REASSEMBLY Newsletter

Reassembly of species interaction networks – Resistance, resilience and functional recovery of a rainforest ecosystem (DFG Research Unit FOR 5207)

#1, 2022

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Nico Blüthgen, TU Darmstadt

¡Qué año!

Our joint endeavor called **Reassembly** officially began in November 2021 with our **team** involving forty-four people. Now, as I write this column, our research unit has become a one-year-old toddler, at least formally when discounting the many years of planning and individual research beforehand. Along the lifetime axis of a human being, one year may be short – but we know how quickly animal communities change and trees grow in a tropical forest, best seen along the chronosequence of our study site, and likewise our experience, insights and data grew quickly. One year full of scientific opportunities and observations, personal experience and privileges.

Resilience of research and forests, wounds and minds

Exciting opportunities rarely appear without challenges and obstacles, unexpected problems and drawbacks. Biological networks may be hard to predict – scientific collaboration networks can be even challenging. The nodes are individual persons with individual characters, and the resulting links and outcomes may sometimes appear stochastic. But all problems have been solved and settled shortly after their appearance – which again was achieved by a great team effort from many sides. Reassembly not only investigates how biological parameters recover after disturbance, our scientific exercise itself goes through small disturbance – recovery cycles. This includes a first grant application draft submitted in 2018 which then had to be revised and turned into a second one before it was supported later by the DFG. As expected, the original idea and proposal actually recovered in a much better shape than the previous one.

Now, one year after implementation, all empirical subprojects finished at least one round of work on all the 62 chronosequence plots, despite daunting efforts and exhausting long distances. We experienced many cases of physical or mental exhaustion, wounds, diffuse bacterial infections, leishmaniosis, hepatitis, a wave of covid, a car running over Connie's leg. The team has overcome all of them. Again, recovery can be fast, not only for a forest ecosystem.

Great data collections, compilations and analyses have been achieved and interpretations are in progress – overall, this looks fantastic. Some first results were discussed in a lively workshop last October in the Bavarian Forest. It is highly motivating to see such new insights across the chronosequence and across subprojects, and some earlier efforts by Phil and Karen have now also resulted in the first publications. See our big and small success stories and **news on our homepage**.

Only our main experiment, P-REX, received too little attention by many of us in the beginning, mainly due to logistic problems and over-commitments. This has been disappointing, as P-REX represents not only a very laborious team effort so far, implemented a year ago over several weeks, but also a particularly important scientific element of Reassembly to understand the disturbance-recovery dynamics. However, now we defined a solution that will be more inclusive and we re-disturb part of the treatment next year. Even the re-disturbance itself is an opportunity to measure components that have already regrown after one year. Another wound that seems to be healing quickly.

Resistance of optimism

Most of us share an optimistic view about such recoveries and wound-healing from minds to forests, and the science and the progress that can be achieved in this research unit. How do I revive my optimism when problems appear and drawbacks seem to constrain the progress? I remind myself that it is an incredible privilege to learn from all other participants of the large Reassembly network. Our network nodes include knowledgeable local co-workers, young enthusiastic researchers and experienced colleagues. The links between us are particularly rewarding and this exchange grows – the network becomes more and more connected, and the importance of each link grows.

To see the rainforest through a different perspective and to participate in thinking, observing and measuring of another person is highly rewarding. Each of us learns from young and from experienced researchers, from a skilful expert of another taxon or from a seemingly naïve thought of an outsider of your own expertise. One of my highlights last year was a night with an enthusiastic Santiago mist-netting bats, and I vividly remember several night walks with Mark-Oliver and Yadira finding dozens of frogs, day walks with Connie learning from her about the species composition of a mixed bird flock and discussions with David looking at ant colonies.

I can only encourage everyone in the project to take some time, say a few hours on a day or night, to join another researcher during his or her work in the forest, and to see the ecology of a forest again through a different lens. Take this as your 'homework' for 2023: join at least two other researchers on their field trip.

And breathe! On a quiet moment during a forest walk, an armadillo was crossing the path close to my rubber boots, on another walk spider and howler monkeys were close and calm enough to be filmed **(see here)**. Rain cooling the sweat of my body after a long hike, a particularly pretty creek crossing or another beetle species on a leaf that I never saw before. All these little things help to resolve problems and recover a previously stressful, hectic, or troubled mind or angry mood. This may sound inappropriately romantic or esoteric when you read this in your office, but I am confident that most of us who were or still are in the forest agree on such mental or spiritual ecosystem services of a recovering or intact tropical forest. It is rewarding, peaceful, and very exciting.

¡Qué oportunidad!

Our joint project is a basic scientific opportunity, but also allows us to appreciate how effective restoration and conservation can be. Jocotoco is an amazingly successful non-profit organisation, involves highly engaged people locally at each reserve as well as in the headquarters, is well respected regionally and nation-wide, and even in an increasingly crucial role to meet global goals for biodiversity and climate change. While occasionally interactions with Jocotoco or demands of ecotourism or donors may not always appear to run smoothly to everyone – remember the unique chance offered by our collaboration with this conservation foundation. Conducting such a large-scale research endeavour would be absolutely impossible without Jocotoco, without their past and continuous vision and wisdom – starting already around 2000 to buy farmland to let it grow back into rainforest – which we now study as a well-resolved chronosequence. The opportunities include the knowledge and support by the people, the safety and logistics, the hiking trails, the accommodation and huts. Impossible without the help of the current, highly motivated and supportive staff – Katrin, Bryan, Lady and Leo among several other great shorter-term helpers. And an amazing research station – the Chocó lab - that was built and implemented at an unprecedented speed and now seems to serve all our needs and even more. It was largely funded by an anonymous donor, made possible through immense personal effort by Adela and others, and we co-funded parts of it via the TU Darmstadt. I am very, very thankful for all of these opportunities. Many thanks are also due to our paradise-like "substation" Tesoro Escondido and the personal effort and friendship by the team led by Tali, Yadi and Adri. Now our endeavour has even grown with an additional funding allocated to study rainforest recovery in another great Jocotoco reserve, Buenaventura. But this may be a separate topic and can be left for the next newsletter. Of course, my annual thanks include all 44 ($\pm$10) team members and all 62 ($\pm$2) plots.

Last but not least I am indebted to Connie for her unique commitment in solving all these major scientific and mental issues very professionally in all multiple dimensions you can imagine in the first year of Reassembly. Connie has left a very lasting mark on the project, and we all wish her the best for her fantastic new position as a Conservation Scientist at the British Royal Society for the Protection of Birds (RSPB). My last recovery metaphor here is that we will even survive and recover from this disturbance – with a new scientific coordinator starting already early next year. Her name stands for optimism: Felicity.

When Martin and I had the crazy idea of such a research project – in 2017 on his veranda in Freiburg during our Easter holidays – we hadn't yet envisioned such an exciting and huge research unit that we all

now are. The first year has been full of great achievements. This clearly became my favourite research endeavour ever [please don't tell the others in which I am involved or even leading ;)]. I am looking forward to see the scientific and ecological networks grow, and to see the collaboration networks tighten within and outside our research unit. And now I am very eager to read this exciting Reassembly newsletter No. 1.

Figure 1: *Ectatomma tuberculatum* ant on a extrafloral nectary (*Inga*)

Figure 2: Orchid bee *Euglossa*

SP Coordination Module

Connie Tremlett, TU Darmstadt

What everyone should know about my work

The Coordination Module (CM) is responsible for the scientific coordination among sub-projects, the provision of baseline data, and logistics, organisation and support. CM is headed up by Nico Blüthgen, and includes the station managers and the field team at Canandé, the two project postdocs, and the Co-PIs. This section is written by the scientific coordinator.

The CM module had a lot of work to do in the first year of the project! This included to:

1. Finalise the selection and implementation of the chronosequence plots;
2. Implement the Perturbation-Recovery Experiment (P-REX);
3. Start the tree inventory project;
4. Start the collection of baseline data from plots;
5. Organise the kick-off meeting (hosted in Frankfurt, Germany), the first workshop (hosted at the Chocó Lab, Ecuador), and an autumn meeting (in the Bavarian Forest, Germany;
6. Define policies and procedures for the project (data management, publications).

Plans and Reality

1. The selection and implementation of the chronosequence plots

The selection of the chronosequence plots actually took several years – many of the plots were initially selected by Nico, Martin and Karsten Mody over field trips in 2018 and 2019. However, the size of the plots increased from 25 m² to 50 m² during the review process for the project funding, and so many of the initially selected plots were too small. As those of you that have visited the project site will know, the terrain can be quite tough – steep hills and a LOT of mud – so finding 50m² areas that are relatively convenient for fieldwork is quite a challenge. This was particularly key for the subset of plots that will be more intensively studied as part of the P-REX. We also wanted to address an elevation bias in the initial plot selection which resulted in a tendency for active agricultural plots to be found at a lower elevation and old-growth forest plots at a higher elevation (the higher, least accessible parts of the forest were less likely to be logged or cleared for agriculture).
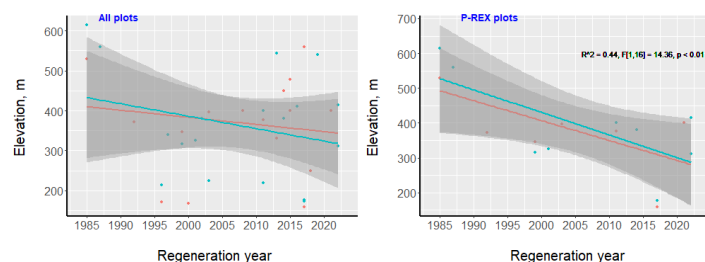
Figure 3: Regeneration year and elevation for regenerating plots along the chronosequence, for all plots (left) and P-REX plots (right).

This meant searching out new plots at different elevations. We optimistically thought we could make the final selection during a five week trip to Ecuador in September and October 2021, but in fact this process continued until early April 2022. There is now no statistical difference in elevation between plot types, but we do still have an effect of regeneration age within the P-REX subset – the higher plots were more likely to have been abandoned a long time ago (Fig. 3).

We decided this was a worthwhile trade-off in exchange for more accessible plots (the possible alternatives were much further from the main shelters).

2. Implementation of the Perturbation-Recovery Experiment (P-REX)

The P-REX plots were marked out and fenced between October 2021 and February 2022. This in itself is a hard job! The first challenge was finding areas that could fit a 50 m² plot, accurately mark the boundary, and select four 10 m² subplots that were as convenient for placing experiments as possible. The fences are made of a thick mesh material, which is cheaper and easier to transport than metal. This means that it is flexible and will not break when a tree lands on it, though the integrity of the fence is sometimes compromised when it is squashed by mud or branches. We are therefore replacing plastic tubes with wooden fence posts. We implemented the P-REX disturbance work in the plots between the 5th of February and March 24th (Covid-19 delayed the beginning of the work). It then took a full day to implement the P-REX work at each plot.

Figure 4: PREX plot with all litter removed, featuring litter traps in white and fence in black. Blue line marks "holy area".

We removed and quantified: all trees and palms with a circumference at breast height (cbh) of below 25 cm (we recorded individual cbh of trees/palms with a cbh of between 6-25 cm, and count of individuals and wet weight of trees and palms [as separate categories] with a cbh of below 6 cm); seedlings (classified as all plants below knee height, count of individuals and collective wet weight); grasses (collective wet weight); vines (classified as any plant with a creeping or scandent growth habit that is not a woody liana, collective wet weight); and other monocots (count and collective wet weight). Across all the plots, this alone added up to 8,700kg! We also removed leaf litter and dead wood but did not quantify them (we had planned to do so at first but found it was too time-consuming). Everything was dumped outside of the 50x50m plot, which caused unavoidable disturbance along the way. Comparison of the wet weights for vegetation between sites will be complicated by variation in rainfall. We also measured the circumference of larger trees and palms, and woody lianas, but did not remove them from the plot. Implementing the P-REX was pretty gruelling and we were all glad when

it was finished! We also, unsurprising, encountered many snakes along the way, and I was very thankful that there were no accidents.

3. Start of the tree inventory project

The first step to the tree inventory is tagging the trees. This work was started in December 2020, and is still continuing - see the next section from Sebastián, who is leading the botanical fieldwork. The team mark the trees with aluminium tags and nails, before taking measurements. Our main problems here were sourcing the tagging materials. We bought 2000 aluminium tags from the USA that got stuck in customs for 5 months, and we had to pay an additional fee to release them. It is also very difficult to source aluminium nails in Ecuador, so we unfortunately had a few months where we were working with steel nails - this is problematic as the steel can oxidise and be harmful to the tree.

4. Start the collection of baseline data from plots

We placed all the iButtons out at the plots by the end of April, which record data on temperature and humidity once per hour. One datalogger is placed in each plot in a position thought to be generally representative, at a height of 1.5 m, and in the shade to avoid overheating in the sun. The data needs to be downloaded from the iButtons every 150 days, and we are trialling a handheld reader that downloads the data from the loggers in the field, which so far seems to work nicely. Preliminary data shows, as expected, a generally very high humidity everywhere (mean of >90%), with active agricultural plots the least humid and old-growth forest plots the most humid (Fig. 5).
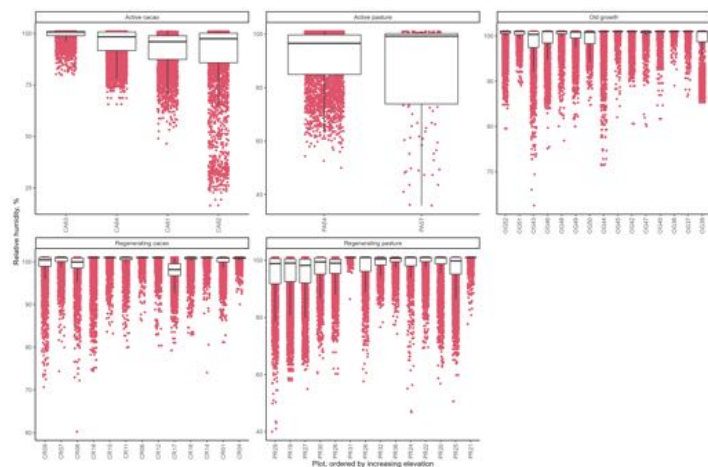

Figure 5: Relative humidity, % recorded by iButtons at plots, with plots ordered by increasing elevation, showing clockwise from top left: active cacao, active pasture, old-growth, regenerating cacao and regenerating pasture plots

Mean temperatures at plots were between 22 and 25 degrees, with lowest temperatures in old-growth forest and highest temperatures in active agricultural plots. Temperature decreases with increased elevation (Fig. 6). A further challenge here is loggers going missing, particularly in active agricultural plots.

The next step is to decide which further meteorological data we would like to collect (e.g. rainfall) and buy the equipment, and to organise analyses of soil chemistry and composition. The soil analysis in particular is no small task, and to do it properly requires following a very thorough sampling protocol using professional (and expensive) equipment. We plan to measure, at a depth of both 0-10cm and 40-50cm: soil pH, various elements (including nitrogen, phosphorous, potassium,

calcium, magnesium, sulphur, iron, copper, manganese, zinc, borium), organic carbon and the C/N ratio.
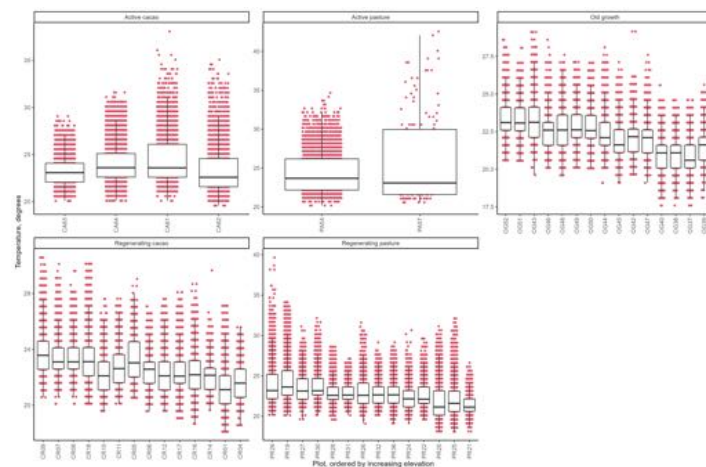

Figure 6: Temperature °C recorded by iButtons at plots. Plots are ordered by increasing elevation, showing clockwise from top left: active cacao, active pasture, old-growth, regenerating cacao and regenerating pasture plots

5. Organise meetings and workshops

We had the kick-off meeting for the Reassembly project at the Palmengarten in Frankfurt, back in November 2021. It was great to get lots of the team together (lots of old friends, but lots of new faces too, including me) to officially start the project, introduce the study site and team, and to welcome the new PhD researchers who all gave a short talk to introduce their projects. Our first more applied workshop was held in the Choco Lab at the end of March 2022, where Nico and Carsten introduced data standards, entry and sharing, and gave an introduction to RMarkdown, Github and network analyses. The internet connection was the main challenge at both events; it is important to design programmes that can cope with the Wi-Fi disappearing for a few hours. We then met in the beautiful Nationalpark Bayerischer Wald for our autumn workshop, which we kept quite informal as an opportunity to meet together and talk, discuss forests and restoration, and particularly to encourage integration among sub-projects. We are also busy organising the first annual meeting for the project, which will be held in January 2023 in Germany.

6. Define policies and procedures

Another important and time-consuming activity has been to define policies and procedures for the project. This has included developing a strategy for data management and storage, and defining the Reassembly project Rules of Procedure – our policies for sharing data, co-authorship, synthesis works, collaborations, and other important things to support conducting science with the ethos of fair, open and transparent science.

Impressions from the field

We are very lucky to have such a beautiful and biodiverse field site, with a fantastic research lab, and such a wonderful local team! But it is also challenging. My main practical insight from spending time in the field is that it is important to plan 'extra' time for fieldwork to allow for the inevitable setbacks from weather, landslides, health issues, limited staff time and transport etc.

SP Coordination Module

Sebastián Escobar, Universidad de Las Américas

What everyone should know about my work

The Coordination Module (CM) is responsible for obtaining baseline data that will be used on its own, but can be also used by other students or researchers in their own projects. I am one of the two postdocs in the project and work at Universidad de Las Américas (UDLA) with María José Endara, who is an expert in tropical ecology and evolution. Besides her, I also coordinate activities with Nico Blüthgen, Connie Tremlett, and Juan Guevara. With the help of two parabiologists, we carried out several activities during 2022. First, we are collecting data on forest structure and tree diversity. We have also collected samples from all tree species to develop a genetic barcode library, and for phylogenetic and chemical analyses. CM also facilitates the obtention of all the permits required by students and researchers to develop their research.

Plans and Reality

1. Forest structure and tree inventory

This activity looks to understand how biomass production and tree diversity vary along a gradient of forest regeneration. We expect that these parameters increase along the chronosequence as forests recover from disturbance. In addition, we will analyse our forest structure and tree data along with climatic and soil data. This activity has been the most time-consuming of all three during the first year of the project and it is still not finished. This work cannot be carried out by myself alone. I have received the valuable assistance of two parabiologist in the field, Fredi and Franklin; although, Johan has also stepped in when Franklin has been busy with other activities. The two parabiologists are the first ones arriving to the plots. They tag all trees with a circumference at breast height > 25 cm and measure the circumferences of their stem and height. If multiple stems are present, as it is the case of cacao or some palms, they measure the five biggest stems.

Doing this job was a little complicated at the beginning of the project because, as Connie mentioned in the previous section, we did not have the necessary materials to do so. And as you can imagine, getting specialized materials in Ecuador is difficult, expensive, and can take many, many months. To start with, we did not have available metal plates when the project started because they were stuck at Ecuadorian customs during several months. We therefore had to borrow some plates from collaborators, who luckily agreed to provide some until we got our own. If the plates did not arrive, the aluminum nails did not either. Thus, the trees in the first plots were tagged using 'regular' steel nails that could damage the individuals because of the formation of oxide. This is, of course, not the optimal solution but helped to start with tree tagging during the last months of 2021. It is worth mentioning that no tree was killed or seriously damaged due to the use of steel nails. Later, we were suggested by Jocotoco to use nylon string to tie plates around trees. Aluminum nails finally arrived at the end of June, and then we started using them. During following visits to the plots, we have observed that some plates attached with steel nails or nylon string have already fallen off and need to be re-attached with aluminum nails. This should be done in at least 27 plots to ensure that tags will remain in place for new measurements during following years.

In total, 46 plots have been tagged and measured so far. Only four plots (Casa Rosero) are left to be tagged because we are not doing it in the 12 pasture and cacao active plots.

Once tree tagging and measuring is done in a certain plot, we visit it again to identify and sample the marked trees. Since I have been a 'palm guy' for more than ten years, the learning curve of tree diversity at Canandé was steep during the first field campaigns. Luckily, I have received valuable help in this matter from Juan, who is an expert in tropical tree taxonomy, and from Fredi and the parabiologists who know the local flora. Tree identification and sampling could take several days depending on the type of forest and its stage of regeneration. For instance, a plot in a regenerated pasture or cacao could take one day or less. But a plot in an old-growth forest could take between three and five days, depending on the number and diversity of trees, the time needed to arrive to the plot, the weather, and the terrain.

The most difficult part of this activity is that we need to observe closely (fresh) leaves and branches to identify the trees species. Finding fruits and flowers also helps species identification. But trees are tall. And leaves are usually high. Thus, we use different techniques to take a close look on those leaves. We first use binoculars for identifying the tree species based on the shape, colour, and vein patterns of the leaves. This means that we are constantly looking up but also trying to pay attention to our surroundings because we do not want to step on a snake or fall into a hole. If we cannot identify the tree that way, we need to take the leaves down. We mainly use a telescopic pole pruner for this purpose, which also took several months to arrive in Ecuador. With this tool we can reach leaves up to 10 m height. For taller trees, we use a small lead fishing weight tied to a roll of string. The weight is thrown over a branch and then recovered so we can pull the branch with the string. A practical and easy solution for reaching leaves up to 15 m height. However, extra precaution must be taken when throwing the weight as it can fall down abruptly and provoke an accident. Sometimes the weight gets stuck among branches and we need to think ways to take it down. Thus, it is a good idea to bring more than one with us. If we cannot reach the tree leaves with any of these methods, we look for dry (sometimes green) leaves on the ground. But if any of this works, we then cut a small portion of wood for genetic identification using barcodes.

Leaf samples that cannot be identified in the field are taken to the National Herbarium to do so. Those samples need to be prepared first, which we do every day after returning from the field. We place a leaf sample within a sheet of newspaper and stack many to make them flat. In addition, we cut a small leaf portion for genetic and chemical analyses which are stored in paper bags with silica gel to get dry. At the herbarium, the samples are pressed once again using pressure straps and then dried in a large oven at 65–70°C for 24 hours. Once the samples are dry, we can compare their morphology with samples at the herbarium for a correct identification. Getting the necessary materials for this activity has not been difficult, with exception of newspapers whose production gets reduced with time. Therefore, we buy as much as we can when we find it.

We have identified and sampled trees in 32 plots, being ten old-growth forest, ten cacao regeneration, and 12 pasture regeneration. A total of 4740 individuals have been surveyed and 3907 of these (82.4%) have been fully identified up to species level. Seven hundred fifty-six samples have been collected up to date belonging to 469 species.

2. Genetic barcode library

CM is also preparing a barcode library using the samples collected during the tree inventory. The idea is that students and researchers working with seeds, pollen, and seedlings can identify the species they are working with by comparing their barcode sequences with ours. We will barcode our tree samples using four genes commonly used for this purpose (ITS, matK, rbcL, and trnH-psbA). All lab materials and reagents have

been bought and already arrived at UDLA. This, of course, took many months as well. We have just started grinding leaf samples, which is a necessary step for DNA and chemical compounds extraction. We could not start this activity earlier because we were waiting for specialized reinforced tubes to arrive. But now they are in our hands. Thus, we expect to perform DNA extractions and PCR amplifications massively during the following year. At first, we planned sequencing the barcodes at the facilities of UDLA. However, the Sanger sequencer there works with DNA fragments up to 500 bp and all our barcodes are longer than that. Thus, we will sequence our samples at Macrogen in Korea, which is even 30 percent cheaper if we send the samples in plates instead of in tubes. Related to this genetic work, we will also barcode samples from students that require it. We will particularly assist barcoding seedlings and seeds collected by students, but we could also barcode animal samples if needed. Seedling samples have been already delivered at UDLA and will be processed next year along with the tree samples.

Additionally, we can use barcode sequences to build phylogenetic trees. Thus, we could build a phylogenetic tree for each plot and study how phylogenetic diversity changes along the chronosequence of forest regeneration. Phylogenetic diversity is a measure of biodiversity that integrates the evolutionary history between the species of a community and could be considered a more explanatory measure of biodiversity than alpha diversity measures such as species richness [1]. We expect that phylogenetic diversity will increase with forest regeneration. Nevertheless, it is possible that the highest levels are not in the oldest stages of regeneration because an arborescent fern, which is evolutionary distant from seed plants [2], is only present in regenerating forests. Whether ferns, lianas, and other plants that are not technically trees but present thick stems (palms?) are kept in the inventory remains to be discussed.

3. Research permits

Another activity in which CM is involved is obtaining different permits that students and researchers need. We did not need to obtain a sampling permit (Contrato Marco) because it was already managed by UDLA. Nevertheless, we need to request mobilization permits every time samples are moved from the Canandé reserve to Quito. We have obtained 20 mobilization permits for 2353 samples/lots during this first year. We are also in charge of obtaining export permits which are mandatory to take samples out of the country. To get an export permit, we first need to sign a Material Transfer Agreement (MTA) between UDLA and the German university where samples will be moved to. This step can take a couple of weeks plus two or three more weeks for the processing of the export permit. Therefore, I would like to emphasize again that students and researchers need to send a filled MTA at least five weeks before their planned trip. Otherwise, there could be delays (and there have been) in obtaining an export permit. The tricky part with these permits is that we need a mobilization permit for requesting an export permit, but not for signing the MTA. Thus, your samples should be in Quito two or three weeks before you leave the country so we can start with the export process.

Impressions from the field

After spending a year divided between the humid forests of Canandé and the office at UDLA, I definitely suggest students and researchers to always leave extra time in your planning for unexpected delays. This applies for obtaining all necessary permits, getting materials and reagents, and for the sometimes unpredictable conditions of field work.

First results

Based on the data collected so far, tree Alpha diversity (Shannon Index) significantly increases with time of regeneration (Fig. 7). This was observed in cacao (Adjusted R^2 = 0.566, $p < 0.01$) and pasture regeneration plots (Adjusted R^2 = 0.518, p = 0.011), as well as in both together (Adjusted R^2 = 0.539, $p < 0.001$). This agrees with our expectation that tree diversity increases as forest recovers from disturbance, and apparently this is independent from previous land use.
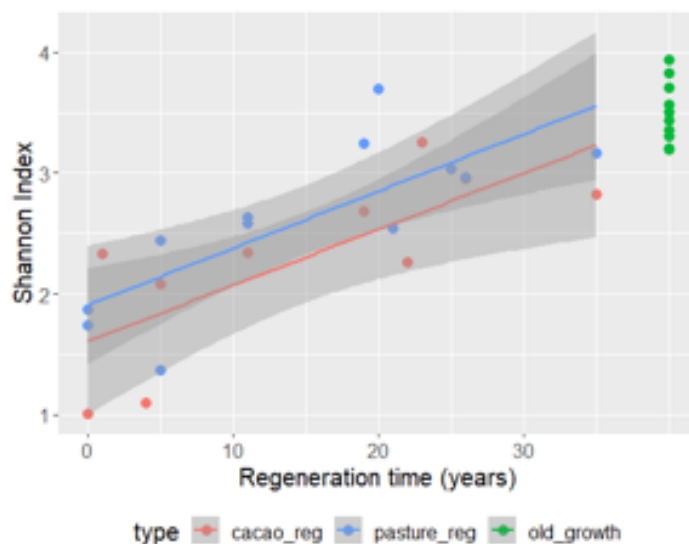

Figure 7: Tree Alpha diversity (Shannon Index) significantly increases with time of regeneration in cacao and pasture regeneration plots. Data on old-growth forest are displayed for comparison.

Plans for 2023

The tree inventory is expected to finish within the first semester of the following year and herbarium work may last several months after finishing the inventory. Then, tree and forest structure data will be analysed along with climatic and soil data to prepare a first manuscript. Genetic samples will be also processed at UDLA's facilities during 2023 to prepare the barcode library and a second manuscript on phylogenetic diversity.

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[2] S.-D. C. M. Palmer, J.D. The plant tree of life: and overview and some points of view. *American Journal of Botany*, 91(10):1437–1445, 2004.

SP 1: Theory and modelling

Timo Metz, TU Darmstadt

Who we are

SP1 is the theoretical subproject of REASSEMBLY. While many of the other subprojects focus on certain animal groups or interaction types, our theoretical approaches try to identify general patterns and overarching rules of network assembly for various groups of animals and interactions. We thereby are exchanging a lot with all the other subprojects to find out how to apply our models best to the needs of empirical scientists. Our subproject consists of two PIs and two PhD students. The PIs are Barbara Drossel, who is a theoretical physicist with a long background in the modelling of biological and ecological systems, and Carsten Dormann, an ecologist with a strong focus on statistical analysis. My PhD student colleague is William Castillo, who is originally from Ecuador and also studied physics there, but now focusses on the statistical analysis of ecological networks in Carsten Dormann's group. Finally, there is me, Timo Metz, who studied physics and biology and now combines these two fields of studies in Barbara Drossel's research group, to model the assembly of ecological networks. In my opinion, REASSEMBLY presents an exciting opportunity to build a bridge between empirical and theoretical ecologists.

Aims

My work deals with the theoretical aspects of ecosystem restoration and species interaction network reassembly. I use models and computer simulations to find out how ecological communities recover and especially how the interactions between species change and develop in time when ecosystems are restored. I want to work together with empirical scientists to explain observations from the field with theories how they could have emerged. Furthermore, I want to supplement the empirical work with theoretical expectations. With that, I try to broaden the knowledge about what helps ecosystems to recover and which building blocks are essential for successful ecosystem recovery.

How I work

Ecological systems such as tropical rainforests are very complicated and characterized by an confusingly high amount of species, individuals and interactions between species and individuals. As a theoreticist, I want to understand this complexity by simplifying it first and then gradually working my way up towards higher complexity to understand which new building block leads to which effect.

Fortunately, often it is not necessary to describe the whole system in all its detail to gain insights about very important processes. What I rather want to achieve is, to identify the main building blocks that characterise the system and its properties, so that my model reproduces general patterns that can also be observed in nature. Thereby, models can help us understand how and why general patterns that we observe emerge, because if our model is able to reproduce it, chances are good we are also able to understand it on a process-based level. If the model is good at representing the actual ecosystem, we can also observe patterns in the model, and find out if we also observe these patterns in the field. Thereby, the models might help us guiding empirical work into new directions. But what would be an example of such a "general pattern" that I keep talking about? Since I am interested mostly in species interactions, for instance, a general pattern would be a trend over time in the way species interact with each other. Are species at an early stage of

restoration interacting with a larger variety of other species (i.e. they are more generalized) compared to those at a later stage? Which species are only there at a later stage and why? Is this trend dependent on the type of interaction or on some specific environmental circumstances? What happens if we look at species that engage in multiple different types of interactions? Does one interaction get stronger than the other?

What I studied so far and how my model works

A type of interaction that I studied a lot during the first phase of my PhD was mutualism. Mutualistic interactions are beneficial for both interacting species. In terrestrial systems mutualistic interactions happen often between a plant and an animal. Examples are pollination (where the animal obtains pollen or nectar and the plant is pollinated) or seed dispersal (where the animal eats a fruit and the plant's seed is dispersed). In my model, a species is characterized by a trait and its possible range of interaction. In case of plant-seed disperser interactions, a trait that is very important for the interaction would be a bird's beak size and the plant's seed size. If they match well, an interaction is likely. If they match less well, but an interaction is still possible (for example if we have a large bird and a small seed) the interaction is less likely and less beneficial. Species can either be specialized if they interact only with few other species, or generalized, if they interact with many other species. Furthermore, they can be dependent on the interaction (for example a bee that needs nectar to survive) or not dependent on the interaction (for example a bird that does not only eat nectar or fruits but also insects). I call these animals non-obligate in contrast to "obligate" mutualists, which are dependent on mutualistic interactions for survival. Since we model the interaction network assembly in a recovering patch, these non-obligate mutualists can either be not dependent on their mutualistic partner in the sense that they are facultative (forage on something different than what is produced by the plant mutualistic partners) or non-resident (eat something produced by a mutualistic partner, but the partner is outside the investigated patch). It is important to note here that my model does not yet intend to model specific animal groups such as birds, bats or insects. Important is only what the species does, which is for example seed-dispersal of large or small seeds and how dependent the species is on the interaction for survival. Future works could, however, also look at different animal groups if they differ in their properties and if there is an interesting question to look at which needs this additional complication.

My model incorporates the restoration process by having repeated immigration attempts from the surrounding habitats (the intact rainforest or as a physicist would call it rather conceptionally: the species pool) over time. Species that are in the recovering habitat are then subject to competition and natural mortality (which decreases the population), but also natural population growth which is additionally increased due to mutualistic interactions with partners. Thereby, the model features two main properties of ecosystem dynamics: the "assembly dynamics" (which defines how the network develops) and the "population dynamics" (which defines how the populations of the species develop). Species can either be successful if their population grows and they establish in the habitat, or they can be unsuccessful if they go extinct (if they have too little interactions and too much competition). Thereby, the recovering habitat changes over time, with new species successfully entering the network and old ones disappearing. Thereby, some important metrics change over time, such as the number of species, and the properties of the species (for example, the mean number of interactions that species in the network have). Furthermore, the assembly process itself is interesting. It is possible to observe whether species have higher or

lower probability of establishing successfully, depending on how the community looks like at the time of immigration. Simply speaking, with our model we perform "virtual experiments". We for example ask: what changes in the simulation output, if we change this thing in the model or if we incorporate this new effect. Thereby, we can learn what drives different changes in this "model system". In the results section, there will be an example of what changes if we have species in the network that are not dependent on their interaction partner for survival (which I called "non-obligate"). However, our model not only shows what changes, but by looking at different metrics of our model output, we can also find out why these changes happen. If we then compare the model's results to observations from the field, we can try to find out whether our model is right, and thereby gain a process understanding of the system.

Results

Recently, I submitted the first paper of my PhD and also published the paper on bioRxiv [2]. In the paper I investigated animal mutualists with a low dependence on mutualistic interactions and their influence on mutualistic network assembly. I found two major results. First, I could find that mutualistic network assembly was quicker in the presence of non-obligate animal mutualists compared to when they are not there. One can see this quite nicely in Fig. 8. There, the network development over time is shown for a model with only obligate animal mutualists (upper row) and with non-obligate animal mutualists additionally (lower row). Especially at the early stage (left two pictures), one can see that the network is in a far more developed stage, owing to a decreased competition and increased immigration success of immigrating plants and animals (see figures in the publication [2]).

The shown result is also a nice example of how theoretical ecology generally works. Carlo and Morales [1] could show empirically, that diet and habitat generalist birds promote tropical forest regeneration by enhanced seed dispersal. Our model suggests that the facilitation effect of diet and habitat generalists is not only valid for seed dispersal but in fact for any type of mutualism and not only for birds but for any kind of animal group which engages in a mutualistic interaction.
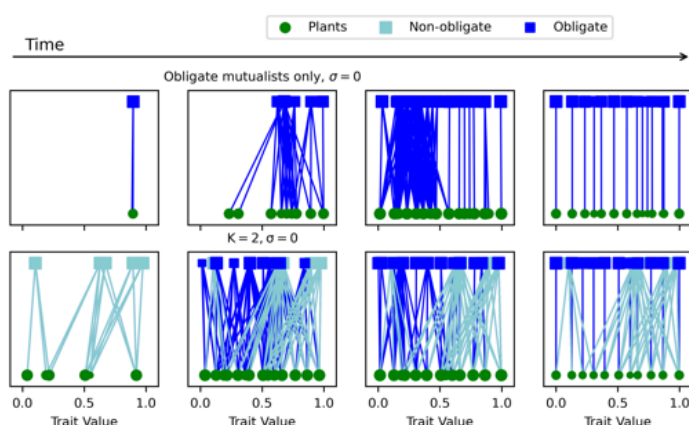
Figure 8: Snapshots of the network development over time. Upper row are snapshots from a model with only obligate animal mutualists. Below are snapshots from a model with non-obligate animal mutualists additionally

Another result that we obtained with our model is depicted in Fig. 9. There one can see on the left side that the contribution of non-obligate animal mutualists to the growth of plants due to mutualism (i.e. the

reproductive service) is initially 100% but decreases over time and eventually vanishes. Therefore, the obligate species take over and provide the full reproductive service in the long term. This result suggests that we have a gradual increase of specialized species in the ecosystem over time. While very generalist species help the network to assemble at the early stage, they become less important later on. However, on the right side, on can see a plot of the same model just with demographic noise turned on. Demographic noise represents random fluctuations in the population densities of species due to stochastic fluctuations in birth and death processes and also in interactions. It is visible that non-obligate animal mutualists still provide a non-zero fraction of reproductive service. A simulation with demographic noise is more realistic than a simulation without, and so is also the result: The obligate species, which are more specialized, provide more reproductive service in the long-term than the non-obligate species. Nevertheless, non-obligate species are still important and provide stability to the ecosystem by being more resistant to demographic noise.
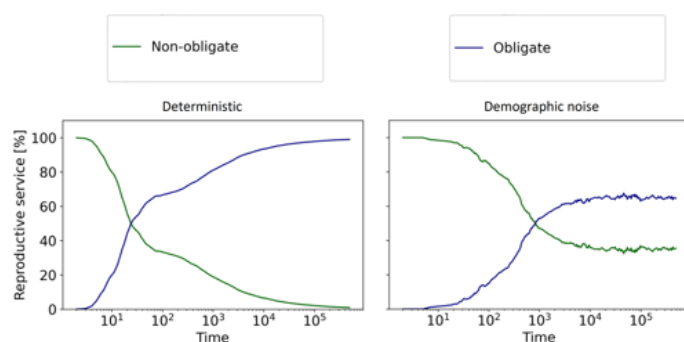
Figure 9: Reproductive service (contribution to plant growth) of the non-obligate and obligate animal mutualists. The left picture shows a simulation where population densities where not subject to demographic noise, which are small random fluctuations in the population density. On the right, demographic noise was turned on additionally.

Next steps

The type of model that I currently use can easily be adapted to other types of interactions. Currently, I work also on antagonistic interaction network assembly. Antagonistic interactions are beneficial for one partner, but detrimental to the other partner of the interaction. An example would be a plant-herbivore interaction, where the herbivore eats the plant. A long term goal, after understanding the assembly of antagonistic networks, is to assemble coupled networks of mutualistic and antagonistic networks. However, I would be interested in exchanging with the other SP's which work on antagonistic or coupled mutualistic-antagonistic interactions. Thereby, we could find out which topics would be most interesting to look at.

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SP 1: Theory and modelling

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What everyone should know

In the last few years tropical deforestation has occurred at a rate of over 7.6 million ha per year [8]. The Chocó rainforest is one of the most vulnerable and biodiverse ecosystems in Ecuador. Less than 2% of the original vegetation remains and the deforestation rate is the fastest in the country. Timber industry, extension of road infrastructure and conversion of forest to agricultural land are main causes of deforestation [1]. Then, understanding the dynamics of ecosystem regeneration is a pressing issue in ecology and society as well. In accordance with this problem, one of the main inquiries that this project aims to address is: if whether a disturbed forest is capable of reaching old-growth conditions [4].

The most common ideas who attempt to describe the development of ecological systems are based on descriptive data obtained by observing changes in biotic communities over long periods, or on highly theoretical assumptions (unfortunately, very few of the generally accepted hypotheses have been tested experimentally). For instance, in a recent perspective paper on long-term restoration of degraded ecosystems and their complexity, Moreno-Mateos et al. [5] brought this expectation to the point: regeneration will be fastest for species diversity, intermediate for species composition, and slowest for complex species interaction networks. Reassembly of even more complex features such as meta-community interaction networks will be more delayed than simpler networks (Fig. 10).
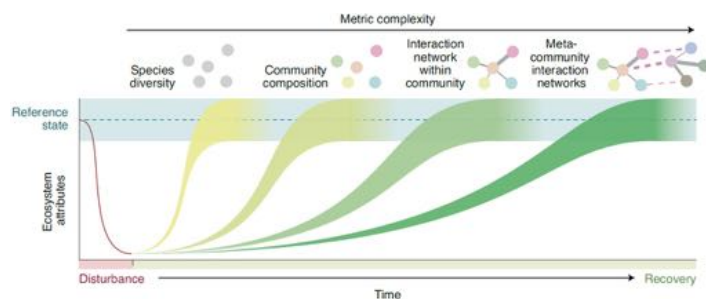

Figure 10: Prediction of trajectories of long-term recovery of an ecosystem, including four of its components with increasing complexity [5].

Nevertheless, as far as the evidence suggests, ecological regeneration is not a single straightforward idea (As Fig. 10 illustrate); in actual fact, it entails a strong interacting and complex process leading to collective behaviour. It is the result of modification of the physical environment by the community; that is, regeneration is community-controlled even though the physical environment determines the pattern, the rate of change, and often sets limits as to how far development can go [6].

By way of illustration, it is not entirely clear how to translate smaller-scale processes (predation, pollination, herbivory, tree seedling recruitment, etc.) into main characteristics of an ecosystem (abundance/distribution of organisms, ecological succession, human impact, etc.). The presence of many scales or, even worse, the confluence of scales and lack of a characteristic scale that would allow the breakdown of the problem into subproblems makes a standard reductionist micro-macro approach difficult. This property often makes systems sensitive to initial conditions, boundary conditions and small changes in the control parameters [7, 2, 3].

Plans & Reality

Although the importance -and beauty- of properties of individual species and their interactions remain in many aspects. Statistical physics and thermodynamics have taught us that not all microscopic details are equally important if a macroscopic description is our aim [7, 3]. Just as statistical mechanics provides a framework to relate the microscopic properties of individual atoms and molecules to the macroscopic properties of materials, ecology needs a theory to relate key biological properties at the individual scale, with macroecological properties at the community scale.

Still and all, this step is more than a mere generalization of the standard statistical mechanics approach. Indeed, in contrast to inanimate matter, for which particles have a given identity with known interactions that are always at play, in ecosystems we deal with entities that evolve, mutate, and change, and that can turn on or off as well as tune their interactions with partners.

Outlook

Since I started working from the perspective of complexity science, I have worked in geology, meteorology and currently ecology. In these disciplines we had worked with data that were not collected for the specific analysis we performed. This decreases the quality of data and increases uncertainty of systems which are already complicated. Therefore, one of our main challenges is producing data under the conditions and assumptions of the models under test. In sympathy with the classical experiments, design experiments to answer a specific question. This is a laborious task; data recollection is expensive and hard work. As well as, most of the systems that show complex behaviour are unrepeatable. Here, intensive interaction with empirical scientists is required.

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SP 2: Food webs and alkaloid defenses - frogs and litter fauna

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What everyone should know about my work

Who we are

SP2 investigates the structure of original and reassembled predator-prey networks, with a particular emphasis on the role of alkaloids as drivers for trophic interactions among anurans and their arthropod prey. The core research unit comprises of two PIs, two external collaborators and two PhD students, one of whom being myself. Our subproject includes researchers from a variety of backgrounds providing, in my opinion, a very holistic perspective on the research theme. With regards to the PIs, Michael Heethoff is a chemical ecologist and passionate acarologist and Mark-Oliver Rödel is an ecologist, conservation biologist and fearless herpetologist. Our collaborators are David Donoso, an ecologist, entomologist and, in particular, a Neotropical ants' expert and Ralph Saporito, a chemical ecologist and evolutionary biologist with leading expertise in the field of poison frogs' research. Finally, there are Karla Neira - a highly skilled biologist and amazing colleague – and myself, Arianna Tartara, a chemist with experience in environmental analysis and monitoring. Personally, I find it extremely exciting to work in such an interdisciplinary team, engaging in a work programme which includes a variety of research methods and conducting highly interlinked tasks with the final purpose to eventually answer a big common biological question.

Aims

The objective of SP2 is to investigate the role of alkaloids for the reassembly of a predator-prey networks of leaf-litter organisms – poison and non-poison anurans and their arthropod prey, centring on ants, oribatid mites and alkaloid-producing taxa. In fact, as by the "dietary hypothesis", some families of leaf-litter frogs (here, Dendrobatides) steal their defence mechanism, i.e. toxic alkaloids, from some of the arthropods they feed on [2]. Being a very intricate network with plenty of variables to examine, we split the work as follows: on the one hand, Karla focuses on the anuran assemblages and the prey items identification from the frogs' stomachs, on the other hand I examine arthropods communities and the alkaloids analyses. Along our chronosequence, we hypothesise the predator-prey networks to be most complex in old-growth forests and least complex in the actively-disturbed areas. We expect alkaloids to play a key role in structuring the respective food webs with the composition of alkaloid-synthesising prey communities influencing alkaloid profiles of sequestering anuran predators [4, 6]. In general, we expect species and functional diversity to increase in recovering forests [3], likely with non-poison frog - generalistic prey network recovering faster than the more specific poison frog - alkaloid-synthesising prey network. Furthermore, within the latter we also expect differences among species depending on which prey item they depend on (either ants or oribatid mites, millipedes or staphylinid beetles)[1, 5].

What I do

Within such an exciting framework, my role as a PhD student is to:
1. analyse the respective assemblage changes in leaf-litter arthropods across the chronosequence and along a shorter time-scale on the P-REX plots. On these experimental plots I will also monitor what environmental factors (e.g. small mammalian, canopy openness, vegetation, humidity) may affect arthropods during the recolonisation process;
2. based on the identity of prey items (Karla Neira), try to comprehend these specific predator-prey networks, how they disentangle from undisturbed to disturbed plots, and observe if and to what extent they reassemble in recovering forests and P-REX plots;
3. evaluate to what extent alkaloids - produced by specific arthropod prey - affect network reassembly.

In practice, this is done by:
1. characterising the arthropods communities that habitually live in each parcel of the chronosequence;
2. perform a litterbag experiment in the P-REX plots to examine the recolonisation process and evaluate the decomposition potential;
3. analyse for alkaloids the anurans skin samples and the allegedly alkaloid-synthesising prey;
4. network analysis.

Methods

In short, the methods included sampling of leaf-litter arthropods from every plot following an in situ extraction with Berlese funnels. In parallel, the litterbag study was run in the experimental plots and the samples were processed in the same manner. The litterbags design composed of a mesh of 0.5 cm to "size-exclude" the prospective poison-frogs prey, filled with a defined amount of locally-collected sterilised leaves. Every treatment of the P-REX plots contained three litter bags which are individually collected at regular intervals (5 weeks) for a total of 15 weeks. Upon evaluating what arthropods live in each plot along the chronosequence, the objective is to build a database of the leaf-litter mesofauna to later compare with the arthropods retrieved in the frogs' stomach (Karla Neira). It ensues the alkaloids analysis of the frogs' skin accompanied by the inventory of the prey items identified as part of the diet of the poison frogs. Subsequently, the following field season will focus on the collection of those very same arthropods considered to produce the cleptotoxins with the final aim to merge the data to elucidate the reassembly of the complex frog-arthropod network driven by the alkaloids.

Current status

In the moment of writing, I am exactly one year into the project and I can call myself satisfied with what has been achieved so far. I successfully completed my first field season and I am currently delving into the identification of the arthropods in the ca. 500 samples collected. I engaged with the setting up of the PREX with the CM and some members of the other subprojects. Coordinated with Karla's sampling schedule, we collected the frogs skin samples which I will soon analyse for alkaloids with the method developed by our collaborator Ralph Saporito. Besides the strictly scientific achievements, I think almost all subprojects can say that we have come together as a nice team of researchers. We have been supporting each other morally as well as collaborating with some practical duties. Most importantly, we are developing the best ways to communicate with each other as well as with the field assistants, the stakeholders and the research station staff.

Plans & Reality

In my imagination, fieldwork life as a PhD would have been extremely solitary; I was picturing myself alone, tackling all the difficulties of a new project and work environment, the dangers of the Choco forest, the stressful situations, the adaptation to a different lifestyle, the tropical

diseases.… Yet, no. Since the very beginning I was surrounded by passionate researchers and lovely human beings with whom I shared work strategies, emotions, difficulties, laughter… and tropical illnesses. In practice, I organised most of my sampling campaign combined with my colleagues – not only due to logistical reasons, but especially because of the convenience of teamwork. An example of the sense of community that I perceived dates back to the beginning of my field work, when I had to actually handcraft ca. 500 litterbags. A lot of project members helped me in this tedious and lengthy task, not only saving me time but also laying the basis for wonderful cooperation.

An unforeseen difficulty was the logistics of organising the rapid transport of the freshly-gathered samples to the research station to be extracted, especially for the furthest plots. The key point was not to let too much time pass between the collection and the processing of the sample due to possible decay and/or prolonged within-bag predation hence generating a bias in the abundance of extracted arthropods. Other unexpected variables were the recurring blackouts at the research station, meaning that the electricity would go off and the light-extraction of the samples would be interrupted for an unpredictable amount of time, sometimes days, leading to unpleasant consequences such as, once again, rotting and/or predation. This issue was finally solved with the use of a small generator connected to the Berlese funnels allowing a stable and smooth processing of the samples.

A further divergence from the original setup was part of the decomposition data that I planned to retrieve through the litterbag experiment, by calculating the weight loss between the dry weight of the samples before and after being in the field for the 3 different time intervals. In practice, in the perturbation treatments of some plots located on slopey terrain the data was spoiled by the unexpected mud slides. Such events occurred in old-growth forests as well as cacao and pasture regeneration; on the other hand, active plots are strategically located on flat land hence they were unaffected. The mud avalanches were induced by the deforestation as part of the P-REX - they swamped the litterbags so the respective leaf-litter content was plunged in dirt, inevitably influencing the later weight measurements. This bias also affected the practicality of the mesofauna identification of the respective litterbag samples. Indeed, plenty of soil granules fell into the Berlese funnels due to gravity, making it very slow and laborious to separate and classify the arthropods. In this regard, the parabiologist Jefferson played a key role in terms of help and patience. He spent a consistent amount of time at the stereoscope assisting me in "cleaning" the Berlese extracts hence separating the soil-dwelling organisms from dirt.

Where I work - Field Impressions

The field station of Canandé could be defined as heaven and hell simultaneously. In the beginning, both researchers and station staff experienced strong difficulties; but after a few months of adaptation, things started to run more smoothly until the Reserve became basically a second home to me. However, since the very beginning I was amazed by the enthusiasm, strength and availability of the parabiologists Leo, Lady, Jefferson, Franklin and Jordi and the plot manager Bryan. Coupled with the station manager Katrin Krauth, their assistance really made a difference in my work and without their help I would have not been able to complete the field season. Most importantly, not only they were essential workforce, but they also provided amazing moral support and kept up my spirit during tough times.

From a naturalistic perspective, Reserva Canandé is outstanding. I managed to observe wildlife as never before in my life both in terms of diversity and proximity. I now store in my memory a bank of unique experiences and encounters in the forest surrounding the station that

I will hardly forget: from the frightening grunts of the pecaris to the scent of gorgeous orchids, from the howls of the monkeys playing on the branches to the stunning colours of birds feeding undisturbed on tropical fruits … The community around Canandé was very generous and hospitable. Our parabiologists, field assistants and station staff were from the most part from these very same settlements, and they openly shared with us their knowledge and their time. They were all restless workers and irreplaceable companions – for me, spending moments with them was the real highlight of the field season.

Results

After exporting the samples to Germany, I am currently identifying the arthropods retrieved in these samples with the help of a Bachelor student Annika. I expect to soon have an overview of the arthropods' communities in each plot and within the P-REX. For those plots that were not affected by the mudslides I was able to measure the decomposition rate (Figure 11), which translates in the fact that I could not calculate the decay from the perturbation treatments of secondary and primary forests.
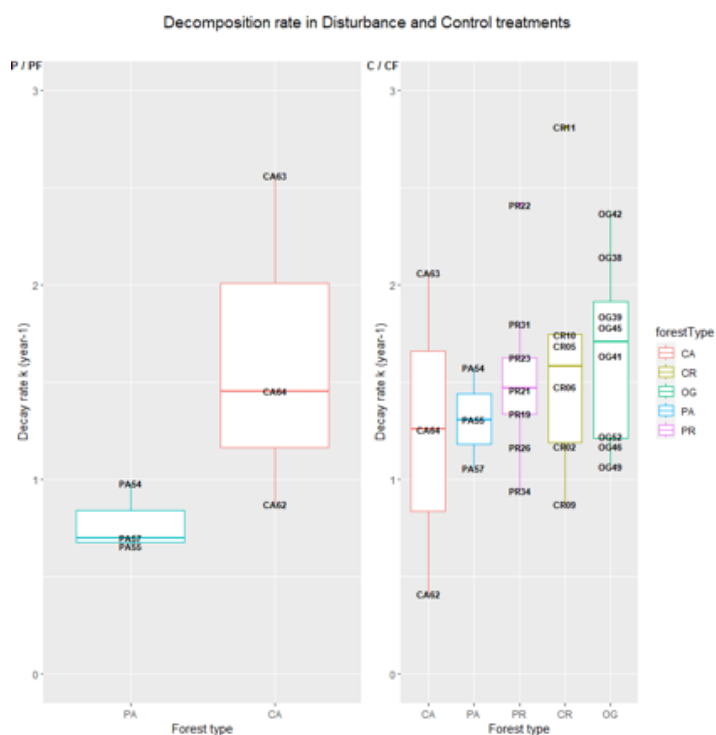

Figure 11: Boxplot of decay rates k. The values of the individual plots are reported. It should be noted that as by experimental design, the litterbags had a mesh size of 5mm hence excluding some decomposers.

In the control treatments (without differentiating the presence of the fence) the decay rate is highest in the old-growth forest with $k = 1.675$ yr^{-1}, and slowest in active cacao plantations and pastures with $k = 1.205$ yr^{-1} and k = 1.310 yr^{-1}, respectively. In the disturbance treatments only the data from the agricultural land was usable for calculating the decomposition rates. Pastures have a lower k after the perturbation compared to the control whereas litterbags decomposed faster in the disturbed cacaos than in the control. In both treatments, the cacao fields' decay rates have a huge variance, which I assume it can be explained by plot data like understorey microclimate and humidity. In general, I am looking forward to correlate the decay rates with the

outcome of the arthropods' community assessment as well as the data from other subprojects, such as canopy openness, soil parameters etc.

Within the more tangible realm of citizen science and public outreach, SP2 does have a first outcome (see the ReAssembly webpage). It was actually the local community who inspired us to attempt to return at least a bit of the openness and kindness they were spoiling us with. We thought of ways to integrate the research unit with the confined reality and we decided to involve the local school for a scientific workshop at the research station. The pupils and some respective parents engaged enthusiastically with the various educational activities SP2 proposed. Personally, I think of it as a success: we managed to lay the foundations of the bridge between foreign researchers and the local people. Explaining our work duties, showing the newly-built laboratory from the inside and exploring altogether the forest surrounding Canandé definitely helped to explain, or perhaps clarify, our role in the area.

Outlook

In the field

Once the identification process will be over, I will have a broader picture of the effect of the mudslides on the soil-dwelling organisms colonizing the litterbags affected by the deforestation simulation upon comparison with the control treatments. For the upcoming sampling season, I have already drafted an experiment to lay out in occasion of the "reperturbation" that the CM has planned for the beginning of next year. The design will follow the same litterbags method, it will include less time points but it will be sturdier and hopefully more resistant to the mudslides. In fact, I plan to repeat the experiment in the parts of forest types affected by the mudslides i.e. old-growth, cacao and pasture regeneration and protect the litterbags from the avalanches with a barrier up-hill. Moreover, I will install pitfall traps in the P-REX plots in order to assess the community of bigger (> 5 mm) leaf-litter mesofauna. The sampling will have a duration of 48 hours, making it logistically challenging. Once again, collaboration with the CM and other subprojects will be crucial for a successful outcome.

In the lab

In addition, chemical and molecular analyses are next to come. I will soon analyse the frogs' skin for alkaloids as well as get the individual arthropods barcoded. Very likely the chemical analysis will be performed in Quito with Sebastian Taco from the chemical engineering department at EPN. This consists of a wonderful opportunity to consolidate the partnership between Ecuadorian and German institutions as well as it will spare me the hassle of export permits, international shipping procedures and higher chances of spoiling the samples.

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SP 2: Food webs and alkaloid defenses - frogs and litter fauna

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What everyone should know about my work

SP2 is an interdisciplinary and intercultural team consisting of the PIs Michael and Mark-Oliver (MO), the major collaborators David and Ralph (who are introduced in detail in the section written by Arianna), and the doctoral students Arianna and Karla. Arianna is an Italian chemist with experience in environmental analysis in very remote areas of the world. And I, Karla, am an Ecuadorian biologist exploring the fascinating world of herpetology and ecology, mainly of amphibians since my undergraduate studies.

Together we aim to understand the reassembly of predator-prey networks. Leaf-litter frogs and the arthropods that make up their diet can provide us with an exceptional opportunity to learn more about reassembly processes. Arianna focuses on the leaf-litter arthropods' communities and alkaloids, while I study frogs' assemblages and their diet.

Frog-arthropod networks comprise a wide range of interactions. Some frog species ingest their prey randomly (generalists) and others consume only specific arthropods from those available in the environment (specialists) [5]. Although dietary specialization in frogs is rare, various poison frogs sequester alkaloids by feeding mostly on alkaloid-containing arthropods [2, 1]. For example, dendrobatid frogs are aposematic, diurnal, and actively hunt specific prey that includes mites and ants [4]. In contrast, species considered generalists are often cryptically colored, sit-and-wait predators that feed on fewer, but larger prey [4]; this later group includes various species of direct-developing frogs of the genus *Pristimantis*.

Figure 12: Example of a generalist and a specialist frog species from Canandé and Tesoro Escondido. a. *Pristimantis chocoensis*, (Strabomantidae); b. *Oophaga sylvatica*, (Dendrobatidae).

After forest disturbance, frog and arthropod assemblages change, and thus, the corresponding predator-prey networks might too. Moreover, diet specificity may result in some networks exhibiting tighter pair-wise interactions than others. I am interested in understanding how frog assemblages change from active areas to old-grown forests and the reassembly of predator-prey networks between specialists' and generalists' frogs along the chronosequence.

Fieldwork

In order to achieve the main goals of my project, the first step is fieldwork. The processing of the frogs is done *in situ*. We set up the station in the field, then we actively search for frogs through visual encounters, capture them, and then process them. First, we identify the species, age class, and sex. Then we take morphometric measurements and weight. To obtain the stomach contents - the prey we will later identify for network analysis - we perform a stomach-flushing. If we capture a species that might contain alkaloids, we collect the alkaloids from its skin by a non-invasive electro-stimulation technique, TAS (Transcutaneous Amphibian Stimulator) [3]. Finally, the frogs are released into their environment unharmed.

Figure 13: Karla, Jaime and many frogs to process.

Plans & Reality

Adjustments to the initial plan

The original intention was to visit each of the 64 plots, ten times during the daytime and ten times during the night, half in the dry season and a half in the wet season. This was considered assuming that it would be possible to visit six plots per day. Arianna and I realized after the first week in Canandé, during the PREX implementation, that the original plan was overly ambitious and nearly unachievable given the field conditions. The rain, the distances to the plots, the time required to process the frogs *in situ*, and the overall logistics made it extremely difficult to fulfill the initial plan. After discussing with MO and Nico, we agreed to adjust the sampling design. I decided to sample all PREX and add six more plots: three active cacao and three active pastures. Thus, I have a total of nine regenerating pastures (PR), nine regenerating cacao (CR), eight old-grown forests (OG), six active pastures (PA), and six active cacaos (CA).

In 2022 I conducted two diurnal and two nocturnal repetitions, during the dry season as well as the rainy season. The four rounds were alternated, a day round followed by a night round, and so on. The remaining plots will also be sampled once to meet the objectives of the network analysis.

The sampling strategy was another decision we had to make in the field. My initial idea was to do transects following the plot guide ropes, that is, a 200-meter transect around the plot. However, landscaping conditions in many plots made this task quite challenging. We opted to sample within the plot while respecting the experimental zones.

Challenges in the field

The main challenges I had in the field were identifying *Pristimantis* and finding frogs during daytime sampling. February 2022 was the first time I visited the Chocó Forest in Esmeraldas. Some species with wide distributions were known to me, but there were others that I had to learn to identify as I went along. The most difficult (and also the most fascinating to me) are the frogs of the genus *Pristimantis*. Though these direct-developing, often cryptically colored, sometimes very similar frogs between species can be very challenging, learning to identify them has been very satisfying.

Regarding daytime sampling, Leo, the parabiologist who aids me, is the best at finding very tiny and cryptic frogs in the morning. Leo formed his own method: he doesn't move the leaf-litter or stir it up; he simply walks very slowly, keeping an eye out until he observes any frogs jumping. The juvenile frogs are so small, they can easily be mistaken for crickets or grasshoppers, "but the way they jump is completely different", says Leo. I have learned his method, which currently allows me to find very small frogs during the day.

Impressions of the field

The Chocó Forest is extraordinary, and I consider myself extremely fortunate to be able to work there. There was not a single day – or night – in the field that we did not find some amazing amphibian or reptile species on the plots or on our way to them. Although the plots appeared to be too far away at first - I recall Nico's words "later you will thank me", now I agree with him – it is true that only by reaching those remote places will we be able to achieve our goals.

We are also very fortunate to have the field conditions we have in Canandé and Tesoro Escondido. Having a comfortable bed, prepared meals, and an amazing staff always willing to help, are certainly a great privilege. Also, being able to work in the ChocoLab, in the middle of the forest with all the conditions any laboratory has in the city is fantastic.

The people

Working with my field team has been great. Arianna and I got along very well from the beginning, we did the work together and were a great support to each other. MO has been to Canandé twice this year, I learn a lot from him, working with him in the field means not only finding every animal, but it's basically like walking in the forest next to a living encyclopedia. An undergrad student, Jaime, helped me in the last phase of the fieldwork. He was very enthusiastic, reliable, and responsible. He takes great pictures and never complained about the hikes, which is very important when choosing a student! Leo has been extremely important to our fieldwork. One of my greatest satisfactions this field season was when Silvia, Leo, and I went to Casa del Medio. Silvia is afraid of frogs and snakes. However, Leo explained the whole process to her, from identification to stomach-flushing, and they did it together. That was a delightful surprise for me!

Moreover, all doctoral students in the project are outstanding professionals and extraordinary people. We have formed a very supportive team and we encourage and motivate each other. The ChocoLab managers, first Adriana, then Chiara, and Katrin who is still with us, have worked very hard to make everything run smoothly in the lab. Despite all the obligations Katrin has, she always finds a way to help and support me in absolutely everything.

One of the best experiences I keep with me is the happiness of having shared with the parabiologists, who are now my friends: Leo, Jordy, Lady, Silvia, Jefferson, Franklin, and the plot manager, Bryan. They are young people from villages near Canandé. Their job is to assist research in the field and in the laboratory. However, their activities go beyond their duties. They are always very enthusiastic and try to find solutions to any problem we have. I have had the opportunity to work with each of them, and I believe that by sharing our experiences and knowledge has allowed me to extensively learn not only about the forest, but also about life.

The implementation of spaces that go far beyond science, contributing towards the development of young people from local communities, is undoubtedly an extraordinary opportunity provided by Reassembly and Jocotoco, and a great step towards the conservation of the region's forests.

Outlook

I plan to begin analyzing amphibian community data next year. In January and February, I will be working in Rödel's lab at the Museum für Naturkunde in Berlin. We will return to Canandé in March, along with MO and a master's student, who will investigate dendrobatid microhabitats. I will continue sampling in the same manner as last year, and I will help Arianna as well. I'll also need to evaluate prey items from the frogs' stomach contents for later identification *via* metabarcoding.

Giving something back to the communities where you conduct research is essential to me. So next year, during my time in Canandé, I aim to offer a basic ecology course for the parabiologists and rangers, if the guys agree. My idea is to provide them with additional technical tools for their present and future work.

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SP 3: Plant-pollinator interactions

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What everyone should know about my work

SP3 is the pollination subproject of REASSEMBLY. The group is formed by the PIs Sara Diana Leonhard (TU München), Alexander Keller (LMU München) and Gunnar Brehm (U Jena), which amass a large amount of knowledge on tropical insects and their ecological interactions, and by me, a PhD candidate based on the TU München and leading the collection of data in the Ecuadorian Chocó. Our project aims at understanding how diurnal and nocturnal pollination networks re-assemble after disturbances (i.e. deforestation) in the biodiversity hotspot of the Ecuadorian Chocó. Most angiosperms in tropical ecosystems are reliant on animals for pollination [4], and these mutualistic relationships results in complex interaction networks. Their structure provides insight into the stability of network mediated processes [1], e.g., pollination of plants and provisioning of pollinators [2]. These processes are in turn affected by variation in the composition of communities, as well as their taxonomic and functional trait diversity. In fact, there is increasing evidence that the structure of tropical pollination networks is driven by niche-based processes and thus more by deterministic factors (e.g. functional traits) than by stochastic processes (e.g. abundance) [3]. Using a functional trait perspective, our main objective is to shed light on the traits shared by plants and pollinators that allow them either to withstand disturbances or to colonize newly-disturbed habitats and restore complexity to the community. Within this framework, we are also investigating how networks reassemble at different spatial levels: the understory and the canopy, the latter a rarely sampled space within the forest [5]. Aside from exploring the effect of disturbance on community-wide interaction patterns, we will also explore how secondary processes are affected, such as pollination service (pollen transfer and fruit set) and pollinator provisioning (bee population dynamics and survival) to extract conservation-oriented conclusions.

What we do

In order to sample insects and their interactions with plants, I set a variety of traps onto the plots, both in the understory, and in the canopy (when present) using a bow-and-arrow method. This method comprises shooting a rope onto branches between 20 and 30 m above ground in order to for a pulley to raise traps. To capture bees, which require a unique and standardized way of being sampled, we use a combination of mixed color vane traps, scent traps, and trap nests, as well as active netting within the plot (understory only). For moths, we use mixed UV light traps designed by Gunnar Brehm. All these traps stay active for one entire day or night and come equipped with a chloroform evaporation mechanism that kills insects without the use of liquids, thus decreasing the chance of pollen contamination. We will also include bat-plant interactions in our nocturnal network, which are being sampled by SP4's Santiago Erazo. Insects will be properly identified by specialists, and a variety of interaction and dispersal traits will be measured for all species (e.g. proboscis length, size). Additionally, I will perform a thorough assessment of the abundance of plants consumed by insects in the sites, while collecting their morphological and resource-related traits for our species-trait framework. Interactions with plants will be identified by pollen loads collected from insects, via next-gen metabarcoding sequencing performed in the LMU München. We also have two additional working packages (WP) the require fieldwork in the Chocó,

which will be carried out in 2023: the pollen flow WP, consisting in an intricate in-situ experiment where we collect reproductive success data from phytometers transplanted into the field, and from dioecious trees naturally found in the research area; and the pollinator provision WP, which consists in the installation of trap nets to sample nesting bees, which will have their fitness measured within the chronosequence.

Plans and reality

The first year of fieldwork was a success. This first stage, subdivided into two intense two-month field campaigns, consisted in installing the canopy rope mechanisms and the two first trap types: the moth light traps, and the bee vane traps (Figure 14).

Figure 14: Insect traps installed in the Reassembly plots during the first fieldwork stage. Upper left: a mixed coloured bee vane trap, containing blue and yellow vanes; lower left: a mixed UV-light trap, which employs both UV and visible light to attract moths. Right: The traps installed in the canopy with the aid of a bow and arrow

Installing the canopy ropes was a challenge on its own, and arguably the hardest and most unpredictable part of the work. The bow-and-arrow method requires a lot of fine tuning (and luck) to work, as well as a perfect branch not too cluttered by vegetation and at a good height (20-30 m). Therefore, while some plots were delt with fairly quickly, some others required several hours (and arrows) to finally have it's canopy ready for sampling. But ultimately, all REASSEMBLY plots that had a significant canopy layer (N=28) were successfully prepared with the rope mechanisms. As for the traps, both types worked well and yielded a very large diversity and abundance of moths and nocturnal bees (light traps), and a decent number of bees (vane traps), although the latter mostly limited to stingless bees (tribe Meliponini). Therefore, the data collection project is fortunately moving mostly according to plan. I would rather say, in fact, that we were overly cautious with the planning, as we had planned repetitions for e.g. the light trapping in order to increase samples size that, as current numbers show, will not be necessary. The only setback so far appears to be the low functional diversity of bees captured by the vane traps. I can thus cite two major changes in the sampling design so far, based on the outcomes of this year: (i) initially planned to be repeated for one additional year, moth sampling will be restricted to 2022 only, as we already acquired an impressive abundance

and diversity of moths, and in order to give time for the other parts of the project; (ii) instead of repeating the color vane trapping in 2023, this trap will be replaced by other capture methods, i.e., active netting within the plots' understories, and the installation of odor traps in both levels to attract orchid bees (Euglossini), which are incredibly diverse in the area. Additionally, the next year will also encompass the work on the other WPs (as described above) and the collection of plant abundance and trait data. In terms of interaction sampling, our first metabarcoding test sequencing runs in Germany worked perfectly, which means that soon the network will start to take shape!

Where I work - field impressions

Canandé is an impressive demonstration of tropical nature. The landscape dazzles at a distance, with its cloud-like formations emanating from the forest in rainy days that can be seen from the reserve's viewpoint a couple of kilometers away from the lab, or from the lab's second floor itself. Up close, the lifeforms therein are a spectacle of shapes and color. Looking at the insects that I have collected, for instance, I cannot cease to be impressed by the moths and bees gilded by shades of bright pink, deep blue, metallic green, or flaming red. Or perhaps the proboscis from *Manduca* hawkmoths, that may surpass 10 cm in length and may reach into the deepest flowers (Figure 15).

Figure 15: A display of the incredible diversity of colours and shapes of moth and bee species found in the Canandé reserve. Upper left: a diurnal moth from the Uranidae family, lower left: a *Manduca* hawkmoth with its long proboscis exerted; Upper right: a display of several species of orchid bees (Euglossini); lower right (counter clock-wise): Tiger moths (Arctiinae): *Elysium* sp., *Idalus* sp., *Gorgonidia* sp., and an unknown Sphingidae species.

These treats to the eyes are one of the many gifts that gives the necessary energy to work in the beautiful, albeit challenging Chocó. The 10 km strides to and back from far away plots, the swamp trails in rainy days, the weary backs from carrying trap materials and the army of stingless bees seeking your eye fluids (especially when trying to operate a bow) are a few of the daily obstacles to be overcome. Not unlike other tropical environments, fieldwork in Canandé has proven to be hard, but rewarding.

Results

After the first visit to all plots, I was able to get 91 successful light trap samples and vane trap samples. Although only approximately 65% of samples have been processed, these have yielded so far ca. 1400 bees (mostly Meliponines and nocturnal Megalopta), and ca. 7000 moths from our focal groups (Erebidae: Arctiinae, and Sphingidae). Bees still require due identification, but due to the thorough moth catalogue prepared by Gunnar Brehm and collaborators made with moths from the reserve, we are already able to take an initial peek into trends of moth diversity and abundance in the site. With ca. 40% of moth samples processed only, we may see the emergence of a hunch-backed curve with a higher moth diversity and abundance in plots with an intermediate period (Figure 16). Perhaps a support to the Intermediate Disturbance Hypothesis? Too soon to tell and more data is needed, but results are promising!
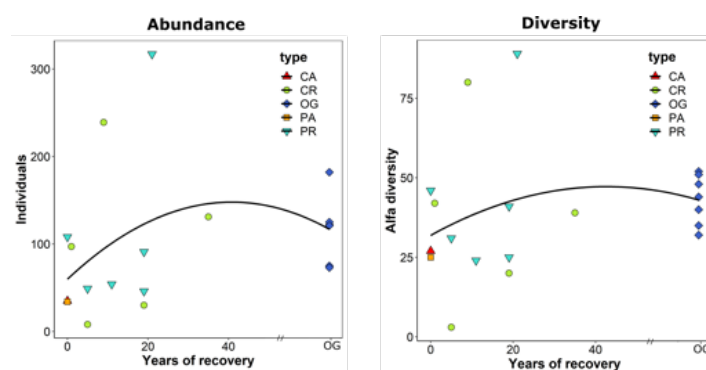

Figure 16: Preliminary trends in the diversity and abundance of moths from the Reassembly plots, perhaps suggesting a support for the Intermediate Disturbance Hypothesis. OG: Old-growth forest, CA: Active cacao, PA: Active pasture, CR: Cacao regeneration, PR: Pasture regeneration.

Outlook

Now, the challenge of condensing several steps of the project into next year's schedule arises. That means setting the two new trap types, netting bees in the understory, recording the abundance of flowering plants and their morphological and resource traits on the plots, and transplanting the phytometers. It will be a lot of work, but with proper planning and enough time in the Reserve, we will hopefully be able to finish all parts of the project. Moreover, metabarcoding sequencing runs are constantly taking place in Munich, which will soon yield our first interactions and thus the first peeks into the network. Joint efforts with Gunnar Brehm's team in Jena are also leading to a more complete moth catalogue and better understanding of the moth diversity in Canandé. After fieldwork, I also plan to start the endeavour of identifying and taking functional traits of the bees that I collected in Ecuador. Lots of work to come in the next years!

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SP4: Seed dispersal by birds and mammals

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What everyone should know about my work

SP4 is studying the reassembly and recovery of seed dispersal by birds and mammals during unassisted forest recovery. Our sub-project is composed of two PhD students, three PIs, two collaborators and two parabiologists. While Marco Tschapka, Santiago Burneo, Santiago Erazo and Jefferson Tacuri focus on seed dispersal by bats, Matthias Schleuning, Eike Lena Neuschulz, Boris Tinoco, Jordy Ninabanda and I study dispersal by birds and other frugivorous mammals.

Figure 17: Anna, Jordy and a Baudó Guan (*Penelope ortoni*).

Seed dispersal is fundamental for restoration, as it allows plants from natural habitats to recolonise degraded ones [2]. However, studying the reassembly of seed dispersal can be a great challenge, as while plants depend on frugivores for recolonisation, frugivores depend on the presence of the fruits they consume for survival [5]. Furthermore, the presence of both frugivores and fruiting trees is contingent on other environmental drivers, such as precipitation and temperature [1]. Thus, seed dispersal reassembly is shaped by a complex interplay of plant and animal communities' and species' responses to biotic and abiotic factors.

Seed dispersal is also a complex ecological process, with many demographic transitions between dispersal, seedling establishment and plant growth into an adult tree [9]. Many factors influence the probability of these transitions. While some are only indirectly related to frugivores, such as seedling emergence and survival, others have a direct relation, such as dispersal distance and the effect of gut passage on seedling establishment [7]. Different frugivores provide different services as dispersers, in the sense that they consume and disperse different fruits, but also that they provide different qualities of dispersal [6]. In a restoration scenario, for instance, a larger bird will consume larger fruits and disperse seeds over longer distances.

With this in mind, I want to understand:

1. The reassembly of communities of frugivores, fruiting plants and their interactions during forest recovery.

2. How seed dispersal functions re-establish along forest recovery.

Plans & Reality

In order to achieve these goals, I am collecting data of interactions between frugivorous birds and mammals on all forest strata, which requires two different methods. To record the interactions on upper strata of the forest, I am observing fruit consumption by birds and mammals using binoculars with the help of a parabiologist. At each plot, we observe interactions for 5 hours, starting at sunrise, during 3 consecutive days. Most interactions on upper strata are expected to be realised by birds. To record interactions on the forest ground, we establish four feeding stations with the fruits available in the plot and set up one camera trap at each station (see Figure 18). In this case, most interactions are expected to be realised by rodents and ground-dwelling birds. Camera traps are left to record interactions for 6 consecutive days on each plot.

Figure 18: Sampling ground frugivory with camera traps. A) Fruits available in the plot are displayed in front of the cameras. B) Agouti feeding on displayed fruit. C) Fruits that were left after 6 days in the ground.

For the first goal, in the field, we collect information of functional traits of the observed plant species. Next year, I will obtain data of animal traits from the literature and museums' collections. I focus on traits that are known to be relevant for trait matching in seed-dispersal interactions (e.g. avian wing shape and plant height, avian beak width and fruit width [3]). Finally, I will combine the information of species' interactions in different stages of forest restoration with the collected trait information to understand how the reassembly of seed dispersal network is mediated by the traits of frugivores and plants, and identify which species are least likely to recover along the chronosequence.

Figure 19: Examples of frugivorous birds found in Canandé. Above, on the left, a male and, on the right, a female of Green Honeycreeper (*Chlorophanes spiza*). On the bottom, on the left, a Pale-Mandibled Araçari (*Pteroglossus erythropygius*) and on the left a Squirrel Cuckoo (*Piaya cayana*). First three photos from Heike Feldhaar and last photo from Santiago Erazo.

For the second goal, I will build on the interaction and trait data and will additionally include functional traits that express the quality of seed dispersal. Important functional traits for seed dispersal quality are the effect of the passage through the gut on seeds as well as animal movement [8]. These trait data can be combined with empirical seed dispersal networks to estimate how different species in a community contribute to seed dispersal [4]. I will combine data of seed dispersal networks in different stages of forest recovery with these functional traits at different stages of the chronosequence of forest recovery in order to compare seed-dispersal functioning.

Where I work - Field Impressions

The Ecuadorian Chocó has a beautiful and diverse community of frugivorous birds and mammals. It is always a nice surprise to see different species in the field. Regarding bird species, some of our favourite moments so far were seeing grey-and-gold tanagers (*Tangara palmeri*) and scarlet-breasted dacnis (*Dacnis berlepschi*). With the camera traps, some of our favourite footage related to frugivory are with white-collared pecaries (*Tayassu pecari*) and rufours-fronted wood quails (*Odontophorus erythrops*), as well as some non-frugivorous surprises, such as with ocelots (*Leopardus pardalis*) and pumas (*Puma concolor*).

Our fieldwork also implies many challenges, as we start very early in the morning -before sunrise - and survey plots for three consecutive days. This means that we stay for a week or more on the remote huts and sometimes even need to camp in the forest. While this can be challenging, it also allows beautiful experiences. Some of my favourites are when we have a beautiful river near where we are camping, so we can have nice baths and enjoy some diving or when we stay with local families, who are always so receptive and share a bit of their stories and culture with us.

Figure 20: Making *mantequilla de maní* at the Velazquez's house, where we stayed for a week.

Outlook

So far, we have sampled 33 out of the 62 plots. Next year we will sample the other 29 plots, some of which will be especially challenging. For example, staying at Casa Rosero for two whole weeks! We will split our work into two field seasons, from March to June and then, from September to December of 2023. We will also start identifying some plant species that are too small and thus, are not included in the botanists' sampling.

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SP4: Bats and Seed dispersal

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What everyone should know about my work

In the SP4 work team, we focus on seed-dispersal by frugivorous birds, bats and other mammals. We are a diverse group, made up of three PIs, two main collaborators and two PhD students working on several topics together, but we are also divided into two subgroups. One group focusses on seed-dispersal by frugivorous birds and non-flying mammals, formed by Matthias Schleuning (PI), Eike Lena Neuschulz (PI), Boris Tinoco (main collaborator), and Anna Rebello Landim (PhD student). The other group focusses on seed-dispersal by bats, formed by Marco Tschapka (PI), Santiago Burneo (main collaborator) and I (PhD student). In addition, we have been able to count on the valuable collaboration of the parabiologists at different stages of the project, and in particular with the indispensable participation of Jefferson Tacuri and Jordy Ninabanda.

Seed dispersal is one of the bases for the initial processes of natural regeneration [4]. In Neotropical rainforests, the seed dispersal by frugivorous vertebrates is a crucial ecological process [3]. In addition, the ecosystem service of seed dispersal is a mosaic of subservices performed by distinct groups of frugivores [6]. Frugivorous birds and bats are considered the main dispersal agents, and dominate the initial stages of forest succession [5, 1]. Differences between bat–fruit and bird–fruit networks in structural properties have been recorded, and support the hypothesis that those two disperser groups form different mutualistic modules [6]. In this context, we aim to provide an understanding of the reassembly of seed dispersal networks based on response, interaction and dispersal traits in a forest recovery gradient (Old growth forest, cacao and pasture) in the Chocó region.

The Chocó biogeographic region, located within the Tumbes-Chocó-Magdalena hotspot, is very important for its diversity and endemism [7] and faces constant and increasing threats generated mainly by deforestation for agriculture and timber activities [2]. In order to contribute to the knowledge of this important region and to promote its conservation, this study is being carried out in the Ecuadorian Chocó.

Considering this background, our working group focused on seed dispersal by bats has the following objectives:

1. Determine the diversity of bats and their main conservation threats in the Ecuadorian Chocó region.
2. Study the taxonomic and functional composition of bats to understand the characteristics and responses of their assemblage in a forest recovery gradient.
3. Analyze bats as seed dispersers, their response, interaction and dispersal traits, in order to establish how they influence the reassembly of seed dispersal networks in a recovering forest gradient.

Plans & Reality

To meet the different objectives, our plan is to sample the 62 plots previously established, over a period of two years. For the capture of the bats, we are using six mist nets of 6 m over three consecutive nights (18:00–24:00). To analyze bat–fruit interactions, we collected fecal material directly from bats (defecating at the mist net) or by keeping them for up to 1 hour in a clean cloth bag. Functional traits are measured in bats captured in the field. If necessary, data will be complemented by measurements in museum collections.

Figure 21: Methodology: bat capture and functional trait measures

Figure 22: Methodology: camping

In this first year, in compliance with our plans, it was possible to sample 33 plots. Among the sampled plots we captured more than 1200 bat individuals, and among them, as interactions, we recorded more than 500 fecal samples and also collected pollen samples of nectarivorous species. Among the individuals captured we recorded about 50 species, some common species, but there is a significant number of rare species, possible distribution extensions and possible new species. These preliminary results demonstrate the important diversity of bats

in the Chocó region, which is why we plan to submit a proposal to be declared by RELCOM as an "Área de Importancia para la Conservación de Murciélagos" (AICOM).

Figure 23: Jeff and our field office

As first appreciations, we observed that some species are widely distributed in most of the plots, other species are more selective to the characteristics of each plot and the presence of others has been limited to one plot. We have also been able to observe variations in the seeds dispersed, depending on the bat species, the community structure and the characteristics of the plot. These preliminary observations meet our expectations in a positive way.

Figure 24: Bat diversity: *Gardnerycteris keenani*

Impressions of the field

The fieldwork has been extensive and although often exhausting, it is impossible not to enjoy Chocó. In the different points where we did the field work we were surprised by spectacular landscapes, sunrises and sunsets, which, in addition to being engraved in our minds, are also captured in photographs. In addition, we were able to observe and photograph a small sample of the great biodiversity of this region, including mammals, birds, amphibians, reptiles, among others.

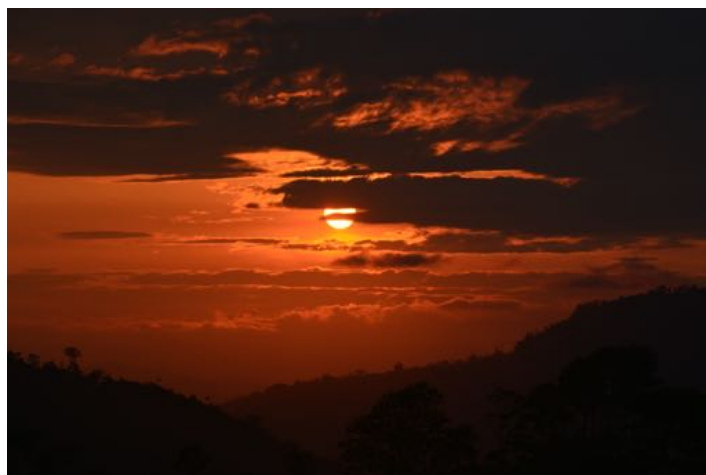
Figure 25: Bat diversity: *Trachops cirrhosus*

Figure 26: Sunset in Chocó

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Figure 27: Chocó diversity: *Potos flavus*

Figure 28: Chocó diversity: *Leopardus pardalis*

Figure 29: Chocó diversity: *Penelope ortoni*

SP5: Tree seedling recruitment and herbivore interactions during forest recovery

Eva Tamargo López, Philipps-Universität Marburg

What everyone should know about my work

The team SP5 studies the recovery of tree seedling communities and their interactions with herbitores along a tropical forest chronosequence. Our team is formed by 3 PIs, Nina Farwig, a conservation ecologist, focusing on biotic interactions, ecological networks and ecosystem processes, Katrin Heer, specialised in evolutionary ecology of tree species, and Sybille Unsicker, an expert in plant environment interactions and chemical ecology, as well as me, Eva Tamargo, PhD student at Philipps-Universität Marburg and Lady Condoy, an amazing parabiologist at Canande Reserve. Addiotionally, the project involves master students. In the first year, Lukas Werner and Elis Martinelli, from Marburg University, joined us. Their help and encouragement has been a strong motivation for me in the start of this project, I hope I can take with me their enthusiasm for the rest of my PhD.

Together, we aim to shed light on assembly rules in space and time, since such insights into the processes determining the resilience of tree seedling interactions in recovering tropical forests can enhance global restoration programs. To do so, SP5 will record the seedling germination, survival and growth in an Ecuadorian lowland Choco forest, as well as monitor the intensity and diversity of herbivory in the seedling community. To increase our comprehension of this phenomena of the seedling-herbivore interaction, we will sample the insect herbivore community in the successional gradient, as well as determine the physical and chemical response and interaction traits of the main tree seedling species.

Increasing the knowledge in tree seedling interactions in tropical forest

Habitat quality and connectivity strongly shapes tree seedling recruitment of secondary forests [1], yet the succession of interactions with herbivores are hardly understood. Herbivore interactions are complex, influenced by the vegetation complexity [1, 3, 2, 4], and associated with its functional diversity [5] as well as the herbivore predators, which play an important role in shaping the leaf damage patterns in tropical forests [6, 7]. Taking this into account, it is important to comprehend the status of both the plant and the insect community for understanding the seedling-herbivore interactions trends.

In this sense, we will assess **how the seedling community, its structure and dynamics will respond to forest recovery**. Specifically, we aim to know **how the response, interaction and dispersal traits of the seedlings will respond to forest recovery**. We expect to see a turnover in the response traits (as germination and growth rate) of the community, from early to late succesional species, as the age of the forest increases, as well as an increase in the functional diversity on the interaction traits (leaf thickness and toughness, and leaf chemistry). This chemical diversity (in relationship with antiherbivores defences) might be correlated with the network specialization along the gradient of forest recovery; plant communities with high species diversity lead to a highly specialised herbivore community, due to the phytochemical diversity in herbivore defences. Having said so, it is clear that **the structure and dynamics of seedling–herbivore interactions respond to forest recovery**, and we aim to understand the patterns behind this process in tropical forests. Network complexity should increase together with the

forest recovery, however, species diversity usually recover more quickly than the interaction network. As said, the interaction traits diversity tend to increase with forest recovery, and this could be as well related with an increase in specialised herbivores in the network. Additionally, since specialised herbivores, as gallers, usually recolonize more slowly the forest, its expected to find more specialised herbivory patters in old forests, with a better forest recovery.

Seed dispersal and seed rain network are essential for seedling recruitment and the resultant seedling community. With the purpose of better understanding of this process, we will identify and map the parental trees in the surrounding of the treatments of our PREX plots. With this information we will comprehend better the spatial seed dispersal (vertical or horizontal) in our study area, and how this dispersal changes along the different forest stages. We expect the diversity of the seed rain and seedling recruitment to increase while the forest recover. We also hypothesise that the density and diversity of seedlings will be higher when excluding terrestrial mammals. We expect the vertical contribution of dispersal to exceed the horizontal one and that the seed bank will also play a minor role. At the same time, excluding terrestrial mammals will preserve the seedlings from being trampled or eaten. We expect this effect to be stronger in early successional stages of the forest recovery gradient.

Plans & Reality

From January to December 2022 we did the first monitoring year across 39 of the 64 plots. These comprise 32 PREX plots and 7 non PREX plots. The plots were monitored one time right before the clearance was carried (in the "disturbed" treatments only) and three times post-perturbation, every 3-4 months.

Monitoring pre-disturbance

We took the chance of collecting information on the seedling community before the disturbance started. To do so I travelled to Ecuador in early January to start the sampling. For monitoring the PREX plots, it was necessary to do 50 continuous days of work in the field and laboratory in which I collected information about: number of individuals per morphospecies, size of these individuals and amount of herbivory per class in the tree seedlings. Since I was the only SP5 member in this first monitoring, acquiring all this information required too much time per day, so the majority of the functional traits sampling was done in the second round.

It was in this monitoring when we decided to redefine the term seedling. Originally we defined seedling as a plant individual that has cotyledons and/or first or second sets of true leaves and absence of a woody stem. Instead, we are considering a seedling a tree individual that ranks from 2-10 mm of RCD (Rooth collar diameter). Even knowing that RCD can be not the best way to define a tree age, this allowed us to exclude the tiny seedlings that add a lot of uncertainty to the data, due to the difficulty of identification.

Monitoring post-disturbance

Once the perturbance was done, the post-disturbance sampling started. From this point the data collection was carried in the four treatments of the PREX plots, and also on the 7 additional non-PREX plots too.

At this point the team included, in addition to myself, Elis Martinelli, Lukas Werner and Lady Condoy. Together, we completed two consecutive monitoring from March to August 2022. In this monitoring, all the individual seedlings, as well as 6 leaves per individual were marked with a numbered tag, and mapped with coordinates, allowing

us to track the survival and herbivory rate per individual and leaf. When marking, we decided to tag also seedlings smaller than 2mm of RCD, for having into account their moment of germination and learning about the appearance of the tree species at this stage. A total of 1000 seedlings have been tagged in the 39 plots (Figure 30).

For all these seedlings, we recorded the RCD, height, number of leaves, whether the stem is woody or not and the herbivory per leaf. For easing the species identification work, we also prepared a seedling field guide, with perspectives of improving with the barcoding results and further work. In these months, a functional trait sampling was also conducted, recording traits as leaf thickness and toughness, and specific leaf area.

Figure 30: Seedling number 1131, morphospecies 081

Additionally, we accomplished a macroinvertebrate herbivore hunting trial in the plots. This was just a visual hunt, with 1 person searching for 1 hour per plot search, in order to know what to expect regarding the herbivore insect fauna in the plots (Figure 31), the interaction herbivore-seedling and prepare a proper methodology for the next campaign.

Figure 31: Generalist herbivore collected in a young forest plot

Impressions from the field and area

Even if I had already worked in nearby forests in Ecuador, I was surprised about how extremely beautiful Canande and Tesoro are (Figure 32). We are all lucky to be working in this location. In addition, thanks to my early arrival, I was able to know all the parabiologists of the project, since we got to spend so much time together while the perturbation was carried. I really appreciate to have been able to share so many experiences with all of them, as well as my colleges in the project, this enriched my experience in the amazing and beautiful Ecuadorian Choco Forest, making it fun, dynamic and approaching it to the local realities. To be present at the set up of the PREX experiment was a nice experience for me. Even if I was only able to help the perturbation work in counted occasions, this made me understand how hard this work is, from the installation of the plot, to the fencing, and the perturbation itself which can take several hours of strong physical work, under all kind of weather conditions. And I understand better now how many different and small things one need to take into account when preparing a field experiment. I am looking forward to be again in the Choco Forest of Canande and start the next sampling season.

Figure 32: Amazing river at Tesoro Escondido

Results

Now as we have a whole year of monitoring data at hand, we are currently exploring the data on species diversity of the seedling communities along the chronosequence and the different forest types.

Additionally, 355 individuals corresponding to 38 morphospecies were sampled for functional trait information. For these individuals, we recorded the leaf thickness and toughness, leaf dry weight and we took pictures in order to obtain later on the specific leaf area. We are currently obtaining the leaf area information from the pictures in the field so we can also start to depict the variance in the functional diversity community along the gradient.

Also, since the identification of seedlings can be really complex, and it gets worse as we want to dig deep in the taxonomy, we collected individuals of approximately the half of morphotypes we found in the forest for barcoding and we are currently waiting for these results.

Outlook

We will start soon our first manuscript, hoping we can finish the draft before March. This paper will describe the tree seedling community along the forest successional chronosequence, and the turnover of species between the previous existing species community and the community recovered after the perturbance.

At this moment we are also preparing the next field season, that will start in March 2023 and will have a duration of six months, from

March to August, after which our parabiologist Lady Condoy will do an extra round until December 2023. In this next field season we will implement new sampling protocols to acquire more information. We will start a herbivore sampling, to increase our information about the interactions between specific insect species and the main tree morphospecies in our seedling community, which will give us a better impression of the seedling-herbivore network.

For a better understanding about vertical and horizontal dispersal, and how they respond to forest recovery, we will start recording and mapping the adult tree community in a 10 m circle around our subplots. With this information we will understand better where the seedling species of our communities come from.

A sampling regarding chemical components of the leaves will be also carried out, focusing on the 5 most common morphospecies in the study area. All these samples will be then prepared and send to the Max Plank Institute, in Jena, Germany, where we will then obtain information about the chemical components in the leaves as well as anti-herbivore defenses. To finish, we will also start a trial implementation of volatile components monitoring in the leaves of these important morphospecies in our plots.

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SP 6: Dung beetle community reassembly

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What everyone should know about our work

In SP 6 we focus on the community reassembly and trophic niches of dung beetles across the chronosequence. Our team there are three PIs, Nico Blüthgen a network ecologist, Thomas Schmitt a chemical ecologist, Diego Marin a dung beetle taxonomist, and one PhD student Karen Marie Pedersen a PhD candidate with a general background in tropical ecology and conservation. We also have had three bachelor's students Jan Johann, Maira Elora Bradler, and Silvia Canelos. Our focus on the dung beetles is impart due to their amusing behaviour (it is well worth watching them as they fight over a particularly tasty dung pile), but more because of their ecological importance. Ecologically their consumption of animal feces, carrion (vertebrate and invertebrate), rotten fruit, millipedes, and snail mucus, make dung beetles important decomposers [9, 10, 17, 8, 18]. They are present on all continents excluding Antarctica with over 6000 described species [9]. Decomposition is a important process within ecosystems. Ecologists have often focused on dung beetles as decomposeres of mammal dung, because it has many beneficial contributions to the ecosystem. Including reduced pathogen load for other mammals, soil aeration, increased dung surface area for bacterial decomposition, and the secondary dispersal of seeds [13].
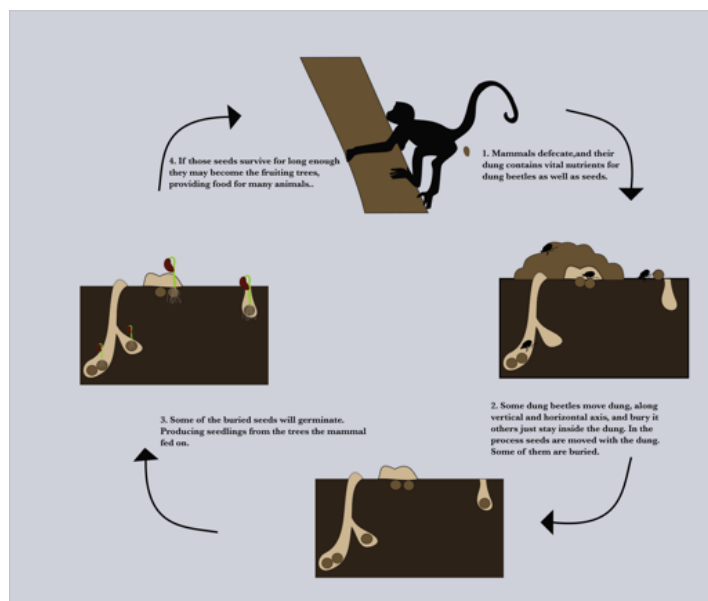

Figure 33: Illustration of secondary seed dispersal by dung beetles.

Dung beetles are sensitive to microclimatic changes that result from habitat conversion from natural state to agriculture and back this suggests that the role of dung beetles as a group might shift as the forest (in our ecosystem) recovers from conversion to agriculture. Agriculture, particularly pasture has different microclimatic conditions compared to the old growth forest. This might change the community composition and thus the efficiency of dung beetles as decomposers as the forest regenerates. Within this paradigm there are many questions that naturally follow, first what is the local dung beetle community composition, second is there a change in community composition, third does this change result in any measurable community trait shifts, do these shifts result in any changes in dung removal by dung beetles, and is there a shift in dung beetle diets within species across the chronosequence. While these questions relate to the role of dung beetles as decomposers they are also secondary seed dispersers.

The removal and burial mammal feces dung beetles contributes to secondary seed dispersal by moving seeds with there dung balls to a new location (Figure 33). This relocation of seeds depends in part on the seed traits, including size, surface and odor [12, 1]. When dung beetles bury seeds even 1 cm below the soil surface they may reduce seed predation by 90% [19]. Seed predation and pathogens are hypothesised to be major sources of seed mortality. In a regenerating forest it is interesting to understand which interactions promote seedling establishment and survival. For this reason secondary seed dispersal is a major question in our sub project.

We study dung beetles within the context of community reassembly, across the chronosequence. Community reassembly implies the reassembly of networks, and ecological processes. We asked many questions surrounding this enumerated below.

1. How does the dung beetle community composition change across the chronosequence?
2. Does the speed of dung removal change across the chronosequence?
3. Which species are responsible for dung removal and does that change across the chronosequence?
4. Does dung beetle diet breadth as in the number of acceptable resources, (carrion, dung, etc) across the chronosequene?
5. Does volatile attractiveness of compounds found in dung change across the chronosequence?
6. Do dung beetle mammal networks change across the chronosequence?
7. Which seeds might be subject to secondary seed dispersal by dung beetles, and does this shift across the chronosequence?
8. Are dung beetles important in seed dispersal across the chronosequence?

Data collection what worked and what did not work an ecologists tale

Field season and methods development 2019 (Playing with poo)

The first field season was a longer and I (KMP) was a bit unsure exactly what I was doing. However, I had five months in my first field season I was able to experiment with a lot of different types of methodologies to get a feel for what would work and what was possible within our field site.

Bait Selection It was clear that if I was going to do any real work I needed to understand the dung beetle community, and learn some of the common species. We used traditional pitfall traps which I originally wanted to bait with cow dung because it is much more pleasant to work with than human, but after running a transect with cow and human dung spaced 50 m apart along the trail all I needed was a visual inspection of the vials of beetles from each trap was enough to tell me that human was the way to go if I cared about the dung beetle community (Figure 34). However just to be sure that the first transect was not a fluke I repeated the transect three times, and the results were pretty clear in traps baited with human dung I collected a total of 617 dung beetles on human bait, 16 species of dung beetle, in contrast the traps baited with cow collected 139 dung beetles belonging to 12 species (identification by Diego Marin). Despite my initial feelings about using human dung I could not dispute the results and my subsequent pitfall trapping using human dung as bait.

Figure 34: Vials of beetles collected from pitfall traps baited with either human or cow, vials from the same location and are placed in front of each other the first row in small vials are traps baited with cow dung the row behind are vials from traps baited with human dung. The number and size of vials makes it visually clear that human dung captures more dung beetles.

Pitfall Traps After determining the bait to be used we were able to set up pitfall traps in a preliminary plot selection. I wanted to try and capture dung beetle activity windows. With this goal in mind I attempted to empty the traps every few hours, then dropped the visits to just day and night, however with a regular field schedule I found caring for the additional traps to be very hard both physically and logistically, and abandoned the idea, at least for the first field season.

Gut content When I first started reading the dung beetle literature I remember finding a paper showing that horse DNA could be extracted from dung beetles gut contents when dung beetles were collected on horse dung [7]. This made me think it should be possible to extract mammal DNA from the guts of dung beetles more generally not just those collected on directly on dung. In addition this seemed to me to be a less bias way of generating a picture of dung beetle diets. It seemed to me that than using dung from zoos or the sparse field observations as had been done previously [9, 6, 5, 16] had an inherent bias in that it is logistically almost impossible to represent the full local mammal community with dung from a zoo. In addition, dung in a natural setting would not be evenly spaced, continuously available, or all have the same volume and weight. For these reasons I suggested that studying gut contents with molecular techniques might be a better way to study dung beetle mammal networks. This was probably one of the methods I was most excited about in the beginning of the project. However, like with all new methods we ran into a couple issues in the preliminary attempts to generate a new method. The first obvious issue was human DNA contamination. All our sequences except for one from our first trials were human DNA. Humans are mammals and our DNA is ubiquitous, it took several trials over the course of a year to generate a lab protocol that allowed us limit the human DNA contamination, one of the key changes was a washing protocol to remove DNA from the outside of the beetles, in addition to several other hygiene steps to reduce human DNA contamination in the environment. However, after this almost nothing amplified and I suspected PCR inhibition. However, this was almost a year after we had started to develop the protocol and it was clear that to me that we would need to spend a lot of money trying different methods to overcome the inhibition or we could ask a lab that already had those

material on hand for help. So we took our preliminary attempts with all the tested protocol and all the tested primers (6 primer pairs) and sent all them to the company Sinsoma GmbH. They confirmed that there was indeed PCR inhibition and were able to test some protocols to limit inhibitors and finalise the proof of concept trials. We were then able to take this protocol and apply it to several beetles caught in pitfall traps both in Ecuador and Germany over the next year. This paper was submitted in December 2022 to PeerJ.

Dung choice Of all the methods I tried in my first year I would say this was by far the least successful. I took dung from the brown headed spider monkey *Ateles fusciceps fusciceps* and dung from the mantled howler monkey *Alouatta palliata* and laid them out in front of me and waited for dung beetles to arrive. It was horrible boring and not at all like the activity I observed sitting under a tree waiting for the monkeys to defecate and then watching the beetles arrive. Instead I was there for hours and not a single beetle landed on the dung of either monkey species dung. It was frustrating and I was covered in mosquito bites and had no data to show for it. However despite the utter failure of the first attempt I tried more three times and decided that I did not want to continue to suffer or use my time staring at dung if it did not yield any data.

Monkey dung and dung balls We were interested in secondary seed dispersal by dung beetles. It seemed like observing what dung beetles did with fresh dung would be key to understanding them as secondary dispersers in our forest. I took advantage of the frequent monkey encounters in the forest to collect defecates and then watch dung beetles create dung balls then collect both the beetle (sometimes beetles), and the ball together. With these defecates and dung balls we were able to quantify seed morphospecies, seeds per dung ball and seeds per defecate. We were also able to measure seed mass within dung balls a defecates. Further able to collect trait data about the seeds included dung balls and monkey defecates including seed lengths, and seed width, seed shape and seed surface. This resulted in a a natural history observation with *Oxysternon conspicillatum* moving large pubescent seeds like a dung ball. This lead us to hypothesize that seed surface and shape might also be important for secondary dispersal of seeds by dung beetles. Our first attempts to publish the observation as a natural history note were unsuccessfully, so it eventually became a paper after the collection and analysis of seed trait data 36 [14].

Rolling distance Along with the collection of dung balls I began to measure the distance dung balls were moved from the original location in the defecate (Nico was insistent that if we wanted to understand secondary dispersal of seeds we really needed to know how far they were being moved). This involved watching the dung beetles, and waiting patiently for them to form their ball, then marking the location in the defecate the ball was formed from, then watching as they rolled the ball away. This is also a little frustrating because there are so many beetles and you want to watch them all, because they all represent data you are missing as you focus on one beetle. However, if you fall into this temptation you miss your original beetles path and it rolls under a leaf and then vanishes, as there are so many beetles you cannot tell if this beetle under this leaf is your original beetle and then you have no data. So you have to patiently only watch one beetle at a time until it buries the dung ball. I did not record many of these observations in the first year. Partly because it was a bit hard to do well and partly because the temptation to collect the beetles before they buried their dung balls was really hard to resist.

Seeds/beads with strings I read about a series of two papers about secondary seed dispersal by by dung beetles using string attached to seeds that were contained in tamarin defecates [2, 3]. The method as described is 25 cm of string tied around a seed of 1 cm or larger with 5

cm flagging on the end that is labelled so that each seed can be tracked both horizontally and vertically (horizontally by the amount of string above the soil surface and vertically by the distance from origin). The fate of these seeds was then tracked for survival and germination rate. I was very enthusiastic about trying this because it seemed to beautifully solve many problems with previous studies, which only measured either dispersal distance (horizontal and or vertical) or germination rates and not both at once. The first time I tried this method I tied strings to too many seeds too close together and they became so tangled that they had formed almost a solid mass that I could not move and I was sure could not be moved by a dung beetle. I was not sure the data that resulted was any good. I also realised some seeds had a surface that made attaching the strings and keeping them attached very hard. However, I did have enough success with it when I attached the string to only three larger seeds that I thought maybe it could be interesting to try and do on a larger scale. However, the student Taly gave the project to had a pretty difficult time and I have not pursued it since then either.

Field season and methods development 2021 (working with poo)

Dung beetle rolling distance During this second field season we continued the observation of dung beetles as they rolled their dung balls and measured the horizontal distance on the ground. Sometimes they will drop from the leaves on to the ground, which does seem like it could be important because seeds on leaves are unlikely to survive. This was frustrating and resulted in a lot of mosquito bites but we did get more measurements of rolling distance, it was particularly helpful to have Jan Johann in the field helping because it double the number of possible observations.

Decomposition Gradient For this second field season Nico had really emphasised that we should be focusing on more than just dung because dung beetle diets are more diverse than that. So we came up with the idea to offer more a variety of baits. We thought it would be more important to understand if they would eat something than if they preferred a particular type of food. For this reason we knew the traps needed to be at least 40 m apart to avoid trap confusion, rather than placing baits next to each other which would have resulted in a choice experiment[11]. However, there were still a lot of questions about the bait. We wanted to measure the community that came to dung, rotten meat, rotten fruit, and dead millepeds across the chronosequence. The design needed to be standardised that meant we needed to make some decisions about what we would use to represent the types of resources. First, there was a lot of discussion about which fruit to use, we finally settled on bananas because we could get enough of them, and we could get them to a similar fermented state in large quantities. We also need to decide how rotten the rotten meat (carrion) should be, we chose cow meat because that was also something we could get in large enough quantities. To try and decide how long we should allow the meat to rot before using it as bait for our pitfall traps we set up three transects, spaced the traps 50 m apart, and rotated meat and millipede in each pitfall trap, we checked the traps everyday for seven days and counted the number of beetles and morphospecies in each trap. This allowed us to settle on two days of rotting time for the meat because this was the stage that would allow us to most fully capture the community attracted to rotting meat and for millipedes.

Dung beetle niche breadth first half We took our baits and then set up or experiment from the second half of September through the early part of November. During this time a bachelors student Jan Johann took the lead on this question for his bachelor's thesis. We still had a preliminary selection of plots, though we were given updated plot lists through out our sampling period. Over the three months we sampled

38 plots six cacao plots, four pasture plots, 11 cacao regeneration plots, seven pasture regeneration plots, and 10 old growth plots. There were five pitfall traps per plot each trap with a distance of 50 m from the other traps, baited with cow dung, carrion (one day rotting time), Juliforma millapede, and banana (one day fermentation time), or nothing respectively (Jan was unconvinced that banana pit fall trap would look any different from the trap with nothing). Trap order was rotated in each plot. Traps were collected 48 hours after placement in the field, and the contents stored in 95% ethanol. Sample sorting was done in 2022 and will be finalised in 2023.

Dung beetle observation chambers Observing the behaviour of the species of dung beetles we have in our field site allows us to better understand each species possible contribution to the ecosystem and how this shifts as the dung beetle community shifts. Dung beetles like to burrow under the soil surface, they often do this to create nests for their young, the depth and structure of the nests is different for different functional groups, some dung beetles roll ball, some tunnel near the dung and some dwell within the dung (Figure 33). For this reason we thought it would be interesting to observe the underground tunnel structure for the species in Canandé as there are not too many species (ca. 23), and this has implications ecologically for any seeds and other things they bury while making their nests. The basic idea is that the chamber is not much wider than the dung beetle so you get a vertical slice of the underground view of their behaviour through the panes of plexiglass which have soil between them. We also gave the dung beetles a food resource for them to bury. The plexiglas observation chambers it took a few trials to find a methodology that worked. It is better to make the chamber little wider that the dung beetle so it can turn around on the top layer of soil, even if that means you do not see all the tunnel because some of them will refuse to burrow under such unnatural conditions. We first gave two beetles of the same species more tightly packed soil and more loosely packed soil to see if this was important. The more tightly packed soil not only showed clearer tunnels but the beetles seemed to burrow more readily. I think this would be worth pursing in a more systematic fashion in the future, because it was very nice to see the tunnel structure and it would provide some additional natural history information that might helps make inferences about any shifts in community across the chronosequence.

Filming dung removal While dung beetles are admittedly a well studied beetle group, we still lack a lot of species specific information about natural history. There are a lot of assumptions made that genera have similar natural history characteristics. These assumptions seem to be made from very few documented observations that I have found. By filming dung piles we could possible capture at a finer scale activity windows, the speed of dung removal across the chronosequence, and which species or functional groups of dung beetles are responsible for the removal of dung. This has the advantage over laying out dung and leaving because it provided more precises data, without the need to have an observer in the field for 24 hours straight. To this end we tested a Bushnell NatureView Cam HD which has the advantage of coming with a camera lens with a shorter focal length than that of the standard wildlife cameras. This allowed us to film insects with more precision. It also has and a screen that allows you to see how the camera is positioned in real time which makes setup and the mico-adjustment's easier.

However, even after testing a few times the camera would not run for 24 hrs. The problem was most pronounced in the evening, when the camera pause for a few minutes between videos which resulted in a disproportiate loss of information at night. While data could be collected in this way, we might be able to develop a better method with a modular camera set up.

Field season and methods development 2022 (poo professional)

Dung beetle niche breadth second half In the most recent field season from the end of March 2022 to the beginning of May 2022, the pit fall trap set up from the previous field season studying dung beetle niche breadth across the chronosequence was finished by bachelors student Silvia Canelos and Diego Marin. We followed the same protocol as before but eliminated the unbaited trap, because it captured only one dung beetle and represented additional resources, time, and cost. Diego's involvement in this field campaign means that the species identifications for the second half of the data set. In addition, bachelor student Maira Elora Bradler also sorted the dung beetles from the original community composistion campaign in 2019 to morphospecies and where possible species. The beetles she sorted came from 8 cacao plantation, 10 pastures, 12 cacao regeneration sites, 17 pasture regeneration sites, and 19 primary forest plots. She then measured morphological traits for the most common ten species and organised a literature review of some important behavioural traits like nesting behaviour and activity.

Results

This results section is more of a highlights reel than an exhaustive description of the results so far, in part because some of them are already published or submitted and in part because three years of results is a thesis and not a newsletter.

Community Composition As expected both dung beetle abundance and diversity per trap increase across the chronosequence. My current list of dung beetle species has 21 named species and some additional morphospecies. Such a small number of species means that we can learn a lot about each species if we take the time to do natural history observations.
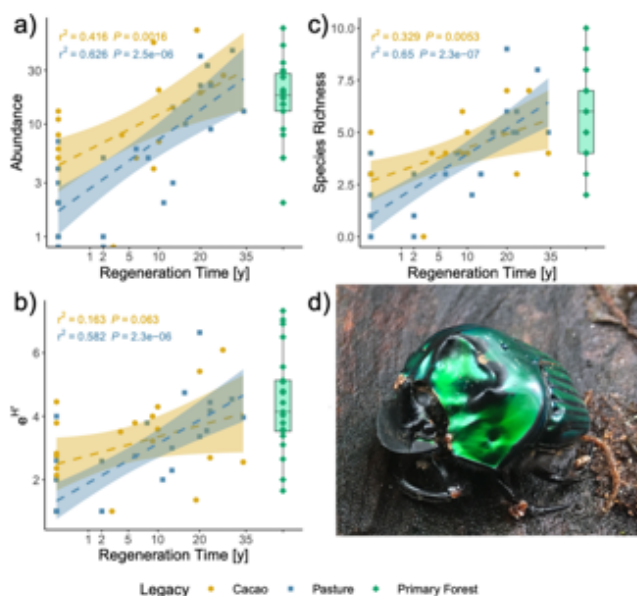

Figure 35: Linear models of dung beetle abundance, diversity across the chronosequence and photo of *Oxysternon conspicillatum* nicknamed "Richards" by Nico Blüthgen. Figures from the Bachelor thesis of MEB.

Secondary seed dispersal We found that most dung beetles secondarily disperse seeds of their body length or shorter. Thus dung beetles act as a filter for which seeds from the dung they will include in their dung balls. They also filter based on seed surface with larger seeds more

likely to have a pubescent or hairy surface that grips dung probably making it harder for them to distinguish between dung and seed (Figure 36).
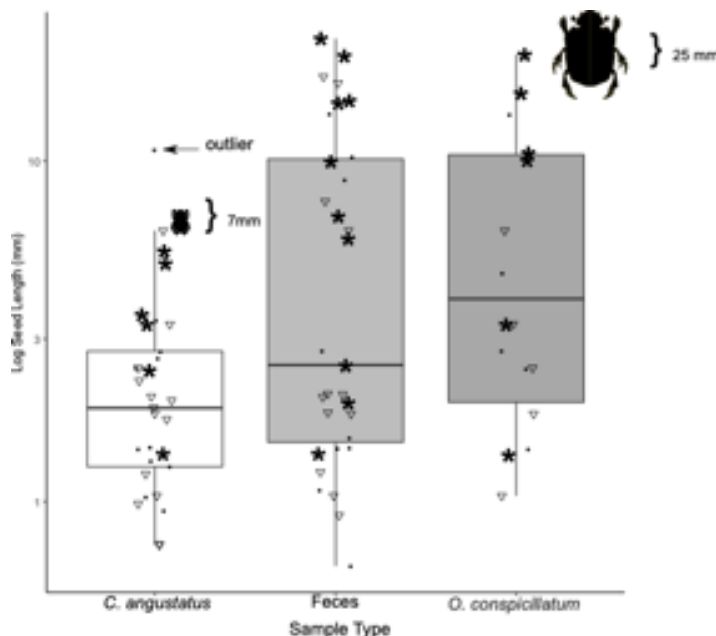

Figure 36: Distribution of seed size across samples. Each plant morphospecies is represented by a data point. Pubescent (hairy) morphospecies are denoted with an asterisk, smooth morphospecies are denoted by a filled circle, and striate morphospecies are indicated with an inverted triangle. Beetles are placed along the x-axis at a height that indicates their body length. Seed length is plotted on a log scale. The arrow points to an outlier seed in *Canthon angustatus* dung balls which was flat, and possibly pubescent. Figure from [14].

Niche breadth Within our design of pitfall traps baited with rotten meat, dung, millipedes, and fermented banana, we did see that the community did shift from using primary dung to using more carrion in the forest. This does seem to be a shift in species in composition from the agriculture to the forest. Dung comes out as a resource with higher specialisation in our site then carrion. Interestingly the species in the agriculture represent a subsection of the forest species (Figure 37).
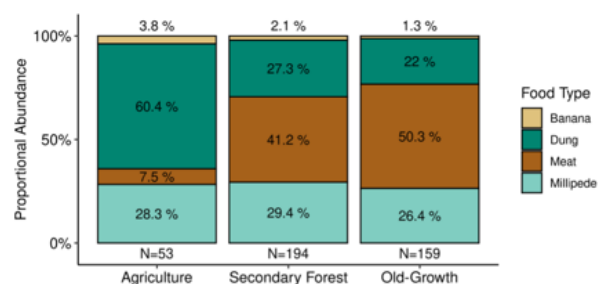

Figure 37: Shift in dung beetle resource use from agriculture to forest. Figure for the Bachelor's thesis of JJ
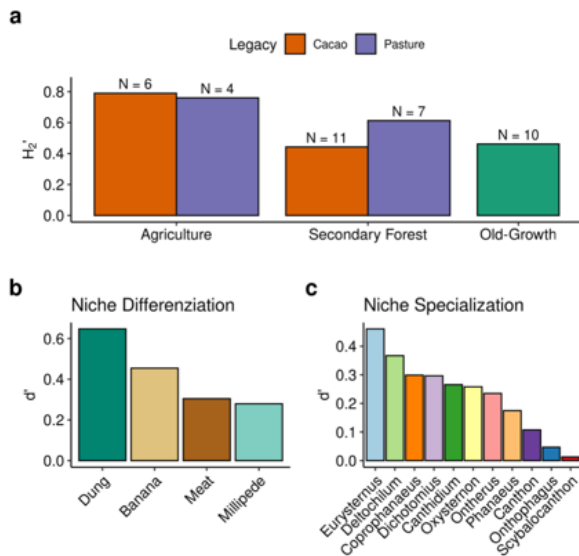
Figure 38: a) The community specialisation in agriculture, secondary forest, and old growth forest. Specialisation is higher in the agriculture and lower in the forest. b) Niche exclusiveness by bait type. c) Resource exclusiveness at the genus level. Figure from the Bachelos's thesis of JJ

Outlook

The up coming field season will expand upon our preliminary work with secondary seed dispersal. Using dung with a known mixture of seeds of various sizes. We will use the P-Rex plots to lay out two dung piles per P-Rex treatment, then exclude dung beetles from half of them. This will allow us to quantify how many seeds of each size are still present when we return. After 48 hours we will return to count remaining seeds. Then we will return 2 weeks and four weeks after we placed the dung and seed mixture to track germination and short term survival if any in the secondary dispersed and not dispersed (dung beetles exclosure) seeds. This has the possibility to set us up for longer term monitoring of seedling survival with and without secondary seed dispersal by dung beetles.

While the trait data from the seeds in the monkey dung and dung balls is published [14] along with the data sets [15], the identification using molecular methods later this year to species level would allow generate a tripartite network of seeds, monkeys and dung beetles. The proportion of the dung balls made of seeds, the rolling and burial distance could also be included here. This could generate data about the diets of both the critically endangered brown headed spider monkey and the mantled howler monkey, which might be useful for policy decision of both Reservá Canandé and Tesoro Escondido.

We are also currently working to try and get a working prototype of a modular time lapse camera like that being used with pollinators in [4]. This would allow us to quantify the time it takes to remove dung and the activity windows of our dung beetles across the chronosequence. However, there is currently a shortage of Raspberry Pis so this may not be feasible. I am trying to create a working prototype with the readily available but less user friendly ESP32-Cams for the next field season.

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SP7: Deadwood and saproxylic insects

Ana Falconi, Universität Würzburg
Nina Grella, Universität Bayreuth

Who we are

In SP7 we investigate deadwood decomposition and the interactions between saproxylic insects, fungi, and deadwood along the forest recovery gradient. Our subproject consists of three PIs and two PhD students (Figure 39). Nina Grella, the first PhD student, is based at the University of Bayreuth and supervised by Professor Heike Feldhaar. The second PhD students, Ana Falconí, will spend her first three years at Universidad de las Américas in Quito and will complete her dissertation under Professor Jörg Müller's supervision in her final year at the Julius Maximilian University in Würzburg. Professor David Donoso is a specialist in Neotropical ants at Escuela Politécnica Nacional in Quito and is the third principal investigator. Together, our diverse expertise allows us to work toward the common goal of unraveling the interactions between saproxylic insects, fungi, and deadwood. During their fieldwork, Nina and Ana collaborate closely and perform the majority of the sampling and experiments together. Ana focuses on saproxylic beetles and fungi, whereas Nina explores eusocial insects like ants and termites.

Figure 39: PhD students. Ana Falconí and Nina Grella

What everyone should know about my work

Deadwood plays a crucial role in nutrient cycling and carbon storage. During the ongoing twin crisis of biodiversity loss and climate change, research about deadwood and its capacity for carbon storage is vital. Former studies estimate that the net effect of insects on carbon flux may account for 29 per cent, whereas 93 per cent of carbon released from deadwood globally originates from the tropics. Yet many aspects of the relationship between saproxylic insects, fungi, and deadwood are still unknown. With five work packages (WPs) in our subproject, we want to investigate different aspects of deadwood. In our first WP, we conduct a deadwood survey where we record the amount and diversity of deadwood in all plots and analyze the data with regard to the different forest recovery stages. We want to assess if secondary forests can recover the deadwood storing capacity of old-growth forests. In our second WP, we collect ants, termites, and beetles in five different deadwood objects in each plot as well as from the surroundings. We want to analyze the co-occurrence of the different insect species in deadwood and compare them with the communities in the surrounding habitat. Doing this we will focus on potential interactions like priority effects or competitive exclusion in the different forest regeneration stages. In our third WP, we expose wood logs from five different tree species on each plot and analyze the ant, termite, beetle, and fungi communities that colonize the deadwood. The resulting interaction network gives important insight into which insect and fungus species are most important in colonizing and decomposing deadwood in the different recovery stages. In our fourth WP, we will analyze the functional traits of the specimen collected in WP2 and WP3 and we aim to detect which functional traits depend on forest age. Finally, in WP5, we want to measure the contribution of termites, ants, beetles, and fungi to deadwood decomposition. We perform an insect exclusion experiment on the PREX plots and measure the contribution of the single taxa depending on forest age, disturbance, and the exclusion of large mammals. With these experiments, we want to shed light on the relationships between species identities, functional traits, and contribution to deadwood decomposition of saproxylic insects and fungi in the different forest regeneration stages.

Figure 40: Logistics with Jocotoco staff. Meeting with Katrin and Bryan

Plans and Reality

This year our SP had two field campaigns. The first was conducted from February to April and the second from mid-August to October. During these five months, we gained a lot of experience about pitfalls and challenges that can occur during fieldwork, but also how to find creative solutions; especially with logistics. During the first field cam-

paign, we conducted the deadwood survey (WP1) and the collection of insects from deadwood and the surroundings (WP2). We had to improvise often during rainy weather because ants are less active then. But during that time the disturbance of the PREX plots was ongoing. In a project of this size, we could not just change our plans but we had to find solutions together with the station managers and with Connie, our scientific coordinator. Fortunately, we became a good team and found our way to solve the challenges and problems. When estimating the time that it takes to measure all the deadwood objects and to collect the insects with many different sampling methods, we were too optimistic in the beginning. Luckily we have a team of great parabiologists that often helped us with the collection. After spending much time in the forest, it is now much simpler to estimate the amount of time needed for experiments and the walking distances to the plot.
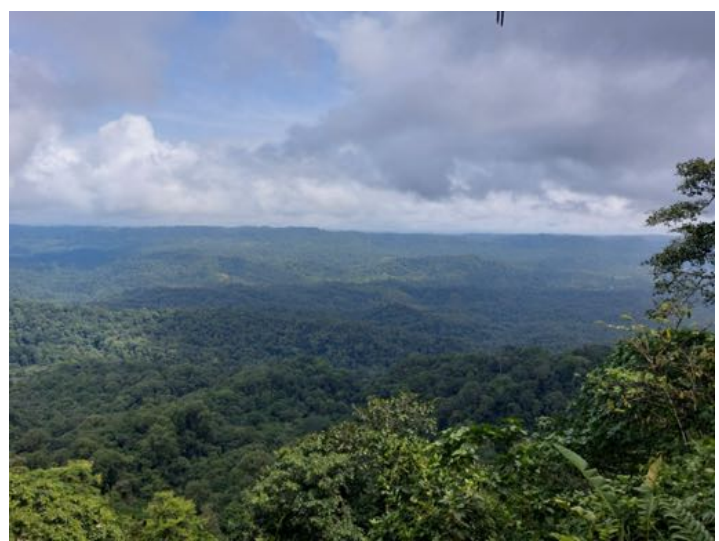

Figure 41: Mirador de Canandé

The second field campaign was logistically very challenging. Initially, we were planning to conduct the experiments for WP3 and WP5 at the same time. That would have meant placing 320 pieces of wood on the 64 plots for the first experiment and additional 1024 pieces of wood on the 32 PREX plots for the second experiment. For the latter, we needed aluminum trays and insect exclusion cages, but the shipping from Germany to Ecuador took longer than expected. After more than eight weeks our equipment arrived, but we had already started with the first experiment and decided to postpone the PREX experiment. In hindsight, this was a blessing in disguise, because we realized that the preparation of the wood and the transport to the plots take a long time and much effort (Figure 43).

In this campaign Silvia, a new parabiologist from La Yuca, joined our team (Figure 42). Together we overcame the many challenges with the wood logistics and she helped us with the handling of Tarsilo, our project mule. Although Tarsilo behaved literally very mulishly, he helped us a lot with carrying our wood.

Ana Falconi, Universität Würzburg

Impressions from the field

In the Chocó region of Ecuador, there is a reserve that was created to protect one of the most important biodiversity hotspots in the region: Canandé (Figure 41). The reserve is located in the province of Esmeraldas and was created with the intention of conserving one of the last remnants of Ecuador's tropical forests that are threatened today by extractive

and agricultural activities. The Jocotoco Foundation, an Ecuadorian environmental organization that protects areas of critical importance for conservation, led the process of creating the reserve, although with the help of other organizations such as Rainforest Trust, International Conservation Fund of Canada and American Bird Conservancy.

Figure 42: Silvia, our great support

Figure 43: Experiments. a. Deadwood species handling, labelling and cutting; b. Exposed five deadwood from different tree species on each plot; c.WP5 insect exclusion experiment on the PREX plots

We are fortunate to have been able to carry out our research in 64 plots distributed in two reserves: Canandé and Tesoro Escondido; we also had the support of some people from the community to access plots of cacao and active pasture. The first few weeks were learning on both sides, as we were coordinating all the logistics for our project (Figure 40). Also, we were able to visit nearby plots and practice with some experiments (Figure 43). The hikes to most of the plots were very long

and exhausting, but we made it to all 64 plots. Our great support were the parabiologists, whom with their knowledge and guidance took us safely to all the plots.

For me it has been a very enriching experience. I have been able to meet great people and professionals. We have learned how to react and adapt to changing situations. I really enjoy sharing with the other PhDs students and getting to know a bit more about their projects, being able to support them when they need it and laugh at the meals we have together or on the field trips we share.

Results

In this first year of the Reassembly project and having been in the field for almost 5 months, I have collected data on the diversity of dead wood and beetle larvae and adults in the 64 plots. Next year I am planning to barcode them and get their species identities. Figure 44 gives an overview of the diversity of deadwood in active and regenerating pastures, active and regenerating cocoa plantations, as well as in old-grow forests.
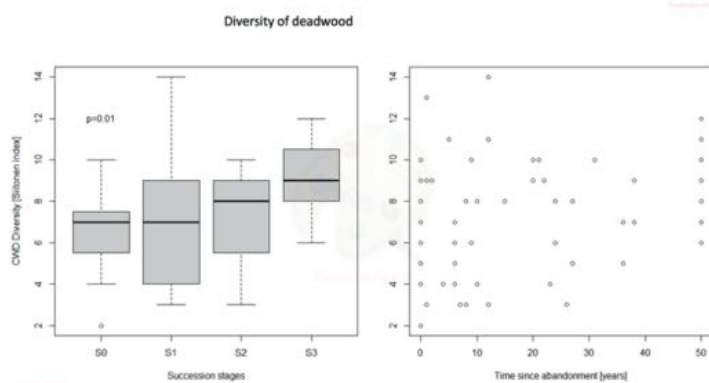

Figure 44: Diversity of Deadwood

Coarse and fine woody debris increase along the gradient. Fine woody debris (FWD) increases faster than coarse woody debris (CWD) – legacy effect (Figures 45, 46).
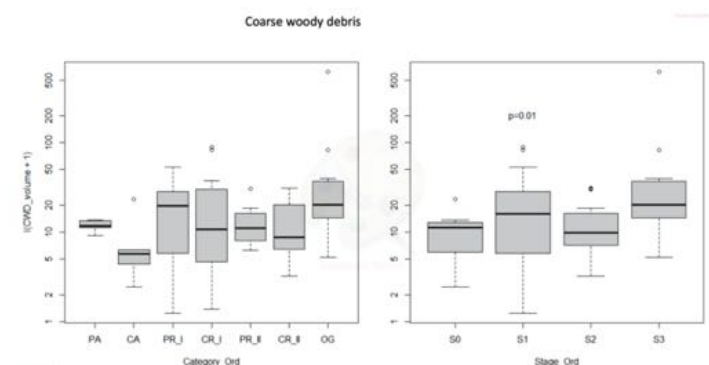

Figure 45: CWD increase along the gradient

Outlook

Next year we are planning to have two more field campaigns in Canandé and Tesoro Escondido. We have to remove the wood that we put in the plots in October and put them in emergence chambers. In these chambers we will capture emerging insects and identify the species. Afterwards, we plan to carry out our last experiment, in which we will

measure the contribution of different insect and fungal taxa to the decomposition of dead wood (WP5).

We are confident that our experience with the wood logistics from last October will help us in organizing the next field season. In addition to the fieldwork, I have to barcode my beetles and fungi samples. Also, I will identify the beetle species via morphology.
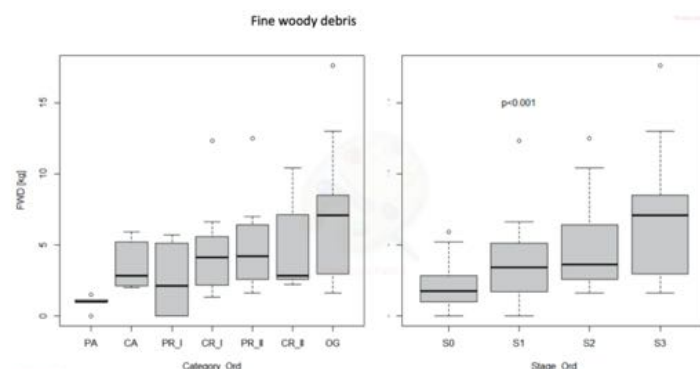

Figure 46: FWD increase along the gradient

Nina Grella, Universität Bayreuth

Impressions from the field

During my first stay, many Reassembly members started with the fieldwork at the same time and it became quite crowded in the Chocó lodge. However, looking back it was a great experience starting the project with so many other Ph.D. students and being able to help each other during our ups and downs. For me, the Chocó rainforest is a breathtakingly beautiful place. During my stay, I had the opportunity to meet amazing and inspiring people and experience an incredibly diverse nature.

Figure 47: In WP4 we exposed five deadwood pieces from different tree species on each plot. Next year we will collect them and explore the insects and fungi that colonized them

Most fascinating for me was watching army ants during their raids as well as observing the hard-working leaf-cutter ants carrying plant material into their nest. During our work on the plots we often had to hurry to get to the next plot and get our work done before it was getting dark. Hence, I enjoyed joining the herpetologist's night excursions and having some time to take photos and look at the animals for a little longer.
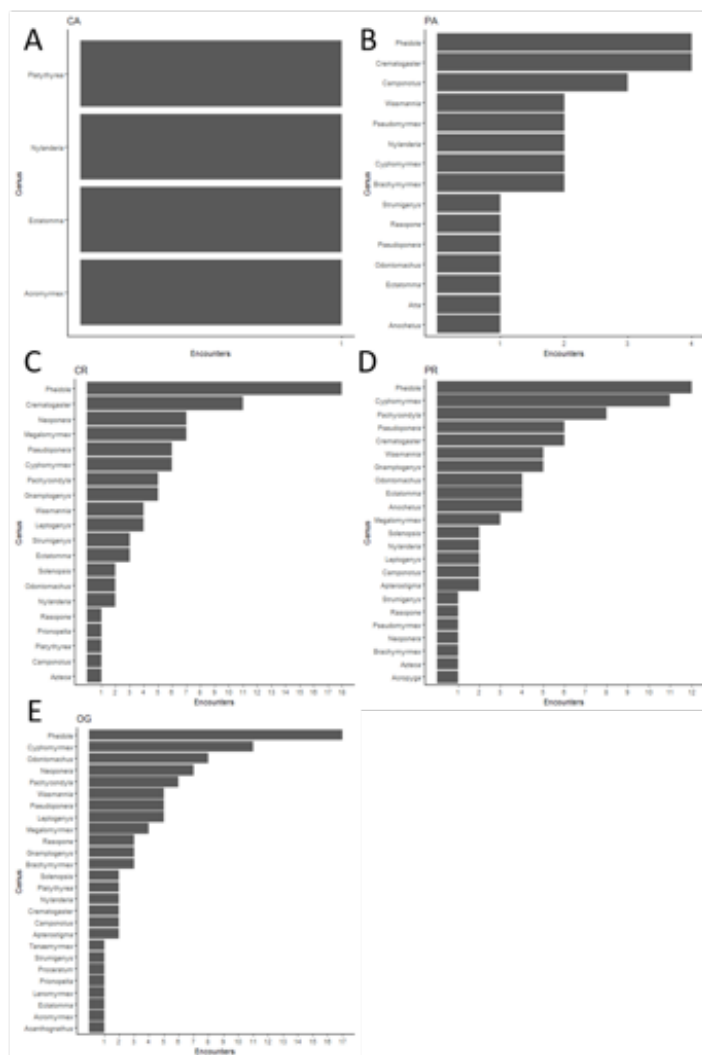
Figure 48: Encounters of ant genera colonizing deadwood in different forest regeneration categories A) active cocoa plantations B) active pastures C) regenerating cocoa plantations D) regenerating pastures E) old-growth forest.

Outlook

Next year I am planning to have two more field campaigns in Ecuador at about the same time as this year. We need to get back in the wood that we placed on the plots in October and put them into emergency chambers. In these chambers, the emerging insects will be captured and we will identify the species. Afterwards, we are planning to conduct our last experiment where we will measure the contribution of different insect and fungi taxa to deadwood decomposition (WP5). Now, that we have almost all our equipment in Ecuador, hopefully everything runs smoothly. I am confident that our experience with the wood logistics from last October will help us in organizing the next field season. In addition to the fieldwork, I have many ants and termites in Bayreuth waiting to be barcoded. I have already gained an overview of the genera and I am optimistic that I can identify the species next year via barcoding and morphology.

Figure 49: Tarsilo helping us to carry wood to the plots

Results

This year I have been in the field for five months and I already collected many ants and termites. Next year I am planning to barcode them and get their species identities. Between my two field campaigns, I identified the ants colonizing deadwood to genus level to get an overview of which genera do occur in general and in which forest regeneration ages. Figure (48 gives an overview of the ant genera colonizing deadwood in active and regenerating pastures, active and regenerating cocoa plantations, as well as in old-grow forests. We collected five deadwood pieces per plot if possible. Encounters were defined as the sum of how many dead wood pieces were colonized by a genus and not as the number of individuals.

As one can already see (Figure 48), most ant genera were found in the old-growth forest, followed by the two regenerating forest categories. The lowest number of genera was found in the two active agriculture categories. Interestingly the active and regenerating pastures bear a higher number of genera than the active and regenerating cacao plots. However, these first findings just give a first impression and the results of the species identification analyses will deliver important insight into species richness, species diversity, and functional traits depending on forest regeneration and wood traits.

Imprint

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