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Green Ocean Amazon 2014/15 Terrestrial Ecosystem Project (Geco) Field Campaign Report

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June 2016

ARM CLIMATE RESEARCH FACILITY

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Principal Investigator

June 2016

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Office of Science, Office of Biological and Environmental Research

Acknowledgments

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Acronyms and Abbreviations

AGU	American Geophysical Union
ARM	Atmospheric Radiation Measurement Climate Research Facility
ATTO	Amazon Tall Tower
BVOC	biogenic volatile organic compound
CCN	cloud condensation nuclei
CESM	Community Earth System Model
DMS	dimethyl sulfide
DOE	U.S. Department of Energy
DSI	dynamic solution injection
ESM	Earth System Model
GC-MS	gas chromatograph-mass spectrometer
Geco	Terrestrial Ecosystem Project
GoAmazon	Green Ocean Amazon 2014/15
INPA	Instituto Nacional de Pesquisas da Amazonia
JBEI	Joint BioEnergy Institute
LBA	Large Biosphere Atmosphere, an INPA research program
LBNL	Lawrence Berkeley National Laboratory
m	meter
MEGAN	Model of Emissions of Gases and Aerosols from Nature
OSSEPP	Off-Site Safety and Environmental Protection Plan
PDFF	Dynamic Biology of Fragmented Forest
ppm	parts per million
ppt	parts per thousand
PTR-MS	proton transfer reaction mass spectrometer
ROS	reactive oxygen species
SFA	
TD-GC-MS	thermal desorption-gas chromatograph-mass spectrometer
TES	Terrestrial Ecosystem Science
VOC	volatile organic compound

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1.0 Project Overview

In conjunction with the U.S. Department of Energy (DOE)'s Atmospheric Radiation Measurement (ARM) Climate Research Facility GoAmazon campaign, the Terrestrial Ecosystem Science (TES)-funded Green Ocean Amazon (GoAmazon 2014/15) terrestrial ecosystem project (Geco) was designed to:

- evaluate the strengths and weaknesses of leaf-level algorithms for biogenic volatile organic compounds (BVOCs) emissions in Amazon forests near Manaus, Brazil, and
- conduct mechanistic field studies to characterize biochemical and physiological processes governing leaf- and landscape-scale tropical forest BVOC emissions, and the influence of environmental drivers that are expected to change with a warming climate.

Through a close interaction between modeling and observational activities, including the training of MS and PhD graduate students, post-doctoral students, and technicians at the National Institute for Amazon Research (INPA), the study aimed at improving the representation of BVOC-mediated biosphere-atmosphere interactions and feedbacks under a warming climate. BVOCs can form cloud condensation nuclei (CCN) that influence precipitation dynamics and modify the quality of down welling radiation for photosynthesis. However, our ability to represent these coupled biosphere-atmosphere processes in Earth system models suffers from poor understanding of the functions, identities, quantities, and seasonal patterns of BVOC emissions from tropical forests as well as their biological and environmental controls. The Model of Emissions of Gases and Aerosols from Nature (MEGAN), the current BVOC sub-model of the Community Earth System Model (CESM), was evaluated to explore mechanistic controls over BVOC emissions. Based on that analysis, a combination of observations and experiments were studied in forests near Manaus, Brazil, to test existing parameterizations and algorithm structures in MEGAN. The model was actively modified as needed to improve tropical BVOC emission simulations on a regional scale.

Geco studies, currently reported in 10 peer-reviewed papers, have addressed key questions about mechanistic controls over leaf- and landscape-scale tropical forest BVOC emissions, and the driving biological and environmental factors that are expected to change with a warming climate. Together with a number of trained MS and PhD students at INPA, we are actively working on finalizing addition high-impact papers.

In conjunction with the DOE's Atmospheric Radiation Measurement GoAmazon campaign, the TES-funded Green Ocean Amazon (GoAmazon 2014/15) terrestrial ecosystem project (Geco) was designed to evaluate leaf-level algorithms for biogenic volatile organic compounds (BVOCs) emissions in Amazon forests near Manaus, Brazil, and to conduct field studies to characterize biochemical and physiological processes governing leaf- and landscape-scale tropical forest BVOC emissions, and the influence of environmental drivers that are expected to change with a warming climate.

While the majority of GoAmazon studies focused on atmospheric processes (blue lines in figure below), Geco studies focused on characterizing terrestrial emissions and ecophysiological processes (yellow lines in figure below).

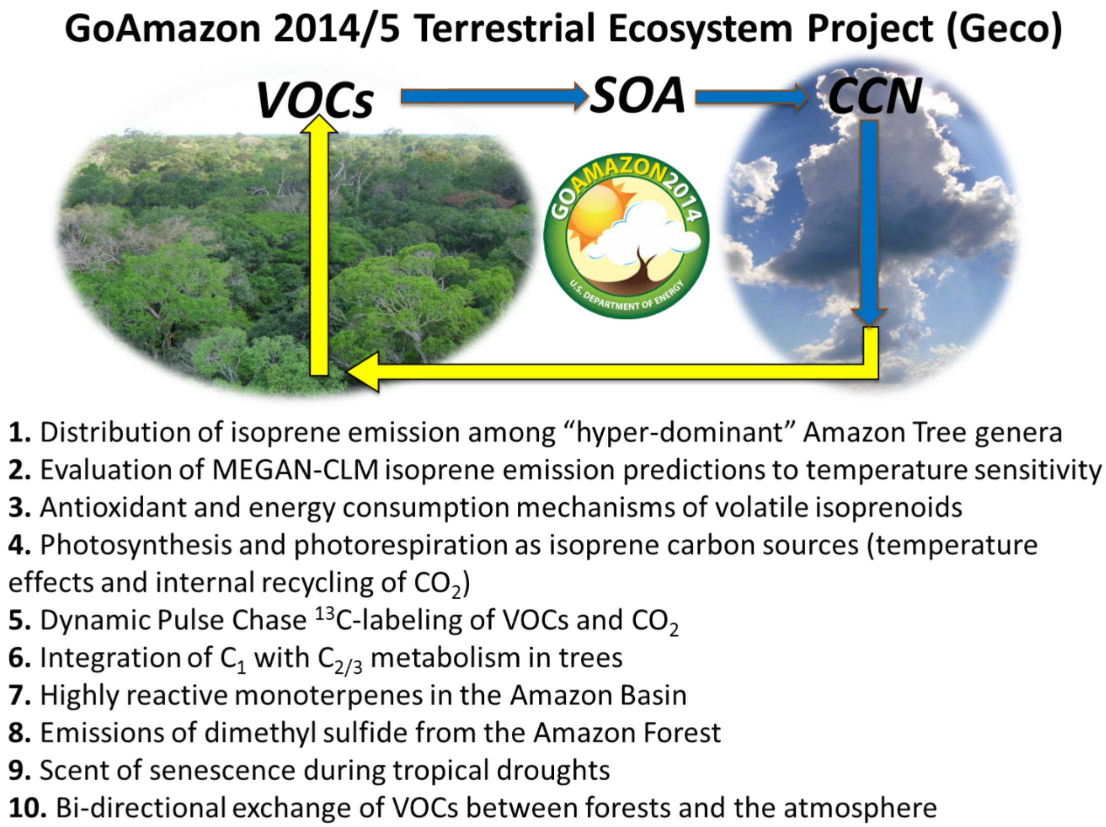

Figure 1. Overview of the GoAmazon 2014/15 Terrestrial Ecosystem Project.

2.0 Objectives

The Green Ocean Amazon terrestrial ecosystem project (Geco) is focused on biogenic volatile organic carbon (BVOC) emissions from forests in the vicinity of Manaus, Brazil. The project is exploring the influence of tree species as well as leaf temperature and light, soil-to-canopy moisture status, and leaf physiology on leaf-level and understory-to-canopy BVOC emissions using a variety of methods. BVOC emissions have been related to net forest CO_2 exchange, water and energy fluxes, and variation in light quality as measured at a co-located eddy covariance site. Geco results have informed algorithm development for the Model of Emissions of Gases and Aerosols from Nature (MEGAN) in the Climate and Earth System Model (CESM). Thus, Geco has addressed key questions on controls over leaf and landscape-scale tropical forest BVOC emissions, with attention to those biological and environmental factors that are expected to change with a warming climate. The LBNL TES SFA effort is closely linked to the DOE Atmospheric Radiation Measurement Facility's Green Ocean Amazon campaign in terms of infrastructure, observations, and questions.

3.0 Personnel

Table 1. Geco key research staff.

Personnel	*Institution*	*Research Roles and Responsibilities*
Jeffrey Chambers	LBNL	• PI
Kolby Jardine	LBNL	• Leads BVOC leaf-level emission study • Leads leaf- and ecosystem-level field measurements
Jennifer Holm	LBNL	• Leads BVOC MEGAN modeling study • Evaluation of CLM-MEGAN driving parameters
Rob Rhew	UC Berkeley	• Alkene/alkane leaf emissions and relaxed eddy covariance
Key External collaborators. Not funded by this SFA or parallel FWPs		
Antonio Manzi	INPA	• Co-PI and eddy covariance tower support
Niro Higuchi	INPA	• Co-PI and permanent plot forest dynamics support
1. Valdiek Menezes 2. Sabrina Garcia 3. Vinicius Fernandes de Souza 4. Angela Jardine 5. Ana Paula Florentino 6. Luani Piva 7. Raquel Zorzanelli 8. Bruno Gimenez 9. Clarissa Fontes 10. Flavia Durgate 11. Vilany Carneiro 12. Ana Maria Serrano 13. Eliane Alves 14. Giordane Martins 15. Priscila Moraes 16. Andrea Teixeira 17. Cilene Palheta	UFAM INPA INPA INPA INPA INPA INPA INPA UC Berkeley INPA INPA UC Berkeley INPA INPA Max Planck INPA Stanford	1. Undergraduate student; BVOCs in tree resins 2. PhD student in leaf-level BVOC plant physiology 3. PhD student in leaf-level BVOC plant physiology 4. PhD student in ecosystem-scale BVOCs (K34 tower) 5. PhD student in climate and environment 6. MS student in BVOCs in tree resins 7. MS student in isoprene distribution among plants 8. Field technician and logistics 9. PhD student on plant hydraulics 10. PhD student on tree identification with BVOCs and IR spectra plus logistics support 11. Postdoc in plant identification (ZF2) 12. Postdoc in ecosystem-scale BVOC emissions (ATTO) 13. Postdoc in leaf level BVOC emissions (ATTO tower) 14. Postdoc in ecosystem- and regional-scale fluorescence 15. Analytical chemistry technician and logistics 16. Analytical chemistry technician and logistics 17. Analytical chemistry technician and logistics
Alex Guenther	UCAR	• Collaborator on developing MEGAN algorithms

4.0 Performance Milestones and Metrics

4.1 Major Milestones

Over the period of the Geco project, we have made some significant progress with key deliverables including the establishment of an analytical laboratory at the ZF2 field site, 19 current peer-reviewed publications reporting recent field and laboratory results, evaluation of environmental sensitivities in the Model of Emissions of Gases and Aerosols within the Community Earth System Model (MEGAN), development of analytical infrastructure at ZF2, presentation of results at local and international meetings, and education and outreach efforts in the US, Europe, and Brazil. In this section, we review scientific progress toward achieving the Geco program milestones (Table 2), highlight published peer-reviewed papers, and describe our education and outreach activities through presentations, graduate student mentoring, and instrumentation training and laboratory/field site development in Brazil (Table 3).

Table 2. Geco milestones for FY14.

Geco Tasks	Status
1-year extension of visas for Geco researchers and equipment	Completed
Safety training updates for Geco	Biological vaccinations and disease prevention and safety with electricity, first aid, chemicals, high-pressure gases, ergonomics, heat, and tower work.
Establishment of new Geco collaborations	1) Initiated new collaboration with the Dynamic Biology of Fragmented Forest (PDFF) project to initiate work at ZF3 on BVOCs from secondary forests. Collected initial atmospheric data set. 2) Initiated new INPA-LBA-EMBRAPA collaboration by traveling to Belem, Brazil and conducting a new VOC experiment within an EMBRAPA oil palm plantation. Manuscript in preparation. Formed new collaborations with analytical chemists, plant physiologists, and atmospheric scientists (e.g., Alexandro Araujo, who also manages K34 tower data). 3) Initiated new interdivision LBNL collaboration between the Geco project within ESD and the Joint BioEnergy Institute (JBEI) through the expression of a tropical monoterpene synthase gene in *E. coli*. Applications include fundamental plant physiology of reactive monoterpenes, monoterpene synthase enzyme kinetic mechanisms under climate change variables, and next-generation biofuel production. 4) Initiated a new collaborative project with one of the founding fathers of isoprene biochemistry and photosynthesis physiology, Thomas Sharkey. We are seeking to inhibit the isoprenoid pathway using fosmidomyocin to examine the impact on photosynthesis under high light and temperature conditions of tropical forests. 5) Initiated collaborations with LBA hydrologists to integrate our Geco project into their hydrologic network at ZF2.
Installation, calibration, and maintenance of field and laboratory equipment	1) Installed, repaired, regularly calibrated, and maintained high sample throughput of analytical chemistry and plant physiology equipment in the Geco laboratory specializing in VOCs at the National Institute for Amazon Research and the ZF2 forest preserve in Manaus, Brazil. This includes a high-sensitivity proton transfer reaction mass spectrometer (PTR-MS) and a gas chromatograph-mass spectrometer (GC-MS). 2) In collaboration with the Large Biosphere Atmosphere program (LBA) at INPA, we installed a climate-controlled instrument container with 24/7 power at ZF2 K34 tower. Acquisition and installation of sap flux sensors, leaf temperature probes, data logger, and light sensor as new permanent instruments on the K34 tower. Installation of C/N/H elemental analyzer at INPA, acquisition (from INPA colleagues) and training of leaf fluorimeter, and method comparisons of reactive oxygen species (ROS) quantification in plant tissues.
Canopy access development	Canopy access methods for in Manaus have been achieved including K34 walk-up tower, and canopy access walkways. These methods will be further

Geco Tasks	Status
	developed, and incorporated into our Off-Site Safety and Environmental Protection Plan (OSSEPP) at LBNL.
Geco Leaf-level and wood resin BVOC survey	Completed 60-species survey of tree species evaluated for VOCs with full light curves (leaves) and trunk resins.
Examples of completed Geco research projects with publications including INPA professors, graduate students, and technicians as coauthors.	1) Characterized methanol and isoprene emissions from the fast-growing Tropical Pioneer Species *Vismia guianensis.* Provided the first data to validate a photosynthesis-linked, energy-based model of isoprene emissions. 2) Applied newly developed technique of dynamic ^{13}C-puluse chase to tropical trees and discovered mechanism for the integration of C_1 and $C_{2,3}$ metabolism. 3) Discovered highly reactive light-dependent monoterpenes in the Amazon Basin (ecosystem-scale gradients and leaf emissions). Discovery of linear temperature sensitivity of monoterpene composition during the 2015 Amazon drought. 4) Discovered dimethyl sulfide in the Amazon Forest (previously assumed to derive from marine sources). 5) Collaborated with Max Planck researchers on Diel and seasonal changes of biogenic volatile organic compounds at the Amazon Tall Tower (ATTO) site. 6) Discovered that isoprene carbon sources as a function of leaf temperature reflect leaf photosynthetic and photorespiratory/respiratory behavior. 7) Evaluated MEGAN-CLM parameter sensitivity to predictions of isoprene emissions from an Amazonian rainforest. Highlighted the high sensitivity of isoprene emissions to leaf temperature. 8) Highlighted the role of bidirectional exchange of biogenic volatiles with vegetation in a review paper. 9) Demonstrated the effects of temperature on isoprene emission from the abundant tree species *Eschweilera coriacea* during leaf phenology in the central Amazon. 10) Demonstrated the differential fates of the C_1 atom and $C_{2,3}$ of pyruvate in biosynthesis and emission of acetyl-CoA and CO_2.
Final Geco projects with goal of completion in 2015	1) The ‘Smell of Senescence’ in the Amazon Forest 2) Leaf isoprene biosynthesis from internal recycling of respiratory and photorespiratory CO_2 3) Development of a unified theory of carbon and energy requirements for isoprene production 4) Leaf Photosynthesis and Volatile Isoprenoid Emission patterns among three dominant Central Amazon Tree Families Lecythidaceae, Sapotaceae, and Burseraceae 5) Volatile organic compounds in wood resins in abundant tree species in the Amazon as species-specific “fingerprints” 6) $^{13}CO_2$ labeling of 12 monoterpenes emitted from banana leaves reveals a common substrate with strong connections to photosynthesis 7) Development of a portable leaf photosynthesis and VOC emission system 8) Cis-β-ocimene and Trans- β-ocimene and as tracers of New World versus Old World tree species 9) Secondary tropical forests as extreme sources of BVOCs in the atmosphere 10) Carbon tetrachloride land-surface exchange in the Amazon Basin

Geco Tasks	Status
	11) Within plant isoprene oxidation associated with post-mid-day inhibition of photosynthesis and transpiration: a new ecosystem-scale indicator of oxidative stress? 12) High fungal sesquiterpene emissions from leaf litter in the Central Amazon 13) Bidirectional exchange of oxygenated VOCs in Amazon 14) Seasonal variation of carbon uptake in a primary forest ecosystem in southeastern Amazon
Geco Model Evaluation	**Status**
Tropical leaf temperature and BVOC emission ecophysiology analysis in CLM	Evaluation of leaf temperature sensitivities for isoprene emissions from the Amazon in MEGAN-CLM Make parameter and structural changes to MEGAN-CLM mechanistic modeling of BVOC dynamics

Table 3. Geco education collaboration, and outreach initiatives.

Activity	Status
Outreach to INPA students and scientist working within or as collaborators of the Geco project in Manaus, Brazil.	1) Trained laboratory and field technicians and supervised their work as a part of the Geco project and GoAmazon 2014/15 collaborations (see key external collaborators list). 2) Assisted with GC-MS instrument repair and student/technician training at UEA (Jose Fuentes GoAmazon 2014/15 project) and aided in repair and training of GC-FID at INPA Casa 20. 3) Formed collaborative research interactions with GoAmazon 2014/15 PIs Scot Martin, Jose Fuentes, Scot Saleska, Joe Berry, Allen Goldstein, Paulo Artaxo, and Antonio Manzi. 4) Developed online website describing our GoAmazon 2014/15 project (https://voc-amazon.wikispaces.com/). 5) Held weekly science training seminars for INPA graduate students and engaged in Geco data sharing; helped with instrument repair, importation issues, and paper writing. 6) Hosted two research training courses on the interactions between Terrestrial Ecosystems and the Atmosphere for GoAmazon 2014/15 PIs, postdocs, researchers, graduate students, technicians, and collaborators. The meeting involved a 2-day research training intensive at the Park Suites hotel in Manaus, Brazil with the primary goal of practicing scientific presentations, initiating collaborations, and training in data analysis tools and analytical chemistry instrumentation methods. 7) Co-taught a 1-month field course on Forest Management throughout the month of July and August at the ZF2 forest preserve near Manaus, Brazil for undergraduate students from all over Brazil and INPA graduate students. Geco components involved lectures, field experiments, and lab studies on ecophysiology with

Activity	Status
	intensive sessions with 5 students for three days (10 groups in total). 8) Formal and informal research advisor to a number of UFAM, INPA, University of California–Berkeley, and Stanford University undergrads (1), graduate students (10) and postdocs (4). Helped create Geco special issue in *Atmospheric Chemistry and Physics* as a part of GoAmazon2014/15 and served as Editor of *Biogeosciences*.
Participation in INPA MS and PhD proposal and defense committees	1. Sabrina Garcia 2. Vinicius Fernandes de Souza 3. Angela Jardine 4. Luani Piva 5. Raquel Zorzanelli 6. Joao Vitor Ceron 7. Ana Maria Serrano 8. Eliane Gomes Alves 9. Giordane Martins 10. Andrea Teixeira
Attended International meetings and gave talks and posters.	AGU, GRC, ESS PI meeting, TES-review, IGAC, and GoAmazon Harvard meeting, and GoAmazon Joint PI meeting.
Set up collaborative Wiki page.	Initial set up and invitation of colleagues from Germany, Brazil, US, and UK to share ideas and data sets.
Actively publish the latest Geco results from experimental field and laboratory research.	Published 14 papers in international journals including *Plant Physiology*, *Global Biogeochemical Cycles*, *Plant, Cell & Environment*, *Atmospheric Chemistry and Physics*, etc.

In section 5 below, we list a few examples of papers which were published during the Geco project

4.2 Leaf- and Ecosystem-Scale VOC Emissions Measurements

Within plant canopies, ambient concentrations of biogenic VOCs are usually very low (few ppbv to pptv) and often a part of complex mixtures with anthropogenic VOCs and secondary photochemical oxidation products (Atkinson and Arey 2003). Studies of ecosystem metabolism of VOCs in forest ambient air, and their biological and atmospheric significance, requires highly specific and sensitive instrumentation to accurately identify and quantify the complex VOC mixtures. For the Geco project, we configured a coupled gas analysis system with high selectivity and sensitivity for VOCs and their oxidation products: a high-sensitivity proton transfer reaction mass spectrometer (PTR-MS; Lindinger and Hansel 1997) and thermal desorption-gas chromatograph-mass spectrometer (TD-GC-MS; Massold et al. 2005) (Figure 2). Coupled to a variety of gas samples from ambient air within forests and environmentally controlled plant chambers, the analytical configuration allows for the identification and online quantification of a wide range of VOCs in ambient concentrations as low as a few pptv (Table 4).

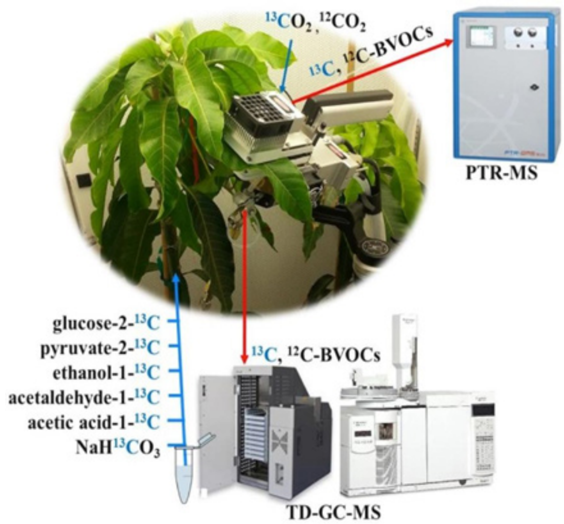

Figure 2. Experimental setup for Geco leaf-level study.

Table 4. Plant-generated volatile organic compound classes and examples of individual compounds.

Volatile isoprenoides	isoprene, monoterpenes, sesquiterpenes
Oxidation products	methyl vinyl ketone, methacrolein, nopinone
Green leaf volatiles	3-hexenal, 3-hexenol, 3-hexen-1-yl acetate, formic acid, acetic acid
Sulfides	hydrogen sulfide, dimethyl sulfide, methyl mercaptan
Aromatics	benzene, toluene, phenol, cresol, benzaldehyde
Alkenes and alkanes	ethane, propene, ethane, propane

The potential power of volatile ecosystem metabolomics studies stems from the fact that many biological processes leave unique volatile fingerprints that can be studied by quantifying VOC profiles across a wide range of temporal scales (seconds to years) and spatial scales (cells to whole ecosystems and regions). A PTR-MS (Ionicon Analytik, Austria) for online analysis of VOCs with 10s of pptv detection limit for most compounds has been upgraded for use in this Geco study by increasing the vacuum system pumping power by a factor of two, which was achieved by integrating EcoCube pumping stations (Pfeiffer Vacuum, Germany) into the system. In addition, an automated thermal desorption autosampler interfaced with an Agilent 5975C gas chromatograph-mass spectrometer (TD-GC-MS) has been developed for use in this Geco study to identify and quantify VOCs in air samples down to a few parts per trillion. A TD-100 thermal desorption system (Markes International, UK) was interfaced with a 5975C series gas chromatograph/electron impact mass spectrometer with a triple-axis detector (Agilent Technologies). Samples (0.5-3.0 L) are pre-concentrated on an external sorbent tube and automatically analyzed at INPA in Manaus, Brazil. Supporting this 'volatile metabolomics' approach is a calibration technique developed by Geco scientist Jardine termed dynamic solution injection (DSI; Jardine et al. 2010a). The DSI technique enables rapid TD-GC-MS and PTR-MS calibration to pptv to ppbv standard atmospheres of complex gas-phase lipid mixtures. Commercial VOC standards in nitrogen diluted in purified air was cross-validated using this system. The use of the combined TD-GC-MS with PTR-MS molecular weight + 1 ions (protonated parent ions) allowed us to use a system with high temporal resolution (PTR-MS) while gaining compound identification and quantification abilities using TD-GC-MS, including the speciation of structural isomers like monoterpenes and sesquiterpenes (Jardine et al. 2015).

Figure 3. Portable photosynthesis and VOC emission system.

4.3 Dynamic Leaf Gas Exchange System

The goal of Geco leaf-level studies is to characterize the relationships between leaf biological and physiological variables (tree species, net photosynthesis, stomatal conductance, transpiration, and VOC emission rates) with environmental variables (light, leaf temperature, CO_2, and moisture). A modified Li-COR 6400XT portable photosynthesis system together with VOC measurements using PTR-MS and new field-portable automated TD-GC-MS methodology has been developed (Figure 3; Jardine *et al.* 2015, manuscript in prep).

Novel $^{13}CO_2$ and ^{13}C-positional-specific metabolite labeling techniques (see Figure 1) have been applied in primary and secondary metabolism studies (Jardine et al. 2010, Jardine et al. 2012, Jardine et al. 2013, Jardine et al. 2014a, Jardine et al. 2014b) using dynamic ^{13}C-pulse chase labeling experiments (Jardine et al. 2014b). These experiments have been used to track carbon through VOC biosynthesis and emissions and to gain insight into how fluxes through primary and secondary metabolic pathways change with environmental conditions. Positional-specific isotopically labeled substrates were provided via the gas phase (e.g., $^{13}CO_2$) or as aqueous solutions (5-10 mM) delivered to leaves and branches via the transpiration stream (e.g., pyruvate-2-^{13}C, glycine-2-^{13}C, $H^{13}CO_3^-$, acetate-2-^{13}C, methanol-2-^{13}C).

Individual leaves have been analyzed for relationships photosynthesis and isoprene emissions as a function of light, temperature, and CO_2. We found that while photosynthesis and isoprene emissions increases together with light, there is a strong uncoupling across leaf temperatures (Figure 4). We applied

$^{13}CO_2$ to track intra- and extra-cellular carbon sources for isoprene biosynthesis together with glycine-2-^{13}C labeling to demonstrate, for the first time, the tight connections between isoprene emissions and both photosynthesis and photorespiration, and their differential control as a function of leaf temperature (K. Jardine et al. 2015, *Plant Physiology*). Although legacy studies revealed a close connection with photorespiration and isoprene as environmental conditions change, more recent studies dismissed this possibility. Thus, our study reopens this discussion.

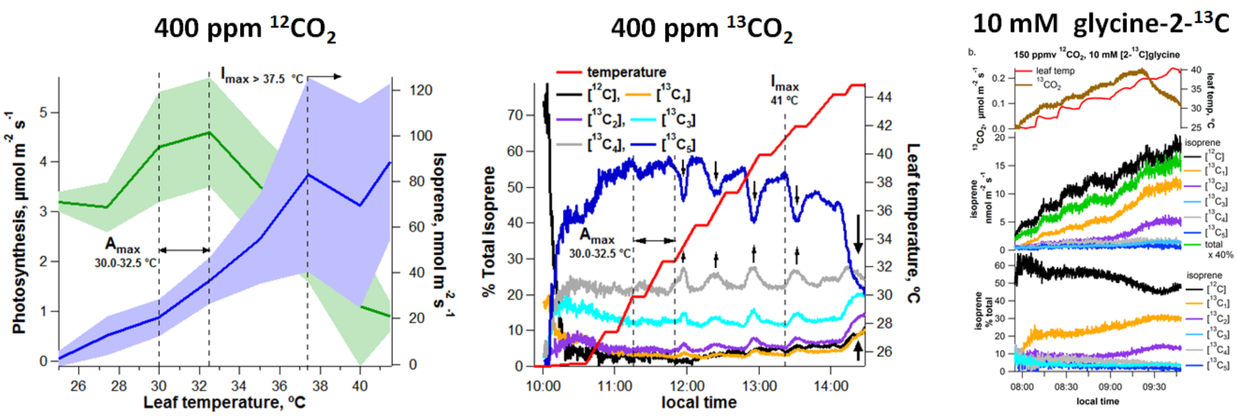

Figure 4. The uncoupling of leaf isoprene emissions and net photosynthesis as a function of leaf temperature. $^{13}CO_2$ labeling reveals the decrease in ‘photosynthetic’ carbon sources are replaced with ‘stored or alternative’ carbon sources at high temperatures. Glycine-2-^{13}C.

Our observations demonstrate that isoprene biosynthesis uses ‘stored carbon’ sources to maintain high emission rates at temperatures beyond the maximum for net photosynthesis. Our isoprene labeling method may also provide new information on the independent gross fluxes of photosynthesis and photorespiration/respiration in leaves, which are difficult to separate using conventional methods. Our observations suggest that the decrease of net photosynthesis in leaves at high temperatures and other abiotic stresses like drought may not be due to a decrease in gross photosynthesis rates, which may actually increase, but rather due to a strong stimulation of internal CO_2 sources such as respiration and photorespiration. Our observations suggest that photosynthesis is using these stored carbon sources under stress to maintain the energy consumption pathways and minimize photoinhibition and photooxidation: key processes that can initiate leaf senescence. Thus, these processes are critical to understanding carbon cycling in forests under climate change, including increased surface temperatures and frequency and duration of droughts leading to enhanced tree mortality.

In recent work, we gained further evidence for the role of internal leaf cycling of CO_2 as ‘alternative’ carbon for isoprene under stress conditions (S. Garcia and K. Jardine et al. 2015, in preparation for *New Phytologist*). We isolated the ‘alternate’ carbon source for isoprene by studying emissions under CO_2-free atmospheres as a function of temperature and light (Figure 4). When compared with emissions under ambient CO_2, isoprene emissions under 0 ppm CO_2 were stimulated in a similar fashion and represented between 10-30 % of emissions under standard conditions of light and temperature. This is exactly the same range of previous reported ‘alternate’ carbon sources for isoprene. Moreover, inhibition of photosynthesis in the dark or using a specific photosynthesis inhibitor DCMU, isoprene emission under 0 ppm CO_2 were eliminated. Finally, isoprene emissions were 13C-labled under 0 ppm CO_2 when $H^{13}CO_3$-was delivered to the transpiration stream, confirming the role of internal CO_2 reassimilation as an ‘alternate’ carbon source for isoprene.

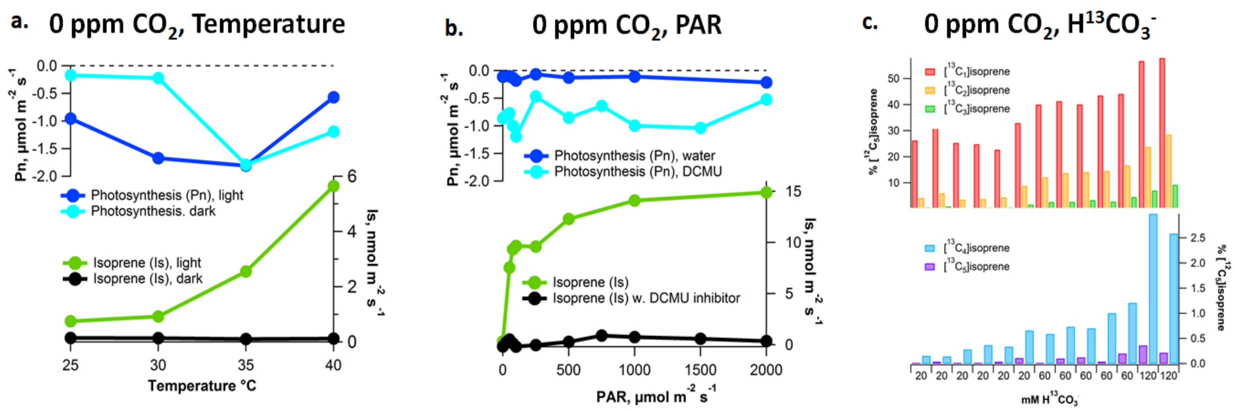

Figure 5. Isolation of 'alternate' carbon for isoprene emissions under 0 ppm CO_2 suggests re-assimilation of internal CO_2 generated by photorespiratory and respiratory processes.

4.4 Isoprene Emissions from Pioneer Tree Species

In a paper entitled "Light-dependent partitioning of energy between photosynthesis and the MEP pathway for isoprene production and the role of leaf phenology in isoprene and methanol emissions in a fast-growing tropical pioneer species *Vismia guianensis*," we test the hypothesis that fast-growing pioneer species, which dominate large rainforest disturbance gaps in the Amazon Basin, do not invest carbon and energy resources into isoprene emissions during photosynthesis, but partition this carbon instead towards growth. Our observations at the leaf and ecosystem scale do not support this hypothesis. For the first time, we observed high ambient concentrations of isoprene (up to 11 ppb) above a secondary Amazon rainforest ecosystem and characterized light-dependent isoprene emissions rates from the highly abundant pantropical pioneer tree species *Vismia guianensis*. We found high rates of isoprene emissions that account for up to 2% of the net photosynthesis rate. A non-linear relationship between isoprene emissions and Pn was found in *V. guianensis* leaves and is consistent with an increased dedication of photoassimilated carbon to isoprene biosynthesis via the methylerythritol 4-phosphate (MEP) pathway under light-saturating conditions of Pn,, possibly due to the use of excess available energy and reducing equivalents. These observations suggest that volatile isoprenoids offer substantial protection to the photosynthetic machinery against photoinhibition and oxidative damage under stress conditions such as the high-light and leaf temperatures that are regularly experienced in secondary forest environments. In the case of *V. guianensis*, we observed that isoprene emissions and photosynthesis rates increase together through leaf development while methanol emissions decreased. These observations are consistent with a function role for methanol production in the establishment of photosynthetic machinery and a defense role for isoprene and monoterpene production to help protect this photosynthetic machinery against the abiotic stresses that are commonly experienced in secondary rainforest ecosystems.

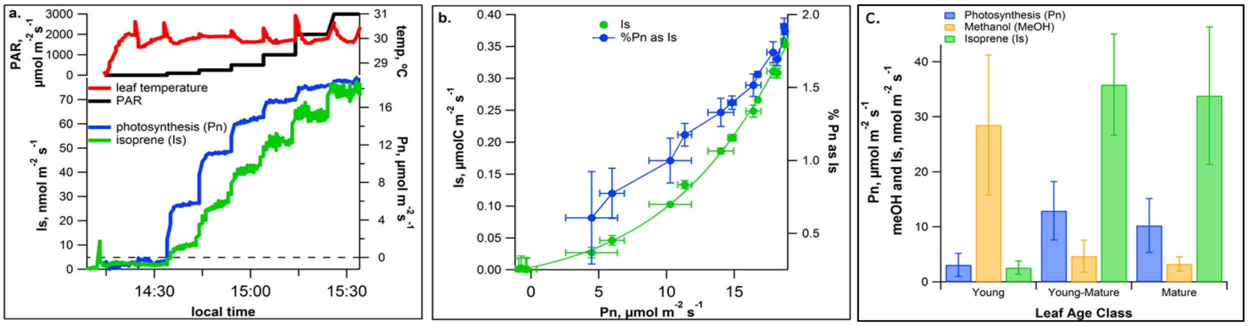

Figure 6. Isoprene emissions from the highly abundant pantropical pioneer species *Vismia guianensis* increases non-linearly with net photosynthesis (Pn) as a function of photosynthetically active radiation (PAR). This supports a functional role of isoprene biosynthesis through consumption of excess ATP and NADPH under photo-inhibition conditions. During leaf development, isoprene emissions increase together with photosynthetic capacity whereas methanol emissions decrease (K. Jardine et al. 2015, in prep for *Biogeosciences*).

4.5 Highly Reactive Light-Dependent Monoterpenes in the Amazon Rainforest

Despite their orders-of-magnitude differences in atmospheric reactivity and their great diversity in biological functioning, little is known about tropical monoterpenes—volatile organic compounds that are emitted by trees into the atmosphere and that may be involved in insect-plant and microbe-plant interactions, as well as playing anti-oxidant roles to assist photosynthesis during abiotic stresses at times of environmental extremes. In addition, monoterpenes are thought to be involved in the formation of secondary organic aerosols and therefore link the biosphere with the atmosphere through cloud formation and water recycling (Figure 6).

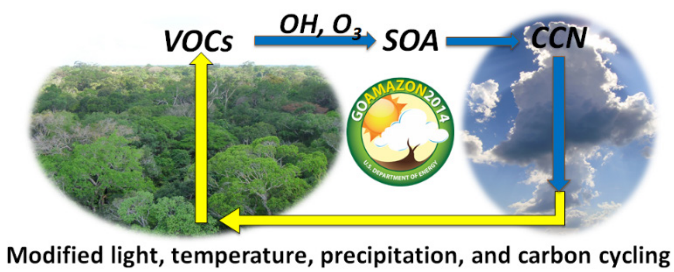

Figure 7. VOC-mediated biosphere-atmosphere interactions.

In a recent Geco study published in *Geophysical Research Letters* (Jardine et al. 2015), we identified and quantified monoterpenes in both ambient forest air and as emissions from leaves in relation to photosynthesis in the Amazon Forest. We investigated monoterpene light-dependent leaf emissions from a variety of tropical trees at a central Amazon field site by developing a new field-portable photosynthesis and monoterpene emission auto-sampling system (Figure 2). We determined their atmospheric concentrations within and above a primary rainforest canopy, using a new rapid vertical-profiling technique. We found light-dependent leaf emissions of highly reactive monoterpenes from a number of abundant tree species, with emissions representing up to 2% of photosynthesis. Moreover, for the first time, we observed the buildup of these highly reactive monoterpenes in the ambient air within the tropical forest canopy (Figure 7). Our results suggest that the emissions of highly reactive monoterpenes from plants protect photosynthesis during stress by acting as powerful anti-oxidants. Similar oxidation chemistry occurs in the atmosphere through monoterpene atmospheric oxidation, which generates low volatility oxidation products that can serve as secondary organic aerosol precursors. Thus, our discovery of highly reactive monoterpenes at the ecosystem scale represent a new uncharacterized local source of secondary organic aerosols, which play critical roles in climate through their interactions with radiation and clouds (Figure 6). We suggested that Amazon trees able to produce highly reactive monoterpenes may have an advantage over isoprene-producing trees in future climate and land-use change scenarios. The research is expected to stimulate a large number of studies in plant biochemistry and physiology, atmospheric chemistry and climate, and land-use change in the tropics.

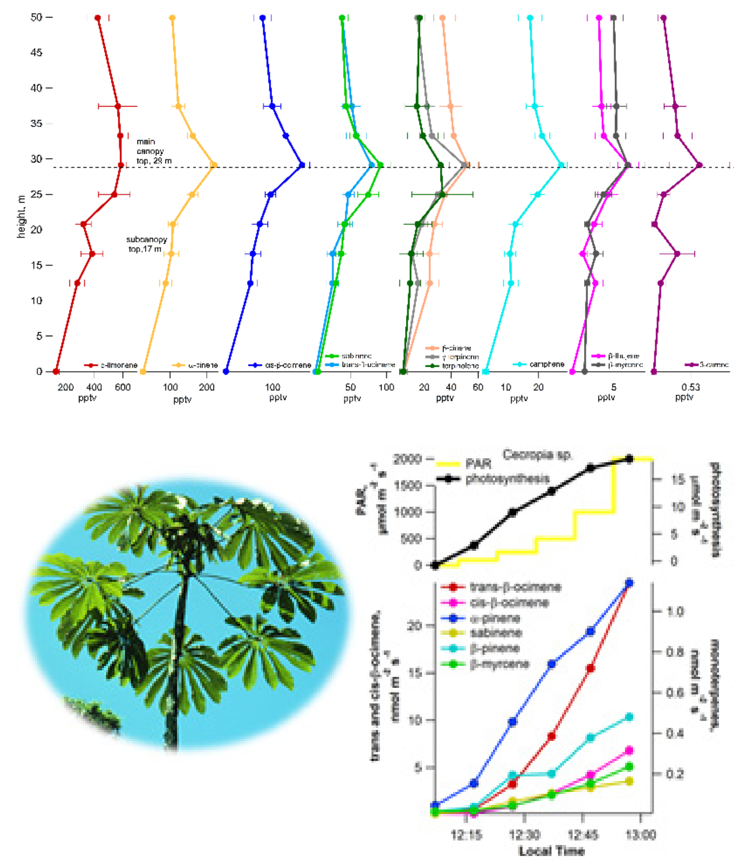

Figure 8. Ambient levels of highly reactive monoterpenes cis- and trans-β-ocimene reflect forest structure, are light-dependent and linked with photosynthesis, and represent an estimated 18% of the total atmospheric monoterpene composition, but 37% of the total monoterpene ozonolysis rates (c-d).

4.6 Integration of C_1 and $C_{2,3}$ Metabolism in Trees

C_1 metabolism in plants is involved in photosynthesis, photorespiration, and the methylation and biosynthesis of metabolites and biopolymers. Although the flux of carbon through the C_1 pathway is thought to be large, its intermediates are difficult to quantify. In this study, we demonstrate that methanol initiates the C_1 pathway in trees and for the first time show a tight connection between the C_1 pathway and the biosynthesis of central C_2 compounds. Delivery of [^{13}C]methanol and [^{13}C]formaldehyde solutions to detached branches of the tree species *Inga edulis* through the transpiration stream rapidly stimulated volatile emissions of C_1 ([^{13}C]methanol, [^{13}C]formaldehyde, [^{13}C]formic acid, $^{13}CO_2$), C_2 ([$^{13}C_{1\text{-}2}$]acetic acid, [$^{13}C_{1\text{-}3}$]methyl acetate) and C_5 ([$^{13}C_{1\text{-}2}$]isoprene). Upon transition into [^{13}C]methanol or [^{13}C]formaldehyde, acetic acid emissions were replaced by [$^{13}C_1$]acetic acid emissions, which in turn were rapidly replaced by [$^{13}C_2$]acetic acid. Delivery of [2-^{13}C]glycine (a photorespiratory intermediate) stimulated emissions of [^{13}C]formic acid, $^{13}CO_2$, [$^{13}C_{1\text{-}3}$]isoprene, and [$^{13}C_1$]acetic acid, confirming the role of the C_1 pathway in photorespiration. The results suggest the integration of the C_1 pathway with photorespiration in fast-growing plants may provide an alternate carbon source for glycine methylation, enhancing CO_2 concentrations within chloroplasts, and increasing the production of key C_2 compounds (K. Jardine et al. 2015; *Plant Cell and Environment*, in review).

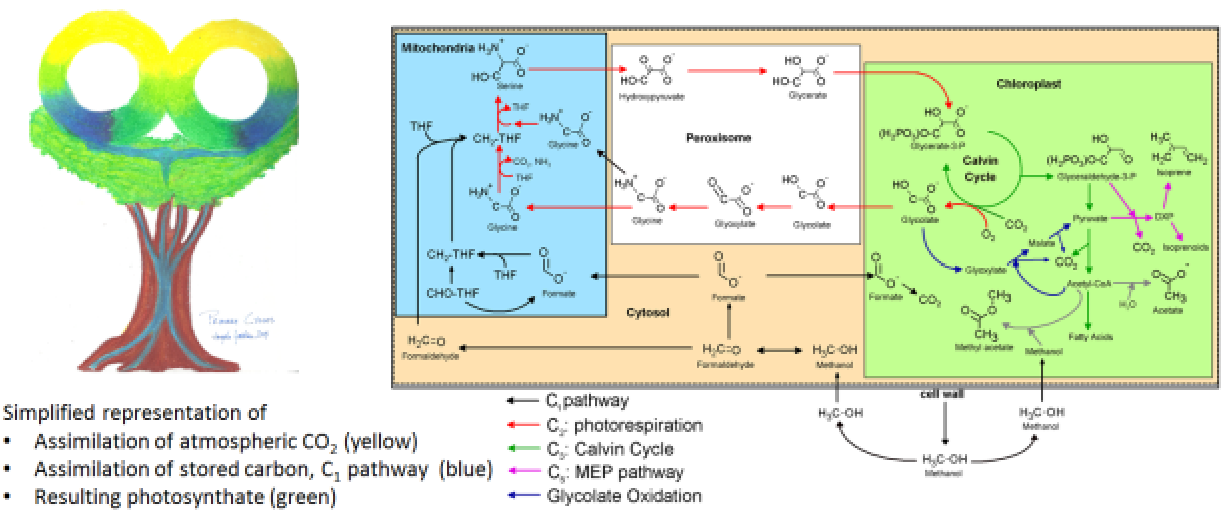

Figure 9. Methanol initiates the C_1 pathway in trees that integrates into 1) photorespiration (C_2 cycle) via the production of 5,10-methylene tetrahydrofolate (CH_2-THF; 2) photosynthesis (C_3 cycle) via the reassimilation of CO_2 derived from formate oxidation; and 3) glycolate oxidation to form acetyl-CoA/acetic acid.

4.7 MEGAN-CLM Parameter Sensitivity Analysis for Isoprene Emissions from the Amazon Rainforest

In this modeling study, we investigated the biophysical parameters that strongly contribute to variability in isoprene emissions estimates from the Amazon Rainforest (J. Holm *et al.* 2015, *Atmospheric Environment*, in prep). We evaluated the strengths, weaknesses, and sensitivities of a widely used leaf-level BVOC emissions model in the land component of an Earth System Model (ESM) in order to determine the largest uncertainty in emissions. We also compared key driving variables like temperature in MEGAN-CLM to in situ measurements. Upon evaluating the sensitivity of 19 parameters in the Community Land Model (CLM) that currently influence isoprene emissions by using a Monte Carlo analysis, we determined that up to 66% of the uncertainty in mean isoprene emissions was caused by the uncertainty in the parameters related to leaf temperature. Leaf temperature was strongly correlated with isoprene emission activity factor (R^2 = 0.89). However, when compared to field measurements in the Central Amazon, CLM failed to capture the upper 12 °C of leaf temperatures (i.e., failed to represent ~33 to 45 °C), and the spread observed in field measurements was not representative in CLM. This is an important parameter to accurately simulate due to the non-linear response of emissions to temperature.

Due to both the strong influence of leaf temperature as well as the insufficiencies of the CLM in being able to capture tropical forest leaf temperature based on the current model structure, this has inspired a field campaign effort in which a thorough investigation of tropical canopy leaf temperatures will be measured. This field campaign starts in June 2015 in Manaus, Brazil, at the K34 tower. A more up-to-date and extensive field campaign to capture current trends in leaf temperature and ecosystem-scale isoprene emissions in parallel could greatly improve modeling capacity and predictability.

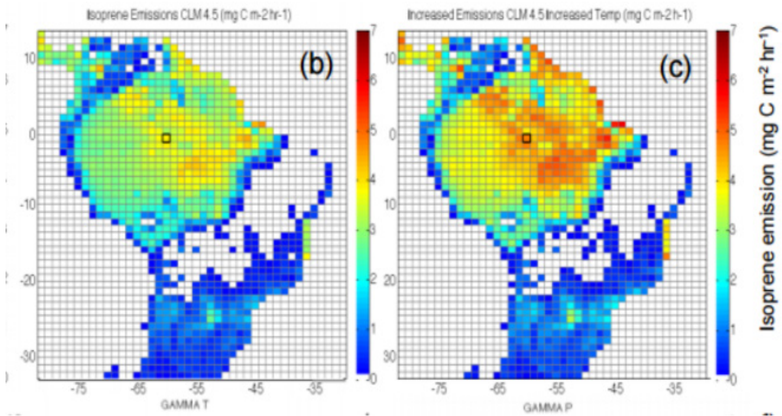

Figure 10. CLM 4.5 isoprene emissions from South America are highly sensitive to a 1.0 °C leaf temperature increase.

4.8 Dimethyl Sulfide in the Green Ocean Amazon

Surface-to-atmosphere emissions of dimethyl sulfide (DMS) may impact global climate through the formation of gaseous sulfuric acid, which can yield secondary sulfate aerosols and contribute to new particle formation. While oceans are generally considered the dominant source of DMS, a shortage of ecosystem observations prevents an accurate analysis of terrestrial DMS sources. Using mass spectrometry, we quantified ambient DMS mixing ratios within and above a primary rainforest ecosystem in the central Amazon Basin in real time (2010-2011) and at high vertical resolution (2013-2014). Elevated but highly variable DMS mixing ratios were observed within the canopy, showing clear evidence of a net ecosystem source to the atmosphere during both day and night in both the dry and wet seasons. Periods of high DMS mixing ratios lasting up to 8 hours (up to 160 ppt) often occurred within the canopy and near the surface during many evenings and nights. Daytime gradients showed mixing ratios (up to 80 ppt) peaking near the top of the canopy as well as near the ground following a rain event. The spatial and temporal distribution of DMS suggests that ambient levels and their potential climatic impacts are dominated by local soil and plant emissions. A soil source was confirmed by measurements of DMS emission fluxes from Amazon soils as a function of temperature and soil moisture. Furthermore, light- and temperature-dependent DMS emissions were measured from seven tropical tree species. Our study has important implications for understanding terrestrial DMS sources and their role in coupled land-atmosphere climate feedbacks.

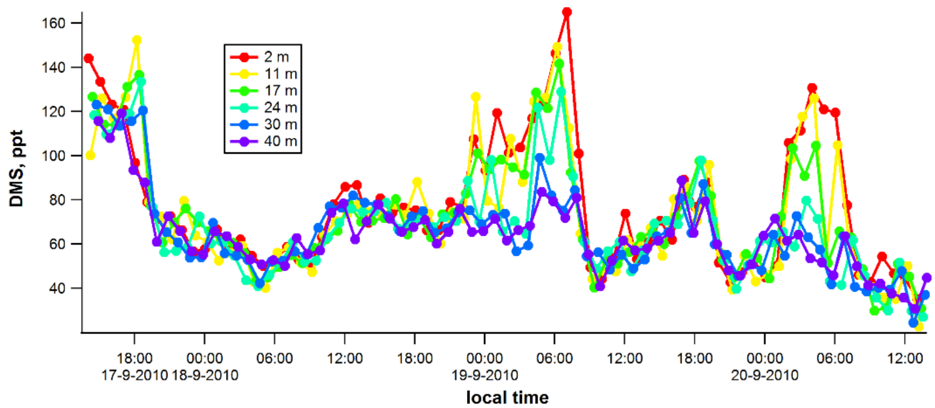

Figure 11. Example time series plots showing real-time ambient DMS mixing ratios within and above the canopy at the TT34 tower in the Central Amazon during the dry season (from 17 September to 20 September, 2010). Note the small buildup of DMS mixing ratios in the afternoon and the strong buildup at night. Note the minimum at early mornings just prior to sunrise due to imitation of vertical mixing and dilution (height < 30 m).

5.0 Publications

Geco studies have to date (June 2016) resulted in 19 high-impact peer-reviewed papers as listed below. Additional papers are currently being prepared for submission in 2016/17. Current peer-reviewed papers with a number of INPA students, technicians, post-docs, professors, and other collaborators include:

Alves, EG, P Harley, JF Gonçalves, CE da Silva Moura, and K Jardine. 2014. “Effects of light and temperature on isoprene emission at different leaf developmental stages of *eschweilera coriacea* in central Amazon. “*Acta Amazonica* 44(1): 9-18, http://dx.doi.org/10.1590/S0044-59672014000100002

Alves, E, K Jardine, J Tota, A Jardine, A Yáñez-Serrano, T Karl, J Tavares, B Nelson, D Gu, T Stavrakou, S Martin, P Artaxo, A Manzi, and A Guenther. 2015. “Seasonality of isoprenoid emissions from a primary rainforest in central Amazonia.” *Atmospheric Chemistry and Physics* 16: 3903-3925, http://dx.doi.org/10.5194/acp-16-3903-2016

Garcia, S, K Jardine, F Souza, A Manzi, N Higuchi, J Chambers, and J Gonçalves. 2016. “Leaf isoprene emission from de novo assimilation of leaf internal CO_2 sources.” (In preparation).

Holm, JA, K Jardine, AB Guenther, JQ Chambers, and E Tribuzy. 2014. “Evaluation of MEGAN-CLM parameter sensitivity to predictions of isoprene emissions from an Amazonian rainforest.” *Atmospheric Chemistry and Physics Discussions* 14: 23995-24041, http://dx.doi.org/10.5194/acpd-14-23995-2014

Jardine, KJ, K Meyers, L Abrell, EG Alves, AM Yanez Serrano, J Kesselmeier, T Karl, A Guenther, JQ Chambers, and C Vickers. 2013. “Emissions of putative isoprene oxidation products from mango branches under abiotic stress.“ *Journal of Experimental Botany* 64(12): 3669-3679, http://dx.doi.org/10.1093/jxb/ert202

Jardine, K, F Wegener, L Abrell, J van Haren, and C Werner. 2014. "Phytogenic biosynthesis and emission of methyl acetate." *Plant, Cell & Environment*, 37(2): 414-424, http://dx.doi.org/10.1111/pce.12164

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Jardine, A, K Jardine, J Fuentes, S Martin, G Martins, F Durgante, V Carneiro, N Higuchi, A Manzi, and J Chambers. 2015. "Highly-reactive light-dependent monoterpenes in the Amazon Basin." *Geophysical Research Letters* 42, http://dx.doi.org/1010002/2014GL062573

Jardine, K, AM Yañez-Serrano, J Williams, N Kunert, A Jardine, T Taylor, L Abrell, P Artaxo, A Guenther, CN Hewitt, E House, AP Florentino, A Manzi, N Higuchi, J Kesselmeier, T Behrendt, PR Veres, B Derstroff, JD Fuentes, ST Martin, and MO Andreae. 2015. "Dimethyl sulfide in the Amazon rain forest." *Global Biogeochemical Cycles* 29(1): 19-32, http://dx.doi.org/10.1002/2014GB004969

Jardine, K, and A Jardine. 2016. "Biogenic volatile organic compounds in Amazonian forest ecosystems." Chapter 4 in *Interactions between Biosphere, Atmosphere and Human Land Use in the Amazon Basin*, Springer, Ecological Studies, Editors: Nagy, L, B Forsberg, and P Artaxo. http://dx.doi.org/10.1007/978-3-662-49902-3

Jardine, K, A Jardine, J Holm, D Lombardozzi, R Negron-Juarez, S Martin, J Chambers, and N Higuchi. 2016. "Monoterpene 'thermometer' of tropical forest response to climate warming." (Submitted to *Nature*).

Jardine, K, B Gimenez, A Araújo, R Cunha, J Felizzola, L Piva, J Chambers, and N Higuchi. 2016. "Diurnal pattern of leaf, flower and fruit specific ambient volatiles above an oil palm plantation in Pará State, Brazil." *Journal of the Brazilian Chemical Society* (In press).

Jardine, K, J Chambers, P Oikawa, J Fuentes, V Fernandez de Souza, S Garcia, J Concalves, A Manzi, N Higuchi, M Bill, R Porras, and U Niinemets. 2016. "Integration of C_1 and $C_{2,3}$ metabolism in trees." *Plant Cell and Environment* (In review).

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Niinemets, U, S Fares, P Harley, and KJ Jardine. 2014. "Bidirectional exchange of biogenic volatiles with vegetation: emission sources, reactions, breakdown and deposition." *Plant, Cell & Environment* 37(8): 1790-1809, http://dx.doi.org/10.1111/pce.12322

Yañez-Serrano, AM, AC Nölscher, J Williams, S Wolff, E Alves, GA Martins, E Bourtsoukidis, J Brito, K Jardine, P Artaxo, and J Kesselmeier. 2015. "Diel and seasonal changes of biogenic volatile organic compounds within and above an Amazonian rainforest." *Atmospheric Chemistry and Physics* 15: 3359-3378, http://dx.doi.org/10.5194/acp-15-3359-2015

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