4 Methodology and quality control EcoRespira-Amazon

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High average air temperatures combined with very high average air humidity strains people and material (▶ 2). Therefore, tiredness and exhaustion may easily occur during fieldwork – and must not compromise the quality and diligence in sampling and sample preparation. The environmental conditions are hard on materials, electronics and mechanics – with resulting potential issues in malfunctioning and high maintenance demand. Whatever is not achieved in the field cannot be compensated for later. This general perspective defined the approach taken with utmost discipline of the team and with specifically designed tools and equipment to ensure the best possible quality of all work packages. While this may appear as something to be taken for granted, it cannot be emphasised enough.

To avoid misunderstandings and possible misinterpretation at this state of project affairs (we are still getting in additional results and this will go on for several months beyond this May 2017 version of the report), we here communicate almost exclusively median values (where applicable) in order to report robust results. This inevitably (at this point) compromises important details that particularly lie in more extreme data, which can only partly be touched in this report – but will be in focus in forthcoming publications.

Locations

Project locations are situated in the central and south-western Amazon Basin in northern Brazil. Samples were taken in the north-south orientation from 02°31'59.7" S to 09°45'01.5" S and in the east-west extension from 067°11'53.7" W to 058°49'53" W (Figure 06).

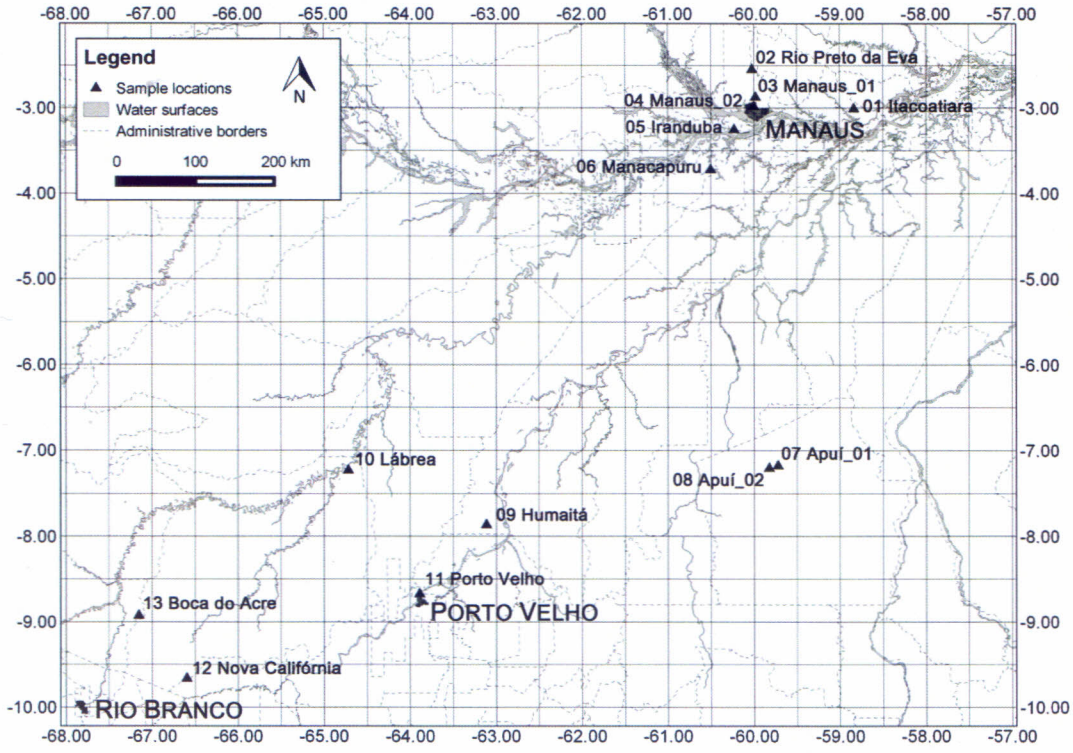


***Figure 06**. Section of Amazonas state with the 13 sampling locations (Map: Lennart Kieschnik). Each location represents at least two sites with forest and post-forest land cover*

The pilot project, having started with fieldwork in February and March 2016 aimed at six locations in the central Amazon region near Manaus and another seven locations in the southern reaches of the Amazon basin in the state of Amazonas. The first six locations also served as training ground, assessing measuring and sampling time as well as team building. The 13 locations, including as of phase 02 the forest biosphere reserve Adolfo Ducke near Manaus as reference, were re-visited until February and March 2017 to repeat the sampling procedures at all sites in order to build a more robust and reliable database for soil respiration data.

All locations were accessible at all seasons by car. They were selected to obtain a representative cross section of locations at least typical for the central and southern reaches of Amazonas state. During the 1st field campaign we observed that all forests were secondary (in different stages); no primary forest was detected in the accessible areas. The post-forest land-cover sites included pastures, orange (*Citrus sinensis* L.), Brazil nut (*Bertholletia excelsa*) Jacaréuba (*Calophyllum brasiliense Cambess.*), Açaí (*Euterpe oleracea*), Cassava (*Manihot esculenta*) and Corn/Maize (*Zea mays*) plantations.

On-site (field) measurements and sampling

The team selected and prepared three spots for soil and ecosystem respiration measurements and related gas sampling at each site. These were defined following with intensive prospection walks to define exemplary spots. These spots represent an area of roughly 10.000 square metres (≈ 100 x 100 m. 1 ha). The spots were geo-referenced (GPS coordinates) and re-visited in all subsequent campaigns. Soil humidity was determined around each spot. Soil samples were taken in close proximity (TOP, BOT). Additional litter samples (ORG) were collected over the entire site (Figure 07).

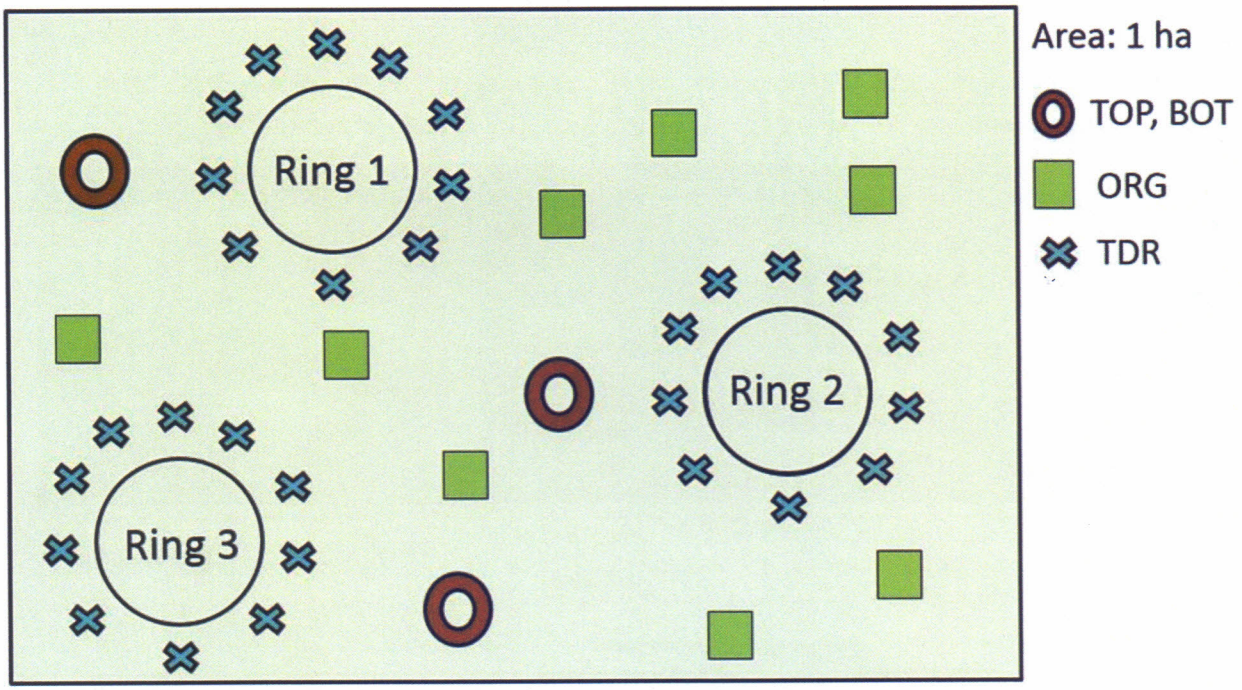


***Figure 07**. Sampling strategy at each site for respiration, soil (TOP, BOT) and litter (ORG) samples as well as for soil humidity (TDR)*

Soil humidity determinations

We determined soil humidity during each of the three field campaigns, both within the SEMACH-FG chambers (MAS-1) and around each measuring spot (TDR; ▶ 4; Figure 07). We determined soil humidity area-representative at all locations and sites around each individual ring (3 per site) by Time Domain Reflectometry (TDR probe, HH2 moisture meter, Delta Devices, England) in the upper 10 cm of mineral soil. Ten to 20 measurements were taken with the sensor in a radius of approximately 10 m around each ring. Median values were calculated later for each ring and each site. The fortunate circumstance that our three campaigns represented the full range of extreme conditions from very dry to very wet soils, allows for both a characterization of average conditions and of extremes with their related consequences.

The ThetaProbe TDR ML2x-sensor measures volumetric soil moisture (or water) content (VWC, q_v), using the established method of responding to changes in the dielectric constant. These changes are converted into a direct current (DC) voltage, virtually proportional to the soil moisture content. Volumetric soil moisture content is the ratio between the volume of water contained in the sample and the total volume of the soil sample. This dimensionless value is expressed as percentage (% vol.) or ratio ($m^3\ m^{-3}$). Consequently, a completely dry soil has the value of 0.0 $m^3\ m^{-3}$; a value of 1.0 $m^3\ m^{-3}$ corresponds to pure water. The probe produces a 100 MHz sinusoidal signal, which is attached to an internal transmission line that extends into the soil by means of an array of three steel rods. The impedance of the rod array affects the reflection of the 100 MHz signal. These reflections and the applied signals combine with each other, forming a voltage standing wave along the transmission line. The output of the ThetaProbe is an analogue voltage that is proportional to the difference in amplitude of this standing wave at two points. This allows a precise measurement of soil water content. The accuracy at temperatures between 0 and 70°C is ± 0.05 $m^3\ m^{-3}$ (Delta-T Devices 1999).

We controlled accuracy and precision of this approach by comparative measurements in the laboratory (gravimetry and TDS in soil within buckets with defined humidity's) and in the field.

Soil sampling

Three types of composite soil samples were taken at each site of every location: the litter layer with high organic matter content (**ORG; n = 75**). a surface mineral soil sample (**TOP: 0–20 cm; n = 79** and a bottom soil sample (**BOT: 30–50 cm; n = 80**), both together largely representing the root zone. Each location represents two to three sites with different land cover (Table 03). In Phase 01, the composite samples were split (aliquots) in the Embrapa-Sede soil laboratory in Manaus by our team and were analysed for most of the periodic system, including high resolution CNS in Freiberg (the latter has been done for all phases). Additional analysis was performed in the Embrapa-Sede soil laboratories in Manaus. Results are constantly being updated and can be found on the bilingual project web site (**http://blogs.hrz.tu-freiberg.de/ecorespira**).

***Table 03**. Number of soil samples from the three field campaigns EcoRespira-Amazon*

	ORG	TOP	BOT
Phase 01	23	24	24
Phase 02	27	28	29
Phase 03	25	27	27
Total	**75**	**79**	**80**

- **Step 1**: Organic debris (litter. ORG) was sampled with glove-protected hands, representative for the entire site. Only larger living plants parts, stones and larger roots were removed from the sample. This ORG material was collected, transported and stored in labelled cotton bags (to allow for air-drying).
- **Step 2**: Cleaning of the Sondaterra® soil auger was done prior to sampling by drilling nearby the final drill site to the desired depth, discarding that sample and manually cleaning the auger with disposable glove-protected hands before taking the "real" sample.
- **Step 3**: The first 20 cm of mineral soil were drilled using the pre-cleaned auger (TOP sample). Small visible stones or larger roots were discarded. The remaining "good" mineral soil material was filled into pre-coded RILSAN® sampling bags. These were labelled with waterproof markers. After sampling at least three bore holes, bags were tightly sealed (no gas or water exchange), double bagged and opened in the laboratory only.
- **Step 4**: Drilling to 30 cm depth, discarding that material nearby (later to fill the hole). This "overdrilling" largely prevents any cross-contamination between TOP and BOT samples.
- **Step 5**: Coring 30–50 cm (BOT sample), subsequent sample retrieval and bagging as above (Step 3).
- **Step 6**: After retrieving the last sample, each site was "cleaned", refilling excess material into the dug hole and camouflaging the sub-sites with organic litter material to prevent accidents. This last step shall not serve beauty, but rather follow the codex: *Take only memories, leave only footsteps*. While we take samples, we shall not make this very visible to avoid any kind of impression that we are making a mess or destructive holes that could injure animals.
- **Step 7**: After each of the sampling steps, the full, labelled and double-bagged RILSAN® bags were transported into a vehicle and carefully stored.

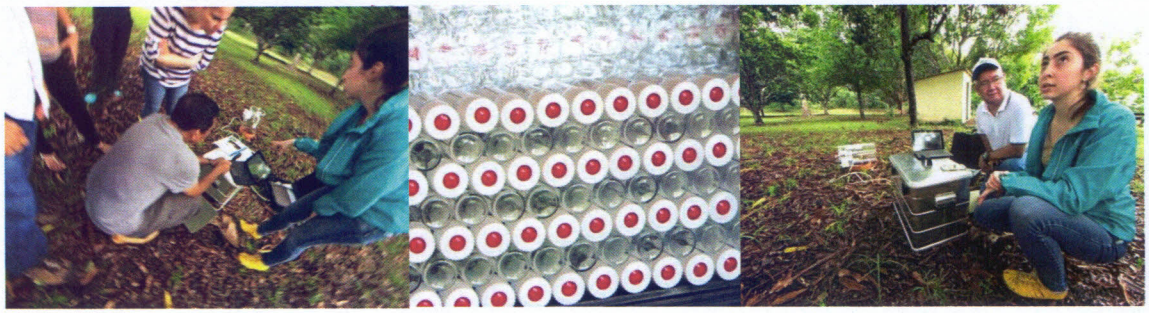
Soil sampling by auger (José Maria Brito Garcia and Jörg Matschullat), storage and transport in Rilsan® bags; Phase 01

Respiration measurements and gas sampling

At each site three PVC-rings were installed, representing an area of circa 1 hectare for in-situ CO_2-determination and gas sampling. Rings were installed at a distance of at least 300 m to the next (access) road or other disruptive elements. Rings were placed on ± flat terrain to obtain comparable conditions everywhere.

Earliest after 24 hours of ring settling time, soil/ecosystem respiration was measured directly (CO_2). On operation of the SEMACH-FG dynamic closed chamber system please refer to the respective manual. A first gas sample was taken right at the beginning of the CO_2 measurements (sample 0 min). After CO_2 measurements were finished for each site and subsequent chamber "rinsing" to get back to initial CO_2 concentrations, a 30-minute gas sampling started with samples taken in 5-minute intervals. Using the Luer-lock interface on top of the chamber, a 20 mL disposable syringe and fine (0.25 mm) needle, samples were taken and injected into 12 mL evacuated Exetainer® vials (Labco Ltd. England) for subsequent laboratory analysis (CO_2, CH_4 and N_2O by gas chromatography). After each location, syringes and needles were discarded and a new pair used for the next location. Gas fluxes were calculated using linear regression. Residuals of the linear regression were tested for normal distribution. This contributes to a better understanding of sink or source qualities of land-use

and land-cover types – a contribution to climate models and to sustainable management practices of the biome.



***Left**: Training unit for the SEMACH-FG chamber system. **Center**: Exetainer® vials for gas sampling. **Right**: Laura Medeiros Braga teaching colleagues on operating the chamber system; Phase 01*

Sophie von Fromm taking soil respiration measurements at Reserva Adolfo Ducke; Phase 02

Orthophotography and remote sensing

All sites in the central part of Amazonas state (locations 01 to 06) were photographed by drone (Quadcopter DJI Phantom 3 advanced) from an elevation of 50 m above ground. Flight tracks were calculated with DJI software to cover each site with 90% overlap of the images (ca. 100 pictures per site). These orthophotographic images bridge the gap between ground observations and satellite imagery. The locations 07 to 13 could not be covered by orthophotography because we temporarily lost the drone in the canopy of site 0420 (Reserve Ducke).

Land Cover Classification was carried out for two areas of interest (AOIs), covering all EcoRespira-Amazon research sites. The northern AOI included locations 01–06, the southern AOI 07–13 (Figure 06). Classifications range from 1990 to 2015 in 5-year steps with one exception in 2010 for the northern AOI, which had to be moved to 2011 due to excessive cloud

cover. LANDSAT 5, 7 and 8 imagery were classified using a Random Forest algorithm in Google Earth Engine, a cloud-based imagery analysis platform (https://earthengine.google.com/).

Classes included were: "Forest" (= relatively dense crown cover), "Secondary Vegetation" (= less dense forest crown cover and lower average tree height; "Sec. Veg."), "Pasture" (= pastureland), "Cropland" (= corn or manioc fields), "Plantation" (= Açai, Brazil nut, Orange, Jacaréuba), "Water" (= water bodies) and "No Vegetation" (= barren land, No veg.).

Generally, classification results for the southern AOI are somewhat better than for the northern AOI, both visually and in terms of their overall accuracy (Table 04). This is mostly due to the lacking class "Plantation" in the southern AOI. Similarly, the largest source of uncertainty for the northern sites is between the Plantation and Secondary Vegetation classes, reducing overall accuracy.

***Table 04**. Overall accuracy of Areas Of Interest (AOI)*

	1990	1995	2000	2005	2010	2011	2015
North	83%	78%	75%	76%	/	63%	77%
South	87%	86%	86%	88%	79%	/	84%

The obtained results for each individual research area are presented, based on a 5 km radius; ▶ chapter 11.

NDVI Time Series

The normalized difference vegetation index (NDVI) is a simple graphical indicator. It is used to analyze remote sensing measurements, typically but not necessarily from a space platform to assess whether the target being observed contains live green vegetation or not. Live green plants absorb solar radiation in the photosynthetically active radiation (PAR) spectral region. PAR is used as energy source in photosynthesis. Leaf cells have also evolved to re-emit solar radiation in the Near Infrared spectral region (which carries approximately half of the total incoming solar energy), because the photon energy at wavelengths longer than about 700 nanometers is not large enough to synthesize organic molecules. A strong absorption at these wavelengths would only result in overheating the plant and possibly damaging the tissues. Hence, live green plants appear relatively dark in the PAR and relatively bright in the near-infrared. By contrast, clouds and snow tend to be rather bright in the red (as well as other visible wavelengths) and quite dark in the Near Infrared. The pigment in plant leaves, chlorophyll, strongly absorbs visible light (0.4–0.7 µm) for use in photosynthesis. The cell structure of the leaves on the other hand strongly reflects near-infrared light (0.7–1.1 µm). The more leaves a plant has, the more these wavelengths of light are affected. Since early instruments of Earth Observation, such as NASA's ERTS and NOAA's AVHRR, acquired data in visible and Near Infrared (NIR), it was natural to exploit the strong differences in plant reflectance to determine their spatial distribution in these satellite images.

The NDVI is calculated from these individual measurements as follows:

$$NDVI = (NIR - RED)/(NIR + RED),$$

where RED and NIR stand for the spectral reflectance measurements acquired in the red (RED, visible) and Near Infrared (NIR, invisible) regions, respectively (http://earthobservatory.nasa.gov/Features/MeasuringVegetation/measuring_vegetation_2.php). These spectral reflectances are ratios of the reflected over the incoming radiation in each spectral band individually; hence they take on values between 0.0 and 1.0. By design, the NDVI itself thus varies between -1.0 and +1.0. It should be noted that NDVI is functionally, but not

linearly, equivalent to the simple infrared/red ratio (NIR/VIS). The advantage of NDVI over a simple infrared/red ratio is therefore generally limited to any possible linearity of its functional relationship with vegetation properties (e.g., biomass). The simple ratio (unlike NDVI) is always positive, which may have practical advantages, but it also has a mathematically infinite range (0 to infinity), which can be a practical disadvantage as compared to NDVI. Also in this regard, note that the VIS term in the numerator of NDVI only scales the result, thereby creating negative values. NDVI is functionally and linearly equivalent to the ratio NIR / (NIR+VIS), which ranges from 0 to 1 and is thus never negative or limitless in range. But the most important concept in the understanding of the NDVI algebraic formula is that, despite its name, it is a transformation of a spectral ratio (NIR/VIS) and it has no functional relationship to a spectral difference (NIR-VIS).

In general, if there is much more reflected radiation in near-infrared wavelengths than in visible wavelengths, then the vegetation in that pixel is likely to be dense and may contain some type of forest. Subsequent work has shown that the NDVI is directly related to the photosynthetic capacity and hence energy absorption of plant canopies (slightly modified after https://en.wikipedia.org/wiki/Normalized_Difference_Vegetation_Index).

The NDVI (Normalized Difference Vegetation Index) time series were calculated for each individual measurement site based on 32-day LANDSAT NDVI composites, ranging from 1990 to 2015. Exemplary charts are given for sites 01 and 07 below. Data gaps are mostly due to cloud cover, except for the gap between October 2011 and June 2013, where no LANDSAT data was acquired due to satellite failure.

Laboratory (analytical) work

All ORG, TOP and BOT samples as well as all gas samples were transported to the Freiberg laboratories. There, soil solution electrical conductivity (λ in $\mu S\ cm^{-1}$) and pH-values were determined, soil chemistry (CNS, major, minor and trace elements) quantified and soil gas (CO_2, CH_4 and N_2O) analysed. Soil physical characteristics were largely determined in Embrapa-Sede laboratories in Manaus but on Phase 01 samples only.

Physical soil parameters

All related work has been performed according to norms developed by Embrapa. For more information, we refer to the methodological manual for soil analysis (Manual de Métodos de Análise de Solo – Embrapa 1997). This manual is made available on the EcoRespira-Amazon project web page and on the final (RIU) workshop materials (CD-ROM) from June 05, 2017.

Soil solution conductivity and pH-values

The soil samples were ground with an agate mortar and pestle to a grain size < 2 mm and sieved (2 mm) for the determination of electrical conductivity and soil pH values. Aliquots of 15 g of sieved soil were weighed in with a SCALTEC SBA 42 balance (Max: 120 g, d=0.001 g) into 125 mL LDPE containers (Nalgene).

Each sample was weighed-in twice (15 g each), since pH determinations were done for pH in 75 mL of deionized water (pH_{H2O}) and in 75 mL of 0.01 M calcium chloride solution (pH_{CaCl2}). The samples were shaken lying in a shaker (THYS 2, company MLW, Germany) for one hour, avoiding any sedimentation in the sample containers. Thereafter, the suspension was left standing still for one hour prior to measurements.

Prior to all measurement series, the electrodes were calibrated. The conductivity probe (Cond 3110, company WTW, Germany) was tested against the standards 84 and 1413 $\mu S\ cm^{-1}$. The

pH electrode (EGA 161, company Meinsberger Elektroden, Germany) with the pH-meter 340 (WTW, Germany) was calibrated against bulk solutions of pH 6.86 and pH 4.01. This electrode is fit for measurements in sludge and suspensions as well as solutions with low electrical conductivity. Each electrode was thoroughly rinsed in deionized water prior to each use.

Those samples that were prepared for pH_{H2O} determinations were first used for the determination of electrical conductivity, since the pH electrodes are stored in 3 M KCl solutions and would inevitably contaminate the sample solution with largely increased conductivities.

Any liquid phase separation/stratification in the solution was dissolved prior to the pH determination by light swivelling. During the determination, the sample solution was left still, e.g., to avoid any uptake of atmospheric CO_2. The pH value reading was done after a 5-minute time interval. The determinations in $CaCl_2$ solution were done by pH electrode only. Here, the determination was made once the signal stood stable. Water temperatures during all measurements were permanently logged.

Soil chemistry

Following thorough soil homogenisation and separation soil samples, were dried in an oven until constant weight (30°C over 24 to 48 hours, according to need). TOP and BOT material was milled in a planetary ball mill (Pulverisette 7, Fritzsch, Idar-Oberstein, Germany) and a rotating disc mill (RS 200, Retsch, Haan, Germany) to analytical grade (<63 µm), both with agate materials. ORG material was cut to small pieces with ceramic scissors, then homogenized and milled in the same rotating disc mill (RS 200) to analytical grade. Sample powder was tested for effective homogeneity by sieving through nylon sieves. If too much material showed fine fibres, then the material was additionally milled in an ultra centrifugal mill (ZM 1000, Retsch, Germany).

Elemental analysis (CNS). 20 mg of analytical grade ORG, TOP and BOT material of each sample (n = 420) were weighed into small tinfoil containers using an analytical balance (Sartorius, Germany). 60 mg of tungsten$^{(VI)}$oxide catalyst was added and the tinfoil tightly sealed. This carefully sealed container is thereafter placed in a sample carousel of the Elemental analyser (El Cube, Elementar Analysensysteme, Hanau, Germany) to determine the concentrations of total carbon (C_{tot}), total nitrogen (N_{tot}) and total sulphur (S_{tot}). To obtain the concentration of organic carbon (C_{org}), another aliquot needs to be treated with a drop of 10% HCl solution. After the gas release (CO_2), the sample will be treated as before and the resulting C-concentration equals the amount of C_{org}, prerequisite to calculate the C/N ratios. Calibration and daily factor determination were done using 4-aminobenzenesulfonic acid. Certified reference material (ORIS, BHA-1) and an in-house standard for tropical soil (BraSol) were added as unknown samples and treated accordingly. The limits of quantification were C: 0.04, N: 0.003, S: 0.003 wt.-%. Sample duplicates reproduced identical values for carbon (RSD ≤1%); deviations remained under 5% RSD for nitrogen and sulphur. The reference materials were always reproduced within their recommended ranges.

Loss on ignition (LOI). To determine the volatiles, a powdered sample aliquot is weighed in precisely into a porcelain beaker. The sample is then heated in a muffle oven at a temperature of 900°C. After cooling to room temperature, the sample is weighed once again. The weight difference equals the amount of volatiles (= LOI).

Total element concentrations (WD-XRF). We used Wavelength-Dispersive-X-Ray Fluorescence spectrometers (Axios, PANalytical, The Netherlands, and S8 Tiger, Bruker, Germany) with their respective software packages. Our lab regularly partakes in national and international

round robin tests and fares very well. The powdered sample (<63 µm) undergo two tracks of analysis with WD-XRF, glass fusion disks and pressed powder pellets.

a) **Glass fusion disks**. A precise amount of roughly 100 mg inweight is thoroughly mixed with a defined amount of lithium tetraborate and filled into platinum vessels. This powder mixture is to be heated to a completely homogenous melt and then filled into platinum receptacles. The resulting glass fusion disk is rapidly cooled and labelled on its rougher side. This sample will then be analysed by WD-XRF for major and minor and some trace elements (the volatiles are gone). The amount of volatiles is put individually per sample into the program and (depending on the total concentration of each element in a specific sample) the total concentrations from lithium (Li) to uranium (U) will be determined quantitatively. A precise instrument calibration, appropriate reference materials and other lab quality control procedures are paramount.

b) **Pressed powder pellets**. Again, a precise amount of powdered sample (ca. 1 g) is mixed thoroughly with a wax (ca. 5 g). This admixture is then pressed into a solid powder pellet with a pressure of 20 tons per square centimetre. The resulting pellet can then be used directly in the WD-XRF to determine the total concentrations of all elements, including the volatiles. Mind that highly volatile elements such as mercury (Hg) may be underdetermined due to Hg-losses in the entire chain from sampling to analysis.

Quality control is based on external (analysis of various components with another independent technique) and internal exercises (monitor samples, sample duplicates, a suite of standard reference materials as unknown samples, multiple determination of the same sample over an extended time period). Generally, we follow the regulations of Good Laboratory Practice (GLP) and all steps are documented.

Trace and ultra-trace element concentrations (ICP-MS and ICP-OES). Following multi-acid full digestion of sample aliquots (ca. 100 mg each), the diluted and acid-free fumed aqueous solution was analysed by inductively-coupled plasma mass-spectrometry (ICP-MS Elan 9000, Perkin Elmer, Perkin Elmer, Germany). The very low limits of determination permit quantifying even minute element concentrations (ultra trace elements).

Since various generally major and minor elements such as Ca, Mg, K and Na occur in rather low concentrations in Amazon soils. These are quantified jointly with Mn and Sr by ICP-OES (Perkin Elmer) and not ICP-MS to avoid disturbances such as mass overlaps, etc.

Quality control is based on external (analysis of various components with another independent technique) and internal exercises (calibration control, blind values, sample duplicates, a suite of standard reference materials as unknown samples, multiple determination of the same sample over an extended time period). Generally, we follow the regulations of Good Laboratory Practice (GLP) and all steps are documented.

Soil gas determinations

Jointly with quality control gas samples, the original Exetainer® flasks were equilibrated to room temperature in the laboratory in Freiberg. Thereafter, the samples were measured with a calibrated gas chromatograph (SRI 8610C, SRI Instruments, USA; ▶ data below in Table 05) and concentrations of CH_4, CO_2 and N_2O quantitatively determined. The standard gas material demands 3.88 ± 0.08 ppm_v for CH_4, 414.6 ± 8.3 ppm_v for CO_2, and 359 ± 36 ppb_v for N_2O. Our standard re-production was 3.81 ± 0.06 ppm_v for CH_4, 413.7 ± 5.9 ppm_v for CO_2, and 361.2 ± 8.5 ppb_v for N_2O. Following the analysis, the results were recalculated in their logical sequence to flux rates of the three gases.

The GC was calibrated using three calibration gases (Cal. 1: CO_2: 257.5 ± 5.2 ppm_v, CH_4: 2.49 ± 0.05 ppm_v, N_2O: 249 ± 25; Cal. 2: CO_2: 414.6 ± 8.3 ppm_v, CH_4: 3.88 ± 0.08 ppm_v, N_2O: 359 ± 36; Cal. 3: CO_2: 1940 ± 39 ppm_v, CH_4: 9.98 ± 0.2 ppm_v, N_2O: 1010 ± 100; Air Liquide).

Table 05. *Parameterization for the gas chromatograph SRI 8610 C (SRI Instruments, USA)*

Parameter	**Characteristic**
Carrying gas	Nitrogen 6.0
Detector 01	FID
Analysed gases	Methane, carbon dioxide
FID temperature	360°C
Separation column	Hayesep D
- Length	3 m
- Inner diameter	2 mm
- Outer diameter	1/8“
Detector 02	ECD
Analysed gases	Nitrous oxide (N_2O)
ECD temperature	330°C
Pre-column	Parapak Q
- Length	1 m
- Inner diameter	2 mm
- Outer diameter	1/8“
Separation column	Hayesep D
- Length	3 m
- Inner diameter	2 mm
- Outer diameter	1/8“
ECD Make-up gas	Argon 95%-Methane 5%

Root biomass determination

Sites at locations Manaus_01 (03), Iranduba (05), Itacoatiara (01), Rio Preto da Eva (02) and Manacapuru (06) were chosen to study the dynamics of root biomass (Figure 06). Five root samples were taken in depth of 0–15 cm with a soil auger (diameter 8 cm; Figure 08).

Fine roots (< 2 mm) were separated from mineral soil material by flotation (Nepstad et al. 1994). Samples were dried at 65°C to obtain the net biomass. In total, 180 root samples were collected and processed in the Embrapa laboratory for Studies and Analyses of Forests at Embrapa Amazônia Ocidental, Manaus.

Figure 08*. Root sampling in central Amazonas, location 01 (Itacoatiara; Fotos: R. Lima 2016)*