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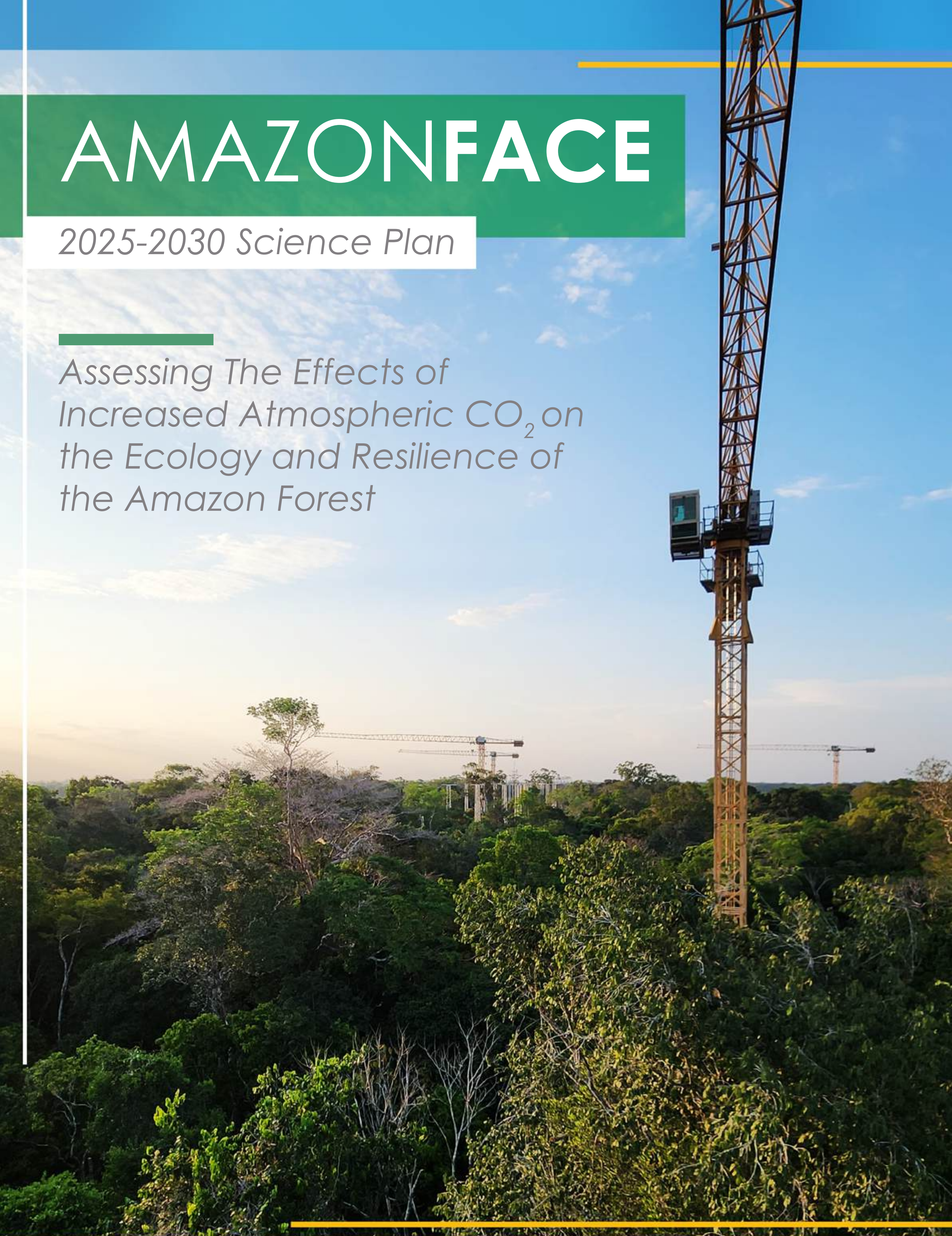
2025-2030 Science Plan

Assessing The Effects of Increased Atmospheric CO_2 on the Ecology and Resilience of the Amazon Forest



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MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E INOVAÇÃO

GOVERNO FEDERAL BRASIL UNIÃO E RECONSTRUÇÃO

Assessing The Effects of Increased Atmospheric CO_2 on the Ecology and Resilience of the Amazon Forest

2025-2030 SCIENCE PLAN

Coordinating lead authors:

David M. Lapola, Universidade Estadual de Campinas - UNICAMP, Brazil
Iain P. Hartley, University of Exeter, UK
Richard Norby, University of Birmingham, UK
Carlos A. N. Quesada, Instituto Nacional de Pesquisas da Amazônia - INPA, Brazil

Lead authors:

Adriane Esquivel-Muelbert - Univ. Birmingham, UK
Anja Rammig - TUM, Germany
Bruno Takeshi T. Portela - INPA, Brazil
Carolina Blanco - UNICAMP, Brazil
Flavia Santana - INPA, Brazil
Iokanam S. Pereira - INPA, Brazil
Izabela Aleixo - INPA, Brazil
Katrin Fleischer - Free University Amsterdam, Netherlands
Laynara F. Lugli - TUM, Germany
Lucia Fuchslueger - Univ. Vienna, Austria
Lucy Rowland - Univ. Exeter, UK
Maíra C. G. Padgurschi - CNPEM, Brazil
Marko S. Monteiro - UNICAMP, Brazil
Sabrina Garcia - INPA, Brazil
Tomas F. Domingues - USP, Brazil

Contributing authors:

Adriana Alcântara - UK Embassy, Brazil
Aline Radaelli - UFRGS, Brazil
Anna Carolina Martins - INPA, Brazil
Andy Wiltshire - MetOffice, UK
Bárbara R. Cardelli - UNICAMP, Brazil
Bart Kruijt - Univ. Wageningen, Netherlands
Bianca F. Rius - UNICAMP, Brazil
Crisvaldo Cassio Souza - INPA, Brazil
Eráclito Sousa Neto - UNICAMP, Brazil
Florian Hofhansl - IIASA, Austria
Gabriela Sophia - Max Planck Institute for Biogeochemistry, Germany
Gustavo Spanner - INPA, Brazil

Joe Davies - MetOffice, Brazil
Juliana Schietti - UFAM, Brazil
Julyane Pires - INPA, Brazil
Keith Lewin - MetOffice, UK
Leonardo R. de Oliveira - INPA, Brazil
Leonor Patrícia C. Morelatto - UNESP, Brazil
Lina Mercado - Univ. Exeter, UK
Maria Clara Ferreira Guimarães - UNICAMP, Brazil
Maria Juliana Monte - INPA, Brazil
Martin De Kauwe - Univ. Bristol, UK
Moara C. Teixeira - INPA, Brazil
Nathielly P. Martins - TUM, Germany
Richard Betts - MetOffice, UK
Roberta Souza - INPA, Brazil
Sara A. Andrade - UNICAMP, Brazil
Simone Vieira - UNICAMP, Brazil
Tiago Jacaúna - UFAM, Brazil
Thorsten Grams - TUM, Germany

Reviewed by:
Maria Clara Ferreira Guimarães
Matheus Schwab
Luciana Cafasso

Design by:
Felipe Furlan
Fábio Palomo

Front cover picture by:
Bruno Takeshi T. Portela

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Contents

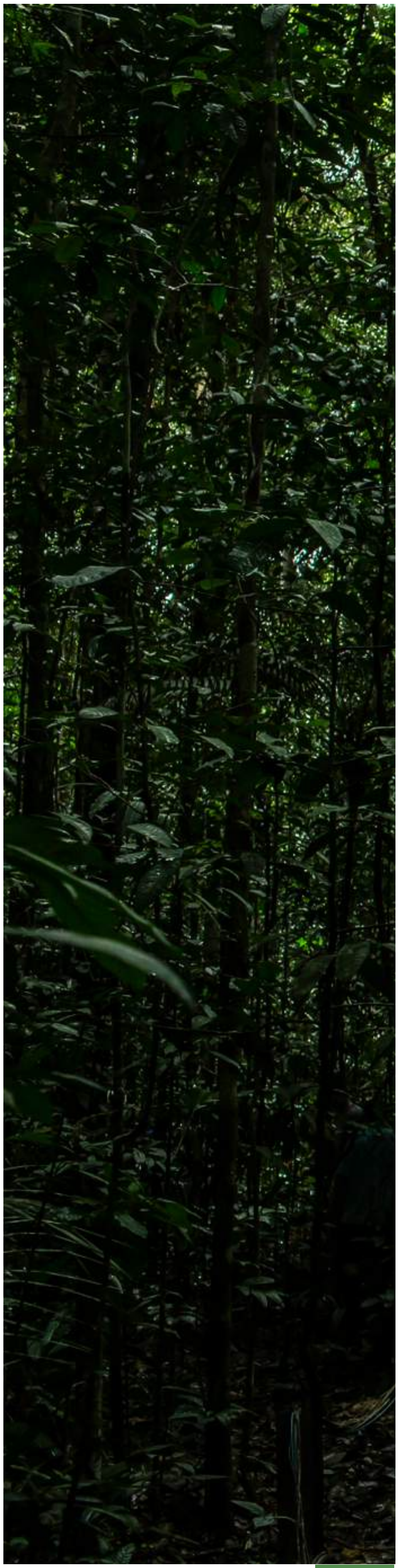
1. Preface and acknowledgements

It is with great satisfaction that the AmazonFACE Programme launches this new Scientific Plan, inaugurating the second phase of the Programme, in which there is the full implementation and operation of the first ecosystem-scale fully replicated FACE experiment Free-Air CO_2 Enrichment in a tropical forest. This document is a broad update of the first Scientific Plan launched in 2014, when the Programme was established. Since then, important lessons have been learned regarding the organisation and scientific planning of the Programme, such as the new distribution of the scientific research topics into Carbon, Nutrients, Water, Biodiversity, Socio-environmental and Modelling Research Areas. It is worth mentioning that the Biodiversity and Socio-Environmental Research Areas are completely new for a FACE experiment and that they assume key relevance in the case of Amazon. Since the launch of the first Scientific Plan in 2014, there has also been an extensive collection of preliminary data for the experiment, which is extremely important for analysing the effect of the increase in atmospheric CO_2 in the forest.

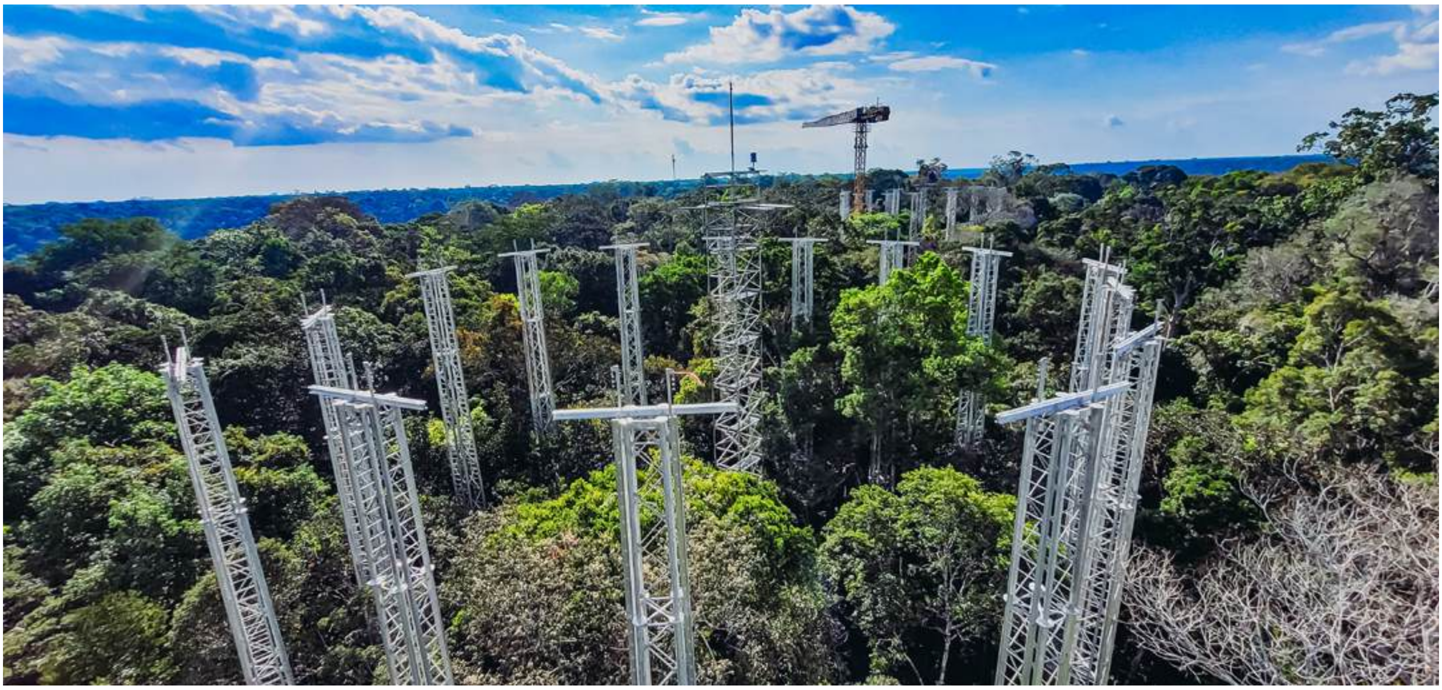
This Scientific Plan does not intend to be exhaustive in outlining the possibilities of scientific research that can be conducted within AmazonFACE, but to identify the key processes and parameters that must be measured or monitored during the experiment. These measurements and observations are the evidence that will help to answer the Programme's central questions, and it will be up to AmazonFACE's core team of researchers and technicians to ensure that these essential examination are being made during the experiment. It is worth remembering that AmazonFACE is a community research structure and that the participation of research groups from Brazil, other Amazonian nations, and other countries is very appreciated.

We thank Brazil's Ministry of Science, Technology and Innovation (MCTI) for the long-lasting continuing support for AmazonFACE as one of MCTI Research Programmes and the Government of the United Kingdom through its Foreign, Commonwealth & Development Office (FCDO) for the key partnership. These two institutions are making possible the execution of one of the most awaited experiments in the fields of Ecology and Climate Change Sciences. We are also thankful to Brazil's National Institute for Amazon Research (INPA) for hosting and coordinating this huge scientific effort, the University of Campinas (UNICAMP) and the UK Met Office for also coordinating this endeavour. Finally, we thank the members of the AmazonFACE Scientific Steering Committee and all the other scientists, technicians, administrative personnel and students who have contributed to or promoted the project for making this dream come true.

David M. Lapola
Iain Hartley
Richard Norby
Carlos A. Quesada



2. Executive summary



The AmazonFACE Programme is directed toward resolving a key source of uncertainty about the future of the Amazon forest: the potential for rising atmospheric CO_2 concentrations and to preserve tropical forests against the deleterious effects of climate change by stimulating forest growth and resilience to drought. The core task of AmazonFACE is a CO_2 enrichment experiment of unprecedented scope and importance, conducted in a primary, old-growth forest in central Amazon. The experiment will simulate the future atmospheric CO_2 composition in order to attempt to answer the question: "How will rising atmospheric CO_2 affect the resilience of the Amazon forest, the biodiversity it harbours, and the ecosystem services it provides?"

Rapid changes in the Earth's climate caused by the burning of fossil fuels and deforestation pose a severe threat to the forests of the Amazon basin. While current Earth system models tend to project a strong CO_2 fertilisation effect (stimulation of plant productivity due to increased atmospheric CO_2) that counteracts the effects of warmer temperatures and drier conditions on the forest, long-term observations have identified the Amazon carbon sink is weakening. The response of tropical forests to long-term climate change remains, therefore, highly uncertain, ranging from modelled scenarios of increased carbon storage capacity to the so-called 'Amazon tipping point', in which substantial areas of rainforest could be replaced by seasonal forest or savannah. Reducing this uncertainty is critical to steering future development policies for the Amazon region, as well as global assessments of ecosystem vulnerability to climate change. This updated science plan presents the rationale for the implementation and operation of a Free-Air CO_2 Enrichment (FACE) experiment in an old-growth forest in the Amazon basin near Manaus, Brazil. FACE technology has proven to be a valuable method to determine long-term, ecosystem-scale responses of forests to elevated CO_2 in temperate regions. However, no such experiment has ever been attempted in a tropical forest, despite the long-standing recognition in Science and policy communities of the need for such effort.

The experiment is composed of six 30 m diameter plots, three of which are maintained at ambient CO_2 concentrations and the other three are kept at elevated (+200ppm) CO_2 concentration for at least ten years. The research site is a plateau at the ZF2 research station, with vegetation and soil representative of a dominant fraction of Amazonia's forests. Experimental plots comprise stands of 30-m tall trees on deep, well-drained clay Ferralsols. Managed by Brazil's National Institute for Amazon Research (INPA), the site has supported a long tradition of research on tropical forest ecology, forest management and biosphere-atmosphere interactions.

Six research areas, Carbon, Nutrients, Water, Biodiversity, Socio-Environmental impacts and Modelling, are the focus of the Programme. Since 2014, a multi-disciplinary team of scientists have been employing state-of-the-art tools and methods to investigate the dynamics of the forest, from deep in the soil to above the forest canopy, to establish a baseline characterization of the experimental forest. The resulting data set from the experiment starting now will be valuable resources for a broad community of scientists and for improving model projections of the future of the Amazon. Significant scientific products from this experimental effort will derive from a strong interaction between empirical and modelling data. The cascading impacts of climate change and elevated CO_2 on the forest and its effects on human populations in the region are investigated concomitantly with the field experiment. AmazonFACE is a flagship scientific endeavour that stimulates the scientific empowerment of research institutions in Brazil and strengthens cooperation with foreign research groups. Results from this project will be disseminated through peer-reviewed scientific journals and communicated to the public in general, government agencies and decision-making bodies, with the goal of reducing the uncertainty of predictions about the vulnerability of the Amazon forest to climate change, helping to steer future development policies for the Amazon region.

3. Introduction: the scientific basis

3.1 Rising atmospheric CO_2, climate change and Amazonia

Humans have increased Earth's atmospheric CO_2 concentration ([CO_2]) by 50% since the late 19th century, owing mainly to the large-scale burning of fossil fuels and, to a lesser extent, land-use changes [1]. The current [CO_2] of approximately 420 parts per million of volume (ppmv) has no precedent in at least the last 3 million years of Earth's history [2]. The atmospheric [CO_2] by the end of this century could reach as high as 1.135 ppmv in the highest emission scenario, but the projections are quite variable, depending on assumptions about energy use, population growth, and other physical, biological, and socioeconomic factors [3]. Because atmospheric CO_2 is the primary substrate for all terrestrial productivity via photosynthesis, this substantial increase undoubtedly is affecting the metabolism of the Amazon forest and other ecosystems worldwide [4].

In fact, about one-third of all the CO_2 released annually to the atmosphere via human actions is currently absorbed by terrestrial ecosystems, being tropical forests and mainly the Amazon responsible for a large fraction of that carbon sink [1,5,6]. The magnitude, duration and global extent of an increase in CO_2 uptake by the terrestrial biosphere in response to rising atmospheric CO_2 concentration, the so-called CO_2 fertilisation effect, are largely unknown, representing a major source of uncertainty that limits the capacity to understand tropical forest processes, assess their vulnerabilities to climate change and improve the representation of these processes in Earth system models. This uncertainty surrounding tropical forest responses to elevated atmospheric CO_2 (eCO_2) and climatic change is especially critical given the large impact that the forests of the Amazon basin have on global carbon and water cycling and on the climate.

The Amazon basin also harbours a considerable fraction of the world's biodiversity and provides substantial ecosystem services to humankind. The Amazon forest is projected to face particularly severe climatic change in the next decades [7–9], compromising the provision of those services [10–12]. The potential for a CO_2 fertilisation effect will have a key role in the response of the forest to climate change [13–21], and so it must be evaluated. Much is known about the effects of eCO_2 on biochemical and physiological processes in leaves, including leaves of tropical trees under tropical conditions [22–25]. However, the primary responses to eCO_2, especially the stimulation of photosynthesis, do not necessarily reveal the ultimate responses of ecosystem productivity, biomass stocks, carbon cycling and biotic interactions.

Free-Air CO_2 Enrichment (FACE) experiments in temperate forests, including young plantations and older native stands, have revealed many higher-order responses and emphasized the importance of interactions and feedback between CO_2 and other environmental resources (namely soil nutrients), stand development, and integration across time and space [26–28]. No such experiments have ever been conducted in a tropical forest, despite such forests account for one-third of the total metabolic activity of the Earth's land surface [29]. Tropical and temperate forests differ substantially in plant species, plant diversity, forest structure, soils, and climate. These variations severely limit our ability to use results from temperate zone studies to predict tropical forest responses [30,31]. Hence, current land surface schemes and vegetation models are highly uncertain in their predictions of tropical forest responses to rising CO_2 and the feedback that vegetation-eCO_2 interaction provides to the global climate system [20,32].

Nevertheless, analysis of the vertical profile of CO_2 concentration in the atmosphere [5,33] and field observations from long-term plot networks [6] both indicate that non-disturbed tropical forests are a strong sink for CO_2 and, through their biological productivity, provide a crucial negative feedback to the accumulation of CO_2 in the atmosphere. However, particularly in the Amazon, the strength of such a carbon sink has declined by 30% since the 1990s [6], raising concerns about the Amazon forest reaching a tipping point of escalating climate-forest degradation [34,35]. The importance of this carbon sink feedback for understanding the unfolding of climate change, the Amazon tipping point, and the impacts on human well-being over this century is indisputable, and the need for direct observational evidence on the Amazon forest response to eCO_2 is compelling.

3.2 Knowledge gaps on tropical forest responses to elevated CO_2

Experimental Evidence

Observed increased growth and recruitment rates recorded in tropical forests over the last three decades are best explained by eCO_2 (with higher near-surface temperature and increased frequency of droughts explaining the 30% decline in the Amazonian carbon sink since the 1990s) [6]. Although there have been no FACE experiments in the tropics, the lessons from temperate FACE experiments [26,27] can highlight some critical areas of uncertainty that must be resolved to improve predictions of tropical ecosystem responses to atmospheric and climatic change. A stimulation of photosynthetic CO_2 uptake is the initial interaction between rising eCO_2 and a forest tree, and in most ecosystems, an increase in the net primary productivity (NPP) is the expected result. In previous FACE experiments in younger temperate forests, NPP increased on average 23% in eCO_2 [36]. The critical question then is whether increased NPP results in the accumulation of carbon in perennial tissue (i.e., wood) or rather is cycled quickly through the ecosystem and released as CO_2 back to the atmosphere [37].

NPP represents the input of organic matter into an ecosystem but by itself does not predict ecosystem carbon storage, a process that depends on how carbon is partitioned to different plant and soil pools and the turnover times of those pools. We must understand the relative partitioning of carbon to the production of leaves, wood and roots, to storage compounds, to respiration and other losses to assess the destination of carbon in the ecosystem. Hence, an important uncertainty that must be resolved is whether NPP stimulation in the tropics results primarily in increases in woody biomass or increased detrital input into soil. In the Oak Ridge FACE (ORNL-FACE) experiment in a temperate forest in Tennessee, USA, the stimulation of NPP was primarily associated with increased fine root production, and although fine roots turn over rapidly and do not contribute to ecosystem C storage, their input of C into the soil resulted in increased soil organic C [38]. Our understanding of root system responses in tropical forests is especially weak and must be improved given the many intersection points among roots, plant growth, carbon, water, and nutrient cycles in tropical forest ecosystems.

Temperate zone experiments also revealed the importance of nutrient availability and feedback between carbon and nitrogen cycles in modifying responses to eCO_2. In the ORNL-FACE experiment, the initial stimulation of NPP in eCO_2 declined due to a progressive nitrogen limitation that was accelerated in the eCO_2 plots [39]. Many tropical forests may not be nitrogen-limited, as strong evidence indicates that NPP in tropical rain forests is phosphorus (P) limited [40–45]. While major differences exist between Eucalyptus forests and tropical rainforest, the fact that P limitation is thought to be the primary reason that there was no NPP response to eCO_2 in the EucFACE experiment in Australia [28] further emphasizes the crucial need to quantify P cycling responses to eCO_2 in Amazonia. New observational data on the interplay between eCO_2, P limitation and drought are thus needed to inform models on P limitation of tropical photosynthesis and mechanisms whereby P limitation might be attenuated under eCO_2, such as increased phosphatase enzyme activity to stimulate soil phosphorus availability [46] and increased root and mycorrhizal exploration of the soil profile that enhance the ability of trees to increase access to less available forms of phosphorus under eCO_2 [47,48].

Interactions between eCO_2 and the water cycle could be very important to tropical forests in a future high-CO_2 world, especially in the Amazon, where a substantial amount of rainfall is dependent on the water flux vapour from the forest to the atmosphere [49]. By increasing photosynthesis and/or decreasing water use via reductions in stomatal conductance, water-use efficiency (WUE; carbon uptake per unit water loss) usually increases in response to eCO_2 [50]. Depending on other factors, especially responses in total leaf area, increased WUE may or may not result in decreased water use [26], but increased WUE potentially could confer increased drought tolerance to trees in eCO_2 [24]. Increased soil moisture has been associated with eCO_2 in some experiments, with subsequent effects on soil respiration and nutrient turnover [51] as well as potential consequence for the flux of moisture to the atmosphere [32].

Interactions between CO_2 and light derive from the capacity of eCO_2 to increase light-use efficiency in photosynthesis and decrease the photosynthetic light compensation point [52]. Although plants in the deep shade of a closed tropical forest will have slow growth, their relative response to eCO_2 can be dramatic [53,54]. Hence, eCO_2 has the potential to facilitate the expansion of plants into deeper shade [23,54] and alter the species composition that results after a canopy opening. This issue is critical in determining the response of leaf area index (LAI, m^2 leaf area per m^2 ground area) and the associated change in biosphere-atmosphere interactions under eCO_2 conditions.

Few data are available describing the differential sensitivity to eCO_2 among tropical species, but if important differences exist at large scales, they could represent a significant influence on forest structure resulting from revegetation of a forest gap or abandoned agricultural land. Lianas (woody vines) are increasing in Neotropical forests, representing one of the first large-scale compositional changes documented for old-growth tropical forests. Some research indicates lianas, woody legumes and early-successional species may be particularly sensitive to eCO_2 [55–60], and this could potentially have far-reaching consequences for ecosystem carbon storage.

Insights from Models

Models are the primary tools for interpreting ecosystem measurements, understanding their relationship to environmental variables and placing those observations in a larger spatial and temporal context. Models have been used to interpret past and current responses of terrestrial ecosystems to atmospheric CO_2. They are especially useful for projecting responses to future scenarios of eCO_2 and the exchange between the land and the atmosphere, which may alter future climate. Confidence in such model predictions depends on the models being well-informed by both process-level and large-scale observations and responses to experimental manipulations [61].

Global models that incorporate a whole ecosystem heuristic illustrate the potential importance of eCO_2 to tropical carbon cycling and the exchange from the tropics to the global climate [13,62]. Carbon cycle predictions of different dynamic global vegetation models (DGVMs) are partly consistent with contemporary global land carbon budgets and can diverge considerably when forced with the future climate predicted by general circulation models (GCMs), CO_2 emission scenarios and different parameterizations on the effects of increasing atmospheric $[CO_2]$ on photosynthesis and photosynthetic water-use efficiency by plants. Importantly, current DGVMs do not represent well the fluctuations of the carbon sink in the Amazon, and in general they consider that the forest carbon sink will continue indefinitely in the future due to CO_2 fertilisation [21].

Past studies constrained the likely range of sensitivities of tropical land carbon fluxes to climate change by current observations, suggesting that tropical forests, and especially the Amazon forest, are more resilient to climate change than previously thought, assuming CO_2 fertilisation effects are as large as suggested by current vegetation models [16]. In the LPJ dynamic global vegetation model (DGVM) the enhancement of NPP driven by eCO_2 was shown to be more pronounced in the tropics (35% NPP enhancement), than in temperate forests (26% NPP enhancement) at an atmospheric CO_2 concentration of 550 ppm relative to that at 370 ppm [30]. This latter result was derived primarily from the expression of photosynthesis in the model, which shows greater stimulation by eCO_2 at higher temperatures (due to changed CO_2/O_2 specificity of the RuBisCO enzyme at higher temperatures). It is important to emphasize that potential nutrient limitations were not included in the model. A more recent model intercomparison between models considering nutrient cycling showed that the lack of soil P can reduce biomass gains due to the CO_2 fertilisation effect on average by 50% in the Amazon, conditional on how the P cycle is represented [20,46,63].

The FACE Model-Data Synthesis project used data from the Duke and Oak Ridge FACE experiments (after their conclusion) to benchmark model predictions of temperate forest responses to eCO_2, also exploring in a detailed pattern the underlying reasons for model behaviour under ambient and increased CO_2 [64,65]. Fundamental insights were provided on the model assumptions that best capture the responses of temperate forest vegetation to

eCO_2, such as the dependence of leaf-atmosphere coupling for better capturing changes in plant water use [50] or the use of allometry-based carbon allocation methods for better representing changes in biomass [66].

Many other studies using different vegetation models have highlighted the key role of the CO_2 fertilisation effect for counteracting the likely deleterious effects of climate change on vegetation, maintaining the Amazon forest biomass relatively unchanged and resulting in the tropical land being predicted to be a net sink for carbon rather than a net source over the 21st century [14,15,17,18,20,21,62,67–71]. Exceptions were found for extreme climate scenarios – extreme increases in temperature or decreases in annual rainfall – for which even a strong CO_2 fertilisation effect is not sufficient to avoid the modelled loss of biomass. Thus, the possibility of climate change causing a substantial loss of Amazon rainforest cover and carbon stocks and amplifying the climate-carbon cycle feedback – the so-called "Amazon forest dieback" or "Amazon tipping point" [34,72] – is still an open question because of the potential resilience that eCO_2 might confer to vegetation and the lack of experimental field studies to constrain the vegetation models with respect to this resilience.

However, many uncertainties related to the effects of eCO_2 on tropical forests remain to be better addressed by models, such as the limitation of NPP and tree growth by P availability [20,46,73], the integrated flux of moisture in the soil-forest-atmosphere continuum under eCO_2 and the concurrent effect of droughts [74,75], or the hypothetical dampening role that the hyperdiversity of trees found in tropical forests may have on the ecosystem-level responses to eCO_2 [68,69]. As of today, model predictions on those processes are based on limited information and omit what are likely to be critical modifying processes. Considering the nexus between functional characterization of the plant community at the experimental site, their relationship with ecological and biogeochemical processes [76,77] under eCO_2 and the cascading effects on ecosystem services and human well-being [12,78], such uncertainties could hamper the adaptability of human populations to those changes [51]. The proper integration between AmazonFACE experimental data and models, with in-depth analysis of model assumptions [64], has an enormous potential to leapfrog our knowledge on the tropical forest responses to eCO_2 and the resilience of the Amazon forest and its populations to ongoing climatic changes.

3.3 Why do we need a FACE experiment in Amazonia?

The critical need to address the many substantial scientific issues concerning the response of the Amazon forest to rising atmospheric CO_2 is the primary justification for a long-term and large-scale FACE experiment in the Amazon. Modelling studies indicate that there is a substantial, though uncertain, risk of wide-spread dieback, or tipping point, of the Amazon rainforest under future climate change [14,15,17,18,20,21,62,67–71]. This occurrence would have a key impact on the natural resource base of Latin America and would represent a significant threat to the region's economy, for example, via changes in the region's water circulation patterns and the cascading impacts on agricultural outputs, hydropower supply, ultimately leading to financial losses in the order of US$ 8.2 trillion, migration and other socioeconomic hardships [12].

As outlined above, some of the negative effects of climate change on forests may be mitigated by the CO_2 fertilisation effect stimulating forest growth and increasing resilience to drought. However, if mitigation through CO_2 fertilisation does not occur, then tropical forests are predicted to be much more vulnerable to climate change and the risk of crossing a tipping point would increase. Currently, models cannot provide sufficient confidence in future projections for the Amazon forest without field-based experimental evidence on the ecosystem-scale responses of tropical forests to eCO_2. Therefore, reducing uncertainty in this area is critical to steer future development policies for the Amazon region.

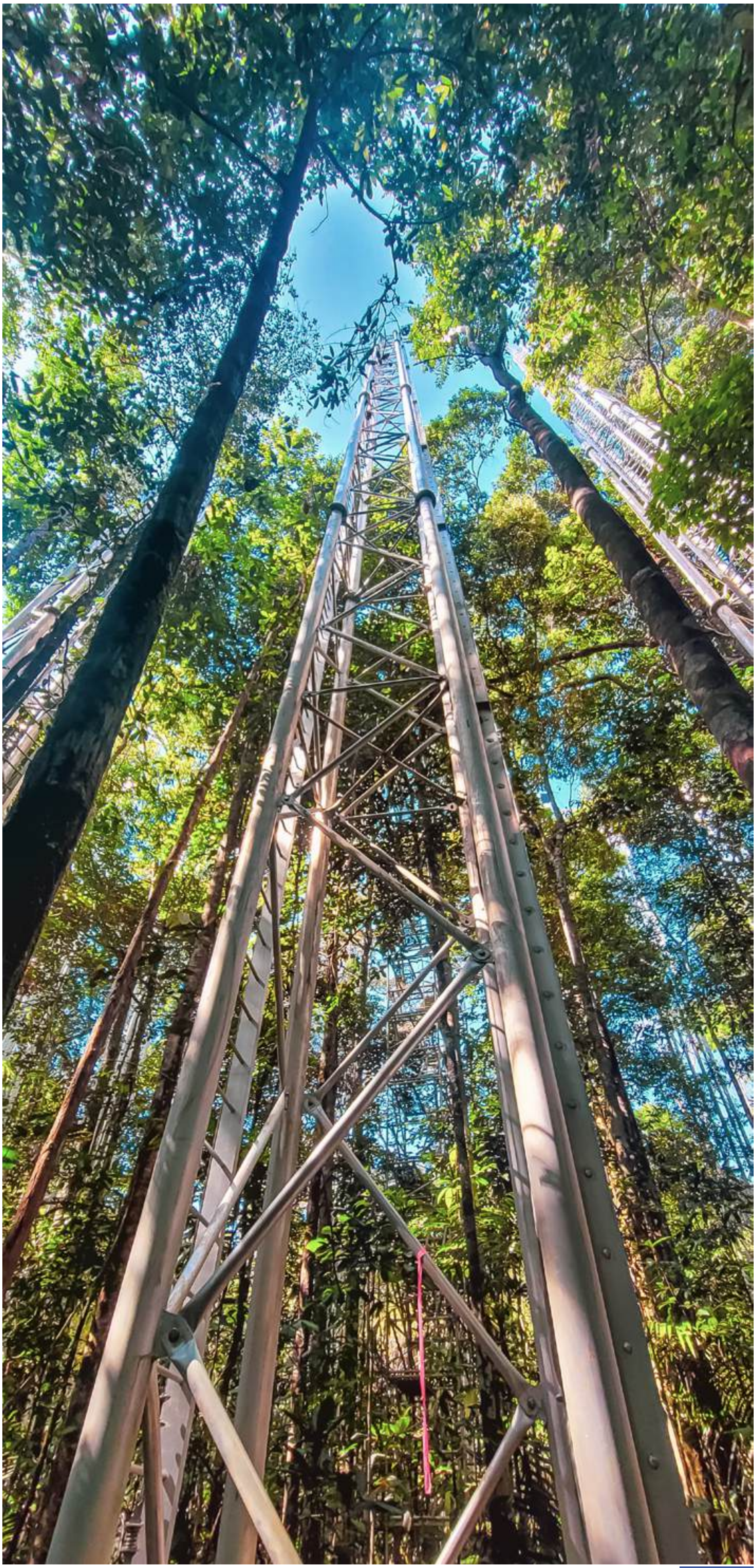
The responses of forests to eCO_2 have not been tested in the Amazon or anywhere else in the tropics, and there is a compelling need to reduce this uncertainty. A FACE experiment is the most direct and robust scientific approach for accomplishing this. The AmazonFACE experiment will provide primary scientific information that advances our knowledge and understanding of the physiological and ecological effects of $e[CO_2]$ in tropical forests. It will provide data needed for parameterizing and improving predictive models of the long-term effects of elevated CO_2 on carbon cycle and climate change. Several reasons make a FACE experiment in the Amazon forest especially relevant:

- The forests of the Amazon basin – the largest extent of tropical forest in the world – have a large impact on the global atmosphere, carbon and water cycles, comprise the world's largest repository of biodiversity and provide substantial ecosystem services to humankind. For instance, the Amazonas river outflow represents 20% of the global flow of fresh water to the oceans [11]. All these functions will be affected by eCO_2 [32], and then it is important to predict the role Amazonia will play in the next decades for the global carbon and water cycles, climate regulation and biodiversity conservation.
- In addition to its key relevance for the global carbon and water cycles, biodiversity and traditional human populations, the Amazon forest is also the only tropical forest region considered a "tipping element" of the climate system [34]. The ultimate impact of or recovery from threats occurring in the world's largest tropical forest, such as deforestation, forest degradation and namely climate change, will strongly depend on the direct physiological response of the forest to eCO_2.
- The Amazon basin is home to about 28 million people, and if the forest dieback (or tipping point) indeed takes place, there will be considerable consequences for the region's social welfare and economy [12].
- Existing data and infrastructure: There is already a well-maintained, coherent network of forest plots throughout the basin in which biodiversity and forest dynamics have been studied and catalogued, and tree growth have been monitored [6], in a few of which there is the co-occurrence of eddy flux towers, most of them from the LBA (Large Scale Biosphere-Atmosphere

Experiment in Amazonia) project [79].

- Institutional capacity: The Amazon region and Brazil have built top-quality expertise in the field of biosphere-atmosphere interactions in tropical forests during recent decades, with strong scientific collaborations with European and US American institutions and research groups.

Even before its commencement, AmazonFACE is a flagship scientific endeavour with existing high visibility in the international scientific community and media [80]. In addition to the primary scientific justification for the proposed experiment, there are numerous ancillary benefits. The analysis of the CO_2 fertilisation effect in the Amazon forest should have many significant economic and environmental implications for the Amazon basin and for global carbon and water cycles. It is expected that the experiment will also have direct implications for issues such as biological conservation, forestry practices, land use and climate policies, and the provision of ecosystem services from the Amazon forest. The multi-disciplinary research team already involved in the programme will advance the scientific empowerment of developing nations (Amazonian and other tropical forest countries) through education and training, hands-on research experience, and international collaboration. The experiment will provide a forum for outreach and education on climate change issues and tropical forest ecology for stakeholders, policy makers, and the public in general.



4. Objectives and major research question

The AmazonFACE research programme is directed toward resolving a key source of uncertainty in climate change science: the potential for rising atmospheric CO_2 concentrations to prevent tropical forests and the ecosystem services they provide against the negative effects of climate change by stimulating forest growth and increasing resilience to drought.

The core aim of the project is the planning, implementation and execution of a CO_2 enrichment experiment of unprecedented scope and importance in a hyperdiverse mature tropical forest located 80 km north of Manaus, Brazil. The experiment will simulate the atmospheric CO_2 composition of the future[1] to help answer the overarching question:

"How will rising atmospheric CO_2 affect the resilience of the Amazon forest, the biodiversity it harbours, and the ecosystem services it provides in light of climate change?"

AmazonFACE will allow advancement in six key relevant research areas: Carbon, Nutrients, Water, Biodiversity, Socio-Environmental and Modelling – the Biodiversity and Socio-Environmental areas definitely aggregates, in relation to past FACE experiments given, respectively, that this is the first FACE in a highly diverse ecosystem, and the potential impacts of climate change and eCO_2 on several of the region's socio economic sectors [12]. Such research advancements are also punctuated, since the beginning of the Programme, by modelling activities, with the main goal of improving vegetation, climate and Earth system models with respect to the effects of eCO_2 in tropical forests.

[1] The concentration of ~615 ppmv is predicted to be reached by the 2070's in the SSP2-4.5 scenario, which, as of 2023, seems the most plausible emission trajectory [4].

5. Expected outcomes and broader impacts

The major expected outcome of this project will be an improvement of our scientific knowledge about the future of the Amazon forest in the context of atmospheric and climatic change: how the Amazon forest can support humanity to diminish carbon emission, as well as how vulnerable the forest will be to ongoing climate change. This improved knowledge will be delivered through multiple products. Data sets describing physiological and ecological responses will be made publicly available and will provide invaluable inputs for parameterizing, testing and improving vegetation, climate and Earth system models used to predict terrestrial responses to eCO_2, climate change and other disturbances. The results from both field experiment and modelling exercises will serve as the basis for understanding how the changes occurring in the forest in response to eCO_2 and climate change will impact different socioeconomic sectors of the Amazon basin and neighbouring regions. In addition, it will assist in creating climate mitigation and adaptation strategies at the local and regional levels.

Results of experimental and modelling activities will be published in peer-reviewed scientific journals, including synthesis papers in high-visibility international publications, with a target of having a significant fraction of the Programme's publications as open access. The scientific products also will be prepared in close collaboration with scientific press professionals in a format appropriate for informing society and decision-makers (including active social media channels) and providing input into sustainability initiatives in the Amazon.

Another important outcome of the AmazonFACE Programme will be in the scientific training of Brazilian students and capacity building of Brazilian institutions. Successful implementation of this project will require the participation of many students in various disciplines: Plant Biology, Experimental Field Ecology, Ecological Modelling, Soil Science, Microbiology, Meteorology, Data Analysis, Engineering, Social Scientists, Anthropologists and Scientific and Public Communication. The Programme expects to host at least 30 postdoctoral researchers, about 50 PhD students, and similar numbers of field and lab technicians and master students throughout its duration. Such a generation of students trained through AmazonFACE will be prepared to use these skills, for example, in future – perhaps even more ambitious – research programmes, government policy analysis and nonprofit organisations promoting sustainability.

AmazonFACE has enormous potential to foster innovative science in Brazilian institutions, especially in Manaus and Amazonas, regions that have historically suffered from a shortage of specialised scientific personnel and infrastructure. In addition to training local students and embedding them in the exchange of research methods between UK, European, US-American and Brazilian research communities, AmazonFACE will create transformative research that can change our understanding of the Amazon region, during a period of lingering environmental crisis in Amazon.

Large, integrated field experiments and infrastructures have always led to technological advances in techniques for monitoring, and AmazonFACE can be expected to deliver substantially in this area. New developments can be expected in remote sensing, automated canopy observation techniques, automated plant physiology measurement, analysis of soil and root biochemistry, and modelling soil-vegetation-atmosphere interactions, as these will be especially useful for AmazonFACE. The project will actively seek collaboration with regional (Amazonian or Brazilian) engineering companies and research centres in Brazil to jointly develop innovative approaches in such fields.

6. History (2011-2024)

The scientific importance of conducting such CO_2 enrichment experiment in a tropical forest has been highlighted since the early 1990s [81], when the notion of carrying out such an endeavour in the Amazon forest also began to be considered. A first attempt to implement a FACE experiment in the Amazon forest, more specifically in Rondônia, in the mid-1990s was obstructed by both financial and logistical limitations. The very first discussions that later culminated in AmazonFACE were held in a hybrid meeting on October 12th and 13th, 2011 at INPA in Manaus. Discussions continued during the Rio+20 Summit in 2012, and AmazonFACE was effectively materialised during a dedicated workshop held at the Inter-American Development Bank (IDB) headquarters in July 2013 (which was featured as a story in Nature magazine) [82]. In 2014, it became an official R&D Programme of Brazil's Ministry of Science, Technology and Innovation (MCTI) under the execution of the National Institute for Amazonian Research (INPA).

Initial financial support came through cooperation agreements between MCTI and the Inter-American Development Bank (IDB), Amazonas Research Foundation (FAPEAM) and the Coordination for the Improvement of Higher Education Personnel (CAPES) with the objective of writing the first Science Plan & Implementation Strategy [83], delimiting the experimental plots, conducting a baseline ecological characterization of the experimental area (which started in 2015), developing formal engineering plans, generating hypotheses from modelling exercises [12,20,84], as well as conducting a first-order large-scale evaluation of the socioeconomic impacts of the Amazon forest dieback [12]. Other institutions such as São Paulo Research Foundation (FAPESP), Brazil's National Council for Science (CNPq), U.S. Agency for International Development (USAid), and the Serrapilheira Institute provided funding for smaller projects conducted within AmazonFACE.

In 2017, support from IDB/MCTI was discontinued, and the Programme focused on a smaller-scale eCO_2 experiment in the forest understory with Open-Top Chambers (OTC) installed a few hundred meters away from the FACE plots (see section 7.7), the results of which are now becoming publicly available [54]. In the meantime, several of the baseline measurements conducted in the FACE plots were continued, especially tree stem growth.

In 2021, significant investment from the UK Government, matched by MCTI, was announced at COP26 in Glasgow, Scotland. These investments secured the purchase and installation of all needed infrastructure in the experimental area, including towers, cranes and tanks. The first two AmazonFACE plots were concluded and tested by mid-2024, and the fully replicated experiment is expected to start by late 2024.

AmazonFACE gathers a community of approximately 130 people comprised of researchers from different disciplines, students, administrators, social scientists and even journalists and artists from about 40 different institutions in Brazil, UK, Europe, USA and Australia. Due to the importance of the topics it explores, the size and quality of the involved community and its interdisciplinary nature, AmazonFACE is considered one of the most relevant scientific efforts taking place in the Amazon region.

7. Summary of baseline results

7.1 Photosynthesis and stomatal conductance

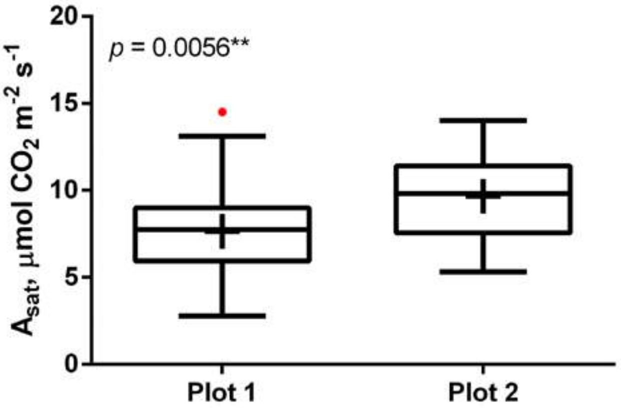


Figure 1. Pre-experimental net CO_2 assimilation at saturating light (A_{sat}, Mmolm^{-2} S^{-1}) of eight species in plots 1 (treatment) and 2 (treatment) the experimental area of the AmazonFACE programme.

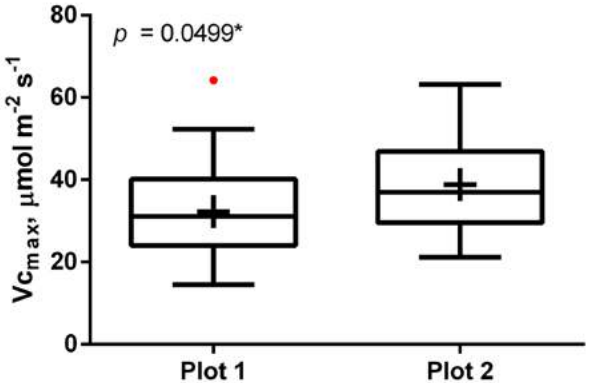


Figure 2. Pre-experimental maximum carboxylation rate of rubisco (Vcmax, Mmolm^{-2} S^{-1}) of eight species in plots 1 (treatment) and 2 (treatment) the experimental area of the AmazonFACE programme.

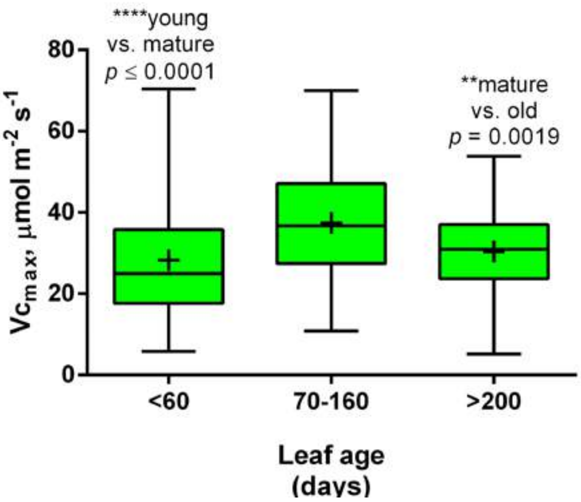


Figure 3. Leaf age effect on maximum carboxylation rate of RuBisCO (Vcmax, µmolm^{-2} s^{-1}) among leaf age classes of nine tree species (n = 213). The boxplots represent the age categories, which are divided into young (< 60 days, n = 61), mature (70 < x < 160 days, n = 86) and old (> 200 days, n = 66). Horizontal lines indicate the median Vcmax, while the boxes represent the interquartile range (the middle 50% of measurements). Whiskers extend to 1.5 times the interquartile range, and black dots denote outliers beyond this range. Leaf age has a margin of error of ± 30 days.

7.2 Aboveground biomass

Aboveground biomass and production have been measured annually, providing essential baseline data for subsequent evaluation of these critical responses to eCO_2. Given the large spatial variability in biomass and production, differences between plots in response to CO_2 enrichment would be undetectable without the ability to separate CO_2 effects from pre-existing differences. All trees > 2 cm DBH have been measured in October each year since 2015 in six plots (approximately 1,400 trees). However, one of those plots (#5) is no longer part of the experiment and has been replaced by plot #7. Wood dry mass of those trees within 13 m from the plot centre (i.e., excluding a 2 m buffer from the circle defined by the vent pipes) is calculated using the allometric equation published [85].

On a subset of trees, height data are available, permitting the use of the allometry based on both DBH and height. Otherwise, an equation based only on DBH is used. In both cases, wood density of the species is an additional factor. Wood density values were obtained from the Global Wood Density database using the BIOMASS package from R. If the species is in the database, the average of all observations of that species is taken. If the species is not in the database, or if only the genus of the tree in the plot is known, then the average of all observations of that genus is taken. If the genus is not known or not in the database, the average of all values of AmazonFACE trees (0.69) is used in calculations. Standing biomass is expressed as the total of all trees divided by plot area of 531 m².

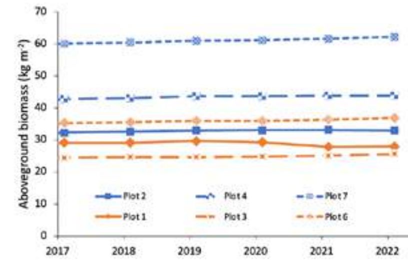


Figure 4. Standing Aboveground Biomass Variation in the AmazonFACE plots.

Standing aboveground biomass varied by a factor of 1.8 over the six AmazonFACE plots, and plots to be assigned to aCO_2 (blues lines) had greater total aboveground biomass than plots for eCO_2 (orange lines). One should notice that the selection of plots was made back in 2013 to minimise differences in productivity, avoiding areas with emergent trees and

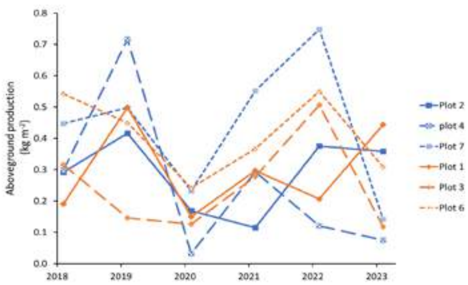


Figure 5.a

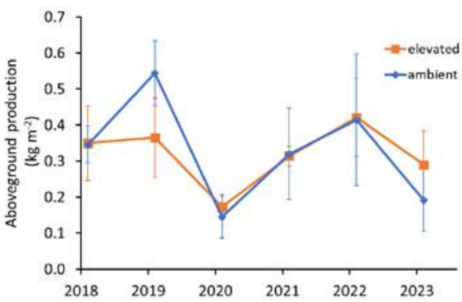


Figure 5.b [2020 to 2022] "Elevated" and "ambient" means, the plots that will receive, respectively, CO_2- enriched and ambient air once the experiment is running.

Annual wood production is calculated as the difference in each tree's biomass from one year to the next. Trees that died during the year are excluded from this calculation. (a) Productivity in 2021 varied by 3.2 times, and there was substantial year-to-year variation, which is not consistent across plots. This emphasises the importance of having multiple-year baseline data. These data can be used to adjust subsequent productivity data to a common baseline. (b) Fortunately, the mean of the three replicates of the two treatment groups are very similar from 2020 to 2022.

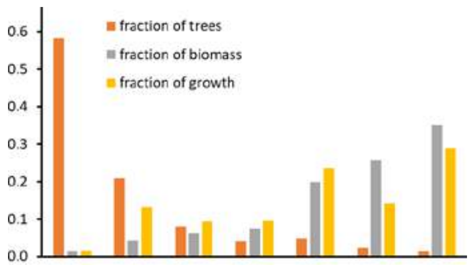


Figure 6. Standing biomass, trees productivity and growth.

71% of the standing biomass and 66% of the productivity was in trees with DBH > 20 cm, although these trees represented just 8.9% of the trees. 58% of the trees had DBH < 5 cm, but these small trees had only about 1.5% of the total biomass and productivity.

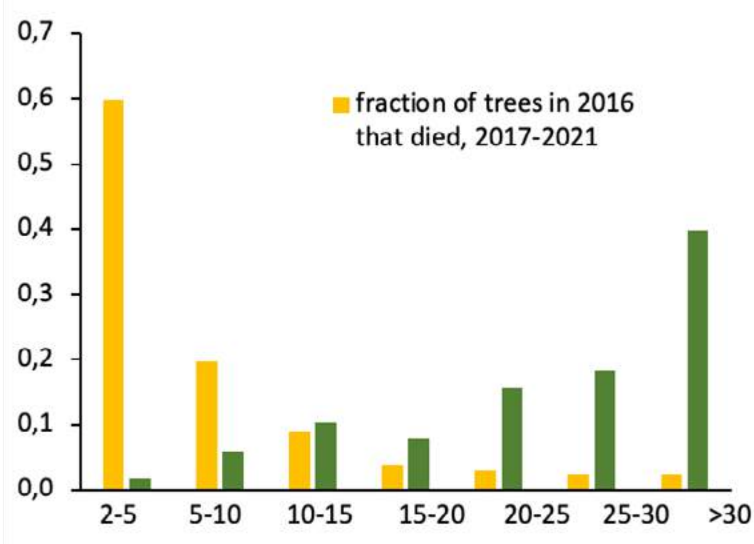


Figure 7. The annual change in forest biomass is equal to new production minus mortality.

In 2021, over 6 plots (including plot 5 but not plot 7), tree biomass increased 1.72 g m^{-2}, but this was offset by tree mortality of 1.83 g m^{-2}, for a net loss of 0.11 g m^{-2} in aboveground biomass. 132 trees of the total alive in 2016 died from 2017 to 2021 (8.4% or 1.7% per year). 10 trees accounted for 74% of the lost biomass. It is important to note as rare events, since accurate assessments of mortality require larger plots than the ones we are using in AmazonFACE.

7.3 Leaf and fine root production

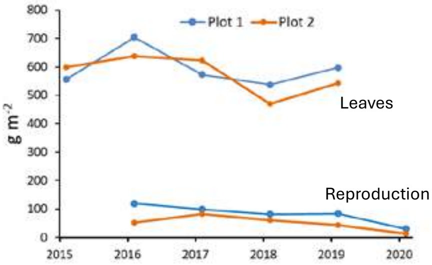


Figure 8. Leaves, fruit, flowers, and twigs collected biweekly from twelve 0.25-M² litter baskets per plot, oven-dried and weighed. The calculations assume that litter fall represents the previous year's leaf production based on careful tracking of leaf phenology. The total for the year (October 1st – September 30th) is corrected for change in mass per area of green leaves vs. litter (1.4%). Plot 1 is assigned to eCO_2 and plot 2 to aCO_2.

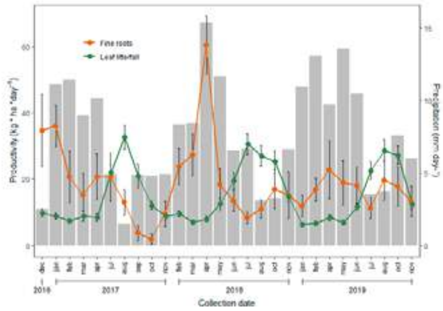
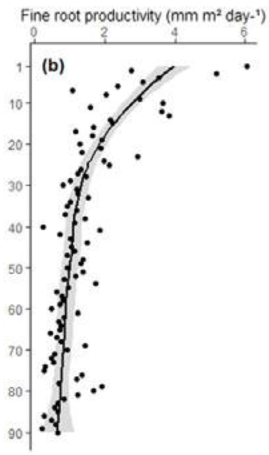


Figure 9. Fine root production was measured with minirhizotrons in plots 1 and 2 from December 2016 to November 2019. Annual fine root production was 6.7 Mg ha^{-1}, with greater production in the wet season than in the dry season. There was a distinct asynchrony between fine root production and leaf litterfall. Since new leaf production occurs at the same time as litterfall, this asynchrony represents a tradeoff in allocation between leaf and fine root production.



Depth	Fraction of standing stock	Fraction of productivity	Turnover (year^{-1})
0-30	0.51	0.60	1.02
30-60	0.38	0.26	0.61
60-90	0.12	0.13	0.40

Figure 10. Fine root standing stock and production fine root standing stock and production were greatest in surface soils, but there was nevertheless a substantial fraction of roots deeper in the soil. Fine root turnover decreased with depth [86].

7.4 Net primary productivity

With the additional data on leaf, reproductive tissue, and fine root production in plots 1 and 2, net primary productivity could be calculated for 2017-2019. The year is defined as October through September. Leaf production is considered litter production of the previous year × 1.014. This factor takes considers that dry matter is resorbed from leaves as they senesce. Mass of twigs collected in litter traps is added to the aboveground wood of the previous year. Reproductive tissue in litter traps is for the current year. Coarse root production is set to 21% of aboveground wood production following.

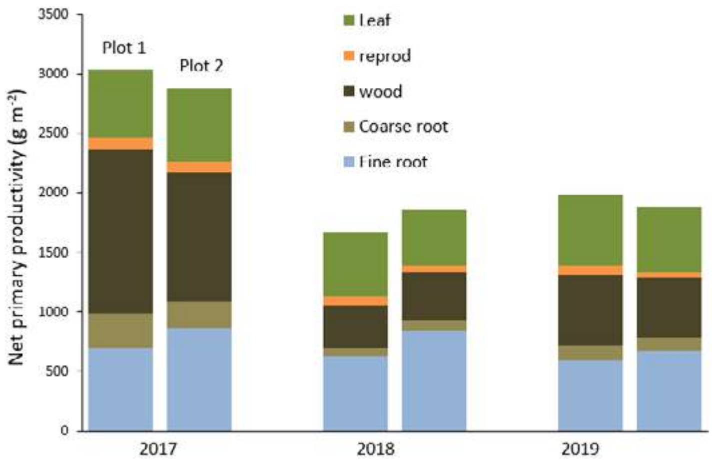


Figure 11.a. NPP was 2,740 g m^{-2} in 2017, 1,776 g m^{-2} in 2018, and 1,769 g m^{-2} in 2019; average over the three years was 2,065 g m^{-2}.

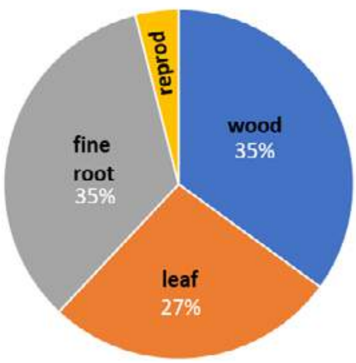


Figure 11.b. Allocation to fine roots was especially high, and to woody biomass lower than in other forest ecosystem studies.

7.5 Root depth distribution

Construction of the towers surrounding each plot required digging of deep pits for the concrete bases. This provided an opportunity to collect valuable and hard-to-obtain data on root distribution, root morphology, and soil physical and biogeochemical characteristics. 1,200 samples were collected in three campaigns. Here, we present preliminary data on root distribution from five pits in plot 1 and five pits in plot 2, collected in October 2022 from the plot-facing side of the pits. Fine roots (0-1 mm and 1-2 mm diameter) were extracted from soil samples of controlled volume at depth intervals of 0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm, 30-50 cm and 50-100 cm. Additional samples were collected with a soil auger from the bottom of the pit to depths of 100-150 cm and 150-200 cm [87].

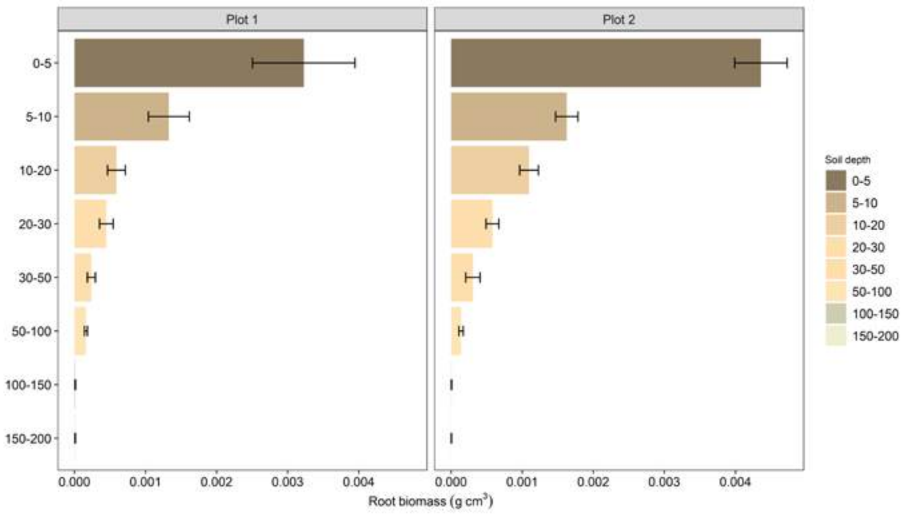


Figure 12. The density of fine roots < 1 mm diameter was greatest in the top 5 cm and decreased with depth (Fig. 12). Multiplying the density by the layer thickness yields the contribution of each layer to total column fine root mass (Fig. 13).

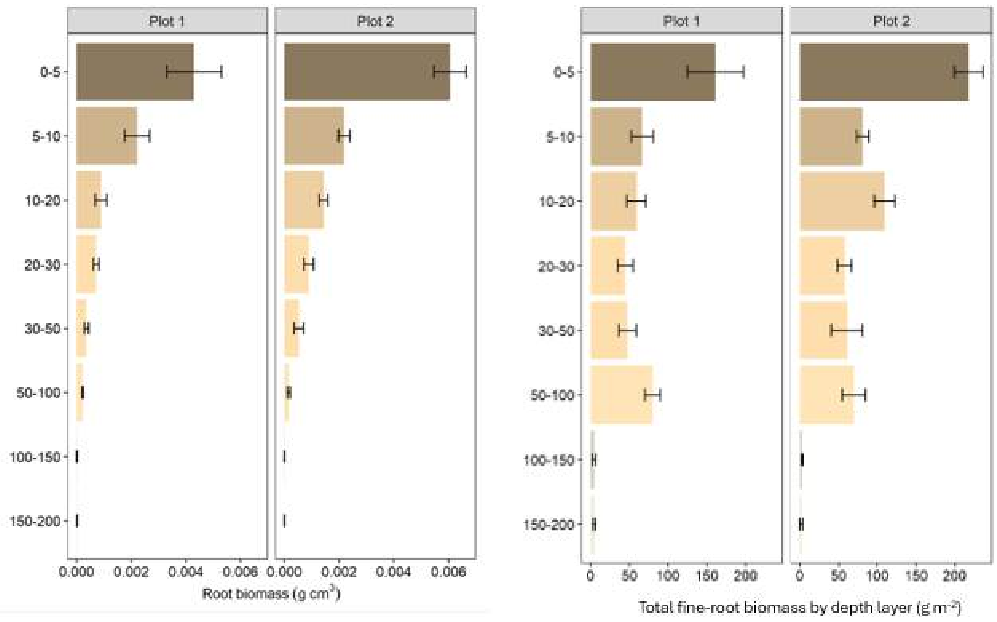


Figure 13. Fine root biomass in AmazonFACE plots 1 and 2. Biomass of fine roots (<1 mm diameter) per unit soil volume (a) and per unit ground area (b). Data are the means ± SE of five pits in each of plots 1 and 2. Graphs courtesy of N. Martins.

The depth distribution can be modelled by the beta function developed by Gale and Grigal (1987) [188], in which cumulative fraction of root mass = $1-\beta^D$, where D is depth (Fig. 13). The value of ß (0.944) for plots 1 and 2 is within the range of the ß values of the world's biomes reported by Jackson et al. (1996) [88] but indicates a more surficial root distribution than the average value for tropical evergreen forests (0.962). A better fit is provided by the equation developed by Zeng (2001), which was developed to better represent deep water uptake by tropical forests: cumulative fraction = $1 - 1/2 \times [\exp(-a \times D) + \exp(-b \times D)]$, and fit to plots 1 and 2, a = 0.02477 and b = 0.1673.

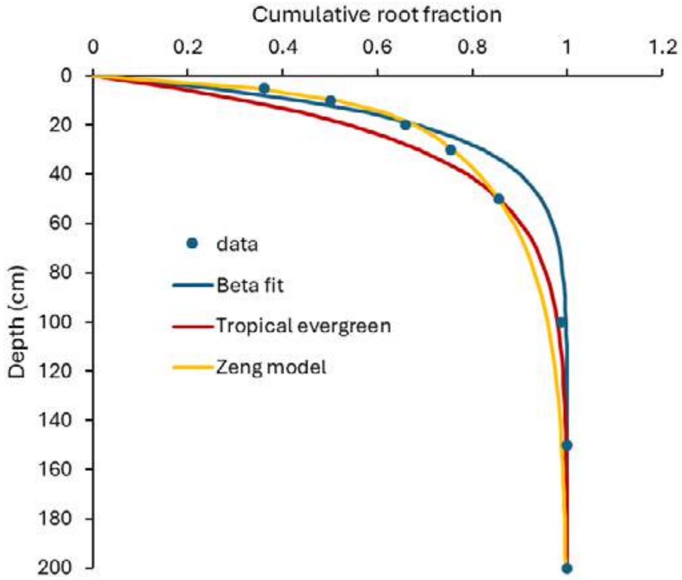


Figure 14. with higher soil C concentrations in the upper 30 cm, but almost 75% of C is stored in the first metre (Figure 15)

These data and the modelled fits to the data will be important in the analysis of fine root production data from minirhizotrons, biogeochemical cycling, water uptake, and ecosystem modelling.

7.6 Soil nutrient composition and soil microbial dynamics

The soils at the AmazonFACE site contain around 180.5 Mg C ha-1 in the first two metres. Total C concentrations show a similar distribution along the soil profile as roots, with higher soil C concentrations in the upper 30 cm, but almost 75% of C is stored in the first metre (Figure 14). The soils are characterised by rather low total and available soil P with values only reaching around 150 µg g-1 of soil in the upper 15 cm [89]. On the one hand, this causes a tight cycling of mineral nutrients, such as P and K by plant roots from the litter layer [90]. It also strongly influences labile organic and inorganic P availability over the course of a year in the soil [89]. In addition, the soil microbial biomass presents high C:P ratios indicating that the soil microbial community may be P, rather than N limited. Moreover, soil extracellular enzyme rates, which can be used as proxy for soil microbial activity, are highly dynamic and respond to fluctuations in available C and mineral nutrient supply [91,92].

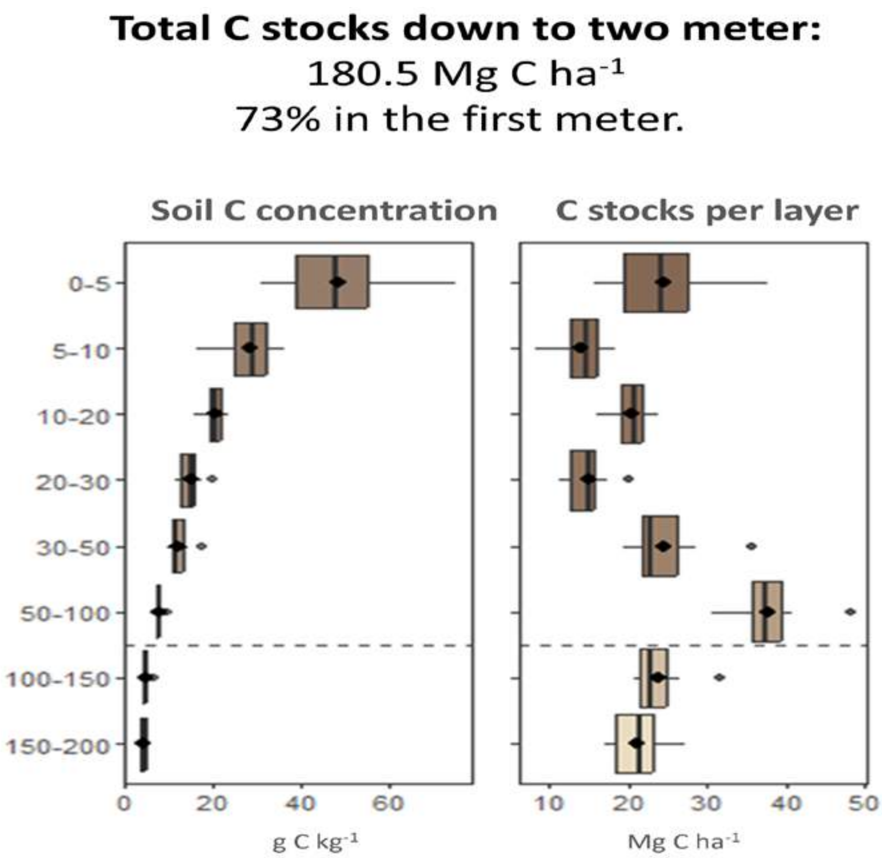


Figure 15. Soil carbon concentrations along a soil profile (left) and total stocks per soil layer per ground area (right). Boxplots show the mean (circle) and median (line) as well as the first and third quartile (n = 10 pits).

7.7 Effects of eCO_2 on the forest understory

The main vegetation responses to increased CO_2 in Open-Top Chambers placed close to the AmazonFACE plots (see section 8.7) can be divided into aboveground and belowground responses. Belowground responses showed an increase in root length and root area inside OTCs under eCO_2 followed by an enhancement in biochemical phosphorus (P) mineralization in the litter layer [90]. Similarly, soil fungal and bacterial communities shifted in response to eCO_2. The aboveground responses were expressed as an increase in assimilation rates (A_{sat}), maximum electron transport rates (J_{max}), apparent quantum yield (Φ) and water-use efficiency (WUE), not followed by any significant response in stomatal conductance or transpiration (Fig. 15). Also, an increase in leaf area and base diameter in trees under eCO_2 was observed [54].

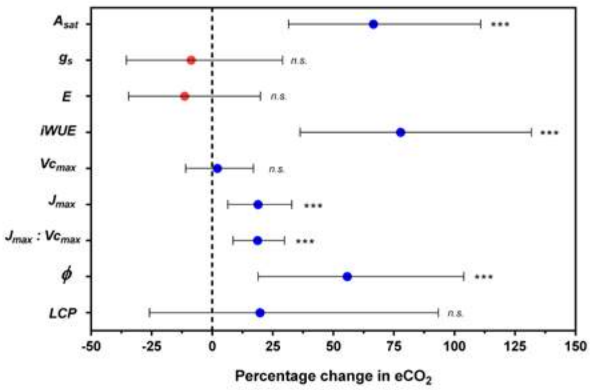
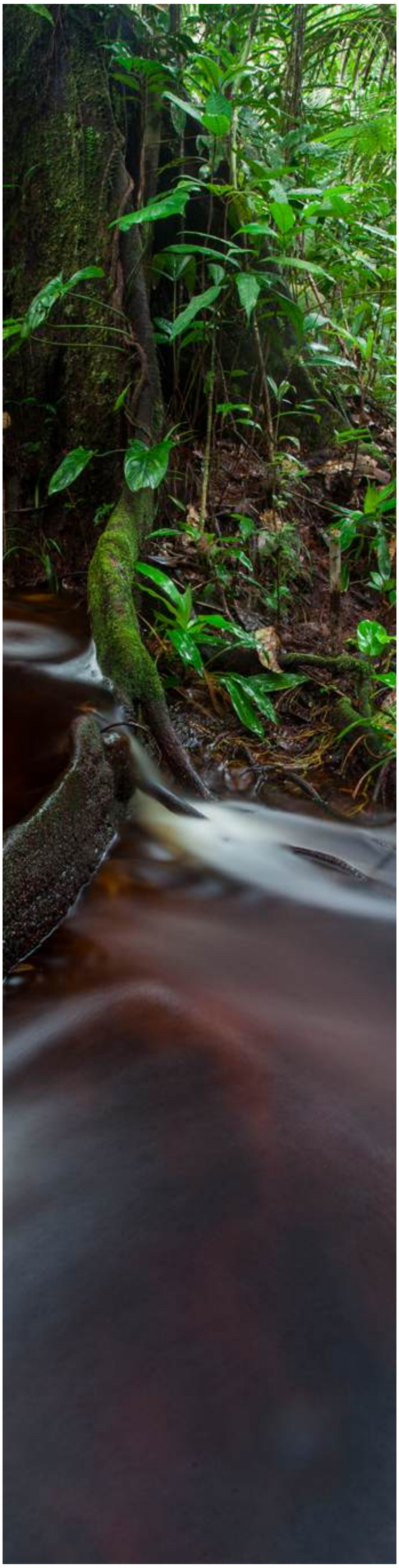


Figure 16. Mean response to eCO_2 of understory plants inside AmazonFACE open-top chambers.

Mean response to eCO_2 (n = 8, ±95% CI) of understory plants inside AmazonFACE Open-Top Chambers: net CO_2 assimilation at saturating light (Asat, µmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), transpiration (E, mmol m^{-2} s^{-1}), intrinsic water use efficiency (iWUE, µmol mol^{-1}), apparent maximum carboxylation rate of RuBisCO (Vcmax, µmol m^{-2} s^{-1}), apparent maximum electron transport rate for RuBP regeneration under saturating light (Jmax, µmol m^{-2} s^{-1}), Jmax:Vcmax ratio, apparent quantum yield (Φ, µmol m^{-2} s^{-1}) and light compensation point (LCP, µmol m^{-2} s^{-1}). The dashed line represents no change, black circle (•) an increase and open circle (○) a decrease under eCO_2. The asterisks indicate significant treatment effect (***$p \leq 0.001$) and n.s. = no significant, n = 8 OTCs (4 –aCO_2 and 4 –eCO_2).

In an experiment with Inga seedlings in pots under eCO_2 and with or without the addition of soil P, the main results were that plants invested mainly in light-capture-related traits and more resistant leaves, suggesting that P availability can be a strong factor in carbon sink for tropical species [94]. Additionally, plants allocated more biomass to fine roots and nodules under eCO_2, rather than increased phosphatase exudation per root unit [95].

7.8 Ecosystem modelling



An exercise involving fourteen different DGVMs showed that the consideration of phosphorus cycling limits biomass gain driven by eCO^2 on average by 50% [85] (Fig. 16). However, in a few of the employed models, biomass gain due to eCO_2 was in fact null or close to null because of phosphorus limitation. Such an intercomparison highlighted the current large variation of methods to represent the phosphorus cycle, and the need of more field-based data on the P cycle to improve its representation within models. Another modelling exercise estimated that tropical plants in low fertile soils like AmazonFACE's may invest up to 29% of their NPP to P acquisition under eCO_2, which would imply major changes in the carbon and possibly water cycles. These studies also pointed out key variables and processes that should be measured – like tissue stoichiometry and phosphatase activity in the soil – to reduce uncertainty on P cycle and interacting effects with eCO_2.

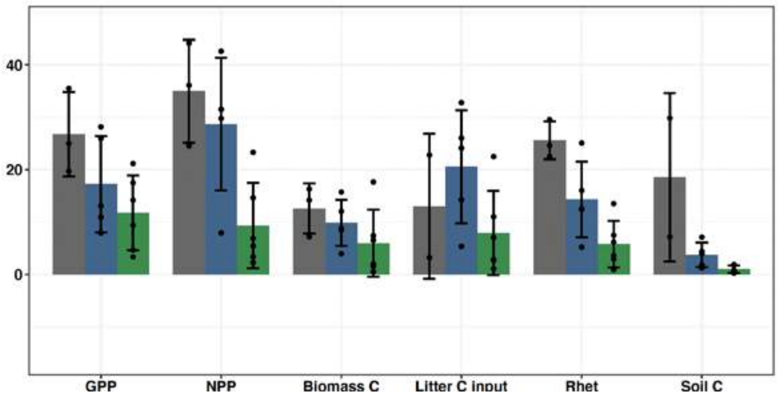


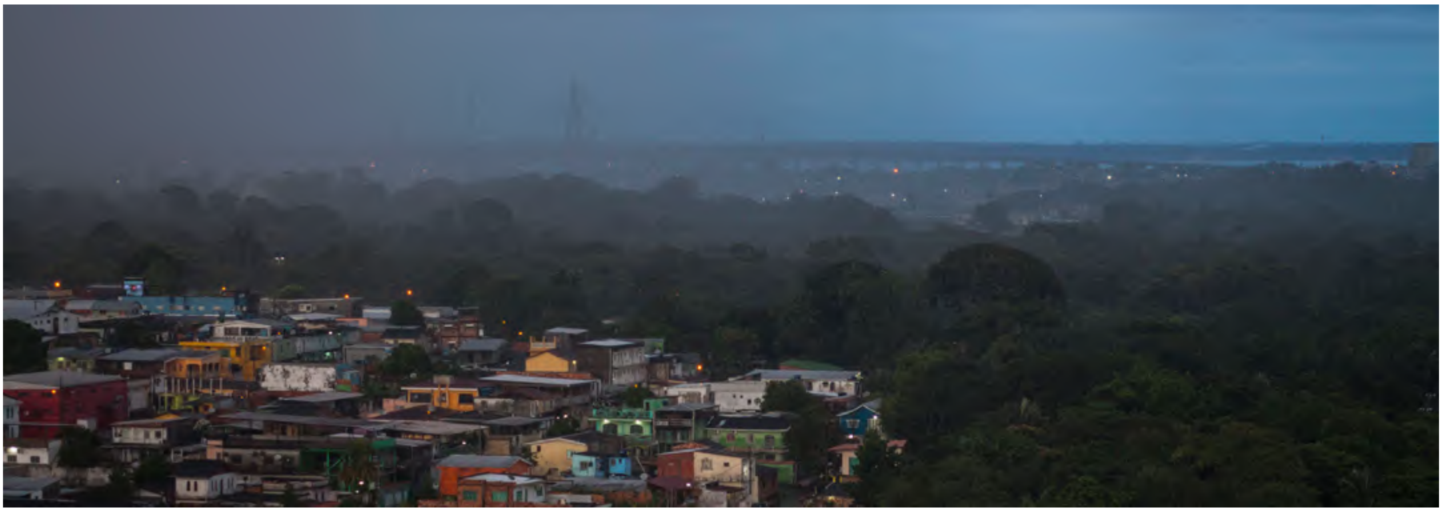
Figure 17. The 15-year modelled response of eCO_2 on productivity (GPP and NPP), biomass C, litterfall, heterotrophic respiration and soil C for dynamic global vegetation models considering C, CN and CNP cycles in the AmazonFACE grid cell. Responses to eCO_2 are the differences between the elevated and ambient model run, shown as mean and s.d. (black lines) per model group, with individual model results as dots.

The consideration of a higher functional diversity inside a vegetation model leads to the improvement of representation of Amazon forest total biomass and increases the forest resilience to drought [97]. These results suggest that the functional diversity found in the AmazonFACE experimental site may play a key role on the ecosystem responses to eCO_2.

In another study, a coupled biosphere-atmosphere modelling exercise showed that the physiological effect of eCO_2 leads to reduced stomatal conductance, reduced transpiration and ultimately causes a 12% reduction in basin-wide precipitation, which is equivalent to the reduction of precipitation found in a scenario where 100% of the forest is substituted by pastures (9%) [32]. Mechanisms behind the large-scale reduction of precipitation due to eCO_2 pass through changes in the heat balance of the planetary boundary layer, indicating that it is extremely important to measure transpiration (and other water-related variables) in the AmazonFACE plots to understand large-scale changes that eCO_2 may cause to the regional water cycle.

7.9 Socioeconomic implications of the Amazon tipping point

An AmazonFACE paper published in 2018 presented estimated costs of the so-called Amazon forest dieback or tipping point. It demonstrated that no action or later action about the tipping point would result in major social impacts that may influence migration to large Amazonian cities through a causal chain of climate change and forest degradation leading to lower river-water levels that affect transportation, food security, and health. Net present value socioeconomic damage over a 30-year period after the tipping point is estimated between USD $957 billion and $3,589 billion (compared with Gross Brazilian Amazon Product of USD $150 billion per year), arising primarily from changes in the provision of ecosystem services. Costs of acting now would be one to two orders of magnitude lower than economic damages. However, while tipping point mitigation alternatives such as curbing deforestation are economically attainable (USD $64 billion), their efficacy in achieving a forest resilience that prevents the tipping point is uncertain. Concurrently, a set of 20 adaptation measures proposed in the study is also attainable (USD $122 billion) and could bring benefits even if the tipping point never occurs.



7.10 Ecosystem services and adaptation to climate change

Among the 423 tree species identified inside the AmazonFACE plots, approximately 60% already have reported human use in the literature, mostly as raw material, for medicinal use or food. In fact, human populations from the Amazon recognize a vast diversity of nature benefits, but food (including planting fruit trees, gardening, and cultivating vegetable gardens, both for subsistence and trading), wild food (extractivism), habitat and biodiversity maintenance and water flow are the highest cited in terms of relevance to their diet (Fig. 18). In terms of food/wild food, 26% are classified as food provision ecosystem service, and fruit is the main part used (Fig. 17.c). Raw material usage (43%) and medicinal use (31%) are also benefits found in the literature for the tree species from the plots and 13% of the 220 tree species have multiple use, i.e., either as raw material, medicinal use and food ecosystem services (Fig. 17.a and 17.b). From de total, 24% (59) are species listed as hyperdominants. [98]

Of the species above mentioned and identified for human use, eight are most vulnerable to extinction according to the IUCN Red List: *Tabernaemontana muricata* (Endangered), *Couratari guianensis*, *C. tauari*, *Mezilaurus itauba* and *Sorocea guilleminiana* (Vulnerable), and *Lecythis retusa*, *Minquartia guianensis* and *Pouteria platyphylla* (Near threatened). The results presented underscore the importance of advancing scientific understanding regarding the trajectory of the Amazonian forest amidst climate change, thereby mitigating uncertainty in this domain to inform prospective developmental policies for the region.

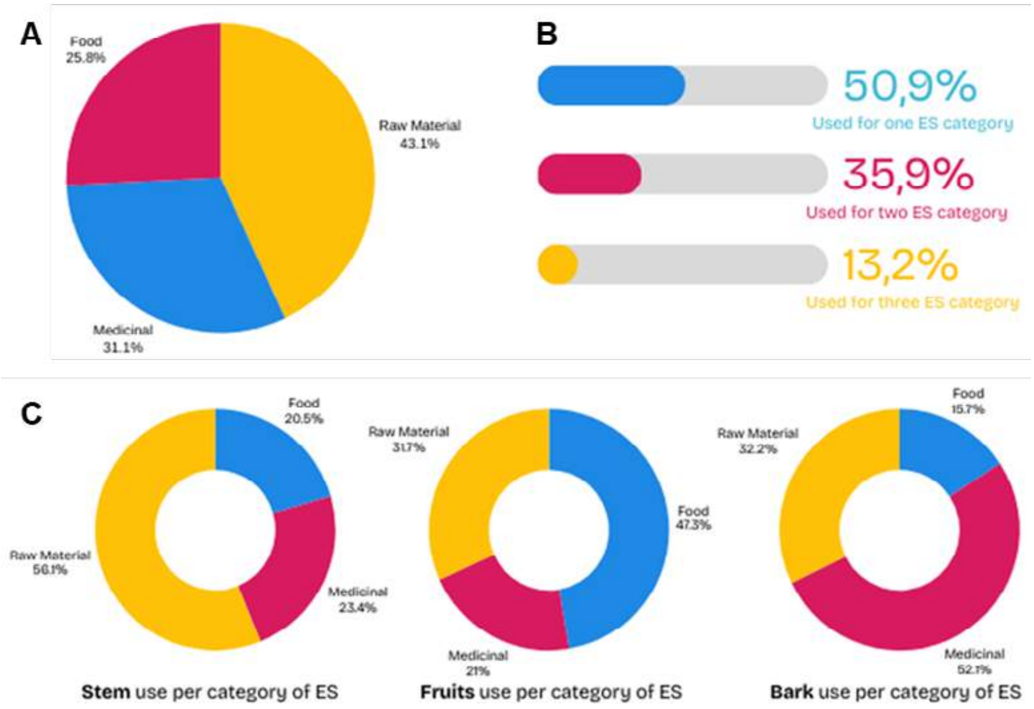


Figure 18. Tree species from AmazonFACE plots categorized by Ecosystem Services (ES) categories. (a) The percentage of use register founded by ES category; (b) The percentage of species used simultaneously for one, two or three categories of ES; (c) Percentage of use of the three most common plant parts cited per category of ES. Taken from: B. O. Tristão MSc. thesis

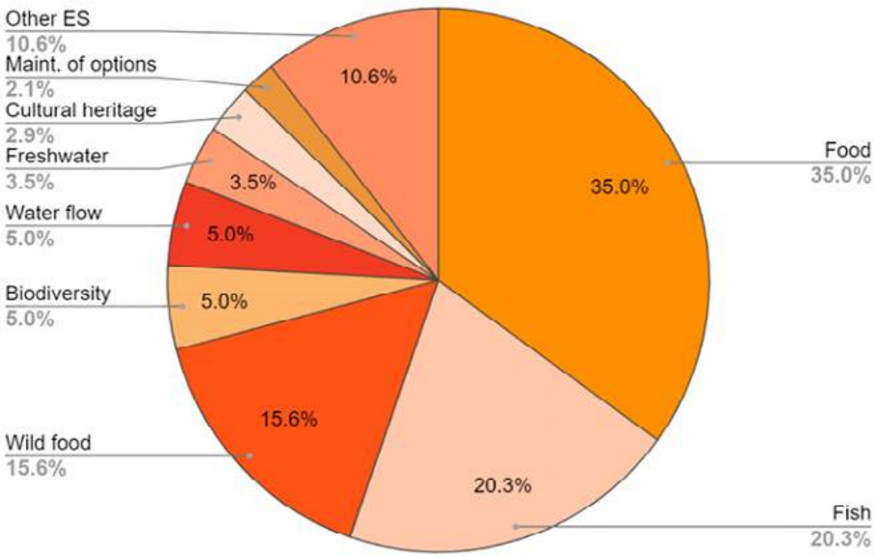


Figure 19. Amazon riverine populations perception of Ecosystem Services (ES) with the percentage of citations for each ES linked to food. Taken from: A. L. C. Cruz MSc. thesis

Temperatures in the region of the experiment have been rising (Fig. 19), and preliminary results show that local inhabitants are not only perceiving this change, but often already having to adapt to it. Results from ongoing research connected to the Socio-Environmental Research Area expose that rising temperatures affect, for example, agricultural practices, with reports showing how riverine communities are changing the times of work in the fields. Also, with changing water cycle dynamics and the occurrence of extreme events, some communities adapt by changing the location of houses (usually near rivers), with river cycles also changing access to these communities to transportation, and, therefore, to trade incursions to Manaus and other urban centres, and to their social networks.

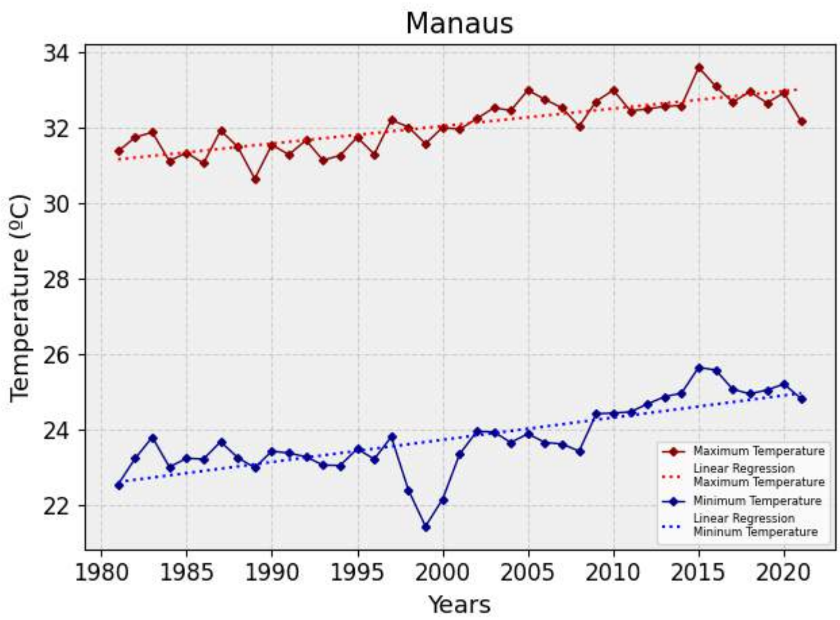
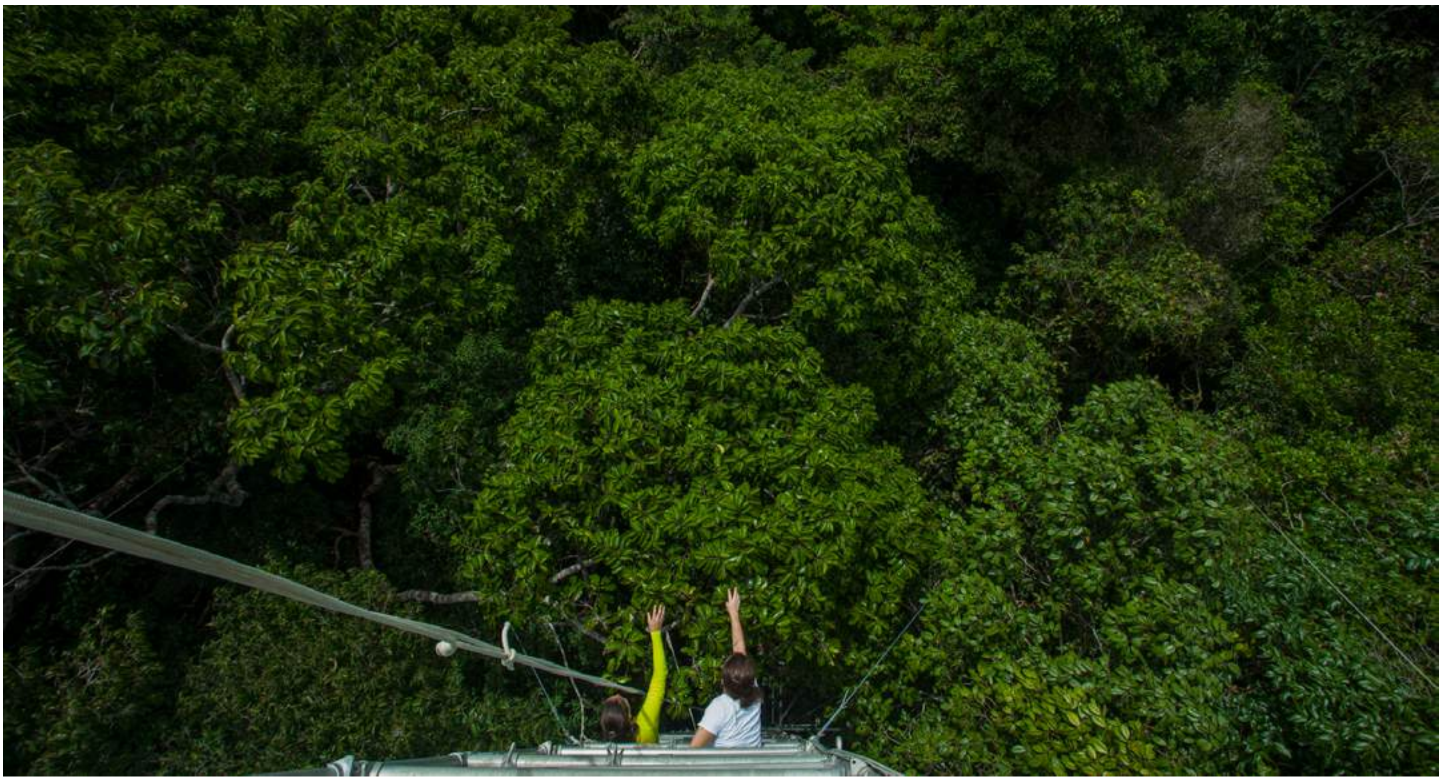


Figure 20. Average Annual Maximum (Red) And Minimum (Blue) temperature (°C) of the municipality of Manaus for the period 1981-2021.



8. The AmazonFACE field experiment

8.1 Study area and support infrastructure

The experiment is located in the Central Amazon at the Cueiras (ZF2) Research Station bordered on the North by the ZF2 (Zona Franca 2) road and situated approximately 80 km north of Manaus. The FACE site has access via the BR-174 paved road (50 km) and the ZF2 unpaved road (34 km). The site is administered by Brazil's National Institute for Amazonia Research (INPA) and has a long tradition of research in tropical forest ecology, forest management and biosphere-atmosphere interactions. Long-term projects at the Cueiras Reserve started in 1979, and Large Scale Biosphere-Atmosphere Experiment in the Amazon (LBA) project activities started in the 1990s, having resulted in a large scientific literature about the site. Since 1999, there has been nearly constant monitoring of the forest-atmosphere exchange of CO_2, water vapor, sensible and latent heat, momentum transfer, and other meteorological variables from flux towers installed on the site. There is also valuable knowledge on the site's soil composition and soil CO_2 efflux characteristics, long-term trends in forest structure and dynamics, basic leaf physiology, water balance and nutrient constraints.

The vegetation is old-growth closed-canopy terra firme (non-flooded) forest. The forest type (formally classified as Lowland Dense Ombrophylous Forest) and soil found on plateau forests along ZF2 (Ferralsol / Oxisol) are representative of ~32% of the forests occurring in the Amazon basin (~60% of Brazilian Amazonia). Local variations in soil type, topography and drainage status have created distinct patterns in forest vegetation composition. On the plateaus, well-drained clay soils favour high biomass forests 30 m in height with emergent trees over 45 m tall: typical terra firme forest. Along the slopes, where a layer of sandy soil deepens towards the valley bottom, forest biomass is lower, and canopy height is around 20-35 m with few emerging trees. In the valleys, the sandy soils are poorly drained and usually remain waterlogged during the rainy season, supporting lower biomass and lower tree height (20-35 m), with very few emerging trees. Mean air temperature is 26 °C, and the average annual rainfall is about 2,400 mm, with a distinct dry season during July, August and September, when there is less than 100 mm rainfall per month.

The proximity to Manaus (a city of 1.8 million inhabitants with a large industrial park, an international airport, and research institutions) made ZF2 an attractive option for locating the experiment when considering the provision and transportation of the CO_2 needed for the experiment (see Section 5.3). The proximity of INPA is also an advantage for both the scientific and technical management of the experiment. There is a long-existing research station (camp) 500 m away from the AmazonFACE site, at ZF2 road km 34, which can host small groups of scientists and students for short periods. Additionally, there are preliminary plans for constructing a new building that can host AmazonFACE technicians, researchers and students on a permanent basis and serve as a hub for training courses and events at the experimental site.

A 36 m^2 advanced field laboratory for sampling trial and preliminary storage and analyses is installed adjacent to the AmazonFACE experimental area. Toilets, satellite internet connection as well as 360 kVA of electricity from multiple diesel-powered generator [2] are available exclusively for AmazonFACE usage. The ZF2 access road has been extensively improved in 2022/2023 with the addition of red soil on its surface all the way to the experiment, providing safe conditions for the transit of 15-ton CO_2 trucks throughout seasons and even two-wheel drive vehicles. Small repair services on this road are necessary every two years to keep its high-quality trafficability.

2 The nearest power grid line at the ZF2 site is located 34 km to the East, along BR-174 paved road. Initial estimates indicate that the costs for pulling an electrical cable from BR-174 over the entire unpaved road would be far more elevated than using diesel--powered generators. There are ongoing studies on fulfilling at least part of the energy demand with solar-generated power.

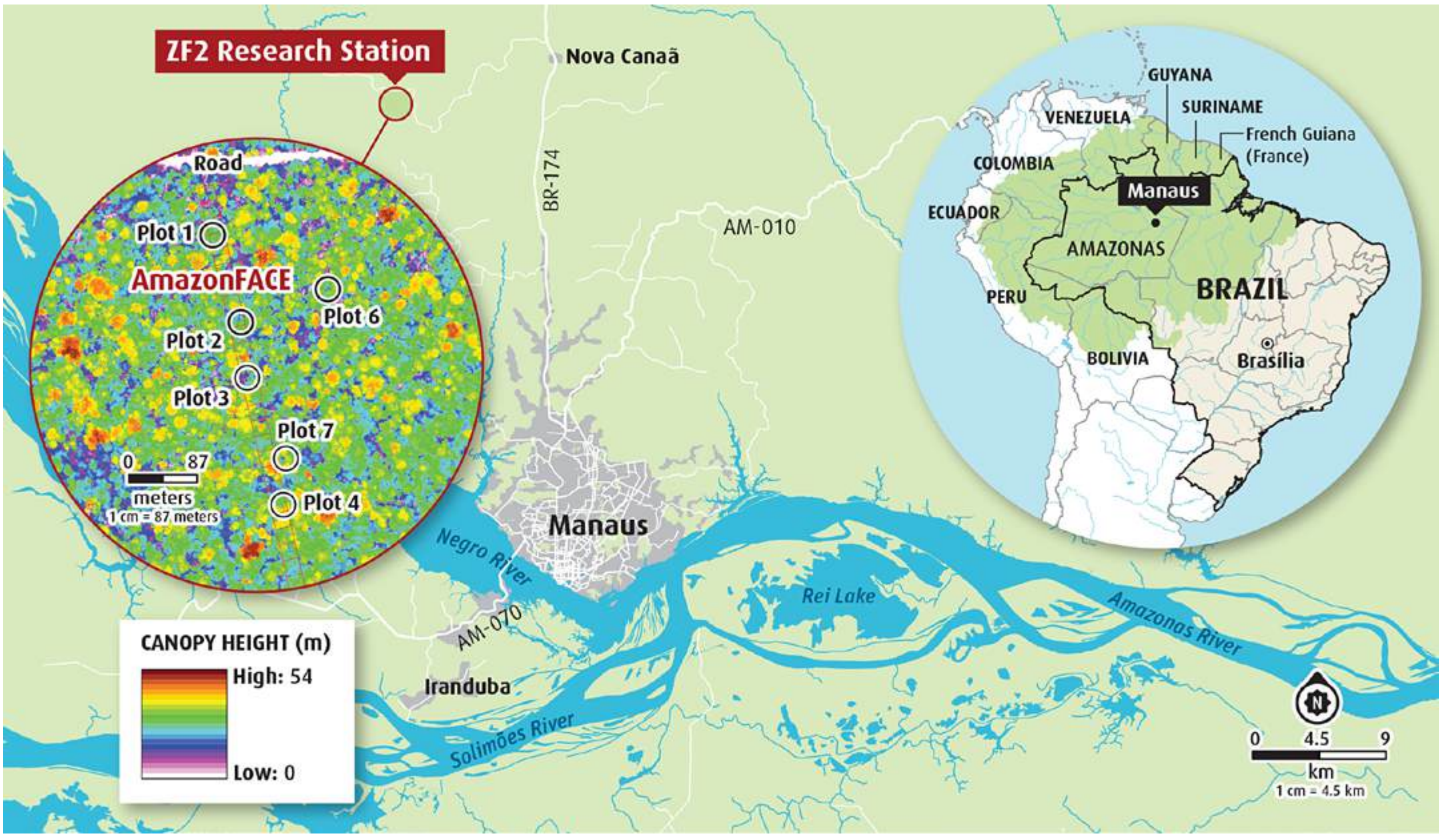


Figure 20. The exact location of AmazonFACE's experimental plots are shown in the map above

The exact location of AmazonFACE's experimental plots are shown in the map above. Part of these FACE plots take advantage of a long-term study initiated in 1996 by the Jacaranda Project. That project included two transect plots, each comprising 0,02 × 2,5 km (5 ha total) permanent plots oriented in North-South (NS) and East-West (EW) directions. Four of the AmazonFACE plots (#1, 2, 3, 4) are located on the initial plateau forest of the NS transect. Therefore, a number of trees inside these four FACE plots have been monitored since the 1990s (1996, 2000, 2002, 2004, 2006, 2008, 2010, 2011, 2012, 2013, and annually after 2015).

In the recensus, all the trees with DBH ≥ 2cm identified at the start of the experiment in 2016 are remeasured to calculate the annual increment, and those trees that have died are identified as dead in the spreadsheet. In 2023, a new survey was carried out inside the plots to identify recruits which were new individuals inside the plot with DBH ≥ 2 cm, for instance. A subset of trees has been outfitted with manual dendrometer bands, which are measured monthly to estimate seasonal variation in growth rates. Many other projects have been carried out on these transect plots, including an ecosystem respiration study and comparison with tower-based eddy covariance data, a characterization of soil properties and soil carbon cycling dynamics at plateau (Oxisol) and valley (baixio) (Spodosol) sites, a pan-Amazon comparative study of forest structure and aboveground carbon cycling dynamics, a tree growth rate and radiocarbon age-structure study and a variety of synthesis studies, technical reports, and INPA Masters and PhD theses. This previous work serves as an excellent foundation for the AmazonFACE experiment.

8.2 FACE technology

Free-Air CO^2 Enrichment (FACE) is a technology which allows the elevation of the atmospheric CO^2 concentration in large field plots with minimal disturbance to the natural ecosystem [116,117]. This is done by releasing CO^2 on the upwind side of a circular research plot and allowing the CO^2 to be carried across the plot, diluted by ambient wind. Computer-controlled feedback and feedforward algorithms maintain a target CO^2 concentration within the plot volume.

The first successful application of FACE technology to a tall forest was accomplished in 1994 by Brookhaven National Laboratory (BNL) at the Duke University Research Forest in North Carolina, USA. This initial study was expanded to a fully replicated experiment that operated from 1996 to 2010 [118]. Additional temperate forest FACE facilities were constructed using this design in Oak Ridge, Tennessee, USA [119], and Rhinelander, Wisconsin, USA [120]. BNL updated the FACE facility design for use in a eucalyptus forest in New South Wales, Australia, (the EucFACE project) [121] and an old growth oak forest in the United Kingdom (BIFoR FACE) [122]. The FACE technology used in the Amazon forest FACE experiment is based on the designs successfully used in the Duke University, EucFACE and BIFoR FACE facilities, modified to accommodate the unique conditions encountered in this tropical forest. Specific challenges to establishing a FACE experiment at this location include the relative remoteness from infrastructure such as paved roads, the electrical grid and industrial sources of liquid carbon dioxide and uniformly elevating the atmospheric CO^2 concentration in this tall (up to 35 m) and dense forest canopy.

8.3 Cranes and towers

The six experimental plots are equipped with tower cranes that support the construction of the plot hardware and provide scientists with canopy access during the experiment. Depending on the distance between two plots, a unit can serve two plots. A total of four Liebherr® 85 EC-B units are installed in the experimental area. One unit serves plot #1, and another unit provides concomitant access in plots #2 and #3. Plots #4 and #7 are served by a third crane, and a fourth unit is placed next to plot #6[3] . Crane jib-arms are placed at a height of 45 m and are 50 m long, covering an area of approximately 1 ha or forest both inside and outside the FACE plots. Baskets for lifting a maximum of three people are used at the tip of the jib-arms hook, and cranes can be operated either from the tower cabin, from remote control on the ground or inside the basket. Operations and safety procedures strictly follow Brazilian regulations on work at height. These cranes are currently the only ones used for canopy research in the whole Amazon forest.

A walk-up style (scaffold), guy-cabled, 37 m-tall modular tower is placed in each plot centre to allow placement of the required sensors and instruments within and above the canopy and to provide researchers with close access to some trees below the canopy.

[3] AmazonFACE originally had delimited 8 plots, one of which (#5) was lost in 2022 due to a treefall, and the other (#8) is not used due to its location in a sloped terrain, at the edge of the experimental area plateau.

8.4 Experimental design

The experiment design of AmazonFACE consists of three elevated CO_2 (eCO_2) and three ambient CO_2 plots (or arrays). Each plot is approximately 30 m in diameter, but the precise shape depends on the location of tree stems and canopies. All plots, irrespective of whether they will receive eCO_2 or not, have a ring of 16 towers constructed on their outer edge. Thus, ambient plots are set up as infrastructure controls. The towers are 35 m tall but have the potential to be extended up to 40 m as the trees grow. Liquid CO_2 will be stored in large and insulated tanks at ~25 bar pressure and vaporised using passive ambient air vaporizers and distributed to the treatment plots through a network of pipes. In the eCO_2 plots, the CO_2 is then mixed with ambient air before being released through vertical pipes containing multiple holes along their full height that extends from the soil surface to the canopy top (two pipes per tower, 32 per plot).

The control system maintains the enrichment in CO_2 relative to concentrations in ambient plots using feedback and feedforward algorithms based on measured CO_2 levels, wind speed and direction [117]. The CO_2 flow to each individual plenum and the number of open holes and their locations can be adapted to maintain target CO_2 levels. The target increases in CO_2 concentration above ambient in the centre of each plot is 200 ppm, with fumigation running from sunrise to sunset.

Within each plot, the 5 m section closest to the towers is considered the buffer zone where no physiological measurements are made and only trees with stems located within the central 20 m are considered within the plot for productivity monitoring. Cranes (see 7.3) facilitate canopy access to all trees in the plots, allowing ecophysiological measurements to be made on attached leaves, as well as allowing for remote sensing of canopy dynamics and processes (e.g. leaf temperature and solar-induced chlorophyll fluorescence, see Research Areas). In addition to the ecophysiological monitoring, core measurements within the plots include:

1) all key components of NPP (canopy, stem and fine root) and their nutrient contents;

2) soil nutrient and carbon dynamics including available N, P and cations, soil respiration and changes in microbial, root and mycorrhizal processes;

3) the water cycle including soil moisture, sap flow, hydraulic and ecophysiological traits (see also Research Areas and Appendix Table).

AmazonFACE will not install infrastructure control plots but will instead undertake monitoring of the specific trees across the plateau to maximise the number of species that are studied under both ambient and elevated CO_2. This approach contrasts with previous FACE experiments but is in response to the high levels of tree biodiversity found at the site. Critically, the cranes that allow access to the trees within the plots are equipped with 50 m horizontal booms that make it possible to access the canopies of many trees on the wider plateau, including those which are suitably remote from CO_2 fumigation, especially for the cranes supporting the ambient plots. The cranes, thus, provide a level of canopy access that is almost unparalleled in tropical forest research and will help researchers tackle questions related to the high levels of biodiversity and the potential for species-specific or functional group-specific responses to eCO_2.

Overall, AmazonFACE experimental design has been developed to ensure the facility can determine how carbon, water and nutrient cycles respond to eCO_2 in a biodiverse tropical rainforest, across scales from microbial to tree to ecosystem.

8.5 Meteorological measurements

Meteorological variables are measured continuously for all three levels below, inside and above the forest canopy. Above the canopy, each plot will have sensors for precipitation and global and diffuse radiation. Below the canopy, on the ground, there will be sensors measuring heat fluxes, humidity, relative dielectric permittivity and soil temperature. Inside the canopy, the meteorological towers will be equipped with profiles of air temperature, relative humidity, wind speed and direction, Photosynthetically Active Radiation (PAR) and infrared radiation.

To complement the meteorological measurements, an integrated CO_2 and H_2O atmospheric profile system with six different levels along the canopy is installed. Also, as part of the FACE control system, a multi-port sampling system is deployed inside the control shed of each plot to measure [CO_2] throughout the three-dimensional space of the plot. All these meteorological measurements are managed with rugged data loggers and immediately uploaded to the AmazonFACE database.

8.6 CO_2 demand and provision

The current estimate of the CO_2 requirement for each CO_2-enriched AmazonFACE plot, under a CO_2 treatment of +200 ppm above ambient, daytime-only treatment, and average wind speed above the canopy of 1.25 m s^{-1} is estimated to be 3.0 metric tons per day, or approximately 1,100 Mg (= metric tons) per year. These quantities are based on actual CO_2 use rates at three FACE experiments with plot dimensions similar to those planned for this study. Taking 1,100 Mg per plot per year as a reference value, the CO_2 requirements for the long-term full experiment (three FACE plots with elevated CO_2) would reach 3,300 Mg y^{-1}. Better constrained estimates will be obtained during the testing phase with a pair of control/treatment plots by mid-2024.

Currently there are two CO_2 vendors in Manaus – CarboMan and Carboxi – which produce CO_2 out of the burning of natural gas. Although it is the easiest way for acquiring CO_2 for the testing phase, their price as of April 2024 was in the order of USD $1,500 per Mg of CO_2. Nevertheless, the two vendors are not capable today of providing the quantity of CO_2 required for the full long-term experiment, but they could expand their production to meet AmazonFACE demands. Other vendors have offered either to start their activities in Manaus to serve the experiment or even bring liquid CO_2 from Northeast or Southeast Brazil at competitive prices. The most likely way forward, as recommended by specialists on the Brazilian CO_2 market, is that multiple (2 or 3) contracts will be set up with CO_2 vendors to avoid any shortage of CO_2 in case a vendor faces problems in their production unit.

Six 650 m^3 h^{-1} vaporizer banks were sized for the full experiment, and six 25 Mg CO_2 storage tanks owned by AmazonFACE were installed by the ZF2 road, in front of the experimental site.

8.7 Open-top chambers

An experiment aiming to expose patches of the forest understory to eCO_2 using Open-Top Chambers (OTCs) in the AmazonFACE area was initiated in 2018 and has been operational since then. The experimental design consists of twelve small circular areas surrounded by trenches to constrain roots external to the mini plot. OTCs are present in eight of these areas, whereas the other four serve as non-infrastructure ("blank") controls. The employed OTCs have an octagonal shape, with a diameter of 2.40 m and a height of 3.0 m, and are aluminium-made, with transparent polycarbonate walls that allow the entrance of light (Fig. 20). The operation consists of keeping the $[CO_2]$ in the treatment OTCs (i.e., with eCO_2) approximately 200 ppmv above the $[CO_2]$ of the control OTCs (i.e., 200 ppmv above the ambient $[CO_2]$) between 6:00 a.m. and 6:00 p.m. This OTC experiment is located adjacent to the area where the AmazonFACE plots are located (Fig. 21). This is the first in-situ experiment exposing Amazon forest understory plants to eCO_2, and represents an important scientific study considering the understory is responsible for a considerable fraction of the forest NPP, leaf area and evapotranspiration [123,124].

The focus of the OTC experiment is to analyse and understand carbon, nutrient and water relations under eCO_2 in the plants (tree saplings, juvenile lianas and herbs) that occur naturally inside the OTCs. In addition, another experiment took place inside the OTCs, using potted seedlings, seeking to understand how the low availability of phosphorus (P) in the soil affects carbon assimilation by plants under ambient and eCO_2. Six pots with seeds of Inga edulis were allocated to each OTC, three in natural soil (-P) pots and three in soil fertilized with phosphorus (+P).

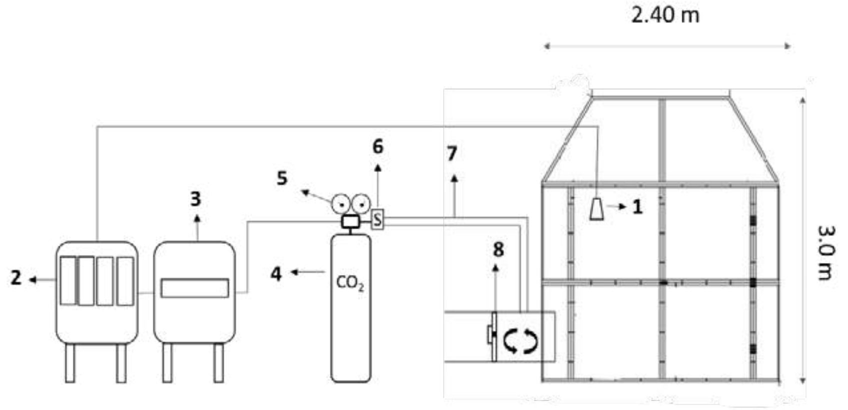


Figure 21. AmazonFACE's Open-Top Chamber Design Structure, Operation Scheme and Its Components. An inlet collector of air samples (1) to analyse CO_2 concentrations in infrared gas analysers (2). (3) A datalogger stores data and operation software for CO_2 aspersion. (4) Pressurized CO_2 cylinder and (5) manometers to control CO_2 exit pressure. CO_2 release is controlled by solenoid valves (6), channelled through a rubber tube (7) and mixed with the ambient air through a fan (8) before entering the OTC with eCO_2.

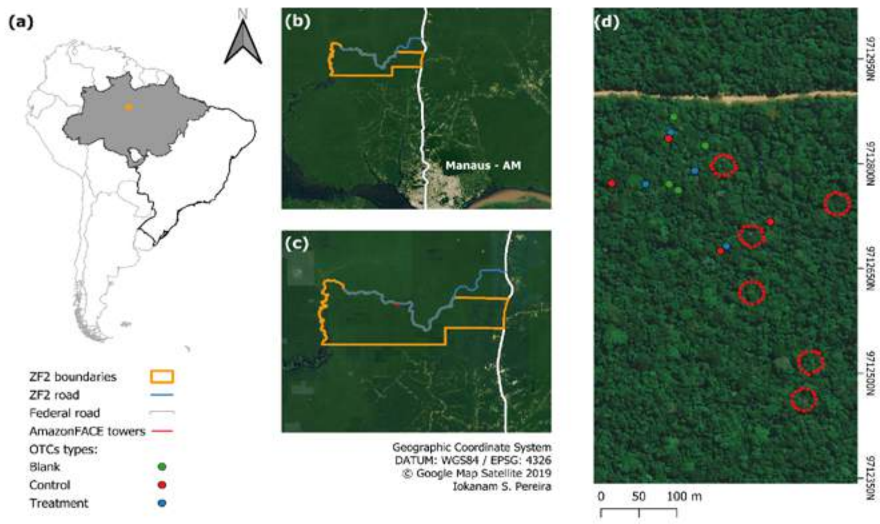


Figure 22. Large- and small-scale geographic context of the open-top chamber experiment, located in the AmazonFACE experimental area.

The OTCs system designed in 2018 was able to maintain the [CO_2] above the setpoint, with the main issues being engineering failures due to the harsh conditions found in the tropical forest environment and problems with the supply of CO_2, which ultimately compromised 20% of the operation time of the OTCs in the 2018-2023 period. Some of the published results attained with the OTCs are presented in section 7.5.

At the time this Science Plan was written, there were ongoing studies to evaluate water fluxes inside the OTCs, with emphasis on stomatal conductance and understory-canopy integrated estimate of transpiration under eCO_2 occurring naturally inside the OTCs. With the commencement of the AmazonFACE experiment, in which the understory will also be exposed to eCO_2, the OTCs might be used as a testbed for more manipulative small-scale experiments, for example, with planted lianas and/or other functional groups, and potentially including the addition of soil nutrients. Although scientifically innovative and logistically simpler, the OTC experiment cannot answer the questions which we aim to address in the full ecosystem-scale FACE experiment.

9. Research areas

9.1 Research area 1: carbon

Background

The many responses of forest ecosystems (or any terrestrial ecosystem) to elevated CO_2 (eCO_2) start with the uptake by leaves of CO_2 from the atmosphere. All subsequent responses, such as increased tree growth, adjustments in leaf area, changes in nutrient and water cycles, or altered soil microbial populations and activity, are secondary or tertiary responses to a response of leaf-level gas exchange. Theory predicts that it is likely that leaf-level photosynthesis at AmazonFACE will be enhanced in eCO_2, and this critical response must be documented and quantified. However, this fundamental leaf-level response may or may not scale to greater annual CO_2 uptake by the whole forest canopy, depending on adjustments in leaf area, leaf responses throughout the canopy and season, and supply of resources such as nutrients and water.

The key question that must then be addressed is the fate of the increased C taken up from the atmosphere, i.e., how the C is allocated to different plant organs and processes, and how much and how fast C returned is to the atmosphere. It has long been recognized that "the initial effect of eCO_2 will be to increase NPP (the total amount of C fixed into biomass and made available to consumers) in most plant communities. "(...) a critical question is the extent to which the increase in NPP will lead to a substantial increase in plant biomass. Alternatively, increased NPP could simply increase the rate of turnover of leaves or roots without changing plant biomass." [37]. This plant-centred analysis will be extended to the ecosystem level, recognizing that increasing allocation to fast-turnover pools (leaves and fine roots), and an increase in turnover rates themselves can lead to increased C flux to the soil and the potential for sequestration into longer-lived C pools.

Research questions

1) How much additional C is taken up by the forest (through photosynthesis) in response to eCO_2? Is the response sustained over the course of the experiment?

2) How does C allocation to different ecosystem pools and fluxes change? What is the fate of any additional C allocated?

3) If there is additional C transferred to the litter and soil layer deposited through leaf and root litter production and root exudation, does any of this accumulate in long-lived soil organic matter or is it all respired back to the atmosphere?

Carbon cycle processes are altered by the nutrient and water environment, and analyses described here must be closely connected to the Nutrients and Water Research Areas.

Objectives and tasks

Objective 1.1. Determine Gross Primary Productivity Responses of the Forest Stand to eCO_2

Gross Primary Productivity (GPP) describes the integration across space and time of the uptake of CO_2 from the atmosphere by the forest canopy. GPP at the whole canopy-scale cannot be measured directly, but one can take independent approaches to estimate it.

Task 1.1.1. Measure leaf-level photosynthesis.

Photosynthetic carbon assimilation and stomatal conductance of multiple individuals and at different canopy strata will be measured and the data analysed in relation to light, temperature, humidity, nutrient content (coordinated with Research Area 2), foliar carbohydrate content and secondary metabolites, leaf structure, phenology, and season. Preliminary surveys and guidance from Objective 4.1 will help to determine the optimum sampling strategy. Measurements should focus on net carbon assimilation rates at prevailing $[CO_2]$ with seasonal surveys of A-C_i and light response curves. Simultaneous measurements of stomatal conductance will support estimation of leaf-level instantaneous water use efficiency, affecting actual CO_2 availability to chloroplasts as well as in support of Research Area 3 (Water) objectives.

Task 1.1.2 Scaling up leaf level measurements.

Leaf-based assessments on CO_2 uptake can be used in models to scale up to GPP of the forest canopy using a scaling model that accounts for vertical light interception and leaf and canopy temperature and optimality assumptions. The spatial distribution of climate drivers (longwave, NIR and shortwave radiation, surface and air temperature, humidity, wind) will be continuously monitored throughout the canopy profile using automatic weather stations. Various methodologies can be considered for turbulence analysis, inducing budget methods, variance methods, and 'surface renewal' approaches.

Task 1.1.3. Calculate GPP as NPP plus autotrophic respiration

Combine NPP measurements (Objective 1.2) with scaled-up measurement of stem (bole and branch) CO_2 efflux (after accounting for soil CO_2 in sap flux), leaf dark respiration, and root respiration rates (Task 1.2.4). Scaling to annual GPP will require integration of tree and stand structure, variation among individual trees, and environmental controls on respiration rates.

Task 1.1.4. Canopy spectral analysis

A FluoreSens10 sun-induced chlorophyll fluorescence (SIF) system (Campbell Scientific) will be installed on a tower above plots 1 and 2 prior to initiation of CO_2 treatments and maintained for two years of CO_2 fumigation. The system comes with a high-resolution spectrometer covering the atmospheric O_2A and O_2B oxygen absorption bands. With proper calibration, this system can provide continuous monitoring of GPP. The system also includes a second spectrometer covering wavelengths 350-1,000 nm from which indices of canopy structural seasonal dynamics (e.g. NDVI), canopy physiology (e.g., photochemical reflectance index, PRI), and through partial least squares regression, estimates of photosynthetic activity (V_{cmax}, J_{max}), canopy biochemistry (e.g., N, C, $\delta^{15}N$, and fiber content), and leaf morphology (leaf mass per area, LMA). Upward and downward facing PAR line sensors will also be part of the installation.

Objective 1.2. Determine NPP And The Allocation Of GPP To Autotrophic Respiration And Production Of Plant Biomass Pools Of Different Turnover Rates

Objective 1.2 will measure net primary productivity (NPP) and address the fate of the increased C taken up from the atmosphere, i.e., how much of the C is allocated to different plant organs (leaves, stem, fine roots) and processes, and how much and how fast C is returned to the atmosphere. NPP is estimated by integrating measurements of the annual production of wood, leaves, reproductive material, and fine roots, and some additional smaller components (root exudation, volatile emissions from the canopy, shed bark, and losses to herbivores). Turnover rates of leaves and fine roots will also be determined.

Task 1.2.1. Stem wood production

Annual surveys will measure tree circumference of all trees with DBH > 5 cm. The surveys will be conducted in May, at the end of the wet season, when the trees are fully hydrated, and diameter is expected to be most stable. A subset of trees will be fitted with automatic dendrometers that will provide hourly data from which seasonal dynamics of tree growth can be determined. Trees with DBH of 2-5 cm will be measured annually in three 1.5 × 1.5 m subplots, which is relevant for Objective 4.2 in Research Area 4. The annual diameter increment of each tree is converted to dry matter increment (DMI) using allometric equations combined with species-specific wood density.

Generic allometric equations from Chave et al. based on diameter, or diameter and height when tree height data is possible is currently used, with supplemental locally derived equations for trees with diameter at breast height (DBH) of 2-10 cm. The use of this or any other published allometric equation entail many uncertainties, including differences among species, accounting for broken tops, and changes in response to eCO_2. Collaborative research conducting Terrestrial Laser Scanning (TLS) of the plots should provide reliable estimates of tree height and canopy structure. A site-specific and tree-specific allometry equation will be developed from TLS determination of tree volume. Wood growth should be analysed in relation to phylogeny, tree size, canopy position, or aboveground traits, in coordination with the Biodiversity Research Area.

Task 1.2.2. Leaf production

Leaf litterfall will be collected in litter traps monthly. Litter mass production can be related to leaf mass production through analysis of leaf turnover and change in Leaf Mass per Area (LMA) during senescence. Leaf Area Index (LAI) can be estimated by integrating the litter mass and LMA data with hemispheric photos. An NPP response to eCO_2 can then be separated into a functional component (photosynthesis per unit leaf area) and a structural component (LAI and the distribution of leaf area in the canopy). A TLS survey of the site will provide information about canopy structure. Reproductive tissues (flowers and fruits) and twigs collected in the traps will be separated from leaf litter and quantified.

The distributed automatic weather stations with spectral radiation sensors will also support the quantification of leaf and woody area distribution. Branch litter will also be measured. Changes in LAI and leaf area distribution will be related to changes in the canopy radiative distribution (PAR, reflected PAR, NIR, reflected NIR at various heights), as well as to changes in profiles of temperature and relative humidity. These changes will in turn be related to change in vertical distribution of photosynthesis and stomatal conductance.

Task 1.2.3. Fine root dynamics

Fine root production, mortality, and standing stock (and calculation of turnover) will be measured through minirhizotrons, as well as by sequential collection of soil cores and in-growth cores. Minirhizotron tubes should be installed at a 60-degree angle from horizontal to a depth of approximately 1.2 m. Fine root biomass in the litter layer and in soil cores allow the analysis of standing C stocks and support upscaling of minirhizotron data. In-growth cores (also in the litter layer) can provide fine roots of known age to estimate C allocation and nutrient analysis (Task 2.1.1), and another estimate of fine root production when coupled with turnover estimates from the minirhizotrons.

Task 1.2.4. Autotrophic respiration

Respiration rates of tree boles, branches, leaves, and roots should be measured at a time-step and with sufficient ancillary data to support model integration of these processes to a whole-plot, annual value. Stem chambers to measure CO_2 efflux should be installed on boles and branches of subset of trees with differing wood density, growth rates, or other traits, as guided by analyses in the Biodiversity Research Area. Dark respiration rates of leaves can be measured with cuvettes on leaves maintained in the dark prior to measurement at the end of day. Root respiration should be measured in cuvettes on excavated fine roots in addition to separation of components of soil CO_2 efflux (Task 1.3.4).

Objective 1.3. Determine fluxes of C into and out of soil and changes in SOM pools

Although allocation of carbon to fast-turnover tissues (leaves and fine roots) instead of wood may not lead to increased carbon storage in tree biomass, those fast-turnover tissues increase carbon flux to the soil, with the potential for sequestration into longer-lived C pools. The input of detritus into the soil system will be quantified in Tasks 1.3.2 and 1.3.3. Increases in soil carbon stocks are very difficult to document because of the size and spatial heterogeneity of the pool, so carefully controlled measurements and model integration are needed.

Task 1.3.1. Carbon transport speed

The speed at which carbon molecules move from initial uptake from the atmosphere by leaves to roots and their subsequent release from roots to mycorrhizal hyphae, rhizosphere microbial communities and exudation will be measured by tracking the ^{13}C signature. This can be accomplished by capturing the ^{13}C pulse induced by fumigation with ^{13}C-depleted CO_2 at the start of the CO_2 exposure. Depending on the turnover rates of the respective pools, it may take days (e.g. leaf sugars) to years (e.g. SOM) until they are uniformly labelled with the altered ^{13}C signature). The degree of change allows an estimate to which the pool has been supplied with recently fixed C, which will be an invaluable source of data for parameterizing turnover processes in soil and vegetation models.

Task 1.3.2. C flux to exudation, mycorrhizae, and soil microbial communities

Measurements of mycorrhizal colonisation, hyphal production, and exudation of low-molecular weight organic C compounds into the rhizosphere and rhizosphere microbial communities are especially important as they are important C fluxes and can be modulated in response to plant nutrient demands. Quantification of exudation rates per unit root length can be combined with minirhizotron measurement of total root length to generate ecosystem scale rates that can be included in NPP quantification. Mycorrhizal quantification based on the percentage of root length colonised can be similarly scaled, aliquots of roots will be archived for species identification (see also Research Area 4). Mycorrhizal hyphae can be quantified from minirhizotron images and in ingrowth bags.

The soil microbial community C pool is an important C sink, but also responsible for large heterotrophic C fluxes (up to 75% of total soil CO_2 efflux) from the soil to the atmosphere and can release large amounts of C from older and longer-term stored SOM (e.g. via priming) induced by higher exudate inputs. Microbial biomass C (as well as N and P) will be determined using the chloroform fumigation extraction method (see Research Area 2), additional microbial physiological parameters (e.g. growth and respiration) will be determined using small scale lab incubations.

Task 1.3.3. Decomposition of plant organic matter

Decomposition rates of different plant components (leaves, wood, roots) will be measured by sequential re-collection of the respective litter placed within mesh bags on the soil surface (leaf and woody debris) or in the soil (root litter). The effect of initial litter nutrient concentration, which might be affected by eCO_2, on decomposition rates will be evaluated. (also see Research Area 2)

Task 1.3.4. Soil CO_2 efflux

CO_2 efflux from soil will be measured in monthly campaigns, including ancillary data on soil temperature and moisture. CO_2 efflux represents a combination of autotrophic (i.e., root/mycorrhizal respiration and heterotrophic (microbial) respiration. CO_2 efflux will be separated into autotrophic and heterotrophic components using root-free soil collars, supplemented with direct measurement of root respiration (Task 1.2.4). The relative importance of VOC emissions will be evaluated.

Task 1.3.5. Soil organic carbon pools

Monitor changes in soil organic matter pools, including particulate and mineral associated organic matter fractions and their respective C and N contents will:

i. allow estimation of soil C turnover rates and long-term C sequestration;

ii. provide critical inputs to soil C models, particularly those that account for mineral-association as a critical soil carbon stabilisation [126,127].

Task 1.3.6. Carbon budget

Develop an ecosystem-level C budget, including allocation fractions among plant biomass components and CUE (NPP/GPP) and to soil compartments, and compare with model projections (e.g., Jiang et al. [2020] at EucFACE). Methods to continuously monitor the bulk carbon exchange (NEE, GPP, Reco) of the experimental plots will be developed and tested.

Model-data integration

NPP and its components provide important benchmark data for ecosystem models, and it is essential that the critical inputs for the models used at AmazonFACE are measured and coordinated with the modelling research strategy. CO_2 effects on primary productivity and carbon allocation will be combined with independent estimates of tree mortality to evaluate process-based land models and generate long-term and larger-scale predictions of vegetation carbon sequestration (see "Modelling Strategy Section").

Models will need to consider whether observations demonstrate downregulation of photosynthetic capacity parameters (Vcmax, Jmax) or stomatal conductance parameters to elevated CO_2 [128–133], i.e acclimation to CO_2 and whether there is thermal acclimation of these parameters under possibly higher canopy temperatures experienced under elevated CO_2.

Temperature acclimation and acclimation potential will be evaluated by contrasting modelled response (trained on "average" year with data) vs. year(s) with heat extremes, if heat extremes happen to occur during the experiment. Given the conditions experienced during the baseline monitoring, it is almost certain that heat extremes will be encountered during the CO_2 fumigation period. The hypothesis is that suppression of photorespiration in eCO_2 leads to larger CO_2 responses in warmer regions [30,134].

9.2 Research area 2: nutrients

Background

Research Area 2 aims to determine how the forest's responses to elevated CO_2 are controlled by links between carbon and nutrient cycling. Incorporating nitrogen (N) into Earth systems models has reduced predictions of future rates of terrestrial carbon uptake due to elevated CO_2 by as much as 50%. Tropical forests productivity, particularly that of the Amazon forest, has been shown to be more strongly constrained by phosphorus (P) availability than temperate forests [20,45]. The biogeochemical cycling of N and P fundamentally differ; for N, there are often substantial atmospheric inputs into terrestrial ecosystems through N deposition and biological N fixation, but also substantial N gaseous and aqueous losses.

Therefore, under eCO_2, total ecosystems N stocks may increase if N input rates increase or N losses decline, which could partially alleviate potential N limitation. In contrast, for P, the main input is from rock weathering, with such inputs reduced to zero in many ancient tropical soils [135], so that low levels of dust input, including from the Sahara, represent the main input of P into many Amazon forest soils [136]. Furthermore, there are no gaseous P losses, and aqueous losses are generally limited due to rapid recycling and low phosphate mobility in soil. This suggests that low P availability may be critical in constraining tropical ecosystem responses to elevated CO_2.

The lack of substantial P inputs and outputs means that total ecosystem stocks should not change, and thus, sustained forest growth responses to P will only be possible if trees can:

i. use P more efficiently; or

ii. gain greater access to pools of soil P that were not available under ambient CO_2.

Although such predictions come from theoretical and P-based models [20,96], in situ evidence for this in tropical rainforests is scarce [20,96]. Key soil P pools that could become accessible for plants may include:

i. inorganic P that is currently bound to mineral surfaces in the soils (e.g. associated with iron and aluminium oxides that are abundant in central Amazon oxisols); or

ii. organic P pools within the litter layer and soil matrix. The potentially low availability of mineral-bound P suggests that organic P pools may offer the greatest potential for enhanced access under eCO_2, although their accessibility to plants also greatly varies.

In this context, microbial biomass may be a particularly important organic P pool as they can contain more P in their biomass than all the trees in a forest [138]. In addition, plant roots are colonised by symbiotic arbuscular mycorrhiza that can enhance nutrient uptake from smaller soil pores inaccessible to roots, and associated microbial communities in the rhizosphere can accelerate organic matter mineralization (e.g. via the priming effect or nutrient mining) and increase nutrient availability. Therefore, the outcome of the interactions between plants and soil microbes for P under eCO_2 may be especially important [137,139].

In summary, fundamental differences exist between the cycle of N and P, and this Research Area 2 aims to complete a detailed assessment of the effects of eCO_2 on nutrient cycling at AmazonFACE and to identify whether the availability of N, P, or other elements constrains the overall forest C uptake and sequestration potential and response to eCO_2.

Research questions

1) How much do tropical rainforest trees increase nutrient uptake, particularly of N × P, under elevated CO_2?
2) To what extent can tropical rainforest trees use nutrients more efficiently under elevated CO_2?
3) How does elevated CO_2 influence ecosystem nutrient cycling and nutrient budgets?

Objectives and tasks

Objective 2.1. Determine the Influence of eCO_2 on Plant Nutrient Uptake

Task 2.1.1. Plant tissue-level nutrient concentrations

All NPP components (in concert with Research Area 1; stem wood, leaves, fine roots, litterfall) will be analysed for key nutrients and changes in tissue chemistry. For leaves in particular, sample age will be considered to determine nutrient retranslocation as an indicator for potential augmenting nutrient limitation by comparing green versus recently senesced leaves. In addition, leaf-level nutrient concentrations are critical in quantifying (ideally species-specific) photosynthetic nutrient-use efficiency (measurement of leaves used in photosynthetic campaigns, Research Area 1 and Research Area 4). Combined with NPP measurements, plant nutrient uptake and changes in plant nutrient allocation between tissues will be quantified. The calculations of total nutrient uptake and nutrient uptake per unit biomass produced are crucial for evaluating whether eCO_2 changes tree nutrient uptake rates and/or plant nutrient use efficiency, with the latter also considering the change in allocation.

Task 2.1.2. Nutrient acquisition adaptations at the root and rhizosphere level

Plants can adjust their root morphology, exudation, enzyme excretion and microbial associations (e.g. symbiosis with mycorrhizal fungi and/or N_2-fixing bacteria) to foster nutrient uptake and to respond to different availability of inorganic and organic soil nutrient (P) pools. In coordinated sampling campaigns (with Research Area 1) conducted every wet and dry season, standing root biomass stocks (in the top 30 cm of soil), fine root morphology, fine root phosphatase activity, root mycorrhizal colonisation rates and root C and nutrient concentrations will be analysed. Small root aliquots will be archived for potential plant and mycorrhizal fungi identification (see also Research Area 4).

Moreover, fine root productivity will be monitored using ingrowth cores (in three-month intervals) and analysed for similar parameters as described for root stocks. In special campaigns, root exudates will be collected (baseline, then once every two years) for quantification and chemical and isotopic characterization of compounds released into the rhizosphere. In synergy with Research Area 1, this can be used to approximate plant C costs for nutrient uptake, wherein potential increases may be associated with exacerbation of plant nutrient limitations under eCO_2.

Objective 2.2. Determine the Effects of eCO_2 on Soil Nutrient Cycling

Task 2.2.1. Soil nutrient pools and fluxes

If plant nutrient uptake or nutrient-use efficiency changes under eCO_2, it is essential to determine the underlying mechanisms. Excess C could enhance plant nutrient demand and tighten nutrient cycling between plants and soil. Nutrient deposition rates will be analysed to account for external inputs, and due to the increasing importance of organic P cycling, the release rates of nutrients from leaf-litter decomposition under eCO_2 will be monitored. In addition, detailed measurements of total and available soil N and P pools and fluxes are essential, as they are tightly linked to quantify changes in C cycling (e.g. soil C stocks and soil respiration, Research Area 1). Soil organic and mineral N and P (available P and Hedley-fractions) pools will be measured in the top 30 cm every wet and dry season to estimate net changes over time. While P responses to eCO_2 represent the key focus, other important cations (K, Ca, Mg, Mn) will also be determined. Moreover, plant-available nutrients will be analysed using anion and cation resins.

Task 2.2.2. Soil microbial biomass and community mediating soil carbon and nutrient cycling

Soil microbial communities have crucial roles in ecosystems for releasing CO_2 and other greenhouse gases, depolymerizing large and complex organic compounds and mineralizing nutrients. They also serve as a nutrient pool and are crucial for controlling soil carbon sequestration (see also Research Area 1). Regular analysis will be conducted on microbial biomass and C:N:P stoichiometry as an index for potential enforcing nutrient limitations under eCO_2, and investigate their role as organic/ inorganic P source or sink, i.e. if they are acting as competitors for plant P availability. Small-scale lab incubations will evaluate microbial community physiological parameters such as community level growth and respiration rates based on microbial phospholipid-fatty-acid (PLFA) and DNA turnover allowing to estimate microbial community/biomass turnover rates.

These data will be linked with soil microbial community composition (integrated with Research Area 4). Fungal and bacterial community changes will be investigated using targeted DNA-based amplicon sequencing or untargeted metabarcoding, extensive soil samples will be archived frozen. Additionally, this Research Area will make use of microbial phospho- and neutral lipid-fatty-acids as a more quantitative community fingerprinting method of (arbuscular mycorrhizal) fungal and bacterial dynamics (in connection with Research Area 1 and 4).

Task 2.2.3. Soil extracellular enzymes

Soil enzyme assays provide potential activities of enzymes, generally acting on the chain ends of polysaccharides, chitin and organic P, each specific substrate responsible for the rate-limiting step in C, N and P decomposition. In soil, extracellular enzyme production depends on nutrient availability and follows resource or substrate supply and demand principles. Soil extracellular activity rates in the upper 30 cm of soil will be analysed every wet and dry season and use the stoichiometry of extracellular enzymes (targeting C, N and P-containing compounds) to assess nutrient limitations of soil microbial communities in response to eCO_2.

Objective 2.3. Determine the role of nutrient recycling from plant litter under eCO_2

Task 2.3.1. Litter nutrient stocks

Ground litter stocks will be collected twice a year from predetermined areas to calculate the standing litter mass, and will be analysed for their C, N, P and macro-, micronutrients contents. Subsamples will be used for litter colonising root stock determination, as well as for determining meso or microfauna and fungal/bacterial community composition (in alignment with Research Area 4)

Task 2.3.2. Litter decomposition

The leaf litter layer can become an important source for (mineral) nutrients that have not been reallocated before leaves have been shed, particularly if plants have a higher nutrient demand under eCO_2 conditions. A litter decomposition experiment (see also Task 1.3.3.) will be conducted to trace the mobilisation of nutrients from the litter layer, as well as their fate (e.g. litter mass loss, colonisation by plant roots, microbial biomass, soil organic matter formation).

Objective 2.4. Calculating a forest stand nutrient budget

Task 2.4.1. Nutrient deposition and forest internal nutrient deposition

External nutrient inputs could be crucial sources to balance the higher plant nutrient demand by eCO_2. Wet and dry deposition as well as throughfall rates will be measured.

Task 2.4.2 Forest stand nutrient budget

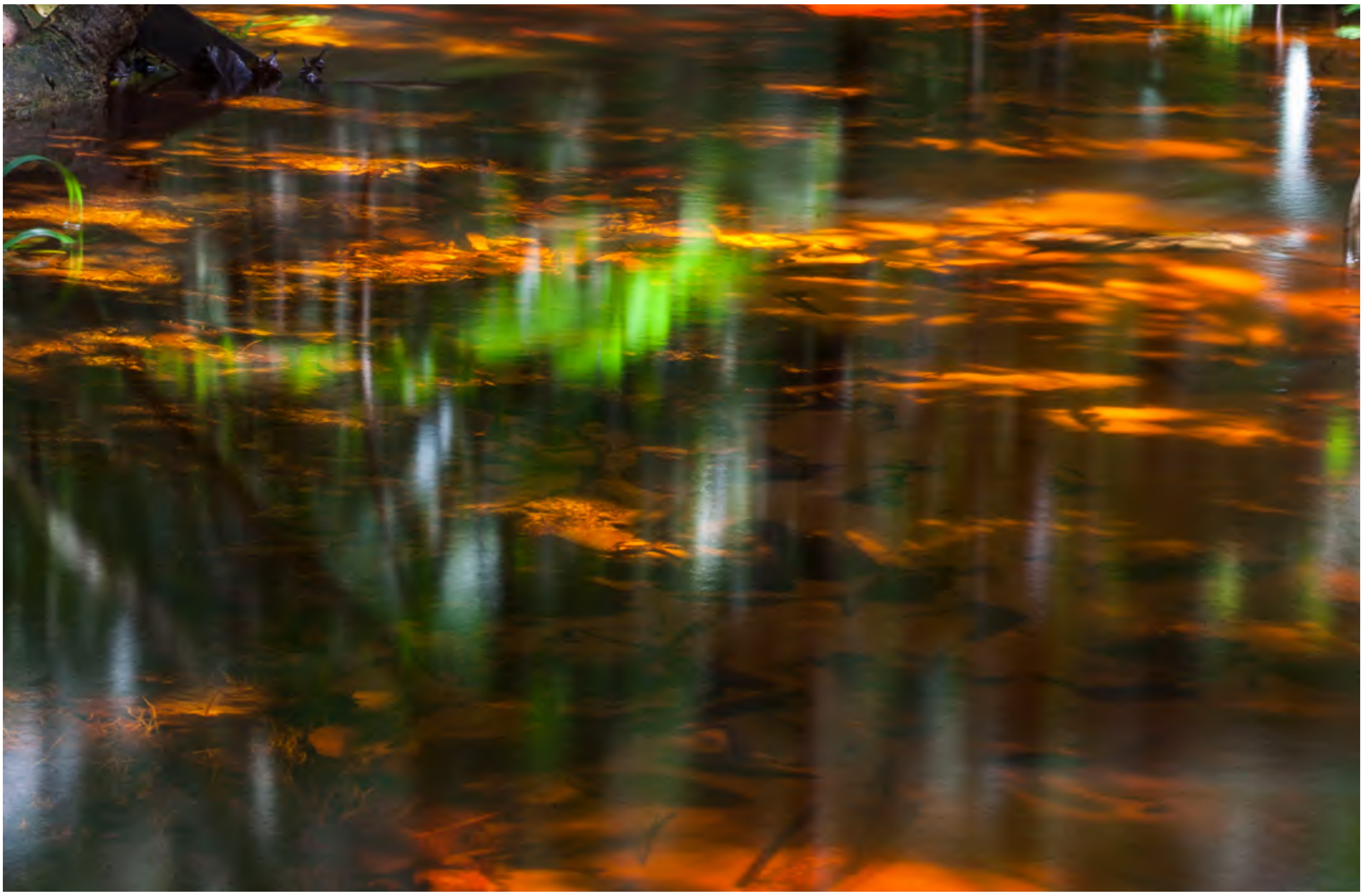
Finally, the effects of eCO_2 on the total forest nutrient budget will be measured by calculating nutrient stocks of all ecosystem components derived from the previous Objectives (leaves, stem, roots, soil, soil microbes).

Model-Data integration

Detailed measurements of nutrient stocks, stoichiometry and process rates in the ambient and elevated CO_2 plots can help develop model representations and parameterization of C, N and P cycling and interactions between the different elemental cycles and serve as benchmark data for model evaluation. Nitrogen- and phosphorus-enabled models will be parameterized with key measurements made at the AmazonFACE site, such as nutrient stocks and plant and soil microbial biomass turnover. Soil-microbial explicit models will be of particular interest to represent the plant-soil-soil microbial interactions and nutrient exchange. Measurements of soil enzymes, root morphology, and characterization of the soil microbial community under eCO_2 will be invaluable for the model development of these critical processes.

Model development of unaccounted but critical processes and experiments, such as sensitivity analyses and inter-model comparisons, will refine measurements on key uncertainties. Nutrient feedback to eCO_2 at the AmazonFACE site, mediated by plant adaptations and soil microbial communities, will provide input and understanding for previously unquantified processes in models.

9.3 Research area 3: water



Background

There is extensive literature suggesting that stomatal conductance declines under eCO2, but there is limited evidence for tropical forests and mature tropical trees [22,54,140]. Stomatal conductance is an optimisation of maximising carbon gain through photosynthesis, whilst simultaneously controlling damage to the plant hydraulic system [141,142]. When a plant is exposed to eCO2, it is reasonable that C assimilation (in comparison with ambient CO2) occurs with a lower stomatal conductance, potentially driving a higher temperature of leaves due lower evaporative cooling. If the carbon acquisition is maintained, this would mean that the leaf water potential, a key measure of plant hydraulic stress, could be maintained at higher (less stressful) levels, as a result of the restricted transpiration. Consequently, the plants would be able to minimise an often-observed midday transpiration depression and maintain greater hydraulic safety, meaning they are likely to be less vulnerable to episodic drought events. However, if leaf area increases [54,143] and/or plants increase photosynthesis in response to eCO2, it is possible that the demands on the plant hydraulic system become greater.

In this instance, with the absence of increased investment in root water uptake, plants may operate with lower (more stressful) leaf water potentials and aim to increase hydraulic conductivity, potentially making them more vulnerable to drought-induced mortality. In tropical forests where there is very high competition for resources between species this outcome may be more likely, if other resources, such as nutrients are not limiting. There is still limited evidence for plasticity in plant hydraulic responses in woody plants [144] and therefore the likelihood for imperfect adaptation to eCO2 in the context of future drought events is possible. Another important consequence of changes in g_s is the energy balance of leaves. Lower g_s restricts the evaporative cooling and, as the photosynthetic process is based on enzyme activity and gas diffusion, temperature has a modulating influence over carbon assimilation rates. Exploring trade-offs associated with water and carbon use within plants is critical to evaluate whether rising CO_2 will offset the negative impacts of predicted future increases in drought and heat events across Amazonia [32].

Given this, the water cycling research area is organised around six key questions.

Research questions

1) Does leaf-level water use efficiency increase under eCO_2 and alter transpiration?
2) What are the effects of eCO_2 on the soil water balance in a mature Amazon rainforest?
3) Can an increase in CO_2 alter the drought vulnerability of a tropical forest?
4) What is the effect of eCO_2 on leaf or canopy temperature under normal conditions but also during drought events?
5) Will plants adjust their wood anatomy (as related to their capacity to transport water) under eCO_2?
6) Will the stomatal sensitivity to atmospheric vapor pressure deficit change under eCO_2?

Objectives and tasks

Objective 3.1. Determine If Water Loss Through Stomata Declines Under eCO_2

Task 3.1.1. Measure water use efficiency

To evaluate whether plants will display increased water use efficiency under eCO_2, leaf level stomatal conductance (g_s) will be measured in combination with carbon assimilation rates (see Objective 1.1). Portable photosynthesis systems (eg. Licor 6800) will be used to gather ecophysiological data from leaves with the aid of canopy cranes. Survey (spot) measurements will be carried out and also leaf-level response curves of g_s to leaf-to-air vapor pressure deficit. The spatial and temporal integration of the g_s signal will be aided by the assessment of photosynthetic discrimination against $^{13}CO_2$ ($\delta^{13}C$) via stable carbon isotopes. Changes in leaf temperature resulting from changes in g_s shall also be measured.

Objective 3.2. Determine the Soil-Plant-Atmosphere Water Fluxes

Task 3.2.1. Measure canopy-scale transpiration

To evaluate the impact of changes in g_s and leaf area, the water flow through trees must be evaluated at the community scale. Xylem sap flow will be monitored, as an integrated measure, which can be scaled to whole-tree transpiration [145]. Sap flow sensors will be installed across a subset of trees, aiming to cover the largest trees (largest contributors to plot-level water use) and a range of trees across size classes (needed for modelling and upscaling water use). Using relationships between tree size and water flux will allow scaling up sap flow to calculate whole canopy tree transpiration. Sap flow will be measured continuously on an hourly timestep.

Objective 3.3. Evaluate How Soil Water Store and Availability Changes Across Depths

Task 3.3.1 measure Soil Volumetric Water Content

If plants change their water use habits, it is likely to feed back to alter the available soil moisture with depth. If transpiration decreases under eCO_2, then improved water conditions will likely influence organic matter decomposition rates, and plant community activity during the dry season. This will be monitored using time-domain reflectometry sensors installed at nine depths from the surface (5, 10, 20, 30, 40, 50, 60, 75 and 100 cm) within each plot in AmazonFACE.

Task 3.3.2: Measuring Soil Water Potential

Soil water potential is determined jointly by volumetric soil water content and the capacity of that water to be withdrawn from the soil, which is in turn determined by soil structure. Soil water potential can be calculated through measuring soil water retention curves or through the co-location of soil water potential sensors with volumetric water content sensors to evaluate soil water potential. In addition, the soil water potential to which the trees are exposed can be assessed using predawn leaf water potential measured monthly (see Task 4.1 below).

Objective 3.4: Determine If eCO_2 Drives Plasticity in Plant Hydraulic Traits

Task 3.4.1: Measure leaf water

potential

To evaluate plant water stress status, monthly to bimonthly leaf water potential measurements will be undertaken on the trees with sap flow sensors installed Measuring predawn leaf water potential indicates the stress imposed by soil water availability, and midday leaf water potentials measure the combined soil and atmospheric stress. Leaf water potential will be sampled on a minimum of three leaves per tree.

Task 3.4.2: Determine changes in vulnerability to embolism and hydraulic safety margin

The minimum annual midday leaf water potential will be combined with a one-off measurement of P50, the water potential at which a branch loses 50% of its conductivity, to calculate the hydraulic safety margin. This is a key measure of the vulnerability of a plant to drought-induced embolism. P50 will be calculated using a vulnerability curve, which will be measured for all studied trees once at the start of the experiment on a branch of ~1-2m in length. Initially 1 measurement is likely to be sufficient, as plasticity in P50 is likely to be low and given the size of the branch needed, it will minimise damage to trees.

Task 3.4.3: Determine If maximum hydraulic conductance (K_{smax}) changes with eCO_2.

Hydraulic conductance is likely to be more plastic than P50, however in response to eCO_2 it may increase if water demand increases (e.g. elevated leaf area) or decline if water demand declines (reduced stomatal conductance). Monitoring this is essential to determine if water supply capacity is changing, but also to understand potential trade-offs between the efficiency of water transport for the whole tree and the hydraulic safety of the water transport system. K_{smax} will be measured on small (3–5 cm long) distal branch samples using the hydraulic setup described by Sperry et al. (1988) [187].

Model-Data integration

Assessing feedback with the carbon and nutrient cycles, and with the atmosphere

Leaf temperatures may increase with stomatal closure and lower transpiration rates, associated with overheating of leaves at times and thus, reduction of photosynthetic activity. Higher soil moisture could change microbial activity and nutrient uptake. On the other hand, an increase in leaf area could compensate for the reduction in transpiration rates. In summary, the ecosystem-level effects of eCO_2 on water fluxes remain unknown at large spatial scales, despite the recognition that the Amazon is the tropical forest with the highest dependence on rainfall recycling by vegetation (Kooperman et al. 2018) [49]. The measurements will be used for integrating leaf- to canopy-level responses and will be integrated in vegetation modelling to assess larger-scale impacts of eCO_2 on the Amazon water budget. Model-data fusion will be used to synthesize soil moisture data, precipitation and other parameters, merging data gaps and ensuring consistency in temporal and vertical monitoring of soil moisture.

Establishing the water budget

A detailed stand-scale model, locally parameterized for canopy structure and vertical profile of leaf area density, will be employed to scale water flux from the leaf to the canopy from measurements. MAESPA will be employed to derive the stand-scale water budget which is then used for benchmarking the process-models.

Effects of eCO_2 on plant hydraulics and drought response

Building upon previous model intercomparison activities from Fleischer et al. (2019) [20], this Research Area will apply the assumption-centred approach regarding water-related process-representation at the AmazonFACE site to identify the needs from the modelling side regarding relevant processes and potential changes under eCO_2. Using an ensemble of hydraulic dynamic vegetation models that incorporate plant-water regulation strategies, and the measurements derived from this task will allow assessing species-specific sensitivities to water availability via plant hydraulics and the link to the carbon cycle. Such modelling exercises will also simulate longer-term responses and assess model assumptions and develop existing hydraulic models further.

9.4 Research area 4: biodiversity

Background

Amazonian forests are amongst the most diverse ecosystems on the planet. These forests are home to around 15,000 tree species [146], each of which may respond differently to the change in climate and increasing atmospheric CO_2 concentration. Tropical forests also have a huge but understudied mycorrhizal, saprotrophic and bacterial diversity [147], which might influence the responses of this system to changes in CO_2 concentration. Changes to the plant and microbial functioning driven by the extra CO_2 are likely to have consequences to the whole trophic cascade [148–150], influencing multiple ecological interactions and ultimately ecosystem-level diversity. AmazonFACE is the first FACE experiment in a tropical, highly diverse ecosystem, allowing us to understand how different species and their strategies may respond to additional CO_2. This understanding of how the huge diversity of this system responds to eCO_2 is critical if we are to predict the future of Amazonia.

Plant species are expected to respond differently to CO_2 fertilisation depending on their life-history strategy (pioneer or shade-tolerant), their capacity to fix nitrogen and life form (lianas, trees and palms). These are hypotheses derived from theory [151,152] and are supported by trends observed in long-term monitoring studies [55,153,154] or greenhouse experiments [155]. However, whilst observations are unable to control for other drivers, such as local disturbances and changes in climate, greenhouse experiments cannot capture the complexity and interactions that are intrinsic to such diverse systems. AmazonFACE will allow us to finally test these long-standing theoretical predictions within such a complex ecosystem.

Due to the high diversity within the plots of AmazonFACE a species-level analysis is unlikely to be feasible or meaningful. A total of 394 tree species and 55 botanical families were identified within the 1,305 stems (DBH ≥ 2cm) within the AmazonFACE plots. From these, half of the stems (657) belong to

49 species, whilst the other half of stems were distributed across 345 species, of which 190 are singletons. Despite its high diversity, AmazonFACE will not represent the taxonomic/phylogenetic diversity of the whole Amazon Forest. A functional diversity-oriented analysis shall be conducted across the different research areas within AmazonFACE. This Research Area aims to systematically link key plant traits with ecophysiological processes indicative of plant performance. Such a functional-based approach for interpreting responses of Amazonian diversity and its interaction with increased CO_2 will favor the connection with modelling and the upscaling of results from the experiment to larger spatial scales–considering that vegetation, climate and Earth System models work with plant functional groups and not species.

Changes in the functioning of the plant community and its ecophysiology in response to eCO_2 are expected to impact other trophic levels in the ecosystem. An increase in CO_2 concentrations is known to directly affect ecological interactions [156,157]. Whether and how changes in biodiversity and the strength of biotic interactions may result in changes in ecosystem processes remain unclear in this CO_2 elevation scenario. It is already accepted that tree leaves under eCO_2 may reduce foliar nitrogen (N) concentration which leads to an increase in the C:N ratio, which would impact all consumers-resource interactions over the trophic chain. AmazonFACE will thus allow us to investigate the impact of eCO_2 on biodiversity across the ecosystem and trophic chain [158].

Research questions

1. Is the response of tropical woody plants to eCO_2 predictable based on their functional traits?
2. Are responses of woody plants to eCO_2 predicted by local environmental characteristics? If so, are these more important than the plant's functional traits?
3. Does eCO_2 alter the trophic cascade (herbivores, predators, pathogens and symbionts) through altering the abundance of keystone species and diversity across the cascade?
4. Can eCO_2 affect the interaction between plants, microbes and insects with consequences to ecosystem-level processes?

Objectives and tasks

Objective 4.1. Characterise the AmazonFACE functional space and plant diversity into functional groups

Task 4.1.1 Characterize the different amazonian plants by their functional traits

It allows us to have a mechanistic understanding of how the plants respond to elevated CO_2.

i. carbon assimilation, allocation and plant growth;

ii. nutrient acquisition and use; and/or related to

iii. water fluxes/use efficiency (Table 1).

For 392 species, the AmazonFACE team measured wood density, specific leaf area, chlorophyll content, and leaf thickness; for 316 species, the stomatal density, stomatal size and leaf vein density were measured. Hydraulic traits have been measured by Research Area 3 during the baseline phase. Belowground traits will be measured from previous soil samples (in collaboration with Research Areas 1, 2 and 3). The tracing of species identity for root traits is desirable through DNA barcoding. This will allow for a better understanding of the coordination between below and above-ground strategies. Given the challenge of characterizing diversity, traits at the species level instead of the individual level will be measured. Traits for different life-history stages and canopy positions for the most dominant species will be measured.

Traits will be used to classify or ordinate the experiment's functional plant diversity either in groups or in a continuum and help provide a mechanistic understanding of the responses of Tropical Forests to eCO_2.

Table 1. Ecological functions and associated key traits to evaluate the ecological performance of plants under eCO_2 during the AmazonFACE experiment.

Ecosystem processes	Specific ecological function	Key trait(s)	Ecological performance indicator(s)	Key references
Carbon Assimilation and Allocation	Gross primary productivity (GPP)	Maximum carboxylation rate (Vcmax)	Photosynthetic efficiency	Walker et al. (2014)
	Net primary productivity (NPP)	Specific leaf area; Leaf N and P content.	NPP, leaf economics spectrum, leaf turnover, leaf area, leaf age	Díaz et al. (2016); Domingues et al. (2010)
	Plant growth, NPP (carbon storage)	Stem wood density; Potential size Belowground and aboveground carbon allocation.	Growth rate, mechanical support, longevity	Chave et al. (2009); Menezes et al. (2021)
	Reproduction	Seed mass/number Fruit size and mass	Reproductive success	Moles et al. (2018); Venable et al. (1992)
Nutrient Acquisition and Use	Nutrient use	Nutrient content and in tissues and retranslocation, starting with N and p	Tissue stoichiometry, dark respiration, tissue turnover	Domingues et al. (2010)
	Nutrient uptake	Specific root morphology; Mycorrhizal association (quantity & type); enzymes and organic acid exudation	Nutrient foraging; nutrient mining	Carmona et al. (2021); Reichert el al (2022)

Task 4.1.2. Evaluate the functional representativeness of AmazonFACE plants within the whole amazonian trait space

Functional trait data from existing databases [152] will be used combined with species composition data from the Amazon Tree Diversity Network (ATDN) to provide a full functional characterisation of the trait space across Amazonian. This will allow us to have a solid evaluation of the representativeness of the plants occurring within the AmazonFACE plots in relation to the functional diversity of the entire Amazon forest [170].

Task 4.1.3 Create functional groups

The understanding of the functional diversity of trees in AmazonFACE will be used to create functional groups that can inform model development representing the diversity of tropical systems. Integration between below and aboveground is desirable, but likely to be challenging.

Objective 4.2. Integrate ecophysiological performance under eCO_2 to the functional space

Task 4.2.1. Understanding the Performance of Trees

The responses of trees to eCO_2 across the functional space will be evaluated. This could provide indications of the effect of eCO_2 on the composition and diversity of Amazonian forests. As performance parameters, individual growth and recruitment rates will be monitored, initially targeting aboveground woody biomass of adult trees but potentially monitoring other plant tissue such as leaves and roots, if possible, to trace tissues to individuals (together with Research Area 1). While it will not be possible to quantify changes in adult tree mortality throughout the course of the experiment given the low sample sizes, it is possible to evaluate their risk of death from changes in canopy condition, hydraulic safety margin and non-structural carbohydrates.

Task 4.2.2. Local environment of the plants

Any response in performance should be evaluated under the context of the position of the plant across the trait multidimensional space and the local environmental condition. Local environmental conditions, which should be related to the canopy position of the individual, must be considered when investigating any responses. Individuals, rather than plots, should be considered the main level of investigation, and analyses may also consider the growth rates of the different plants.

Task 4.2.3. Effects on germination and Seedling Growth

Changes in germination and seedling growth rates within the AmazonFACE plots will serve to indicate shifts in floristic and functional composition, at least in this life-history stage. Small subplots, without any disturbance, will be established to monitor seedling dynamics.

Task 4.2.4. Response of plants, other than trees, to eCO_2

Quantify the performance of lianas and palms in concert with Research Areas 1-3. A particular focus will be put on lianas, which competitive performance is expected to increase under eCO_2. Thus, growth and physiology of lianas will be monitored to test for changes. Studies on bryophytes, herbs and ferns are desirable for both epiphytes and ground flora.

Objective 4.3. Evaluate Cascading Impacts Upwards and Laterally in the Food Chain

Task 4.3.1 Invertebrates community composition

While herbivores may respond directly to changes in plant chemical quality driven by eCO_2 (bottom-up) it is also known that the population of many herbivores may also be regulated by invertebrate predators (top-down). Moreover, from the plant's perspective, other invertebrate groups, such as pollinators and agents of biotic defences against herbivores, would be indirectly affected by the changes in plant nutritional conditions due to elevated CO_2. Diversity, abundance and biomass of invertebrates are desirable information and could be assessed in collaboration with external research groups.

Task 4.3.2. Soil microbial community composition

Integrated with Research Area 2, use techniques of DNA-based amplicon sequencing or untargeted metabarcoding to explore species diversity of bacteria and fungi communities in soils and litter layer. Communities from leaf surfaces are desirable. Additionally, analyse samples from roots targeting the identification of bacteria-roots symbiosis (rhizobia) and fungal-roots symbiosis (mycorrhizas and possibly dark septate endophytes) in concert with Research Areas 1 and 2.

Objective 4.4. Determine the herbivore contribution to NPP and nutrient fluxes

Task 4.4.1. Herbivory measurements in the canopy and leaf litterfall

Herbivory rates would be accessed with two different approaches. The first one is not destructive; by calculating leaf area loss using scanned images of leaves from the litterfall samples using protocol suggested by Metcalfe [171] [this can be combined with analyses on litter decomposition by fungal saprotrophs]. This approach can be used as a monitoring data of herbivory since litterfall materials are monthly collected. The second approach would involve branch samples of some individual trees in each plot. Using a standardised protocol [172], leaves of one branch of each individual canopy tree will be collected, and leaves will be numbered, pressed, oven-dried, and digitised. For both methods, leaf area loss will be determined using ImageJ software. Herbivory will be estimated in percentage as the ratio of leaf area losses over the leaf lamina by total leaf area.

Task 4.4.2. Estimate foliar production removed by herbivory

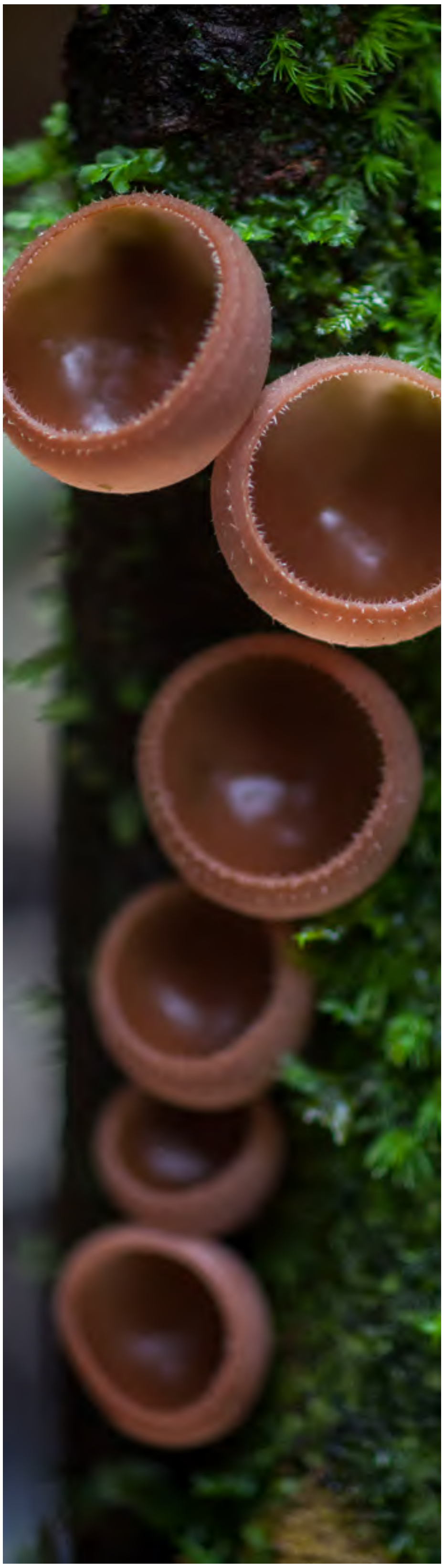
Herbivory loss will be scaled to the whole plot using total foliar litterfall dry mass (Mg ha-1 year-1) and integrated into the NPP calculations (in concert with Research Area 1; leaf production). Therefore, total foliar biomass production would be calculated as total foliar litterfall dry mass divided by 1-Herbivory loss.

Task 4.4.3. Estimate foliar nutrient fluxes resulting from herbivory

Using the nutrient concentration of leaves (in concert with Research Area 2; leaf-level nutrient concentration), nutrient fluxes will be calculated considering herbivory. Nutrient fluxes will be estimated by multiplying dry biomass by live foliar C, N and P concentrations (g g^{-1}). Foliar nutrient fluxes will then be multiplied by herbivory to calculate the mean plot of foliar nutrient fluxes removed by herbivores (Mg ha-1 year-1).

Model-Data integration

Integration with modelling should aim to represent AmazonFACE's functional diversity under ambient conditions and its response to eCO_2, both from a short- and long-term perspective. Our approach will evaluate respectively the ecophysiological performance of the different plant functional groups/entities and eventual longer-term shifts in community composition that will not be possible to observe throughout the course of the experiment but can be captured in modelling applications. A suite of varying trait-based DGVMs [69,97,173,174] as well as data-assimilation / site-specific models [175–177] should be employed for that purpose. AmazonFACE will provide invaluable data to parameterize and evaluate such models. Multi-model intercomparison and assumption-based model analyses should be encouraged.



9.5 Research area 5: socio-environmental

Background

Elevated CO2 and its consequences may have profound impacts on people from the forest, rural and urban areas [12] through, for example, changes in ecosystem properties, processes, biodiversity [178] and ultimately on ecosystem services (EC)[5][179]. Changes in the NPP process, for example, are influenced by modifications in specific leaf area, roots and canopy's architecture and size [76]. Such traits, in turn, could be affected by eCO2 influencing the resulting biomass and associated food productivity (an ES). In short, any implications of eCO_2 for the provision of biodiversity-based ecosystem services will affect the well-being of people that rely on these services, imposing the need to adapt to the reality of those changes or be impacted by it.

Research Area 5 aims to understand such "climate-forest-people" nexus, focusing on how knowledge emerging from the AmazonFACE experiment will help coproduce [180] our understandings and actions related to climate change in the Amazon[6]. About half of more than 400 tree species occurring inside the AmazonFACE plots have a previous register of being used by humans. Therefore, the AmazonFACE field experiment will allow for a broader understanding of how the effects of eCO_2 and climate change on the forest may unsettle the region's social-ecological systems through shifts in the provision of ecosystem services. Combined with the experiment, the results of this Research Area will also allow for a glimpse into future trends, enabling policies of adaptation and mitigation to develop in a better-informed manner.

[6] The concept of ecosystem services will be used under the broader perspective of the Nature's Contributions to People concept.

Research questions

1) How do changes in the forest caused by eCO_2 impact social-ecological systems in the Amazon?
2) How can human populations of the Amazon adapt to the changes in the forest caused by the increased CO_2 and climate

change according to the impacts verified in question 1?

3) How is the AmazonFACE experiment impacting, coproducing and influencing policy?

Objectives and tasks

Objective 5.1. Evaluate the Impacts of eCO_2 and Climate Change on the Provision and Use of Ecosystem Services

The provision, demand and governance of ES[5] depend on ecological processes as well as on the way people perceive, value and interact with nature. Therefore, interdisciplinary and multimethod approaches are valuable to gain deeper understandings of impacts of climate change. The impacts of eCO_2 on ES will be assessed by using the Essential Ecosystem Service Variable (EESV) classes [181] and indicators emerging from the other research areas, such as NPP and fruit biomass (ES: food provision and cultural services), NPP and carbon sequestration (climate regulation), decomposition rate and soil microbiota (soil fertility) [76]). These results will be upscaled to Amazon basin by integrating with the modelling research area and combined with the societal sphere of ES through socio-economic assessments, ethnographic research as well as geographic, economic and sociological research about the impacts of climate change, among others.

Task 5.1.1. To map the impacts o eCO_2 on the delivery of ecosystem services

Through linking ecological processes/functions to ES, in close collaboration with Research Area 4, Objective 4.2.

Task 5.1.2. identify people's demand [7]

Including those of "diffuse beneficiaries" like C storage, and analyse how dependent they are on those ES.

Task 5.1.3. Analyse how people[4] perceive the changes in the delivery of ES and how such changes affect them

Through both academic and local knowledge approaches.

Task 5.1.4. Analyse how changes in ES may affect social-environmental systems in the future scenarios through modelling

Measured by data from other research areas and from people's perception.

Objective 5.2. Investigate how populations adapt to interrelated climate and forest changes

Research on this topic involves collecting data about people's adaptations, including practices already developed and implemented or being developed. It also includes investigating what adaptation is being conceived in these contexts. Data collection can be done through usual quantitative and qualitative methods, but also through more active engagement with local communities, including coproduction of knowledge and policies with local stakeholders.

Task 5.2.1. Analyse adaptation strategies to climate change

Strategies people are already putting into practice.

Task 5.2.2. Investigate the drivers of such adaptations

If by climate-change self-experience (e.g., perception of changes in rain patterns and adaptation accordingly) or via institutions.

Task 5.2.3. Classify the types of strategies

If nature-based, technological and/or other

Task 5.2.4. Evaluate the effectiveness/success of such adaptation strategies

Objective 5.3. Investigate How the FACE Experiment Will Interface with and Impact Policies and Governance, and to Promote Engagement and Policy Advice

innovations[8]

Research to achieve this Objective will engage with policymaking and analyse practices where decision-making, scientists and knowledge interact. This will involve qualitative methods (interviews, document and policy analysis, ethnography, focus groups, surveys, among others) that enable the analysis of data including official documents, participant observation, interviews and media. Events in Manaus and other sites of interest will be organized to

[7] e.g. local governments, communities, NGOs, etc.

[8] Sustainable agricultural practices, and alternative management practices or IPLCs practices with potential to be understood as adaptation are also discussed as ways to adapt to a changing climate

promote engagement, contacting stakeholders and promoting workshops, focus groups and other forms of dialogue between FACE scientists, decision makers and societal actors interested in eCO_2.

Task 5.3.1. To map and analyse climate/environmental policies

Those will be potentially impacted by knowledge emerging from AmazonFACE.

Task 5.3.2. To map and analyse agents involved in policy and governance schemes related to the amazon biome

They can be decision-makers, experts, local communities, NGOs etc.

Task 5.3.3. Promote engagement between scientists and stakeholders involved in policy, decision-making and governance of the Amazon biome

Task 5.3.4. Prepare policy briefs and other material to policymakers about climate change, eCO_2, biodiversity, ecosystem services and other phenomena studied by the experiment

This is an integrative task which will involve input and interaction from all other research areas.

Model-data integration

The findings of the Socio-environmental Research Area, namely those related to Objective 1, will be upscaled both in space and time with the support of the modelling projections predicted in the Modelling Research Area. That will allow a better understanding of how the cascading impacts of eCO_2 and climate change on the forest will impact ES at the large scale now and in the future. Such projections can potentially be used in present-day policies and governance of the Amazon at different scales, from local to global. The production of knowledge through the interfacing between AmazonFACE models and existing models which take into account socio-economic variables, such as Integrated Valuation of Ecosystem Services and Tradeoffs (InVEST) will be sought, as a way to map, quantify and assign value to

[9] Available at: <https://naturalcapitalproject.stanford.edu/software/invest>.

9

9.6 Research area 6: modelling

the studied ES.

Background

Models are the primary tools for interpreting ecosystem measurements, understanding their relationship to environmental variables, and placing those observations in a larger spatial and temporal context. They are especially useful for diagnosing observed behaviours (e.g., via factorial experiments) or for projecting responses to future scenarios of elevated atmospheric carbon dioxide (eCO_2) and potential feedback to the atmosphere and climate. Model outputs play a crucial role in informing government policy, for example they have been used to calculate the remaining carbon budget to reach the two-degree target of the Paris agreement. Confidence in such model predictions depends on the models being well grounded in by both process-level and large-scale observations and responses to experimental manipulations. Global models highlight the importance of the effects of eCO_2 on tropical carbon-, water- and nutrient-cycling and the feedback from the tropics to the global climate.

Research questions

1. What do current models (e.g., DGVMs and land surface models) project for the future of the Amazon under climate change?
2. Are the underlying assumptions made in the models consistent with insights gained from AmazonFACE?
3. When constrained by the experiment, do models simulate over- or under-estimation of climate change impacts and feedback (including water), and carbon budgets for policy targets?
4. What key processes have model-observation mismatch identified that require revision?

5. What long-standing hypothesis was the FACE experiment unable to test? What additional measurements/experiments are now needed?

Objectives and tasks

A suite of process-based models, like standard DGVMs, trait-based DGVMs, site-specific data-driven models, and atmosphere-vegetation coupled models, will be applied to synthesise and upscale the observed effects of eCO_2 on the Amazon forest related to carbon assimilation and allocation, nutrient acquisition, water fluxes and functional diversity-driven responses.

Inspired by previous FACE efforts, a dedicated programme is developed that accompanies observational efforts in AmazonFACE with dedicated modelling studies and developments with the following objectives:

i. rapid synthesis of emerging experimental results;

ii. in-depth analysis of model behaviour with a focus on challenging prevailing model assumptions (assumption-centred model evaluation);

iii. generation and testing of alternative hypotheses (e.g. on belowground processes, carbon-water relationships) developed by experimentalists and modellers to provide further guidance for experimental needs while the experiment is still running.

iv. development of a framework for upscaling key measurements from the site-level or leaf-tree-soil level (e.g., leaf-scale to canopy-scale photosynthesis; tree-level to plot level evaporative fluxes).

v. application of modelling tools – grounded in the experimental testing – to larger scale predictions (e.g. basin-wide carbon/ water fluxes and others).

Tasks

Task 6.1: Synthesis and upscaling of measurement data to the stand/ plot level

It will be done in the individual research areas by using a sub-selection of models (for example, detailed 3D stand-scale model to translate leaf-level photosynthesis into canopy-scale estimates, or Bayesian modelling approaches to propagate uncertainty across whole-ecosystem carbon, nutrient and water budgets [28]; see for example Jiang et al. 2020) (+[28]).

Task 6.2: Carry out model ensembles to analyse assumptions and generate hypotheses

Existing models will be run to inform experimental work, evaluate underlying hypotheses (i.e. model-data integration) [64] and identify knowledge gaps. We will apply and run model ensembles of different model types (e.g. dynamic vegetation models, trait-based models, Earth system models) within individual research areas to help inform experimental work, as done in Fleischer [20], where we already identified alternative model assumptions and measurements needed for model improvement related to phosphorus cycling. Similarly, model ensembles will be run in other research areas, to identify key measurements and make sure that all necessary data are collected in the experiment, including new data that might be identified as needed from new model evaluations. The experimental results will also be used to assess model performance of existing model ensembles such as CMIP6.

Task 6.3: Recommendations for improvements of large-scale models

It will be elaborated that are available for the modelling community with the aim to ensure the experimentally derived understanding links to broader patterns and observations across the Amazon. Based on the results from model-data integration (Task 2), new processes to be implemented and revision of existing processes will be identified. Links with other field experiment programmes in the region (e.g. ATTO, AFEX, drought experiments) will be established and models will be improved collaboratively.

Task 6.4: Deliver model projections for the future development of the Amazon rainforest using understanding from AmazonFACE

Based on that, the goal is to have new insights and understanding from AmazonFACE integrated in improved coupled models and a future generation of a major model ensembles, e.g. CMIP, ISIMIP and to better constrain the CO_2 response in future simulation runs.

10. Data integration and synthesis

A successful FACE experiment will largely depend on successful integration. Ecosystem experiments always encompass myriad interactions between carbon, water, and nutrient cycles. Those interactions affect the system state and system processes that are key to understanding how the ecosystem functions and how it will respond to a perturbation. The FACE experiment starts with the perturbation of the carbon cycle by the increase in CO_2 concentration, but the ultimate response to the perturbation will be shaped by secondary effects on nutrients that feedback on photosynthesis and the carbon cycle, or on the uptake and use efficiency of soil water that may confer increased drought resistance.

Over the longer term, differential responses of species may alter competition and biological diversity. Because complex interactions are involved, integration across multiple data streams is necessary to obtain a comprehensive understanding of ecosystem function and its response to elevated CO_2. Hence, we have emphasised the need for integration in all the research tasks in this science plan. For example, net primary productivity, which is a fundamentally important ecosystem metric that is expected to be responsive to eCO_2, requires at a minimum integration of separate data streams on leaf production, wood production, and fine root production, as outlined in Research Area 1. NPP is expected to be modified by nutrient status.

As outlined in Research Area 2, construction of a nutrient budget requires integration of some of the same biomass data as NPP, in addition to nutrient concentrations in different tissues and ecosystem inputs and outputs of nutrients. Close coordination between researchers measuring production of different plant tissues and those measuring their nutrient concentrations is required. Hydrologic investigations (Research Area 3) include quantification of the distribution of roots in relation to sources of soil water, as measured by carbon cycle measurements and representation of root distribution in models (see pretreatment responses). Quantification of gross primary productivity (Research Area 1) requires integration of detailed data on leaf photosynthesis, canopy structure, and meteorological variables, all of which provide input into a model.

A common thread through all these integration products is the overarching need for each project participant to understand how their individual measurements fit into the whole scientific enterprise and a commitment to follow certain guidelines on how their research is conducted.

These guidelines include open and timely sharing of all data within the project; acceptance of standardised formatting of data; frequent discussion to ensure all critical measurements are made and are made at the best temporal and spatial scale. Team leaders and researchers responsible for producing integration products will be expected to make sure these guidelines are followed. An especially important activity will be close communication and coordination between empiricists and modellers. Are the modellers representing the field data correctly? Do the empiricists recognize the critical data needs of models? While all these requirements may be demanding on the individual researcher, they are also what makes ecosystem science especially rewarding.

Finally, a key ingredient for the due integration and synthesis needed in a research effort like AmazonFACE is a proper database, promptly accessible to the internal community (and after the due time to the external community too). The AmazonFACE Programme database is currently under construction following the FAIR principle for scientific data: findable, accessible, interoperable and reusable. More details on the Programme Data Policy and database can be found on Section 16.

11. Communication and outreach

The full communication strategy of AmazonFACE is made explicit in the Programme's Communication Strategy and is composed of mainly three components. It is needed to address each one with its own specificities, due not only to differences in communication obstacles, but also to their different aims. Every communication action requires an understanding of the public, media and objectives. AmazonFACE can also be a reference in terms of public outreach given not only its association with standing forests and technology, but also the cutting-edge science in the field of climate change it represents.

Internal (peer-to-peer) communication

This aims to overcome the challenges of having the research community based not only in Manaus, but in other parts of Brazil and the world. It also involves the virtual and in-person meetings of AmazonFACE Scientific Steering Committee, as well as the production and circulation of a newsletter to keep the internal community aware of the latest developments. Both an intranet portal and the AmazonFACE data portal also serve the purpose of exchanging information and data internally.

Communication with the external community (scientific and non-scientific)

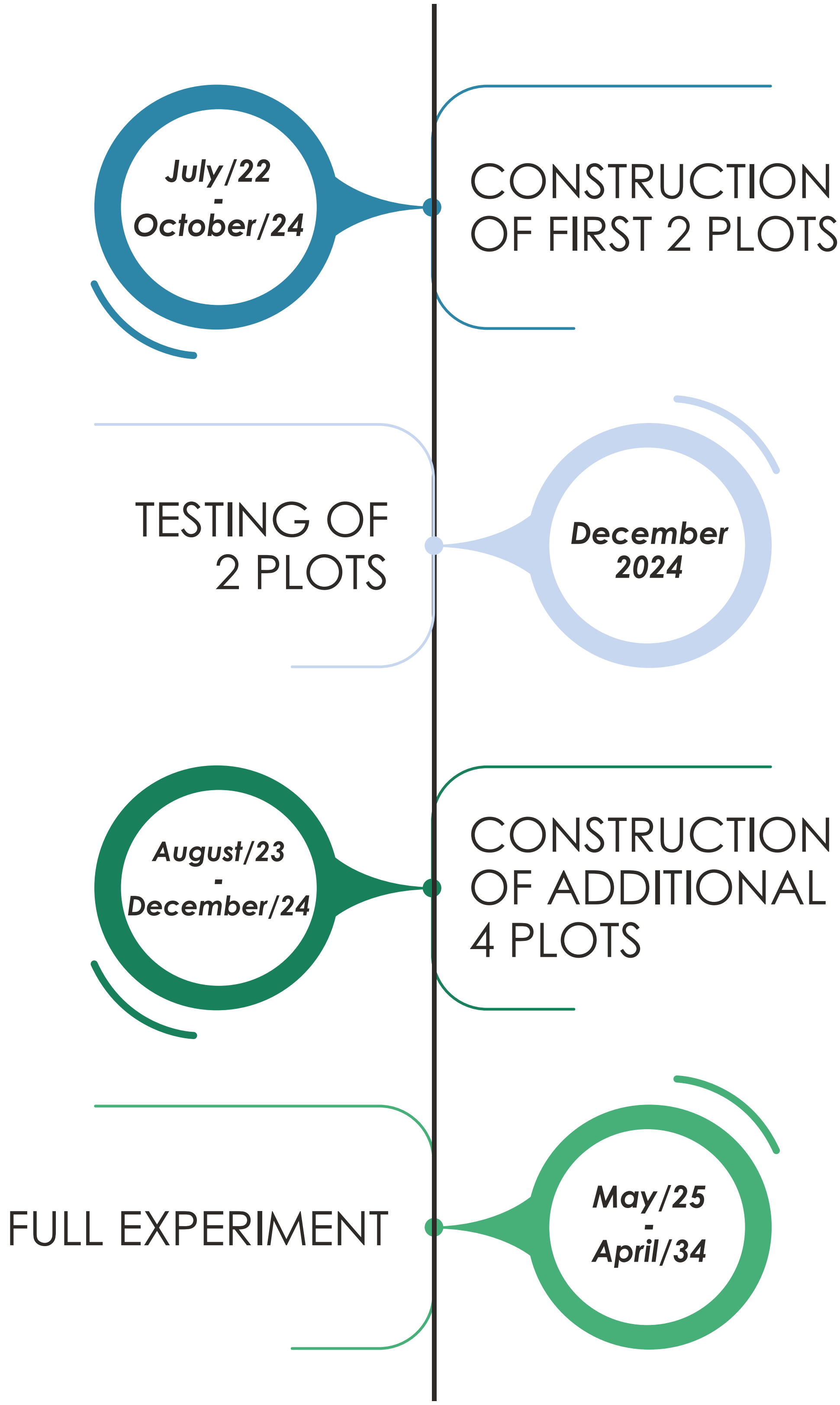
This communication will be effectively made via a webpage portal, social media channels, open workshops, press releases, press conferences, and elaboration and dissemination of FAQs about AmazonFACE, understanding that a transparent and accessible scientific process can help people, especially children to see science in a less abstract way.

Interactions with decision makers

Considering that AmazonFACE Research Area 5 (Socio-Environmental) is concerned with communication with the usage of AmazonFACE results and discoveries by stakeholders, such that impact policies and climate and conservation governance is based on state-of-the-art scientific data (see Research Area 5 tasks 5.3.3 and 5.3.5).

12.Intended timeline

Baseline measurements
2015-2024



(Subject to changes due to climatic, logistic or financial conditions)

13. Externalities and their mitigation

Impact on local environment and population

The area surrounding the Programme's site is tropical rainforest. It is important to highlight that for local/traditional populations, the forest, beyond the utilitarian aspects which guarantee their survival, has a symbolic meaning. ZF-2 site is governed by Resolution RE No. 004/2017, which presents the regulation and general rules for visiting and using INPA's research bases, whether there are stations, reserves or floating sites. Based on available information, there will be no need to submit the project to the environmental licensing process. The following traditional communities live in the area close to the Programme's experiment (southwest of the experimental site, inside the Puranga-Conquista Sustainable Development Reserve):

- Barreirinhas (14 families), 14 km away;
- Boa Esperança (11 families), 27 km away; and
- Nova Esperança (22 families), 34 km away.

From a human and cultural perspective, it is important to consider these populations as protagonists and subjects in biodiversity conservation and recognise the weight and strength of their ancestry.

Although the Programme will not require the ILO 169 recommendation of 'prior consultation and dialogue' as mandatory, the AmazonFACE team have set up a line of communication with these communities as early as possible and before construction began. In addition, the Programme team has presented an outline of the project to the local communities and planned several potential community focused projects (subject to funding), so that they can understand the impact of climate change on the river and resources sustainability.

Permits

The land used for the experimental site known as ZF2 is owned by INPA and permission has been granted to undertake the experimental build and run many years of experimentation. Pertinent permits for foreigners wishing to conduct research in the AmazonFACE area are properly requested from Brazil's National Council of Research and Development (CNPq).

Impact of construction

Recruiting the right contractor to implement construction plans is crucial in both the short- and long-term success of the Programme. The project team reviewed several potential contractors to undertake construction work to build the demonstration pair of rings and groundworks for the entire site. The contractor chosen demonstrated proven experience of working on many projects in the Amazon Rainforest, showed an understanding of the sensitivities to preserve the forest, flora and fauna of the site to an absolute minimum, and limited the impact of the construction process on the local environment.

The contractor has been imaginative and innovative in conjunction with support from the AmazonFACE team to be creative in methods used to transport machinery to the site, install towers, use structures built for the project as camp bases to limit the need to travel to and from the site on a regular basis. The contractor understood and complied with the need to limit the disturbance to soil and flora within the site when installing towers, storage tanks etc.

Road improvement

The Programme has significantly improved the health and safety whilst traveling the final 34 km to the site, through the restoration of this road. Signage has been used to denote the site and check point barriers with security used to monitor access.

Health and safety

AmazonFACE team has implemented several measures to ensure the safety of workers, researchers and visitors to the site. This has included the recruitment of an expert consultant who has provided a site review, recommendations, and training to staff and contractors. Equipment has been purchased, and is operational to ensure safe operation of machinery, sample collection by technicians, and maintenance of cranes and towers throughout the life cycle of the experiment. The main field laboratory contains medicines, emergency equipment, such as a satellite phone, and a star link is being installed to provide daily and emergency communications. As per recommendation of the aforementioned consultant, field campaign involving more than ten people counts on the permanence of a paramedic and ambulance.

Carbon emissions

The carbon footprint of AmazonFACE has the potential to cause a negative perception of the Programme, especially as it is aimed at improving understanding of the Amazon forest environment. As a result, the Project team has commissioned an independent carbon emissions report to include the construction, experimental and de-commissioning phase of the experiment. Throughout the remaining lifecycle of the experiment the project team will review the options, and subject to funding, will implement a local carbon offset plan for the Programme or a commitment to balancing the carbon footprint of the Programme against positive carbon actions. Such plans include but are not limited to reforestation and geological carbon burial. Any scheme chosen will need to be credible and meet the needs of the Programme.

14. Institutional arrangement

Since 2014 AmazonFACE is a Research Programme of Brazil's Ministry of Science, Technology and Innovation (MCTI) based at and coordinated by the National Institute for Amazon Research (INPA) and co-coordinated by the University of Campinas (UNICAMP). MCTI and the United Kingdom's Foreign, Commonwealth & Development Office (FCDO) are currently the major funders of AmazonFACE. FCDO resources are made available to AmazonFACE via the UK Met Office, which is also a major scientific partner of AmazonFACE. Should funder regulations allow, all financial resources are managed by Arthur Bernardes Foundation (FUNARBE). That does not preclude in any way the support from research funding agencies such as FAPESP, FAPEAM, CAPES or CNPq.

Many other institutions have scientists and students participating in the project such as the University of São Paulo – USP, University of Exeter, University of Birmingham, Technical University of Munich – TUM, Wageningen University and the Federal University of Amazonas – UFAM, among others. New requests for collaboration are examined by the Scientific Steering Committee (through a specific form available in the AmazonFACE website), following the logic that AmazonFACE is a community infrastructure (registered in MCTI's National Platform on Research Infrastructure – PNIPE) open to the development of all relevant science, but also trying to avoid significant impact and conserve, as far as possible, the forest ecosystem for the experiment that should last at least 10 years.

Synergies with and stimuli to other ongoing related scientific projects are encouraged, especially with other forest FACE experiments and with climate-change related projects taking place in the Amazon. There are obvious points of interest, either in technical and scientific terms, between AmazonFACE, BiFOR-FACE in the UK and EucFACE in Australia. There is currently a notion of establishing a Global Forest-FACE Hub, formally congregating the three ongoing experiments and setting a common platform for sharing technical information, hard-coding a single operation software, promoting the exchange and training of researchers and students, and fostering co-participation in field campaigns, modelling exercises and publications.

There are also relevant complementarities between AmazonFACE and the Amazon Fertilisation Experiment (AFEX) regarding limitations of primary productivity imposed by the lack of soil phosphorus in Central Amazon [45] and how it may constrain the forest response to eCO2. Synergies also exist with the Amazon Tall-Tower Observatory [183], for example, with respect to the effects of eCO2 on water and energy fluxes in the leaf boundary layer and how it upscales to the canopy and planetary boundary layers, with cascading impacts for the region's rainfall and energy balance [184]. Another example comes from interaction between AmazonFACE and the ESECAFLOR rainfall exclusion experiment in Pará [185], to help understand the interactions between eCO2 and trees' resistance to droughts in the Amazon forest, especially from the perspective of ecosystem modelling.

15. Organisational structure

The management structure of AmazonFACE is made simple to prioritise the flow of information, the operation of the experiment and quick solving of eventual problems. Such an organisational structure is presented formally in a Decree of Brazil's Ministry of Science, Technology and Innovation (MCTI), made available in the Programme web portal. MCTI has a supervision role, more specifically through its General Coordination of Climate Science (CGCL).

Coordination of the Programme is made primarily by INPA, with the possibility of other institutions co-coordinating it conditional on specific agreements firmed with INPA (currently UNICAMP is a co-coordinating institution). Both communications and data management sectors are directly linked to the Programme Coordination. An executive office, composed of three managers (administrative, operational and technical) guarantees the proper execution of the Programme in financial and logistical terms. A Scientific Steering Committee, the most participative instance in the management structure of AmazonFACE is composed of approximately 20 members, from Brazil and other participating countries, seeking a gender balance and the inclusion of young scientists.

The Research Areas detailed in this plan in Section 8 are nested within the SSC, having one or two members of the SSC that also act as Research Area leaders. These Research Area leaders, the president of the SSC and the Programme Coordinators are in constant contact with the managers of the Executive Office to ensure that the collegiate decisions taken at the SSC meetings are properly implemented or attended with practical actions. Ideally the SSC holds monthly online meetings and at least one in-person meeting per year. More details on the organisational structure of AmazonFACE can be found in the MCTI Decree.

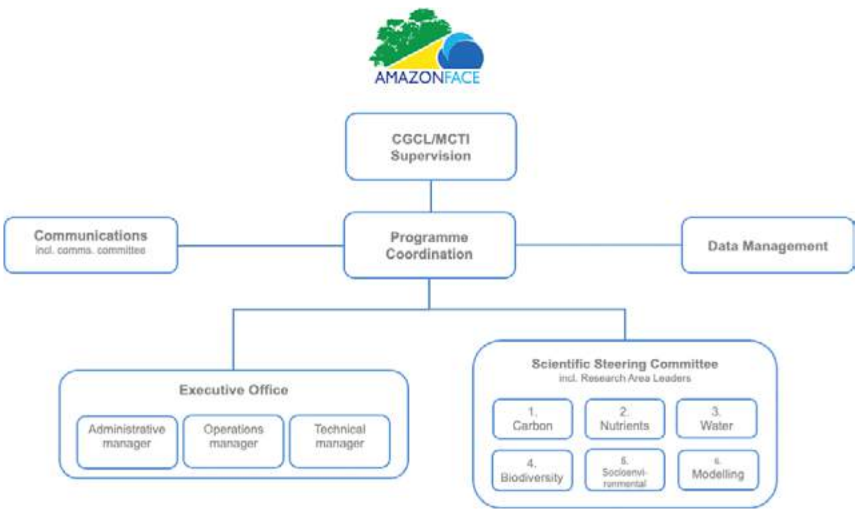


Figure 22. Schematic representation of AmazonFACE organisational structure.

16. Code of conduct and data policy

The AmazonFACE Scientific Steering Committee approved the first version of the Code of Conduct (CoC) for the programme In January 2023. The CoC established rules outlining the norms, responsibilities, and proper practices of individuals and institutions within the AmazonFACE programme.

The CoC applies to all members, associates, and collaborators of AmazonFACE and to all spaces and instances where research and activities of the programme are conducted. The document establishes people's unnegotiable commitment to a safe, respectful, and welcoming environment and zero tolerance for harassment of any kind. The CoC details expected professional behaviour and unacceptable conduct and outlines procedures for reporting, investigating, and solving possible misconduct. Authorship guidelines for fair, appropriate, and transparent authorship of scientific publications arising from AmazonFACE are established in the CoC, as well as the rules for intellectual property, and the resulting guidelines for sharing data and material of the programme. The CoC is subject to periodic review and updates, and the SSC welcomes suggestions and feedback from members to always ensure the highest social safety measures possible.

In 2019, the AmazonFACE Scientific Steering Committee approved and published in the AmazonFACE website a Data Policy document for the Programme. The document is subject to periodical review of the Programme's regulations related to data availability and sharing, but at the time this Science Plan was prepared the policy predicts, among other issues, that data should be made available to the internal Programme community within six months of collection, and to the external community in 12 months after collection. Such data will be made available through an accessible and comprehensive data portal– which is under development–, using the FAIR principle for scientific data: findable, accessible, interoperable and reusable. The current Data Policy also predicts specific recommendations on co-authorship of papers derived from AmazonFACE data.

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18. Appendix


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Carbon			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 1.1.1	Net C assimilation at prevailing [CO_2]	twice a year (wet and dry season)	One-point measurement, light response curves, A-Ci response curves
Task 1.1.4	Plant photosynthetic activity (continuous GPP monitoring)	continual	Solar Induced Fluorescence (SIF) system
Task 1.1.4	Leaf fluorescence	twice a year (wet and dry season)	Saturating light pulse in light- and dark-adapted leaves
Task 1.2.4	Leaf dark respiration	twice a year (wet and dry season)	One-point measurement in dark-adapted leaves
Task 1.2.2.	Leaf production	biweekly	Leaves from litter traps
Task 1.2.4	Bole and branch CO_2 efflux	monthly	CO_2 efflux measured through bole chambers installed in trees with DBH > 20 cm, using a portable CO_2 gas analyzer
Task 1.2.2, Task 4.2.1	Leaf dry mass per area (LMA)	monthly	Leaves from litter traps / area meter - oven dry - weigh
Task 1.2.3	Fine-root productivity and turnover	monthly	Minirhizotron measurements
Task 1.2.4	Root respiration	once in year 2	Respiration collar flux partitioning and root respiration on excavated fine roots using cuvettes
Task 1.3.4	Soil CO_2 efflux	monthly	Soil chamber measurements, CO_2 efflux will be separated into autotrophic and heterotrophic components using root-free soil collars
Task 1.3.4	Soil volatile organic compounds (VOCs)	once after 1 year of eCO_2	Air sampling from soil chambers using adsorbent cartridges
Task 1.3.5	Soil organic matter (SOM) fractions	every 2 years	Soil cores - in the top 30 cm, size and density fractionation and elemental analysis
Task 1.3.1	Carbon transport speed	in pretreatment testing period	Sampling of ^{13}C - respired CO_2, leaves, roots, mycorrhizal hyphae, rhizosphere microbial communities and exudation (Picarro and IRMS)

Carbon, Biodiversity			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 1.1.1, Task 4.1.1	Biochemical photosynthetic parameters (Vcmax, Jmax)	twice a year (wet and dry season)	Light-saturated CO2 assimi-lation (A-Ci) response curves
Task 1.1.1, Task 4.1.1	Light response curve para-meters	annually	Light response curves at prevailing [CO_2]
Task 1.1.1, Task 4.1.1	Temperature response curve parameters	annually	Temperature response cur-ves at prevailing [CO_2]
Task 1.1.1, Task 4.1.1	Leaf carbohydrates	twice a year (wet and dry season)	Sampling of mature leaves from trees where gas ex-changes were measured
Task 1.2.2, Task 4.1.1	Leaf area	twice a year (wet and dry season)	Sampling and scanning of mature leaves
Task 4.1.1	Green leaf volatile organic compounds (VOCs)	annually	Air sampling from LiCor leaf chamber using adsorbent cartridges
Task 1.2.2, Task 4.4.1, Task 4.2.1, Task 4.4.3	Leaf lifespan	monthly	Leaf-level demographic monitoring
Task 1.2.2, Task 4.4.1, Task 4.2.1, Task 4.4.3	Leaf area index (LAI)	monthly	Hemispheric photos
Task 1.2.1, Task 4.2.1, Task 4.2.2	Stem wood production of trees with DBH < 5 cm	annually	Diameter of trees with DBH < 5 cm in three subplots of 1.5 x 1.5 m per plot
Task 1.2.2, Task 4.2.1	Branch litter	monthly	Branch traps on the forest floor

Carbon, Nutrients, Biodiversity			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 1.1.1, Task 2.1.1, Task 4.1.1	Green leaf nutrients and isotopes	twice a year (wet and dry season)	Sampling of mature leaves from trees where gas exchanges were measured, and supple-mented with extra leaves of differing ages
Task 1.3.2, Task 2.2.2, Task 4.3.2	Soil microbial biomass and nutrients	twice a year (wet and dry season)	Soil cores - in the top 30 cm, CFE and C and nutrient analysis of aqueous samples
Task 1.3.2, Task 2.2.2, Task 2.3.1, Task 2.3.2, Task 4.3.1	Invertebrates community composition	twice a year (wet and dry season)	Ground litter stocks, manual ac-tive searching, flight interception trap (5 - 7 days per season)

Carbon, Nutrients			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 1.2.3, Task 2.1.2	Fine-root productivity	twice a year (wet and dry season)	In-growth cores
Task 1.2.3	Root biomass stocks	annually	Roots in soil cores
Task 1.3.2	Root exudation	every 2 years	Sampling of ≈ 5 trees per plot. Compound class identification
Task 1.3.2	Root mycorrhizal colonisation	twice a year (wet and dry season)	In-growth cores, staining and microscopy
Task 1.3.2, Task 2.2.2	Mycorrhizal hyphae biomass	twice a year (wet and dry season)	In-growth cores, soil samples. Lipid biomarkers
Task 1.2.2, Task 2.3.1	Standing litter mass	twice a year (wet and dry season)	Ground litter stocks total mass
Task 1.2.2, Task 2.2.1	Litter nutrient stocks	twice a year (wet and dry season)	Ground litter stocks nutrient contents with multiple laboratory analyses including elemental analysis, UV/vis spectrophotometry and AAS
Task 1.2.3, Task 2.3.2	Litter colonising root stock	twice a year (wet and dry season)	Ground litter stocks, root biomass measurements in subsamples
Task 1.3.3, Task 2.3.2	Litter decomposition	set up in year 3	Litter decomposition experiment - sequential re-collection of the respective litter placed within mesh bags on the soil surface (leaf and woody debris) or in the soil (root litter).
Task 1.3.5, Task 2.2.1	Soil organic and mineral N and P pools	twice a year (wet and dry season)	Soil cores - in the top 30 cm, multiple laboratory analyses including elemental analysis, UV/vis spectrophotometry and AAS
Task 1.3.2, Task 2.2.2, Task 2.2.3	Soil microbial physiological parameters	annually	Soil cores to small-scale lab incubations - Fungal and bacterial community level growth, respiration and turnover rates
Task 1.3.2, Task 2.2.3	Soil enzymes	twice a year (wet and dry season)	Soil cores - in the top 30 cm, analysed using the stoichiometry of extracellular enzymes (fluorometric potential activity assays, targeting C, N and P-containing compounds)
Task 1.3.5, Task 2.2.1	Bulk density	every 2 or 3 years	Soil cores

Nutrients			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 2.1.1	Stem wood nutrients and isotopes (C,N)	once after 2 years of eCO2	Stem sampling of 5 to 10 trees per plot. Multiple laboratory analyses including IRMS and elemental analysis, UV/vis spec-trophotometry and AAS
Task 2.3.1	Litter nutrient contents and isotopes (C, N)	twice a year (wet and dry season; composite samples from biweek;y collections)	Litter traps, multiple laboratory analyses
Task 2.1.2	Root morphology	twice a year (wet and dry season)	In-growth cores and roots in soil cores. Root scanning
Task 2.1.2	Root nutrients and isotopes	twice a year (wet and dry season)	In-growth cores, roots in soil co-res. Multiple laboratory analyses including IRMS and elemental analysis, UV/vis spectrophotome-try and AAS
Task 2.1.2	Root phosphatase	twice a year (wet and dry season)	In-growth cores. Fluorometric potential enzyme assays
Task 2.2.1	Available soil nutrients	twice a year (wet and dry season)	Soil cores - in the top 30 cm, multiple laboratory analyses including UV/vis spectrophoto-metry and AAS
Task 2.4.1	Nutrient deposition	monthly	Wet deposition and throughfall rates - funnels installed above and below the forest canopy to collect precipitation, Dry de-position - surface accumulation method, laboratory analysis

Carbon, Biodiversity, Socio-environmental			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 1.2.1, Task 4.2.1, Task 4.2.2, Task 5.1.1	Stem wood production of all trees with DBH > 5 cm	annually	Diameter of all trees with DBH > 5 cm, automatic dendrometers, terrestrial laser scanning (TLS)
Task 1.2.2, Task 4.1.1, Task 5.1.1	Green leaf dry mass per area (LMA) / Specific leaf area (SLA)	twice a year (wet and dry season)	Sampling of mature leaves from trees where gas exchanges were measured / area meter - oven dry - weigh
Task 1.2.1, Task 4.2.1, Task 4.2.2, Task 5.1.1	Stem wood production of trees with DBH > 20 cm	monthly	Diameter of trees with DBH > 20 cm, automatic dendrometers, terrestrial laser scanning (TLS)
Task 1.2.1, Task 4.2.1, Task 4.2.2, Task 5.1.1	Tree mortality	annually	Inventory of all trees with DBH > 5 cm
Task 1.2.1, Task 4.2.4, Task 5.1.1	Liana biomass	annually	Diameter of all lianas with DBH > 5 cm

Biodiversity			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 4.1.1, Task 4.2.1	Leaf thickness	twice a year (wet and dry season)	Micrometer
Task 4.1.1	Chlorophyll content index	twice a year (wet and dry season)	Chlorophyll content meter
Task 4.2.4	Liana leaf area index (LAI)	annually	Drone photos
Task 4.4.1, Task 4.2.4	Liana Huber value	annually	Analysis of wood sample

Water, Biodiversity			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 1.1.1, Task 3.1.1, Task 4.1.1	Water use efficiency	twice a year (wet and dry season)	One-point measurement, light response curves, A-Ci response curves, Leaf- to-air water vapor pressure deficit (VPD) response curves
Task 3.4.1, Task 4.1.1	Leaf water potential	twice a year (wet and dry season)	Pressure chamber
Task 4.1.1	Stomatal anatomy	once after 2 years of eCO2	Sampling of mature leaves - preparation of histological slides for measuring stomatal size and density
Task 4.1.1	Leaf vein density	once after 2 years of eCO2	Sampling of mature leaves - preparation of histological slides for measuring leaf vein density
Task 3.4.2, Task 4.4.1, Task 4.2.1	P50 and hydraulic safety margin	once after 2 years of eCO2	Vulnerability curves in branches of ≈ 1 - 2 m in length

Water			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 3.2.1, Task 4.4.1, Task 4.2.1	Xylem sapflow	continual	Sap flow sensors installed in 12 trees with DBH > 20 cm per plot
Task 3.4.3, Task 4.4.1	Maximum hydraulic conductivity (Ksmax)	once after 1 year of eCO2	Sperry et al. (1988) method in small branches of ≈ 3 - 5 cm in length
Task 3.3.1	Soil moisture and temperature	continual	Soil moisture, electrical conductivity, and temperature profile at nine depths (5, 10, 20, 30, 40, 50, 60, 75, and 100 cm)
Task 3.3.2	Soil water potential	once	Soil water retention curves

Nutrients, Biodiversity			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 2.1.2, Task 4.1.1., Task 4.2.1	Mycorrhizal fungi identification	twice a year (wet and dry season)	In-growth cores. Genomics
Task 2.1.2, Task 4.1.1., Task 4.2.1	Root plant identity	once	Barcoding of roots in soil cores
Task 2.2.2, 4.3.2	Soil microbial community composition	twice a year (wet and dry season)	Soil cores, genomics

Biodiversity, Socio-environmental			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 4.2.3, Task 5.1.1	Palms and epiphytes inventory	annually	Inventory of all Palms and epiphytes in the plots
Task 5.1.1, Task 4.2.1, Task 4.2.2, Task 4.2.3	Recruitment rates	annually	Three subplots of 1.5 x 1.5 m per plot

Biodiversity, Nutrients, Socio-environmental			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 1.2.2, Task 2.1.1, Task 5.1.1	Production and morphology of seeds and fruits	biweekly	Seeds and fruits from litter traps

Carbon, Water, Biodiversity			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 1.1.1, Task 3.1.1, Task 4.1.1	Stomatal conductance at prevailing [CO_2]	twice a year (wet and dry season)	One-point measurement, light response curves, A-Ci response curves, Leaf- to-air water vapor pressure deficit (VPD) response curves, porometer measurements

Carbon, Water			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 1.1.2	Leaf and canopy temperature	continual	Infrared Radiometers installed in the central tower

Carbon, Nutrients, Socio-environmental			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 1.2.1, Task 2.1.1, Task 4.2.1, Task 4.2.2, Task 5.1.1	Tree height	biennial	Metric tape measure, terrestrial laser scanning (TLS)

Carbon, Nutrients, Biodiversity, Socio-environmental			
Task Number(s)	**Measurement**	**Frequency**	**Samples needed and measurement method**
Task 1.2.2, Task 2.1.1, Task 4.1.1, Task 4.4.3, Task 5.1.1	Plant phenology	monthly	Leaf-level demographic monitoring

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