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ECOLOGY

Elusive tropical forest canopy diversity revealed through environmental DNA contained in rainwater

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Exploring the biodiversity hidden in tropical rainforests canopies represents a major frontier in biodiversity research yet remains challenging. Environmental DNA (eDNA) can revolutionize this field as it did already in various ecosystems. Here, we test the hypothesis that eDNA contained in canopy throughfall could be used to monitor this elusive diversity and detect anthropogenic disturbance. Using custom-made, low-cost rain collectors, we sampled rainwash eDNA in a mature Amazonian forest and a nearby tree plantation. We successfully detected eDNA from tropical woody and epiphyte plants, vertebrates (mammals, birds, and amphibians), and insects (e.g., mosquitoes, ants, and beetles). The taxonomic composition and diversity reflected disturbance, with significantly lower diversity in the plantation. Crucially, rainwash eDNA integrated biodiversity over a 10-day period in passive collectors and provided a local signature. This approach has thus potential for establishing a cost-effective monitoring system for tropical moist forest canopies, applicable in impact assessments and sustainable management.

INTRODUCTION

Tropical rainforests are home to a rich and largely unexplored biodiversity (1, 2). They also play an essential role in fundamental services such as food supply, water quality regulation, and global climate regulation (3) but are increasingly threatened by tropical deforestation, biological invasions, and climate change (4–6). Within these ecosystems, the forest canopy is a particularly dynamic and important zone, representing the active interface between the terrestrial biosphere and the atmosphere, and a recognized hotspot of biodiversity that still represent a frontier for the discovery of biological diversity (7). However, forest canopies are difficult to access, which limits their scientific exploration and the effectiveness of monitoring programs. It is therefore important to improve methods for biodiversity monitoring in these particular ecosystems.

In recent years, the amplification and sequencing of taxonomically informative DNA fragments from environmental samples (8–10), i.e., environmental DNA (eDNA) metabarcoding, has revolutionized bio-monitoring in all domains of life. The approach relies on the fact that all organisms naturally release DNA through tissue/cell renewal, excretion (e.g., urine and feces) or the release of other material that can adsorb onto environmental matrices. These eDNA sources can thus

be used for biomonitoring. Environmental matrices such as soil (11, 12), invertebrates bulk (13, 14), marine and freshwater (15–17), or air (18–20) are now commonly sampled for these purposes. However, these environmental matrices are selective and poorly suited for the sampling of terrestrial aboveground biodiversity, particularly that of the canopy (21, 22). For example, the DNA found in soil samples mainly belongs to belowground organisms, and the probability of detecting aboveground organisms is very low (10, 21, 22). The ingested DNA (iDNA) contained in hematophagous and/or phytophagous arthropod bulks has been used to sample vertebrates or plant communities (13, 14) but is destructive. The filtration of water from local streams or lakes (15–17) or of air (18–20, 23) can alleviate these limitations but often requires active sample collection, which can be difficult to implement in remote areas. Common to all these sampling methods is the difficulty of characterizing the spatio-temporal extent they cover due to the numerous factors involved in eDNA release, transport, and decay, which further vary across organisms, habitats, and environmental matrices (21, 24–26). Given these limitations, continuing searching for alternative sampling strategies remains critical (21).

Here, we explore another environmental matrix for accessing the biological diversity of the mostly inaccessible tropical ecosystem compartment that is forest canopies with eDNA. As in other terrestrial compartments, organisms inhabiting the canopy naturally release DNA that adsorb onto the vegetation surfaces. This source of eDNA has been analyzed in the past using leaf swabs to detect terrestrial vertebrates (27), but scaling this approach to an entire ecosystem appears impractical, especially in hyperdiverse tropical forests where a considerable sampling effort is required. The manual spraying of water has also been proposed used to collect this source of eDNA and study arthropods (28, 29), but this also appear difficult to implement routinely.

Canopy throughfall water, when it contacts with leaves, can transport and concentrate the DNA adsorbed on plant surfaces. Hence, canopy arthropod species have already been detected in the eDNA

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extracted from rainwash collected under the canopy (29, 30). In addition, the collection of rainwash eDNA is amenable to passive and low-cost devices that do not require any power supply (31, 32). These devices can be left onsite for a certain period, making the sampling less dependent on the timing of rainfall events and providing an integrated temporal signal, especially in tropical forests, characterized by abundant rainfall throughout the year. Collectively, these characteristics suggest that rainwash eDNA sampling holds exceptional promise for large-scale eDNA-based monitoring of tropical forest canopies.

Here, we hypothesize that rainwash eDNA is a suitable method to sample a broad spectrum of plants, vertebrates, and insects from the canopy. We test whether this method captures changes in diversity and composition caused by anthropogenic disturbance. We expect rainwash eDNA to yield a spatially constrained signature due to limited horizontal eDNA transport in this matrix. Last, we hypothesize that this eDNA accumulates over several days, thus providing a temporally integrated signature. To test these hypotheses, we sampled rainwash eDNA from an old growth Amazonian rainforest and a nearby rubber and rosewood plantation (Fig. 1A). To collect canopy rainwash water in a low-cost fashion, we repurposed umbrellas (fig. S1) and equipped them with four types of passive eDNA samplers (Fig. 1B). To estimate the temporal window covered by passive rainwash eDNA sampling, we conducted a first campaign (the “temporal experiment”), measuring the dynamics of plant, vertebrate, and insect diversity and composition using eDNA metabarcoding (see Materials and Methods and table S1). We also estimated rainwash eDNA persistence by spraying carrot juice on canopy leaves at the start of the experiment and quantifying carrot eDNA over time. To estimate the spatial window covered by rainwash eDNA, we conducted a second campaign (the “spatial experiment”), measuring plant, vertebrate, and insect diversity and composition with an increased sampling spatial grain.

RESULTS

Rainwash eDNA captures elusive canopy taxa

We tested whether the taxonomic composition detected from rainwash eDNA corresponded to canopy diversity. To this end, we used as DNA markers a subregion of the chloroplastic gene *rbcL* for plants, a portion of the mitochondrial 12S ribosomal RNA (rRNA) gene for vertebrates, and a portion of the 16S rRNA gene for insects to describe our sample diversity. This choice was a trade-off between several criteria that we discuss in Material and Methods.

We retrieved a total of 10,687 molecular operational taxonomic units (MOTUs), assigned to a total of 562 different taxa [of any taxonomic rank, as we used an assignment method based on the least common ancestor (LCA) approach, see Materials and Methods]. Of these, the plant and vertebrate taxa identified to a fine taxonomic level were known to be present in South America (Fig. 2 and data S1). Note here that the taxonomic annotations retrieved by eDNA should be interpreted as indicative only, as they are inferences representing the closest matches in current reference databases constrained by the database completeness, generally low for tropical organisms (see Materials and Methods), and the DNA marker resolution.

For plants, the taxonomic assignments retrieved encompassed 88 families, and 56 of them were provided at the species level. Thirteen of these taxa corresponded to mosses and liverworts, 14 to ferns including the arborescent ferns family, 3 to gymnosperms, and 16 to green algae. The remaining taxa corresponded to flowering plant families known to occur in the study area, such as Lecythidaceae, Melastomataceae, Fabaceae, Chrysobalanaceae, Vochysiaceae, Euphorbiaceae, Annonaceae, Clusiaceae, and, in much lower proportion of sequencing reads, epiphytes (e.g., Bromeliaceae, Orchidaceae, Pteridaceae, and Polypodiaceae).

For vertebrates, after careful exclusion of common contaminants such as human, beef, and pig (33), the retrieved taxonomic assignments spanned 21 vertebrate orders and 28 families that included

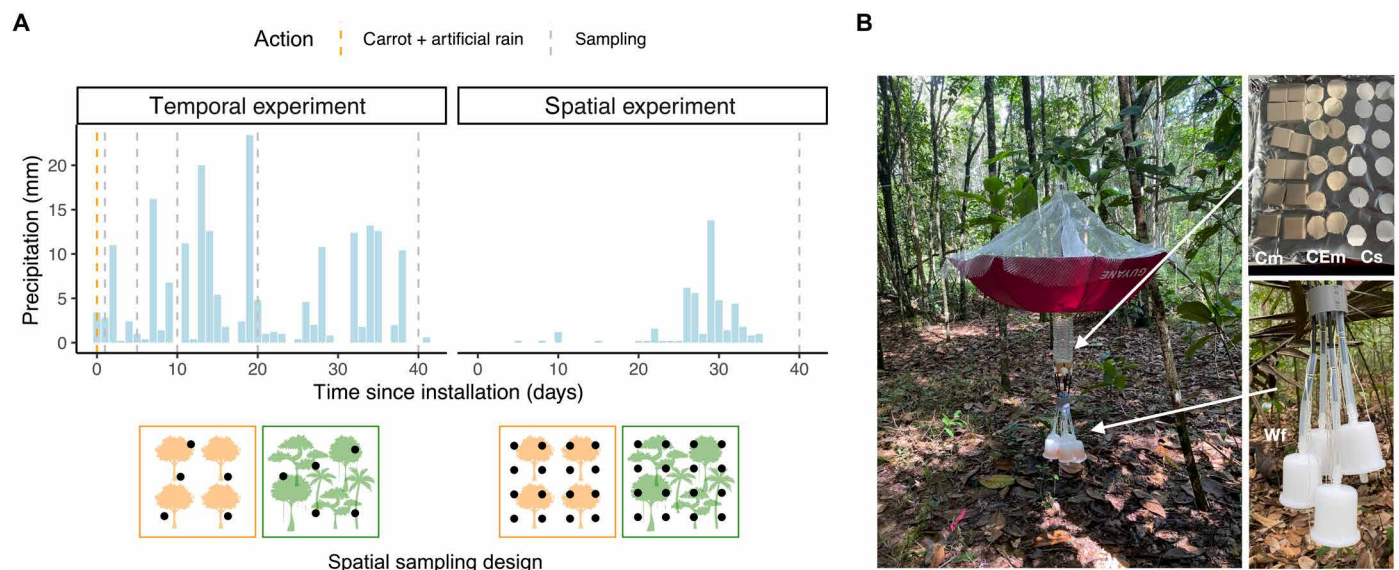


Fig. 1. Overview of the study experimental designs. (A) Description of the temporal and spatial experiments in terms of precipitation received throughout the sampling periods, initial setting (i.e., addition of carrot spike-in or not), and the spatial and temporal distribution of the sampling points. Orange plots: Tree plantation. Green plots: Old-growth forest. (B) Pictures of a canopy rainwash sampler (see also fig. S1) and of passive eDNA samplers through eDNA fixation (Cm, ceramic mosaics; CEm, cellulose ester membrane; Cs, cotton strips) or through rainwash water filtration by gravity (Wf, Waterra filters). Image credits: A.I., CNRS.

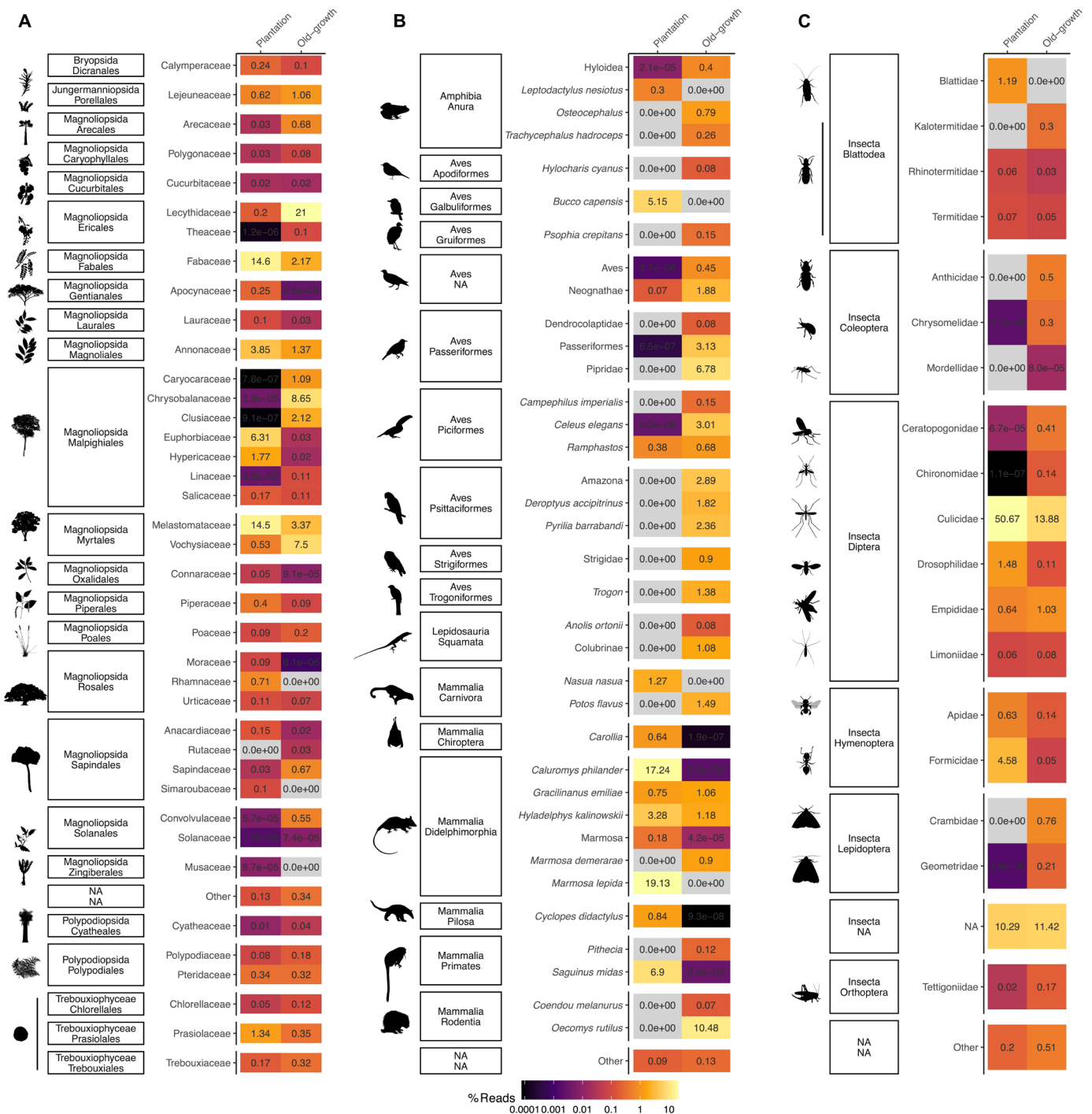


Fig. 2. Taxonomic composition of canopy rainwash eDNA in the tree plantation and the old-growth forest plots for plants and animals. (A) Plants, (B) vertebrates, and (C) insects in the temporal experiment. Only taxa with a relative abundance (in terms of % reads) > 0.5% in the total dataset are shown (summarized at the family levels for plants and insects). The percentage of reads reported here corresponds to sequencing reads summed across all samples of the same condition ($n = 25$ per forest type, i.e., five spatial and five temporal sampling points). Gray cells correspond to null values and black ones to very small and yellow ones to high values. NA, not applicable.

11 amphibian taxa, including the frog *Leptodactylus nesiotus*, known to occur in the area (34); 24 bird taxa (including, e.g., parrots and toucans); 4 squamate taxa; and 32 mammal taxa. Most mammal taxa were not only attributed to opossums and rodents but also comprised genera of neotropical monkeys (*Saguinus* and *Pithecia*), of sloth (*Choloepus*), of anteater (*Cyclopes*), of bats (*Carollia* and *Rhinophylla*), and of procyonids (*Potos* and *Nasua*). All these taxa are described from the local fauna, and they are strictly or occasionally arboreal (35). We also retrieved MOTUs assigned to fish taxa and the domestic cat at relatively high sequence similarity scores, but these are not included in the above counts.

For insects, the retrieved taxonomic assignments encompassed 20 orders and 107 families. The most represented insect orders in terms of diversity and proportion of sequencing reads were Diptera (including several species of fruitflies, sandflies, and mosquitoes), Coleoptera, Hymenoptera, and Lepidoptera, which are the most diverse orders in these ecosystems. We also detected cockroaches, termites, true-bugs, grasshoppers, or dragonflies. We lastly found five noninsect arthropod taxonomic assignments: two spiders, two springtails, and one isopod.

We also investigated the relevance of the approach from a conservation point of view. Among the 204 taxa identified to the species level, few corresponded to species with a vulnerable to critically endangered status. However, a careful consideration of their identity and of their distribution range reveals that they do not occur in French Guiana, and most likely correspond to the misassignment of sister taxa occurring locally but that are not in reference DNA databases, leading to an erroneous taxonomic assignment at the species level (see Materials and Methods, text S1, and fig. S2 on this point). For instance, the local species belonging to the genera *Campephilus*, *Dipteryx*, and *Swartzia* species were wrongly assigned to *Campephilus imperialis*, *Dipteryx alata*, and *Swartzia bahiensis*, respectively. This illustrates the importance to carefully interpret the list of taxonomic assignments retrieved from eDNA surveys. The rest were either found without IUCN (International Union for Conservation of Nature) status or were explicitly not evaluated.

The taxonomic diversity and composition the old-growth Amazonian rainforest relative to the nearby tree plantation were consistent with our expectations (Fig. 2). The taxonomic assignments were 1.3 to 1.9 times more diverse in the old-growth forest plot relative to the plantation, with assignments corresponding to 155 plant taxa (versus 111 in the plantation), 61 vertebrate taxa (versus 32), and 276 (versus 153) insect taxa. The old-growth forest plot also harbored higher relative abundance of Lecythidaceae, Chrysobalanaceae, Vochysiaceae, and Caryocaraceae, as well as the frog *Trachycephalus hadroceps*, taxa that are typically found in old-growth forests (36, 37). It also harbored more MOTUs and sequences assigned to phytophagous taxa such as Lepidoptera and Coleoptera. Conversely, the tree plantation yielded a higher proportion of reads assigned to the rubber tree family (Euphorbiaceae), with its most representative MOTU corresponding to the rubber tree itself (*Hevea*; data S1). We also detected a higher relative abundance of termites belonging to the genus *Coptotermes*, to which the rubber tree termite belongs, and of mosquitoes, ants, and cockroaches in the tree plantation. Last, the tree plantation exhibited higher relative abundance of the rosewood family (Lauraceae); of Fabaceae, Melastomataceae, Hypericaceae, and Piperaceae; and to the frog *L. nesiotus* that are often associated with disturbed or more open habitats (34, 37).

Optimizing the sampling of rainwash eDNA

Next, we tested whether rainwash eDNA-based biomonitoring can be operationalized, i.e., based on low-tech and cost-effective strategies that limit the number of sampling campaigns and that generate biodiversity inventories that are spatially and temporally representative of the study area. The requirements are even more stringent for an effective implementation in tropical countries, where challenges are compounded.

Rainwash eDNA can be effectively collected passively

We tested five low-cost eDNA collection methods where rainwash eDNA accumulates passively (Fig. 1B). After 1 day of immersion in rainwash water, filtration by gravity with Waterra filters captured higher MOTU diversity for plants and to a lesser extent for vertebrates compared to the other passive captors (fig. S3A and table S2). A similar trend was observed for invertebrates but was not significant. This collection method was also more effective in fixing the carrot spike-in DNA (fig. S3B and table S2) but also for longer immersion periods (fig. S4). Hence, this collection method holds great potential in tropical rainforest environments. Passive sampling through eDNA fixation on cotton strips, which was the most affordable method tested here, could be considered as an effective alternative collection method but requires greater fixation surface and/or more passive sampler replicates per rainwash water samplers to reach a level of detection similar to passive filtration method we present here.

Rainwash eDNA persists over 2 weeks

We analyzed the eDNA dynamics by sampling rainwash eDNA at different time intervals (Fig. 1A) using the passive filtration method discussed above. We assessed these dynamics through three criteria: MOTU diversity dynamics, MOTU compositional temporal turnover, and spike-in DNA decay.

First, we analyzed how MOTUs diversity of each target clade varied with the duration of the eDNA collection. We measured MOTU richness on rarefied data. We fitted several functions modeling population dynamics (38) on the temporal trends of MOTU diversity and found that the Ricker function, which exhibits an optimum, was the best fit (Fig. 3A). The fact that the temporal dynamics of MOTU diversity exhibits an optimum can be attributed to the reduced influxes of new eDNA molecules through time resulting from fewer and less intense rain events (Fig. 1A), coupled with a stable eDNA degradation. Based on this model, we estimated the initial MOTU accumulation rate, which ranged between 5 and 10 MOTUs per day after installation and the time at which the diversity is maximal (Fig. 3B and table S3), here between 8 and 20 days after the installation of rainwash eDNA samplers, depending on the marker.

We also calculated the decrease of MOTUs compositional similarity with time (fig. S7). The observed diversity dynamics of MOTUs we retrieved for plants and vertebrates exhibited such a relationship, suggesting a balance between eDNA persistence in the matrix and influx of new molecules. This relationship was weaker for animals, and statistically nonsignificant for insects. This may be explain at the very high richness of the samples, hence a low similarity.

Last, we estimated the decay rate of the carrot spike-in DNA using a negative exponential function (39), from which we estimated the time of decay to 5% of its initial quantity to about 29 days (Fig. 3, A and B, and table S3).

Rainwash eDNA captures localized diversity

Last, we assessed the spatial sampling effort needed to capture diversity within an area of typically 1 ha, alongside the community's spatial

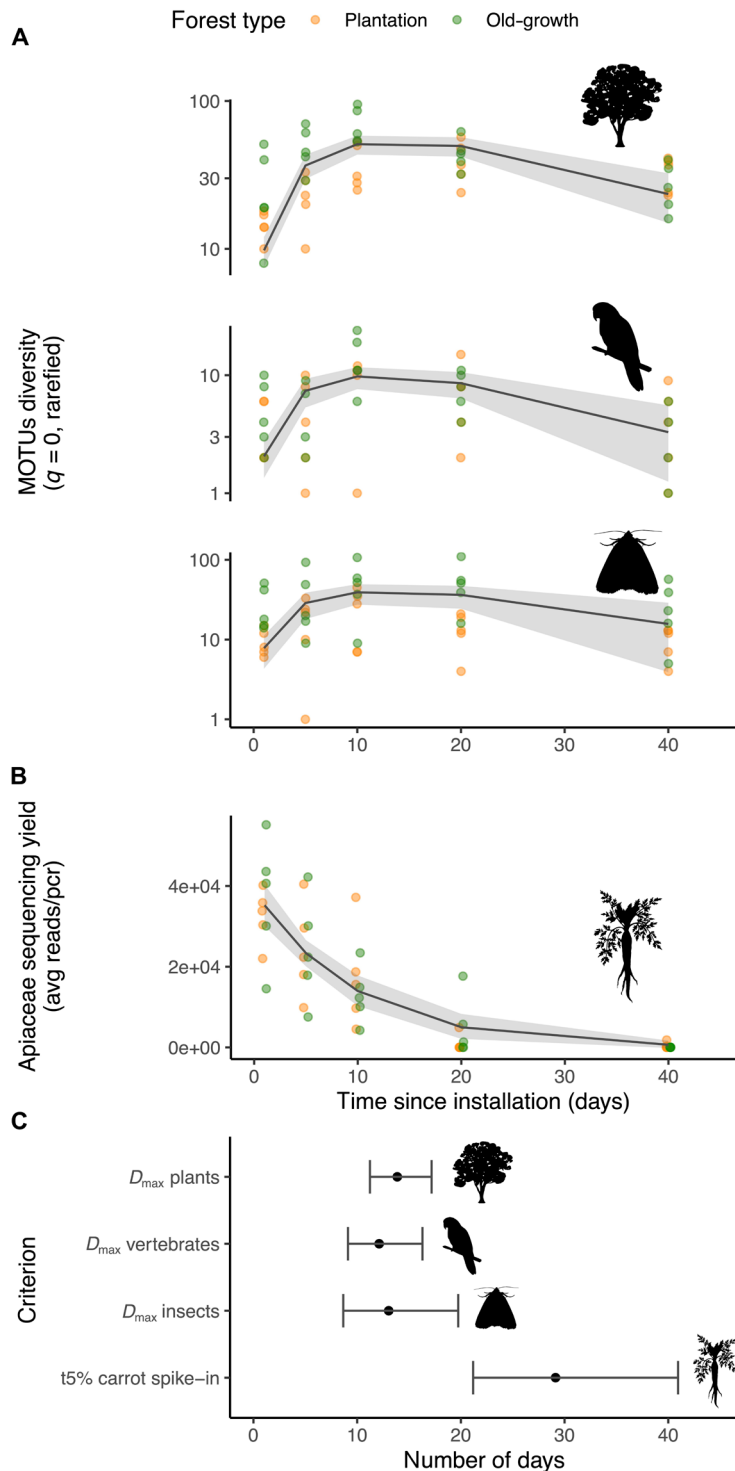


Fig. 3. Dynamics and optimal temporal sampling window of rainwash eDNA. Dynamics of (A) MOTU diversity ($q = 0$, rarefied data) of plants, vertebrates, or insects and (B) amount of carrot spike-in DNA. Gray lines and gray transparent ribbons in (A) and (B) correspond to the models' fits (see also table S3) and 95% confidence interval. (C) Temporal window of detection in terms of maximum diversity for each target clade or of 5% of spike-in initial concentration, with confidence intervals at 95%.

compositional turnover. We hypothesized that, given the predominantly vertical nature of rainfall and its capacity to transport eDNA from canopy leaves (29, 30), the eDNA signal collected via rainwash should be spatially punctual. To test this, our spatial experiment (Fig. 1) involved sampling rainwash eDNA every 20 m in each plot, with rainwash collectors left on site for 40 days.

After excluding vertebrate data, of which signal was insufficient (see Materials and Methods), we built rarefaction curves on which we fitted species rarefaction models (40) and estimated different properties (Fig. 4). The extrapolated rarefaction curves showed higher plateau values in the old-growth forest for both target clades.

In terms of coverage, plant diversity was relatively well covered with the proposed sampling strategy (i.e., >70%). By contrast, at best, 46% of the insect plot-scale diversity was detectable. Reaching 90% of the plot-scale diversity would require at least 40 samples for plants and significantly more for invertebrates (Fig. 4 and table S4). While marker resolution difference cannot be entirely excluded, this result is consistent with the large insect diversity relative to plants in tropical rainforests (41). Last, we found very low to null MOTU compositional similarity between sampling points and the absence of clear distance-decay patterns (fig. S6).

DISCUSSION

Our study presents rainwash eDNA as a promising, noninvasive tool for monitoring the elusive biodiversity hidden in tropical forest canopies. We demonstrate its capacity to effectively capture organisms typical of the canopy environment that are otherwise difficult to monitor. For instance, most retrieved taxa lacked an IUCN status or were explicitly not evaluated. This exemplifies the usefulness of rainwash eDNA to document the distribution of data-deficient taxa and highlights its relevance for conservation efforts, as over half of data-deficient species are projected to face extinction (4). Our sampling protocol also proved capable of detecting shifts in plant and animal

diversity and composition between sites of differing anthropogenic disturbance. Specifically, both taxonomic and MOTU diversity was significantly higher in the old-growth rainforest, and the taxa recovered in each forest was congruent with their distribution and ecology. This suggests that our method is valuable for both basic research—to better document and understand patterns of diversity in tropical forests canopies across habitats or in a context of global change—and for more applied purposes such as environmental health assessment or forest restoration success monitoring.

We further establish a baseline for optimal passive sampling of rainwash eDNA by proposing a low-cost and power-free collection method designed to maximize eDNA capture and by providing objective measures of its temporal and spatial dynamics. The passive filtration of rainwash eDNA effectively integrated biological signals over time, covering a temporal window from 8 to 40 days depending on the initial eDNA concentration at the site. This integrative property makes our protocol less susceptible to the “snapshot” effect of one-shot samplings, typical when collecting soil, water, air, or leaf swabs, which require repeated sampling campaigns to mitigate this effect. This temporal integration substantially enhances the feasibility of routine analyses, reducing the need for frequent on-site visits and enabling monthly sampling for real-time tracking of temporal biodiversity trends and shifts in community composition.

Our spatial analysis indicates that while our sampling strategy was sufficient to detect nonrare taxa at the plot scale, the signal retrieved remains largely localized (tens of meters). This pattern is consistent with observations from soil eDNA (21, 22) and temperate rainwash eDNA (29, 30). This localized nature can be viewed as a limitation for large-scale assessments compared to stream/lake water or air eDNA, which can cover larger spatial extents (tens of kilometers) (42–45). However, it offers clear advantages when highly resolved local inventories are required and contamination from neighboring sites must be minimized, such as in environmental health

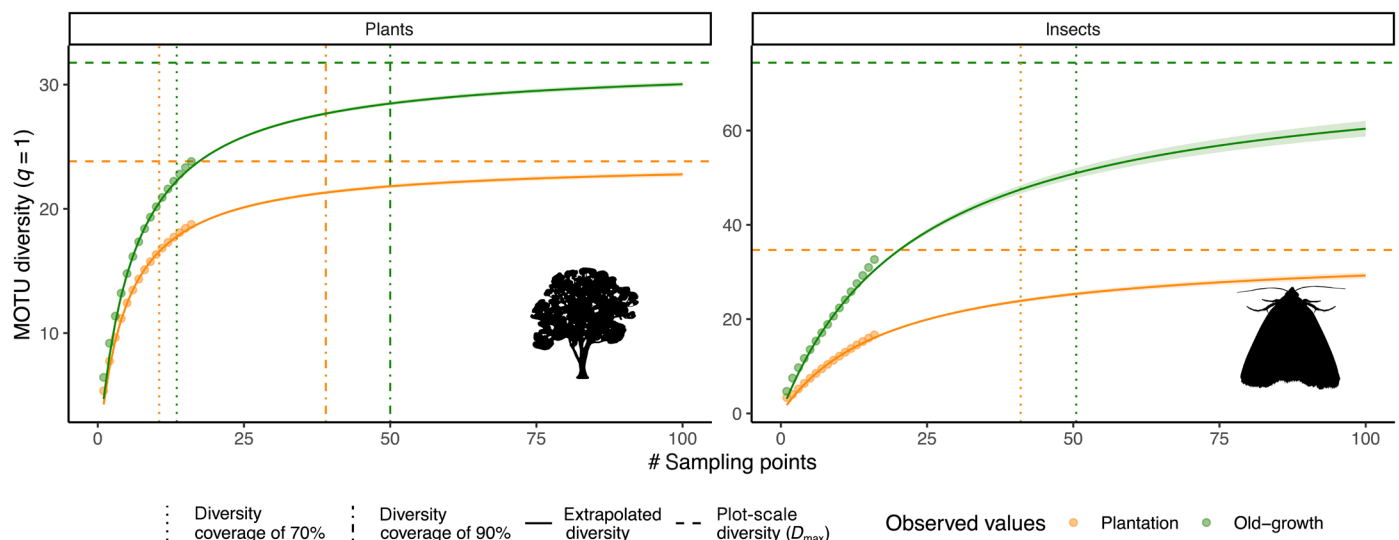


Fig. 4. Accumulation of rainwash eDNA diversity with increasing sampling effort for plants and insects. Points correspond to observed diversity values. The different line types represent parameters or curves derived from the Monod models' fits (see also table S4). Vertical lines correspond to the number of samples required to sample 70 and 90% of the plot-scale diversity. For insects, 90% of the diversity requires exceeds 100 samples (see table S4). The transparent ribbons represent the confidence intervals at 95% of extrapolated diversity.

monitoring or assessment of forest restoration success. In these cases, rainwash eDNA is likely more appropriate.

Our methodology overcomes the challenges of working in remote and hyperdiverse environments where species distribution is spatially strongly heterogeneous both horizontally and vertically (45–47), and where eDNA is not transported as easily as in aquatic ecosystems. Rainwash eDNA provides an effective nondestructive alternative to iDNA retrieved from arthropod bulks used for the same purpose (13, 14). Moreover, our approach relates to aboveground diversity, contrasting with soil eDNA that is largely dominated by plants and soil organisms (21, 22). Collecting both environmental matrices could therefore provide a more holistic view of both above- and belowground diversity.

Unlike water eDNA, rainwash eDNA collection does not require the presence of a standing water body. Compared to airborne eDNA, which has very low concentrations (21, 26) and thus higher risks of contaminations (24, 26), we anticipate rainwash eDNA to be more concentrated because it should be enriched with DNA traces left on leaf surfaces by organisms living in the canopy (29), making it less prone to contaminants. In addition, eDNA in stream/lake water or air transports differently depending on particle size distribution, water/air mass movements, hydrogeomorphological or atmospheric properties, rain events, and other abiotic conditions (25, 26, 43, 44, 48–50). The intricacy of these factors and processes leads to complex fluctuations in the retrieved signal and makes its spatio-temporal coverage currently challenging to predict.

While rainwash eDNA transport likely exists (as suggested by the fish taxa retrieved in our inventories, similar to reports for leaf swabs (27), potentially a digestion product from canopy vertebrates), we expect this transport to be more limited because rainwash eDNA primarily transports vertically and is sampled closer to its source community. These points suggest that the spatial dynamics of rainwash eDNA is more traceable and potentially easier to interpret for characterizing local terrestrial biodiversity in forested areas, especially in the canopy. This localized signal is particularly valuable not only for both applied applications, such as environmental monitoring and land-use management, but also for basic ecological research, enabling targeted sampling directly beneath specific tree species (30) to test with enhanced sampling effort long-standing hypotheses regarding, for instance, interspecific interactions driving species distribution patterns, such as the Janzen-Connell hypothesis (51) or its reverse alternative (52).

While our findings offer a pioneering assessment of rainwash eDNA dynamics in real-life conditions, we acknowledge that they do not cover all the factors and processes governing eDNA dynamics in general (48). As for any eDNA matrix, these include the organisms biology or activity, the source of the DNA detected (e.g., tissues, feces, and pollen) (48), or, in our case, the frequency and intensity of rain events. These rainfall events are often unpredictable, which can undermine both the sampling efforts and blur the spatio-temporal window covered. For example, our second sampling campaign yielded lower diversity levels due to reduced precipitations. This illustrates the fact that infrequent or single rain events (artificial or not) capture insufficient amounts of eDNA for robust downstream analyses. This inherently limits the relevance of deploying the approach during the driest season or in areas receiving lower or more irregular annual precipitation, such as in tropical dry forests, Cerrado forests or Caatinga forests, or temperate forests outside rainy seasons.

However, our passive sampling strategy, using collectors that can be left on site for extended periods, maximizes the chance of

receiving rain water without necessitating returning on site at each rain event in areas exhibiting sufficient precipitation during the sampling campaign. To improve the robustness of our estimates on rainwash eDNA dynamics and downstream inferences, we advocate for more systematic assessments of factors influencing eDNA temporal dynamics using simple approaches. For example, the amount of rain received by the collectors, while not controlled here, can be easily estimated by placing a container at the filter outlet. Likewise, eDNA decay can be assessed using exogenous DNA tracers easy to obtain and monitor as we did here with carrot juice.

Common to all eDNA-based assessments are limitations linked to molecular approaches, including molecular biases and contaminations (10), and molecular markers resolution, reflected by the low number of finely resolved taxa we detect. However, this latter point likely stems from a critical issue in tropical eDNA research: Tropical biodiversity remains severely underrepresented in genetic databases such as GenBank or BOLD. We illustrate this with our gap analysis for the species of French Guiana, where we find notable gaps for reptiles, nightjars, and many plant orders, and with our eDNA data, where sequence identity scores against Genbank are overall quite lower (i.e., below 90%) than in temperate eDNA studies. This partial referencing inherently reduces the accuracy of taxonomic assignments. For example, several fine-resolution taxonomic annotations obtained here corresponded to taxa not expected to occur in the studied area or even in the Neotropical realm. This emphasizes the urgent need to expand DNA reference databases for tropical diversity if we are to fully exploit the power of eDNA in general in the tropics (22, 53).

These different aspects, together with the fact that rainwash eDNA can be easily sampled passively, makes it a promising alternative for eDNA-based biomonitoring of terrestrial ecosystems, especially in remote and inaccessible tropical forest canopies. At present, it does also not cover treeless areas, but this limitation could be overcome by developing rain collectors more adapted to low-stature vegetation. Nonetheless, it is essential to more comprehensively compare and contrast the efficacy of rainwash eDNA against the other eDNA-based substrates for characterizing terrestrial biodiversity. These in-depth comparisons will ultimately clarify the strengths and optimal applications of each matrix, allowing even more optimal eDNA sampling across the different strata of terrestrial ecosystems.

In conclusion, our study demonstrates the potential of rainwash eDNA as a powerful, noninvasive, and low-cost tool for monitoring the elusive diversity of plants and animals populating the tropical forest canopies, a realm notoriously challenging to study with traditional methods. Here, we have established a robust methodological framework for an optimal rainwash eDNA sampling, opening doors for further refinements in community-level assessments across environments or disturbance gradients and for the detection of species crucial for conservation or environmental health. By enabling large-scale and noninvasive monitoring, rainwash eDNA can substantially improve our description of the elusive diversity hidden in tropical forests canopies and inform and assess tropical forest conservation strategies, contributing vital data for the protection of these irreplaceable ecosystems.

MATERIALS AND METHODS

Study area

The study was conducted in the municipality of Sinnamary, which features two types of forest: an old-growth forest and a tree plantation.

The equatorial climate in the studied area is characterized by two climatic periods: a long rainy period from mid-December to mid-August often interrupted by a short drier period between March and April and a longer dry season for the rest of the year. In this area, the annual rainfall is 3041 mm with mean air temperature of 26°C ($\pm 1^\circ$ to 1.5°C across the year). The 1-ha plot (plot P16) of old-growth forest is located in the Paracou Research Station (54) (latitude: 5°18'N, longitude: 52°53'W; <https://paracou.cirad.fr/website>). The Paracou research station managed by the French Agricultural Research Centre for International Development (CIRAD) comprises 500 ha of tropical rainforest in a hilly terra firme region. This site is a long-term research station where plant communities are well characterized, and precipitation is monitored daily (Fig. 1A). The 1-ha plot of tree plantation is located in a former rubber tree (*Hevea brasiliensis*, Euphorbiaceae) plantation also managed by CIRAD, where rosewood (*Aniba rosaedora*, Lauraceae) was more recently introduced and of which the exact current floristic composition is unknown. It is located 5 km away from the Paracou research station (latitude: 5°17'N, longitude: 52°54'W).

eDNA sampling

Temporal experiment

To collect rainwash eDNA, we repurposed conventional umbrellas and plastic bottles into a low-cost rainwash water sampler with integrated passive eDNA sampler holders (instructions described in fig. S1). We equipped these samplers with four types of eDNA passive captors. First, we used Waterra eDNA filter (600-cm² polyethersulfone filter, pore size of \varnothing 0.45 μ m; Waterra USA Inc.), which are commonly used for eDNA sampling in aquatic ecosystems (55) and that we here consider as a reference. We plugged the Waterra filters at the bottom of the water tank of the rainwash water sampler with silicon tubes to passively collect eDNA through gravity filtration (Fig. 1B) (56). We also tested three alternative, cost-effective passive captors based on eDNA fixation. First, we considered mixed cellulose ester membrane filters (\varnothing 47 mm, pore size of 0.45 μ m; Merck KGaA, Darmstadt, Germany), which have recently proved effective for studying aquatic biodiversity with eDNA (31). We also considered ceramic mosaics of 25 mm by 25 mm, as ceramic has DNA binding properties, even over long time periods (57, 58). Last, we considered cotton strips of 25 mm by 25 mm, which also have the potential to passively sample aquatic eDNA (32). Ceramics and cotton strips have also long been used to measure biofilm colonization/productivity rates (59) and decomposition (60, 61), respectively. As biofilms also have eDNA-fixing properties (62), this property may increase the efficiency of these passive eDNA samplers for our purpose. All passive eDNA samplers were placed into the rainwash collector water tank.

A total of five rainwash water samplers were installed in each 1-ha forest plot at randomly selected locations away from the plot edges. We assessed the efficiency of the passive captors and quantified eDNA persistence through time by introducing an exogenous DNA just before the installation of the rainwash water samplers. We chose carrot (*Daucus carota*, Apiaceae) as a spike-in because of its widespread availability and because this plant family is absent from the study area and more generally from the Guiana Shield, except the aromatic plant *Eryngium foetidum*, which is not cultivated near the study sites (63). To do so, we sprayed about 10 ml of carrot juice on canopy leaves at each sampling point with a conventional streamer. About 20 min after spraying, we installed below our rainwash

water samplers and then simulated an initial rain by spaying about 40 ml of water on the canopy. The water was made of rainwater collected on site a few days before the start of the experiment (2:5) and was complemented with bottled springwater (3:5) to obtain a sufficient volume. eDNA passive captors were sampled after 1, 5, 10, 20, and 40 days for subsequent molecular analyses and placed in 50-ml sterile falcon tubes (Waterra filter excepted). We then added 50 ml of Longmire buffer (for 1 liter of buffer: 100 ml of 1 M tris, 100 ml of 1 M EDTA, 2 ml of 5 M NaCl, and 798 ml of molecular biology grade water) in each falcon tube and Waterra filter to store and preserve eDNA passive captors at room temperature (64) before molecular analyses. We thus obtained a total of 200 samples (i.e., two plots, five sampling points, five sampling dates, and four passive eDNA samplers) for the temporal experiment.

Spatial experiment

In the same plots, we conducted another sampling campaign in mid-February of 2024 where we placed one rainwash water sampler every 20 m, resulting in 16 points per plot. Following the results obtained in the temporal experiment, the water samplers were equipped with one Waterra filter each. The sampling devices were left at the field for 40 days (Fig. 1). We thus obtained a total of 32 samples for the spatial experiment.

Molecular analyses

DNA extractions

Each falcon tube containing a passive eDNA sampler immersed in Longmire buffer was shaken with a horizontal shaker at 40 Hz for 10 min to solubilize the DNA. After that, we removed the captor matrix. We also shook the Waterra filters' samples at 40 Hz for 10 min, and after that, we transferred the buffer into 50-ml falcon tubes. We then added 33 ml of absolute ethanol to all tubes and subsequently stored them at -20°C overnight. The following day, the tubes were removed from the freezer, left for 30 min at room temperature, and centrifuged at 8500 for 15 min at 15°C. The supernatant was then discarded, and 720 μ l of ATL buffer (QIAGEN, Hilden, Germany) was added to each pellet. The mixture was then transferred to 2-ml tubes, to which we added 20 μ l of proteinase K (QIAGEN; Hilden, Germany), followed by an incubation at 56°C for 2 hours for digestion. The NucleospinSoil Macherey-Nagel kit was then used, following the protocol from step 6 (adjust binding conditions) onward. The resulting DNA extracts were lastly analyzed using a NanoDrop ND-1000 (Thermo Fisher Scientific Inc.) to determine the yield and purity of the DNA.

DNA metabarcoding

We selected DNA markers to describe the sample diversity of each target group as a trade-off between several criteria. First, (i) the extent of available reference of the marker in DNA databases, noting that the Linnean shortfall and DNA reference databases incompleteness is severe for tropical biodiversity and especially for insects (see below and fig. S2 for a gap analysis of DNA references for the plants and vertebrates known to occur in French Guiana). We also considered (ii) the marker taxonomic resolution; (iii) its taxonomic coverage, i.e., its ability to target most of the groups of interest, without (iv) amplifying off-targets, i.e., its taxonomic specificity; and (v) its ease, lack of taxonomic bias, and consistency of amplification across environmental samples.

For plants, we choose the Sper03 marker, which targets the 5' region of the *rbcl* gene (forward: 5'-CACCACAAACAGARACTA-AARC-3'; reverse: 5'-TCCAYACRGTGTCCATGTACC-3'). We

choose this marker over others located in the nuclear ITS (Internal Transcribed Spacer) or the chloroplastic *trnL* gene because, while less resolute, it remains a valid barcoding region (65). The *Sper03* marker used here is also shorter [~130 base pair (bp)] than ITS markers (~250 to 300 bp), hence more easily amplifiable from fragmented eDNA. It is also more specific to Viridiplantae, as shown by an *in silico* polymerase chain reaction (PCR) amplification on GenBank using the *ecoPCR* program (fig. S7) (66). The amplification of this marker for the target group was thus more easy to amplify and was expected to be more reliable. Last, the *rbcL* gene has been historically more used as a reference barcode for plants of French Guiana (67, 68) and is expected to cover a greater number of ferns families (Polypodiopsida), for which ITS primers often exhibit more mismatches (fig. S7) (69). For vertebrates, we choose the *Vert01* marker (70), which targets a subregion of the mitochondrial 12S rRNA gene (forward: 5'-TTAGATACCCACTATGC-3'; reverse: 5'-TAGAACAGGCTCCTCTAG-3'). This marker is also very short (~100 bp) and highly specific to vertebrates (10, 70). To lower the amount of human contamination, we used this marker together with a human-blocking primer: CTATGCTTAGCCCTAACCTCAACAGTTAAATCAACAAAAGTCT-Spc3. For insects, cytochrome c oxidase subunit I (COI) markers, albeit more prone to amplification biases (71, 72), are often preferred due to their higher referencing in DNA databases and their greater taxonomic resolution. In a pilot experiment using the COI primers B/ArR5 (73, 74) on our DNA extracts, 99% of the retrieved sequencing data corresponded to off-target amplification of bacteria (fig. S8). To choose an alternative, more functional marker, we compared *in silico* the amplification success of another widely used COI primer pair, *fwhF2/fwhR2n* (75), and a more conserved marker located in a subregion of the mitochondrial 16S rRNA gene (*Inse01*, forward: 5'-RGACGAGAAGACCCTATARA-3'; reverse: 5'-ACGCTGTATCCCTAARGTA-3') (10), against mitochondrial genomes from RefSeq (<https://ftp.ncbi.nlm.nih.gov/genomes/refseq/>). This analysis also indicated significant off-target amplification of plants and fungi of the COI marker (fig. S9). As the mitochondrial 16S rRNA marker consider here, despite being less resolute, has been shown to be perfectly valid (76), we selected it here to ensure reliable and specific amplification of insect DNA from our environmental samples.

PCR reactions were performed in triplicate for each sample in 20 μ l. Each reaction consisted of 10 μ l of Ampliqaq Gold 360 Master Mix (Applied Biosystems), 2 μ l of a mix of forward and reverse primers (0.5 μ M each final), 6 μ l of nuclease-free water, and 2 μ l of eDNA diluted 10 times beforehand. For each PCR reaction, primers were labeled with unique combinations of 8-nt-long tags at the 5' end of the forward and reverse primers (77) to distinguish PCR replicates after sequencing. PCR thermocycling conditions are indicated in table S1. The final molecular experimental design thus included for each marker 600 PCR reactions corresponding to samples, 33 corresponding to extraction blank controls, and 30 to PCR blank controls for the temporal experiment. For the spatial experiment, it contained 96 PCR reactions corresponding to samples, 8 to extraction blank controls, and 3 to PCR blank controls. All PCRs of the same marker were pooled in equal volumes without standardizing the PCR products concentration to allow a more quantitative comparison of the abundance of taxa sequencing reads across samples (see below and fig. S10) (78). This pool was then used to build sequencing libraries using the Illumina TruSeq Nano HT (Illumina), following the manufacturer protocol but without PCR enrichment of ligation products to minimize the formation of tag-jumps (79).

Sequencing was conducted at the GetPlage sequencing platform (Toulouse, France) for the temporal experiment and at Novogene (Cambridge, UK) for the spatial experiment, with the characteristics indicated in table S1.

Bioinformatics and data preparation

Our data processing curation process is detailed in previous works (11, 80), and parameters used in this process specifically here are summarized in table S1. Briefly, primary bioinformatics/curation procedures, i.e., reads pairing, demultiplexing, dereplication, exclusion of obvious artifacts and singletons, and clustering of sequences to form MOTUs were made with the *OBITools* v1.2.11 [<https://anaconda.org/bioconda/obitools>; (81)] and the *sumacust* v1.0.31 clustering algorithm [<https://anaconda.org/bioconda/sumacust>, (82)] using a *snakemake* workflow (https://github.com/AnneSoBen/obitools_workflow). The taxonomic assignment was done with the *ecotag* program (81) with the reference databases indicated in table S1. These reference databases were built with the *ecoPCR* program (66) from GenBank. Note that the *ecotag* algorithm used here assigns taxonomic names using an LCA approach, which adjusts the assignment based on sequence similarity, marker resolution, and database completeness, hence resulting in varying taxonomic assignments with varying levels of precision. In other words, it returns fine-level assignments with high similarity, when markers are resolute, and coarser assignments even with high similarity, when markers are less variable or when reference databases are incomplete. Occasionally, the LCA method can yield erroneous fine-level assignments with high similarity when the marker lacks resolution and when the database does not contain the actual taxon but contains a related one with the same barcode sequence or fine-level assignments with low similarity if a clade is too sparsely represented in the database. Next, we performed a secondary sequence curation procedure with the *metabar* R package [<https://github.com/metabarfactory/metabar>; (83)] to further exclude contaminants, tag-jumps, or other degraded sequences from the dataset.

Amazonia hosts a diverse, endemic, and partially undocumented biodiversity, even for relatively well-studied groups such as plants and vertebrates (84, 85). For insects, the situation is even more critical, with current description rates suggesting that up to 80% of insect species in French Guiana remain unrecorded and that completing the inventory would take three centuries (86). A direct expectation from this is a severe incompleteness of DNA reference databases for Amazonian biodiversity. This is supported by the fact that a non-negligible amount of plants and vertebrates known to occur in French Guiana are not represented in GenBank for the DNA markers used here (text S1 and fig. S2). In this context, expecting high similarity matches (e.g., 99%) for all sequences is unrealistic, and the exclusion of MOTUs exhibiting lower similarities against reference databases would result in the exclusion of a vast amount of ecologically relevant signal. Accordingly, our taxonomic assignments across markers showed moderate to high sequence similarities against reference databases for plants and vertebrates (ca. 95% on average) and much lower similarities for insects (81%, on average). Therefore, we adopted less stringent data curation thresholds regarding MOTUs sequence similarity against reference databases, which we determined for each marker based on the distribution of similarity scores in terms of number of reads and MOTUs (table S1). Still, for vertebrates and plant data, we further verified that the taxon names retrieved had occurrence records in South America using the R package *rgbif* v3.8.0 [<https://CRAN.R-project.org/package=rgbif>; (87)] of the Global

Biodiversity Information Facility (www.gbif.org). We also manually verified the distribution of each MOTUs in the PCR plate design to ensure that they did not result from tag-jumps from positive controls. In some cases, we further compared their sequences against GenBank (assessed in August and October 2024) using BLAST (Basic Local Alignment Search Tool) (88). An overview of the dataset size at the main steps of the data curation process is shown in table S5. All data processing codes and diagnostic plots/statistics are available along the data at different stages of the workflow (see Data and materials availability).

Given the above and the inferential nature of sequence-based taxonomic assignment methods, we stress that the taxonomic annotations retrieved here and in eDNA studies in general should be interpreted as indicative, representing the closest matches in current reference databases. As with all eDNA-based approaches, confirming a taxon presence rigorously requires an array of approaches (e.g., specimen collection, images, bioacoustics, etc.).

At the end of the data cleaning procedure, we checked how MOTUs diversity varied with sequencing depth for all markers and samples (fig. S11). While rarefaction curves did not saturate when considering MOTU richness, they did so when weighting MOTUs by their relative abundances using the Hill number framework (89, 90). The Hill numbers, which correspond to an equivalent number of taxa, is more appropriate to analyze diversity with DNA metabarcoding data when its q order is >0 (91, 92). We thus present here alpha and beta diversity analyses for Hill's $q = 1$ in most cases, corresponding to the exponential of the Shannon index for alpha diversity, and the Horn dissimilarity index for beta diversity (89). We did so for all analyses, except for those related to temporal MOTU diversity and compositional dynamics, where accounting for rare MOTUs is important due to eDNA decay processes. For this specific analysis, we computed diversity indices at $q = 0$, corresponding to MOTUs richness for alpha diversity, and the Sorensen index for beta diversity, but only after data rarefaction at 1000 reads per sample. All Hill numbers were calculated with the `hillR` R package v0.5.2 [<https://CRAN.R-project.org/package=hillR>; (93)]. Last, we retrieved the IUCN status—when available—for all the taxa found using the R package `gbif.range` [<https://github.com/8Ginette8/gbif.range>; (94)].

Note that we obtained a reduced diversity per sample overall for the spatial experiment (fig. S11 and table S4) and particularly for vertebrates, most likely due to the rarity of rain events during the spatial experiment (Fig. 1). We further found that while we obtained taxonomic assignments corresponding to several vertebrate taxa known to occur locally (e.g., *Saguinus*, *Oecomys*, or *Ramphastos*), the vertebrate data were excessively contaminated. Because the contaminations pertained after rerunning the PCRs from scratch while most PCR blank controls were negative, we concluded that our DNA extracts were compromised for analyzing vertebrates, due to much rarer traces of local DNA than in the temporal experiment, due to higher amounts of contaminations, or both. Consequently, we excluded this dataset from downstream analyses.

dPCR assays

We also quantified the amount of carrot DNA for each passive eDNA sampler obtained at each sampled spatial and temporal points through digital PCR (dPCR) on a gene specific to carrots. For the dPCR-based approach, we quantified the copy number of the carrot *Dau c1* gene using the QIAcuity Digital PCR System (QIAGEN, Hilden, Germany). The single-copy gene-targeted encodes for an allergen protein and is now routinely traced through PCR. In this study, we

used the primers Dauc1F (forward: 5'-CCAGAGCCATTCACCTC-GAGATC-3') and Dauc1R (reverse: 5'-ACTGTATCAACATCAA-GGACAATGC-3') (95), together with the QIAcuity EG PCR kit (25 ml) and QIAcuity Nanoplate 26k 24-well kits (QIAGEN, Hilden, Germany). Each dPCR reaction was performed in a final volume of 40 μ l, consisting of 13.3 μ l of EvaGreen, 3.2 μ l of the two primers mixed at 0.4 μ M final concentration each, 13.5 μ l of water, and 10 μ l of template DNA. We also performed one positive control per plate, adjusting the above conditions for 1 μ l of carrot juice DNA extract as template DNA. Thermocycling conditions were as follow: an initial denaturation step at 95°C for 2 min, followed by 50 cycles of denaturation at 95°C for 15 s, primer annealing at 58°C for 15 s, elongation at 72°C for 15 s, and a final elongation step at 72°C for 5 min. The results were obtained from the machine with default parameters. For a selection of samples (i.e., Waterra filters obtained at t_1 and t_{20} only), we also conducted dPCRs with the plant *Sper03* marker (see above) using the reaction mix as above except that we adjusted the annealing temperature at 50°C (table S1)

Statistical analyses

All analyses performed with R were done with the R version 4.4.1 (96) and the `tidyverse` package (97).

Taxonomic composition overview

To gain a first overview of the relevance of the taxonomic composition obtained from rainwash eDNA, we summed all MOTUs counts across sampling temporal and spatial points for each forest type of experiment 1 data. We aggregated the data at the plot scale rather than considering each spatial point individually due to the punctual information retrieved in each sample (see below for a more formal analysis on this aspect). We also aggregated the counts of all MOTUs assigned to the same taxonomic name.

Identifying the best passive eDNA sampler

We identified the best type of passive eDNA sampler based on capacity to quickly fix a high diversity of taxa and high amounts of carrot spike-in DNA (i.e., 1 day after passive eDNA samplers immersion in rainwash water). For the analysis of diversity detected by each passive sampler type, we considered the diversity of MOTUs at $q = 1$ as explained above. For the analysis of carrot spike-in DNA, we used both dPCR assay data (expressed as a number of copies per microliter of DNA extract) and a subset of DNA metabarcoding MOTUs classified as *Apiaceae* (expressed as a number of *Apiaceae* sequencing reads per PCR reaction). Both were not normally distributed, which is why the downstream analysis mostly consisted of nonparametric tests. We found the two types of data to be well correlated (Spearman's $\rho = 0.56$, $P < 0.001$; fig. S10A). A similar trend was also observed when comparing the gene copy number of our plant marker and the corresponding total *Sper03* sequencing reads per PCR reaction for a subset of samples (Spearman's $\rho = 0.7$, $P < 0.001$; fig. S10B). This adds to previous evidence that DNA metabarcoding number of reads can be quantitative to a certain extent (78) when PCR products are sequenced together on the same lane and were not subjected to concentration standardization prior sequencing. Nevertheless, we present here the two data types as they do not have the same detection levels, the single-copy nuclear gene *Dau c1* being less detectable than the multicopy chloroplastic gene used for DNA metabarcoding of plants, but the latter being more likely to be distorted by different molecular biases.

We then assessed the difference of the diversity, which also did not comply the assumption of normality or the amount of carrot spike-in DNA detected across passive sampler types with Kruskal-Wallis

tests, followed by post hoc Wilcoxon tests with false discovery rate-adjusted p-values when the former identified significant differences. Based on the results obtained, we considered only the best passive eDNA passive sampler for downstream analyses. These statistical analyses were run with the R package `rstatix` v0.7.2 [https://cran.r-project.org/package=rstatix; (98)].

Rainwash eDNA temporal dynamics

To identify the optimal temporal window for passive sampling of rainwash eDNA, we assessed the dynamics (i) of both MOTUs diversity and composition and (ii) of the carrot spike-in DNA. We did not expect the two plots to differ for these properties and preliminary analyses using mixed models did not retain this random effect. We thus analyzed the data without accounting for this factor.

For this specific analysis, we considered MOTU diversity at $q = 0$ after data rarefaction (see Bioinformatics and data preparation section) rather than at $q = 1$. We did so because it gives more weight to rare species (99) and is thus more sensitive to detect MOTU accumulation through time by better accounting for MOTUs that would become rarer due to eDNA decay. We expected MOTU diversity to accumulate rapidly at the start of the experiment and then either (i) to accumulate more slowly if the carrying capacity of the filter is not reached at t_n and/or if the decay rate of eDNA is lower than that of the influx of new ones (ii) to reach a plateau if the carrying capacity of the filter is reached and/or if an equilibrium between the decay rate of eDNA and the influx of new ones, and last (iii) a decrease of MOTU diversity when the decay rate of rainwash eDNA exceeds that of the influx of new eDNA molecules (e.g., due to reduced rain events). To this end, we fitted several nonlinear functions (41), i.e., power ($D = at^b$), monomolecular [$D = a(1 - e^{-bt})$], or Ricker ($D = ate^{-bt}$) on plants, vertebrates, and insects MOTU diversity (D) patterns through time (t). We selected the models exhibiting the lowest Akaike information criterion (AIC), here built with the Ricker function, from which we estimated the initial diversity accumulation rate (a) and time at which the diversity was maximal ($T_{D_{\max}} = 1/b$). Models predicted values and their associated standard deviations and confidence intervals were obtained with the R package `propagate` v1.0.6 [https://CRAN.R-project.org/package=propagate, (100)].

We also analyzed the MOTU compositional turnover at $q = 0$ (see above) using a distance-decay relationship approach, as we expected samples collected at close sampling date to exhibit higher similarity than those collected at distant sampling dates, due to both eDNA persistence/decay and arrival of new eDNA molecules through time. The temporal distance-decay pattern was studied by considering only the pairwise comparisons involving the same spatial sampling point. It was assessed with a semi-log linear model, which is often used to model distance-decay patterns (101) and permutation tests to assess the slope significance with the R package `lmPerm` [https://CRAN.R-project.org/package=lmPerm; (102)]. To circumvent the log transformation problem on null similarity values, we added the smallest non-null similarity value to all similarity values.

Last, we determined the persistence of rainwash eDNA by estimating the decay of the carrot spike-in DNA using a first-order decay model (i.e., a negative exponential function) of the form $C_t = C_0 e^{-\lambda t}$ where C_0 is the initial eDNA concentration, t is the time in days, and λ is the DNA decay rate constant (per day), from which then derived the time of rainwash eDNA detectability as $t_{0.05} = \log(0.05)/\lambda$, corresponding to the time it would take for the carrot spike-in DNA to fall below 5% of its initial concentration. While more complex decay models have been found to outperform the one considered here to

estimate eDNA decay rate constants (39), our approach allows for cross condition comparisons more easily and appears to be biased only for very slow decay rates (e.g., at low temperature and in absence of light), which we do not expect to be the case here. We fitted the decay model only on Apiaceae average reads abundance per PCR reaction. We did not consider here the number of *Dau c1* copies/ μ l DNA extract, as the detection was too limited to obtain reliable fits and because quantities obtained are expressed in a different unit than DNA metabarcoding data.

Rainwash eDNA spatial coverage

To assess the optimal spatial sampling effort, we built rarefaction curves for plants and invertebrates data obtained in the spatial experiment (Fig. 1A). We did so by averaging all diversity values obtained from all possible sample combinations at each sampling intensity (in number of sampling points per plot) and for each plot separately, as we expected the two plot to exhibit different diversity and spatial accumulation rates (103). Here, MOTU diversity was measured at $q = 1$ instead of $q = 0$ on rarefied data. The main reason for this choice lies in the robustness of the diversity measure at $q = 1$ to differences in sequencing efforts and potential remaining errors (91, 92), as indicated above. In addition, the rate of increase of the uncertainty associated with species addition at $q = 1$ is reduced compared to $q = 0$ (99, 104). Last, unlike the MOTU temporal dynamics analyzed above, we did not seek to detect subtle changes in species abundances with increasing sampling effort.

We then fitted on MOTU accumulation curves two simple functions classically used to describe species-area/accumulation curves that exhibit an upper asymptote to estimate the plot-scale diversity and from this, of our sampling coverage. These functions corresponded to the Monod [$DN = D_{\max}N/(k_N + N)$] and negative exponential [$D = D_{\max}(1 - e^{-k_N})$] models (40), where D is the diversity; D_{\max} the upper asymptote, corresponding here to a theoretical plot-scale diversity detectable with our approach over the period of time considered in our study; N is the number of samples; k_N is the “half velocity constant”, in our case the number of samples required to reach 50% of the plot-scale diversity; and k is the MOTU accumulation rate. As for above, we selected the function with the lowest AIC, which corresponded to the Monod models in all cases. We used these models to predict the number of spatial sampling points required to sample 70 and 90% of the plot-scale diversity. Models' predicted values and associated confidence intervals were obtained with the R package `propagate` (100) as above. Last, we also evaluated the pairwise MOTU compositional similarities at $q = 1$ between samples across or within each forest plot. We also assessed the relationship between MOTU compositional similarity and geographic distances graphically.

Supplementary Materials

The PDF file includes:

Supplementary Text S1
Figs. S1 to S11
Tables S1 to S5
Legend for data S1
References

Other Supplementary Material for this manuscript includes the following:

Data S1

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