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REVIEW

Using DNA-barcoded Malaise trap samples to measure impact of a geothermal energy project on the biodiversity of a Costa Rican old-growth rain forest[1]

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Abstract: We report one year (2013–2014) of biomonitoring an insect community in a tropical old-growth rain forest, during construction of an industrial-level geothermal electricity project. This is the first-year reaction by the species-rich insect biodiversity; six subsequent years are being analyzed now. The site is on the margin of a UNESCO Natural World Heritage Site, Área de Conservación Guanacaste (ACG), in northwestern Costa Rica. This biomonitoring is part of Costa Rica's ongoing efforts to sustainably retain its wild biodiversity through biodevelopmental integration with its societies. Essential tools are geothermal engineering needs, entomological knowledge, insect species-rich forest, government–NGO integration, common sense, DNA barcoding for species-level identification, and Malaise traps. This research is tailored for integration with its society at the product level. We combine an academic view with on-site engineering decisions. This biomonitoring requires alpha-level DNA barcoding combined with centuries of morphology-based entomological taxonomy and ecology. Not all desired insect community analyses are performed; they are for data from subsequent years combined with this year. We provide enough analysis to be used by both guilds now. This biomonitoring has shown, for the first year, that the geothermal project impacts only the biodiversity within a zone less than 50 m from the project margin.

Key words: DNA barcode, geothermal project, Costa Rica, biomonitoring with insects, ICE/ACG/SINAC/MINAE/GDFCF.

Résumé : Les auteurs rapportent les résultats d'une première année (2013–2014) de biosurveillance d'une communauté d'insectes dans une forêt tropicale ancienne, au cours de la construction d'un complexe industriel de génération d'électricité géothermique. Il s'agit de la première année de réaction de cette communauté d'insectes riche en espèces; six années subséquentes sont en cours d'analyse. Le site est situé à la marge d'un site du Patrimoine mondial de l'UNESCO, l'Área de Conservación Guanacaste (ACG), dans le nord-ouest du Costa Rica. Ce travail de biosurveillance s'inscrit dans les efforts en cours au Costa Rica pour maintenir de manière durable sa biodiversité sauvage via une intégration bio-développementale avec ses sociétés. Les outils essentiels en sont les

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besoins en génie géothermique, les connaissances en entomologie, une forêt riche en espèces, une intégration gouvernement–ONG, le sens commun, le codage à barres de l'ADN pour l'identification au niveau de l'espèce et les pièges Malaise. Cette recherche est faite sur mesure pour favoriser l'intégration avec la société au niveau du produit. Les auteurs combinent une vision académique avec la prise de décisions d'ingénierie sur le site. La biosurveillance nécessite un codage à barres de niveau alpha combiné avec une taxonomie et une écologie séculaires fondées sur la morphologie. Ce ne sont pas toutes les analyses de communautés d'insectes qui sont réalisées ; elles le seront pour les données des années subséquentes lorsque celles-ci auront été combinées aux données de cette année. Les auteurs réalisent suffisamment d'analyses pour être utiles aux deux guildes pour l'instant. Cette biosurveillance a montré, pour la première année, que le projet de géothermie touche uniquement la biodiversité située dans une zone à moins de 50 m de la marge du projet. [Traduit par la Rédaction]

Mots-clés : codes-barres à l'ADN, projet de géothermie, Costa Rica, biosurveillance d'insectes, ICE/ACG/SINAC/MINAE/GDFCF.

Introduction

Philosophical

The detailed reactions of the biodiversity of whole terrestrial communities of tropical wild eukaryotic biodiversity, to industrial-scale perturbations, have not yet become a major topic of research interest for the academic or commercial community. The social and technical focus has been on the avoidance or repulsion of such perturbations. The notable exceptions are for single species that are endangered, charismatic, disease carriers, major pests, or have other species-level traits of special interest to humans. The causes for this neglect of a potentially huge research area by the science community are (*i*) centuries of absent capabilities for global database (big data) synthesis, and (*ii*) human inability to simultaneously identify the thousands of insect species in a whole-community analysis.

Widely used electronic data management (e.g., Ratnasingham and Hebert 2007, 2013) and DNA barcodes (Hebert et al. 2003, 2004; Holloway 2006; Ratnasingham and Hebert 2013; Janzen and Hallwachs 2016; Miller et al. 2016) now allow avoiding both of these impediments. Here, we report on the use of electronic species-level biodiversity data management and DNA barcoding of large samples of tropical insects to deliberately and simultaneously (*i*) apply biomonitoring with insects of a species-rich and relatively unperturbed old-growth tropical forest that has been point-source impacted by an industrial geothermal project, and (*ii*) lightly touch on basic research questions about a tropical insect community.

Throughout the terrestrial tropics there are a plethora of industrial perturbations to well-established forests that are generated by roads, mining, hydroelectric dams, river capture, light pollution, water pollution, atmospheric pollution, pesticides, logging, agriculture, and tourism (at the least). Examination of the impacts of these perturbations is almost entirely based on the believed or actual impacts on populations or individuals of single species of vertebrates that are usually charismatic, large, and threatened with extinction by some criterion. These species generally constitute less than 0.1% of the biodiversity present. Measurements generally ignore whole phyla and orders of biodiversity, and tens of thousands of species (e.g., fungi, nematodes, mites, insects, spiders, non-timber and juvenile plants, as well as the microbes that ride on or in them). A basic reason for neglect of this 99.9% of wild tropical terrestrial biodiversity is that the species are not only almost totally undescribed by science, but also that it has been generally impossible to identify them and to communicate to the lay community about them as species and as identified individuals, for whatever purpose. Perhaps worst of all, they are viewed as unimportant to humans and their domesticates, in great part due to human ignorance of the details of their lives, and due to human desires to live distant from the wild world that birthed our genomes.

This paper is meant to outline some of the many pragmatic steps that occur in the melding of academic basic biodiversity research with the pragmatics of biomonitoring an industrial geothermal perturbation, as a module within Costa Rica's new ongoing BioAlfa project to know and accommodate its own national wild biodiversity (Janzen and Hallwachs 2019*a*, 2019*b*), which is in turn a module within BIOSCAN′s efforts to DNA barcode the globe (Hebert 2015; www.ibol.org). This report is neither a review of the literature about the topic, nor anything like the detailed scientific scrutiny deserved by the massive amount of data produced and continuing to be produced. Rather, it is meant as an introduction and exploration of an example of melding pragmatic social desires for production with the curiosity-driven research that is standard within the academic community.

Pragmatic

Many items or facts strategically mentioned throughout this introduction and analysis are details of the kind that are not normally reported in either scientific or engineering analyses, or both. They are included, and many more omitted, because they are the kinds of guild-to-guild accommodations that were used to create a win–win situation for two well-entrenched and well-appreciated sectors of society that traditionally have quite different goals and measures of success. This report deliberately mixes them together because that is the way they occur in reality.

Fig. 1. ICE geothermal drilling platform PL12 (1.5 ha) at the border of government-owned old-growth rain–dry forest (Parque Nacional Rincon de la Vieja, part of Costa Rica's ACG UNESCO Natural World Heritage Site). ACG lies above the yellow line (image taken 7 months after PL12 being inserted into the forest). All the forest slightly downslope from the yellow line is government property of ICE. The open areas in the forest inside ACG in the upper left are natural hot spring sites (Las Pailas). The ICE PL12 drilling site is in the center of this image, with three other geothermal ICE drilling sites to the left. [Image: Luciano Capelli, RIP; text added by D.H.J.]

The portion of the biomonitoring of the geothermal project called Pailas II in northwestern Costa Rica (ICE Group Description 2019) originated at the end of nearly two decades (1997–2013) of neighborhood overlap between two long-term projects: (1) very basic biodiversity science and action as biodiversity inventory of Costa Rica in general, and specifically the inventory of the project's adjacent Area de Conservacion Guanacaste (Janzen and Hallwachs 2016, 2019*a*, 2019*b*), and (2) the quite pragmatic event of government harvest of a geothermal resource 2–3 km underneath the old-growth mid-elevation complex tropical rain forest of the ACG UNESCO Natural World Heritage Site (http://www.acguanacaste.ac.cr) and its contained Parque Nacional Rincon de la Vieja. This several thousand-hectare mid-elevation forest (Supplementary data, File S1[2]) is divided roughly in half by the boundary between the long-established Parque Nacional Rincon de la Vieja in Sector Pailas of Área de Conservación Guanacaste (ACG) of MINAE (Ministerio del Ambiente y Energia) (http://www.acguanacaste.ac.cr). The relevant lower half of this mid-elevation rain forest is likewise government-owned land, but it was purchased by a different agency of MINAE, the Instituto Nacional de Electricidad (ICE). ICE is a National Energy Company operating as a government agency (ICE Group Description 2019). This land is explicitly developed as the geothermal project known as Pailas I and Pailas II (ICE Group Description 2019). The specific study site discussed here is drilling platform PL12 of Pailas II, and its surrounding old-growth forest. Its margin is about 60 m from the ACG boundary (Figs. 1–3, 5).

This geothermal project appears to have the potential to be highly destructive to the adjacent ACG UNESCO Natural World Heritage Site and to any old-growth mid-elevation rain–dry forest in its vicinity. Equally complex, the Pailas II PL12 site lies exactly in the intergrade between dry forest and rain forest. This kind of intergrade tropical forest has been almost totally ignored by biodiversity studies, and contains portions of the biota of both of the larger ecosystems as well as a unique biota (e.g., Janzen et al. 2017).

When the PL12 geothermal site was initiated in 2013, it had all the ingredients of a classical Greenpeace versus commercial industry conflict as a small war about important conserved and conservable old-growth forest between two opposing government agencies in the same Ministry. The various anticipated conflicts, and their classical non-solutions prior to 2013, are not discussed here, as they would require another yet more biopolitical report of equal length and not involve DNA barcoding and its application as as part of the technical solution to the conflict.

History, materials, and methods

On 23 August 2013, several middle-management ICE engineers and planners visited ACG's Área Administra-

[2]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/gen-2020-0002.

tiva to explicitly describe their plans for the geothermal development of Pailas II on the ACG boundary (the yellow line in Fig. 1). This visit was prompted by ICE's middle-management having heard and absorbed a five-year litany from the ACG staff (www.acguanacaste.ac.cr) and that of the Guanacaste Dry Forest Conservation Fund (GDFCF; www.gdfcf.org) as to "why don't we work together to find a definable minimally damaging solution for the harvest of this well-known geothermal resource?". On 23 August, ICE was feeling administratively and biopolitically free to approach ACG, with GDFCF (http://www.gdfcf.org) as a collaborating NGO, in hopes of a response to this conservation question.

At the end of the ICE presentation, GDFCF asked ACG's Director if it would be permissible to introject a pointed observation. He responded, "OK, if you want to". GDFCF asked the ICE presenter for permission to use the ICE PowerPoint slides to explain what ACG/GDFCF would do if it were their geothermal project, a project that we all knew the authors of this report would see made sense to do. The presenter in turn asked the most senior ICE person, who turned out to be the Chief Engineer of Pailas II, and they had their conversation. The Chief Engineer replied in effect "OK, discuss, but this is all unofficial". GDFCF took the presentation and said "imagine that NSF had just given GDFCF/ACG a US$2 million grant to ask a question. "What is the reaction of the biodiversity of a complex tropical old-growth rain forest to having a one-lane access road constructed through it, and an accompanying 1–2 ha drilling platform cut out of that same forest?".

GDFCF proposed a novel project to use lines of Malaise traps (Fig. 5; Supplementary data, File S2[2]) for at least a year and hopefully more, to document the reaction by the tens of thousands of species of flying insects in that forest. A trap will be placed at the road margin, at 50 and 150 m distant inside the forest, and then this three-trap line repeated from two points on the margin of the drilling platform, for a total of nine Malaise traps. The traps would be serviced on the same exact day every week of the first year by the same parataxonomist (Janzen and Hallwachs 2011) and hopefully continue for years after. Every insect captured would be DNA barcoded by the Centre for Biodiversity Genomics at the University of Guelph (http://ibol.org), just as ACG/GDFCF has been doing for years to inventory its own adjacent forests inside ACG (Janzen and Hallwachs 2016). ACG/ICE/GDFCF would then jointly begin to address how has the road and drilling platform perturbed this insect biodiversity and at what distances from the perturbation. In this case, a baseline year of traps running pre-perturbation was not possible, but the traps were set up at the very beginning of the perturbation to the forest.

This proposal was followed by more discussion among the ICE staff. ICE engineering sites are generally off limits to non-ICE personnel and to work-in-progress analysis by others. ICE is frequently attacked by NGOs for real or imagined environmental damage done to conserved or conservable wild ecosystems, and generally attempts to follow the traditional national Environmental Assessment rules from MINAE's SETENA (Secretaria Técnica Nacional Ambiental) for engineering projects. ICE is viewed with envy by many other government entities that have far less funding (in part because ICE produces a huge portion of Costa Rica's electricity). ICE is largely staffed and budgeted with professionals and workers who know very little about wild biodiversity, and can do quite large unintentional damage, thereby attracting the ire of conservationists. ICE is conspicuously a different government agency than SINAC (the National System of Conservation Areas) but a dependency of the same government ministry, MINAE.

At the end of the discussion, ICE took a leap of faith and said "OK" to the challenge. The ACG/GDFCF reply was, "then you need to take us immediately to the forest and show us exactly where the access road will be, and where the PL12 drilling/extraction platform will be". Two days later, ICE did. And the engineering budgeteer then said quite memorably, "but how can we know how to budget it when we will not know how many insects will be caught"? This conundrum was resolved with a guess of US$60 000 per trap-worth of barcoding per year averaged over the nine traps; the trap became the practical unit, rather than species or specimens. After the end of the year, financing was available for analysis of only seven traps (the contents of the other two remain in the freezer), but that was vastly better than none, from both a basic science and an engineering viewpoint.

The members of the ACG/GDFCF Parataxonomist Program (Janzen and Hallwachs 2011; https://www.acguanacaste.ac.cr/programa-de-parataxonomos) were then asked to set the traps as soon as the one-lane roadway was bulldozed and the platform margins were likewise chain-sawed and bulldozed out of the forest.

Why only nine Malaise traps? Because a student researcher from the Centre for Biodiversity Genomics (CBG; http://ibol.org) had visited ACG the year before and left nine Malaise traps in the storeroom. Why 0, 50, and 150 m? Guesswork and a start towards other distances for later planning. Why Malaise traps? Because they could be set and attended by a parataxonomist or other outsider with no formal biological background, or by an ICE engineering staff member, and because there were freezers in ACG in which to store the weekly samples in 95% ethanol. And because CBG had already established the proto-BioScan global Malaise trap sampling scheme (Hebert 2015; http://ibol.org) that was quite experienced at Sanger-sequencing mass Malaise trap samples to build the global biodiversity Eukaryota barcode library in BOLD (http://www.boldsystems.org). ACG/GDFCF had already contributed three Malaise trap-year samples to the proto-BioScan (http://ibol.org) effort for ACG bioliteracy and bioinventory (Janzen and Hallwachs 2016).

The weekly samples were accumulated individually in 300 mL glass bottles in −20 °C flat freezers for a year, then shipped in neoprene bottles by DHL to the CBG at the University of Guelph. This was done under MINAE and CONAGEBIO permits for their collection and export, explicitly for DNA sequencing for their individual barcodes and for basic inventory research. This required formal permission from ICE, the legal owner of all terrain being trapped, except traps #1 and #6 that are positioned just inside ACG (Fig. 5). In the CBG the insects were individually Sanger sequenced, the extracts and cadavers stored for later taxonomy and (or) Costa Rica's CONAGEBIO contracted genome exploration. The barcodes and their collateral are stored on BOLD as global public domain, as authorized by the Government of Costa Rica (e.g., Government of Costa Rica 2019). Further taxonomic refinement of specimen description, and at times identification, is in continuous process (e.g., Janzen and Hallwachs 2016; Espinoza et al. 2017; Fleming et al. 2018; Arias-Penna et al. 2019; Burns et al. 2008; Fernandez-Triana et al 2014, 2015). This process is integrated with the ongoing DNA barcoding of all of the biota of ACG, and now, the cross-society BioAlfa program to DNA barcode all of the million eukaryote species estimated to live in Costa Rica, for national bioliteracy and for integration of their conservation with their society through non-damaging biodevelopment (Janzen and Hallwachs 2019*a*). All project data will become progressively more available to any global user as its processing gains taxonomic and organizational maturity over the next 2–5 years.

But what of barcoding costs? The initial PL12 engineering site preparation and development to production cost roughly US$22 000 000. These funds were part of an US$800 000 000 overseas development loan from JICA (Japan International Cooperation Agency). ACG/GDFCF assumed that it would be important for JICA and ICE not to have an environmental black eye on their international environmental balance sheet. They would therefore be willing to fund the comparatively tiny estimated costs of DNA barcoding the biomonitoring results, especially if voluntary rather than forced by NGO attack. After negotiations, JICA provided the projected US$420 000 to meet the direct cost for the DNA barcoding by CBG for about 145 000 specimens (Fig. 6). ACG/GDFCF initially provided all else pro-bono to the project. The CBG provided its BOLD web site analytical services pro-bono. Small costs (ETOH, bottles, two freezers, rental car fuel, parataxonomist, and PI time) were GDFCF investments in the project, though they were later partly reimbursed by JICA as a lump sum consultant fee.

JICA, their consultants, and middle-management ICE inspected the entire project and found it to fit the JICA concept of a SAPI. A SAPI has been explained as "Projects are prepared with assumptions in the preparation stage that they sometimes face unpredictable events and issues in the implementation stage. In such circumstances, JICA organizes a scheme called Special Assistance for Project Implementation (SAPI) on a grant basis as a countermeasure to seek feasible solutions for a smooth implementation of the project." (M. Hasegawa, JICA, personal communication 15 December 2019). GDFCF received the funding and passed it on to CBG with no overhead charges.

A SAPI and the ACG/GDFCF/ICE involvement contained the first year of environmental planning and inspection for the major ICE project underway, along with standard SETENA (as mentioned previously) requirements. A wide variety of visitor inspections and educational events then introduced entomological trapping, barcode sequencing, taxonomy, and basic ecology through and around the geothermal project and its staff, gradually leading to its insertion into the much larger complexity of the engineering requirements and actions. This was coupled with small but highly significant site adjustments (e.g., open gates through protective fencing, reduced lighting, one lane access road, later platform adjustments considering the actual traps, unobstructed educational and research access by foreigners and Costa Ricans, NGOs, government agencies, and planners). It became an on-site and in-symposia display as part of Costa Rican efforts to integrate necessary environmental perturbations into its green country vision. Its data, because the insects were individually barcoded, are a major starting point on building the BioAlfa national DNA barcode library (Janzen and Hallwachs 2019*a*), and groundtruthing for future changes in the insect community. This paper is one of these efforts. It displays biodiversity facts of interest to biodiversity ecologists, engineers, and biopoliticians, but simultaneously is about research on the socioeconomics of blending industry perturbations with conservation of tropical wildlands, something that ACG and GDFCF have attempted to develop since 1985 as an ACG founding principle (e.g., Janzen 1986*a*, 1986*b*, 2000; Janzen and Hallwachs 2016, 2019*a*, 2019*b*).

Results

Partial GDFCF and CBG data analysis for the first year of weekly Malaise trapping at drilling platform (plazoleta) PL12 of the Pailas II ICE geothermal project (November 2013 to November 2014)

The direct technical subtopics of this report, interspersed appropriately rather than strung together in one package, are the following. All require that the individual insects can be known by their DNA barcodes and by their dates of capture by the Malaise traps in space and time, as performed by the CBG. In most cases for each, the Order, Family, BIN, and barcode were machine-obtained from BOLD, but where that failed, morphological identifications were occasionally enlisted for family-level identification. BINs, as defined with DNA barcoding

(Ratnasingham and Hebert 2013), are treated as synonymous with species, though other more detailed studies (D.H.J. and W.H., personal communication; see Janzen et al. 2009; Janzen and Hallwachs 2016) show that about 10% of them contain two or more closely related species in the same genus. The outcome is that, for example, 1000 BINs from a Malaise trap sample is actually represents about 1100 species.

Introduction to data

Context is everything when it comes to measuring environmental variables such as perturbation. The context of the PL12 biomonitoring is a clear example. As described above, this experimental pilot project to biomonitor the PL12 geothermal site with Malaise traps for flying insects was voluntarily initiated on 23 August 2013 by middle management of ACG/GDFCF and ICE in four different yet overlapping contexts:

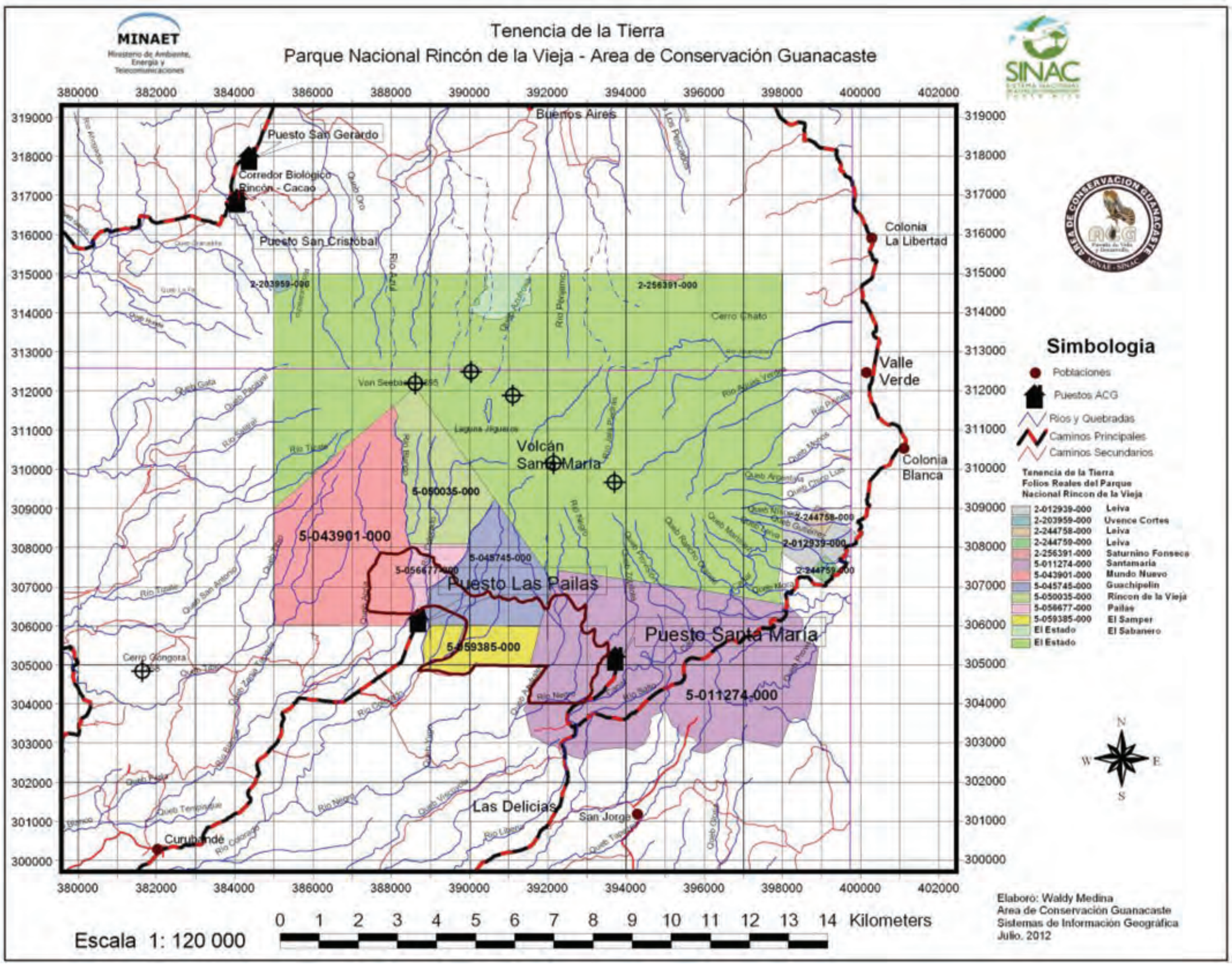
1. Can two different and often competing National Government agencies (SINAC, ICE) and a conservation NGO (GDFCF) collaborate in an actual project to begin to understand, ameliorate, and minimize the environmental damage that unavoidably occurs when portions of an industrial project such as a geothermal development project are inserted into an intact forest of high conservation value, next to the boundary of the sensitive and formally protected Área de Conservación Guanacaste (Fig. 2, a UNESCO Natural World Heritage Site, National Park, and NGO land purchased for conservation) (http://www.acguanacaste.ac.cr)?
2. Can DNA barcoding of tens of thousands of undescribed insects solve a portion of the seemingly insoluble problem of rapid individual and species discriminations (identifications) within massive samples of extraordinarily species-rich tropical insects? If so, this will allow the use of Malaise traps (or any other trapping method) as biomonitors as if they were weather stations monitoring rainfall, with each captured insect being analogous to a drop of rain with its date and temperature. If so, Malaise traps and minimally trained staff can generate a baseline for presence and changes in a species-rich biodiverse community.
3. Is it possible to document a degree of perturbation, and the location of that perturbation, generated by the insertion of a geothermal project into a protected forest? If so, it allows consideration of whether (*i*) such a project is to be allowed by national conservation desires, (*ii*) there is an appropriate form and amount of mitigation, and (*iii*) if the classical engineering structure traditionally used for such a project can be modified to minimize its impact.
4. These considerations allow generic guidelines, with site-specific adjustments, to be developed for the use of Malaise traps (and other trapping devices) as biomonitors for industrial projects that have cause to be sensitive to their impact on their adjacent biodiverse ecosystems, but lack an available community of people professional at biomonitoring. This report is an ad hoc preamble to such guidelines.

The PL12 project has begun to explore all of these topics for the first year of the PL12 biomonitoring project that began in August 2013 (hard data beginning in November 2013), and during the year of SAPI data analysis (March 2016 – March 2017). All four of these topics are, simultaneously, a work in progress and will continue to be for years as more comparable case studies occur in other sites, and as the biomonitoring of PL12 with these Malaise trap sites is continued through at least 2020.

As a key underlying process to all of this report, we distinguish here between the traditional kind of "Assessment"—the "A" in EIA (Environmental Impact Assessment)—and biomonitoring as being conducted for PL12 with Malaise traps. Standard EIAs are usually conducted at approximately one point in time and place, classically near the beginning of the project and to obtain a government permit to proceed. The goal tends to be to detect the presence of threatened species or other charismatic environmental resources, with hopes to avoid or mitigate. The PL12 goal of biomonitoring with insects or other easily monitored biological variables is to notice changes so as to respond to them as the geothermal extraction rolls forward for decades, and to plan other similar integrations of conservation with industry. A camera trap photograph of the jaguar that lives near PL12, or its footprints, during the years to come, tells us only that it is present and nothing of its reactions. A year of Malaise trapping sets a baseline (Fig. 20). The year 2013 begins to document biodiversity changes in response to specific events (onset of drilling, night lights, distance from the site, exhaust fumes, comparison with seasonal changes, consequences of trap placement, site exposure to wind, sun and direct rain, climate change, and restoration). However, such analyses do require entomological, ecological, and taxonomic knowledge following the computerized trapping and barcode analyses, a prime opportunity for collaborations between field biology and industry.

This biomonitoring is tracking ecosystem-level real or suspected perturbations of biodiversity by recording ecosystem or species changes in time and space in relation to the specific perturbation, hopefully throughout the life of the project. It is one of many kinds of biodevelopment towards integration with the societies that own the site. The ACG/GDFCF collaboration with ICE/JICA is based on the concept of at least six total years of biomonitoring and hopefully long after that, both for the benefit of the geothermal project itself (PL12 in Pailas II) and for JICA's goal of "improving geothermal biomonitoring strategies" as a supplementary project goal for other places and other countries. Biomonitoring is therefore viewed here as useful for (*i*) predicting perturbations by as-yet-

Fig. 2. Configuration of the original Parque Nacional Rincon de la Vieja (various colors) within ACG. The yellow property is the first property fund-raised for and purchased (1971) by Sr. Alvaro Ugalde (RIP), the founder/developer of the Costa Rican National Park System. The Pailas II geothermal site occupies a similar-sized area immediately below the yellow polygon, and Pailas I occupies a half-sized polygon to the left (west) of it. The old-growth forest immediately north (upslope) of PL12 (Figs. 1, 5) is the yellow property in ACG. [Image: Created from property titles by Waldy Medina.]



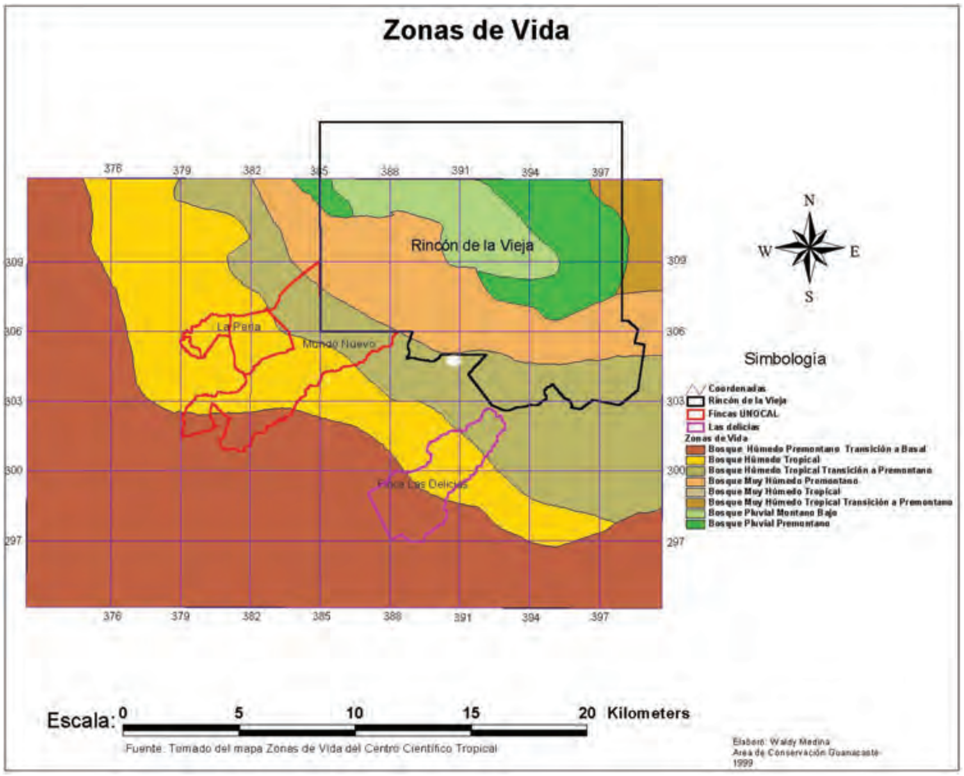
uninitiated other projects, (*ii*) guiding initially unanticipated measures for damage control or mitigation over time, (*iii*) learning about unimagined ecological processes that take years to become evident, and (*iv*) gathering baseline and raw data for quite esoteric basic science such as building DNA barcode libraries for a multitude of other social users including the taxasphere. Equally important as an underlying tenet, biomonitoring may document tracking/invasion/extinction of particular species or interactions, and the use of tracked variables (in this case, the presence of thousands of species of insects) as an indicator of other changes such as global climate change. Over time, the Malaise trapping discussed here will serve these various purposes, though as with any trapping technique, only a subset of the total site biodiversity is obtained with Malaise trapping. How much of the total will only emerge through comparative sampling by other methods from the same forests. Currently, the other methods are light-trapping and litter sampling, both underway since 2013, but with no funding for analysis of the results in the freezer.

Collaborative insertion into the ACG forest and neighbor

This ACG forest as a whole (Sector Pailas and Sector Santa Maria + Pailas II) has long been viewed as having great conservation importance. The portion of forest in ACG's Sector Pailas, immediately north of Pailas II (Fig. 2), was the first piece of private land that Sr. Alvaro Ugalde (RIP), the founder/developer of the Costa Rican National Park System, fund-raised for and purchased in 1971.

In 2011 it was realized that this ecosystem of mid-elevation rain forest is environmentally important for two other reasons, one largely social and the other biological. First, it was named PreCafetal (Supplementary

Fig. 3. The site of PL12 in Pailas II is the white dot in this Holdridge Life Zone map. The black margin is that of Parque Nacional Rincon de la Vieja (Sector Pailas and Sector Santa Maria of ACG, and therefore is the margin of this UNESCO Natural World Heritage Site as well). [Image: Waldy Medina from original coordinates.]



data, File S1[2]) because it is the kind of forest (soil, elevation, climate) that Costa Rica has cleared very thoroughly for 150 years of coffee plantations, a crop indirectly responsible for much of Costa Rica's current environmental awareness. Indeed, this portion of ACG is the only readily accessible large area of this kind of forest ecosystem remaining in Costa Rica, in or outside of a national park. This intermediate-elevation forest is therefore a biological national antique, a reminder of what once was, and what was cleared, in the establishment of the Costa Rican middle class through the developing and developed coffee industry, with all its subsequent consequences. There are still feral coffee trees growing in this forest, escaped from small backyard coffee plantings made by early colonists in Sector Pailas and adjacent Sector Santa Maria (Supplementary data, File S1[2]).

Second, in Costa Rican Zonas de Vida terminology (= Holdridge Life Zones, see Google), the protected Sector Pailas potential geothermal site is Bosque Muy Húmedo Premontano (BHMP) and Pailas Dos itself is Bosque Muy Húmedo Transición a Premontano (BMHTP). The border between these two Life Zones cuts right through the PL12 drilling platform in Fig. 1 (see Fig. 3). Within ACG's Sector Pailas and Sector Santa Maria there are 4525 ha of BMHP and 1654 of BMHTP (Waldy Medina, ACG cartographer, personal communication). However, three years of inventory of the caterpillars, moths, and butterflies by GDFCF/ACG parataxonomists at PL12 indicates that PL12 also lies on the mid-elevation fuzzy boundary between the large ecosystem of Tropical Rain Forest (which once covered lowland Caribbean Costa Rica) and Tropical Dry Forest (which once covered most of the lowland Pacific side of Guanacaste Province).

These blurry intergrades between major Life Zones and ecosystem categories are precisely the kinds of habitats that are heavily deforested, farmed, and ranched elsewhere in Costa Rica and in the tropics in general. They are nearly eliminated and very poorly conserved. They and their biodiversity occupy a severely threatened habitat type, one that is generally ignored when worrying about "saving the rain forest" or "saving the dry forest"; each of these two is its own distinctive ecosystem, and the intergrade between them largely ignored (Janzen 1986*a*, 1986*b*). Ecosystem margins are small, fragile, and easily obliterated, yet occupied by both their own peculiar biodiversity (e.g., Janzen et al. 2017) and by the fluctuating edges of distributions of widespread species. Such places are classical species generators in the evolutionary dynamics of landscapes. This renders their biomonitoring, when perturbed locally by industry or globally by climate change, to be of particular importance.

Unfortunately, and unavoidably, both Pailas II of ICE and Sector Pailas of ACG are perched on top of a very large geothermal energy resource. A geothermal development drills down into this hot water, uses it to run a generator plant, and injects it back into its source. It is a

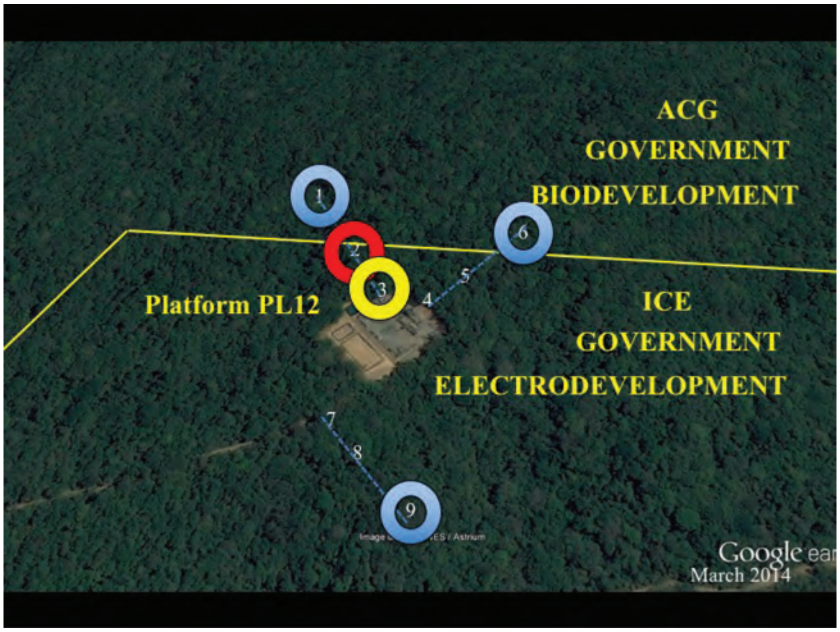
Fig. 4. The ICE/ACG collaboration symbolized by ICE for their presentation in Lima, Peru, for the 2016 MAB programme to "Advance the Mesoamerican Biological Corridor (MBC) in Lima, Peru". It was the last slide in the presentation by Sergio Bermudez of ICE entitled "Practical environmental considerations for geothermal development as a clean energy source adjacent to Área de Conservación Guanacaste (ACG), a Natural World Heritage Site, in northwestern Costa Rica: Joint experiences". We draw attention to the presence of the ACG and ICE logos side-by-side in the upper right corner, arguably the first time ever for such an international expression of collaboration. Simultaneously they represent in-country ABS or Access Benefit Sharing of the Nagoya Protocol of the CBD (https://www.cbd.int/abs/). [Image: Created by ICE artists under guidance by Sergio Bermudez.]

comparatively clean perturbation that can be tightly controlled. The question then becomes can ACG/GDFCF and ICE work together in this project collaboration, instead of having the classical Greenpeace-type confrontation of environmental conservation versus industrial development over a conspicuously valuable natural resource? The goal is, following a general principle of disciplined decentralization by both SINAC and ICE, for ACG and ICE to be allowed to determine among themselves how to get that geothermal resource out from underneath Sector Pailas with minimal ecological perturbation, and appropriate compensation for whatever damage does occur. Biomonitoring of Pailas II is part of the mechanics of the two government agencies, ACG/SINAC and ICE, working together. It also has the potential of limiting the damage to the Pailas II forest to that which is truly necessary for the extraction of the geothermal resource lying beneath it.

Forgetting the various false starts beginning in 1997, the short answer to this key collaboration question, since August 2013, is "yes". A Greenpeace situation has already been firmly avoided. ACG and ICE are working very closely together, and show all indications of continuing to do so. This is actually the first and most important characteristic of successful biomonitoring. It is more important and higher priority than even the technical aspects of Malaise trapping, barcoding, and resulting academic findings. Since August 2013, in great part supported by JICA (Japan International Cooperation Agency) and oversighted by the ERM (Tokyo) consulting firm, GDFCF, ACG, and ICE have collaborated (Fig. 4) in the philosophical and technical biomonitoring of PL12 of Pailas II, and in its associated biopolitics and policy evolution. These are continuing through 2020 and beyond. ICE has now invited ACG/GDFCF to actively participate in the planning of their next geothermal project (Borinquen, well to the west of PL12 but likewise adjacent to ACG) before it has even begun.

The next step in biopolitical demonstration of success is exemplified by the side panel presented by Costa Rica's CONAGEBIO, MINAE, SINAC, and ACG at COP13 of the Convention for Biological Diversity (CBD) on 8 December 2016 in Cancun, Mexico ("Mainstreaming biodiversity in development: A case of geothermal development in Guanacaste, Costa Rica"). This presentation was financed by a collage of different Costa Rican national and international agencies, each thereby supporting their own agendas as well as the collaborative agenda for PL12, and beginning the evolution of guidelines as an export product for other geothermal projects. This is an international example of ABS, with the benefit being biomonitoring know-how and its accompanying biopolitics. This kind of benefit stands out as an example of the kind of know-how benefit that can be shared without creating ownership conflicts among the sharing partners.

Fig. 5. Drilling platform PL12 (~1.4 ha) in March 2014 (after one year of existence). The nearly invisible one-lane entrance road enters the platform from the lower left under the forest canopy. The immediately adjacent old-growth forest appears to be, and largely is, intact. The nine Malaise traps were placed, and have been maintained, as numbered in October–November 2013. The yellow straight line approximates the formal boundary between the UNESCO Natural World Heritage Site Área de Conservación Guanacaste, and Costa Rica's National Electric Company (ICE). [Image: The base image is from Google Earth, March 2014 downloaded by Alex Smith, with indicators added by D.H.J.]



At some time in the close to distant future, the government of Costa Rica may be so eager for clean, reliable, and comparatively cheap geothermal electricity to meet its ever-expanding demand, that the major geothermal resource under this portion of the ACG Natural World Heritage Site will be irresistible to mass development by the government. At that time, there may not be people and groups who would invest the serious time, funds, and biopolitical energy to induce collaboration or chain themselves to trees as can occur with Greenpeace-type confrontations. Should this day come, it is very directly anticipated that the ongoing ACG/ICE Pailas geothermal experience that began in August 2013 will be directly valuable in helping to minimize community-level environmental social perturbation, both technically and biopolitically. In short, the Pailas Dos biomonitoring of PL12 and its access road (see Google image of the site, Fig. 5), is an experiment as if it were happening inside ACG's Sector Pailas on a newly placed drilling platform with its access road.

While it will be well into 2020 before the next big steps in the ACG/ICE collaboration around PL12 will become better defined, it has been recommended by ICE and JICA that the nine traps biomonitoring PL12, and the following five years of samples in the freezer, have their analysis continued, though funds for this have yet to be sourced. Additionally, traps #1, #2, and #3 may remain as the continuation of the beginning of long-term biomonitoring of PL12, now that a baseline has been established. Simultaneously, in the second year ICE expanded the PL12 platform by about 0.5 ha in one direction (north) and added a second 1 ha static processing plant in the other direction (south), both of which desire further biomonitoring. The cost of all biomonitoring of the kind described here will be legitimately included in the normal anticipatory and continuation budgeting for a geothermal project in or adjacent to a conserved wildland, just as are included administration, studies, plans, bridges, roads drilling, generation, and marketing.

Ongoing continued comparisons of the Malaise trap results from PL12 with ongoing ACG internal and national biomonitoring by various methods and BioAlfa national biodiversity inventory (Janzen and Hallwachs 2019*a*) will also occur, irrespective of decisions made about continuation of PL12 biomonitoring. This is because as the years go by, the increasing taxonomic refinement of Malaise trap barcoding and taxonomic results (*i*) continue to increase their value for better comparisons locally, nationally, and internationally, (*ii*) build the regional public barcode library for all users through the internet, and (*iii*) because more comparative DNA barcoding results are constantly being recorded everywhere. All of this contributes to global perception and understanding of biodiversity. In a few years an insect that is currently known only from ACG or PL12 may also become known (by its DNA barcode) from Mexico, Guatemala, or Colombia. The same applies to birds, fungi, and weeds. Every time another site anywhere is biomonitored with Malaise traps or other trapping, the scientific networking of Central (and even South) America is increased, by virtue of the collaborative pooling of this

Fig. 6. Final tally of the first year of DNA barcode success with individualized Sanger sequencing of 144 994 insects that were Malaise trapped weekly in the biomonitoring of PL12 between 21 November 2013 and 14 November 2014 (traps always changed on Thursday). [Image: Screen shot created by multiple coauthors from CBG.]

One year PL12. 13 Nov 2013 – 21 Nov 2014
6 months for laboratory analysis

	Specimens	Trap	Specimens	Barcodes	Records with BINs	BINs	BINs new to BOLD	BINs in just one trap	Cases of contaminants
PL12-1	9829	PL12-1	9829	9172	8898	1836	225	319	108
PL12-2	8488	PL12-2	8488	7724	7535	1556	145	181	42
PL12-3	80124	PL12-3	80124	74520	72394	8650	3286	5220	900
PL12-4	7868	PL12-4	7868	7251	7112	1856	253	382	78
PL12-6	13145	PL12-6	13145	12314	12044	2186	282	384	106
PL12-7	9825	PL12-7	9825	9291	9110	1698	181	267	113
PL12-9	15715	PL12-9	15715	14556	14091	2523	466	647	234
TOTAL	144994	TOTAL	144994	134828	131184	11385	6244	7400	1581

144,994 insects **11,385 BINs** **8,650 in one trap** **91% identified $3.14/insect**

taxonomic information (DNA barcodes with their associated collateral taxonomic names) for all users in all social sectors via the BOLD integrative engine on the internet in the Centre for Biodiversity Genomics at the University of Guelph at http://www.boldsystems.org; http://ibol.org. See below for an example with *Simulium* blackflies ("bocones") that feed on humans (Fig. 8).

DNA barcoding solves identification problems

Malaise traps (Fig. 9) were invented by taxonomists to collect insects for taxonomy or calculating trappable species-richness of a site, rather than for biomonitoring (e.g., Darling and Packer 1988; Flowers and Hanson 2003; Gaston et al. 1996; Brown 2005; Sjoberg and Teal 2014). They are one among many kinds of insect traps used by taxonomists to collect, but have generally been excluded from tropical biomonitoring because of the daunting task of even beginning to morphologically sort the massive number of trapped specimens to species or higher taxa that they collect. Equally daunting, it is obvious that they catch only a large but unknown subset of the insect species in a particular site, as is the case with any trapping system. Additionally, it is generally not the case that the taxonomy-focused collector is stationed in one tropical place for multiple years and interested in faithfully sampling week after week. Finally, taxonomists are usually interested in sorting through Malaise trap samples to find the particular (generally new) species of interest largely to them in their focal taxa, and are usually not interested in the total set of thousands of other species that are caught.

DNA barcoding each individual insect in an entire Malaise trap sample of many thousands of insects by Sanger sequencing or other sequencing methods (e.g., Srivathsan et al. 2019) is, however, a technically superb way of jumping over these barriers. It converts a Malaise trap into an actual biomonitoring device, just as a weather station is a rain-monitoring device. The cost per insect for all of the processing is currently still high. It averaged about US$2.89 per insect (average of US$60 000 per trap for seven traps) for the mass Sanger sequencing and analysis performed in 2016 by the CBG, for the 144 994 insects from the first year of seven Malaise traps from PL12 (Fig. 6). However, this price is substantially cheaper than the US$10–20 per insect cost when the ACG biodiversity inventory began barcoding in 2004. The price today for processing plus sequencing is US$1 per Malaise-trapped insect (P.D.N. Hebert, personal communication; CBG contracts for US$2 million with ACG/GDFCF and the Walder Foundation https://news.uoguelph.ca/2020/01/u-of-gs-centre-for-biodiversity-genomics-awarded-4-million-to-catalogue-life-in-costa-rica/). The biomonitoring samples in the CBG receive a variety of discounts for bulk processing efficiency and because they are simultaneously a contribution to the global insect barcode public service library accumulating in BOLD (http://biodiversitygenomics.net, http://www.boldsystems.org) as part of global BioScan (www.ibol.org), which services Costa Rica and Central America as well. To date,

Fig. 7. Of these 11 385 BINs, 99.93% were successfully assigned to Family by their barcodes without further effort. As the years and decades pass, the numbers assigned to taxonomically recognized groups at the species level will approach 100% as (*i*) the number of barcodes from other places in BOLD increases, (*ii*) taxonomists describe and revise their respective taxa of interest and these names are added to BOLD, and (*iii*) more rapid protocols for scientific descriptions evolve (as they currently are, e.g., Meierotto et al. 2019). A greater level of taxonomic completion is not required for the first set of conclusions reached here and now, though further taxonomic refinement will permit much more use of the data for both pragmatic geothermal biomonitoring, climate change monitoring, and barcoded biodiversity research. [Image: Screen shot created by multiple coauthors from CBG.]

Order	BINs	Identified to Family	Identified to Subfamily	Identified to Genus	Identified to Species
Diptera	4601	4601	362	330	73
Hymenoptera	2443	2439	1013	544	134
Coleoptera	1936	1936	622	142	57
Lepidoptera	1290	1290	596	498	137
Hemiptera	656	654	66	64	32
Psocodea	128	128	10	15	5
Orthoptera	80	79	6	6	2
Thysanoptera	72	71	11	10	3
Blattodea	48	48	3	2	2
Trichoptera	48	48	17	17	11
Neuroptera	36	36			
Isoptera	13	13	4	8	2
Dermaptera	10	10			
Mantodea	7	7	5	3	
Phasmatodea	7	7	3	3	1
Archaeognatha	5	5			
Embioptera	2	2			
Plecoptera	2	2	1	1	
Odonata	1	1	1	1	
Grand Total	11385	11377	2720	1644	459
		99.93%	23.89%	14.44%	4.03%

Costa Rica has contributed at least 45 000 species of insect barcodes to BOLD, out of the perhaps 650 000 species of insects estimated by the BioAlfa project to live in Costa Rica (Janzen and Hallwachs 2019*a*).

The success per insect DNA barcoded and assignment to a BIN code in the 2013–2014 PL12 year, averaged 90.5% across the 144 994 specimens in the first synchronized year of 21 November 2013 to 14 November 2014 (Fig. 6). Of these, there are 11 385 BINs. A BIN approximates one species, with a BIN containing the different individuals with the same barcode (Ratnasingham and Hebert 2013). It is equivalent to a morphologically assigned grouping that assigns individuals to the same species based on their morphological similarity. As with a morphology-based species name, a BIN may contain more than one similar (so-called cryptic) species, each separated by more than simply a ~2% or less barcode difference (e.g., Janzen et al. 2017; Hebert et al. 2004).

About 99% of the insects from PL12 were successfully machine-assigned to insect Order and Family by their barcodes (Fig. 7). With 15 years of experience working with DNA barcodes of 500 000+ ACG insects identified through their nearest neighbor joining phenograms (NJ trees) that are correlated with natural history traits (food plant, host parasitoid, microgeographic location, season, and elevation) (e.g., Janzen and Hallwachs 2016), leads us to estimate that the eventual number of species that will be found to be in this one-year large sample from PL12 will be about 10% more than the number of BINs, or about 12 000 species. This is because experience with ACG samples have shown that about 10% of BINs contain two or even three cryptic similar species that can only be distinguished by very careful morphological examination, correlation with ecological traits, or a deeper genetic analysis (e.g., Burns et al. 2007, 2008; Smith et al. 2006, 2007; Janzen et al. 2017). Of the 11 species of ACG *Astraptes* butterflies found to be hiding inside one scientific name (Hebert et al. 2004), six have now been found to reside in the same BIN.

Most of the unsuccessfully barcoded specimens could likely be DNA barcoded with a second try. However, this would require a more detailed laboratory treatment at a consequently doubled or greater expense. Contaminated specimens could equally be re-barcoded. All specimens have been retained as DNA barcode vouchers at the CBG and a photograph logged for at least one intact specimen from each BIN. These photographs are also available in BOLD by searching for individual voucher codes or taxa names, or BIN codes.

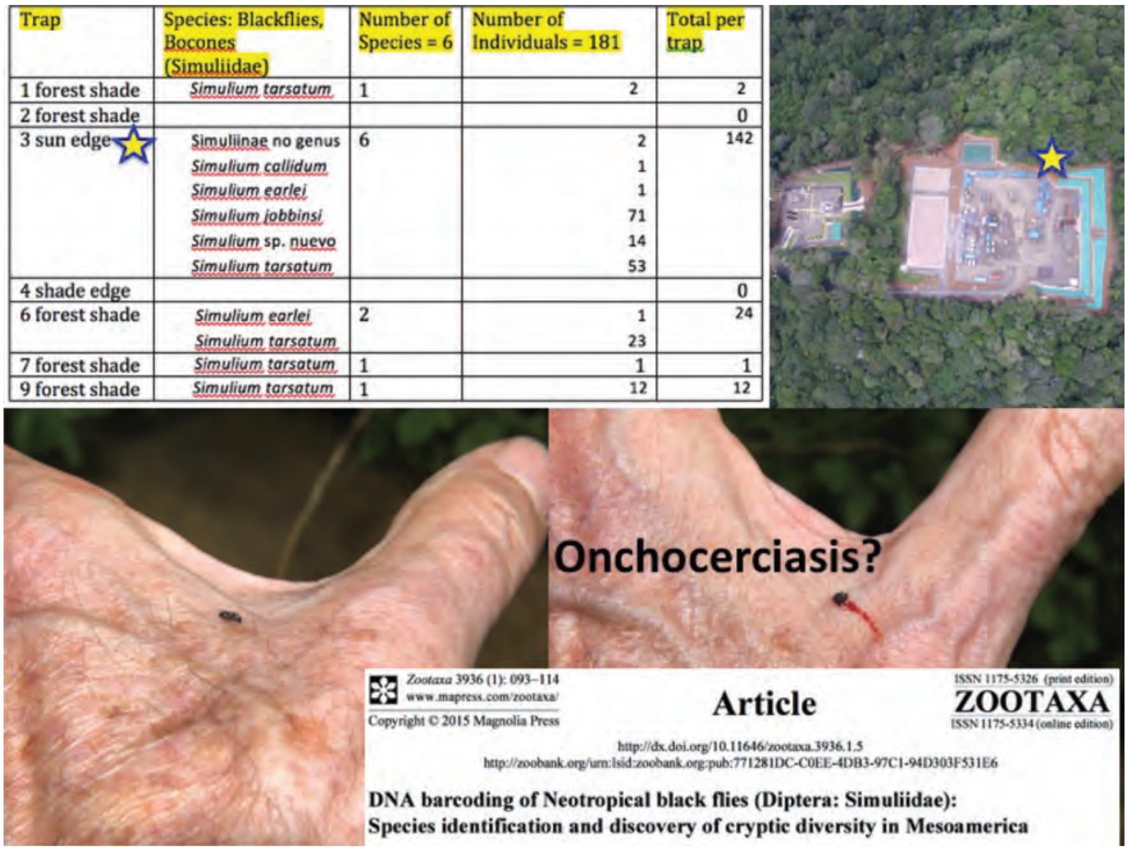
All of this barcoding of mass samples, and therefore national biodiversity inventory (Janzen and Hallwachs 2019*a*), increases the demand and opportunity for taxonomic efforts that are coordinated with the current new possibilities for collecting, curating, and correlating the data offered by DNA barcoding, by BOLD, and by mass collecting techniques. It should be emphasized that the 4.03% figure to the right in Fig. 7 is the percent identified with a real scientific name that is already in BOLD from other sampling and taxonomizing in ACG. Such an identification means that the PL12 insect barcode matches one of an identified insect in BOLD, many of which are ACG specimens laboriously described and therefore scientifically identified during the past 35 years. The percentage in this column is low in large part because the great majority of the captured insects belong to new, undescribed species, and do not yet have scientific names. But ALL of the 11 385 BINs have effectively been identified with a unique BIN code. This almost-taxonomically legal interim name is directly analogous to the human-friendly sorting of pinned museum specimens into species hypotheses by what they look like and giving them an interim nickname. In this context, it is not surprising to find that a BIN can be found to contain multiple species when its barcoded members are matched against other morphological and ecological characteristics (e.g., Janzen et al. 2017).

This allows for national and international comparisons, once its conspecifics from other places are also registered in BOLD. Equally, this species-level (or sub-genus level) taxonomic tag allows each species presence or absence to be recorded per trap and per date (even though it currently has no scientific name), now and over future years.

There is a specific example of these inter-country comparisons. Anyone working in PL12 in the daytime, from visitors to engineering crews, notices immediately that there are often large numbers of small human-biting blackflies or “bocones” (*Simulium* spp., Simuliidae) on the grounds of the drilling platform in the rainy season. These small flies (Fig. 8) are major blood-sucking pests for mammals throughout ecosystems rich in freshwater streams, where their larvae filter out plankton and debris as the water runs past them. The Malaise traps were expected to capture Simuliidae, and indeed, six species were captured at PL12 (including two undescribed species) and identified by their barcodes through BOLD. Because these flies are also carriers of human disease (Onchocerciasis or “river blindness”) their taxonomy has recently been revised for Central America (Hernandez-Triana et al. 2015) and in BOLD.

This identification in turn allowed the beginning scrutiny, through Google, of each of the described species, with a relevant research result from the biomonitoring. On the vegetation-free platform, it is notable that by moving just a few meters from the edge into the shade of the forest, there are no more human-biting blackflies. However, people standing just a few meters away in the open are frequently swatting them as they bite (Fig. 8). One species identified by BOLD by barcoding, *Simulium tarsatum*, was found very rarely in four out of six deep forest traps, but very commonly in the trap on the platform edge (#3). However, the literature reports that *S. tarsatum* feeds on birds and other small animals, and not on humans; the eDNA analysis of their gut contents showed that they feed on humans as well (E. Zakharov, personal communication, Fig. 8). On the other hand, the most abundant of all, *Simulium jobbinsi*, was caught only in the platform edge trap (#3); *S. jobbinsi* is well known to be a species of blackfly that feeds largely on humans. However, their eDNA gut analysis showed that they also feed on many other species of wild vertebrates (birds,

Fig. 8. The presence of species of Simuliidae (blackflies or "bocones") in the seven biomonitoring Malaise traps at PL12 (aerial view on the right). The species figured here, *Simulium jobbinsi*, is erroneously believed to feed largely on humans, and rapidly draws blood, as shown a few seconds later on the right when crushed. However, *S. jobbinsi* gut eDNA showed that it feeds on birds, bats, rodents, and peccaries as well as humans. Despite howler, spider, and capuchin monkeys being normal density in this forest, none tested positive for their blood. Note the capture numbers in trap #3 versus trap #4. [Image: Multiple composite by D.H.J.]



Trap	Species: Blackflies, Bocones (Simuliidae)	Number of Species = 6	Number of Individuals = 181	Total per trap
1 forest shade	Simulium tarsatum	1	2	2
2 forest shade				0
3 sun edge	Simuliinae no genus	6	2	142
	Simulium callidum		1	
	Simulium earlei		1	
	Simulium jobbinsi		71	
	Simulium sp. nuevo		14	
	Simulium tarsatum		53	
4 shade edge				0
6 forest shade	Simulium earlei	2	1	24
	Simulium tarsatum		23	
7 forest shade	Simulium tarsatum	1	1	1
9 forest shade	Simulium tarsatum	1	12	12

bats, and small mammals). It is also a transmitter of Africa-origin Onchocerciasis by the nematode causing river blindness. In other words, the rapid barcoding of the biomonitoring samples allowed understanding of the local distribution of closely related species of flies that are potential disease transmitters, and to highlight that they are therefore a potential health problem for the ICE staff working at the drilling platform, but much less of a threat in the adjacent deeply shady forest understory. It is conceivable that this kind of detailed information will gradually and eventually become available for most, if not all, of the species whose presence has now been documented for the PL12 forest and its drilling platform.

Easily 90% of the species captured by the PL12 biomonitoring Malaise traps are undescribed (= "new") species. As such, they are not accompanied by the species-level taxonomic literature that is characteristic of extratropical species. As a single example of this shortage of collateral information and classical scientific names, to date ACG has collected, by rearing and Malaise traps, about 1100 species of Microgastrinae wasps (very small Braconidae that are parasitoids of caterpillars); prior to their taxonomizing by the ACG/GDFCF inventory of ACG, only about 3% were described species (e.g., Fernandez-Triana et al. 2014, 2015; Arias-Penna et al. 2019).

There is no question as to whether DNA barcoding works for the insects captured in the Malaise traps. It has been demonstrated in ACG since 2003 that it works to identify species and discover new ones (Hebert et al. 2004; Janzen et al. 2009; Janzen and Hallwachs 2016), albeit requiring understanding of its quirks and cautions as is the case with any science or engineering protocol. The PL12 specimens to date have been barcoded by Sanger sequencing. This PCR-based method normally generates barcodes of 500–658 base pair lengths. These lengths have proven to be of very high quality for identification and species discovery for the more than 48 000 species and 500 000 specimens of ACG insects barcoded so far (e.g., Burns et al. 2008; Janzen and Hallwachs 2016; Miller et al. 2016; Smith et al. 2006, 2007; Fernandez-Triana et al. 2014, 2015).

Specific technical comparisons

Compare traps #1, #6, and #9 as representative of deep undisturbed forest understory

Traps #1, #6, and #9 were in the shady old-growth forest understory ~150 m distant from the edge of the

Fig. 9. Malaise trap #9 deep in the forest understory, 150 m from the edge of the access road for PL12, a site ecologically equivalent to that of traps #1 and #6 and 1–2 km distant from them (photo source, GDFCF). The mix of dappled light sunlight and deep shade (moving across the forest floor throughout the day) is the normal lighting of a Malaise trap installed in shady old-growth forest understory, with the insects going into the collection bottle by following the slanted roof upward; the collection bottle is in G. Pereira's hand next to his head in the sun, but normally attached to the trap. [Image: Parataxonomist Program, GDFCF, ACG, and coauthors.]

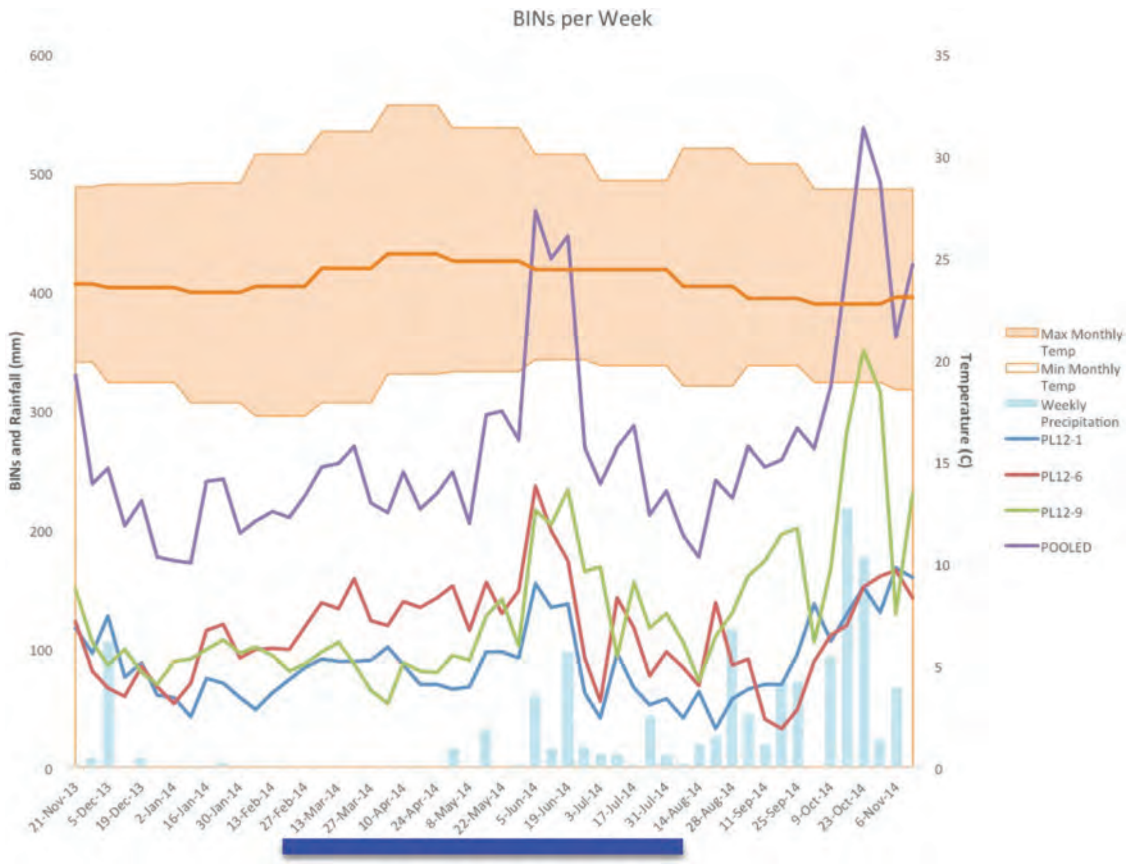
drilling platform PL12 and margin of its access road (Fig. 5). Their positions and distance from the platform were selected, and traps installed, in October 2013, immediately following opening and clearing of the one-lane access road and ~1.5 ha drilling platform. Changes in subsequent years for PL12 will be monitored from this same biodiversity baseline. The traps have been run (and replaced) through 2020, but further samples remained frozen at –20 °C, awaiting funding for processing (which is why this report touches only on the first year of biomonitoring).

The distance of 150 m from the perturbation was chosen because experiences as a tropical field entomologist intimately familiar with these ACG forests and their insects (e.g., Janzen 1973*a*, 1973*b* and subsequent decades) suggest that there will be no immediate impact of the drilling platform and road at this distance from them. Indeed, none was indicated by any of the trapping results (Figs. 10, 11). The perturbation generated no change in numbers of insect species numbers throughout the year; they did change as expected durinng the normal dry season – rainy season cycle: low density in the January–May long dry season, a late May – early June mild peak with the first rains, and a gradual increase during the remainder of the year as adults emerge from the eggs and larvae started at the beginning of the rains and their cooler temperatures. Simultaneously, the additional perturbation of drilling (late February–August) is not evident in the results (Figs. 10, 11). See Supplementary data File S3[2] for a yet more complex but incomplete statistical analysis of this first year.

If the understory forest Malaise trapping were to have been done for only a few months, the striking seasonal changes in the site could easily have been confused with changes caused by the platform perturbation and drilling. Second, only continued Malaise trapping can determine if the platform margin itself will become a source of species and biomass contamination of the adjacent forest interior, though it did not in the first year. Whether it will, is dependent on many other variables.

As expected, the graph of species richness of captures during the long dry season (January to May) was relatively flat and stable, followed by the expected increase of captures with the beginning of the rainy season in May (Figs. 10–11, 20–21). This was followed by the low rainfall (and sunny days) of the August short dry season (and the

Fig. 10. Weekly catches of species by traps #1, #6, and #9, and the three of them pooled (purple line), during the year of 12 November 2013 to 21 November 2014. [Image: CBG coauthors, raw data from study.]



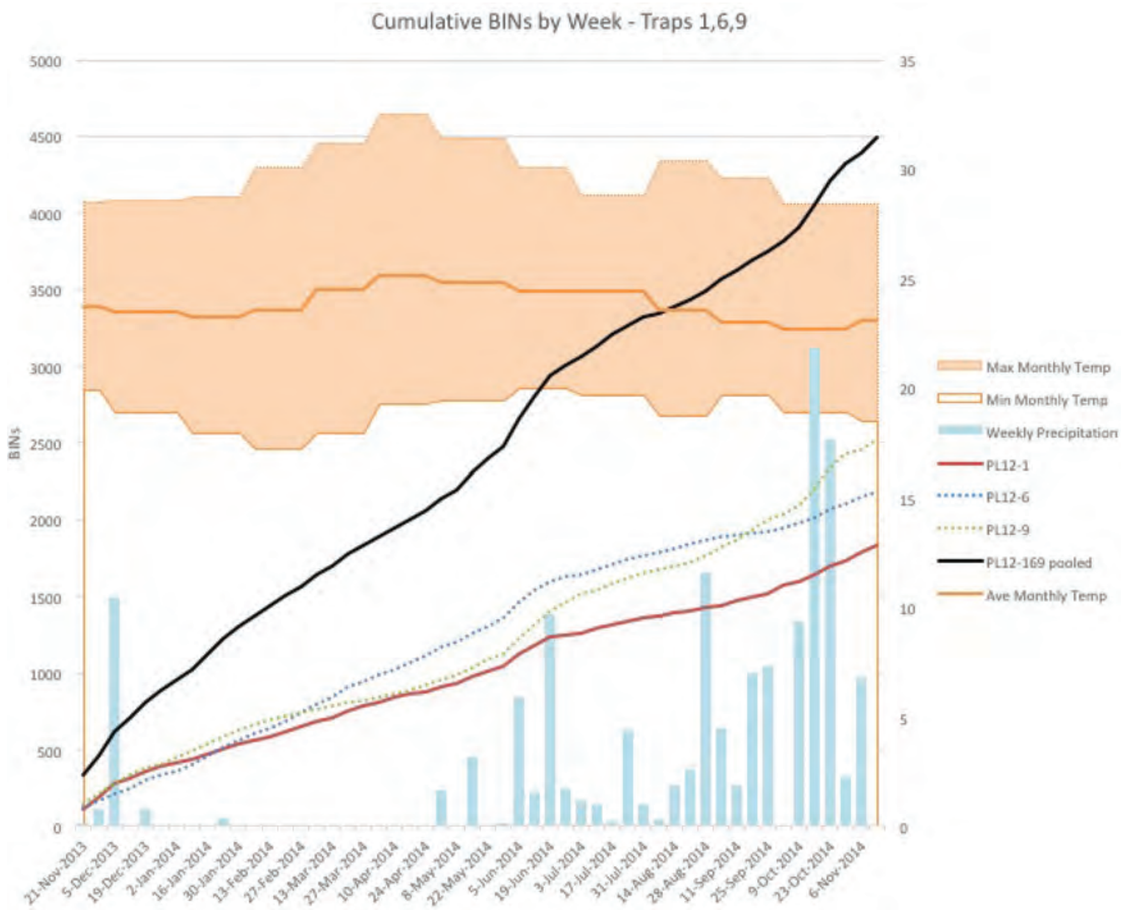
time when many species are in a non-flying egg or larval stage), and then a steady increase of adults through the remainder of the rainy season (Figs. 10–11, 20–21). Trap #9 showed the greatest capture of species after the heavy rains. From late February 2014 to August 2014, the platform drilling (lights, noise, people, vehicles) were present (as indicated by the blue bar in Figs. 10–11, 20–21). There was no visible effect of the site preparation and drilling activity on the usual reaction by the insect community to the first rains of the year. However, a comparison of the two subsequent years of trapping from these three deep forest traps is required to confirm this observation's persistence (to be published when funding is available).

Were these three traps redundant on each other, and therefore just one of them would have conveyed the same message? This question assumes, however, the risk that such a single sample trap would not be destroyed by a tree fall, landslide, wild animal, or vandalism. The patterns of species captured by each individual trap and their sum (Fig. 6), convey the same general message and constitute three deep forest understory baselines for subsequent years (and see Supplementary data File S3[2] for a complex statistical analysis that determines that they are not significantly different from each other). However, without their essentially identical results for this first year, there would be no way to begin to know what is a normal result for this deep forest understory insect community, for comparison with results closer to the platform edges. The other option would be a year of Malaise trapping prior to the beginning of this project, but engineering socio-administration did not allow that. Furthermore, the annual climate in that pre-project year could easily be different from the installation year.

The number of BINs in common among the three traps tells a very different story — only 13% of the BINs captured were caught by all three traps (Fig. 12). If only one trap had been used, the number of BINs would have been 41%–56% of these trappable BINs (by all three traps pooled). If two traps had been used, then about 70%–84% of the trappable BINs would have been captured. It is not possible to estimate the total trappable fauna from these data, since the BIN/trap curve (Fig. 11) is still rising sharply after progressively pooling all three deep forest traps, no matter in which order the traps are pooled.

The trappable insect biota at PL12 is sourced from three quite different microhabitats. First, there are the resident species in the shady understory, many of which have an ecology that is the opposite of heliophiles. Very many of the family most commonly captured, Cecidomyiidae, are in this category, and strikingly did not vary in proportion of species among all seven traps. Sec-

Fig. 11. BIN accumulation per week for traps #1, #6, and #9 for one synchronized year, and the pooling of all three (uppermost curve), showing clearly that there is no approach to an asymptote, and all three traps are accumulating BINS at about the same rate across the year. The BIN accumulation curves show no effect of the platform preparation (all year) or the drilling period (blue bar in Fig. 10). However, there is, as expected, a slight bump upwards in the BIN accumulation curve, with the beginning of the rainy season (blue columns) and its end. [Image: CBG coauthors, raw data from study.]



ond there are those that live in the (insolated, windy, dry) canopy above and are caught at ground level as short-term visitors (e.g., to oviposit where their larvae live in the litter or on low plants) or as waifs. Third, there is the light rain of species represented by single specimens (transients, waifs, migrants, lost, wide-rangers) that arrive on the canopy of the forest, but are derived from the ocean of insect populations and communities in the agroscape that abuts the ragged edge of the Pailas II forest, only 4–8 km distant. These species would normally be carrion or prey for the resident forest inhabitants. That they are captured by this study is the accident of them flying into the Malaise trap in their passage from arrival to being consumed. The *Diabrotica* beetles in Fig. 16 are classic examples of such agroscape species. This category of species can only be recognized through knowing them and their natural history in their home agroscape. Their numbers and seasonality within the insect rain will change in response to microclimates and perturbations in the agroscape, and the distance of that agroscape from the forest being inventoried. A currently unknowable but significant fraction of the species and numbers of the insects in traps #1, #6, and #9 (and #2 and #7), are probably such waifs (which of course may colonize the platform edge). With subsequent years of study of the insect fauna of the PL12 area (as well as of ACG as a whole), many of these will become known for their ecology.

It is notable that 65% of the 11 385 BINs in all seven Malaise traps have been caught by only one trap in this first year (see Fig. 6). There are at least two different (but simultaneously possible) ecological sources for such singleton records.

First, the BIN that is unique to a trap may be represented by only a single specimen captured during the year among all the traps, and therefore can occur in only one trap. Detailed studies of the ACG insect community for the past 35 years (Janzen and Hallwachs 2016) have indicated that in any given year, and often for consecutive decades, a very high proportion (perhaps as many as 75%) of the species of insects indigenous to a site occur naturally at very low density per species, and at even lower densities of trappable specimens. They are simply rare by extra-tropical trapping standards and expecta-

Fig. 12. The degree of overlap between the three PL12 deep forest traps (#1, #6, #9), as indicated by overlap for 4499 BINs. Only 593 (13%) of the BINS were captured by all three traps. If we had used only one trap, the overlap results would have been as follows: trap #1, 41%; trap #6, 49%; trap #9, 56%, of the trappable total this first year. If we had combined two traps, the results would have been as follows: trap #1 + #6, 70%; trap #6 + #9, 84%; trap #1 + #9, 79%. [Image: CBG coauthors, raw data from study.]

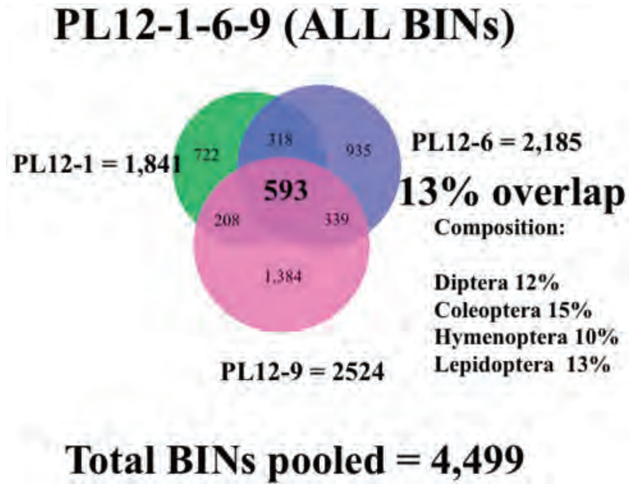
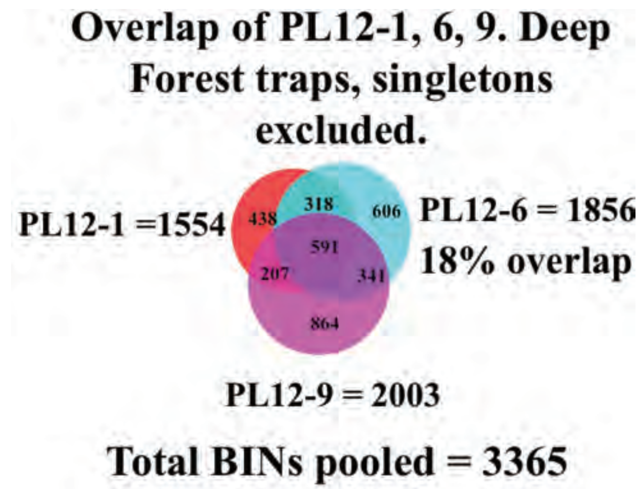


Fig. 13. The degree of overlap (18%) of the three deep forest traps (#1, #6, #9) if singletons (uniques) are excluded. By comparing Figs. 12 and 13 it is obvious that the overlap among the three traps is similarly low, with or without the uniques excluded. Equally, comparing Figs. 12 and 13 shows that uniques make up a large portion of the species captured by a given trap, while the overlaps among the traps with the total are almost identical when uniques are excluded (Fig. 12). [Image: CBG coauthors, raw data from study.]



tions, but at normal abundance by tropical standards and expectations. It is no surprise to capture only one specimen of one of them in a trap during a one-year cycle. When there are a very large number of such species, sampling such a community structure by any method yields a very large number of singletons for a species.

Second, as mentioned above, there are two sources of very low density trappable specimens that come from the outside. The first is that visitors or waifs from the canopy community, even for species that are quite abundant in the canopy, may well contribute only one or very few individuals of canopy-resident species to a trap in a given year. The second is that the above-mentioned rain of insects blown or flying from the agroscape will contain many species at very low density in the specimen rain even if they are common in the agroscape.

On the other hand, whenever considering overlap among or between traps, if the singletons are ignored (since by definition a singleton cannot occur in two or more traps), the degree of overlap among the three deep forest traps becomes slightly more intense, moving from 13% to 18% (Figs. 12–13).

In summary, each of the three deep forest traps tells the same seasonality story and appears to be insensitive to the presence of the drilling platform and road, during the first year of the project. However, if the biomonitoring is directed at any particular species and whether it is influenced by the perturbations, a single trap would at best be capturing (monitoring) about half of the BINs in the pool of 4499 trappable BINs, and two traps pooled would capture about 75% of the BINs in the trappable pool. This speaks poorly to the concept of indicator species but is relevant to indicator communities.

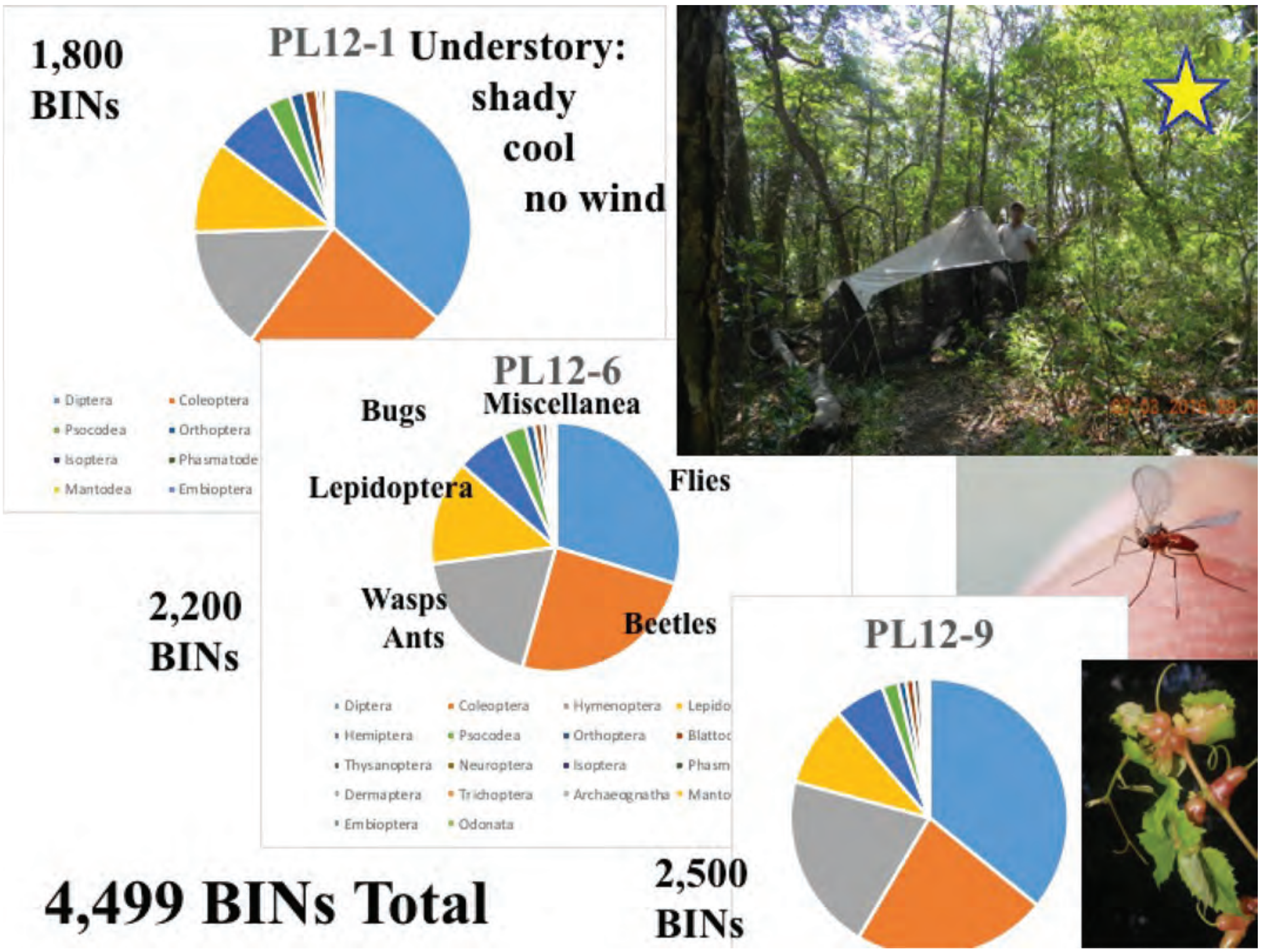
At the Ordinal level, all three deep forest traps tell approximately the same story about the insects captured. As is the case with all ACG and PL12 Malaise trapping, by far the most abundant BINs are flies (Diptera or moscas) (Fig. 14), followed by beetles (Coleoptera), wasps and ants (Hymenoptera or avispas), small moths (Lepidoptera or mariposas), and then lesser numbers of bugs (Hemiptera or chinches).

By comparing Figs. 12 and 13 it is obvious that the overlap among the three traps is similarly low with or without the uniques excluded. Equally, comparing Figs. 12 and 13 shows that uniques make up a large portion of the species captured by a given trap, while the overlaps among the traps with the total are almost identical when uniques are excluded (Fig. 12).

Turning to family-level taxonomic content of the individual three traps, or to all three of them pooled, in all cases they are extremely species-rich (Fig. 15). Cecidomyiidae flies are by far the most abundant BINs.

It is tempting to wonder if biomonitoring just flies would effectively mirror the changes in the entire insect community. This question is provoked by the pragmatic desire by non-entomological users to wish for indicator species. At present, not nearly enough is known about the natural history of these animals to even think about finding indicator species (and, indicators of what, exactly?), or even wonder how representative (and, of what, exactly?) such species might be. It can, however, be concluded that the margin (e.g., trap #3) is rich in species

Fig. 14. Ordinal-level species composition of the trapped content of the three deep forest traps (#1, #6, #9). The shaded trap in the image is #9. The fly is a representative Cecidomyiidae (gall fly), the members of which make up half or more of the species of Diptera captured in PL12, and are numerically dominant in all seven traps. The galls on a grapevine are typical extratropical cecidomyiid galls that have a fly larva inside of each. However, foliage, flower, and fruit galls (e.g., Gagne 1978, Woodley and Janzen 1995) are very rare in this forest; the enormous number of species of Cecidomyiidae must have larvae with a different ecology, such as fungus-feeding and predation. [Image: CBG coauthors, raw data from study.]

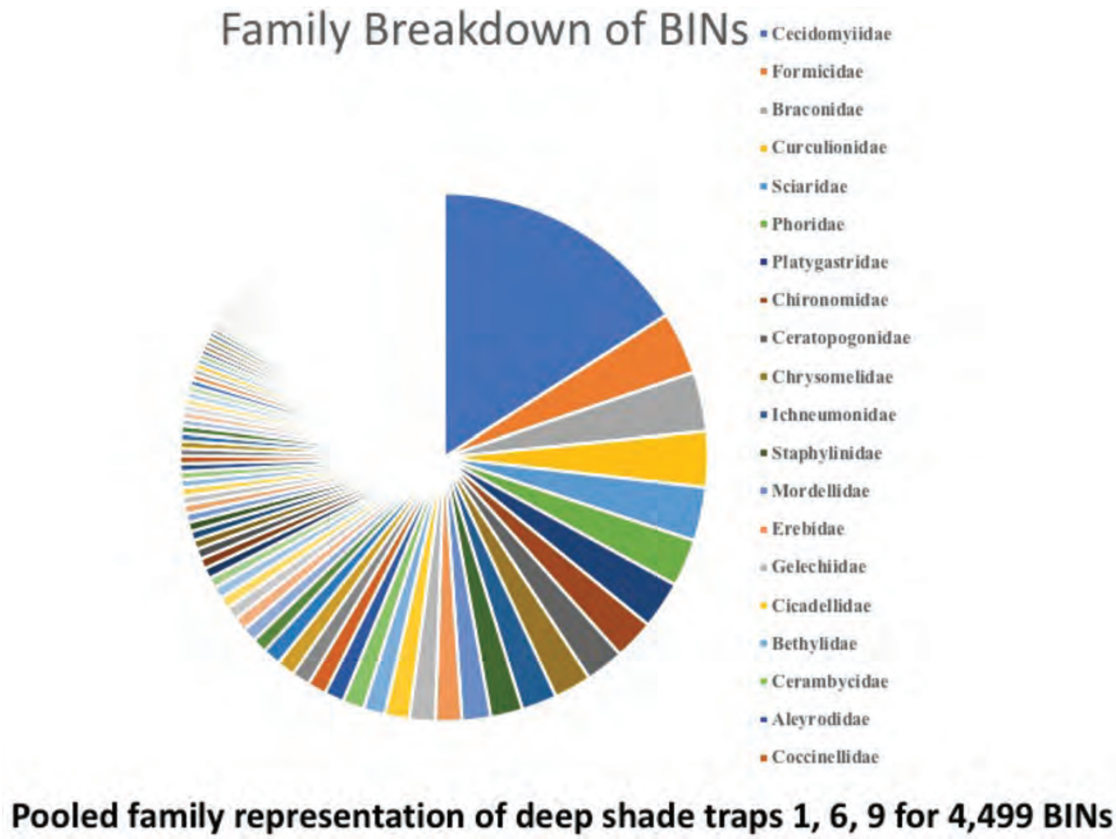


that are not normal or usual inhabitants of the forest understory. The margin is, in its biotic content and physical traits, analogous to a narrow edge of a pasture, roadside, old field, landslide, or insolated riverbank (Fig. 16).

Eventually, looking backwards and laterally through many years of Malaise trap samples from PL12, there will emerge some correlations between perturbations, seasons, and climate change. A few specific taxonomic groups or patterns will emerge as specific indicators of environmental point source and (or) widespread change in Pailas II forests. As for traps #1, #6, and #9, on the level of insect Order, the traps generated essentially identical results with respect to proportions of total numbers of species, and numbers of species of major taxa per week and overall. They are, therefore, a tri-replicate of what is normal for the forest understory at 150 m from the platform perturbation, both at the end of a year and during the year.

It is likely that as many as 10 more understory traps would have delivered the same overall results in this same year. Equally, for traps #1, #6, and #9, at the Family level, the result is about the same as that for the Order level. Each of the three deep forest traps is representative of the trappable insect biodiversity of the deep forest understory (along with its insect rain of currently unknowable sources from the canopy far above and the adjacent agroscape), in terms of numbers of BINs per week and how that parameter behaved for the first year. Since each of the three are the same as each other, and the same as trap #2 (Fig. 5), we conclude that the platform and its drilling have had no effect at all on the biodiversity of the forest at a distance of 50–150 m (and

Fig. 15. Family-level taxonomic representation among the three deep forest traps (#1, #6, #9) pooled for one year. As usual with all ACG and PL12 Malaise traps (except #3), Cecidomyiidae are proportionally the most species-rich family, followed by ants (Formicidae), parasitic wasps (Braconidae), and weevils (Curculionidae). Four of the five next most common families are also small flies (Sciaridae, Phoridae, Chironomidae, Ceratopogonidae). [Image: CBG coauthors, raw data from study.]



further) during this first year. Current ongoing analyses of the first and second years of traps #5 and #8 will reject or reinforce this conclusion.

Whether this is also the case at the level of species behavior remains to be determined. It cannot be determined until when, years from now, the biology or the classical taxonomy of these species is sufficiently well known to be able to attribute natural history traits to most of the species captured (parasite, herbivore, mutualist, migrant, hyperparasite, mimic, and predator). However, that scientific information base is not required to initially address the question of whether the drilling platform or its drilling activity have impacted the adjacent forest understory at 50–150 m or more from the platform and road.

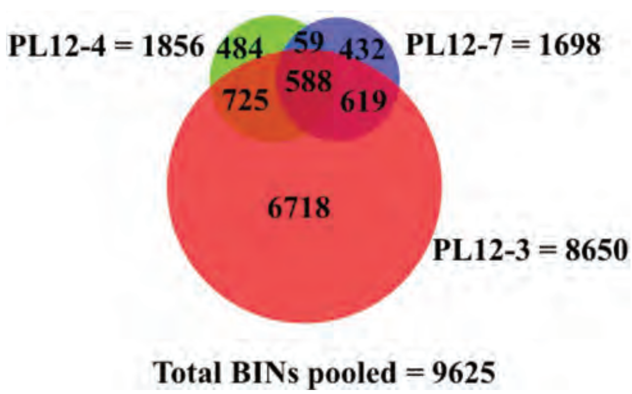
Compare traps #3 and #4 as margins of the drilling platform, and #7 as road edge

As has been noted previously, traps #3 and #4 recorded enormously different numbers of species of insects throughout the year, though the seasonal pattern of trapping is the same for both (Figs. 10–11, 17–21). The cause is that trap #3 serendipitously had its collecting bottle at the trap end pointed towards the sun of the open platform and trap #4 had its collecting bottle pointed towards the deep forest interior shade (and just marginally within that shade immediately adjacent). This happened because taxonomically inclined entomologists, such as ourselves, who normally sample with Malaise traps for taxonomy, usually put the traps in the full open or the full shade. No thought was given to the peculiarities of trapping exactly on an edge. This variable was not considered in the initial positioning of traps #3 and #4, and it has not been considered in any of the published papers describing Malaise trapping (e.g., Darling and Packer 1988). Most trapped light-loving insects escape upwards and are attracted toward light; shade-loving insects escape upwards and toward shade. We suspect that nocturnal fliers will be the same, but they use air temperature and moisture content to orient instead of sunlight. The upward direction of attempted escape is anticipated by the design of the roof of the trap, which slopes upwards towards the entrance to the collecting bottle (see Fig. 9).

Trap #7 (and #4) gave the same basic results as the other three traps that are far from the perturbation in the deep shade, as well as trap #2 which was only 50 m from the edge of the platform (and within sight of it through the forest understory). While the vicinity of trap #7 received a very heavy dose of dry season insecticidal road dust on its surrounding foliage, the trap itself was

Fig. 16. Left: PL12 Platform margin (2016), emphasizing the secondary succession biomass occupying what is in effect, the edge of a pasture, roadside, old field, landslide, or insolated watercourse bank. Right: Two *Diabrotica* (Chrysomelidae) beetles, seeking food on a wind-dispersed *Asclepias currasavica* (Asclepiadaceae) herb in the same margin of PL12; the larvae of this beetle are well-known agricultural pests that feed on the roots of corn plants, and *A. currasavica* is a prominent toxic weed in pastures adjacent to this forest; neither species has a breeding population in old-growth forest understory and both have their biological evolutionary origins in naturally disturbed sites such as landslides and river margins. [Images: D.H.J. and W.H.]

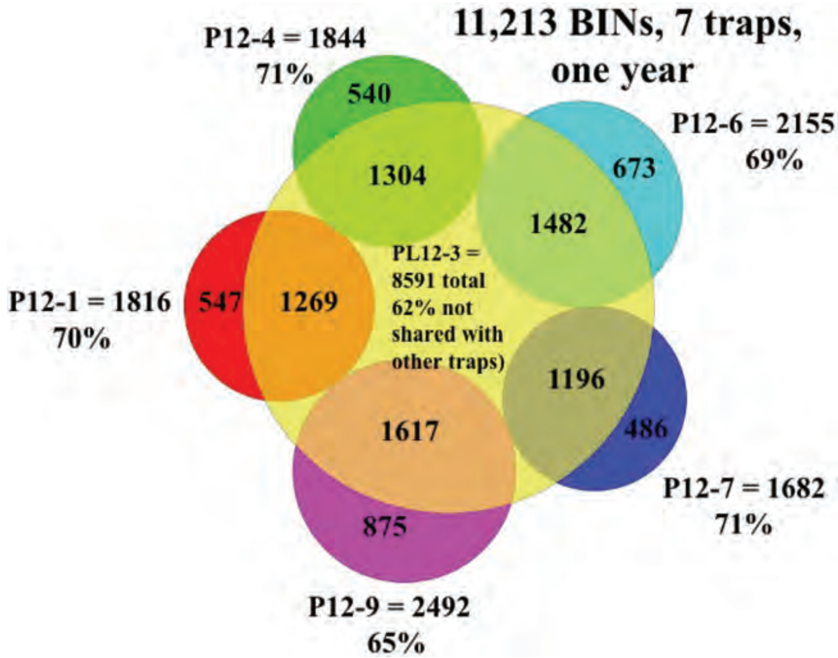
Fig. 17. Numbers of BINS collected in one year of trapping by traps #3 and #4 (edge of PL12 drilling platform) and #7 (deep shade at the edge of the narrow dirt/gravel access road). In total, 71% of the BINS trapped by trap #4 were also trapped by trap #3, but only 15% of the trap #3 BINS were caught by trap #4. Traps #3 and #4 are obviously not capturing the same array of insects, despite seeming to occupy the same position relative to the drilling platform (Fig. 5). [Image: CBG coauthors, raw data from study.]



actually heavily shaded by the nearly intact tall forest canopy on both sides of the one-lane road and therefore in the forest understory ecosystem. Trap #7 showed no significant perturbation effect of the presence of the road during this first year of trapping. While a complex statistical analysis appeared to locate the traps #7 and #4 results somewhat marginal to the cluster of traps #1, #6, #9, and #2, it will require analysis of years 2 and 3 to gain enough of a sample size to know if this is within or without the normal range of variability of these forest edge traps. The road dust unambiguously eliminated foliage-feeding insects (caterpillars, beetles, and bugs), as evidenced by the greenhouse-perfect condition of the foliage near trap #7 (no feeding damage). However, it is not surprising that flying insects of the forest understory (passing through) continued to be caught by trap #7 (and #4). It also needs to be emphasized that the one-lane road itself eliminated a small portion of forest habitat near trap #7 (just as is the case with the platform next to trap #4), which again emphasizes the different kind of perturbation (even if shaded) that it received in the first year. Finally, trap #7, as did #4, had its collecting bottle pointed into the shade of the forest, rather than into the slightly more insolated road.

The detailed statistical analysis of traps #4 and #7 showed a slight difference (more species of Hymenoptera and Lepidoptera) from traps #1, #2, #6, and #9, in the direction of the major difference displayed by trap #3, but not enough to be of concern in practical terms for the first year. If this difference is repeated in the second and third years, then it is an identifiable margin pertur-

Fig. 18. Degree of overlap between each of the other six Malaise traps (#1, #4, #6, #7, #9) and trap #3. In all cases, about 70% of their catches were also caught by trap #3. However, trap #3 captured 5220 BINs that were not caught by any other trap, for a grand total of 11 213 BINs in seven traps in one year. [Image: CBG coauthors, raw data from study.]



bation effect that may be small at one point but if associated with many kilometers of road edge through primary forest, should be considered. The single-lane road itself eliminates a small portion of habitat near trap #7. This emphasizes the different kind of perturbation (even if shaded) that this trap received in the first year, in contrast to the other two margin traps (#3, #4).

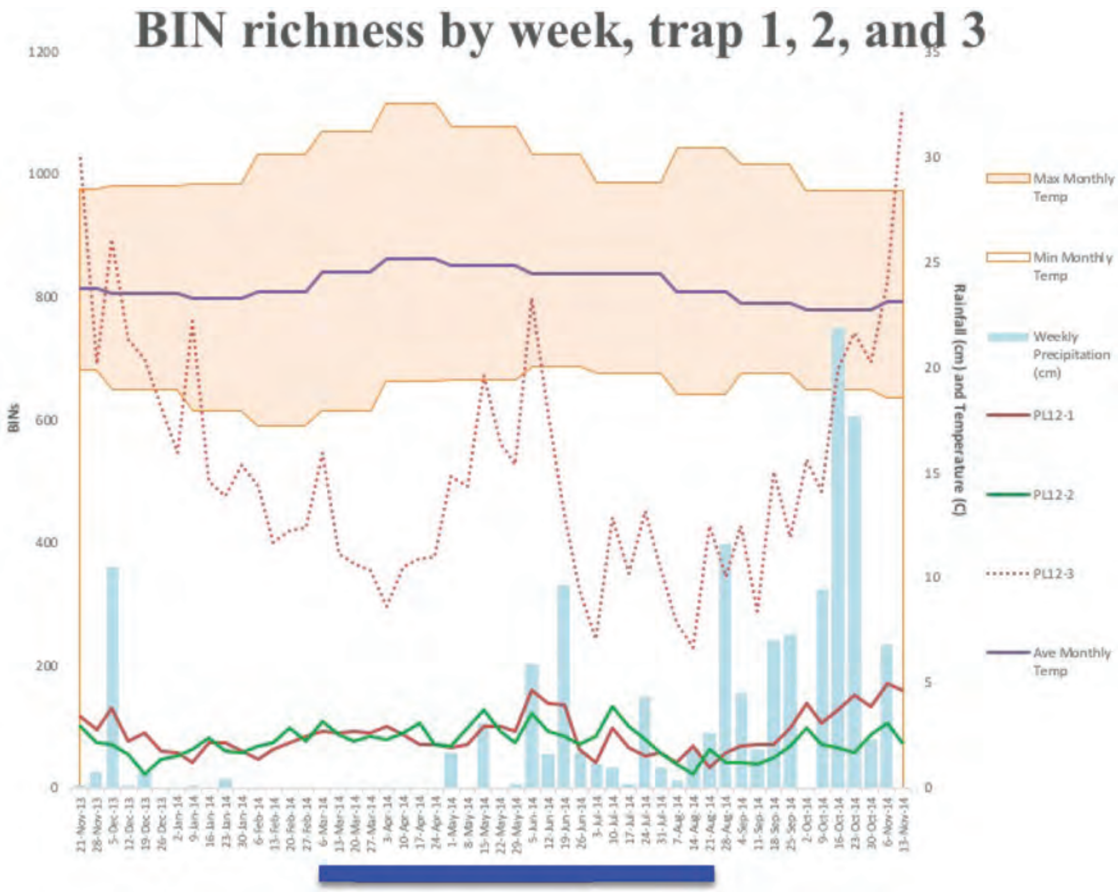
Trap #3 is unambiguously very different from all the others both in numbers of specimens and the numbers of BINs (Figs. 5, 17–18, 20–21). The question becomes, what is the origin of the 5220 BINS not captured by any of the shaded forest traps? The open bare drilling platform is certainly not a living ecosystem that generates them. Traps #4 and #7 are viewed here as forest traps rather than edge traps, given the composition of their contents and the explanation to follow. The great increase in numbers of individuals and BINs in trap #3 is largely contributed by small parasitic Hymenoptera (Platygastridae, Ichneumonidae, Braconidae, Bethylidae) and parasitic and scavenging Diptera (Tachinidae, Phoridae). Quite surprisingly, the proportional increase is not made up of Cecidomyiidae gall-making and fungus-eating larval flies (Fig. 14). Cecidomyiids are the numerically dominant family taxa in all PL12 Malaise traps (and all ACG dry forest and rain forest traps as well). In other words, the proportionally most abundant family of insects did not disproportionately increase in the platform edge. This is probably because the true ecological origin of the Cecidomyiidae flying adults is largely in the forest understory, rather than the canopy or surrounding strongly insolated agroscape. This aspect will be explored in later years when the species composition by Cecidomyiidae in each trap has been more characterized taxonomically. It may also be associated with cedidomyiids being nearly all nocturnal and therefore less affected by 24 h Malaise trap positions relative to sun and shade.

It is highly unlikely that the parasitic and scavenging insects are greatly increased in the platform margin because of a hyper-abundance of food attracting them. In this first year, breeding populations of potential hosts have not had the chance to build up around the margins of the site (if they ever will). This process is especially the case in the first year of perturbation, when the heliophile vegetation of the platform margin is barely developed following the platform clearing and exposing of the forest understory (which was formerly in deep shade and therefore very poor in fast-growing leafy vegetation as food for insects, in contrast to the open insolated agroscape and forest canopy).

There are at least two potential non-exclusive sources for the small sun-loving insects that were so abundantly caught by trap #3. One source is that these are species that normally inhabit the forest canopy with its sunlight, wind, dryness, and heat. When the drilling platform is put into the forest, it introduces that microclimate downward to ground level, and these heliophile insects follow, continuing to search in or just above it, just as they do in the canopy.

The other source is, as mentioned above, the strongly exposed and insolated agroscape adjacent to the Pailas Dos forest. These old fields and pastures are variously beginning to regenerate young second growth forest that generates a steady rain of highly mobile (small) insects onto its surroundings (as waifs, strays, migrants,

Fig. 19. A comparison of traps #1, #2, and #3 throughout one year, for the number of BINs captured per week, along with rainfall and temperature. Trap #1 (150 m into the forest) and trap #2 (50 m into the forest) tell the same story (and the same story as the other shaded traps in the forest (compare with Fig. 20). The blue bar is the period of drilling activity on the platform, which began well before the rainy season and continued to about the end of the insects' first generation triggered and fueled by the rains. [Image: CBG coauthors, raw data from study.]



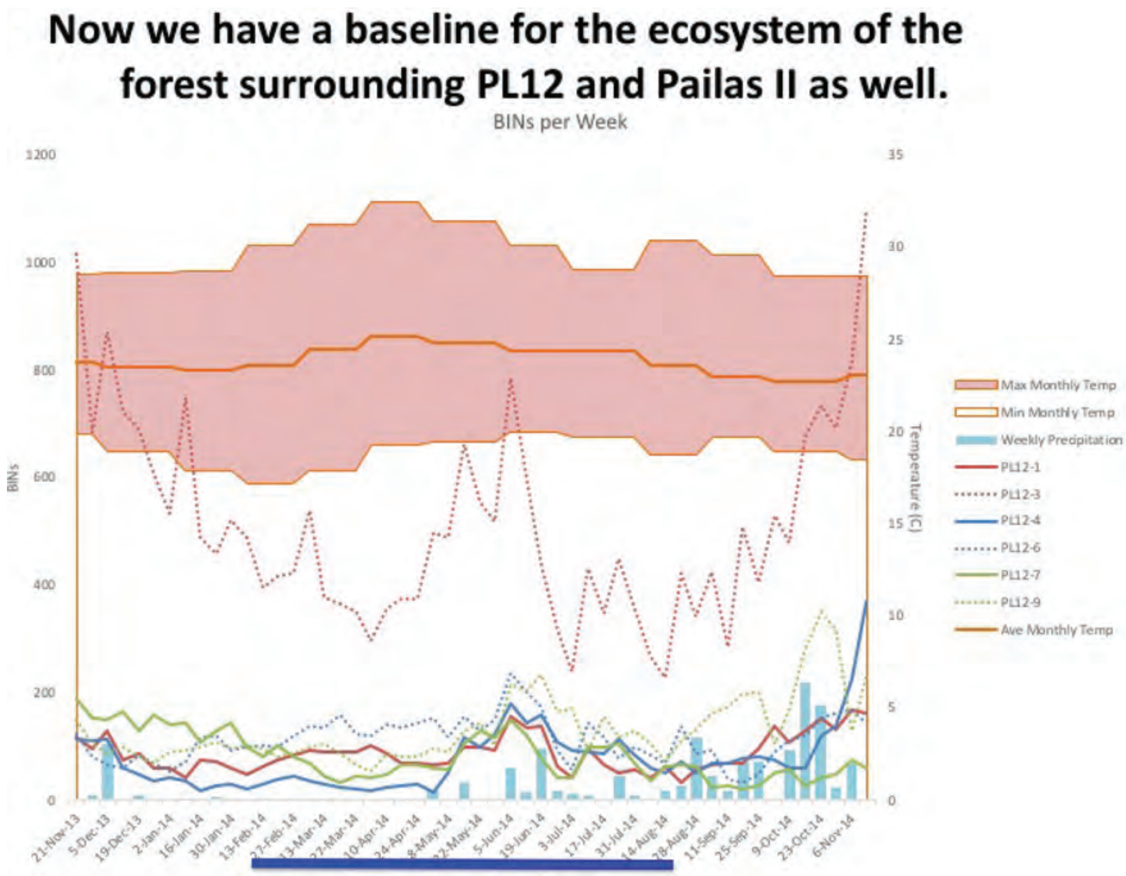
and population explosions). When this insect rain falls on the intact forest canopy, it is largely if not entirely eaten by predators and scavengers and does not establish (due to lack of sunny microclimate and hosts). However, if a large insolated hole, such as the drilling platform, is created in the forest, then these flying insects will be attracted to the disturbance traits of the hole. It becomes an attractive microecosystem for them, irrespective of whether their usual prey is there. In the first year of the platform's existence, conditions for their survival or population establishment are largely absent but we anticipate that as the margin of young secondary successional vegetation builds up around the margin of the platform, they may find progressively better circumstances for establishment and persistence at the PL12 drilling platform edge.

A final source of future change in the forest understory needs to be added to this complexity. Many sampling schemes in the ACG dry forest have shown that during the long dry season (January–May), many species and individuals of insects of secondary succession take refuge as sexually dormant adults in the cool, moist, and shady understory of nearby evergreen or semi-evergreen old-growth forest. This greatly, but just seasonally, increases the species richness in the shady microhabitat. When the rains come in May and there is leaf flush and shoot growth in the insolated (and now moist) secondary succession, these insects abandon the shady (and slow-growing) forest understory and move back into secondary succession (Janzen 1976). This phenomenon is expected in the forest of Pailas II, both near PL12 and near the other roads and drilling platforms. However, whether it has already begun in the first year of perturbation cannot be determined until the natural histories of the species in both forest and adjacent secondary succession are known and followed over subsequent years of sampling.

Compare traps #1, #2, and #3 as a potential gradient from disturbed to undisturbed forest understory, and likely future annual baseline biomonitoring for PL12

As mentioned earlier, it was anticipated that all nine Malaise traps would be analyzed. However, in 2016, funding was available to do only the three deep forest traps (#1, #6, #9) and the three traps at the edge of the perturbation (#3, #4, #7). When the opportunity arose to do one

Fig. 20. Graph of BINs per week for all seven analyzed traps, along with rainfall and temperature. Trap #3, as discussed, is outstandingly different even though it conforms to the same general pattern through the year of the other six traps. Trap #2, even though being only 50 m into the forest behind trap #3, is indistinguishable from the other three deep forest traps, the platform edge trap (#4) with its collection bottle into the forest, and the road edge trap (#7) that is likewise pointed into the forest and also in the shade of the canopy left intact by ICE over the one-lane road. [Image: CBG coauthors, raw data from study.]



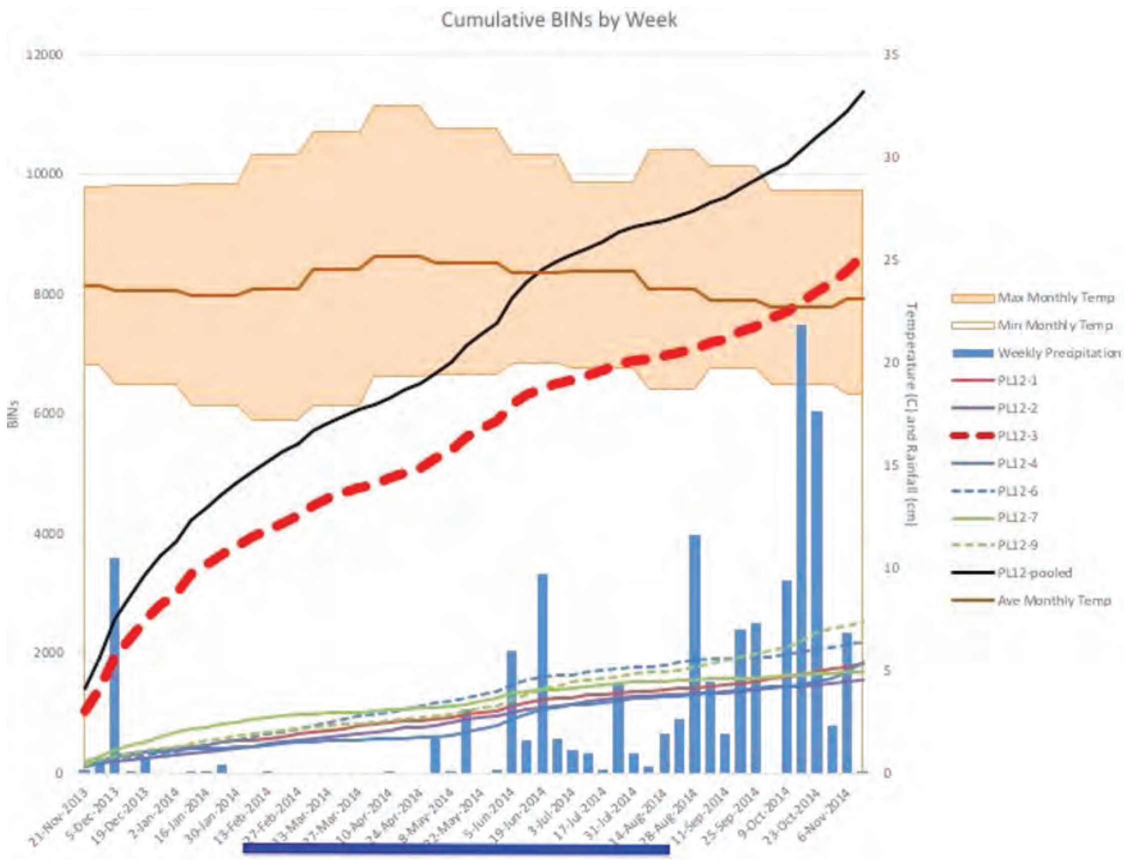
more trap, #2 was chosen out of the three that were available at a distance of 50 m into the forest. This choice was because it is part of the logical biomonitoring line that is likely to continue into the indefinite future. This logical is because the 4-5-6 line was lightly perturbed by ICE expanding the platform in early 2016, and the entirety of the 7-8-9 line may have been impacted by ICE building a 30 m wide road parallel to trap #9's forest at about 30–50 m distance.

In all respects, the contents of trap #2 are the same as the other deep shade traps, at the level of Ordinal, Family, and BIN counts, for this first year, and per week. Despite the drilling platform being conspicuously visible through the forest understory from trap #2, its captured material is essentially the same as that for traps #1, #4, #6, #7, and #9. Strikingly, its species accumulation curve is essentially identical to that of all the other shaded traps and dramatically different from that of trap #3 (Fig. 21). It differs only in that its total number of specimens is at the lower end of the range captured by each of all the other forest understory traps. While it is a sample size of one (to be replicated with future years by analysis of traps #5 and #8 with later funding), this result conforms strongly with the expected. As close as 50 m to the platform, the trappable insect community does not evidence disturbance for the first year of geothermal project activity.

Compare results derived from different intervals of trapping

It is easy to conjecture that more is better when it comes to how often to analyze Malaise trap samples. However, the weekly analysis gives the highest resolution for the purposes of biomonitoring. Whenever the samples were pooled into longer intervals, the resolution was reduced. When only every second week was sampled, or every fourth week was sampled there is a serious loss of resolution (not documented here). However, essentially all sensitivity to the dramatic changes with the rainy season can disappear if the wrong week is chosen. The same qualifier applies to the number of specimens per week. If we only count the number of BINs per month, pooling intervals appears to not matter, but there is very low resolution.

Fig. 21. Raw BIN accumulation curves for all seven traps, as well as their pooled number of BINs (greater than 11 500). It is noteworthy that all curves take a slight bump upwards with the beginning of the rainy season, and three of them increase more than expected as the year approaches the end of the rainy season. Trap #2 is conspicuously the same as the others in the forest understory, even though it is only 50 m from the edge of the PL12 platform. As with other figures, the blue bar indicates the presence of drilling activity at PL12. [Image: CBG coauthors, raw data from study.]



It is also relevant to consider the question as to whether the ecosystem around PL12 can be accurately portrayed through its insect composition by simply recording the number of specimens or their biomass rather than the number of BINs captured by the Malaise traps. As a beginning exploration of this possibility, Fig. 22 compares the numbers of BINs and of specimens in trap #3, the most specimen-rich of all traps. Both graphs (Fig. 22) show the same general pattern, but since there is no taxonomic information included, there is no detail in the baseline against which to compare future years.

Conclusions

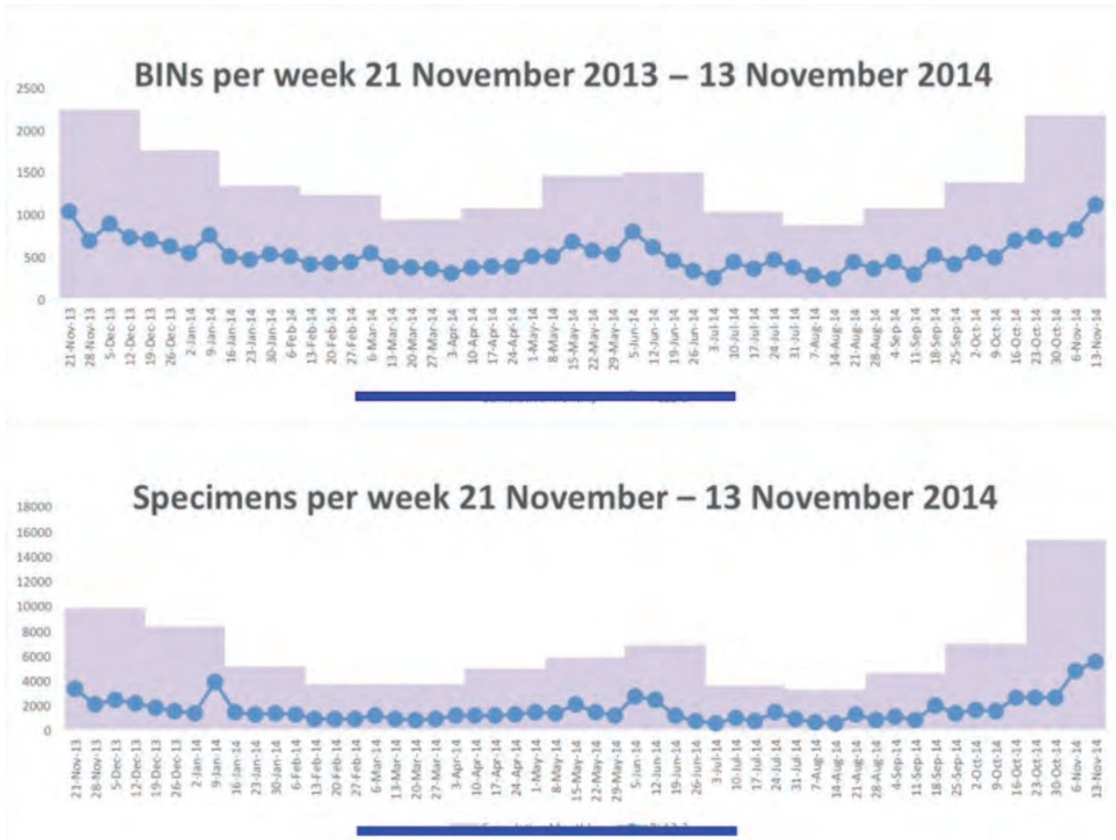
This study is the deliberate melding of incomplete standard and innovative hard science with incomplete industrial necessity of rapid conservation and mitigation on a tropical frontier where massive amounts of wild biodiversity still exist but social desires for forest harvest or elimination are very active and expanding.

This year (2013–2014) of biomonitoring site PL12 of the Pailas II Geothermal Project conducted being developed by ICE was part of the 2016–2017 report to JICA as "*Study on improvement of Environmental Monitoring methodologies for geothermal development in Costa Rica*" (a.k.a. SAPI). The current environmental methodologies used by ICE and Costa Rica's environmental monitoring institution, SETENA of MINAE, are well established, required for ICE and MINAE, and not under analysis here. Rather, the intent of this project by GDFCF/ACG, as part of the SAPI conducted through ERM (Japan) and funded by JICA, GDFCF, and ACG, was to begin to develop a new tool for ICE's and JICA's environmental biomonitoring tool kit.

Primary conclusions from the first year

1. A highly diverse tropical insect community, constituted of tens of thousands of undescribed species, can be used as a simple biomonitoring system to detect changes in biodiversity and abundance that are correlated with the perturbation by the geothermal project, and therefore be an index to the amount and location of that perturbation to biodiversity in general, since insects constitute at least 90% of the animal species-level Eukaryota biodiversity of a tropical forest.
2. Strategically placed Malaise traps capture thousands of species of flying insects per week, and therefore

Fig. 22. Comparison of trap #3, the one with the most BINs and most specimens, along with its cumulative monthly totals. At the level of crude BIN abundance and specimen abundance, mirroring each other and the weather changes (as shown in previous graphs), these results provide maximally simplistic baseline information for each of these traps. The blue bar is the period of drilling activity on the platform. They do suggest several directions for simple biomass and simple eDNA mass analysis, especially once the barcode library is more fully stocked. [Image: CBG coauthors, raw data from study.]



can monitor short-term and long-term ecological responses to biodiversity perturbation; DNA barcoding and its analysis backed by voucher specimens now allows quick, cheap, and accurate identification of the trapped specimens, rather than requiring highly specialized and expensive labor-intensive months-to-years of professional taxonomic analysis (which can emerge later as desired). The process does not require extensive classical identification of the species being tracked. However, as usual, the more finely particulate the data, the more can be built out from it, both for project and generically. Simultaneously, the actual barcodes and their voucher specimens now become a taxonomically tractable barcode reference library and dictionary for all the many other scientific uses society has for an understood biodiversity (Janzen and Hallwachs 2019*a*).

3. Engineers and other geothermal project personnel with no formal biological training can accommodate and understand the required biological aspects of this new kind of biomonitoring, one that is conducted through laboratory-based DNA barcoding of the trapped insect specimens; this renders Malaise traps and what they capture into a technically intelligible methodology, such as engineers and other non-biologist sectors of society are comfortable with applying and learning from.
4. A geothermal project can understand and incorporate biomonitoring with Malaise traps coupled with DNA barcoding analysis as a useful technical component of an industrial project, just as are roads, bridges, drilling, platform sites, personnel, and administration, as well as are the classical kinds of short-term pre-project environmental monitoring (EIA) biomonitoring for endangered species or habitats.
5. It is preferable to collect baseline Malaise trap data for at least one year before any industrial work. This year of effort is also needed for standard EIA pre-preindustrial biomonitoring for endangered species or microhabitats; a year is required so as to encompass the normal seasonal fluctuations in community structure and species presence. However, if a year advance biomonitoring is impossible, results from somewhat distant Malaise traps in the same ecosystem can be proxies for a normal year baseline during the industrial work. Furthermore, these proxies have suffered the same weather as the biomonitors of actual or potential perturbation, thereby avoiding

the problem of a pre-project year being seriously different weather-wise from the year of project initiation.

6. Engineers, planners, and other geothermal project personnel can fruitfully work with ecologists and conservation biologists to minimize the impact of the truly necessary aspects of a geothermal site (e.g., lights (Owens et al. 2020), forest clearing, people presence, earth moving, and noise). Thorough biomonitoring of all kinds may show that some of the anticipated perturbations are more contra-aesthetics and impression-based than actually damaging to the site biodiversity and its other ecological traits.
7. In this particular Costa Rican project, GDFCF (http://www.gdfcf.org) as a conservation NGO, plus its government partner, ACG (http://www.acguanacaste.ac.cr), can work calmly and constructively with the government geothermal company (ICE: https://www.grupoice.com) to create a new kind of long-term biomonitoring process for a geothermal drilling platform, and its access road, in sensitive and conservable old-growth tropical forest. It therefore prepares Costa Rica for consideration of a future within-park situation, inside the adjacent Área de Conservación Guanacaste UNESCO World Heritage Site, if it should ever be legally imposed.
8. Two quite different government agency subcultures (ICE and ACG) and a conservation NGO (GDFCF) collaborated intimately on this complex long-term project even though it required some conspicuous and innovative adjustments to some of the standard bureaucratic traits of these three subcultures, and some site-specific solutions to generic issues. It also required JICA as the fiscal patron for the entire biomonitoring project.
9. During the first year of biomonitoring with Malaise traps of site PL12 at different distances and orientations from the drilling platform, and the three months of drilling itself, there was no detected perturbation of the insect community, except for the actual clearing and its margins. During this first year, the geothermal platform perturbation is ecologically analogous to a 1.5 ha landslide, with respect to the overall biodiversity of the surrounding forest. While a landslide is eventually reoccupied by the adjacent forest, this will not be the case for the access road and platform. Their permanence is most analogous to a river edge within its adjacent forest.
10. In view of these results, a key question emerged: How many "landslides" or "riverbanks" of what size and configuration, and at what distance from each other, with what access roads, would be acceptable in a sensitive and still conservable old-growth forest that its society has major reasons to enter in a controlled manner? The answer varies from forest to forest and will need to be determined by on-site biomonitoring. The use of the same biomonitoring and analysis to track and measure the impact of climate change on highly heterogeneous tropical landscapes also requires serious feedback-rich and site-specific biomonitoring.

All of these 10 project goals were attained for this SAPI project in 2016 and early 2017, for the data from late 2013 to late 2014. They all show observational evidence of continuing to be the case for the PL12 project and other nearby ICE anticipated projects. They are both being used by GDFCF/ACG/SINAC, ICE, and JICA as examples of a positive interaction between conservation and industrial society.

In the process, these 10 goals have created the site- and project-specific result that, for this first year of biomonitoring, the drilling platform and its drilling activity, and its access road, have no visible perturbation effects on forest as close as 50 m distant from the platform edge. However, the platform does remove its biodiversity (except that which flies across it) and has a dramatic effect on its immediate border. Equally, observations by ICE and by GDFCF indicate that various vertebrates treat the PL12 site as a different, though not necessarily negative, microecosystem. There is no evidence that the presence of PL12 has a currently discernable effect on the great bulk of the biodiversity of Sector Pailas of ACG, Parque Nacional Rincon de la Vieja, and the ACG UNESCO Natural World Heritage Site as a whole.

The platform itself, an anthropological analog to a landslide or riverbank that eliminates about 1.5 ha of forest at a particular point, has created a very distinctive microperturbation in the form of a 3–10 m wide border that shares many biological and physical traits with the nearby old-fashioned agroscape and young secondary vegetation (as well as with the forest canopy itself). This narrow strip of heliophile vegetation and its biota is not to be confused or analogized with the sterilized and pesticided modern agroscape of industrialized rice, sugarcane, soya, pasture, or pineapple fields. On the one hand, there is the possibility that this old-time agriculturalization of the PL12 site will spread into the forest, thereby increasing the biological size of the perturbation over time. The ongoing analysis of the current six years of biomonitoring of PL12 of the frozen samples of the nine Malaise traps will document an ongoing presence or absence of such a biological spread. The specimens only lack barcoding budget to move forward with their analysis.

On the other hand, an understanding of the biology and biodiversity of this agriculturalized platform margin suggests possible design and treatments of it so as to minimize the possibility of its impact spreading. Any such modification would best be accompanied by further study of the biodiversity dynamics of tropical complex edge ecosystems, recognizing that each site will be different biologically as well as sociologically—one shirt definitely does not suit all. Ironically, landslides are an omnipresent habitat type in tropical species-rich forests on slopes (as are their flatland and sloping analogues—riverbanks). However, as a microhabitat type themselves, their insect biodiversity has received essentially no attention from tropical field ecologists (but see Krishnadas and Comita 2019 for a lead into plants on edges). A study (Janzen 1983) of a small area of primary old-growth dry forest in Sector Santa Rosa of ACG discovered that birds and other vertebrate seed dispersers move heliophile plants of adjacent secondary succession in abandoned agroscape into natural clearings from tree falls and forest understory. This gradually decomposes old-growth forest into a mix that is neither classic old-growth (with its disturbance by tree falls) nor pure secondary succession following catastrophic deforestation. This provoked the conclusion that for its preservation, a valuable old-growth primary forest might best be bordered by rice fields or clean pasture rather than the secondary succession, rich in vertebrates, much approved by conservationists as a buffer zone. The concept of a buffer zone is very deceptive; in human terms on a map it can look nice and reasonable, but in terms of wildland ecology versus human sociology, it may damage an adjacent old-growth forest more than help it.

Equally, it would be possible to collaborate with geothermal engineers to determine which aspects of standard drilling platforms and access roads could be modified to more exactly mimic the edge of a natural landslide or riverbank and thereby minimize the industrial impact on the forest in which the project occurs. This could involve the detailed configuration and placement of roads and platforms, the timing during the year of doing the clearing and drilling, the absence of lights (or the kinds of lights), and of course the exclusion of hunters or colonists and other unauthorized invaders from adjacent humanity, a highly destructive force that commonly makes use of forest penetration by roads, frontier colonists, and politicians.

There are two major considerations to viewing a geothermal industrial site (and its access road) as a species of landslide (or riverbank). First, it is a well-known concept to ecologists and other observers of nature, that light point-source disturbance to a large expanse of truly intact forest increases many aspects of its biodiversity on many axes. No sheep, species-poor pasture; many sheep, species-poor pasture; few to moderate sheep, species-rich pasture. In other words, how many landslides of what size and duration, and at what distance from each other, can occur in a large conserved forest without negatively impacting its overall biodiversity? The SAPI project focused on PL12 was not designed to directly answer this question, but its results, made possibly only by the CBG DNA barcoding, are relevant. In the practical terms of this particular geothermal site, one, two, or even three replicates of PL12, and its access road, carefully sited within 10 000–20 000 ha of forest of the kind hosting PL12 (note uniformity of forest appearance in Fig. 5), could well show no evidence of being different from several small landslides or a river of similar size. One to three one-time occasional landslides of 1–2 ha size in 10 000 ha of ACG old-growth forest will unambiguously increase that forest's net biodiversity.

Second, there is another biodiversity consideration besides a simple accounting of the number of species and their interactions (a classical measure of biodiversity) as potentially being impacted by one to three geothermal drilling sites. An intact old-growth forest with no roads or other anthropological perturbations is an ever-rarer object on the globe. It is itself a threatened biodiverse object. Just as the human body can of course function equally well (or at times, better) with small and carefully placed surgeon's scars or well-healed bullet wounds, there are parts of the body where even a very small and well-healed surface rupture lowers that human's performance and survival. Location and intensity matter. In the particular case of PL12, the geothermal site and access road were chosen by ICE for their geothermal and engineering considerations before GDFCF/ACG biologists had the chance to collaborate. However, in a fully biodiversity-friendly planning exercise for an industrial geothermal site, the biodiversity traits of the proposed area of perturbation would be well understood biologically and the actual drilling sites and access roads positioned accordingly. Some sites would be simply unavailable because of what they contain or how they influence other sites. It is worth emphasizing that true biodiversity monitoring is concerned with both the immediate impact on focal species and microhabitats, and on the long-term impact on the overall biodiversity interactions of a conserved wildland.

All technical decisions need to consider the social bias against certain kinds of actions, quite irrespective of their measurable impact on the site's biodiversity. It is viewed as quite acceptable to severely perturb 1–2 ha of old-growth forest for ecotourist accommodation and access, education, administrative/protective actions, or research. The same degree, and even kind, of perturbation is viewed with distaste or termed illegal when extracting a geothermal resource, mining, water capture, classical crops, or other quite socially visible commercial purposes. However, all perturbation actions are commercial ventures at some level, but some are declared legally legitimate in a national park and others as illegal. Some are carefully planned so as to not disturb the biodiversity and ecosystem values of the area, and many others are not. A road that subsequently gives access to colonists and hunters into a conservation area is a major threat, while a road with highly controlled use, such as that to PL12 or those throughout ACG, may be hardly more than analogs to small dry riverbeds. A road that transects a unique, and today ever-scarcer, old-growth ecosystem can be a travesty, while the same road in secondary succession on an abandoned pasture can be inoffensive and give legitimate management and approved access, along with society-wide desire and tolerable footprints.

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Supplemental File S1

La biodiversidad del "Bosque Precafetal", Sector Pailas, Area de Conservacion Guanacaste, noroeste de Costa Rica. Unpublished, D. H. Janzen, 7 feb 2013.

Introduccion y ubicacion.

El "Bosque Precafetal" (nombre nuevo) es ubicado en la falda Pacifico, lado sur de la masa de Volcan Rincon de la Vieja, parte de la Cordillera Guanacaste (Fig. S1). Formalmente, es el parte mas al sureste del Sector Pailas del Area Silvestre Protegido de Area de Conservacion Guanacaste (ACG), y algo de lo parte mas suroeste del Sector Santa Maria de lo mismo ACG. Legalmente, el Bosque Precafetal es simultaneamente en el parte central y de elevacion intermedia, del lado Pacifico de Parque Nacional Rincon de la Vieja (PNRV) (Fig. S1).

Figura S1. Area Silvestre Protegido del Area de Conservacion Guanacaste (ACG) en verde y azul. El Bosque Precafetal es la porcion del "perrito" amarillo que es su cuerpo (sin patas, sin cabeza y cuello), en la falda sureste de Sector Pailas y una porcion suroeste de Sector Santa Maria (figura modificada de http://www.acguanacaste.ac.cr, enero 2013).

El Bosque Precafetal es entre 800 y 1000 m elevacion en la ladera boscosa de un juego de volcanes que extienden a 2000 m elevacion, localmente llamados "Rincon de la Vieja". Por su categoria de Zona de Vida, este bosque es "Bosque Muy Humedo Premontano", si uno usa un mapa estandard de las Zonas de Vida para definicion. Sin embargo, el hecho de que este masa volcanica de Rincon de la Vieja es aislado, por el "efecto massenerhebung" el bosque que aqui ocupa 800–1000 m elevacion es lo que uno encontraba a 900–1300 m elevacion en las cordilleras pacificas del parte central de Costa Rica. Y esto es el tipo de bosque que era donde hoy dia estan los cafetales de Costa Rica. Y la biologia indirectamente señala este hecho tambien: hay los restos de chiquititos abandonados cafetales rusticos a la par del sendero de Estacion Pailas (por la pie anterior del perrito) hasta el pico Rincon, a 900–1000 m elevacion (y pasa por donde esta la cabeza del perrito).

En Fig. S2 adelante se puede apreciar el Bosque Precafetal en una foto aereo en Google del año 2010, por su margen rojo — es el cuerpo principal (rectangular) del “perrito” sin piernas y sin cuello y cabeza. “Bosque Precafetal” como nombre del sitio se refiere al hecho de que es el tipo de bosque que hace siglos crecio en los habitats y ecosistemas que hoy en dia estan cubiertas con cafetales y sus restos. Este tipo de bosque esta casi extinguido en Costa Rica hoy dia, y lo poco que hay en ACG es el unico extension grande de este bosque en un parque nacional en todo el pais. Es algo interesante que el habitat y ecosistema que ha sostenido el pais para mas que un siglo es lo menos representado en las zonas protegidas del pais.

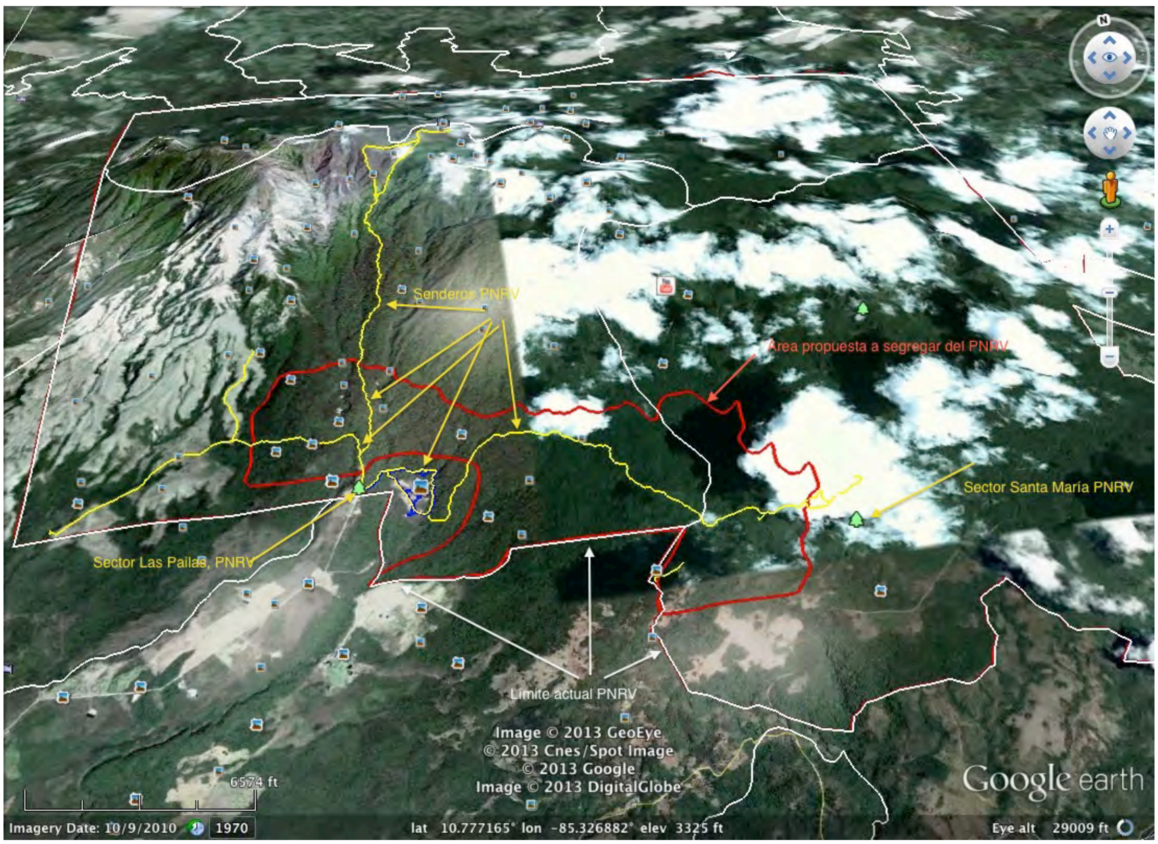

Figura S2. Croquis (adentro de la linea blanca) del Parque Nacional Rincon de la Vieja. En gran parte, la mitad por la izquierda (oeste) es Sector Pailas de ACG y la mitad por la derecha (este) es Sector Santa Maria de ACG. Sector Aguacatales es el parte mas norte de PNRV. Las lineas irregulares amarillos son los senderos principales usadas por unos 60,000 turistas por año a conocer y experimentar las atracciones de la clima, las pailas volcanicas, los saltos de agua, el trepon a la cima y sus actividades actuales volcanicas, la biodiversidad, y los bosques secondarios y primarios donde cruzan los senderos. De lo que hay bordeado en rojo, el Bosque Precafetal es el “perrito” menos sus piernas, cuello y cabeza.

Historia volcanica X bosque.

Volcan Rincon de la Vieja y los otros volcanos en el crater del viejo volcan que exploto hace 1.5 millon de años (Kempter et al 1996, Kempter 1997), arrancaron su crecimiento hace 50,000 años aproximadamente, asi formando una tapa sobre la caldera vieja. Es obvio que la vegetacion en las laderas de todo estas volcanos ha sufrido varios impactos severos cuando hay erupciones. Uno de lo mas obvio en las laderas Pacifico es los restos del lahar de barro que limpio el hombro oeste de Rincon y es muy visible por el lado izquierda en Fig. S2, en gran parte ocupando el parte oeste de Sector Pailas.

Uno tiene que saber y asumir que a intervalos desconocidos, han estados erupciones y lahares como esto que han destruido el bosque totalmente a parcialmente. Entonces cada uno de estos eventos van a producir su propio taza y patron de restoracion, en parte dependiendo en tan completo era la destruccion. Igualmente los lados del evento no van estar impactado igual al parte central, tendiendo a grados de regeneracion y "diferente" tipos de regeneracion. Para complicar el cuadro, hace 15,000–20,000 años, era por seguro glaciers y climas muy frias en las cimas de estos volcanes, que tambien tenian sus impactos en la vegetacion y sus tazas de restoracion natural. Finalmente, los patrones de restoracion van a (*i*) variar con elevacion por un lado, y proximidad a los bosques mas secas debajo Bosque Precafetal por el otro, y (*ii*) tener una gradiente de precipitacion bien visible en el verano cuando el parte mas arriba del Bosque Precafetal es continualmente humedo, mientras que el parte mas bajo refleja el verano en su grado de sequia (y falta de musgos, helechos y plantas epifiticas).

En adicion a esto, la zona bordeando una linea mas o menos recto entre Estacion Pailas y Estacion Santa Maria (Fig. S2) es el margin superior de los terrenos que han sufrido varios niveles de aprovechamiento, cazaria, ganaderia, cultivos, desviaciones de agua, caminos, trafico humano, fuegos y otros perturbaciones. En estos terrenos hay varios niveles de restoracion natural encima de perturbaciones artificiales, y a veces es dificil a saber donde termina la perturbacion humano (ahora en restoracion artificial) y la perturbacion natural (ahora en restoracion natural).

En pocos palabras, el Bosque Precafetal es un complejo de diferentes edades de restauracion natural en fajas elevacionales (de arriba por abajo), con su base concentrado en el parte mas alto (en elevacion) de la restauracion artificial. Sin caer en intentar adivinar mucho detalle, caminando en este bosque desde el oeste al este por el camino medio hemisferico indicado en amarillo entre Estacion Pailas y Estacion Santa Maria, y pasando por el puro centro de Bosque Precafetal (Fig. S2), es facil apreciar esto. Para detallar este unico serie de fajas de restauracion natural requiere por lo menos 1–2 años de estudios detallada y cuidadosa de las plantas, vertebrados y insectos en este bosque.

Pero preliminarmente es facil a ver que mientras que la restauracion artificial en el parte mas bajo del bosque es repetido en otros partes de ACG (lados Pacifico de Volcan Orosi, Volcan Cacao y Volcan Rincon de la Vieja), el parte principal del Bosque Precafetal es unico y virgen en el sentido natural, no importa si el bosque es en sus fajas variando de cientos a miles de años de edad hasta de lo mas reciente perturbacion del volcan. Si fuera que la Meseta Central tenian todavia sus bosques de este tipo, este parche de unos 3–4 mil ha de este bosque seria especial solamente por ser una isla ecological aislado de los otros isletas en Volcan Orosi y Volcan Cacao (y en Volcan Miravalles y Volcan Tenorio), aislada por el mar de tierras bajas y agropaisage que rodea en el lado Pacifico y el bosque lluvioso por el lado Atlantico. Por supuesto, cuando hay una archipelago de islas ecologicas cada “isla” por supuesto tiene su propio fauna y flora, y historia evolucionaria. Pero en este caso la tierra principal de la zona del Meseta Central esta cubierta con cafetal, y el resultado es que el Bosque Precafetal es unico y especial por dos razones — ser “isla” ecologica y ser un restito de lo que era mucho mas comun antes de los Europeos, es decir, una antiguidad que permite uno entender el pasado. Su nombre Bosque Precafetal era escogido en reconocimiento de este ultima realizacion. Tenemos que ser muy, muy agradecidos por donadores privadas temprano en las 1980’s, y el gobierno (RECOPE, IDA), quienes tenian el sabio juicio a guardar este bosque tan importante en la historia de Costa Rica como parque nacional. En este sentido, tiene un valor igual a la Casona Santa Rosa en su representacion de la identidad nacional.

Biodiversidad del Bosque Precafetal.

Para documentar y entender la biodiversidad del Bosque Precafetal, y especialmente la ubicacion exacta de sus partes adentro de este bosque de este tamaño de unos 500 hectareas, costara un par de años de observacion y inventario detallada por personas que son intimamente conocedores de ambos la biodiversidad de plantas, vertebrados y insectos tropicales, y que saben como localizar y documentarlos. No es una tarea facil, ni uno que puede ser ejecutado solamente por gente de la calle, no importa tan profesionales son en sus labores. Por suerte y el hecho que ACG y INBio han pasado tres decades conduciendo este tipo de inventario en otros partes de ACG, y colaborando con biologos y taxonomos nacionales y internacionales en sus labores de llegar a conocer biologicamente ACG y el pais (por INBio), hoy dia existe un equipo bien experimentado en conducir este tipo de estudio con calidad. Para hacer una comparison del Bosque Precafetal con otros bosques en ACG y su vecindad, demandara todavia mas estudio comparativo por el mismo equipo de personas pero puede ser hecho en mucho menos tiempo.

Debido a que el Bosque Precafetal hasta el momento no ha estado todavia el enfoque de inventario dedicado a otros bosques de ACG en el pasado, podemos en este momento mencionar solamente unos de las cosas sobresalientes de este bosque por unos estudios de muy corto tiempo y visitas de relampago en 2012 (rapido, pero muy suficiente a reconocer lo especial de este bosque en grande, pero no suficiente a decir en este momento exactamente donde o como es bosque puede aceptar o tolerar la mas presencia humano que es sufriendo actualmente).

Caracteristicas sobresaliente:

1. Mariposas nocturnas. Durante junio y agosto 2012 empezamos con una comparison entre la fauna de mariposas nocturnas en el parte de Sector Santa Maria donde termina el Bosque Precafetal al este (el parte del posterior del perrito en Fig. S2), y la fauna de mariposas nocturnas en el parte mas oeste del Bosque Precafetal (el pecho del perrito en Fig. S2). En noches de la luna negra, alternativamente, pusimos las luces en el bosque todo la noche en los dos localidades separados por aproximadamente 7 km horizontalmente, pero del mismo elevacion de 850 m. Era cuatro noches en cada sitio. Mientras que colectamos las mariposas de todos tamaños, era posible identificar rapido solamente los grandes especimenes de las familias Saturniidae y Sphingidae.

En terminos generales los resultados fueron muy llamativos y sorprendiente. Por el lado Pailas, las mariposas fueron casi 100% especies comun y residentes en el bosque seco de Guanacaste (y han estado collectado frequentemente en otros años en Estacion Pailas). Sin embargo, por el lado Santa Maria, a nuestro gran sorpresa, en adicion a los especies usuales del bosque seco, eran varios especies que uno normalmente asocia con los bosques lluviosos en las laderas norte de Rincon de la Vieja, mariposas basicamente de San Carlos y nunca encontrado en bosque seco de Guanacaste. Ejemplos son: Sphingidae: *Eumorpha obliquus, Pachylia darceta, Xylophanes belti, Xylophanes cyrene, Xylophanes titana, Xylophanes thyelia, Xylophanes cthulhu, Xylophanes* libyaDHJ01, *Manduca albiplaga, Manduca pellenia, Eumorpha triangulum*, etc. y Saturniidae: *Eacles ormondei, Citheronioides collaris, Citheronia bellavista, Copaxa rufinans, Rothschildia triloba, Leucanella hosmera, Dirphia nora, Automeris zuganaDHJ02,* y otros.

Es que descubrimos que el bosque lluvioso de San Carlos extiende en una peninsula hacia el Pacifico y que medio rodea el volcan en el lado este hasta un punto entre Estacion Pailas y Estacion Santa Maria a esta elevacion. Es decir, el Bosque Precafetal es la fusion y intergredacion abrupta de dos grandes ecosistemas costaricense. El unico otro area que puede ser eqivalente a esto es el noroeste falda de Volcan Orosi, pero este sector (Sector Del Oro de ACG) es en gran parte hoy dia cubierto con cientos de hectareas de plantaciones de naranjas (Fincas de Del Oro). Tal vez vale la pena a mencionar que estas mariposas de bosque lluvioso son “estandard” para San Carlos pero en ninguna manera en los bosques secos de Guanacaste. Y es exactamente en casos como esto donde aparecen nuevos especies — especies del bosque seco evolucionando la capacidad a tolerar y amar las condiciones de bosque lluvioso, y especies del bosque lluvioso evolucionando la capacidad a tolerar y amar las condiciones de bosque seco (e.g., Janzen et al 2012).

2. Mariposas diurnas endemicas. Es tan temprano en el inventario a saber cual y cuantos especies endemicas de mariposas (nocturnas y diurnas) hay en el Bosque Precafetal. Sin embargo podemos decir que es hogar de una muy sana poblacion del morpho blanco (Fig. S3), *Morpho catalina* (Nymphalidae) (Corea y Chacon 1984), muy escaso y endemica a los tres volcanes del Cordillera Guanacaste, la oruga de que se alimienta de las hojas de *Inga punctata, Inga longispica y Inga semialata* (Fabaceae), tres plantas naturalmente encontrado en bosque lluvioso original en el lado Pacifico y Atlantico. Las larvas cuestan 9–10 meses a crecer del huevo (agosto–setiembre) hasta pupar (junio–julio) en la sombra fuerte de bosque original (crecen muy lerdos por la baja valor alimenticia de su comida creciendo en la sombra). Los enormes adultos vuelan solamente en agosto y temprano en setiembre, como un enorme hoja de papel blanco. Mire a <http://www.acguanacaste.ac.cr/paginas-de-especies/178-morpho-catalina-nymphalidae> para conocer la larva (oruga) y Fig. S3 para ver un adulto volando.

Figura S3. Un macho adulto del grande *Morpho catalina* (Nymphalidae) endemica al Cordillera Guanacaste lado Pacifico y altos elevaciones, volando en el puro centro de la foto, como una hoja de papel blanco a 20 m arriba del suelo. Estas mariposas estan adultos volando solamente en agosto y temprano en setiembre (Bosque Precafetal este, 29 agosto 2012).

3. Orugas de mariposas. El programa de inventario de las larvas (orugas) de mariposas diurnas y nocturnas de ACG, en mocion desde 1978 (Janzen et al 2009) y conducido por el Programa de Parataxonomos de ACG (Janzen 2004, Janzen et al 2011), ahora tiene 7 años de inventario del Sector Mundo Nuevo de ACG (Fig. S1), que es la extension de Sector Pailas al su sur y mas bajo en elevacion (300 a 800 m elevacion). Sector Mundo Nuevo, es mas seco que Sector Pailas y mas completamente bosque seco de Guanacaste. Sin embargo, muy sorprendente, este inventario de Sector Mundo Nuevo bosque seco ha encontrado mas que cien especies de mariposas que son "de los bosques lluviosos de San Carlos" (es decir, del lado norte bajo de Rincon de la Vieja en PNRV y Sector Rincon Rain Forest de ACG). Ejemplos son *Euglyphis jessiehillae y Euglyphis gutteralis* (Lasiocampidae), *Archeoprepona amphiktion y* Opsiphanes quiteria (Nymphalidae), *Mimoides clusoculis, Protographium dariensis y Pterourus laetitia* (Papilionidae), *Arsenura batesii* y *Citheronioides collaris* (Saturniidae), *Cerura* rarataDHJ02 y *Hemiceras pallidula* (Notodontidae), *Manduca albiplaga* (Sphingidae), *Dysschema jansonis y Agaraea minuta* (Arctiidae), *Amorbia concavana*, *Anacrusis* spp. (Tortricidae), *Pythonides proxenus, Thracides phidon, Saliana* longirostrisDHJ01 y *Milanion marciana* (Hesperiidae), etc. La influencia de la zona de Bosque Precafetal extiende por lo menos bajo a Sector Mundo Nuevo.

Es claro que la entrada de la fauna (y asumimos la flora) del bosque lluvioso esta infiltrando al bosque seco en esta zona de ACG, haciendolo uno de los pocos lugares en Costa Rica donde uno puede estudiar, observar y entender este caldo de evolucion. En casi todo el resto del pais, cuando bosque seco y bosque lluvioso juntan, uno o ambos de los ecosistemas ha estado convertido a agropaisaje, asi destruyendo este importantisimo ecosistema hibrido y sus consequences en la evolucion de especies.

Y esto ha producido cosas muy complejos. Por ejemplo la mariposa que todo el mundo conoce como *Automeris postalbida* (Saturniidae) y que ocure por todos bosques lluviosos del pais, tambien ocurre en Bosque Precafetal (Fig. S4). Sin embargo, por analysis de las "*Automeris postalbida*" de ACG, el inventario ha descubierto que solamente en ACG hay 5 especies adentro este nombre, 4 de que son no descritas por la ciencia. No es posible discriminarlos sin sacar su "codigo de barra" de su ADN. Esta situacion aplicara a muchos de los especies de insectos en el Bosque Precafetal (por un ejemplo, vea Janzen et al 2012).

Figura S4. La larva (oruga) de la mariposa grande tradicionalmente conocido como *Automeris postalbida* (Saturniidae) y ocurriendo en los bosques lluviosos de Costa Rica. Sin embargo, por medio de sacar los "codigos de barra" de la ADN de estos en el Bosque Precafetal y otros partes del bosque lluvioso de ACG, sabemos que solamente en ACG hay 5 especies bajo este nombre, y es imposible a saber cual de estos es la larva en la foto sin sacar el codigo de barra de ADN de ella (Bosque Precafetal, 29 agosto 2012).

4. Plantas. No hemos tenido chance empezar el inventario de especies de plantas del Bosque Precafetal. Pero hay tres cosas obvias y sobresaliente el momento que uno entra este bosque.

a) No importa que es la misma zona de vida (en el mapa) que son dos otros parchecitos de esta zona de vida en la Cordillera Guanacaste — uno en Volcan Orosi y el otro en Volcan Cacao — los tres sitios cada uno tiene una flora bien diferente en especies y propoporciones de ellos. Sospechamos que es debido al (*i*) tamaño differente de cada uno (Bosque Precafetal es lo mas grande), como ocurria con tres islas en el mar de tres tamaños, (*ii*) diferente historias de perturbacion natural (Bosque Precafetal es claramente 2 y tal vez 3 diferentes edades de succession, con el parte este siendo lo mas viejo), (*iii*) la proximidad de perturbacion reciente del humano por medio de convertir bosque seco a agropaisaje (Orosi es lo peor, Bosque Precafetal es lo major), y (*iv*) proximidad del "continente" que es fuente de las semillas de colonizacion despues de una erupcion (Bosque Precafetal es beneficiado por ser a la par con mucho mas area de bosque seco y bosque lluvioso que es el caso con los otros parchecitos).

b) Hay gran diferencia en especies entre el parte oeste y el parte este del Bosque Precafetal, no obstante que son de la misma elevacion y lluvia (aparentamente). Por ejemplo, hay dos especies del alto arbol comun *Billia* (Sapindaceae), uno con frutos redondas tamaño bola de tenis (*Billia rosea*) que aparentamente occure solamente en el lado este, y el otro con frutos mas elongadas (como un huevo de gallina) (*Billia hippocastanum*) que aparentamente occure solamente en el lado oeste. Digo "aparentamente" por que no he tenido chance hacer una examinacion o inventario detallada del sitio. Igual llamativo son los enormes encinos (*Quercus oleioides*, Fagaceae) adentro lo que aparece ser regeneracion mucho mas joven que ellos (Fig. S5, S6) en la porcion oeste del Bosque Precafetal.

c) Igual como en Volcan Cacao, en el Bosque Precafetal existe una poblacion sano de lo muy escaso arbol *Parmentiera valerii* (Bignoniaceae), que es endemica a la Cordillera Guanacaste y Cordillera Tilaran (INBio, http://darnis.inbio.ac.cr). Este arbol grande de flores cauliflorus blancas polinizadas por murcielagos era visto en mucho peligro de extincion hasta el descubrimiento por accidente por mi de la poblacion sano en Volcan Cacao en 1987 (antes que 1987 era conocido por solamente un arbol vivo en la Meseta Central, donde hoy dia hay cafetal).

Figura S5. Uno de los enormes encinos (*Quercus oleoides*) que ha sobrevivido un minimo de 200 años en el parte oeste del Bosque Precafetal, Sector Pailas, ACG (2 agosto 2012).

de edad (mire Fig. S5 para uno vivo) en el parte oeste del Bosque Precafetal (2 agosto
2012).

Figura S6. Un encino (*Quercus oleoides*) muy viejo (200+ años) que reciente ha muerto de edad (mire Fig. S5 para uno vivo) en el parte oeste del Bosque Precafetal (2 agosto 2012).

c) Y aqui en este bosque primario ocurre plantas muy, muy extrañas. Por ejemplo alli encontramos el primer record para Balanophoraceae de la Provincia Guanacaste (Fig. S7), una planta rara que hemos encontrado solamente en la Peninsula de Osa y el lado norte de

Figura S7. Planta totalmente parasitica (*Helosis cayennensis* en la Balanophoraceae) en las raices de otras plantas "normales" (soto bosque del Bosque Precafetal, 29 agosto 2012).

Rincon de la Vieja. La planta es totalmente parasitica en los raices de otras plantas, y asi no es verde con clorofilo, sino que saca todo su alimentacion de otras especies de plantas normales. Tambien hay cosas lindisimas que son conocido de sitios mas lluviosos pero que no esperaba en este lado mas seco (Pacifico) de los volcanes (Fig. S7).

Figura S8. Por la izquierda es un fruto maduro de una especies de *Cojoba costaricensis* (Fabaceae) similar a *Pithecellobium dulce* (comun de Costa Rica a Mexico) pero el fruto de este especie es caudiflorus, saliendo directamente del tronco bajo, en vez de una ramita en el docel. Las semillas negras son listos a tragar con el aril dulce y blanco por un pajaro grande como un tucan o pavon (y mas tarde regurgitar), y el rojo de la vainica es una bandera anunciando la presencia de un fruto sazon.

Por la derecha son los frutos maduros de una especie de planta epiphitica en el docel, *Anthurium brenesii* (Araceae).

Ambos de estas especies ofrecen alimentacion a los pajaros y mamiferos frugivoros de este bosque primario en el este del Bosque Precafetal (29 agosto 2013).

d) Mientras que no son plantas, mucha gente tratan hongos tipo champiñon como si fueron plantas y el sotobosque del Bosque Precaftal lado este es hogar a unos muy extraños y muy desconocidos a nosotros (por ejemplo, Fig. S9). Otra vez, debido a que son tal vez especies de bosque lluvioso creciendo en el lado Pacifico y bajo la influencia del verano largo, es muy probable que ellos tienen fenologia bien extraño tambien.

Figura S9. Hongo muy extraño con biologia desconocido en el sotobosque del lado este del Bosque Precafetal (29 agosto 2012).

En cuanto diferences en bosque sobre distancias muy cortas, es muy llamativo que el bosque inmediatamente al norte del sitio de las pailas naturales (el cuello del perrito en Fig. S2) es mucho mas viejo que el bosque en el oeste del Bosque Precafetal. Sospechamos que este diferecia es debido a una historia de perturbacion muy diferente que la del Bosque Precafetal. Simultaneamente hay diferencias muy llamativos entre el docel en el oeste (Fig. S10) y el este (Fig. S11, S12). Igualmente la composition de especies y las formas de arbustos, bejucos y arbolitos (adultos) en el sotobosque es muy diferente en los dos extremos oeste y este del Bosque Precafetal, con el este bien similar a partes del sotobosque en bosque original en el lado norte de Volcan Rincon de la Vieja (Fig. S7–S9, S13).

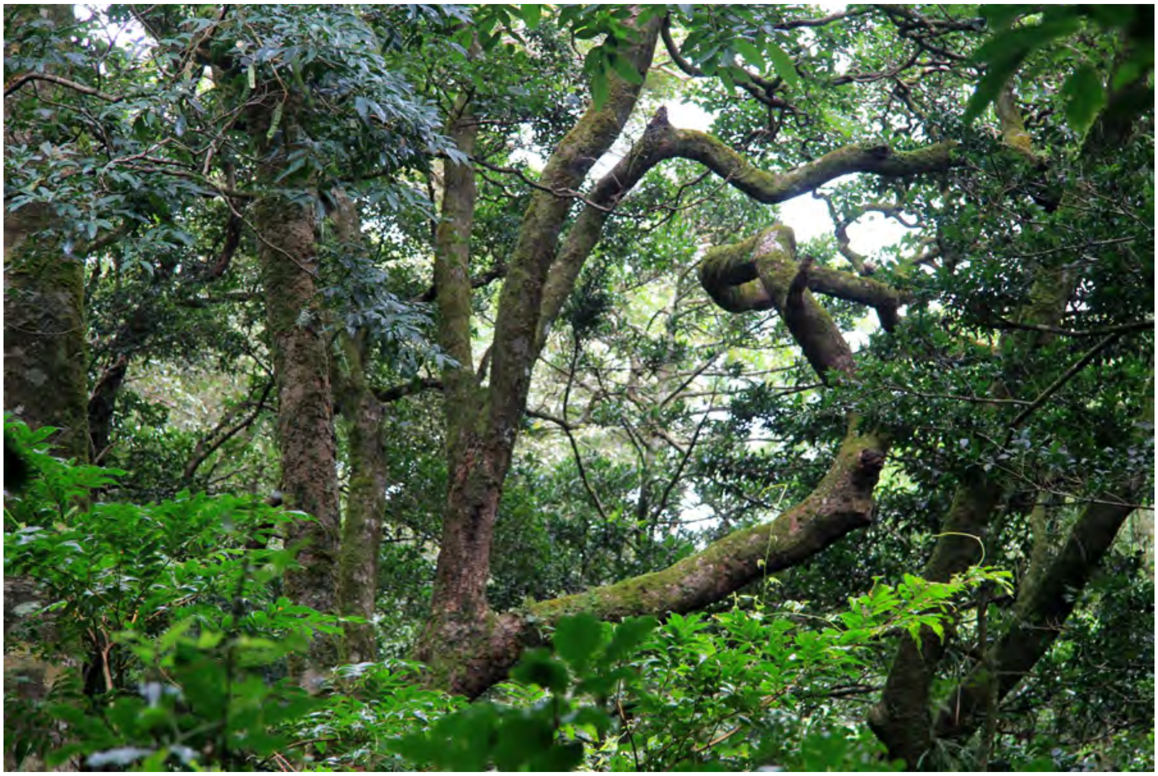

Figura S10. Vista del docel representativa en el parte mas al oeste del Bosque Precafetal, un bosque que es mas seco, mas abierto, mas similar a bosque original en Guanacaste que el bosque lluvioso a unos 2–3 km al este (Sector Pailas, ACG, 2 agosto 2012).

Figura S11. Vista del docel respresentativa (para comparison con Fig. S10) en el parte mas al este del Bosque Precafetal, un bosque que es muy similar a bosque lluvioso de San Carlos, lado norte de Rincon de la Vieja (29 agosto 2012).

Figura S12. Un arbol adulto y representativa de unos 35 m altura en el bosque primario en el este del Bosque Precafetal, expuesto al sol y la camera por la caida/muerte natural de otro arbol alto creciendo a la par (29 agosto 2013).

Figura S13. Vista caracteristica del sotobosque a unos 800 m elevacion en el lado este del Bosque Precafetal. Es muy abierto por la sombra denso (como en Fig. S11) del docel a 30–40 m altura, una sombra que hace la vida dificil para plantulas y arbustos, y resulta en una taza de crecimiento y recuperacion de daño mucho mas bajo que es el caso en el sol de un claro o margen del bosque con el agropaisaje (29 agosto 2013).

5. Vertebrados.

A) Pajaros. No soy pajarologo y costara bastante tiempo observando las especies de pajaros en todos partes del Bosque Precafetal para entender las especies, sus ubicaciones, sus dependencias en tipos de comida, y sus movimientos migratorios por arriba y por debajo en elevacion como cambian las estaciones. Lo que sabemos de muchos estudios de este ultimo fenomeno por La Selva (Boyle 2011) y Monteverde y sus bosques a la par (Powell and Bjork 2004), sabemos bien que varios de los pajaros de las elevaciones arriba tienen que estacionalmente bajar a bosques de bajo y intermedio elevaciones, como Bosque Precafetal, para buscar comida. En este caso especifico, el Bosque Precafetal tiene la ventaja que el transecto de alto hasta 800 m o mas bajo es totalmente intacto, sin perturbacion de aprovechamiento o potreros viejos, como no es el caso por Monteverde.

Es cierto que una investigacion de cerca de los pajaros de Bosque Precafetal va a revelar varios especies inesperado por Guanacaste, especies “del lado Caribe”, que estan utilizando la intergradacion entre este bosque y el bosque seco mas bajo todavia. Igualmente a las plantas y mariposas, van encontrar fuertes diferencias en la composicion de la comunidad de pajaros entre el lado oeste y el lado este del Bosque Precafetal. Otra vez, esta transicion entre bosque seco y bosque lluvioso en bosque original va a decirnos mucho sobre la evolucion de poblaciones de especies moviendo del mojado a lo mas seco, igualmente como es el caso con mariposas (Janzen et al 2012).

Y puedo añadir que en mis pocos visitas al lado este del Bosque Precafetal, ya he visto dos especies de pajaros de tamaño medio, conspicuos y desconocidos a mi, que en mis 50 años de conocer los bosques secos de ACG nunca he encontrado.

B) Vida aquatica. Hay 5 rios que transectan Bosque Precafetal (y 8 rios que transectan el "perrito" en Fig. S2) (Fig. S14, S15). Estos rios de agua pura son condensadas de los nubes. Por el hecho que nunca han estado cultivos o potreros en su cuencas, son totalmente libre de pesticidas y otros agrochemicas (igual como los que fluen de Volcan Orosi y Volcan Cacao) y asi son unicos para los rios de Costa Rica (Standley and Sweeney 1995). Tambien no tienen vados para vehiculos (no hay caminos transitables) y asi no contienen el barro liquido y aceites de lavar carros por debajo en cruzando.

La relevancia es que estos rios descargan al Rio Colorado y Rio Blanco, que llegan directamente a usuarios por Liberia y hasta el Rio Tempisque. La fauna aquatica no ha sido estudiado en estos rios virgenes, pero es una facil prediccion que van estar muchas especies desonocidos (nuevos, sin nombre) por encontrar, incluyendo peces, amphibios, hongos y insectos. Simulateamente, estos organismos aquaticos son exelente indicadores del salud ecologica del Bosque Precafetal, que es especialmente en estos decadas de observar y monitorear el impacto en mocion de la perdida de nubes de la Cordillera Guanacaste, provocado por el cambio climatico.

Estos rios son tambien fuentes de agua para beber para la fauna de vertebrados terrestres durante el largo verano en el Bosque Precafetal y su vecindad, son el sitio de deposito de los huevos y ranacuajos de ranas (Fig. S16) y sapos, hoy dia muy amenazado con extinction, primero por un hongo patogenico *Batrachochytrium dendrobatidis* que es introducido a Costa Rica (de Australia), y segundo con el aumento de la temperatura por el cambio climatico que facilita el hongo.

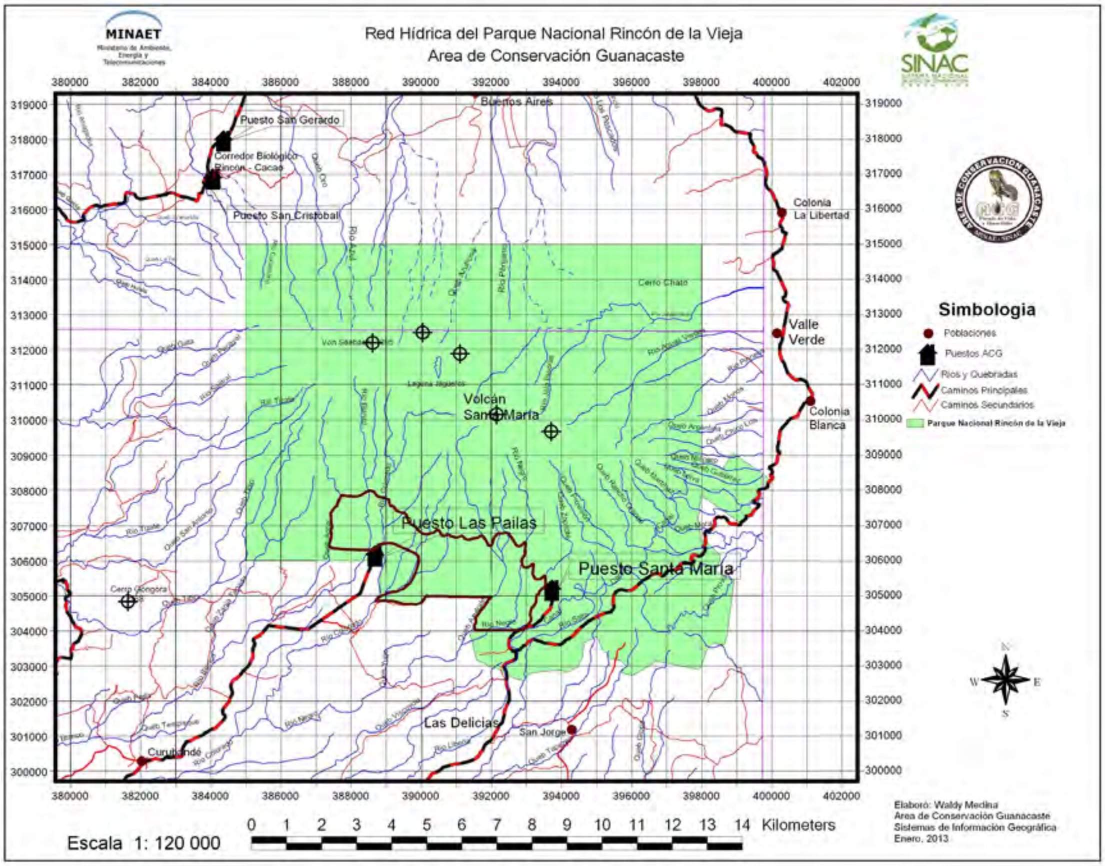

Figura S14. Rios y cuencas que nacen en Parque Nacional Rincon de la Vieja (verde), y que cruzan el Bosque Precafetal (el cuerpo del "perrito" dibujado en rojo en el parte sur de PNRV (Waldy Medina, ACG, 1 febrero 2012).

Figura S15. Dos de los 5 rios que transectan el Bosque Precafetal y desembocan al Rio Colorado para llegar a Liberia. Estos rios de agua pura estan totalmente libre de pesticidas y otras contaminantes del agropaisaje o vados para carros (29 agosto 2012).

Figura S16. Rana (*Eleutherodactylus podiciferus*) en la hojarasca del Bosque Precafetal. Todos ranas y sapos en las volcanes de Costa Rica estan ahora en peligro de extincion debido al hongo *Batrachochytrium dendrobatidis* y el aumento de temperatura debido al cambio climatico. Este especie de rana es indicador de bosque primario y no aprovechado; antes que este foto, no era conocido al norte de Monteverde y nunca de la Provincia de Guanacaste (Savage 2002) (Bosque Precafetal, 29 agosto 2012).

C) Culebras y lagartijas. No existe ningun inventario de culebras y lagartijas del Bosque Precafetal. Sin embargo, por la presencia de otra taxa en el parte este, asumimos que va a tener un rico fauna que es caracteristicamente visto como de bosque lluvioso. Igualmente, el lado oeste muy probable contiene una mescla de especies de bosque seco (por ejemplo, el cascabel) y del bosque lluvioso (por ejemplo el terciopelo). El unico lagartija encontrado en una visita rapida era un individuo de *Norops biporcatus* (Fig. S17) que amigablemente quedo quieta para su foto. Este especie es caracteristica del sotobosque de bosque primario en bosque lluvioso y extiendo a manchas de bosque que son intergradaciones entre bosque lluvioso y bosque seco (Savage 2002).

Figura S17. La lagartija *Norops biporcatus* en la sombra del sotobosque de bosque primario en el este del Bosque Precafetal, siendo fotographiado por una cientifica y ecoturista (29 agosto 2013).

6) Mamiferos.

Los mamiferos que viven adentro, y pasan por, el Bosque Precafetal nunca han estado inventariado, y todavia estan recuperando de la cazaria que impacto estos bosques antes que fueron decretados parque nacional. Es decir, un bosque primario puede aparecer “intacto” pero ser un desierto de la punta de vista de su fauna, si es sujeto a cazaria intensiva. El bosque original tropical, y especial bosque lluvioso, es muy pobre en comida para mamiferos y ellos recuperan muy lerdas de cazaria y de otros perturbaciones a sus poblaciones, en fuerte contraste a zonas extratropicales que pueden sostener mucho depredacion por el humano. Sin embargo, una visita rapida al Bosque Precafetal mostro claramente unas cosas interesante sobre los mamiferos grandes. Mientras que no encontramos otros que lo que mencionado por adelante, es claro que hay perezosos, puercoespines, tepisquintles, guatusas, ardillas, roedores varios, y uno o dos especies de chanchos — sainos, y casi por seguro, chancho de monte o javali (Fig. S18).

a) Monos. El Bosque Precafetal es casa a unas dos o mas tropas de mono congo (*Alouatta palliata*), una tropa por lo menos de mono cariblanco (*Cebus capucinus*) y una tropa grande (por lo menos 20 individuos) de mono araña (*Ateles geofreyi*) (Fig. S18). Los primeros dos son visto como “comun” en Costa Rica, pero mis observaciones personales es cada año son mas escasos fuera de areas protegidas, hasta desapareciendo de areas protegidas pequeñas (pero sobreviviendo en areas silvestres grandes). El mono araña es tan escaso por cazaria y destruccion de bosque en los tropicos que ahora es en la lista mundial de especies en peligro de extincion.

Figura S18. Un mono araña adulto (*Ateles geofreyi*) en el docel del parte este de Bosque Precafetal. El mono araña vive en grupos grandes pero dispersadas y fueron por lo menos 11 individuos en la fraccion de la tropa que contuvo este individuo. Estos monos arañas llamaron la atencion porque no estaban agresivo a nosotros observando ellos, asi indicando que no han tenido malas experiencias con el humano. Este tropa, o un otro grande tropa, circula hasta el bosque al norte de las pailas de barro herviendo por Estacion Pailas de ACG, en la entrada a Sector Pailas, y es igualmente manso y calma (29 agosto 2012, 9 enero 2013).

b). Jaguares. En terminos generales y pensando en todo ACG, parece que ACG contiene una poblacion sano y reproduciendo de jaguares, y hay evidencia de ellos encontrado en todos habitats y ecosistemas. Asi, entonces no es sorprendiente que hay por lo menos uno muy grande que circula en el lado este del Bosque Precafetal, dejando sus huellas en los senderos donde hay barro suave (Fig. S19). Jaguares comen de todo, desde frutos caidos y carroña hasta presa grande recientemente capturado como tortugas marinos en las playas de Sector Santa Rosa hasta caballos de trabajo por Estacion Biologico Cacao en Volcan Cacao. En Sector Murcielago de ACG encontramos una danta medio muerto, que murio, por el ataque por un gato grande, sea puma o jaguar (mas probable un jaguar).

Figura S19. Arriba. La huella de la pata posterior de un jaguar muy grande, tal vez pesando 100 kilos o mas, puro centro del sendero a 1100 m elevacion en el este del Bosque Precafetal. Se ve muy claramente la impression de la palma por la derecha y cuatro dedos por la izquierda.
Figura 19. Abajo. La mano de Petrona Rios, parataxonoma, puesto apenas cubriendo la huella con su palma en la impression de la palma del gato, y sus cuatro dedos en las cuatro impresiones grandes de los cuatro dedos del gato pesado.

c.) Sainos y javali. Mientras que pequeños grupitos de sainos (*Tayassu tajacu*) son encontrado ocasionalmente en muchas partes de ACG, y asumimos que estan manteniendo su poblacion (pero a una densidad mucho mas bajo que en las 1970–1980s), por muchos años era creido que el unico manada de javali o chancho de monte (*Tayassu pecari*) en ACG era en Volcan Cacao-Orosi (Fig. S20), y unos pocos individuos circulando hasta Sector Santa Rosa. El javali es sin duda el mamifero grande mas amenazada con extincion en Costa Rica; dudo que hay mas que 10 manadas en Costa Rica. Sin embargo, los encargados de sector de Sector Pailas y Sector Santa Maria ahora han reportado una manada de javali circulando por la zona de Estacion Pailas y el camino entre Estacion Pailas y Estacion Santa Maria, camino que pasa directamente por el parte central del Bosque Precafetal. Una manada de javali necesita un enorme area de muchos kilometros cuadrados sobre que pueden circular en busqueda de comida (en gran parte cosechas de frutos y semillas de arboles adultos. caidos al sotobosque en bosque primario; la mitad o mas del parte este del Bosque Precafetal es habitat de muy alto calidad para ellos. Afortunadamente la cazaria es ya prohibido por ley en Costa Rica y gradualmente esto va a darles el chance a sobrevivir y ampliar su manada.

Figura S20. Una porcion pequeña de la manada de 200+ chanchos de monte, o javali (*Tayassu peccary*), que ocupa Volcan Cacao en Sector Cacao de ACG. Asumimos que la manada de javali que ocupa Rincon de la Vieja y circula por el Bosque Precafetal aparece igual, pero no tenemos fotos de ellos (photo: Mariano Pereira, Estacion Biologica Cacao, 27 noviembre 2003).

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2 febrero 2013

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Supplemental File S2

First three month report for laboratory analyses

3 June 2016. Daniel H. Janzen on behalf of GDFCF. djanzen@sas.upenn.edu

First (three month) interim report on laboratory barcoding progress for the project:

"SAPI for Geothermal Development Loan: Study on improvement of Environmental Monitoring methodologies for geothermal development in Costa Rica Agreement #0328497.

Dr. Daniel H. Janzen, on behalf of Guanacaste Dry Forest Conservation (GDFCF) fund, as based on recent data from the Canadian Centre for DNA Barcoding (CCDB), now known as Centre for Biodiversity Genomics) of the Biodiversity Institute of Ontario (BIO), University of Guelph, Guelph, Canada.

Professor Paul Hebert of the Department of Integrative Biology of the University of Guelph is the Director of BIO and the CCDB, and now, CBG.

Brief background:

The target philosophy was to begin biomonitoring the PL12 geothermal perturbation site within the ICE Pailas II (Dos) project, on the southwestern lower slopes of Volcan Rincon de la Vieja, from its initiation onward. The ideal would have been to have a year of ground zero calibration sampling before the forest-opening-perturbation, but the biomonitoring opportunity needed to wait until ICE (Instituto Costaricense de Electricidad) collaboratively reached out to ACG (Area de Conservacion Guanacaste) and presented its project plans on 23 August 2013, about 2 weeks before the actual forest clearing began. On 26 September 2013, a Thursday, the first three of 9 Malaise traps (Fig. S1) were set on two lines at 50 m intervals perpendicular to the margin of geothermal platform PL12 in Pailas Dos (Pailas II) of ICE, and a third line equally but beginning at the access roadside (see figure below), with all traps set finally by November. This meant that three traps (#3, #4, #7 in our numbering system) were at the very margin of the perturbation, three traps (#1, #6, #9) were 100 m deep into the forest understory, and three traps (#2, #5, and #8) were at intermediate distance from the perturbation (Fig. S2). This also meant that a "trap-year" did not begin on the same date for all 9 traps, with the consequence that to have one full synchronized year for all traps meant that some traps had to be analyzed for more than a year.

The core question is "What measurable changes in the species-rich insect community are a consequence of this kind of perturbation to a relatively pristine tropical intermediate elevation forested site?" and "Can these changes be used to guide the planning of such projects in sensitive and conservable natural forest, as well as aid in determining what mitigation costs or measures are appropriate?"

This site is immediately adjacent to, and its forest continuous with, the southern boundary (black straight line above PL12 in the diagram below) of the forest of Sector Pailas and Sector Santa Maria of Area de Conservacion Guanacaste, a UNESCO world heritage site. As such, the results may aid in guiding planning for possible eventual introduction of such an industrial project into an explicitly conserved unique forest, and/or serve to suggest what may be the "at-a-distance" impact of such a project near but outside of the conserved forest. In short, the focus is being placed on both measuring direct forest impact and especially, impact on the margin of a large conserved wildland.

Figure S1. Malaise trap #2, PL12.

Figure S2. Pattern of placement of 9 Malaise traps to biomonitor the footprint of PL12 at Pailas II (Pailas Dos).

Every Thursday then, from 3 October 2013, through now, June 2016 (and to be continued until early October 2016), the 9 trap collecting bottles have been collected and frozen in GDFCF facilities in Sector Santa Rosa of ACG (a 1.5 hour drive northwest of PL12) (more exact details to be demonstrated in the final project report). Owing to shortage of available funding from JICA, it was then decided by JICA in late 2015–2016 to at the least analyze only the first year of this biomonitoring with insects for at least the three traps at the edge of the perturbation (#3, #4, #7), and the three traps from deep inside the adjacent forest (#1, #6, #9) (it is anticipated that the final project report will recommend continuing these sample analyses indefinitely once a ground zero has been established). This was formally agreed on 3 March 2016, and actual Sanger sequencing and laboratory subsequent processing at CCDB/CBG Canada began on 1 April 2016.

The analysis process consists of

a) extracting each insect from the weekly sample in ETOH, sorting by eye to suspected order, Sanger sequencing each insect in the trap sample week by week, pinning the larger insects and storing the small ones in individual Lysis plate wells,

b) documenting each insect logistically and then taxonomically to whatever possible level (always Order but generally below),
c) assigning its DNA barcode a species-based species-unique distinguishing BIN code (e.g., BOLD:ACB6865), and if that species already exists in BOLD, capturing its taxonomically detailed information,
d) digitally photographing and permanently storing the insect, and
e) placing the data in BOLD, the BIO/CBG semi-public data management system.

Subsequent interpretive draft analysis of the results both within PL12 and comparatively with previous ACG Malaise trapping results, will be performed by BIO/CBG (Hebert laboratory) and GDFCF (Janzen) between August and December 2016, and finally reported in late January or early February 2017.

On 20 March 2016 the 7 boxes containing the six designated one-year Malaise trap samples (late 2013–late2014) were readied for shipping from Area de Conservacion Guanacaste (ACG), following obtaining all relevant research permits, CONAGEBIO permit (R-008-2016-OT-CONAGEBIO), and export permits (slowed by ICE–CONAGEBIO discussions and Easter Week government slowdowns in Costa Rica and Canada). These permits are issued in the name of Dr. Daniel Janzen on behalf of the Guanacaste Dry Forest Conservation Fund (GDFCF).

On 22 March the samples were DHL-couriered to the CCDB/CBG and finally cleared Canadian customs on 30 March, meaning that they were out of the freezer at room temperature for 10 days. There is no evidence that the sequencing success was impacted by this transition; the individual half-liter containers of weekly samples had been lightly drained of their 95% ETOH before shipping, but the samples were still ETOH-wetted on arrival, and were immediately re-filled with 95% ETOH and frozen until processed. A single 95-well test lysis plate was run on 30–31 March, and 93 of its 95 specimens sequenced successfully. The entire shipment processing of six traps was then initiated on 1 April 2016. The first third of the laboratory payment to GDFCF, $120,000, arrived in the GDFCF account on 19 April 2016 and was immediately paid to the CCDB/CBG. Here we briefly report the first interim numerical results.

Interim results:

The BIO laboratory elected to direct their full DNA barcoding capacity onto the very large number of weekly samples (6 × 52 = 312), thereby temporarily postponing other ongoing barcoding projects. As a result, at the time of this writing in late May 2016, the total task has progressed substantially further and faster than anticipated to be possible. 99.4% of the samples have been sorted to order and barcodeable specimens have resulted so far in 1,248 lysis plates, each containing 95 wells with one insect part or whole insect each (118,560 insects to date). 59.2% of these plates have been sequenced to date. 41.27% of these plates have had their specimens run through the BIN assignment process, but with many tens of thousands of specimens yet to be treated. About 35% of the specimens actually have BINs assigned now. See attached CBG lab report from 20 May 2016. At the least, another 2 months are required for all the lab processing analysis to be completed to the stage where full comparisons will be possible among the traps and weeks of the year (and progression from the start of the perturbation), and between the traps (and the PL12 site taken as a whole), and with the 30,000+ species-level BINs available from ACG as a whole. These analyses will require several months at least, once the entire laboratory operation is completed by August (it is hoped).

Methodologically, the BIO laboratory chemical processing and application of BIO computerized routines for the BIN code, identifying contaminants, and machine-based taxonomy has moved ahead smoothly without speed bumps, though about twice as fast as anticipated (owing to the laboratory unexpectedly being able to turn all of its manpower and machinery power onto this project).

For Dr. Janzen, well-familiar with “reading” the results of ACG barcoding analyses, numerous trends are already suggested by the incomplete data in hand, but it is not wise to attempt to second-guess them now, when in another month or two the complete data set will be available with which to discuss and construct.

Winter 2017 PL12 Malaise results
21 Feb 2017 Alex Smith

10 abril 2014: PL12


10 abril 2014

FOREST UNDERSTORY

PL12-1

PL12-2

PL12-6

PL12-9

EDGE

PL12-7

PL12-3

PL12-4

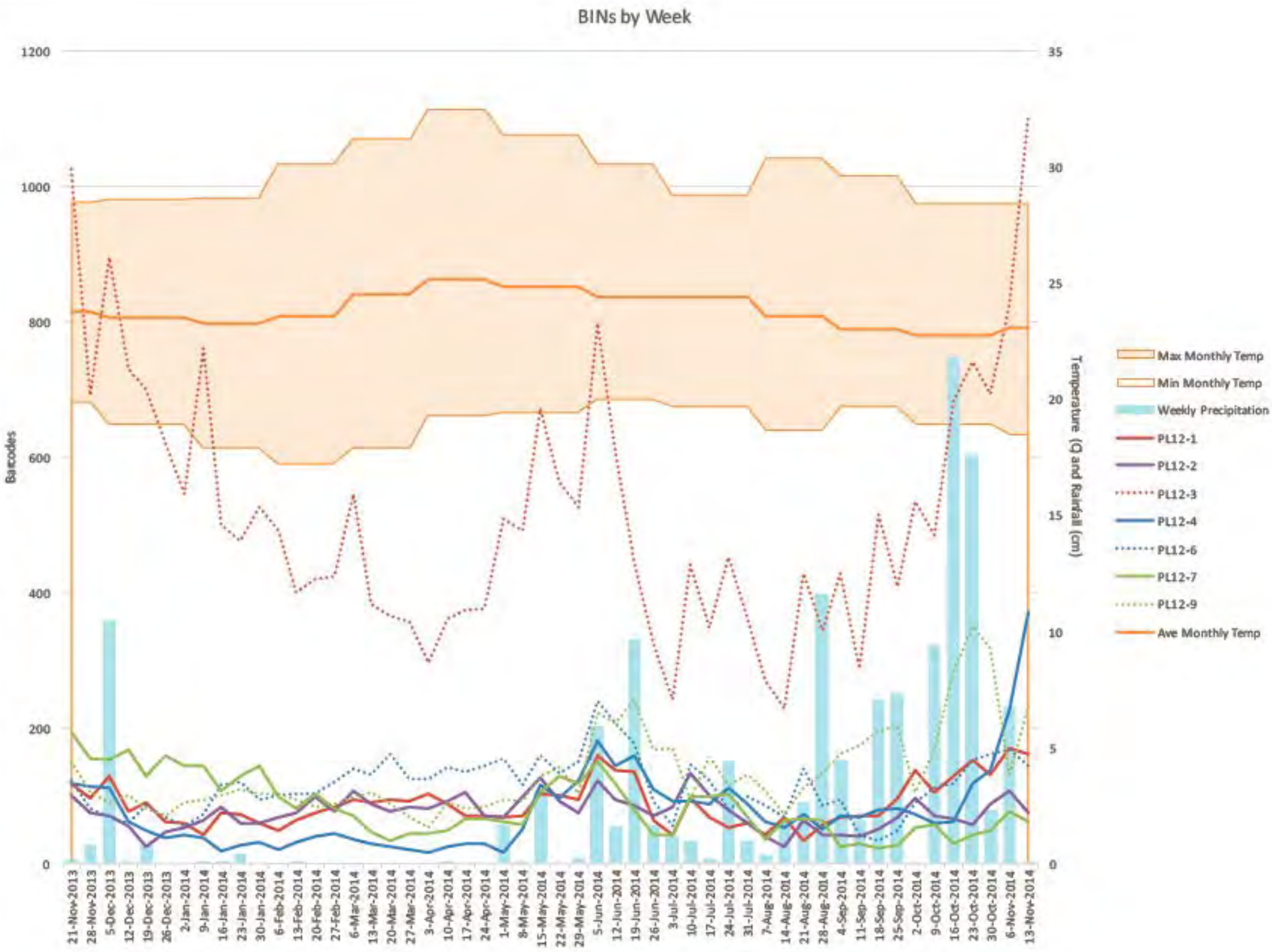
Images dec16
PL12, Pailas II

Newly installed
second generation
Malaise traps , nov16

Alex Smith, fotos

1. **Does diversity and abundance vary by trap?** **(i.e. are traps different?)**

2. **Does diversity and abundance vary through the year?** **(i.e. are weeks different?)**



BINs by Week
Barcodes
Temperature (C) and Rainfall (cm)
Max Monthly Temp
Min Monthly Temp
Weekly Precipitation
PL12-1
PL12-2
PL12-3
PL12-4
PL12-6
PL12-7
PL12-9
Ave Monthly Temp

Constrained ordination via Redundancy analysis (RDA)

on the BIN diversity and abundance of the most abundant Insect orders collected for 52 weeks from seven Malaise traps.

Analysis

- **Reduncancy Analysis (RDA) is a style of multivariate analysis. "Redundancy" is synonymous with explained variance (Legendre and Legendre 2011 p. 579).**
- **An extension of PCA.**
- **Canonical vectors are linear combinations of the response variables.**
- The RDA is performed on the (transformed) species data to test for relationships with explanatory variables. Then biplots displaying the relationships of the species to the explanatory variables are produced.

Methods

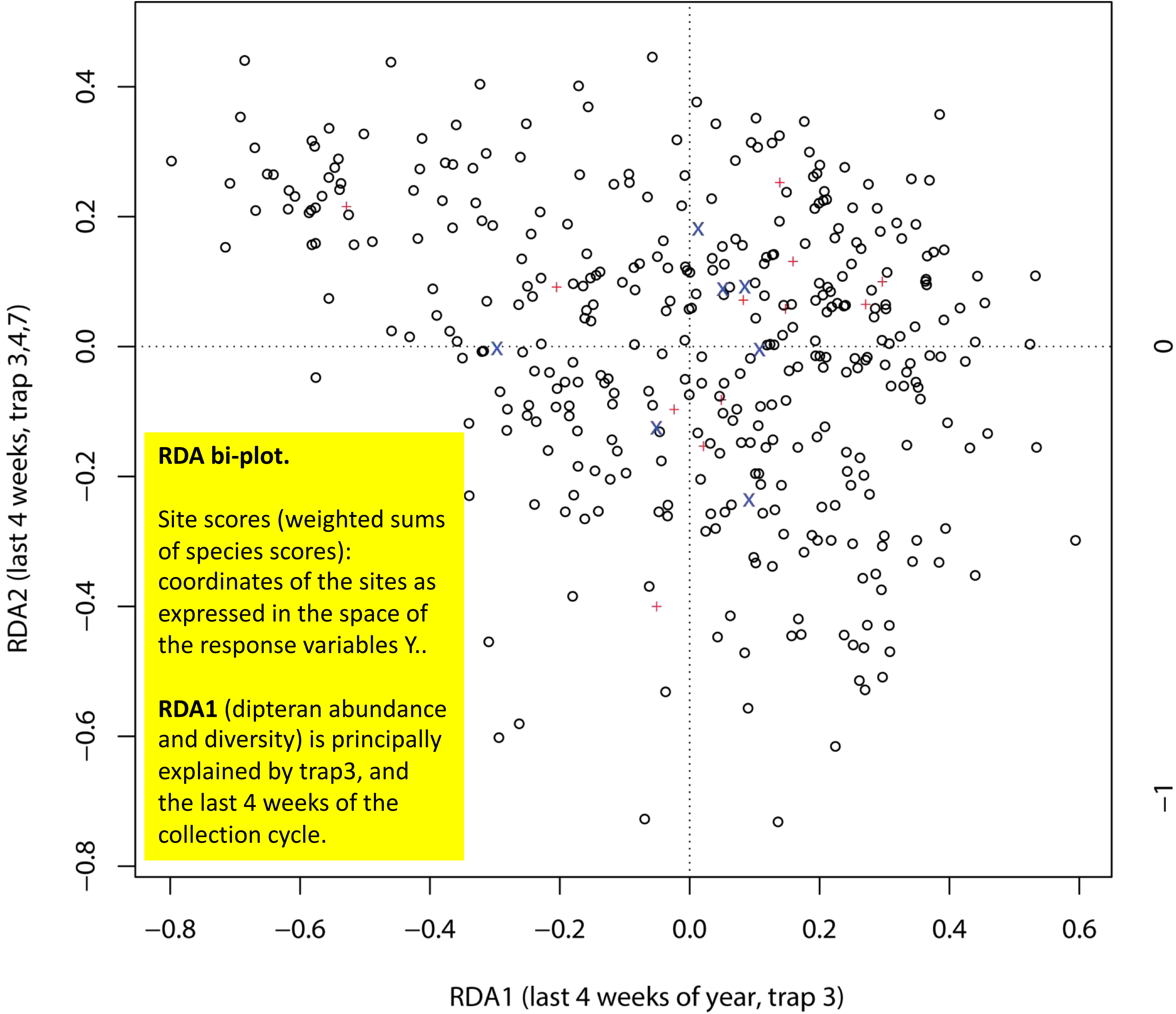
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1		Diptera	Coleoptera	Hymenopt	Lepidopter	Hemiptera	Other	DipteraAbu	Coleoptera	Hymenopt	Lepidopter	Hemiptera	OtherAbur	week	trap	Interior0_Edge1	
2	w01.c1	50	20	11	18	11	7	76	23	14	32	13	12	w01	c1	0	
3	w02.c1	31	22	6	23	10	4	57	24	8	34	10	7	w02	c1	0	
4	w03.c1	54	30	10	14	11	10	111	41	14	17	16	13	w03	c1	0	
5	w04.c1	24	10	9	15	11	7	34	14	11	17	52	9	w04	c1	0	
6	w05.c1	36	19	10	8	9	7	45	26	21	9	9	13	w05	c1	0	
7	w06.c1	12	16	10	12	5	6	28	18	13	13	6	10	w06	c1	0	
8	w07.c1	12	15	9	8	4	11	26	20	14	8	4	15	w07	c1	0	
9	w08.c1	8	11	6	4	6	7	16	15	7	4	9	8	w08	c1	0	
10	w09.c1	19	20	9	6	11	10	36	28	27	13	11	14	w09	c1	0	
11	w10.c1	11	21	18	7	7	9	14	28	28	9	19	11	w10	c1	0	
12	w11.c1	6	22	8	9	9	6	17	32	18	15	19	9	w11	c1	0	
13	w12.c1	7	16	7	5	9	4	10	23	13	9	20	8	w12	c1	0	
14	w13.c1	10	21	17	4	7	5	13	26	21	4	33	8	w13	c1	0	
15	w14.c1	11	24	18	7	7	7	15	30	40	7	57	8	w14	c1	0	
16	w15.c1	8	32	18	9	9	8	11	51	39	13	69	12	w15	c1	0	
17	w16.c1	9	33	17	13	10	11	12	48	38	26	64	13	w16	c1	0	

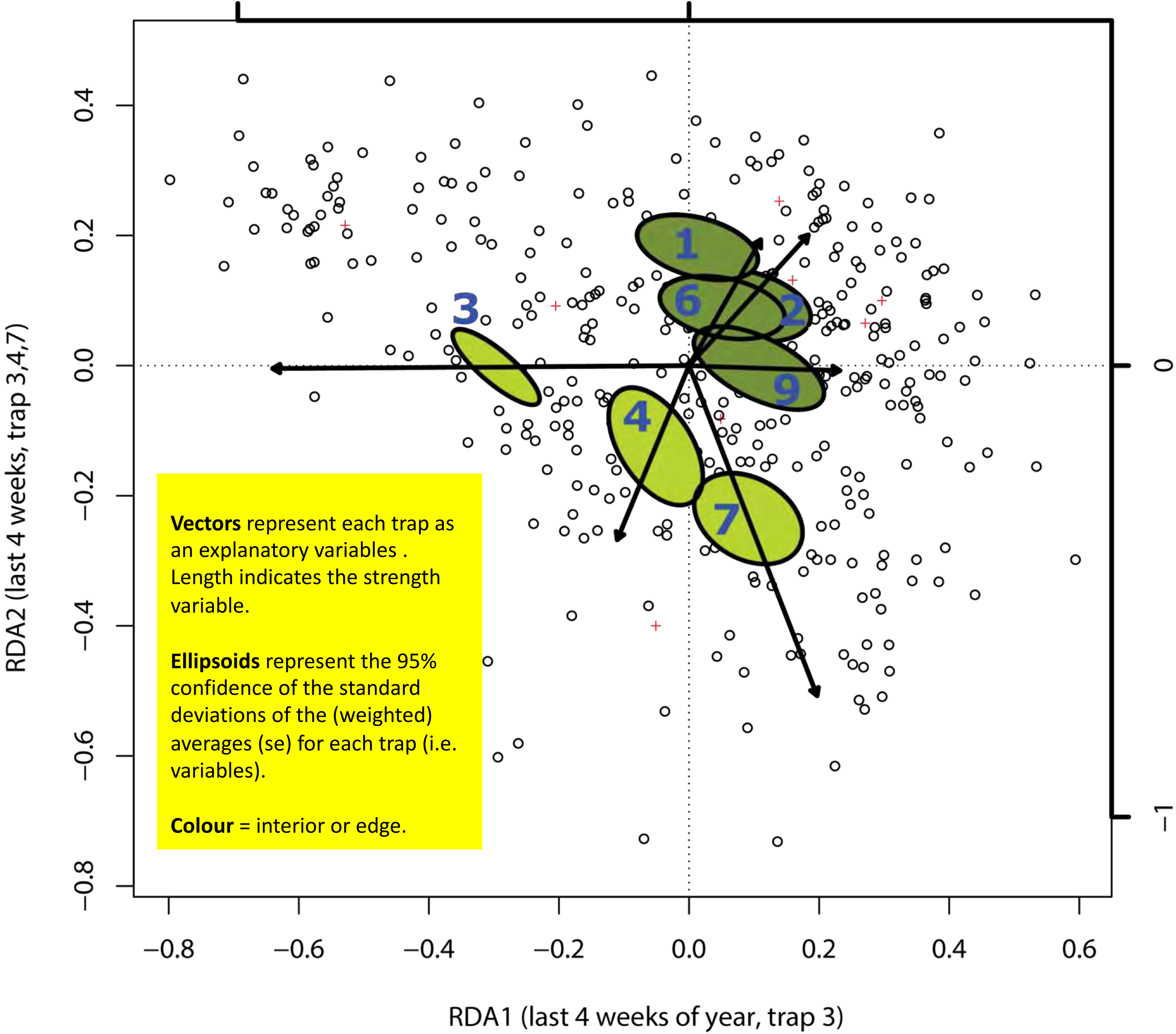
- **Data are in two matrices**: Response variables (Diversity and abundance of 5 orders + other (div and abundance)) and explanatory variables (trap, week and interior/edge state)
- **Transform** abundance and diversity data via the Hellinger transformation (Legendre & Gallagher 2001)
- **RDA** on the transformed data. This is a method of combining regression and PCA. It is a multivariate multiple linear regression followed by a PCA of the table of fitted values. (page 154).

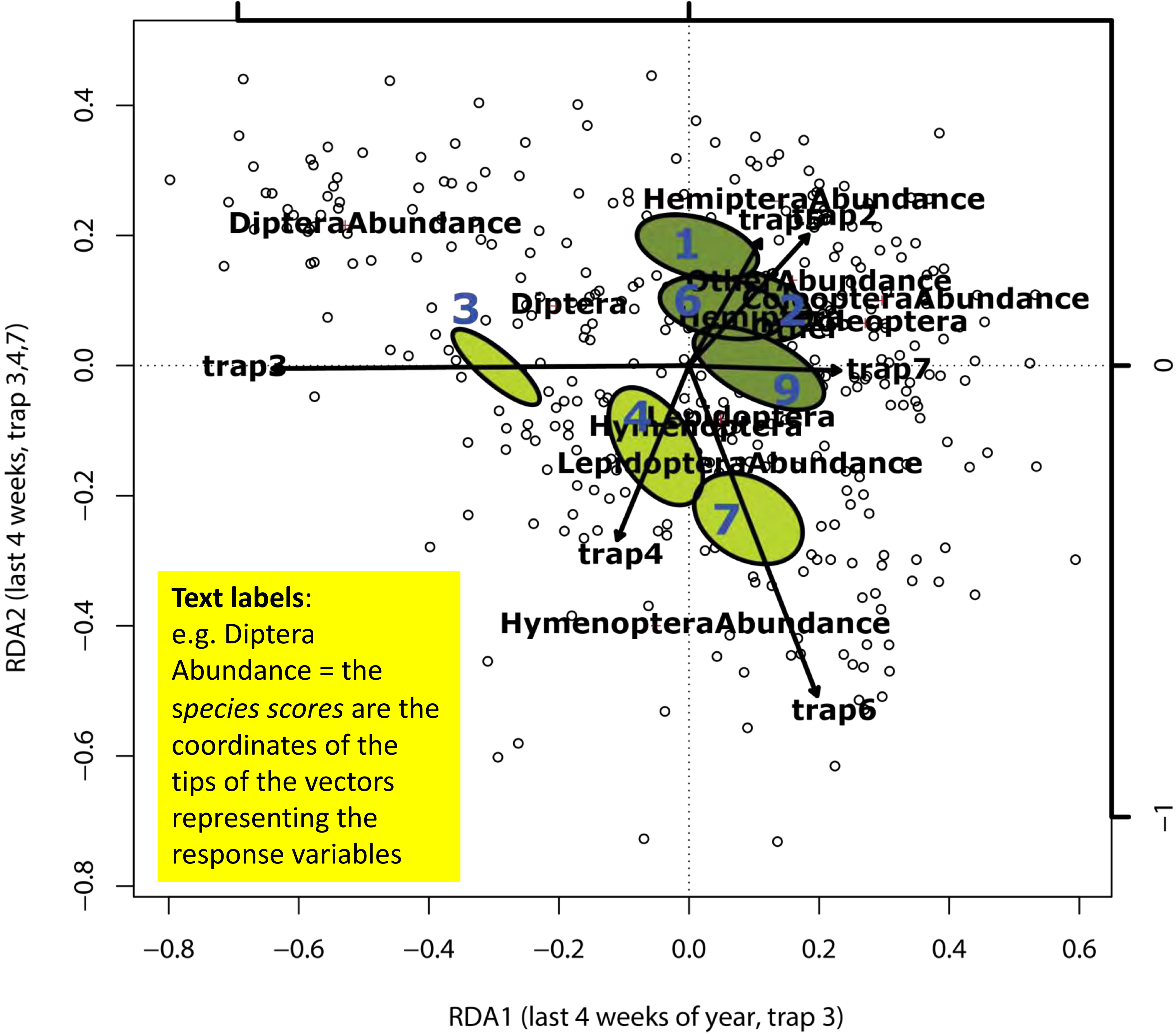
RDA allows you to study the relationship between two tables of variables

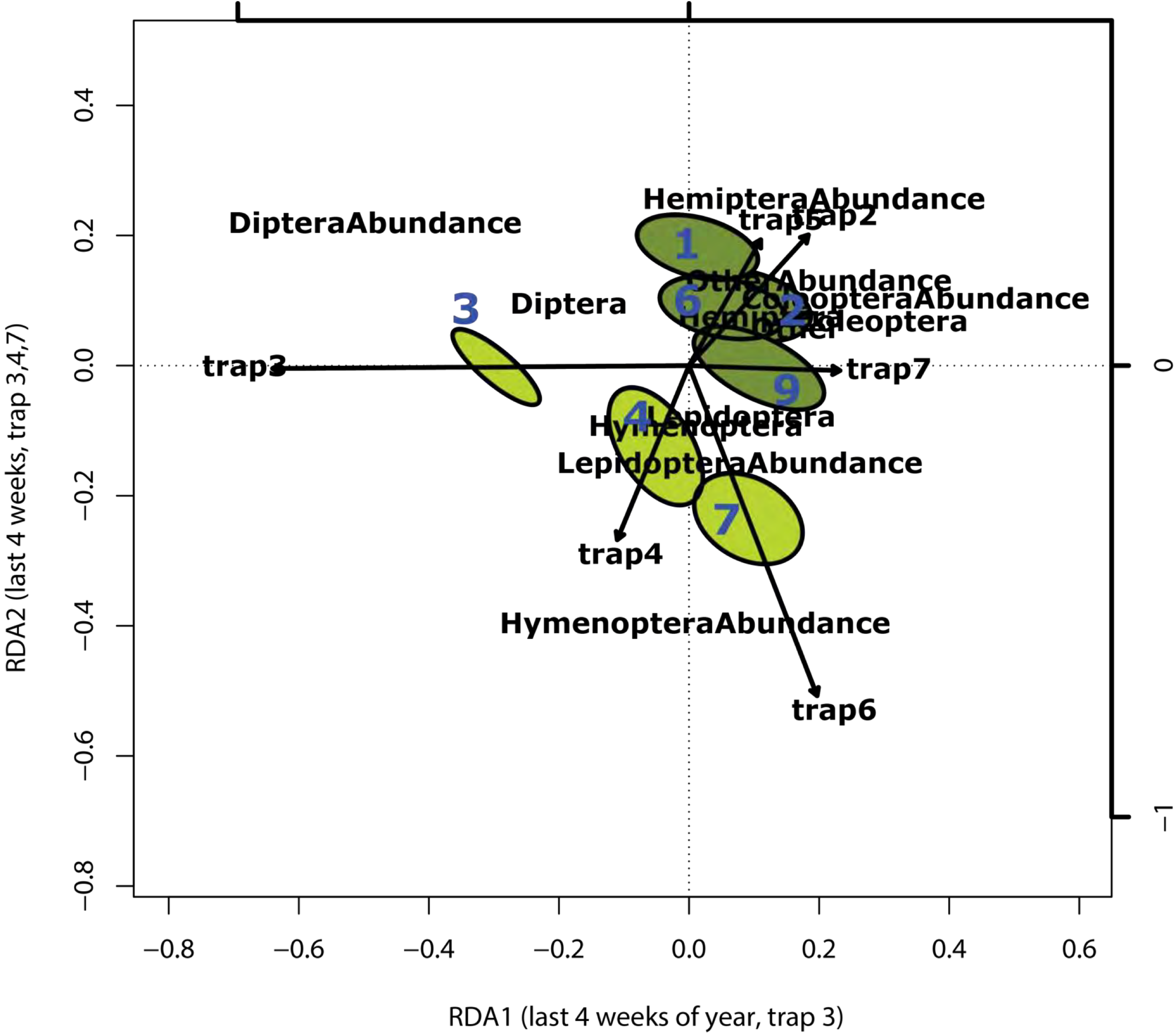
- **An RDA is a constrained ordination that examines how much of the variation in one set of variables explains the variation in another set of variables. It is a multivariate analog of simple linear regression.**
- **In the biplots, the longest vectors are most important in explaining variation in diversity & abundance.**

- **So, in an RDA you have…**
  1. **Y=A set of response variables (here it's ordinal diversity and abundance)**
  2. **X=A set of explanatory variables (here it's trap, week, edge)**
  3. **And a goal of finding those components of Y that are a linear combination of X and represent as much variance as possible.**
- **To do so, you use linear regression to represent Y as a function of X and then use PCA to visualise the results.**

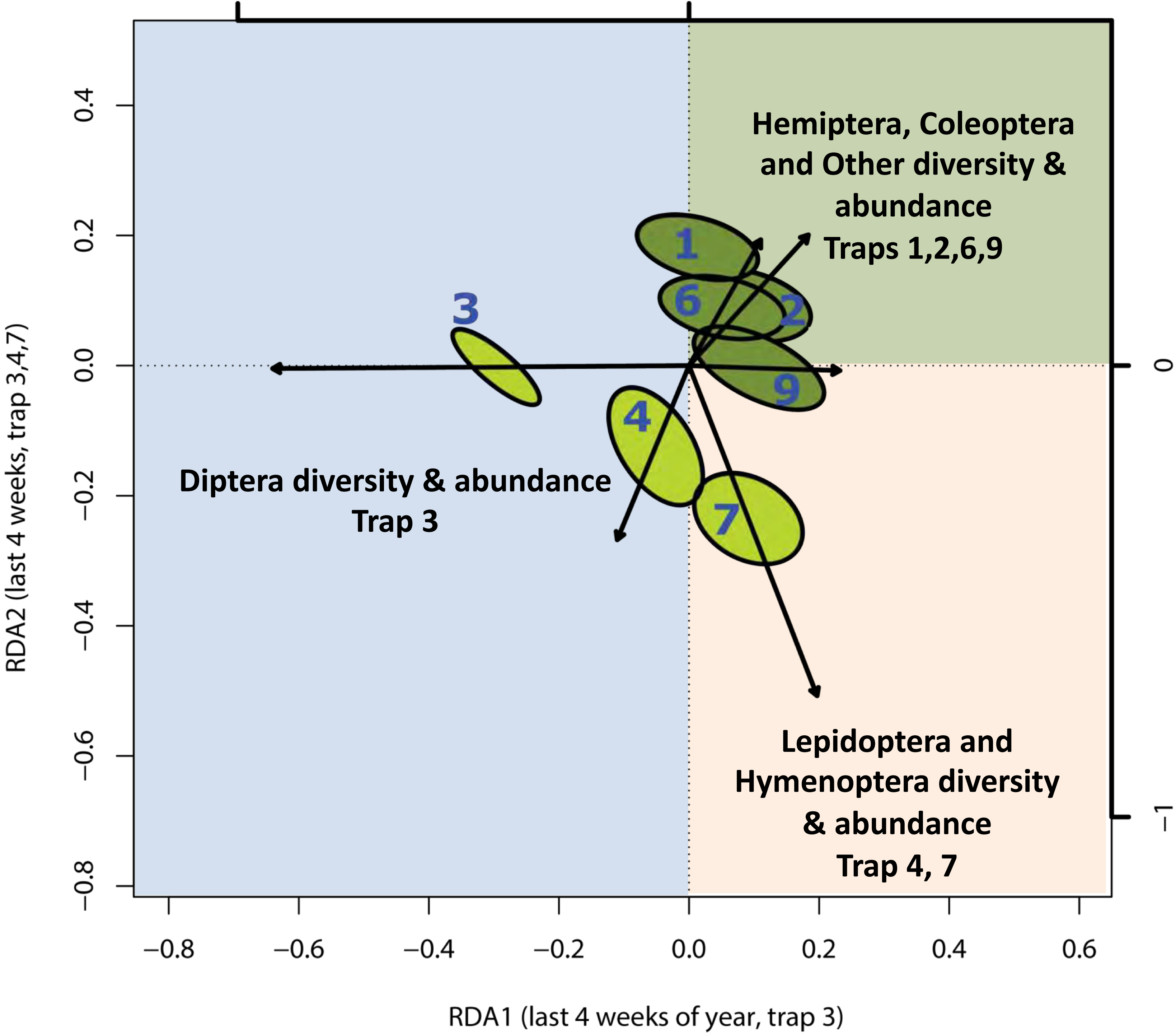

RDA bi-plot.
Site scores (weighted sums of species scores): coordinates of the sites as expressed in the space of the response variables Y..
RDA1 (dipteran abundance and diversity) is principally explained by trap3, and the last 4 weeks of the collection cycle.
RDA1 (last 4 weeks of year, trap 3)
RDA2 (last 4 weeks, trap 3,4,7)
−0.8
−0.6
−0.4
−0.2
0.0
0.2
0.4
0.6
0
−1


Vectors represent each trap as an explanatory variables . Length indicates the strength variable.
Ellipsoids represent the 95% confidence of the standard deviations of the (weighted) averages (se) for each trap (i.e. variables).
Colour = interior or edge.
RDA1 (last 4 weeks of year, trap 3)
RDA2 (last 4 weeks, trap 3,4,7)
1
2
3
4
6
7
9
−0.8
−0.6
−0.4
−0.2
0.0
0.2
0.4
0.6
−1
0

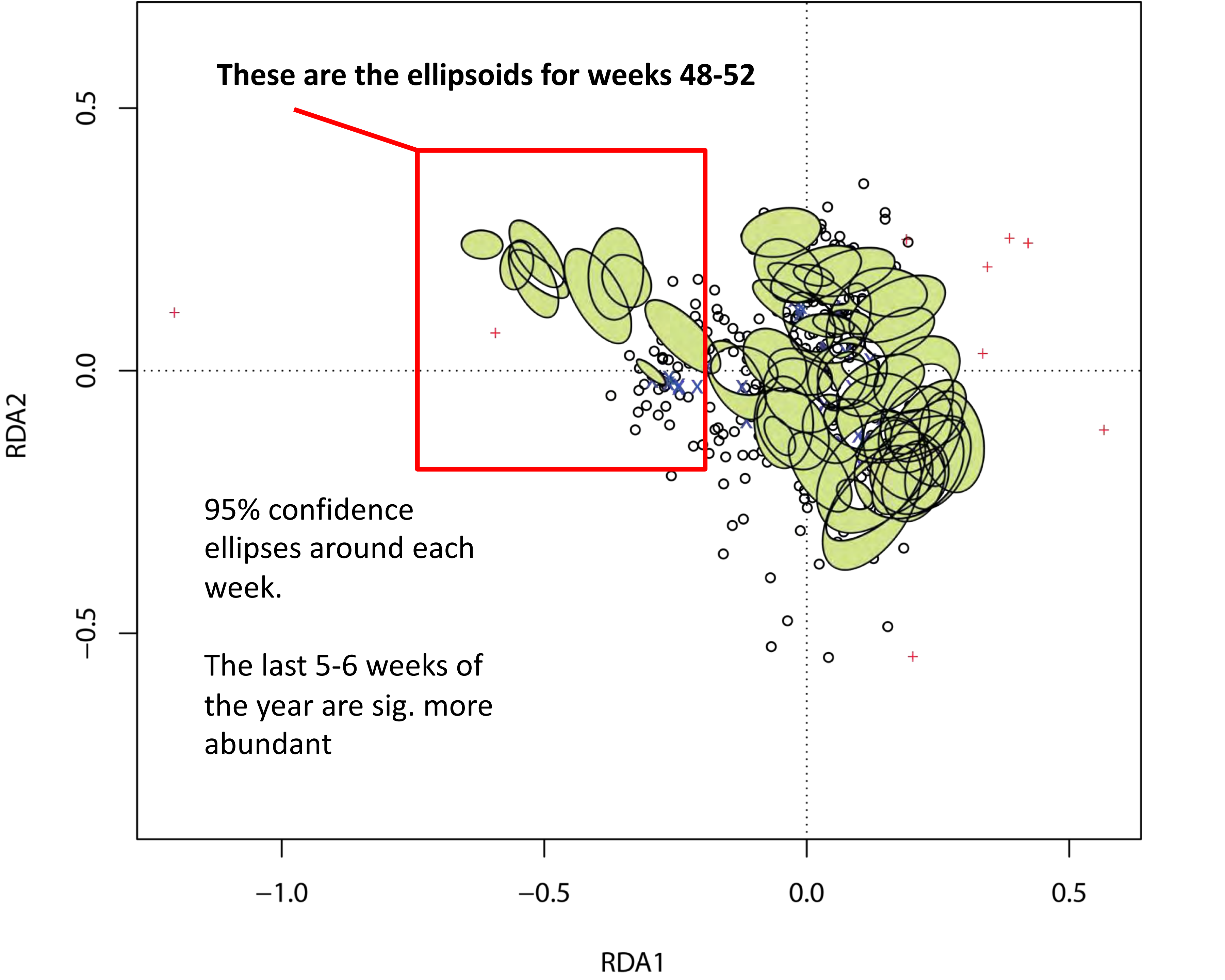

Text labels:
e.g. Diptera Abundance = the *species scores* are the coordinates of the tips of the vectors representing the response variables
DipteraAbundance
HemipteraAbundance
Diptera
trap3
trap7
trap4
trap6
LepidopteraAbundance
HymenopteraAbundance
1
2
3
4
6
7
9
RDA1 (last 4 weeks of year, trap 3)
RDA2 (last 4 weeks, trap 3,4,7)
−0.8
−0.6
−0.4
−0.2
0.0
0.2
0.4
0.6
0
−1



HemipteraAbundance
trap5
trap2
DipteraAbundance
OtherAbundance
ColeopteraAbundance
Diptera
Hemiptera
Coleoptera
Other
trap3
trap7
Lepidoptera
Hymenoptera
LepidopteraAbundance
trap4
HymenopteraAbundance
trap6
1
2
3
4
6
7
9
0.4
0.2
0.0
−0.2
−0.4
−0.6
−0.8
−0.8
−0.6
−0.4
−0.2
0.0
0.2
0.4
0.6
0
−1
RDA2 (last 4 weeks, trap 3,4,7)
RDA1 (last 4 weeks of year, trap 3)



Hemiptera, Coleoptera
and Other diversity &
abundance
Traps 1,2,6,9
Diptera diversity & abundance
Trap 3
Lepidoptera and
Hymenoptera diversity
& abundance
Trap 4, 7
1
2
3
4
6
7
9
RDA1 (last 4 weeks of year, trap 3)
RDA2 (last 4 weeks, trap 3,4,7)
−0.8
−0.6
−0.4
−0.2
0.0
0.2
0.4
0.6
0
−1



These are the ellipsoids for weeks 48-52
95% confidence ellipses around each week.
The last 5-6 weeks of the year are sig. more abundant
RDA2
RDA1
0.5
0.0
−0.5
−1.0
−0.5
0.0
0.5

Venn Diagram of Variance Partitioning

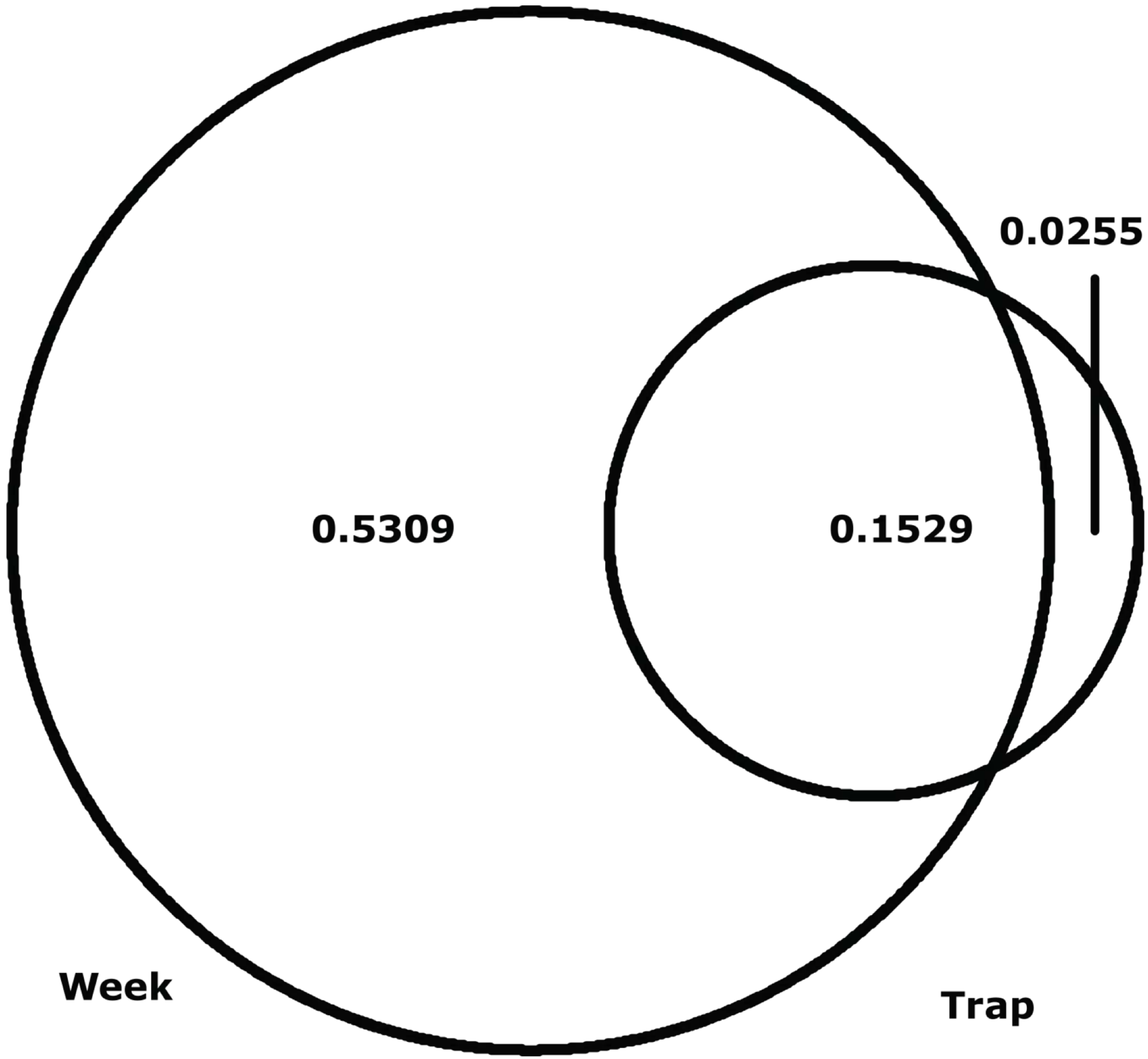
Variance Partitioning:

The variable "week" explained much more (53% variation than did the variable "trap", which by itself only explained a small amount of variation (2.6%).

Most of the variation explained by trap was also explained by week ((15.3%).

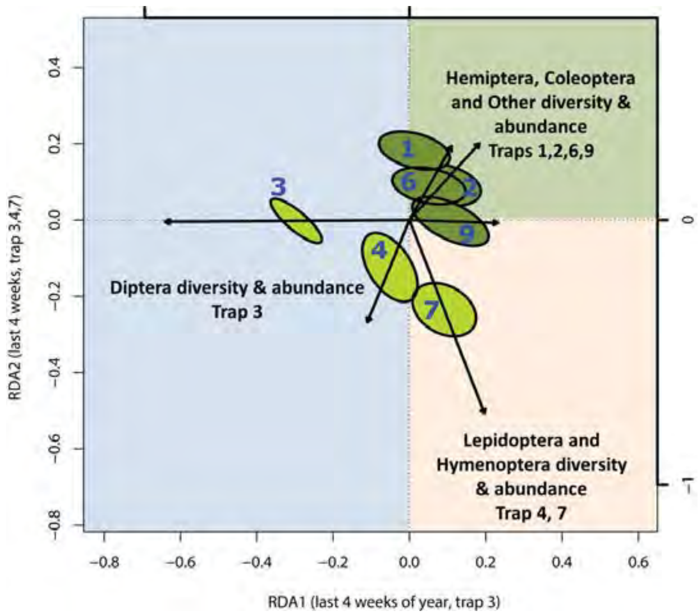
Approximately 30% of the variation in diversity and abundance is not explained by trap or week

Unexplained variance = 0.2907



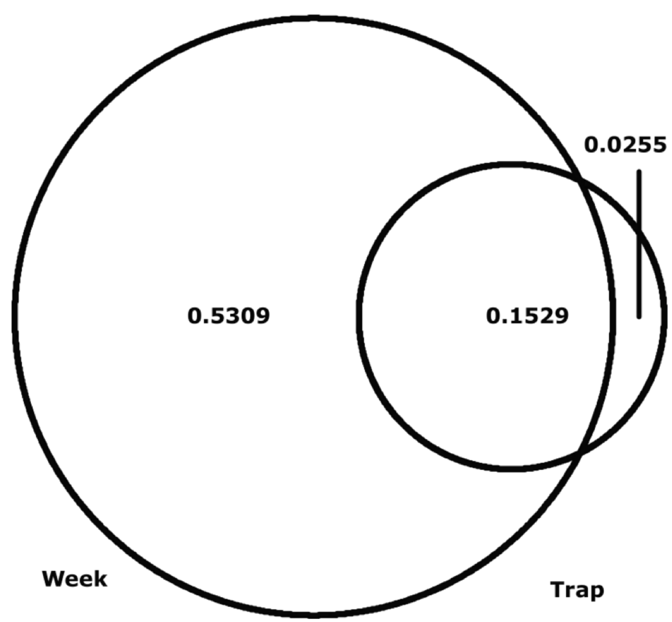
Conclusions

- **Are traps different?**
  - Trap 3 is different ($p<0.01$)
- **Are weeks different?**
  - Last 5 weeks of the 52 are sig different ($p<0.001$)



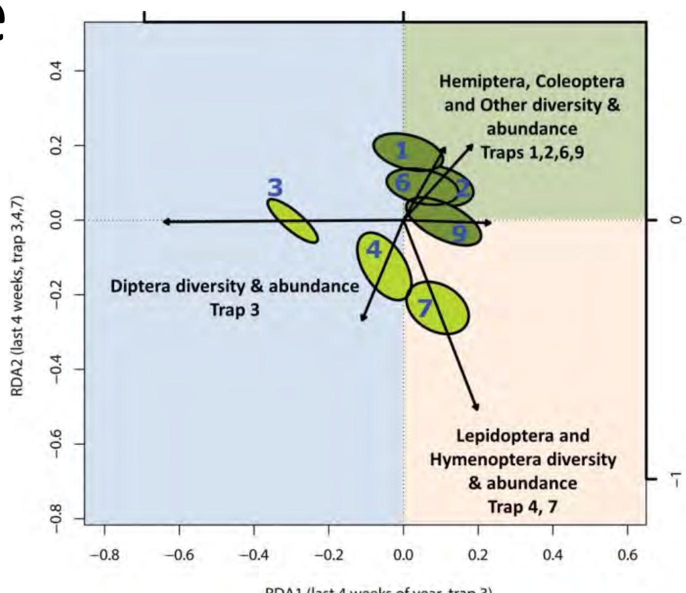
Conclusions

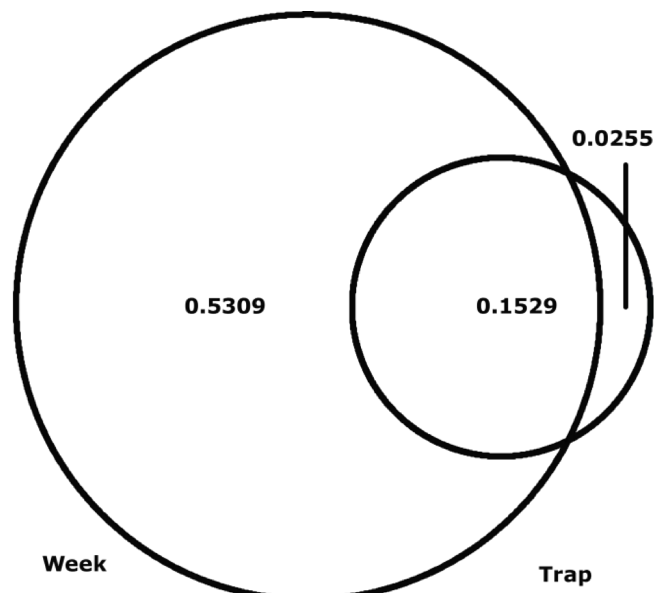
- While one trap is significantly different from the others, the total variation in diversity and abundance explained by traps alone is small (~2%) compared to the variation explained by weeks (>50%) or the interaction between weeks and traps (~15%). 30% of the variation remains unexplained by traps or weeks



Conclusions

- Therefore, while traps alone only explain a small amount of total observed variation, there is a sig. dif. in traps (3). After only one year, the understory/edge status appear different. This suggests that we ought to keep analysing as many traps as possible in the future





Conclusions

- Furthermore, within the weekly trend throughout the year, there is significant variation in abundance and diversity. This suggests the importance of continuing to collect and analyse traps throughout the year in the future.