tag{1}$$

***Anopheles* species diversity and data analyses.** *Anopheles* species diversity analyses included number of collected specimens, species richness, Shannon–Weaver index, Simpson, Equitability, and Dominance. The relationship between *Anopheles* species abundance and land cover types was determined for the entire endemic region by canonical correspondence analysis (CCA).[50] The CCA was conducted per collection site using a matrix of species abundances and land cover areas. To conduct the temporal analysis, time variations were included in the CCA matrix as additional sample sites. To correct possible statistical errors associated with rare or dominant species, a logarithmic transformation was applied to the data matrix. Variance inflation factors were calculated to evaluate the noncollinearity among land cover variables. The statistical significance of the CCA model and canonical axes were evaluated by permutation tests. The model and its significance were estimated under the Vegan library[51] in R Studio v. 3.4.1.[52]

Temporal analysis. Variations in species composition assemblage in each locality and between collection periods was evaluated as a measurement of the dissimilarity of assemblages; more precisely, data on species composition from the initial collections were compared with the composition of the more recent collections using the package Betapart[53] for R.[52] For this comparison the following indices were estimated: the overall dissimilarity between the two time periods (Sørensen dissimilarity, β_{sor}) and its beta diversity components, dissimilarity

TABLE 1
Anopheles mosquitos collected in the Bajo Cauca and Pacific localities in two time periods

Region, locality, and coordinates	Species	Past collections September 2013	Recent collections August 2019
Bajo Cauca/Villa Grande	*An. albitarsis*	2	–
N 7° 32′ 0′′	*An. braziliensis*	1	3
W 74° 42′ 16′′	*An. darlingi*	80	159
	An. nuneztovari	22	110
	An. triannulatus	15	–
	Total	120	272
Bajo Cauca/Puerto Astilla		September 2013	August 2019
N 7° 56′ 31′′	*An. albitarsis*	61	16
W 74° 49′ 45′′	*An. braziliensis*	841	232
	An. darlingi	251	1
	An. nuneztovari	2	24
	Near to *An. peryassui*	40	–
	An. punctimacula	50	–
	An. triannulatus	6	2
	An. albimanus	–	1
	An. pseudopunctipennis	–	1
	Total	1,251	277
Pacific/Córdoba		May 2015	May 2019
N 3° 52′ 19′′	*An. nuneztovari*	716	768
W 77° 4′ 11′′	*An. neivai*	–	1
	Total	716	769
Pacific/La Playa		August 2015	September 2021
N 2° 02′ 33.9′′	*An. albimanus*	54	34
W 78° 40′ 14.3′′	*An. calderoni*	8	1
	An. neivai	–	2
	An. apicimacula	–	7
	Total	62	44

TABLE 2

α-Diversity indices for the *Anopheles* community according to time periods by locality

	Bajo Cauca region				Pacific region			
	Villa Grande		Puerto Astilla		Córdoba		La Playa	
	2013	2019	2013	2019	2015	2019	2015	2021
Species richness	5	3	7	7	1	2	2	4
Individuals	120	272	1,251	277	716	769	62	44
Dominance_D	0.494	0.486	0.497	0.712	1	0.997	0.775	0.625
Simpson_1-D	**0.506**	0.466	0.503	0.288	0	0.003	0.223	0.375
Shannon_H	0.949	0.683	**1.011**	0.622	0	0.009	0.385	0.718
Evenness_e^H/S	0.517	0.660	0.393	0.266	1	0.505	0.735	0.513
Equitability_J	0.589	0.622	0.519	0.319	–	0.014	0.555	0.518
Chao-1	5	3	7	8.5	1	2	2	4

Bold text denotes highest diversity values.

due to species replacement (e.g., turnover, β_{sim}) and dissimilarity due to species loss/gain between sampling units or nestedness (β_{sne})—specifically, collection periods. Finally, the relationship between community composition variation and land cover change was estimated using a linear regression that compared the variation of each land cover with the dissimilarity indices β_{sim} and β_{sne}, respectively.

RESULTS

***Anopheles* species diversity.** A total of 3,511 *Anopheles* specimens were collected, 2,149 in the initial collection period and 1,362 in the more recent collections (Table 1). Of notice, the dominant species were the same in both periods and localities. In BC, *An. darlingi* was the most abundant species in Villa Grande locality and *Anopheles braziliensis* in Puerto Astilla. In PAC, *An. nuneztovari* was the most abundant species in Córdoba and *Anopheles albimanus* in La Playa. Diversity estimates for the *Anopheles* community in each period and locality were low (Shannon-W $<$ 2); however, the α-diversity indices differed between periods (Table 2). At the regional level, the BC localities exhibited the highest diversity values in both periods, showing the highest Shannon-W, Simpson, and Equitability indices and a low species dominance (Table 2). Furthermore, Puerto Astilla-BC exhibited the highest species richness, with seven species in both periods, but their composition slightly differed between periods (Tables 1 and 2). In PAC, Córdoba presented the lowest diversity indices, with only one species in the initial collection period and two in the more recent collections, and *An. nuneztovari* was the dominant species in both periods (Table 2).

Landscape structure. Landscape characterization allowed the identification of the land covers present in the perimeter analyzed, 1.5 km of radius from each sampling site. In both periods, forest was the largest landscape matrix in Córdoba-PAC ($>$ 68%) and Villa Grande-BC (42%). However, a

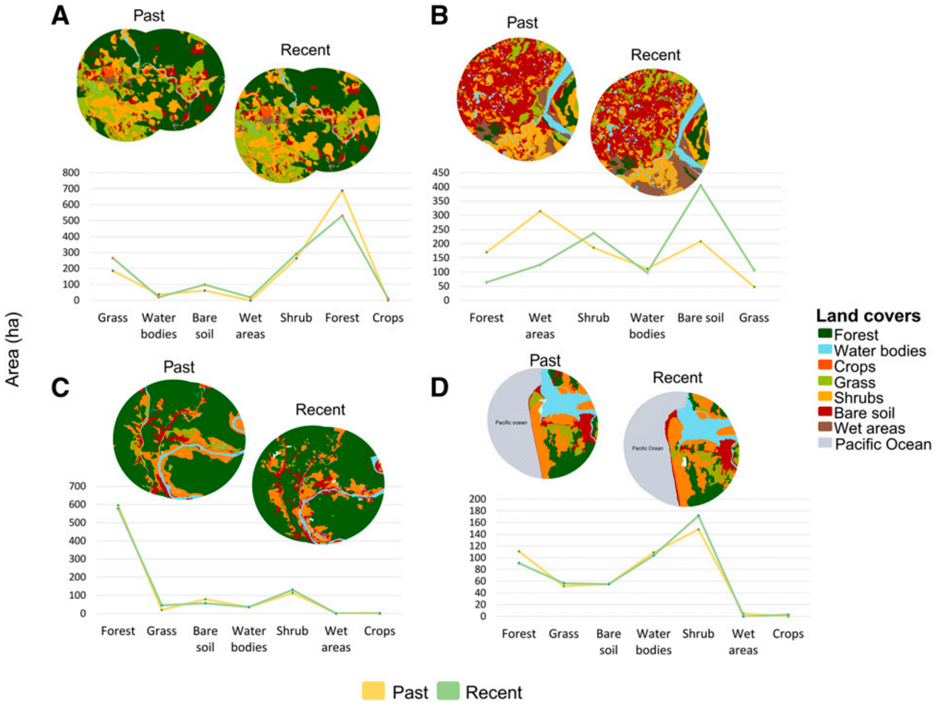

FIGURE 2. Land cover areas registered in both collection periods in the landscapes from (**A**) Villa Grande-BC, (**B**) Puerto Astilla-BC, (**C**) Córdoba-PAC, and (**D**) La Playa-PAC. BC = Bajo Cauca region; PAC = Pacific region.

Table 3

Landscape metrics of the studied areas

		Area (ha)		NP		MPS (ha)		PA (%)		
Region/locality	Land covers	Past	Recent	Past	Recent	Past	Recent	Past	Recent	RC
Bajo Cauca/Villa Grande	Grass	186	265.31	194	18	0.96	32.11	15	21.44	5.91
	Water bodies	37	20.02	97	35	0.38	1.3	3	1.62	−10.23
	Bare soil	62	99.04	269	39	0.23	1.46	5.03	8.00	7.80
	Wet areas	0	19.15	0	40	0	0.89	0	1.55	NA
	Shrub	264	291.63	295	42	0.89	3.1	21.3	23.57	1.65
	Forest	688	530.29	157	1	4.38	0.25	55.58	42.85	−4.33
	Crops	1	11.98	15	1	0.09	0.48	0.1	0.97	41.38
	Total	1,238	1,237.42	1,027	176	–	–	–	–	–
Bajo Cauca/Puerto Astilla	Forest	169.74	63.6	84	10	2.02	6.36	16.38	6.14	−16.36
	Wet areas	314.18	125.77	94	32	3.34	3.93	30.31	12.14	−15.25
	Shrub	185.77	236.81	234	207	0.79	1.14	17.92	22.86	4.04
	Water bodies	111.41	98.25	140	96	0.8	1.02	10.75	9.48	−2.09
	Bare soil	207.82	404.95	118	32	1.76	12.65	20.05	39.09	11.12
	Grass	47.56	106.56	30	70	1.59	1.52	4.59	10.29	13.45
	Total	1,036.47	1,035.94	700	447	–	–	–	–	–
Pacific/Córdoba	Forest	595.45	577.95	23	18	25.89	32.11	70.30	68.23	−0.74
	Grass	19.89	45.48	41	35	0.49	1.3	2.35	5.37	20.67
	Bare soil	78.71	56.92	109	39	0.72	1.46	9.29	6.72	−8.10
	Water bodies	35.38	35.71	109	40	0.32	0.89	4.18	4.22	0.23
	Shrub	112.79	130.25	79	42	1.43	3.1	13.32	15.38	3.59
	Wet areas	1.58	0.25	12	1	0.13	0.25	0.19	0.03	−46.09
	Crops	3.26	0.48	4	1	0.81	0.48	0.38	0.06	−47.89
	Total	847.06	847.04	377	176	–	–	–	–	–
Pacific/La Playa	Forest	110.83	90.85	5	14	22.17	6.49	23.11	18.9	−3.31
	Grass	51.62	56.46	4	7	12.91	8.07	10.76	11.75	1.49
	Bare soil	55.10	54.97	10	5	5.51	10.99	11.49	11.44	−0.03
	Water bodies	109.09	103.97	2	1	109.09	103.97	22.75	21.63	−0.80
	Shrub	148.33	171.69	7	14	21.19	12.26	30.93	35.72	2.43
	Wet areas	4.61	0	1	0	4.61	0	0.96	0	NA
	Crops	0	2.72	0	1	0	2.72	0	0.57	NA
	Total	479.58	480.66	29	42.00	–	–	–	–	–

Ha = hectares; MPS = mean patches size; NP = number of patches; PA = percentage area; RC = rate of land cover change.

reduction in the forest cover was evidenced in all localities; the highest occurred in the BC localities, with Puerto Astilla-BC (16.36%) exhibiting the highest deforestation rate, followed by Villa Grande-BC (4.33%) (Figure 2, Table 3). In Puerto Astilla-BC, the lost forest was replaced mainly by grass and bare soil (land covers) with an increase rate of 24.57%. However, in Villa Grande there was a reduction in the forest land cover (4.33%). The decrease rate in water bodies was higher (10.23%) there, with an increase mainly in crops and bare soil (49.18%). The locality experiencing the lower degree in forest land cover change was Córdoba-PAC, where the reduction rate was 0.74%, with an increase in grass land cover rate (20.67%) (Table 3). In La Playa-PAC, the largest reduction rate was in forest (3.31%) with an increase rate in the shrub land cover (2.43%) (Figure 2, Table 3).

Landscape fragmentation was common to all localities (Tables 3 and 4). The largest degree of fragmentation occurred in the BC compared with the PAC localities in both collection periods. Hence, a higher number of patches was detected in Villa Grande-BC in the initial collection period (1,027), and in Puerto Astilla-BC in the recent period (447) (Table 3). In addition, Villa Grande-BC showed the highest subdivision and splitting index in both periods and a low effective mesh size index, which evidences the large fragmentation occurring in this locality (Table 4). Of note, for all localities except La Playa-PAC, there was a reduction on landscape fragmentation over time, with an increase in the mean patch and effective mesh size indices and a reduction in the number of patches and subdivision and splitting index, which increased in area proportion (Tables 3 and 4). In general, there was a high fragmentation in the initial collection period, with more land cover uniformity during the recent period owing to the increase in land cover area by the connection of isolated patches, and there was an increase in landscape diversity; the highest fragmentation occurred in Puerto Astilla-BC in both periods (Shannon's diversity index = 1.297 and 1.589, respectively) (Table 4).

Species temporal variation. The nestedness component analysis of species temporal variation between the initial and recent collections indicated that, on average, 23% of species were related to the pattern of species loss/gain between periods (β_{sne} mean = 0.23, SD = 0.16). Species turnover was low (β_{sim} mean = 0.07, SD = 0.19), suggesting the effect of loss/gain was dominant over species replacement or turnover. Results of the linear regression that evaluated the relationship between dissimilarity (the beta diversity components β_{sim} and β_{sne}) versus land cover change over time, showed a significant relationship between β_{sim} and forest ($r^2 = 0.9323$, $F_{1,2} = 27.52$, $P = 0.03446$), and β_{sne} and forest ($r^2 = 0.9425$, $F_{1,2} = 32.79$, $P = 0.029$). The comparison of β_{sim} and shrub was not significant, although the P value was close to the cutoff level ($r^2 = 0.889$, $F_{1,2} = 16$, $P = 0.057$) (Figures 3 and 4).

Association between *Anopheles* species abundance and landscape. The CCA analysis allowed identifying five canonical axes. The first two canonical axes explained the main variance of the data; 51% of the variance was explained

TABLE 4
Landscape fragmentation indexes for the land cover types

Region/locality	Land covers	ID		SPLIT		MESH		SHDI	
		Past	Recent	Past	Recent	Past	Recent	Past	Recent
Bajo Cauca/Villa Grande	Grass	96.19	88	26.25	8.34	9.68	31.83		
	Water bodies	79.35	27.94	4.84	1.39	8.41	14.43		
	Bare soil	97.1	94.60	34.46	18.53	2.18	5.34		
	Wet areas	0	84.99	0	6.66	0	2.87		
	Shrub	96.8	88.56	31.23	8.74	11.54	33.37		
	Forest	88.76	75.98	8.9	4.16	93.57	127.37		
	Crops	87.76	46.94	8.17	1.88	0.18	6.36		
	Total	90.99	72.43	18.98	7.10	20.93	31.65	1.217	1.412
Bajo Cauca/Puerto Astilla	Forest	89.17	58.24	9.23	2.39	18.38	26.56		
	Wet areas	69.4	83.10	3.27	5.92	96.13	21.26		
	Shrub	97.97	95.55	49.29	22.47	3.77	10.54		
	Water bodies	36.26	55.16	1.57	2.23	71.01	44.06		
	Bare soil	21.49	11.06	1.27	1.12	163.15	360.16		
	Grass	92.68	88.19	13.67	8.47	3.48	12.58		
	Total	67.83	65.22	13.05	7.10	59.32	79.19	1.297	1.589
Pacific/Córdoba	Forest	79.67	79.74	4.92	4.94	121.06	117.07		
	Grass	94.67	87.27	18.75	7.86	1.06	5.79		
	Bare soil	93.91	65.44	16.42	2.89	4.79	19.67		
	Water bodies	74.51	43.34	3.92	1.76	9.02	20.24		
	Shrub	96.32	85.67	27.14	6.98	4.16	18.67		
	Wet areas	77.69	0.00	4.48	1.00	0.35	0.25		
	Crops	70.13	0.00	3.35	1.00	0.97	0.48		
	Total	83.84	51.64	11.28	3.78	20.20	26.02	0.991	1.027
Pacific/La Playa	Forest	41.95	52.79	1.72	2.12	64.35	42.89		
	Grass	57.78	55.87	2.37	2.27	21.79	24.92		
	Bare soil	49.44	47.39	1.98	1.9	27.85	28.92		
	Water bodies	36.99	103.97	1.59	1	280.85	103.97		
	Shrub	73.53	83.73	3.78	6.15	39.31	27.93		
	Wet areas	0	0	1	0	4.61	0		
	Crops	0	0	0	1	0	2.72		
	Total	43.28	39.96	2.07	2.41	73.13	38.56	1.297	1.543

ID = subdivision index; MESH = effective mesh size; SHDI = Shannon's diversity index; SPLIT = splitting index.

by the first axis and 27% by the second axis (Figure 5). The permutation test was significant ($P < 0.05$) and explained most of the variance. Abundance of *An. nuneztovari* was associated with the forest land cover, whereas *An. darlingi*, *An. triannulatus*, *An. braziliensis*, and *An. albitarsis* abundances were associated to the shrub, bare soil, and wet area land covers. *Anopheles albimanus* and *An. calderoni* were weakly associated to the water bodies land cover.

DISCUSSION

Among the most important economic activities in the Colombian endemic-malaria regions BC and PAC are cattle raising, open-pit mining, small-scale agriculture, and wood extraction. These activities are the cause of extensive deforestation and changes in the land cover and therefore produce significant environmental disturbances that affect mosquito vector and diseases transmission dynamics.[11,25] Previous studies conducted in northwestern Colombia defined the land cover types related to the presence of specific *Anopheles* species[20,32]; however, the temporal variation component was not estimated. This is the first study conducted in Colombia that evaluates the effect of land cover changes occurring over time in the *Anopheles* community assemblage.

Evaluation of beta diversity components, change in species composition and species loss/gain through time evidenced the influence of the nestedness resulting from dissimilarity in local assemblages; this means that there was a loss or gain of *Anopheles* species depending on the locality, over time. Linear regression analysis showed that the change in species composition, loss or gain, was significantly related to variations in the forest land cover ($r^2 < 0.93$) (Figures 3 and 4). These results reinforce that the status of the forest is a relevant factor influencing *Anopheles* community assemblages. In fact, deforestation processes are reported to favor the presence of the main malaria vectors *An. darlingi* and *An. nuneztovari* in endemic regions of South America.[16,32] The relationship between species dissimilarity indices (β_{sim} and β_{sne}) and forest variation demonstrated that modification of the forest land cover affected *Anopheles* community assemblage. In the BC region, increased livestock and mining activities produced a reduction in the forest land cover and an increase in grass and bare soil covers. In this regard, a species loss was evidenced in Villa Grande-BC, where *An. albitarsis* and *An. triannulatus* were detected in the past but not in the recent collection period. These species use breeding sites in riverbanks and water ponds[54,55] that are prone to disappear with an increase in grass and bare soil covers. In the La Playa locality of the PAC region, there was a species gain: *An. apicimacula* was only observed during the most recent collection. Forest reduction in this region was lower than in BC, but there was a moderate increase in grass, shrub, and crop covers, mainly related with predatory logging. Activities such as crop cultivation generate ponds that are larval habitats of *An. apicimacula* and other species of the Punctimacula group.[56–58]

Regarding *Anopheles* spatial community diversity compared at the geographic level, for both ecologically diverse

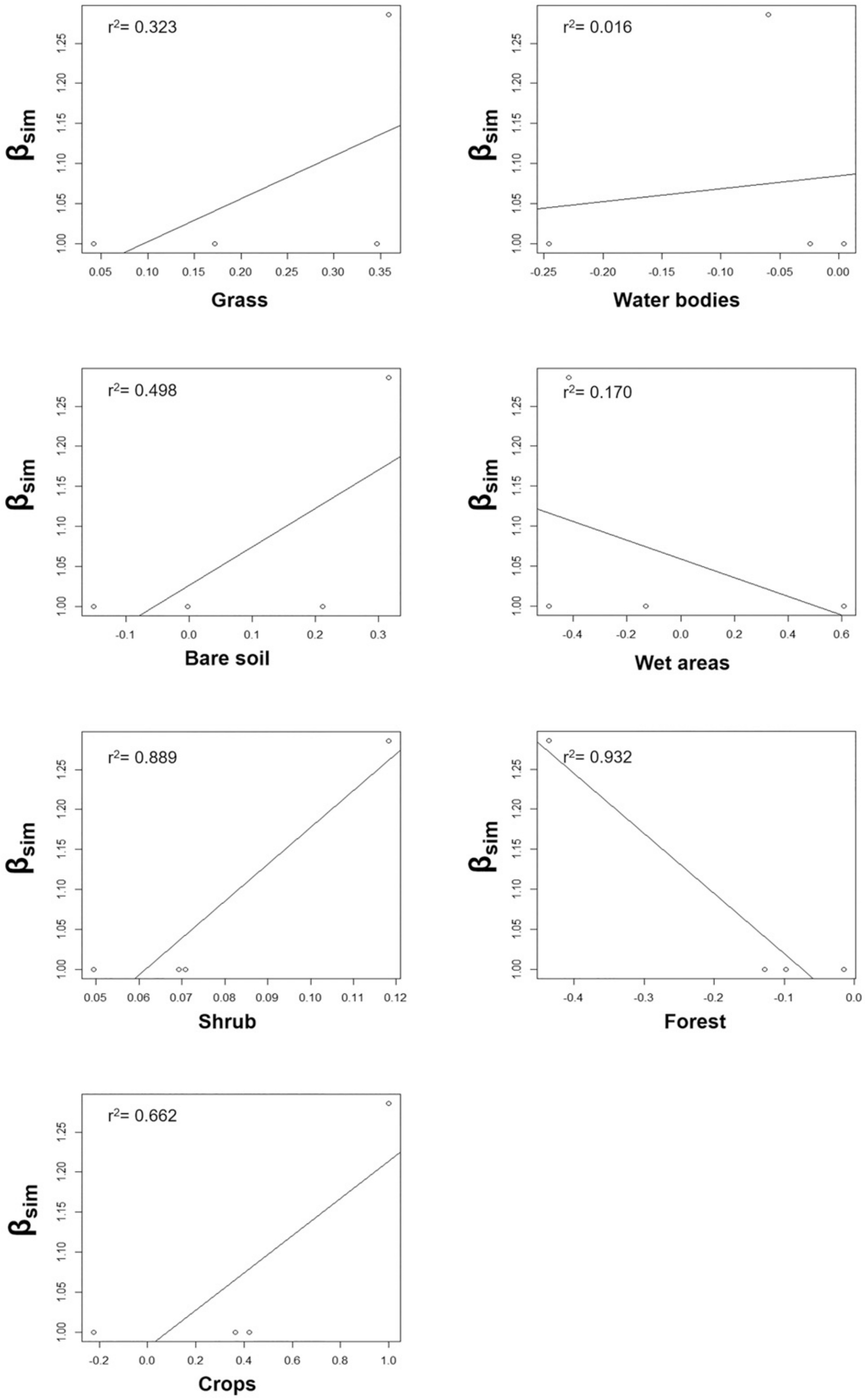

FIGURE 3. Relationship between β_{sim} (value of the turnover component, measured as Simpson dissimilarity) dissimilarity (turnover component, *Y* axis) and land cover changes (*X* axis), determined by linear regression.

regions and in both collection periods, it was observed that the BC localities had the highest α diversity and Shannon's diversity index values. In the PAC region, only in La Playa was there an increase on α diversity in the recent collection period. This seems to be related to an increase in area of some land cover types, such as crops, grass, and shrub. The expansion of a land cover type by the connection of its patches causes a reduction in patch number, leading to an increase in mean patch size. This may propitiate the availability and stability of adequate larval habitats for some

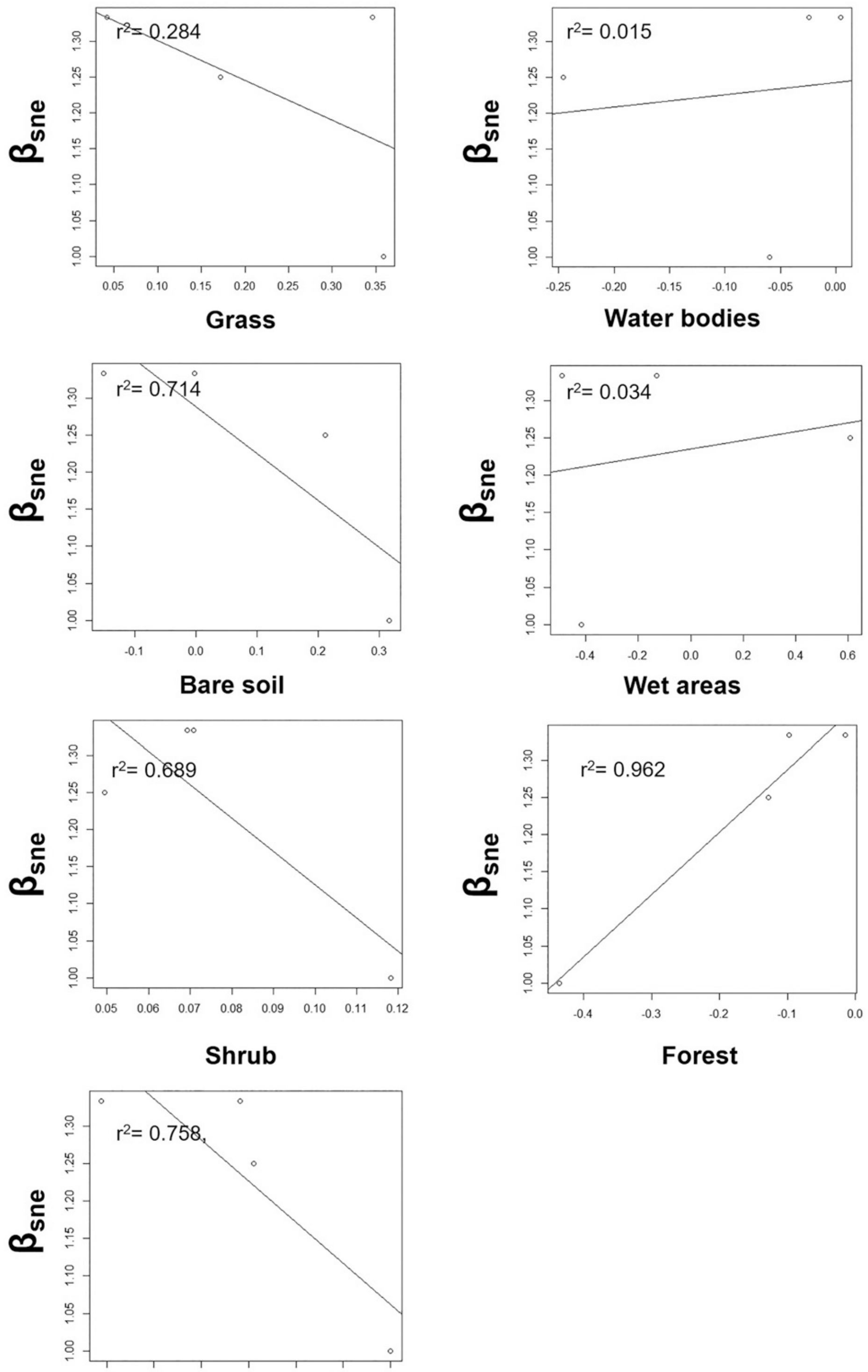

FIGURE 4. Relationship between β_{sne} dissimilarity (nestedness component, *Y* axis) and land cover changes (*X* axis), determined by linear regression.

Anopheles species. Conversely, low α diversity was detected in Córdoba-PAC, owing to the presence and abundance of a single species, *An. nuneztovari.* Historically, BC localities have shown higher *Anopheles* richness compared with those of the PAC region,[20,27,59] which may be due to the higher degree of environmental disturbance in this region. In general, large α diversity values in mosquitos has been related to landscape heterogeneity.[60] The explanation is that heterogeneous landscapes propitiate the formation of a variety of larval habitats, which in turn favors mosquito diversity. Alternatively,

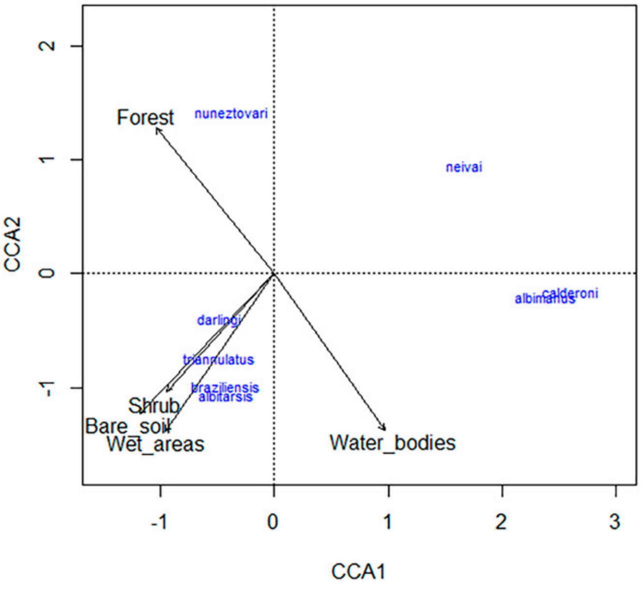

FIGURE 5. CCA assessing the relationship between *Anopheles* abundance and land cover type. CCA = canonical correspondence analysis.

uniformly distributed coverages support larval habitat stability and landscape connectivity, which enables adult dispersal within the landscape.[60] This may be occurring in Córdoba-PAC in the recent collection period, where a reduction in the number of patches with an increase in the area of specific land covers, due to the connection of small patches, was seen; the increase of crop, shrub, and grass land covers with a considerable reduction in the number of patches in forest, bare soil, and wet areas was detected. These environmental characteristics may be influencing the dominance of the important malaria vector, *An. nuneztovari* (Table 4).

Results of the CCA support the association of specific land covers with the presence and abundance of the *Anopheles* species. For example, *An. darlingi* was dominant and abundant in Villa Grande-BC and *An. braziliensis* in Puerto Astilla-BC in both time periods; this seems to be attributable to the transformation of the forest cover to shrub, bare soil, and wet land covers that are known to encourage the formation of suitable larval habitats for these species, such as ponds and shaded and flooded areas.[15,16,20] Other species present such as *An. triannulatus* and *An. albitarsis* may take advantage of these larval habitat types to proliferate.[26,59] *Anopheles nuneztovari*, the dominant species in Córdoba-PAC, did not show dominance in the BC localities, but its abundance increased in the recent collection period; this is probably due to the reduction in forest area. According to previous studies, the transition from forest to other land covers such as grass and shrub favor the presence of *An. nuneztovari*.[20]

In this work, mosquito collections in each locality were 6 years apart for all localities, except for one in the PAC region (Córdoba, 4 years). Despite the limited sampling capability, we could still detect variations in anopheline community composition related to landscape changes. Longitudinal entomological data would be ideal to add further support to our conclusions, particularly in those rural malaria-endemic areas where access was limited for security reasons.

In general, the results of this study, which considered the impact of land cover change occurring over time in the anopheline community composition in localities of the Colombian malaria-endemic regions of BC and PAC, showed differences in the anopheline community assemblages over time. The data evidenced species loss or gain that was specific to the locality and associated with land cover variations. Specifically, the decrease in forest land cover attributed to human activities had the greatest effect in *Anopheles* community variation. The dominance of the main malaria vectors in most localities and over time may be influenced by their tolerance to the anthropogenic transformations; alternatively, the environmental changes may be providing adequate ecological conditions for their presence—for example, the formation of suitable larval habitats.[15,16,27,61] This study, conducted at a macroecological scale, helps to understand the relationships between the *Anopheles* communities and their environment at large spatial scales and over time. Future studies directed at evaluating the ecology of anopheline larval habitats in these localities will help dissect the variables influencing larval productivity more directly, information that is also critical for larval control programs. Finally, the information generated is relevant for understanding the impact that environmental change may have in the dynamics of the neotropical malaria vectors and has implications for disease prevention and control.

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