Review
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Whole-ecosystem experimental manipulations of tropical forests

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Tropical forests are highly diverse systems involving extraordinary numbers of interactions between species, with each species responding in a different way to the abiotic environment. Understanding how these systems function and predicting how they respond to anthropogenic global change is extremely challenging. We argue for the necessity of 'whole-ecosystem' experimental manipulations, in which the entire ecosystem is targeted, either to reveal the functioning of the system in its natural state or to understand responses to anthropogenic impacts. We survey the current range of whole-ecosystem manipulations, which include those targeting weather and climate, nutrients, biotic interactions, human impacts, and habitat restoration. Finally we describe the unique challenges and opportunities presented by such projects and suggest directions for future experiments.

Tropical forest ecosystems as targets for experimental manipulation

The importance of tropical forests

Tropical forest ecosystems are highly complex and heterogeneous, comprising diverse networks of interactions between species within communities, and between those communities and the environment [1,2]. Understanding how these systems function is important because they provide globally significant ecosystem services, including carbon sequestration, climate regulation, supply of water, and conservation of biodiversity [3].

What is a whole-ecosystem manipulation?

Understanding these habitats can require manipulations that target entire ecosystems. We define whole-ecosystem experiments as manipulations aiming to replicate drivers at spatial and temporal scales similar to those occurring in either natural or human-modified ecosystems. We include only studies where either the entire ecosystem is manipulated (e.g., during logging [4]) or where the manipulation is expected to impact on the entire ecosystem (e.g., removal of ecosystem engineers [5]; see Glossary). Our definition is not based explicitly on any spatial or temporal scale because this would be arbitrary, but instead on the scope of the expected impacts. Hence, we include manipulations smaller in scope if impacts are expected to ramify through the ecosystem, or if results can be used to predict ecosystem-wide impacts. For example, excluding ants from a 1 ha area

Glossary

Whole-ecosystem experimental manipulation: a manipulation of an ecosystem in which either the entire habitat itself is experimentally altered, or a subset of the habitat is altered, the response to which is expected to be ecosystem-wide. We also include in this definition some experiments with smaller scope in terms of responses, but the impacts of which can be reasonably extrapolated to entire ecosystems.
Forest fragmentation: isolation of patches of forest by a matrix habitat.
Before-after-control-impact (BACI) design: a powerful experimental design in which measurements are made both before and after a manipulation, and where control replicates are left unmanipulated.
Canopy crane: a method for accessing the forest canopy, allowing thorough and repeated access to a relatively limited area. The most popular design is the tower crane, usually used in construction projects, with a gondola for researchers rather than a hook.
Diversity-ecosystem function relationship: the relationship between diversity of plants or animals, and one of several ecosystem functions, for example, primary productivity.
Ecosystem engineer: a species, functional group, or taxonomic group which has substantial impacts on its environment, hence affecting a large proportion of other species and ecological processes in an area.
Edge effects: changes in the biotic and abiotic components of a habitat resulting from proximity to a habitat edge.
Matrix: in the context of studies of forest fragmentation, the habitat between the remaining fragments (e.g., cropland, inundated areas).
Natural experiment: a study relying on an impact which is not under the control of the investigator, for example, creation of forest fragments during clearing scheduled independently of any scientific program.
Press versus pulse experiments: in a press experiment, a permanent change is caused in the system, such as reducing the density of a focal species or functional group, and maintaining this reduction through the course of the experiment. In a pulse experiment, a temporary change is made and then the manner in which the system recovers (or does not recover) is observed, with no ongoing intervention. For example, the density of the focal species is reduced and then allowed to recover without further manipulation.
Vapor pressure deficit: the difference between the current humidity and the maximum possible humidity at a particular temperature.

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is small in taxonomic scope [5], but the importance of ants in tropical ecosystems means that impacts are expected across all trophic levels. Furthermore, we restrict our definition to purposefully designed experiments with at least some sites in forest, and do not include natural experiments [6] such as monitoring following colonization by invasive species (e.g., [7]). We emphasize that experiments and observations falling outside our definition are still worthwhile but that whole-ecosystem experiments are needed for understanding whole-ecosystem responses.

The need for a whole-ecosystem approach

Disentangling the drivers of functioning in tropical forests usually relies on observed changes following environmental perturbations [6]. These perturbations are often correlated and synergistic, necessitating complementary experimental manipulations if their effects are to be properly understood [8]. However, constraints of cost and logistics mean that manipulations of entire ecosystems are rare. Most experiments are limited in scale and take a reductionist approach, focusing on a tractable subcomponent of the ecosystem (for example the relationship between a particular plant and its herbivores). Owing to the subtleties of interactions at larger scales and across multiple trophic levels in tropical forests [9], manipulations focusing on a specific target taxon, or those conducted at small spatial or temporal scales, are often unable to determine the drivers of ecological processes. For example, environmental variables are often measured at spatial scales too small for optimal prediction of species distributions [10]. A related issue is

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Figure 1. Whole-ecosystem manipulations of tropical forests have been used to investigate a wide range of ecological questions. **(A)** Biological Dynamics of Forest Fragments Project [35] and **(B)** The Stability of Altered Forest Ecosystems project [66], both investigating forest fragmentation effects. **(C)** The Sabah Biodiversity Experiment [42] assessing impacts of replanting logged forest with different diversities of dipterocarp trees (see also Box 2). **(D)** The Large-Scale Biosphere Atmosphere Experiment [20] assessing impacts of drought. **(E)** Canopy ant eradications in Papua New Guinea [5] exploring the roles of arboreal ants in rain forest ecosystems. **(F)** The Agua Salud Project [68] in which different water catchment replanting strategies are being explored. **(G)** The Gigante Litter Manipulation Project [26], in which both litter and nutrients have been manipulated. **(H)** The Canopy Trimming Experiment [50], in which impacts of hurricane damage are experimentally simulated. **(I)** Experimental burning to determine fire-related mortality of trees and lianas [22]. Photo credits: (A) Richard Bierregaard, (B) Ch'ien Lee, (C) Andrew Hector, (D) Dan Nepstad, (E) Vojtech Novotny, (F) Frank Bäse, (G) Didimo Ureña and Emma Sayer, (H) Aaron Shiels, and (I) Jennifer Balch.

that of spill-over effects from the surrounding unmanipulated area, which will be greater at smaller scales, and for plots without buffer zones. Furthermore, during manipulations employing a barrier, populations that are isolated (but otherwise unmanipulated) can behave differently to those in continuous areas [11]. Thus a more holistic approach is needed that borders on a reinvention of systems ecology [12]. Here we advocate such a whole-ecosystem experimental approach (Figure 1) to unravel the complex interactions and processes within tropical forests.

Potential insights from whole-ecosystem manipulations

The diverse, heterogeneous nature of tropical forests, in which many species are rare, means that whole-ecosystem manipulations as defined here are challenging to implement but are vital for understanding these habitats. This approach allows prediction of responses of communities comprising multiple trophic levels and associated ecosystem processes. For example, exclusions of mammalian herbivores from tropical forests suggest that this group is relatively unimportant in controlling vegetation structure [13]. However, manipulations at larger spatial scales in tropical grasslands reveal strong whole-ecosystem responses, with impacts on vegetation structure and cascading effects on other taxa [14]. Understanding whether this reflects a difference in the importance of mammalian herbivores between habitats requires exclusions at comparable (whole-ecosystem) spatial scales in tropical forests (recent extinction of large mammals in tropical forests is one explanation for these differences [15]). Manipulative experiments can also be useful to complement natural experiments in situations where potential confounding factors are present. For example, forest fragments in the tropics are usually left on steep slopes and high areas [16]. Hence, fragmentation is confounded with topographic variables, and only large-scale manipulations are able to disentangle these effects. Furthermore, manipulations allow collection of pre-treatment data, which is not always possible with natural experiments relying on unpredictable events such as fires [17] or hurricanes [18]. Such data allow the impacts of events to be accurately assessed, rather than inferring effects through comparisons between different areas, which can differ for other reasons.

The insights provided by manipulations conducted at whole-ecosystem scales have motivated an ongoing increase in such experiments in tropical forests (Figure 2), with emerging opportunities for collaborative studies. Here we describe the findings of whole-ecosystem manipulations in tropical forests to date, assess the logistic, analytical, and ethical challenges that these projects present, and suggest future research directions. Although this review focuses on whole-ecosystem manipulations in tropical forests, such experiments are useful in other habitats, and many of the points made here are more widely relevant.

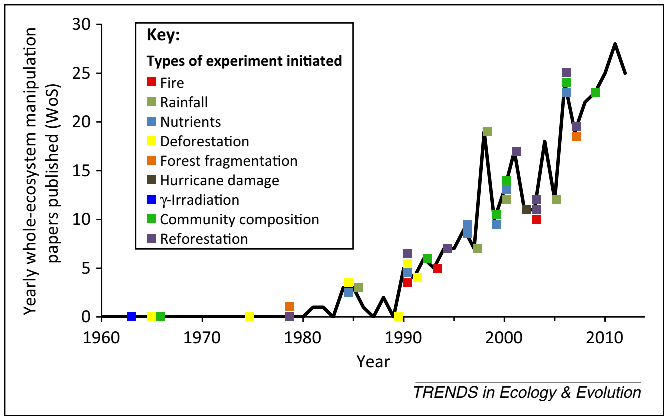

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Figure 2. There has been a recent increase in the use of whole-ecosystem manipulations in tropical forests. This is the case both in terms of the number of new whole-ecosystem experiments initiated (points), and in publications generated as a result of these experiments (unbroken line). Points relate to the references in Table 1. Publication data was generated from a search for 'experiment AND tropical rainforest' in Web of Science, and screening of results to include only those focusing on whole-ecosystem experiments. Note that, as a result, not all publications from Table 1 are included in these counts.

What do whole-ecosystem manipulations reveal about the way tropical forests work?

Whole-ecosystem manipulations (Table 1) comprise those assessing (i) effects of weather and climate, (ii) role of key ecological groups, (iii) importance of nutrients, (iv) effects of direct anthropogenic activities, such as logging, and (v) efficacy of different forest restoration strategies.

The effects of weather and climate

Whole-ecosystem experiments can provide particularly useful insights into climate and weather impacts because these events usually involve a suite of correlated changes, and tropical forests are predicted to be particularly vulnerable to climate change [19]. Only with manipulations can the relative importance of, and interactions between, drivers be disentangled. Water availability manipulations have demonstrated reduced tree growth rates in response to drought [20] and that reduced rainfall is not a proximal cue for leaf fall [21]. Furthermore, separate manipulations of water availability [20] and fire regimes [17,22] offer the potential to partition the impacts of these correlated drivers. Simulations of hurricane damage also show that detritus deposition kills existing saplings, while increased canopy openness stimulates germination from the seedbank [18].

The role of key ecological groups

Direct manipulations of particular taxa have been used to reveal the roles these groups play in structuring tropical forest ecosystems. For example, experiments have demonstrated that mammalian herbivores do not strongly affect vegetation structure in tropical forests [13]. Correlated changes in abundances of taxa do not necessarily imply the existence of causal links because they can be driven by external factors. Hence manipulations of abundances are necessary to directly test the relationships between species, groups of species, and ecosystem processes. This can be important when assessing the dynamics of invasive species, where the simultaneous appearance of non-native species and decline of natives is not sufficient to infer that non-natives have negative impacts, because changes in both taxa can be caused by anthropogenic disturbance [23]. Experiments may also reveal facilitation between invaders. For example, removal of the yellow crazy ant (*Anoplolepis gracilipes*) from parts of Christmas Island has

Table 1. Examples of the range of whole-ecosystem manipulations conducted to date

	Process (manipulation)	Example project name and region	Methods used	Responses measured	Replicate area (ha)^{a}	N	Start	Key Refs	Other comparable experiments^{b}
Weather and climate	**Fire**	Mato Grosso, Brazil	Implemented different fire regimes in three treatment areas (control – no burning, once burned, and annual burning). See also Box 1	Tree and liana mortality; above ground biomass; invertebrates	50	3	2004	[17,22,74]	Experimentally cleared and burned forest in Amazonas and Pará, Brazil [75]
	Rainfall								
	Drought	Tapajós National Forest, Brazil. Part of Large-Scale Biosphere Atmosphere-Experiment in Amazonia	Droughting of treatment plot (reduction of ~40% of rainfall) using plastic panels and guttering, compared to control without treatment in BACI design	Soil and gas flux; tree and liana mortality; net primary productivity; above ground biomass; soil water movement; water uptake	1	2	1998	[20]	Directly comparable with experiments in Caxiuana National Forest, Brazil [76] and in Sulawesi, Indonesia [77]
	Irrigation during dry season	Barro Colorado Island (BCI), Panama	Dry season irrigation in treatment plots compared to control plots	Litterfall	2.25	4	1986	[21]	Irrigation and leaf removal experiment in Brazil [78]
	Hurricane damage								
	Simulated hurricane damage	Canopy Trimming Experiment, Luquillo Forest, Puerto Rico	Canopy thinning and addition of material to forest floor compared to control in factorial BACI design	Seedling mortality and growth; forest floor and canopy arthropods	0.09	12	2003	[18,50]	None
Nutrients	**Nutrient enrichment**								
	N, P, K and other micronutrient additions	Gigante Fertilization Project (GFP), BCI Panama	Addition of N, P, K, other micronutrients or no addition (control) in a factorial design	Litterfall; decomposition; net primary productivity; soil nutrient dynamics	0.16	36	1997	[31,79]	N and P in Hawaii [29], Ecuador [28], and Indonesia [80]
	Leaf litter manipulation	Gigante Litter Manipulation Project, BCI, Panama	Addition and removal of leaf litter from sets of treatment plots compared to controls in BACI design	Trunk growth; litterfall; decomposition rate; nutrient concentrations; arthropods	0.2	15	2000	[25–27]	La Selva, Costa Rica [81]. Has also been compared to GFP results [82]
Communities	**Community manipulation**								
	Liana removal and replacement	Panama	Removal and addition of artificial vines into plots	Ant abundance and diversity	0.64	18	2010	[83]	None
	Removal of arthropods	Florida Bay	Islands covered with tents and fumigated with methyl bromide	Field monitoring (non-lethal) of all arthropod species' populations	0.01–0.05^{c}	9	1966	[84]	None
	Ant exclusion and suppression	Papua New Guinea	Ants excluded and poisoned in treatment plots compared to control plots	Ant abundance	0.06	4	2007	[5]	None
	Suppression of invasive ants	Christmas Island	Application of insecticide bait to three		9.1–35.6	6	2000	[24]	None

Table 1 (***Continued***)

	Process (manipulation)	Example project name and region	Methods used	Responses measured	Replicate area (ha)^a	N	Start	Key Refs	Other comparable experiments^b
			supercolonies of *A. gracilipes*	Ant activity and abundance; scale insect abundance					
	Herbivore exclusion	Gigante Peninsula and BCI, Panama	Paired fenced enclosure and control plots in BACI design	Herb diversity, density and cover	0.14	16	1993	[13]	None
	Mammalian seed predator exclusion	Bladen Nature Reserve, Belize	Live trapping and removal of dominant granivorous small mammal	Fruit removal rate and fate of removed fruits	0.5	6	2004	[85]	None
Anthropogenic impacts	**Deforestation**								
	Selective logging	Gunung Rara Forest Reserve, Sabah, Malaysia	Two modes of selective harvesting compared as well as pre-logging vine cutting in BACI design	Tree diameter breast height, species identity, damage and mortality; skid trail cover	5.76	20	1992	[34]	Experiment investigating the impact of logging intensity and type in Guyana [4]
	Clearance and plantation establishment in river catchments	Mendolong Research Area, Sabah, Malaysia	Clearance of forest and establishment of *Acacia mangium* plantation compared across three extraction methods and two control habitats in BACI design	Precipitation; stream flow and quality; surface runoff; soil moisture; soil physical properties; dissolved nutrients; *Acacia* growth	3.4–18.2 catchments	5	1985	[86]	Experiment investigating the impact of forest clearance and burning in Costa Rica [33]
	Forest Fragmentation								
	Experimental fragmentation	The Biological Dynamics of Forest Fragments Project (BDFFP), Manaus, Brazil	Fragmentation of continuous primary forest into fragments. Includes comparisons with surrounding continuous forest, cattle ranches and regrowth areas	Wide range of studies from biodiversity to ecosystem functions and services	1–100	11	1979	[35]	Forest fragmentation experiment in Sabah, Malaysia [66]
	γ-Irradiation								
	Exposure to γ radiation	El Verde Field Station, Puerto Rico	Irradiation for 3 months with 10 000 Ci source in BACI design	Plant diversity, density, growth, and size	0.07	1	1963	[37]	None
Restoration	**Reforestation**								
	Enrichment planting with different biodiversity mixes of seedlings and vine cutting	Sabah Biodiversity Experiment (SBE), Sabah, Malaysia	Enrichment planting of logged forest with 1, 4, or 16 species of seedlings, compared to 12 control plots and 16 plots with enhanced climber cutting. See also Box 2	Above-ground biomass and carbon	4	124	2002	[42]	Vine manipulation comparable in Oquiriquia, Bolivia, recorded the regrowth of vines and cost-effectiveness of cutting [43]
	Replanting of former plantation with different combinations of seedlings	Reserva Natural Vale, Espírito Santo, Brazil	Factorial design with high and low seedling diversity; low, medium, and high density of	Seedling growth; herbivory; plant defenses	0.25	36	2004	[87]	Biodiversity, Panama [88]

			seedlings; and the inclusion of pioneer species or not						
	Replanting in blocks or patches	Coto Brus County, Costa Rica	Using four native tree species, plots across 14 sites planted with continuous forest, three different sizes of forest patches or nothing (control)	Seedling survival, height and canopy cover; bird communities; arthropod abundance; seed dispersal	0.002–0.25	112	2004	[39–41]	None
	Replanting river catchments	Aqua Salud Project, Panama Canal Watershed Experiment	Planting with native species, teak plantation, compared to natural regrowth, native forest, pasture and invasive grass	Water quality, quantity and seasonality; catchment carbon storage	8–400	12	2007	[68]	None
	Silviculture treatments	Mapane Region, Surinam	Application of silviculture system with different liberation and refinement treatments	Tree basal area, increment and recruitment	1 and 25	30, 1	19651975	[38]	Experiment investigating silviculture treatments, Heredia Province, Costa Rica [89]

[a] Areas refer to the manipulated area, not to the size of sampled plots, which is smaller when an unsampled buffer is employed.

[b] Where multiple experiments have been conducted on the same broad research question, a single example is described in detail, with the final column giving details of representative other experiments on the same topic.

[c] Only island diameter is given here, and areas therefore assume that islands are circular.

demonstrated that the presence of this invasive species facilitates invasion by non-native scale insects [24], rather than both sets of invasions being driven by habitat change.

The importance of nutrients

Manipulations of nutrient availability have allowed the dissection of the drivers of, and responses to, nutrients in tropical forests. Soil inorganic and litter nitrogen (N) increase when leaf litter is added, mimicking hurricane damage or increased natural litter fall, but not when N is added directly, mimicking anthropogenic atmospheric N deposition [25]. Investment in fine root biomass decreases with addition of leaf litter and with potassium (K) [25], and decomposition rate and arthropod abundance in leaf litter are reduced in litter removal plots [26]. However, litter addition does not affect arthropod communities, suggesting that they are not limited by bottom-up control [27]. There are also altitudinal gradients in nutrient limitation in the tropics, with tree growth at higher elevations being limited mainly by phosporus (P), but by N at lower elevations [28]. Nutrient additions along a gradient of primary succession have demonstrated N limitation, but only during the early stages of succession [29]. Additions of N and P also show that nutrient enrichment is likely to negatively impact arbuscular mycorrhizal fungi [30]. Other experiments indicate that, while production of fruits and flowers by trees is N limited, litter fall from dead leaves and woody material and subsequent decomposition is limited by multiple nutrients [31]. It is worth noting that hurricanes are expected to generate single large pulses of nutrients, while increases in litter fall and anthropogenic atmospheric N deposition are expected to cause increases in levels of continuous nutrient input. Hence simulations of these two types of impact (‘pulse’ and ‘press’ experiments [32]) are both valuable in understanding different aspects of environmental change.

Simulations of anthropogenic impacts

Many whole-ecosystem manipulations in tropical forests are motivated by a desire to understand the continuing impacts of humans on these ecosystems. This is because the exploitation of natural habitats usually occurs across large spatial and temporal scales, with a range of correlated drivers and responses. In particular, such experiments allow specific recommendations to be made to reduce the impacts of future exploitation. Experimental slash and burn deforestation has revealed consequent losses in soil elements and increased CO_2 release [33], while reduced impact logging techniques minimize these effects [34]. The experimental creation of forest fragments has demonstrated the importance of edge effects in determining community responses [35], the role of corridors in maintaining diversity [36] in fragmented landscapes, and the long time-periods over which species continue to be lost in isolated fragments [35]. In particular, the Biological Dynamics of Forest Fragments Project in Brazil has given insights into the long-term, large-scale, cross-taxon impacts of forest fragmentation that would not have been possible without a whole-ecosystem manipulation. Experimental setting of fires has allowed the assessment of subsequent vulnerability of forests to future fires (Box 1) [17], which would not

Box 1. Case study: The Mato Grosso fire experiment

Fires caused by humans have the potential to dramatically alter forest ecosystems in the tropics, and can act synergistically with other forms of human disturbance, such as logging and forest clearance. Previous work indicated that areas already affected by fires would be more susceptible to future burns [90]. This motivated a whole-ecosystem experiment investigating feedbacks in fire ecology in Mato Grosso, south-eastern Amazonia [17,22,74] (Figure I, see also Table 1 in main text for other fire experiments), in which different frequencies of burning were applied to three 50 ha plots. Unexpectedly, it was initially found that burn area and flame heights were reduced by prior repeated burning, and consequently there was lower fuel combustion, despite increases in vapor pressure deficit and decreases in litter moisture [17]. This related to an exhaustion of fine litter material by repeated burning. However, repeated burns also increased mortality of trees and lianas, particularly for smaller individuals, although there was wide variation in responses between species [22]. The researchers were also able to assess the synergistic impacts of fire and drought by taking advantage of a 2007 natural drought event, during which there were dramatic increases in tree mortality (up to 426% in plots experimentally burnt every three years), with a consequent transition to grassland [19]. Leaf cutter ants play a role in moderating fires by removing fine-medium fuel close to nesting sites, resulting in larger areas of bare soil, and smaller burnt areas [74]. Experiments involving large-scale experimental burns are necessary, as changes in vegetation structure following burning are likely to have much greater effects on subsequent microclimate when burns are larger in scale because surrounding forest would buffer change relating to smaller burns, and non-experimental burns (both anthropogenic and natural) are often large. Furthermore, dictating the area to be burnt allows baseline data to be collected.

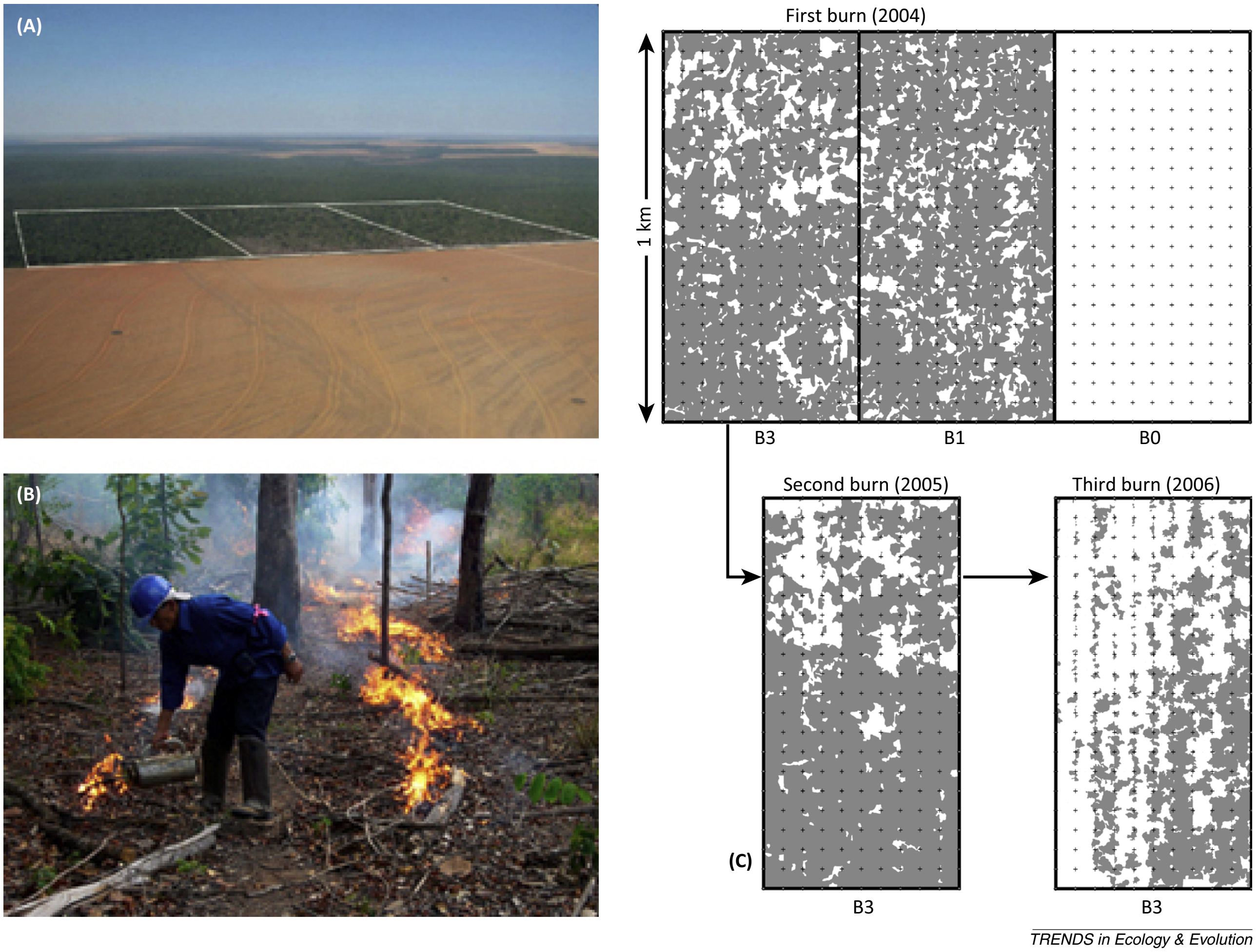

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Figure I. To investigate the effects of fire on transitional tropical forest ecosystems in Brazil, researchers have conducted large-scale burns using an experimental setup of three 50 ha plots **(A,B)** [17,22,74]. **(C)** One plot was burnt once at the start of the experiment (B1), a second was burnt once every year for three years (B3), and a control plot was not burnt (B0). Fires were set in lines (see B0), using a kerosene drip torch. Burnt area is indicated by shading. The effect of these manipulations on fire spread rate, burn area, fuel accumulation, microclimate [17], and tree and liana mortality [22] have been measured. Whether leaf cutter ants affect fuel accumulation and area burnt near their nests has also been investigated [74]. Map reproduced, with permission, from [17]. Photo credits Jennifer Balch.

be possible using a natural experiment because future and current fire risks are expected to be correlated. Perhaps most surprisingly, concerns over use of nuclear weapons led to experimental irradiation of a tropical forest. After initially high plant mortality, regeneration occurred, but more slowly than in natural gaps [37].

Forest restoration

A further series of manipulations have investigated management and restoration of tropical forests, a large proportion of which are now degraded [3]. Because these manipulations attempt to restore previous ecosystem states, assessment of whole-ecosystem responses is

Box 2. Case study: the Sabah biodiversity experiment

SE Asian forests are dominated by trees of the family Dipterocarpaceae, which have high timber value. As a result, forests in the region have undergone repeated rounds of logging, with recruitment of dipterocarp seedlings being compromised. Standard restoration methods involve a combination of enrichment planting and climber cutting (removal of the scrambling vines which often proliferate in logged forest). The evidence base to underpin effective restoration is, however, incomplete; accepted methods remain largely untested and the supposed benefits to biodiversity, seedling recruitment, ecosystem functioning and productivity are presently unclear.

To better understand effectiveness of different replanting strategies on restoration success, over the past ten years a large-scale, long-term rain forest restoration experiment has been established in Sabah, Malaysian Borneo – the Sabah Biodiversity Experiment [42]. The experiment involves replanting plots within logged forest with a range of different diversities of dipterocarp seedlings (Figure I). Much of the focus of early work has been on establishing baseline conditions. While the precise role played by the diversity of dipterocarps will only become clear over time, early indications are that the restoration treatments applied have the potential to supplement natural recruitment, improve seedling survival and accelerate the recovery of degraded rain forest.

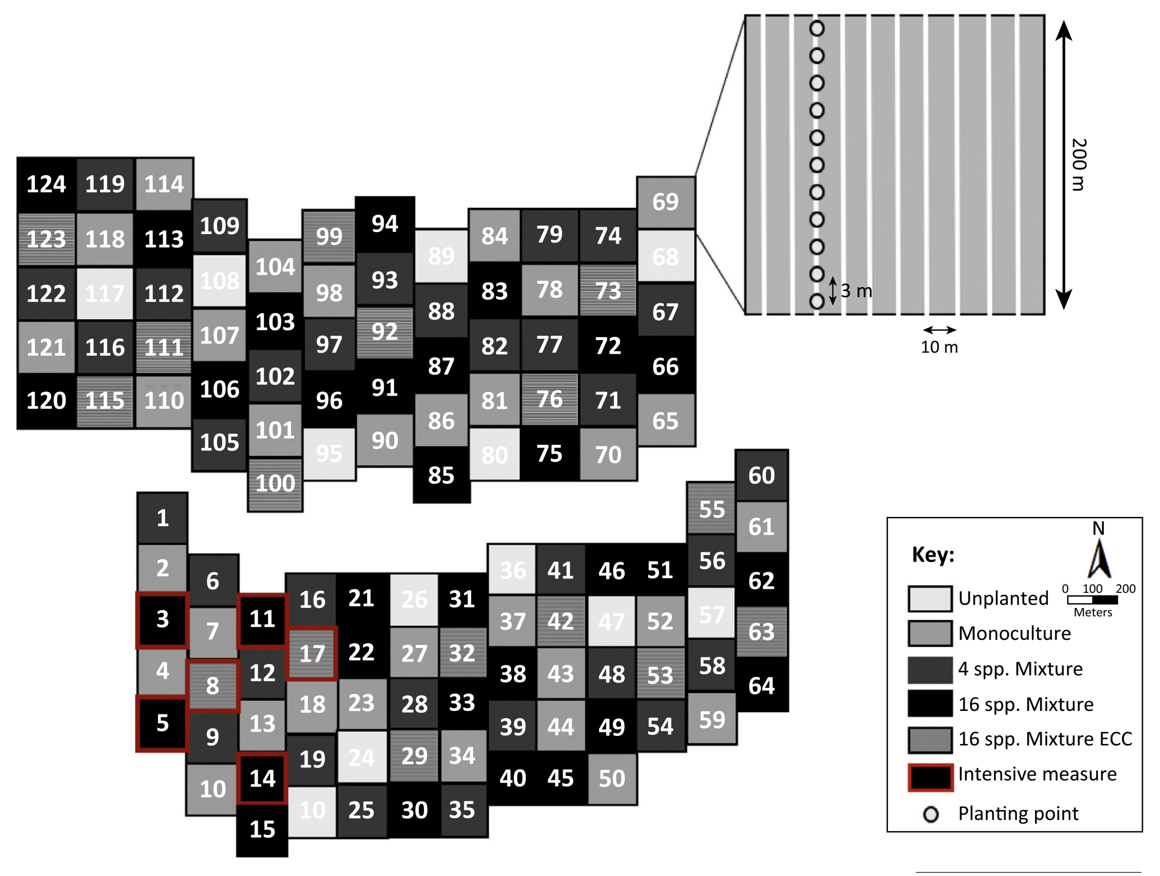

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Figure I. The Sabah biodiversity experiment site covers an area of ~500 ha of logged, degraded forest immediately to the north of the Danum Valley Conservation Area. This comprises 124 plots each of 4 ha (200 m × 200 m), each planted with dipterocarp seedlings. The experiment follows a randomized block design with plots planted at three treatment levels; a high diversity treatment planted with 16 species, 16 different four-species mixtures and single species plantings using each of the 16 species. The main experiment also includes unplanted control plots and plots which receive 'enhanced' climber cutting treatments (ECC). An associated series of comparative transects (10 m × 250 m) has also been established in the Danum Valley Conservation Area and the Sabah Biodiversity Experiment to determine the effects of logging on plant community assemblages [42]. Figure reproduced, with permission, from [42].

necessary. Selective removal of competing trees post-logging can increase yields in subsequent timber extraction rounds [38], while regeneration of forest from pasture can also be achieved, although efficacy depends on spatial conformation of tree planting [39,40], owing in part to differences in attendance of seed-dispersing birds in planted patches of different sizes [41]. Experimental enrichment planting of logged forest with different diversities of native tree species is ongoing (Box 2), with the aim of understanding the relationship between diversity and ecosystem functioning [42]. Manipulations are vital for exploring this relationship because species diversity and ecosystem function can respond in parallel to other, unrelated drivers. Both climber cutting [43] and removal of invasive species [44] are also feasible at whole-ecosystem scales, although impacts of these practices on entire ecosystems have yet to be measured.

Logistic challenges of manipulating tropical forests

The large spatial, temporal, and taxonomic scales required for whole-ecosystem manipulations make such experiments logistically challenging. This is especially true in tropical forests, where single trees can have large ecological footprints and complex canopies, leading to high levels of horizontal and vertical heterogeneity [45]. Tropical experimental plots are particularly susceptible to outside influence because they support large numbers of species [2], many with small, unviable populations (singletons in the extreme [46]). Hence, larger replicates are often required for responses to be detected (see 'Experimental design and interpretation of results'). This is reflected by the smaller commonly used vegetation plots in temperate forests (0.02 ha) [47] compared with tropical ones (0.1–1 ha, up to 50 ha) [48]. Vertical stratification of habitat structure and species composition [49] also complicates the required experimental setup. For example, loss of forest canopy and increase in litter deposition due to hurricane damage [18] can affect ground-level invertebrate leaf litter communities [50]. Different components of an ecosystem can also vary dramatically in their time to respond after manipulation, necessitating long-term monitoring (e.g., [35]), although this will be less problematic for experiments with high setup costs but low maintenance costs (e.g., forest recovery post-experimental logging). For example, understorey insect herbivores show responses only 1 year following logging [51], but it takes at least 15 years for large forest fragments to lose even 50% of their bird species following fragmentation [52]. Furthermore, functional assemblages differ between tropical regions, thereby limiting our ability to design global experiments. However, the greatest challenge is the typical lack of pre-treatment information on the study system, particularly on species interactions. For example, in the tropical rain forest of New Guinea a local food web was estimated to comprise 200 tree and 9600 insect herbivore species, engaged in 50 000 distinct plant–herbivore interactions [1]. It is not clear how many of these interactions need to be documented pre-treatment to interpret experimental results correctly. Designing manipulations that overcome these challenges can require time and financial support on a scale beyond many traditional funding streams.

Experimental design and interpretation of results

Because whole-ecosystem manipulations are so resource-intensive, it is important to maximize the utility of the resulting data. While this is true for any field or laboratory experiment (e.g., [53]), the large spatial and temporal scales over which whole-ecosystem manipulations are conducted make choice of experimental design critical because re-running failed experiments is often not possible. A crucial decision is the trade-off between replication and plot size (Figure 3). Increased replication means improved statistical power but, because plots are smaller, the experiment can fail to capture the process of interest. This is of particular concern because whole-ecosystem impacts are often only detectable at large spatial scales. Furthermore, if species are heterogeneously distributed, division into many smaller plots can result in even greater proportions of species existing as singletons (see above), reducing within-species replication. As a guideline, manipulations should mimic the scale at which the focal process or interaction occurs. This means that disturbances that occur at smaller spatial scales, such as the construction of roads, will be easier to simulate than larger-scale disturbances, such as changes in climate. Plot size should also depend on the ratio of between-plot variance to expected effect size, with more plots being required when variance is large. However, higher replication means that the total area available for sampling will be smaller if a buffer strip (which is manipulated, but not sampled, e.g., [18]) is required due to treatment edge effects, or where the

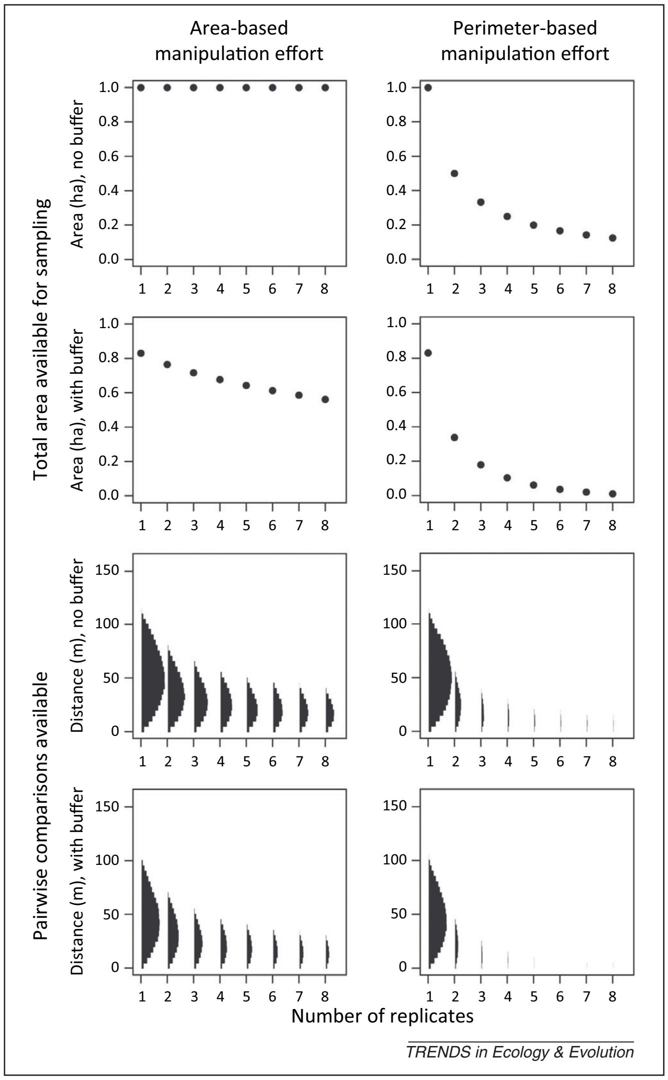

Figure 3. Illustration of the potential trade-offs involved in setting up a whole-ecosystem experiment. Trade-offs can exist between a range of experimental parameters in relation to the resulting manipulated area and potential number of within-plot pairwise comparisons. Because setting up whole-ecosystem manipulations in the field is extremely labor-intensive, maximizing potential utility of the resulting data is important. We present an analysis demonstrating the kinds of trade-offs present when making decisions about experimental design. Particular projects will have their own specific trade-offs, so this illustration is intended to stimulate thinking about trade-offs, rather than to generate specific recommendations. Suppose a project has a given amount of resources available that will be invested in either an area-based manipulation (e.g., leaf litter removal or addition - left hand panels), or in a perimeter-based manipulation (e.g., predator exclusions - right hand panels). The number of plots can be varied, as can the presence of a buffer perimeter strip, which is included in the manipulation, but not sampled. The figure illustrates the trade-off between number of replicated plots and two outputs: total area available for sampling, and distribution of pairwise distances within plots (e.g., for studies of beta diversity, or other spatial analyses). Data for circular plots are presented here, although similar results are observed for square and rectangular plots (data not presented). Inclusion of a buffer strip reduces total area available, with this effect being exacerbated at larger replicate numbers. The number of within-plot pairwise comparisons (assuming sampling units randomly distributed with uniform density, e.g., trees), is more strongly affected by the presence of a buffer strip, with fewer comparisons in total, and no longer-distance comparisons. The distribution of between-plot comparisons depends on the spatial arrangement of the plots and hence is not presented here. When the manipulation is perimeter-based, rather than area-based, increasing the number of replicates has more dramatic negative effects on the area available for sampling, and on the distribution of pairwise comparisons.

manipulation effort scales with plot perimeter (e.g., fenced plots) rather than plot area (e.g., nutrient supplementation) (Figure 3). These negative effects of high replications are exacerbated if the data generated relate not to area but to pairwise within-plot comparisons, as is often the case for spatially explicit analyses (Figure 3). Hence, whole-ecosystem experiments are often best deployed with fewer, larger replicates. If the expected effects are large, and the manipulation is extremely labor-intensive, then experiments with a single manipulated plot and single control can still be informative [5,54], conditional on results being interpreted with caution [55]. Such plots can also serve as proof-of-concept pilot experiments for novel manipulation approaches and inspire future replications elsewhere. The CTFS-ForestGEO 50 ha plots [48] and plots at canopy crane sites [56] are non-experimental examples of this.

Even if a whole-ecosystem experiment is well-designed, the scales in time and space over which the experiment is run will affect interpretation of results. Although experiments will usually only be conducted once, in a single location, it is desirable to extrapolate the results as widely as possible. To ensure that results are extrapolated appropriately, effect sizes can be mapped onto landscapes using geographic information systems (GIS) [57], hence allowing the importance of the process to be inferred, rather than the strength alone [58]. However, the usefulness of extrapolations depends on the similarity in functioning of tropical forests around the globe [59], and they should be treated with caution. Using standard data collection methods (e.g., http://www.rainfor.org/en/manuals) for which there are global, non-experimental datasets, will also assist in placing results within a global context.

Ethics of manipulating tropical forest ecosystems

Experimental manipulation can harm tropical forests, but is also one of the most efficient ways to study and understand these complex systems. Ecological research ultimately benefits tropical forest conservation, even if not designed specifically for conservation goals, because policies need to be based on an understanding of forest ecology. Researchers must therefore balance care of individual organisms, species, and ecosystems with advancing ecological and conservation knowledge [60,61]. We suggest that some damage to tropical forests caused by experiments is acceptable if we are to understand the ecology of these ecosystems [62]. The extent of permissible damage from experiments should be assessed relative to other causes of inevitable forest disturbance, such as logging or agriculture. When placed in this context, ecological experiments, even those targeting whole ecosystems, typically have negligible negative impact, and ecologists already abstain from the few types of ecological experimentation that are potentially harmful on larger scales, such as introductions of alien species. Furthermore, experiments are often labor-intensive exercises that bring employment to local communities and thus contribute to forest conservation despite the damage they might cause [63]. Experimental tropical forest research can be seen as one of the practical uses of tropical biodiversity as a means of biodiversity conservation [64].

More controversially, the ongoing destruction of tropical forests is also providing new opportunities for ecological research. Forests experiencing slash-and-burn agriculture, timber extraction, and conversion to plantations have been used to study the effects of forest disturbance, fragmentation, and predator exclusion [51,65,66]. Although many ecologists have been reluctant to exploit these opportunities, we argue that using ongoing, legal and environmentally informed forest exploitation projects to advance ecological research is acceptable provided that such research does not increase net deforestation or forest disturbance [63], and interpretation of results is independent of any industrial partners that might have conflicts of interest.

Future directions and concluding remarks

Interest in understanding tropical forests is such that several new whole-ecosystem manipulations are now being initiated. These experiments promise to enhance our understanding of human-induced habitat changes by examining tropical forest responses to CO_2 enrichment [67], impacts of habitat degradation and fragmentation on biogeochemical cycles (http://www.nerc.ac.uk/research/funded/programmes/forests/), and impacts of further reductions in rainfall during the dry season when forests are most vulnerable (http://www.tern.org.au/Newsletter-2014-Jan-Daintree-Drought-pg27685.html). The latter experiment is being conducted around a canopy crane to allow assessment of drought impacts at all vertical levels of the forest. Finally, reforestation of the catchments surrounding the Panama canal is allowing investigation of the relationship between replanting strategies and water quality, carbon storage, and biodiversity [68].

Despite the wide range of manipulations already initiated, there remain many avenues for further experiments (Box 3), utilizing a range of strategies. One approach is to implement experiments within established research programs, for example, close to the 53 plots of the global Center for Tropical Forest Science – Forest Global Earth Observatories (CTFS–ForestGEO) network (www.forestgeo.si.edu/) [48]. The International Canopy Crane Network [69], although currently underutilized with some sites ceasing to function, could also serve as a basis for replicated whole-forest experiments, particularly given the ongoing installation of cranes in southern China (http://english.xtbg.cas.cn/ns/es/201212/t20121226_97544.html). These networks already have the requisite infrastructure, trained personnel, and long-term research experience. In terms of human resources, whole-ecosystem experiments often require constant effort to maintain the manipulation, and for continuous monitoring. One strategy is to assemble a team of locally hired and trained permanent research technicians [70]. Where projects require long time-periods for manipulations to take effect (longer than the standard timescale for grants), it is useful to have in-built potential for researchers to conduct other projects not reliant on the manipulation. For example, researchers working at the Stability of Altered Forest Ecosystems (SAFE) Project [66] have been able to exploit pre-existing environmental variation to publish their baseline data in advance of the experimental fragmentation taking place (e.g., [71–73]). This can maintain interest in the project from the scientific

Box 3. Outstanding questions requiring whole-ecosystem manipulations of tropical forests

- Given the ongoing climatic changes affecting global ecosystems, manipulative approaches for testing these impacts are clearly important. Although water availability [54], fire regimes [17], hurricane damage [18] and increases in CO_2 [67] have been simulated, there has been no attempt to alter thermal regimes. Even in temperate regions, such manipulations have only been conducted at relatively small spatial scales [91] and so extending this to the whole-ecosystem level in the tropics would be highly ambitious. However, such experiments are urgently needed if we are to predict the responses of tropical forests to climate change [92].
- We still understand little about how the multitudinous species in tropical forests interact, and further community manipulations provide a powerful tool for investigating this area. In terms of successional dynamics, the rapid rates of growth in tropical forests offer the potential for observing responses to community manipulations over much shorter timescales than their temperate counterparts [93]. Furthermore, the relative importance of top-down and bottom-up processes is still unclear because large-scale experiments have tended to focus only on bottom-up manipulations, rather than looking at effects of herbivores, predators or parasites. A wealth of studies have investigated the impact of canopy predators on ecosystems through experimental exclusions (e.g., [94]), but these have mostly been at the scale of single trees or smaller, with only a few manipulating higher trophic levels at larger spatial scales [5]. Studies investigating the loss of large predators and herbivores in tropical forests have therefore been largely comparative, relying on monitoring the effects of species losses through time following the onset of hunting [15], or losses of larger species as a result of habitat fragmentation [95]. Prey species densities can also be manipulated in order to understand community-level predator-prey linkages. For example, experiments reducing prey movement into and out of temperate streams show the importance of prey subsidies between forest and stream habitats [96] and could be profitably repeated in tropical forests. The importance of pathogens and herbivores in maintaining plant diversity is another developing area; it has taken more than 40 years to test the Janzen-Connell hypothesis, one of the most promising explanations of high diversity of tropical forests, by the exclusion of pathogens and herbivores from tropical seedlings even on a small spatial scale [97] let alone in a forest-wide experiment.
- Although the manner in which nutrient availability affects plant communities has received attention [25,82], there is substantial potential for nutrients to be affected by animal communities, in particular by soil-dwelling invertebrates. Investigating this will require eradication experiments because invertebrate populations are likely to be influenced in turn by soil properties. Furthermore, although litter addition and removal experiments have been conducted [25], to our knowledge, parallel manipulations of dead wood have only been completed in temperate regions, where ground-dwelling arthropods are robust to variation in dead wood deposition [98].
- Understanding how all of these factors act synergistically is clearly also a priority. For example, we need to determine how species interactions, in addition to community composition, are affected by habitat degradation, fragmentation, and a host of other drivers [99] because this will determine ongoing ecosystem stability.

community and encourage the collection of valuable baseline, pre-manipulation data. Indeed, the collection of pre-manipulation data can be easier to fund in tropical forests than elsewhere because even basic ecological observations are likely be novel and interesting. Furthermore, designing experiments such that data can be combined through use of a common sampling design will increase collaborative opportunities [66]. Lastly, funding can be obtained from industry sources, providing that obvious conflicts of interest are avoided.

Studying ecological processes at the ecosystem-scale is crucial for comprehensively understanding tropical forests and making informed management decisions that will sustain multiple ecosystem services, including (but not limited to) the continued preservation of global biodiversity. Tackling these issues is becoming progressively more urgent as anthropogenic modification of the planet continues apace.

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