1. Study area

The study was developed inside the Biological Dynamics of Forest Fragments Project (BDFFP) area, in the KM41 reserve, located ~100 km from Manaus city, in the 41kilometer of the vicinal road ZF-3 of the BR-174 highway (02º 24'S, 59º52'W) (**Figure 1**). Local soils are clay-rich Ferrasols which cover ~ 30% of the Amazon Basin (Quesada et al. 2011). Rainfall ranges from 1900 - 2500 mm annually with a pronounced dry season from June to October (Ranking de Merona et al. 1992). In relation to Forest structural variables was estimated an AGB (above ground biomass) of 322 ± 54 Mg ha^{-1} (ind ≥ 10 dbh) and mean wood density of 0.67 g cm^{-3} (Duque et al. 2017). Regarding species richness, it was found about 280 species (≥ 10 cm dbh) per hectare (de Oliveira and Mori, 1999).

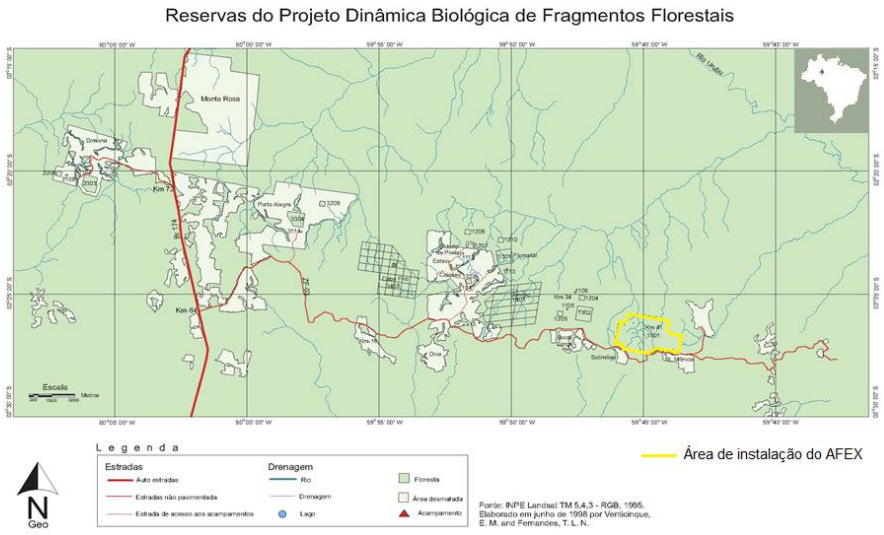

Figure 1 – Location map of the Biological Dynamics of Forest Fragments Project (BDFFP) area. The study was developed in the KM41 reserve (yellow).

2. Sampling design

2.1 – Amazon Fertilization Experiment (AFEX)

AFEX started in March 2017, and consists in a full factorial fertilization experiment. The experiment has eight treatments, with four replicates per treatment, totalling 32 plots, divided into four independent blocks at least 200 meters apart from each other. Fertilisation consists of 125 kg ha^{-1} $year^{-1}$ of N as urea ($CO(NH_2)_2$), 50 kg ha^{-1} $year^{-1}$ of P as triple superphosphate ($Ca(H_2PO_4)_2$) and cations with 50 kg ha^{-1} $year^{-1}$ as potassium chloride (KCl) plus 50 kg ha^{-1} $year^{-1}$ of Ca and 20 kg ha^{-1} $year^{-1}$ of Mg as dolomitic limestone. The treatments are: Control; nitrogen (N); phosphorus (P); cations; nitrogen (N) + phosphorus (P); nitrogen + cations; phosphorus (P) + cations and finally nitrogen (N), + phosphorus (P) + cations Plots size is 50 x 50 meters and all are at least 50 meters apart from each other. All plots were established in areas with similar soil, vegetation and topography (**Figure 2**).

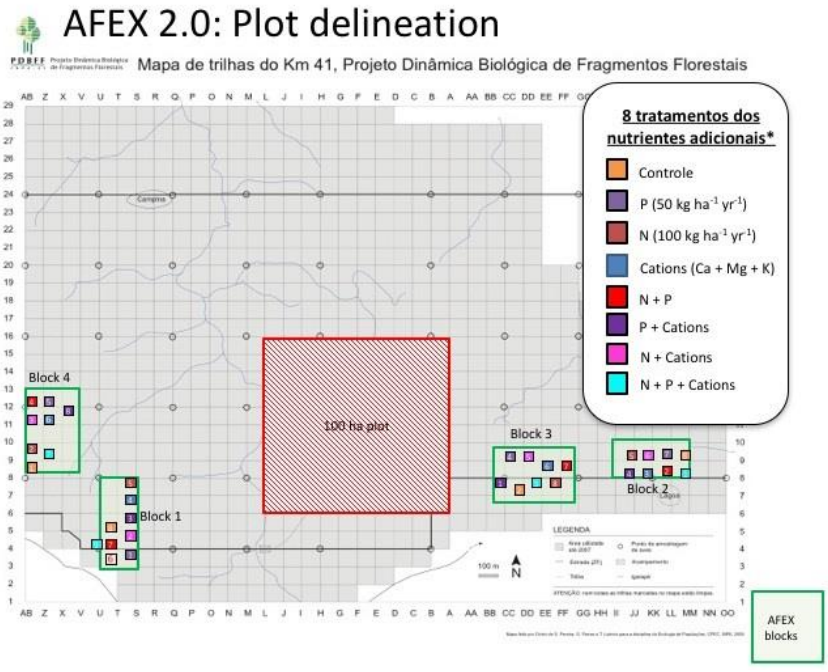

Figure 2. Location map from all fertilized AFEX blocks and plots inside the KM41 reserve.

Fertilization is carried out annually, divided into three applications, in order to mitigate nutrient loss by leaching and runoff. Fertilizers are spread by hand throwing during a systemic walk within the plots.

2.2 Collection methods

Measurements of stem respiration (ppm) were performed on 320 trees with DBH > 25 cm, with 10 trees in each plot, during October 2019. CO_2 stem efflux was measured with an infrared gas analyser (EGM-4, PP systems), where a chamber was placed on to a collar installed in the tree, and detected the rate of CO_2 accumulation for a period of two minutes (**Figure 3**).

Figure 3: A) The collar was sealed with a special glue, and inspections with a flashlight were made to check for any holes B) An EGM-4 portable Infra Red Gas Analyser (IRGA) right and the chamber (left) for measurement of CO_2 efflux.

We also measured soil respiration rates (autotrophic and heterotrophic) using the Li – 8100A soil respiration system (Li-cor, Lincoln, NE, USA), equipped with a 20 cm survey chamber (Model:8100-101). The equipment was set up to an observation length of 1 minute and 30 seconds, and the pre purge and pos purge to 10 seconds.

At two randomly points in the plots, we placed two groups of collars with two types of collars. One tube with short collar (10 cm depth) allowing both heterotrophic and rhizosphere respiration and one tube with longer collars (25 cm depth) with no windows to exclude both roots and mychorrhizae, including some component of disturbance associated with tube installation. The difference in CO_2 efflux between tubes inserted to 25 cm depth and tubes only in the surface is taken as rhizosphere respiration.

Collections dates in 2017 were made in June-July, September, October and November. In 2018 were every month until September and collections dates in 2019 were on January, February, April, July and October.

Data spreadsheet

The spreadsheet contains (**Figure 4**):

	A	B	C	D	E	F	G	H	I
1	TAG	POM	Block	Plot	DBH	Inicial_CO2	Final_CO2	Data	PlotID
2	1257	1.45	B2	P1	38.6	401	405	24/10/2019	B2P1
3	1285	1.5	B2	P1	30.8	388	407	24/10/2019	B2P1
4	1297	1.6	B2	P1	44.6	449	471	24/10/2019	B2P1
5	1301	1.65	B2	P1	58.8	381	412	24/10/2019	B2P1
6	1333	1.5	B2	P1	38.9	405	415	24/10/2019	B2P1
7	1335	1.3	B2	P1	76.9	409	455	24/10/2019	B2P1
8	1351	1.55	B2	P1	44.8	430	472	24/10/2019	B2P1
9	1365	1.4	B2	P1	54.2	378	397	24/10/2019	B2P1
10	1389	1.4	B2	P1	36	386	402	24/10/2019	B2P1
11	1399	1.5	B2	P1	47.4	398	419	24/10/2019	B2P1
12	1424	1.5	B2	P2	31.6	403	422	25/10/2019	B2P2
13	1448	1.4	B2	P2	76.8	395	413	25/10/2019	B2P2

Figure 4. stem CO_2 efflux data spreadsheet deposited in EIDC system.

Colum A – TAG: numerical identification of the trees.

Colum B – POM: Point of measurement of the DAP collected in the forest inventory, the collars were placed approximately 10 cm above this mark.

Colum C – Block: Blocks of the experimental design, which can be B1, B2, B3 or B4, in which the plots are located.

Colum D – Plot: 50 x 50 m plots, ranging from P1 to P8, and containing all fertilization treatments.

Colum E – DBH: Diameter at breast heigh of the selected trees.

Colum F – Inicial_CO_2: CO_2 efflux recorded at the beginning of the measurement.

Colum G – Final_ CO_2: CO_2 efflux recorded at the end of the measurement, 2 minutes later.

Colum H – data: date of collection in day/month/year.

Colum I –PlotID: combination of block and plots.

The spreadsheet contains (**Figure 5**):

	A	B	C	D	E	F	G	H	I	J	K	L
1	Census	File.Name	Date	Date_II	Block	Plot	Group	Type	PlotID	Date_IV	Obs.	Lin_Flux
2	1	AFEX	20170624	July_2017	B2	P1	G1	M	B2P1	24/06/2017 15:01	1	10.46
3	1	AFEX	20170624	July_2017	B2	P1	G1	M	B2P1	24/06/2017 15:02	2	10.32
4	1	AFEX	20170624	July_2017	B2	P1	G1	S	B2P1	24/06/2017 14:57	1	10.95
5	1	AFEX	20170624	July_2017	B2	P1	G1	S	B2P1	24/06/2017 14:59	2	10.56
6	1	AFEX	20170624	July_2017	B2	P1	G1	T	B2P1	24/06/2017 14:49	2	13.09
7	1	AFEX	20170624	July_2017	B2	P1	G1	T	B2P1	24/06/2017 14:48	1	11.92
8	1	AFEX	20170624	July_2017	B2	P2	G1	M	B2P2	24/06/2017 15:27	2	12.9
9	1	AFEX	20170624	July_2017	B2	P2	G1	M	B2P2	24/06/2017 15:26	1	12.38
10	1	AFEX	20170624	July_2017	B2	P2	G1	S	B2P2	24/06/2017 15:34	1	10.07
11	1	AFEX	20170624	July_2017	B2	P2	G1	T	B2P2	24/06/2017 15:20	1	2.75
12	1	AFEX	20170624	July_2017	B2	P2	G1	T	B2P2	24/06/2017 15:21	2	2.68
13	1	AFEX	20170624	July_2017	B2	P5	G1	M	B2P5	24/06/2017 13:42	1	11.73
14	1	AFEX	20170624	July_2017	B2	P5	G1	M	B2P5	24/06/2017 13:43	2	11.21

Figure 5. soil CO_2 efflux data spreadsheet deposited in EIDC system.

Colum A – Census: number of campaigns carried out in the field.

Colum B – File.Name: Name of our project.

Colum C – Date: Year_month_day manually entred by the user during the measurements.

Colum E – Block: Blocks of the experimental design, which can be B1, B2, B3 or B4, in which the plots are located.

Colum F – Plot: : 50 x 50 m plots, ranging from P1 to P8, and containing all fertilization treatments.

Colum G – Group: Group of collars installed on the ground, containing the different type of collars.

Colum H – Type: Type of collars, can be: T = shallow surface collars, which did not cut any roots, and thus contained all soil respiration flux components; M= deep collars (25 cm depth) with a window in their side which were covered with 41 µm mesh which excluded roots, but permitted the ingrowth of mycorrhizae. This type of collar was measured until June 2018, because roots started to enter to the collar. S=deep collars (25 cm depth) with no windows and exclude both roots and mychorrizae.

Colum I – PlotID: combination of block and plots.

Colum J – Date_IV: date and time given automatically by the equipment.

Colum K – Obs: repetition of measurement on the same collar.

Colum L – Lin_Flux: flow in µmol $m^{-2}s^{-1}$

3. References

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