

Response of dung beetles diversity to remediated soils ecosystems in the Ecuadorian Amazon

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Dung beetles are considered to be habitat quality bioindicators. However, studies regarding the Scarabaeinae diversity in remediated ecosystems previously affected by hydrocarbon activity are null. We evaluated the diversity of dung beetles present in remediated soils previously contaminated with hydrocarbons and heavy metals (Agricultural soils and Sensitive ecosystems) and in non-contaminated soils (Natural forest and Palm plantations) in the Ecuadorian Amazon. Four sampling sites within each type of soil were established. At each site, six pitfall traps were installed and eleven samples were carried out monthly over one year. Each month, the traps remained active for 24 hours during a period of five consecutive days, resulting in a total sampling effort of 880 monitoring days with 330 trap-days per site. A total of 7,506 individuals belonging to 13 genera and 37 species of Scarabaeinae were captured. Mean values of abundance, richness, and diversity differed between ecosystems within each month. The non-contaminated soils ecosystems presented a higher abundance, richness, and diversity of beetles than the remediated soils ecosystems. Natural forest and Palm plantations presented higher abundance, richness, and diversity than Sensitive ecosystems and Agricultural soils, respectively. It can be concluded that dung beetle diversity in soils non-degraded (Natural forest and Palm plantations) do not only depend on the characteristics of the ecosystems, but also on the month of sampling.

Response of Dung Beetles Diversity to Remediated Soils Ecosystems in the Ecuadorian Amazon

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Abstract

Biodiversity in remediated soil ecosystems previously affected by hydrocarbon activities is poorly understood. Therefore, bioindicators such as dung beetles could be a valuable tool to elucidate the benefits of remediation processes. We evaluated the diversity of dung beetles on remediated soil ecosystems (Agricultural soils and Sensitive ecosystems) and on non-contaminated soils (Natural forests and Palm plantations) in Sucumbíos and Orellana provinces, Ecuadorian Amazon. The study was conducted at four sampling sites per ecosystem type (a total of 16 sites). At each sampling site, six pitfall traps were installed and monitored monthly for a 120-hour period during one year. We collected 37 species and 7,506 individuals of dung beetles. We observed significant differences in mean species abundance, richness and diversity between non-contaminated soil ecosystems and remediated soil ecosystems, with Natural forests presenting the highest values, and Agricultural soils the lowest values. Regarding sampling month, we also found significant differences among ecosystems. It is remarkable that between the Agricultural soils (remediated soil ecosystems) and Palm plantations (non-contaminated soils) we found the highest species similarity (34.2%). Variation in dung beetle diversity among ecosystems may aid in decision making to improve remediation processes at sites affected by hydrocarbon activities.

Subjects Biodiversity, Ecology, Entomology

Keywords Scarabaeinae, tropical rain forest, degradation, ecological restoration

Introduction

The extraction of hydrocarbon resources has led to the fragmentation of tropical forests around the world (Abrahams, Griffin & Matthews, 2015; Bogaert et al., 2011; Thomas, Brittingham & Stoleson, 2014). In addition, oil extraction processes often cause accidental spills, which result in contamination of soil and water sources, affecting the environment and hence all life forms (Sajna et al., 2015; Souza, Vessoni-Penna & De Souza Oliveira, 2014). In Ecuador, the Amazon Forest is the most affected site by hydrocarbon activities (Villacís, 2016; Villacís et al., 2016a; Villacís et al., 2016b), where crude oil spills of around 650,000 barrels have negatively impacted the diversity of flora and fauna (Yánez & Bárcenas, 2012; Ministerio del Ambiente, 2016). Due to this situation, the Ecuadorian government has remediated 1,200,098 m³ of contaminated soil (PETROAMAZONAS EP, 2018). The remediation process includes the collection and washing of solid waste; the suction and transport of fluids; the cleaning of contaminated soil; and the revegetation of intervened areas (García-Villacís, 2021). After remediation, each site is catalogued as either Agricultural soil or Sensitive ecosystem in accordance with specific permissible levels of hydrocarbons, cadmium, nickel, and lead established in environmental regulations (Ministerio de Energía y Minas, 2010).

García-Villacís et al. (2021) analyzing the benefits of the remediation process in Ecuador included ecosystem variables such as acidification, terrestrial-, and freshwater-eutrophication, and freshwater ecotoxicity. However, organisms that serve as bioindicators of ecosystem alterations were not included. Changes in the abundance, diversity, and composition of these organisms can elucidate the effects of environmental disturbance (Kremen, 1992; Grand et al., 2017). Among the most commonly used bioindicators are dung beetles (Coleoptera, Scarabaeinae), which are distributed across a wide range of geographical locations (Lumaret & Lobo, 1996; Herzog et al., 2013), have high levels of diversity (Espinoza & Noriega, 2018), and are sensitive to microclimatic changes caused by deforestation and forest fragmentation (Campos & Hernández, 2015; Davis & Sutton, 1998; Davis et al., 2001). Dung beetle communities are commonly decreasing under environmental changes that also affect overall ecosystem health (Otavo, Parrado-Rosselli & Noriega, 2013), since these organisms fulfill important ecological functions such as secondary seed dispersal, decomposition of organic matter, and nutrient cycling (Rangel-Acosta & Martínez-Hernández, 2017; Fernandes et al., 2019).

Several studies have been conducted to assess the diversity of dung beetles in degraded ecosystems (Andresen, 2005; Feer & Hingrat, 2005; Nichols et al., 2007; Sánchez-de-Jesús et al., 2016). Therefore, dung beetle diversity could be a useful tool to better understand the impact of remediation activities on sites previously affected by hydrocarbon activities. It was hypothesized that sites with soil degradation (Sensitive ecosystems and Agricultural soils) decrease dung beetle diversity when compared to non-contaminated soils (Natural forests and Palm plantations). To address this hypothesis, changes in abundance, richness, and diversity of dung beetle communities were evaluated in two types of remediated ecosystems.

Materials & Methods

Ethics statement

This study was authorized under research permit 016-2018-IC-DPAO/AVS and authorization for the mobilization of specimens 005-2019-MOV-FAU-DPAO/AVS, both issued by the Ministerio del Ambiente del Ecuador.

Study area

The study was performed in the Sucumbíos (0° 5'S, 76° 53'W) and Orellana (0° 56'S, 75° 40'W) provinces in the Ecuadorian Amazon. The land use of the localities is distributed in 70% for natural forest, 22% for crops and pastures, and 8% for urban and industrialized areas (Ministerio de Agricultura y Ganadería, 2015). The predominant climate is Humid Tropical Rainforest according to the Holdridge climate classification (Holdridge, 1967), with a mean annual temperature of mean 24.5 ° C and heavy rainfall throughout the year (4132 mm). Mean of climate variables during the study period are showed in table 1.

Selection of collection sites

Sampling sites were established after the completion of the remediation process (~1 year) in ecosystems previously affected by hydrocarbon activities (Sensitive ecosystems and Agricultural soils). The designation as sensitive ecosystems or agricultural soils is based mainly on contaminant levels in accordance with environmental regulations (lower in Sensitive ecosystems) but also on the landscape characteristics of each site (Table 2). In addition, two types of non-contaminated soil ecosystems used as controls (Natural forests and Palm plantations) were also included. Before the beginning of the study, a composite soil sample (5 per sampling site) was collected in order to determine the total petroleum hydrocarbon and polycyclic aromatic hydrocarbon concentrations (Table 2) using GC2 014 gas chromatographs (Shimadzu Scientific Instruments, Inc, Columbia, MD, USA). In addition, the concentration of cadmium (Cd), nickel (Ni), and lead (Pb) in soils (Table 2) was determined by using atomic absorption spectrometry (AA-6800; Shimadzu Scientific Instruments, Inc, Columbia, MD, USA) as indicated by the EPA SW-846 method (Le Blanc & Majors, 2001). The analyses were performed at the Soil Laboratory of the Universidad de las Fuerzas Armadas, Ecuador.

Sampling design

Sixteen sample sites (four per each ecosystem type) were established. In each sample site, six pitfall traps baited with pig dung were placed 10 m apart. Pitfall traps consisted of plastic containers of 0.8 L (15 cm depth × 10 cm diameter) buried up to their rims in the soil and containing a solution 50:50 of water with alcohol. The traps remained active at the sites for 120 h per month during one year (February 2018 to January 2019). The amount of dung per trap was ~50 g and was replaced every 24-36 h. In April, 2019 the traps were not evaluated due to conflict with the landowners, who prevented the entrance to the sampling sites in La Joya de los Sachas locality.

Dung beetles were preserved in 70% ethanol, and some specimens were pinned and identified to species using dichotomous keys (Chamorro et al., 2018; Vaz-De-Mello et al., 2011) and comparing with voucher specimens from Museo de Historia Natural "Gustavo Orcés",

(Escuela Politécnica Nacional, Quito, Ecuador). Vouchers were deposited in the Museum of Zoological Researches (Universidad de las Fuerzas Armadas, Sangolquí, Ecuador).

Data analysis

All analyses were performed using the software INFOSTAT (Di Rienzo et al., 2008) in interface with R (R Core Team, 2013). To detect the effectiveness of the inventories, species accumulation curves were created using the Clench method (Moreno & Halffter, 2000; Soberon & Llorente, 1991). In addition, the richness observed in each type of ecosystem was evaluated using the non-parametric estimator Chao 1 (Colwell & Elsensohn, 2014).

The monthly data were grouped to estimate the total species abundance, richness and Shannon diversity index of each plot (Magurran, 1998). Pooled data were used to compare the four types of ecosystems due to the homogeneity among the four sampling sites per ecosystem type.

Abundance, richness, and diversity were analyzed by using repeated measures (by month). Differences between ecosystems were analyzed using analysis of variance with mixed models for a complete randomized design with six replications. In addition, we performed orthogonal contrast for treatments. The first contrast evaluated differences in abundance, richness, and diversity between remediated soil ecosystems and non-contaminated soil ecosystems. The second contrast evaluated the differences between Agricultural soils and Palm plantations, and the third contrast evaluated the differences between Sensitive ecosystems and Natural forests. Furthermore, we tested for differences between ecosystems, months and interactions by using a DGC post hoc test ($P < 0.05$). The normality of the data was verified using the Shapiro-Wilks test, and the homoscedasticity was modeled using independent variances.

The Sørensen index was used to compare the similarity of dung beetle species composition between each type of ecosystem evaluated. Finally, a dendrogram was prepared using this information (Beals, 1984).

Results

We collected 7,506 individual beetles of the Scarabaeinae subfamily, belonging to 13 genera and 37 species (Table 3). Specific abundance varied greatly, ranging from one to 1,502 individuals (an average of $202.86 \text{ individuals} \pm 52.81 \text{ SE}$ per species). *Canthon aequinotialis* (20% of total abundance), *Ontherus sulcator* (13%), *Dichotomius ohausi* (10%), and *Deltochilum howdeni* (9%), accounted for 52% of all individuals collected. Fifty-one percent of the species were classified as rare, with a relative frequency of less than ten percent. Twenty-two percent of the total species collected were found in all four evaluated ecosystems, while 12 others were exclusive to one of them: 11 in the natural forest and one in the Sensitive Ecosystem.

Canthidium aurifex, *Eurysternus atrosericus*, *Ontherus sulcator*, *Onthophagus osculatii*, and *O. nyctopus* are new provincial records, whereas *O. hircus* is a new record for Ecuador (Table 3).

As the sampling time increased, the accumulated richness of the dung beetle decreased from the seventh month in the Natural forests, whereas in the Sensitive ecosystems, Agricultural

soils and Palm plantations, the curves did not stabilize (Fig. 2). The non-parametric estimator of richness (Chao 1) showed that the efficiency of the inventories reached 99.20 % in natural forest, 97.63 % in sensitive ecosystems, 94.34 % in agricultural soils, and 87.35 % in palm plantations.

The average values of abundance, richness, and the Shannon index differed between ecosystems within each month (Table 4).

The orthogonal contrast showed that non-contaminated soil ecosystems contained higher abundance, richness and diversity of beetles in comparison to remediated soil ecosystems ($F_{1, 132} = 313.51$, $P < 0.0001$). Natural forest presented higher abundance, richness and diversity than Sensitive ecosystems ($F_{1, 132} = 313.51$, $P < 0.0001$) and Palm plantations presented higher abundance, richness and diversity than Agricultural soils ($F_{1, 132} = 51.60$, $P < 0.0001$; Fig. 3). The highest monthly average values for abundance (January and November), richness (January, February, September, October and November) and Shannon's index (September and November) were recorded in the Natural forests (Fig. 4).

Cluster analysis showed that Agricultural ecosystems and Palm plantations presented a species similarity of 32.4%, and both were similar to the Sensitive ecosystems at 26.8%. The Natural forests were similar to other ecosystems only at 8.53% (Fig. 5).

Discussion

Our study provides the first quantitative data on dung beetle communities in ecosystems affected by hydrocarbon activities in the Ecuadorian Amazon. For all evaluated ecosystems, the richness of dung beetle communities was greater than 87%, which suggests that minimum changes in species inventories could exist if sampling effort is increased (Feinsinger, 2001).

The species presented in this study represent 17% of the 220 species of dung beetles registered in Ecuador (Chamorro et al., 2018) and more than 50% of previously registered species in the Orellana and Sucumbios provinces. Five species are new for these provinces, whereas *Ontophagus hircus* was recorded for the first time in Ecuador. This demonstrates that beetle diversity must be studied to understand soil disturbance in tropical forests (Beiroz et al., 2017).

Dung beetle diversity

The community structure in the non-contaminated soil ecosystems trends toward high abundance, richness, and diversity when compared to remediated soil ecosystems. The results are similar to those reported by Da Silva, Vaz-de-Mello & Di Mare (2013) and Batilani-Filho & Hernandez (2017), who also found higher values of abundance, richness, and diversity of dung beetles in remnant Atlantic forests (Southern Brazil) and lower values in soils affected by agriculture and deforestation. This could be because natural forests are the habitat of birds and mammals, which provide food resources for dung beetles (Campos & Hernández, 2015; Niero & Hernández, 2017).

Dung beetles are very sensitive to habitat disturbance (Audino, Louzada & Comita, 2014; Campos & Hernández, 2015; Da Silva, Vaz-de-Mello & Di Mare, 2013). Changes in dung beetle

community structure have been reported under low vegetation cover (Nichols et al., 2007) as a result of intense solar radiation on the soil surface, which accelerates the decomposition rate of food sources (Méndez et al., 2019). Moreover, chemical perturbations affect dung beetle communities in several ways such as changes in composition, diversity and population. (Hutton & Giller, 2003; Correa et al., 2022). Therefore, the presence of hydrocarbons and heavy metals in Agricultural soils and Sensitive ecosystems as well as landscape modifications at oil exploitation sites could influence abundance, richness, and diversity of dung beetle assemblages.

Similarly, cluster analyses showed an acute division between the Natural forests and other ecosystems evaluated. This could be because natural forest fragments within human modified landscapes constitute wildlife refuges (Blaum et al., 2009). This trend of decreased dung beetle diversity between the Natural forest and the other ecosystem types follows a general decreasing gradient of diversity and an increase in anthropomorphic disturbances due to contamination and land use (Aninta et al., 2019; McCain, 2005; Stevens & Gavilanez, 2015). For example, Scarabaeinae diversity in Palm plantations was similar to that found in Agricultural soils. This is consistent with previous studies of Fitzherbert et al. (2008) and Harada et al. (2020), which reported that agrochemicals could favor the degradation of soil and nutrients and hence diminish dung beetle diversity.

Temporal variation of dung beetle diversity

The diversity of dung beetles is determined by regional rainfall patterns (Novais et al., 2016). Our results indicated that the diversity of dung beetles in the Natural forests was higher during the month with lower levels of precipitation (October 238 mm month⁻¹) which is consistent with the study of Ibarra-Polesel, Damborsky & Porcel (2015), who studied dung beetles in subtropical ecosystems. However, previous studies demonstrate that higher beetle diversity is linked to the months with elevated values of precipitation (Escobar et al., 2008; Nunes et al., 2016; Rangel-Acosta & Martínez-Hernández, 2017).

Higher dung beetle diversity during the months with lowest levels of rainfall in the evaluated ecosystems may be due to the interference of factors other than rainfall. For example, alteration of microclimates and microhabitats (Medina, Escobar & Kattan, 2002; Noriega & Realpe, 2018; Sánchez-Hernández et al., 2022), changes in trophic structure (Novais et al., 2016), as well as altitudinal gradient effects (Noriega & Realpe, 2018). These factors could affect mainly the mobility, displacement, and genetic flow of organisms between ecosystems (Harvey, Gonzalez & Somarriba, 2006). For example, the Natural forests in the provinces where the study was conducted are under a higher degree of environmental disturbance (deforestation, forest fragmentation, oil spills, population growth, etc.) (Rivera-Parra et al. 2020) and, in general, the entire Amazon basin is under massive degradation due to deforestation (Marin et al. 2022).

Implications for the conservation

The diversity of dung beetles provides a useful tool for assessing the temporary status of remediated sites previously affected by hydrocarbon activities. Although differences in beetle diversity were found between remediated ecosystems, similar recommendations for conservation measures can be made for both Agricultural soils and Sensitive ecosystems. Therefore, Agricultural soils and Sensitive ecosystems should not only be based on the levels of hydrocarbons and heavy metals but also on the diversity of bioindicators.

The presence of dung beetles in the remediation ecosystems provides a guideline for implementing strategies to conserve the existing diversity. However, the conservation of biodiversity in remediated ecosystems depends not only on remediation activities, but also on other anthropogenic activities in the Amazonian tropical forests.

Overall, dung beetle diversity could be used for conservation planning and management of hydrocarbon- and heavy metal-contaminated ecosystems. In addition, our study provides a baseline for future research that may include other environmental variables and activities that modify dung beetle diversity.

Conclusions

Our study shows that in sites where hydrocarbons and heavy metals were present, the abundance, richness and diversity of dung beetles were lower compared to non-contaminated sites. In addition, dung beetle diversity changed throughout the year and was significantly higher in months with low precipitation.

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References

- Abrahams LS, Griffin WM, Matthews HS. 2015. Assessment of policies to reduce core forest fragmentation from Marcellus shale development in Pennsylvania. *Ecological Indicators* 52:153–160 DOI: 10.1016/j.ecolind.2014.11.031.
- Andresen E. 2005. Effects of season and vegetation type on community organization of dung beetles. *Biotropica* 37:291–300.
- Aninta SG, Rocha R, López-Baucells A, Meyer CFJ. 2019. Erosion of phylogenetic diversity in Neotropical bat assemblages: Findings from a whole-ecosystem fragmentation experiment. *Biodiversity and Conservation* 28:4047–4063 DOI: 10.1007/s10531-019-01864-y.
- Audino LD, Louzada J, Comita L. 2014. Dung beetles as indicators of tropical forest restoration success: Is it possible to recover species and functional diversity? *Biological Conservation* 169:248–257 DOI: 10.1016/j.biocon.2013.11.023.

- 286 Batilani-Filho M, Hernandez MIM. 2017. Decline of ecological functions performed by dung
287 beetles in areas of Atlantic forest and contribution of rollers and tunnellers in organic matter
288 removal. *Environmental Entomology* 46:784–793 DOI: 10.1093/ee/nvx091.
- 289 Beals EW. 1984. Bray-curtis ordination: An effective strategy for analysis of multivariate
290 ecological data. *Advances in Ecological Research* 14:1–55 DOI: 10.1016/S0065-2504(08)60168-
291 3.
- 292 Beiroz W, Slade EM, Barlow J, Silveira JM, Louzada J, Sayer E. 2017. Dung beetle community
293 dynamics in undisturbed tropical forests: Implications for ecological evaluations of land-use
294 change. *Insect Conservation and Diversity* 10:94–106 DOI: 10.1111/icad.12206.
- 295 Blaum N, Seymour C, Rossmanith E, Schwager M, Jeltsch F. 2009. Changes in arthropod
296 diversity along a land use driven gradient of shrub cover in savanna rangelands: Identification of
297 suitable indicators. *Biodiversity and Conservation* 18:1187–1199 DOI: 10.1007/s10531-008-
298 9498-x.
- 299 Bogaert J, Barima YSS, Mongo LIW, Bamba I, Mama A, Toyi M, Lafortezza R. 2011. Forest
300 fragmentation: Causes, ecological impacts and implications for landscape management.
301 *Landscape Ecology in Forest Management and Conservation*: 273–296 DOI: 10.1007/978-3-
302 642-12754-0_12.
- 303 Campos RC, Hernández MIM. 2015. Changes in the dynamics of functional groups in
304 communities of dung beetles in Atlantic forest fragments adjacent to transgenic maize crops.
305 *Ecological Indicators* 49:216–227 DOI: 10.1016/j.ecolind.2014.09.043.
- 306 Chamorro W, Marín-Armijos D, Granda V, Vaz-De-Mello FZ. 2018. Checklist with a key to
307 genera and subgenera of dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) present and
308 supposed for Ecuador. *Revista Colombiana de Entomologia* 44:72–100 DOI:
309 10.25100/socolen.v44i1.6545.
- 310 Colwell RK, Elsensohn JE. 2014. EstimateS turns 20: Statistical estimation of species richness
311 and shared species from samples, with non-parametric extrapolation. *Ecography* 37:609–613
312 DOI: 10.1111/ecog.00814.
- 313 Correa C, Ferreira K, Abot A, Louzada J, Vaz-de-Mello F. 2022. Ivermectin impacts on dung
314 beetle diversity and their ecological functions in two distinct Brazilian ecosystems. *Ecological*
315 *entomology* 40(6): 994–1007 DOI: 10.1111/j.1365-2664.2003.00863.x.
- 316 Da Silva PG, Vaz-de-Mello FZ, Di Mare RA. 2013. Diversity and seasonality of Scarabaeinae
317 (Coleoptera: Scarabaeidae) in forest fragments in Santa Maria, Rio Grande do Sul, Brazil. *Anais*
318 *da Academia Brasileira de Ciencias* 85:679–697 DOI: 10.1590/S0001-37652013005000033.
- 319 Davis AJ, Holloway JD, Huijbregts H, Krikken J, Kirk-Spriggs AH, Sutton SL. 2001. Dung
320 beetles as indicators of change in the forests of northern Borneo. *Journal of Applied Ecology*
321 38:593–616 DOI: 10.1046/j.1365-2664.2001.00619.x.
- 322 Davis AJ, Sutton SL. 1998. The effects of rainforest canopy loss on arboreal dung beetles in
323 Borneo: Implications for the measurement of biodiversity in derived tropical ecosystems.
324 *Diversity and Distributions* 4:167–173 DOI: 10.1046/j.1472-4642.1998.00017.x.
- 325 Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW.
326 2008. InfoStat, versión 2018, Grupo InfoStat. Argentina: FCA, Universidad Nacional de
327 Córdoba.
- 328 Escobar F, Halfpeter G, Solís Á, Halfpeter V, Navarrete D. 2008. Temporal shifts in dung beetle
329 community structure within a protected area of tropical wet forest: A 35-year study and its
330 implications for long-term conservation. *Journal of Applied Ecology* 45:1584–1592 DOI:
331 10.1111/j.1365-2664.2008.01551.x.

- 332 Espinoza VR, Noriega JA. 2018. Diversity of the dung beetles (Coleoptera: Scarabaeinae) in an
333 altitudinal gradient in the east slope of los Andes, Napo province, Ecuador. *Neotropical*
334 *Biodiversity* 4:145–151 DOI: 10.1080/23766808.2018.1512199.
- 335 Feer F, Hingrat Y. 2005. Effects of forest fragmentation on a dung beetle community in French
336 Guiana. *Conservation Biology* 19:1103–1112 DOI: 10.1111/j.1523-1739.2005.00087.x.
- 337 Feinsinger P. 2001. Designing field studies for biodiversity conservation. Washington, DC:
338 Island Press.
- 339 Fernandes MG, Costa EN, Dutra CC, Raizer J, Onstad D. 2019. Species richness and community
340 composition of ants and beetles in Bt and non-Bt maize fields. *Environmental Entomology*
341 48:1095–1103 DOI: 10.1093/ee/nvz086.
- 342 Fitzherbert EB, Struebig MJ, Morel A, Danielsen F, Brühl CA, Donald PF, Phalan B. 2008. How
343 will oil palm expansion affect biodiversity? *Trends in Ecology and Evolution* 23: 538–545.
- 344 García-Villacís K, Ramos-Guerrero L, Canga JL, Hidalgo-Lasso. D, Vargas-Jentzsch, P. 2021.
345 Environmental Impact Assessment of Remediation Strategy in an Oil Spill in the Ecuadorian
346 Amazon Region. *Pollutants* 1:234–252 DOI : 10.3390/pollutants1040019.
- 347 Grand C, Pauget B, Villenave C, Le Guedard M, Piron D, Nau J-F, Peres G. 2017. Soil
348 bioindicators as a useful tools for land management and spatial planning processes; a case-study
349 of prioritization of contaminated soil remediation BT - European Geosciences Union general
350 assembly 2017. *Geophysical Research Abstracts* 19:9647.
- 351 Harada LM, Araújo IS, Overal WL, Silva FA. 2020. Comparison of dung beetle communities
352 (Coleoptera: Scarabaeidae: Scarabaeinae) in oil palm plantations and native forest in the eastern
353 Amazon, Brazil. *Revista Brasileira de Entomologia* 64(1): e2019102 DOI: 10.1590/1806-9665-
354 rbent-2019-102.
- 355 Harvey CA, Gonzalez J, Somarriba E. 2006. Dung beetle and terrestrial mammal diversity in
356 forests, indigenous agroforestry systems and plantain monocultures in Talamanca, Costa Rica.
357 *Biodiversity and Conservation* 15:555–585 DOI: 10.1007/s10531-005-2088-2.
- 358 Herzog SK, Hamel-Leigue AC, Larsen TH, Mann DJ, Soria-Auza RW, Gill BD, Edmonds WD,
359 Spector S. 2013. Elevational distribution and conservation biogeography of Phanaeinae Dung
360 Beetles (Coleoptera: Scarabaeinae) in Bolivia. *PLoS ONE* 8:1–11 DOI:
361 10.1371/journal.pone.0064963.
- 362 Holdridge L. 1967. *Life Zone Ecology*. San José, Costa Rica: Tropical Science Center.
- 363 Hutton SA, Giller PS. 2003. The effects of the intensification of agriculture on northern
364 temperate dung beetle communities. *Journal of Applied Ecology* 40: 994–1007 DOI:
365 10.1111/j.1365-2664.2003.00863.x.
- 366 Ibarra-Polesel MG, Damborsky MP, Porcel E. 2015. Copronecrophagous scarab beetles
367 (Scarabaeidae: Scarabaeinae) from Colonia Benitez Educative Natural Reserve, Chaco,
368 Argentina. *Revista Mexicana de Biodiversidad* 86:744–753 DOI: 10.1016/j.rmb.2015.05.011.
- 369 Kremen C. 1992. Assessing the indicator properties of species assemblages for natural areas
370 monitoring. *Ecological Applications* 2:203–217 DOI: 10.2307/1941776.
- 371 Le Blanc G, Majors RE. 2001. A review of EPA sample preparation techniques for organic
372 compound analysis of liquid and solid samples: collecting, preserving, and preparing samples are
373 critical to producing accurate and reliable results in the analysis of organic compounds. *LC-GC*
374 *North America* 19(11): 1120–1127.

375 Lumaret JP, Lobo JM. 1996. Geographic distribution of endemic dung beetles (Coleoptera,
376 Scarabaeoidea) in the Western Palaearctic region. *Biodiversity Letters* 3:192–199 DOI:
377 10.2307/2999676.

378 Magurran A. 1998. *Ecological diversity and its measurement*. NJ: Princeton University Press.

379 Marín FR, Zanón AJ, Monzón JP, Andrade JF, Silva EH, Richter GL, Antolín LA, Ribeiro BS,
380 Ribas GG, Battisti R, Heinemann AB, Grassini P. 2022. Protecting the Amazon forest and
381 reducing global warming via agricultural intensification. *Nature sustainability* DOI:
382 10.1038/s41893-022-00968-8.

383 McCain CM. 2005. Elevational gradients in diversity of small mammals. *Ecology* 86:366–372
384 DOI: 10.1890/03-3147.

385 Medina CA, Escobar F, Kattan GH. 2002. Diversity and habitat use of dung beetles in a restored
386 Andean landscape. *Biotropica* 34(1): 181–187 DOI: 10.1111/j.1744-7429.2002.tb00255.x.

387 Méndez MS, Martínez ML, Araujo PI, Austin AT. 2019. Solar radiation exposure accelerates
388 decomposition and biotic activity in surface litter but not soil in a semiarid woodland ecosystem
389 in Patagonia, Argentina. *Plant and Soil* 445:483–496 DOI: 10.1007/s11104-019-04325-1.

390 Ministerio de Agricultura y Ganadería. 2015. Visor cartografía temática Ecuador SIG.
391 TIERRAS. MaGAP, Ecuador. Available at
392 <http://geoportal.sigtierras.gob.ec:8080/GeoserverViewer/> (accessed 9 July 2022).

393 Ministerio de Energía y Minas. 2010. Reglamento sustitutivo del reglamento ambiental para las
394 operaciones hidrocarburíferas en el Ecuador. Available at
395 [https://www.gob.ec/sites/default/files/regulations/2018-09/Documento_RAHOE-DECRETO-](https://www.gob.ec/sites/default/files/regulations/2018-09/Documento_RAHOE-DECRETO-EJECUTIVO-1215.pdf)
396 [EJECUTIVO-1215.pdf](https://www.gob.ec/sites/default/files/regulations/2018-09/Documento_RAHOE-DECRETO-EJECUTIVO-1215.pdf) (accessed 3 July 2019).

397 Ministerio del Ambiente. 2016. Programa de reparación ambiental y social (PRAS). Available at
398 <https://www.ambiente.gob.ec/programa-de-reparacion-ambiental-y-social-pras/> (accessed 18
399 August 2020).

400 Moreno CE, Halffter G. 2000. Assessing the completeness of bat biodiversity inventories using
401 species accumulation curves. *Journal of Applied Ecology* 37:149–158 DOI: 10.1046/j.1365-
402 2664.2000.00483.x.

403 Nichols E, Larsen T, Spector S, Davis AL, Escobar F, Favila M, Vulinec K. 2007. Global dung
404 beetle response to tropical forest modification and fragmentation: A quantitative literature review
405 and meta-analysis. *Biological Conservation* 137:1–19 DOI: 10.1016/j.biocon.2007.01.023.

406 Niero MM, Hernández MIM. 2017. Influência da paisagem nas assembleias de Scarabaeinae
407 (Coleoptera: Scarabaeidae) em um ambiente agrícola no sul de Santa Catarina. *Biotemas*
408 30(37):37–48 DOI: 10.5007/2175-7925.2017v30n3p37.

409 Noriega JA, Realpe E. 2018. Altitudinal turnover of species in a Neotropical peripheral mountain
410 system: A case study with dung beetles (Coleoptera: Aphodiinae and Scarabaeinae).
411 *Environmental Entomology* 47:1376–1387 DOI: 10.1093/ee/nvy133.

412 Novais SMA, Evangelista LA, Reis-Júnior R, Neves FS. 2016. How does dung beetle
413 (coleoptera: Scarabaeidae) diversity vary along a rainy season in a tropical dry forest? *Journal of*
414 *Insect Science* 16:1–6 DOI: 10.1093/jisesa/iew069.

415 Nunes CA, Braga RF, Figueira JEC, De Siqueira Neves F, Fernandes GW. 2016. Dung beetles
416 along a tropical altitudinal gradient: Environmental filtering on taxonomic and functional
417 diversity. *PLoS ONE* 11:1–16 DOI: 10.1371/journal.pone.0157442.

418 Otavo SE, Parrado-Rosselli A, Noriega JA. 2013. Scarabaeoidea superfamily (Insecta:
419 Coleoptera) as a bioindicator element of anthropogenic disturbance in an amazon national park.
420 *Revista de Biologia Tropical* 61:735–52.

PETROAMAZONAS EP. 2018. Memoria de gestión 2018. Proyecto Amazonía Viva. Quito. Available at <https://www.petroamazonas.gob.ec/?p=10070> (accessed 15 August 2019).

R Core Team. 2013. R: A language and environment for statistical computing.

Rangel-Acosta JL, Martínez-Hernández NJ. 2017. Comparación de los ensamblajes de escarabajos copronecrófagos (Scarabaeidae: Scarabaeinae) entre fragmentos de bosque seco tropical y la matriz adyacente en el Departamento del Atlántico-Colombia. *Revista Mexicana de Biodiversidad* 88:389–401 DOI: 10.1016/j.rmb.2017.03.012.

Rivera-Parra JL, Vizcarra C, Mora K, Mayorga H, Dueñas JC. 2020. Spatial distribution of oil spills in the north eastern Ecuadorian Amazon: A comprehensive review of possible threats. *Biological Conservation* 252: 108820.

Sajna KV, Sukumaran RK, Gottumukkala LD, Pandey A. 2015. Crude oil biodegradation aided by biosurfactants from *Pseudozyma* sp. NII 08165 or its culture broth. *Bioresource Technology* 191:133–139 DOI: 10.1016/j.biortech.2015.04.126.

Sánchez-Hernández G, Gómez B, Chamé-Vázquez ER, Navarrete-Heredia JL, González-Martín Del Campo F. 2022. Dung beetle diversity and community composition along a fragmented landscape in an altitudinal gradient in southeastern Mexico. *Biologia* 77: 1027–1038 DOI: 10.1007/s11756-022-01036-4.

Sánchez-de-Jesús HA, Arroyo-Rodríguez V, Andresen E, Escobar F. 2016. Forest loss and matrix composition are the major drivers shaping dung beetle assemblages in a fragmented rainforest. *Landscape Ecology* 31:843–854 DOI: 10.1007/s10980-015-0293-2.

Soberon M, Llorente B. 1991. Accumulation the use of species for the prediction functions of species richness. *Conservation Biology* 7:480–488.

Souza EC, Vessoni-Penna TC, De Souza Oliveira RP. 2014. Biosurfactant-enhanced hydrocarbon bioremediation: An overview. *International Biodeterioration and Biodegradation* 89:88–94 DOI: 10.1016/j.ibiod.2014.01.007.

Stevens RD, Gavilanez MM. 2015. Dimensionality of community structure: Phylogenetic, morphological and functional perspectives along biodiversity and environmental gradients. *Ecography* 38:861–875 DOI: 10.1111/ecog.00847.

Thomas EH, Brittingham MC, Stoleson SH. 2014. Conventional oil and gas development alters forest songbird communities. *Journal of Wildlife Management* 78:293–306 DOI: 10.1002/jwmg.662.

Vaz-De-Mello FZ, Edmonds WD, Ocampo FC, Schoolmeesters P. 2011. A multilingual key to the genera and subgenera of the subfamily Scarabaeinae of the New World (Coleoptera: Scarabaeidae). *Zootaxa* 2854: 1–73.

Villacís J. 2016. Evaluación de las técnicas de remediación vegetal utilizadas en plataformas petroleras mediante estudios del desempeño de especies y análisis de diversidad funcional. D. Phil. Thesis, Universidad Nacional de Córdoba.

Villacís J, Armas C, Hang S, Casanoves F. 2016a. Selection of adequate species for degraded areas by oil-exploitation industry as a key factor for recovery forest in the Ecuadorian Amazon. *Land Degradation and Development* 27:1771–1780 DOI: 10.1002/ldr.2511.

Villacís J, Casanoves F, Hang S, Keesstra S, Armas C. 2016b. Selection of forest species for the rehabilitation of disturbed soils in oil fields in the Ecuadorian Amazon. *Science of the Total Environment* 566–567:761–770 DOI: 10.1016/j.scitotenv.2016.05.102.

Yáñez P, Bárcenas M. 2012. Tolerance levels to hydrocarbons and phytoremediation potential of four plant species in Baeza-El Chaco, Ecuador. *La Granja* 16:23–47.

Figure 1

Location of collection sites in the Sucumbíos and Orellana provinces

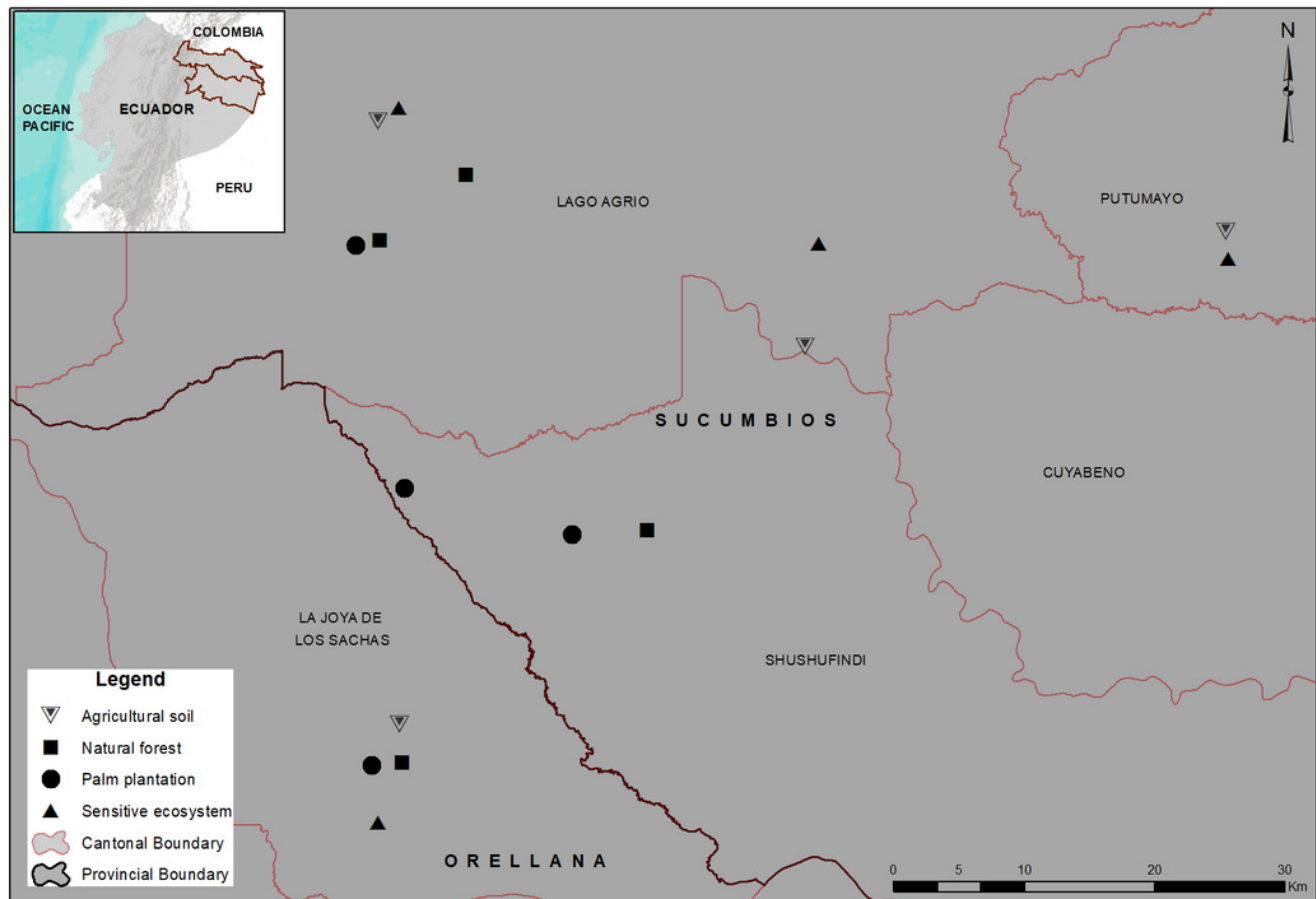


Figure 2

Species accumulation curves of Scarabaeinae communities recorded in remediated soil ecosystems and non-contaminated soil ecosystems in Ecuadorian Amazon

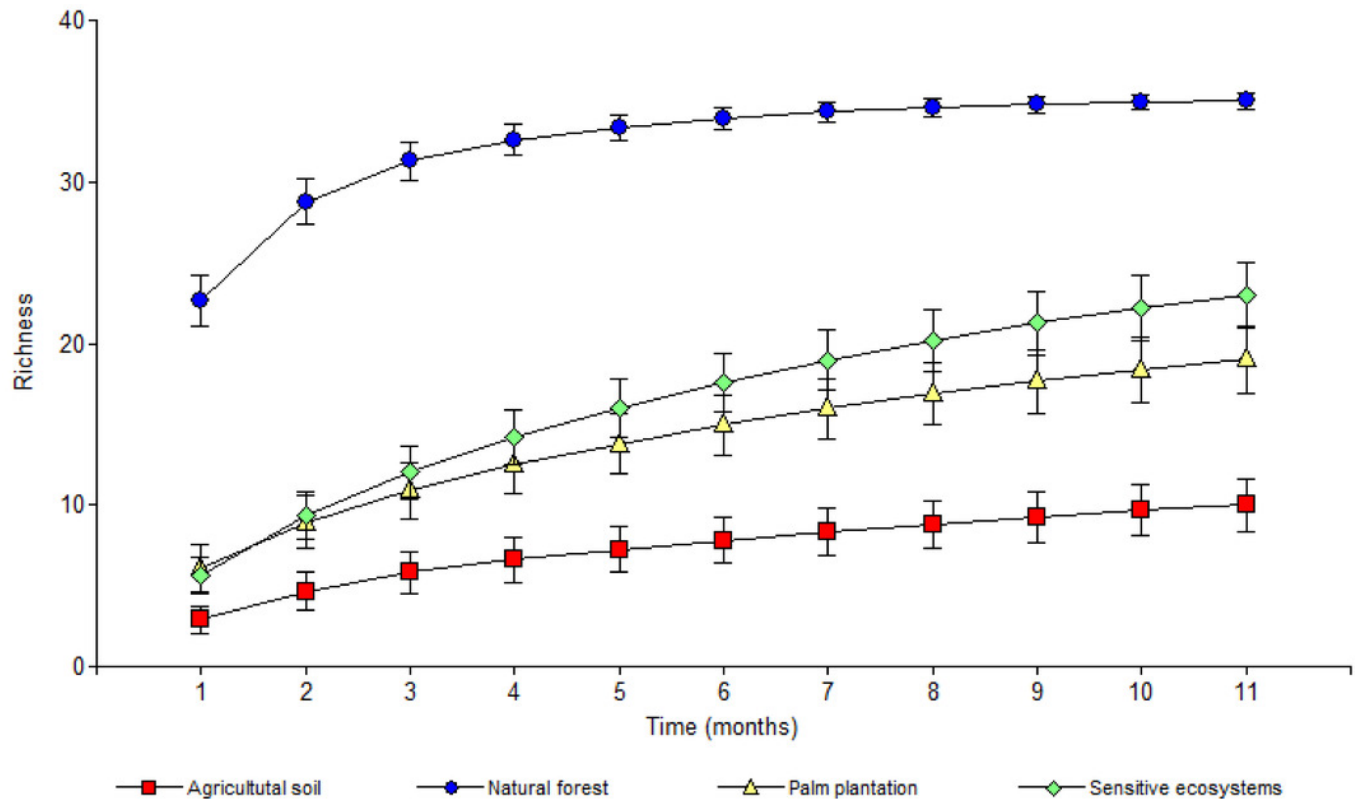


Figure 3

Abundance, richness, and diversity of beetles that were collected in remediated soil ecosystems and non-contaminated soil ecosystems in the Ecuadorian Amazonia

Bars represent means \pm standard error (n = 44)

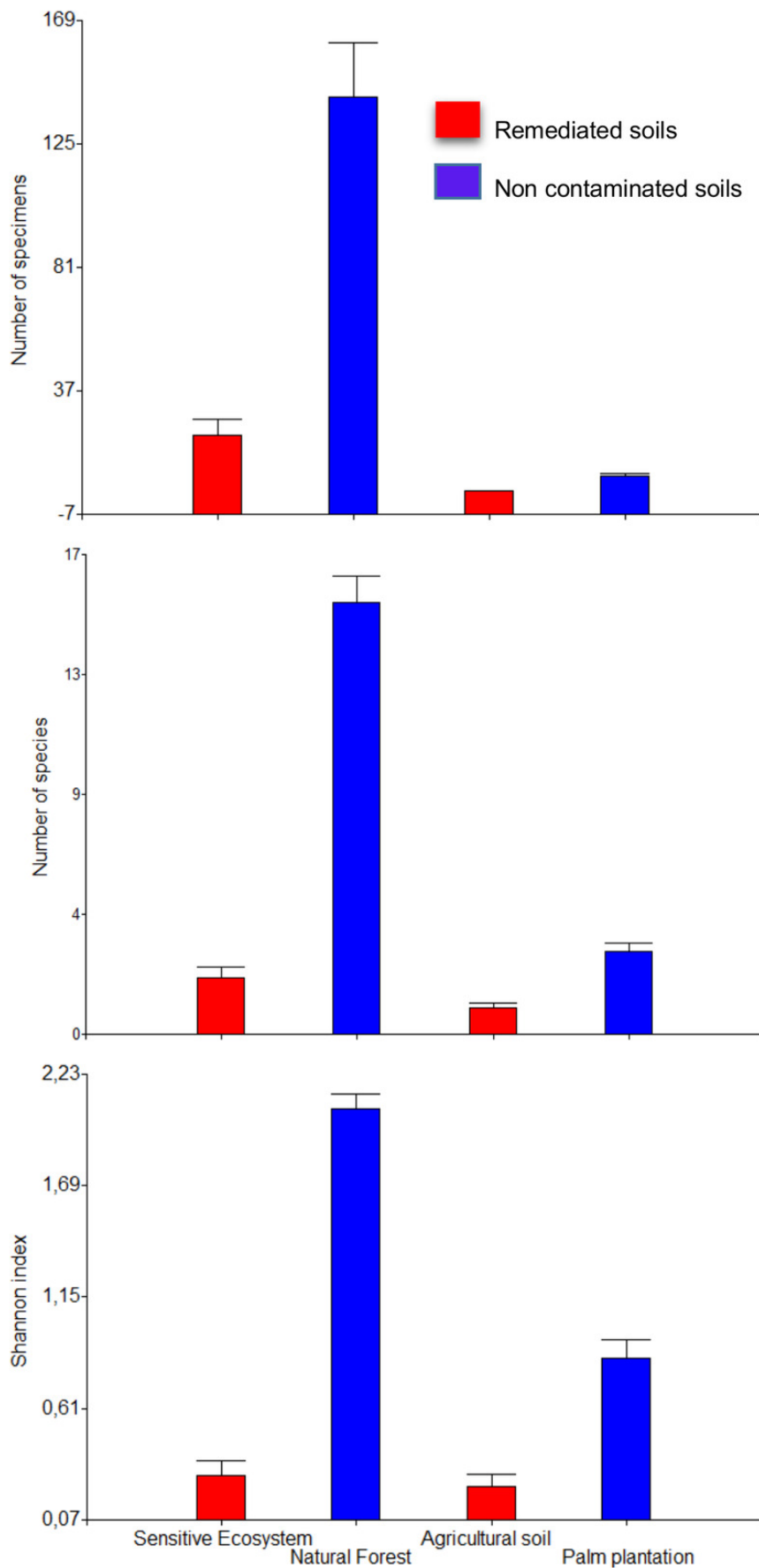


Figure 4

Abundance (top), richness (middle), and diversity (bottom) of beetles that were collected monthly on remediated soil ecosystems and non-contaminated soil ecosystems in the in the Ecuadorian Amazon

Values are means \pm standard error (n = 4). Different letters in each point indicate significant differences (DGC, P < 0.05)

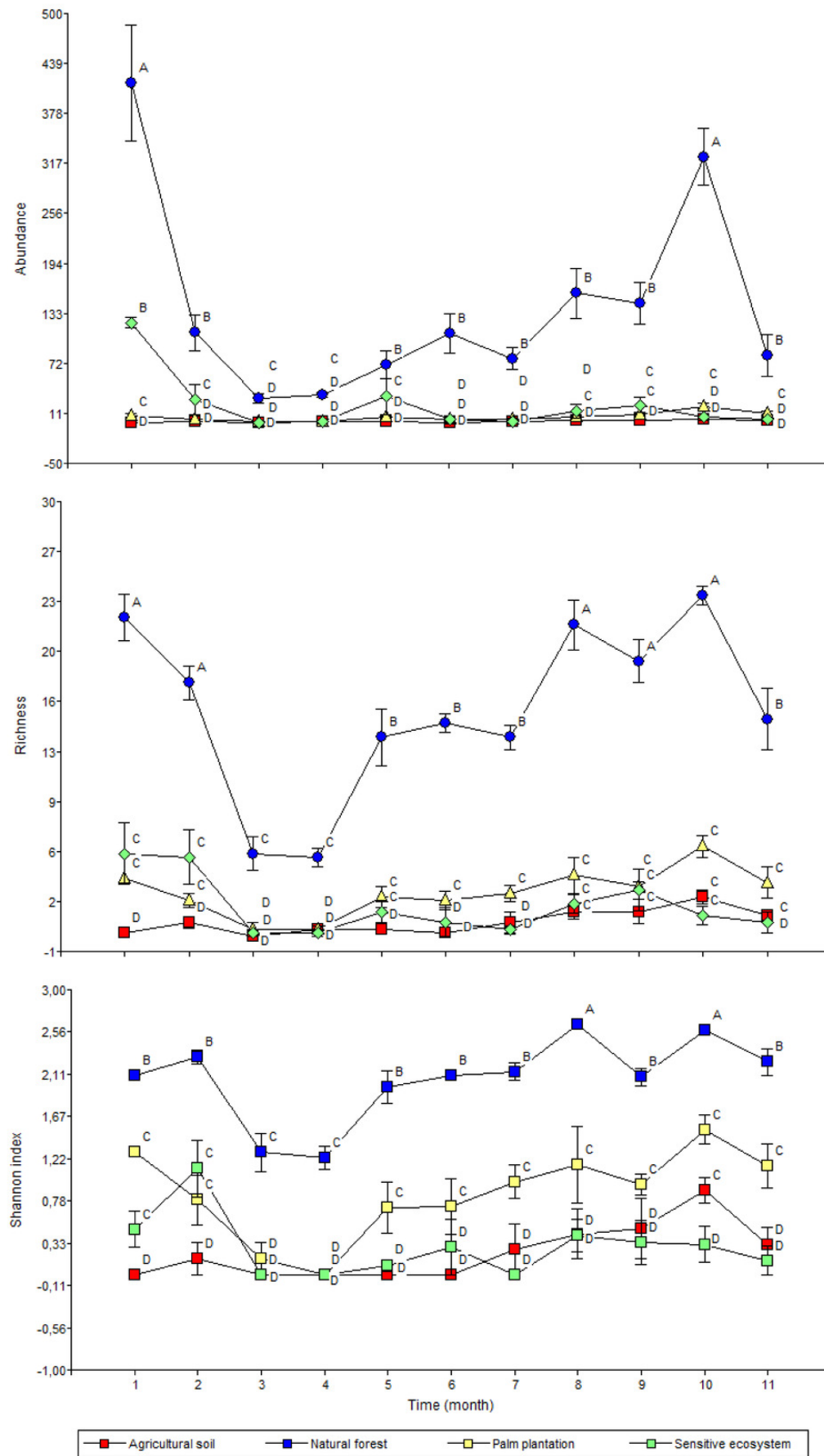


Figure 5

Species similarity clusters based on the Bray-Curtis distance of the Sorensen similarity percentage in remediated soil ecosystems and non-contaminated soil ecosystems in the Ecuadorian Amazon

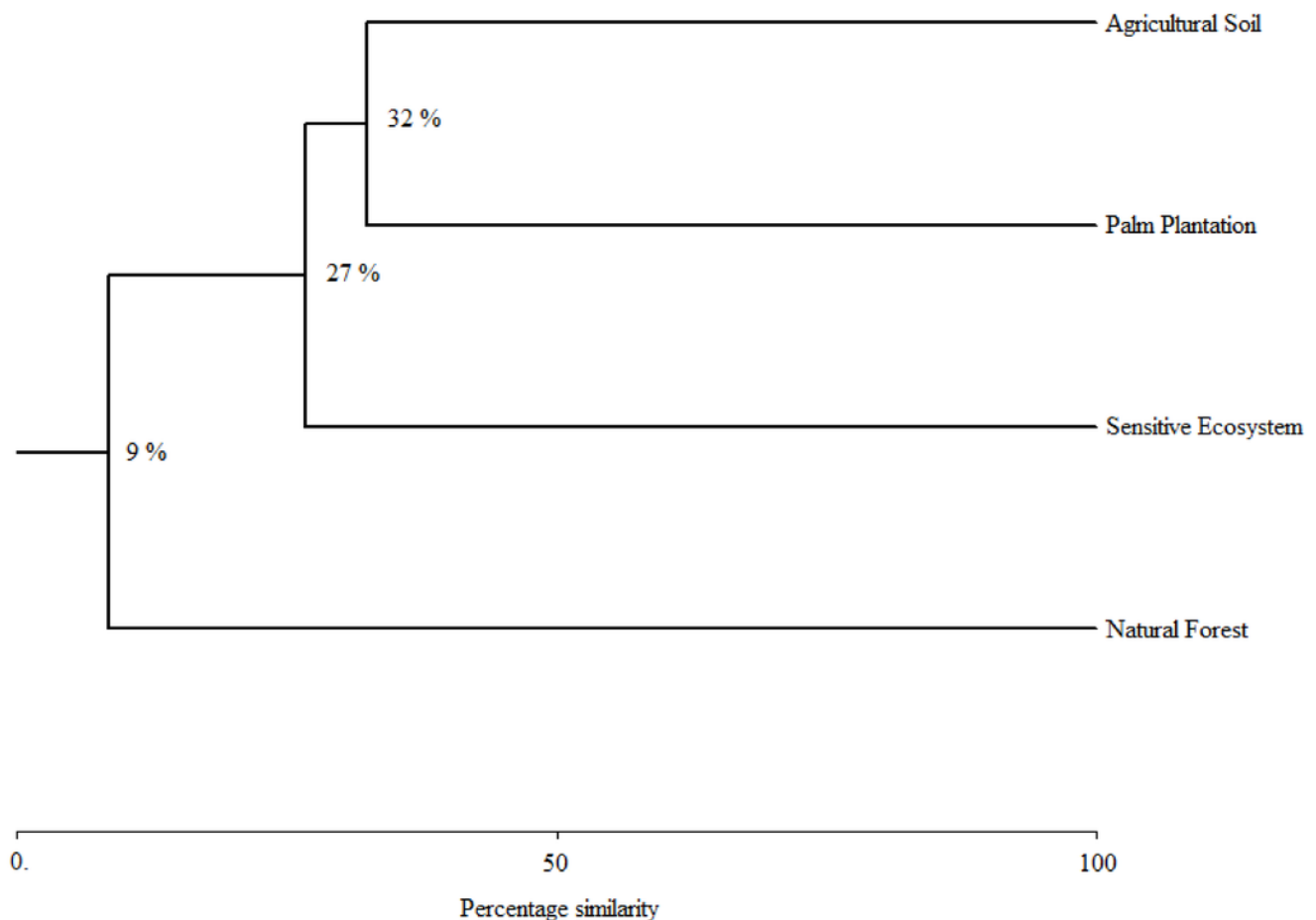


Table 1 (on next page)

Mean values of climatic variables in the ecosystems evaluated

Order	Month/Year	Rainfall mm	Temperature ° C	Humidity %
1	February/2018 ^r	368.81	24.36	90.86
2	March/2018 ^r	429.65	24.2	92.14
3	May/2018 ^r	372.02	24.2	93
4	June/2018 ^r	381.61	23.5	91.43
5	July/2018 ^d	322.25	23.96	90
6	August/2018 ^d	293.75	24.08	89
7	September/2018 ^d	248.15	24.48	88.17
8	October/2018 ^d	238.49	24.93	89.14
9	November/2018 ^d	298.9	24.92	88.5
10	December/2018 ^r	350.52	24.46	89
11	January/2019 ^r	376.31	24.76	89.5

^r= High rainfall, ^d= Low rainfall

Table 2(on next page)

Soil characteristics of the remediated soil ecosystems and non-contaminated soil ecosystems in the Ecuadorian Amazon

1

Variable	Remediated soil ecosystems		Non-contaminated soil ecosystems	
	Sensitive ecosystems	Agricultural soils	Natural forest	Palm plantations
	Application of air and water to the soils with compressors and high-pressure pumps to release crude oil (Petroamazonas EP, 2018)			
Remediation Activities	Yes	Yes	No	No
Plot size (ha)	1	1	1	1
Total hydrocarbons (mg kg ⁻¹)	< 1 0000	< 2 500	No	No
Polycyclic Aromatic Hydrocarbons (mg kg ⁻¹)	< 1	< 2	No	No
Cadmium (mg kg ⁻¹)	< 1	< 2	No	No
Nickel (mg kg ⁻¹)	< 40	< 50	No	No
Lead (mg kg ⁻¹)	< 80	< 100	No	No
Agrochemical Use	No	No	No	Yes (herbicides and fungicides)
Tree cover presence	Yes	No	Yes	No
Number of present strata	2	0	4	2
			<i>Ceiba pentandra</i> , <i>Otoba parvifolia</i> , <i>Pouteria aubrevillei</i> , <i>Inga</i> sp., <i>Nectandra guararipo</i> , <i>Cordia alliodora</i> .	
Most abundant tree species	<i>Otoba parvifolia</i> , <i>Guarea</i> sp., <i>Pouroma</i> sp.	No		<i>Elaeis guianensis</i>
Mean number of trees DAP > 10 cm per ha	3.6	-	21.47	143
DAP mean ± SE (cm)	44.13±1.69	-	45.72±1.61	35.45±4.54
Mean total height ± SE (m)	14.54±0.65	-	16.23±0.25	18.45±2.21

2

Table 3(on next page)

Dung beetle species collected in remediated soil ecosystems and non-contaminated soil ecosystems in the Ecuadorian Amazon

1

No.	Species	Record type	Agricultural soils	Natural forest Total	Sensitive ecosystems Total	Palm plantations	Assemblage
1	<i>Canthon aequinoctialis</i> Harold, 1868	RE		1 490	12		1 502
2	<i>Ontherus sulcator</i> Fabricius, 1775	NR-P	18	135	747	90	990
3	<i>Dichotomius ohausi</i> Luederwaldt, 1923	RE	7	639	17	72	735
4	<i>Deltochilum howdeni</i> Martínez, 1955	NR-P		671	13	1	685
5	<i>Onthophagus haematopus</i> Harold, 1887	RE		493	7		500
6	<i>Eurysternus plebejus</i> Harold, 1880	RE	6	386	4	30	426
7	<i>Onthophagus osculatii</i> Guérin-Méneville, 1855	NR-P		395	7		402
8	<i>Coprophaneus telamon</i> Erichson, 1847	RE	11	307	22	33	373
9	<i>Onthophagus xanthomerus</i> Bates, 1887	RE		246	22	5	273
10	<i>Dichotomius</i> sp. 1	NA		232		1	233
11	<i>Dichotomius mamillatus</i> Felsche, 1901	RE		186	2	1	189
12	<i>Deltochilum amazonicum</i> Kolbe, 1905	RE		175		3	178
13	<i>Eurysternus atrosericus</i> Génier, 2009	NR-P	1	134	5	5	145
14	<i>Eurysternus squamosus</i> Génier, 2009	RE		107			107
15	<i>Canthon luteicollis</i> Erichson, 1847	RE		92	1	1	94
16	<i>Eurysternus caribaeus</i> Herbst, 1789	RE	4	76		13	93
17	<i>Eurysternus wittmerorum</i> Martínez, 1988	RE	1	61	7	10	79
18	<i>Canthidium</i> cf. <i>rufinum</i> Harold, 1867	RE		76			76
19	<i>Onthophagus hircus</i> Billberg, 1815	NR-E	10		40	6	56
20	<i>Onthophagus nyctopus</i> Bates, 1887	NR-P		52	2		54
21	<i>Dichotomius podalirius</i> Felsche, 1901	RE		49			49
22	<i>Uroxys</i> sp. 1	NA		48			48
23	<i>Eurysternus foedus</i> Guérin-Méneville, 1844	RE	3	30	1	6	40
24	<i>Phaneus chalcomelas</i> Perty, 1830	RE		28	3	1	32
25	<i>Scyballocanthon macullatus</i> Schmidt, 1920	RE		31	1		32
26	<i>Oxysternon silenus</i> d'Olsoueffieff, 1924	RE	1	4	1	16	22
27	<i>Eurysternus hamaticollis</i> Balthasar, 1939	RE		19	1	1	21
28	<i>Onthophagus onore</i> Zunino & Halfpiter, 1997	RE		15			15
29	<i>Canthidium</i> sp. 1	NA		10	3	1	14
30	<i>Canthidium aurifex</i> Bates, 1887	NR-P		9	3		12
31	<i>Oxysternon conspicillatum</i> Weber, 1801	RE		8			8
32	<i>Deltochilum carinatum</i> Westwood, 1837	RE		7			7
33	<i>Onthophagus marginicollis</i> Harold, 1880	RE			6		6
34	<i>Scyballocanthon furvus</i> Schmidt, 1920	NA		4			4
35	<i>Canthidium onitoides</i> Perty, 1830	RE		3			3
36	<i>Malagoniella astyanax</i> Halfpiter, Pereira & Martínez, 1960	RE		2			2
37	<i>Canthon angustatus</i> Harold, 1867	RE		1			1
Abundance			62	6 221	927	296	7 506

2
3
4

Richness	10	35	23	19	37
Data in bold indicate species with greatest specific abundance by ecosystem type in the study. RE = registered in the provinces studied, NR-P = newly registered in the provinces studied, NA = not evaluated, NRE = newly registered in Ecuador					

Table 4(on next page)

Analysis of variance for the abundance, richness, and diversity of beetles that were collected monthly in remediated soil ecosystems and non-contaminated soil ecosystems in the Ecuadorian Amazon

Ecosystems were considered fixed factor and months random factor

Source	df	Abundance		Richness		Shannon	
		F	P	F	P	F	P
Ecosystems types	(3)	302.09	< 0.0001	462.21	< 0.0001	314.65	< 0.0001
Remediated soils ecosystems vs. Control ecosystems	1	374.32	< 0.0001	483.13	< 0.0001	313.51	< 0.0001
Agricultural soils vs. Palm plantations	1	991.54	< 0.0001	59.97	< 0.0001	51.60	< 0.0001
Sensitive ecosystems vs. Natural forest	1	289.20	< 0.0001	502.33	< 0.0001	427.16	< 0.0001
Months	10	26.09	< 0.0001	18.63	< 0.0001	9.56	< 0.0001
Ecosystems × Months	30	8.07	< 0.0001	2.41	0.0003	1.90	0.0073

1 df = degrees of freedom; F = result of F-Fisher value; P = result of probability.