

1 *Dear editor,*

2  
3 *Please find attached a new version of our manuscript entitled “Automatic high-frequency*  
4 *measurements of full soil greenhouse gas fluxes in a tropical forest” for consideration as*  
5 *publication in Biogeosciences,*

6  
7 *We have revised the manuscript based on the feedback of the five reviewers and you can find below*  
8 *a rebuttal letters where we give our detailed responses to each point raised by the reviewers.*

9  
10 *You can also find below the a marked-up manuscript version.*

11  
12 *On behalf of the authors,*

13  
14 *Elodie Courtois & Clement Sthal*

15  
16  
17 **Referees' comments:**

18  
19 **RC1, Referee #1 (Remarks to the Author):**

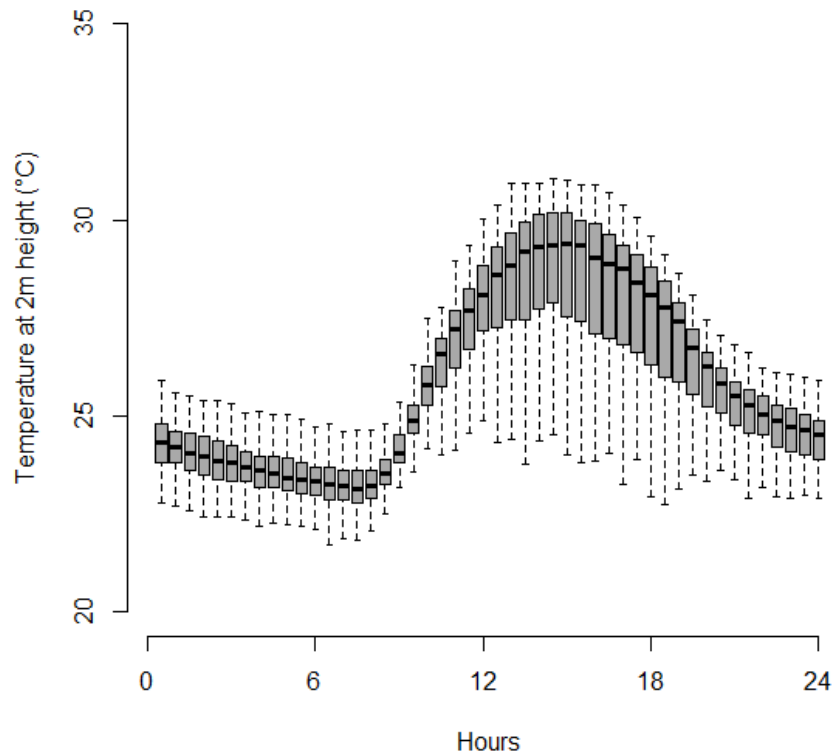
20  
21 This manuscript presents a protocol for measuring three greenhouse gases (CO<sub>2</sub>,CH<sub>4</sub> and N<sub>2</sub>O) at  
22 high temporal frequency in a tropical forest soil using a combination of commercially available  
23 systems. This is a very timely and relevant manuscript, particularly for measurements of CH<sub>4</sub> and  
24 N<sub>2</sub>O. Continuous high frequency measurements with clear sampling protocols will help  
25 researchers capture and model, transient changes in CH<sub>4</sub> and N<sub>2</sub>O fluxes often missed by more  
26 infrequent sampling strategies. This looks like a nice, efficient system for measuring these  
27 important greenhouse gases.

28  
29 *Response: We thank the reviewer for the positive comments and constructive inputs.*

30  
31 The technical write-up is comprehensive and easy to follow however I do have technical questions  
32 regarding their methodology that should be addressed.

33  
34 1. How did the authors keep moisture from affecting sampling, either within the tubing lines or  
35 when moving from IRGA to Picarro? Was there any moisture buildup in the tubing lines?

36  
37 *Response: We also worried about moisture problems prior to our experiment, but such problems*  
38 *never occurred. No significant condensation occurred in our system, we assume because*  
39 *temperature variations were typically small below the canopy, varying from 22°C in the night to*  
40 *28°C during the day (see Figure attached). We regularly checked that no liquid water*  
41 *accumulation occurred in the sampling tubes. Moreover, both analysers measure water vapor and*  
42 *account for its effect on concentration of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O .*



44  
 45 *Figure 1: Daily temperature variation at 2m height under the canopy cover during the study period*  
 46 *(June-September 2016).*

47  
 48  
 49 ***Changes in the manuscript:** Temperature variations are typically small below the canopy due to*  
 50 *the shadowing by dense canopy crown and microclimatic conditions. During the study period,*  
 51 *temperature at 2m height varies during the day from 22 °C in the night to 28 °C during the day.*  
 52 *The presence of water condensation inside the tubing lines was carefully checked every week and*  
 53 *never occurred during the study period.*

54  
 55 2. Were the instruments kept within operating temperature ranges: specifically the upper end of  
 56 operating ranges? Where there diel changes in Licor and Picarro instrument temperatures?

57  
 58 ***Response:** In our study site, all the systems were operating below dense understory vegetation and*  
 59 *canopy cover, which naturally create a buffer maintaining air temperature relatively constant over*  
 60 *the year (i.e. at daily and seasonally time scale). There were no diel changes in Licor and Picarro*  
 61 *inside operating temperature: Picarro temperature was monitored and remained between 44.99*  
 62 *°C and 45.01 °C and Licor temperature at 51.7 °C.*

63  
 64 3. How often were the instruments calibrated?

65  
 66 ***Response:** All the systems used at this time, i.e. from June to September 2016, were new and*  
 67 *received from the manufacturers. We therefore did not re-calibrate them during the study period.*  
 68 *We are confident that the data of the gas concentrations recorded by our analysers were robust.*

69

70 4. The authors subsampled from the flow downstream of the Li-8100A-IRGA into the Picarro  
71 G2308. What was the flow rate through the Picarro analyzer- was it 2.8L/min or some lowerrate?  
72 I don't see the flow controller in Figure 1a diagram- is it built into the external pump?  
73

74 *Response: The flow rate of 2.8L/min corresponds to the flow between the chambers and the*  
75 *multiplexer which cannot be adjusted. This high rate inside the chamber allows to achieve a*  
76 *sufficient air mixing in the chamber headspace during the measurements. Flow rates in the*  
77 *subsampling lines (Li8100 and Picarro) were lower and set between 1.5 and 1.7L/min as*  
78 *recommended by the manufacturers.*

79  
80 *Changes in the manuscript: Flow rates in the subsampling lines (Li8100 and Picarro) were lower*  
81 *and set between 1.5 and 1.7 L min<sup>-1</sup> as recommended by the companies.*  
82  
83

84 5. What is the sampling volume inside the Picarro G2308? My concern is that if there is  
85 subsampling from a high flow rate at the T piece subsampling loop (Figure 1 a) through a secondary  
86 instrument, and then flow is re-merged downstream of the external pump and returned to the closed  
87 chamber there may be a dilution effect. This might not impact a 2 minute sampling time period  
88 but may have a greater impact on a 25 minute time period.  
89

90 *Response: The volume of the PICARRO analyser together with tubing from the subsampling loop*  
91 *represents 130 cm<sup>3</sup>. It represents only 2% of the total volume (6088 cm<sup>3</sup>) and the dilution effect*  
92 *was therefore limited.*  
93

94 6. Did the authors also use their Picarro G2308 to measure CO<sub>2</sub> as well and if so how does that  
95 compare to the LI-8150 analyzer? This would provide confidence in running the two systems  
96 inline.  
97

98 *Response: One step of our data quality check/ quality control consisted in comparing the soil CO<sub>2</sub>*  
99 *effluxes measured by the PICARRO with the soil CO<sub>2</sub> effluxes measured by the Li-8100A. This*  
100 *method was also used to control that there were no leaks. Overall, both systems agreed very well.*  
101 *However, while the PICARRO G2308 analyser automatically reports dry mol fraction of CH<sub>4</sub> and*  
102 *N<sub>2</sub>O, it only reports CO<sub>2</sub> uncorrected by H<sub>2</sub>O concentration. As the precision of measurement was*  
103 *better for CO<sub>2</sub> using the Li8100 and as it also automatically reports dry mol fraction for this gas,*  
104 *we decided to use Picarro estimation for CH<sub>4</sub> and N<sub>2</sub>O and Li8100 estimation for CO<sub>2</sub>.*  
105

106 Specific questions:

107 Pg 2 line 26: are the reference [17,18] in the correct format? This is the only location that lists  
108 reference numbers as opposed to first author.  
109

110 *Response: This has been corrected*  
111

112 Pg 3 line 23: the authors used an external pump, however the LI-8150 has an internal diaphragm  
113 pump- was this turned off or was it used in line with the external pump?  
114

115 *Response: The system was always operating with the two pumps, the internal diaphragm pump of*  
116 *the Li-8150 and the external pump of the PICARRO, turned on, which limited the risk of water*  
117 *condensation inside the tubing line.*

118

119 Pg 3 line 28: the CRDS is not the “only” method that can detect low concentrations of N<sub>2</sub>O. Can  
120 you change to “one of the only” as opposed to “only”.

121

122 *Response: This has been corrected.*

123

124 Pg 4 line 14: the flow rate of 2.8L/min is very high. Was this flow rate tested to determine if  
125 pressure within the closed chambers was altered? I assume that the Licor 8100 chamber tops had  
126 their patented pressure relief valve installed?

127

128 *Response: The flow rate of 2.8L/min corresponds to the flow between the chambers and the*  
129 *multiplexer which cannot be adjusted. This high rate inside the chamber allows to achieve a*  
130 *sufficient air mixing in the chamber headspace during the measurements. Flow rates in the*  
131 *subsampling lines (Li8100 and Picarro) were lower and set between 1.5 and 1.7L/min as*  
132 *recommended by the manufacturers.*

133

134 Figure1b) are all the tubing lines from the 16 chambers to the multiplexer 15m (as in the text)in  
135 length? A small point but they look like different lengths in the diagram.

136

137 *Response: Yes the length of tubing was 15 m for all chambers but as chambers are installed in a*  
138 *grid around the instruments, they are not all 15m away from the instruments. The length of the*  
139 *tubing lines (15 m) is cited in the caption.*

140

141

## 142 **RC2, Referee #2 (Remarks to the Author):**

143

144 The authors measured in situ soil fluxes of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O continuously with a commercially  
145 available automated chamber system coupled with a CRDS analyser in a tropical forest for four  
146 months. The manuscript is focused entirely on the methodological aspect of these measurements,  
147 stressing the importance of adjusting chamber closure times for the different gases for reliable flux  
148 calculations. The effect of closure times on flux calculation results were studied by trying different  
149 chamber closure times in the field and by adjusting the number of data points used for the actual  
150 flux calculation. This is a well-designed study, and overall, the manuscript is well written and  
151 structured. Also choosing appropriate chamber closure times and the operation of automated  
152 chamber systems are important topics for the soil flux community. However, I recommend  
153 publication of this manuscript after major revision because I have some general concerns with this  
154 manuscript.

155

156 *Response: We express our deep thanks to reviewer for his positive comments about our manuscript*  
157 *and constructive remarks. We have addressed, see below, our answers to each comment / remark.*

158

159 1. Soil Flux Pro provides for each chamber measurement linear and non-linear flux calculations.  
160 Why did you choose to use only the linear flux calculation results?

161  
162 *Response: We firstly decided to use linear flux calculations only because we thought that the*  
163 *saturation effects characterised by a plateau after a certain time would be low. However, following*  
164 *your comment and comment from other reviewers, we changed flux calculations in the new version*  
165 *of the manuscript to use exponential estimations.*

166  
167 The underestimation of fluxes with linear regression due to saturation effects is well-known. That's  
168 why numerous non-linear calculation schemes have been developed. Could you have significantly  
169 reduced the chamber closure time for the N<sub>2</sub>O flux calculation if you had used non-linear flux  
170 calculation?

171  
172 *Response: See previous comment.*

173  
174 The selection of the flux calculation scheme can change the MDF at least for chamber  
175 measurements with only few gas samples over time. Does this effect disappear with high-  
176 frequency analysers, i.e. selection of the flux calculation scheme becomes less crucial in that  
177 regard? Would there still be a significant difference between the SHORT and LONG flux  
178 calculation for the different gases when using non-linear flux estimates?

179  
180 *Response: The standard error approach that we used (Nickerson, 2016) is a first order*  
181 *approximation for the MDF from high-frequency measurements and the “true” MDF is a function*  
182 *of the chamber time-series fit type as well (i.e. Linear, exponential, quadratic). Nonetheless, while*  
183 *the use of linear regression resulted in systematically smaller fluxes as compared to exponential*  
184 *regression. It is therefore recommended to initially calculate fluxes with linear regression to*  
185 *determine the threshold for “low” fluxes and to recalculate them using exponential regression*  
186 *(Korkiakoski et al., 2017).*

187  
188 *Korkiakoski, M., Tuovinen, J.-P., Aurela, M., Koskinen, M., Minkinen, K., Ojanen, P., Penttilä,*  
189 *T., Rainne, J., Laurila, T. and Lohila, A.: Methane exchange at the peatland forest floor—automatic*  
190 *chamber system exposes the dynamics of small fluxes, 2017.*

191 *Nickerson, N.: Evaluating gas emission measurements using Minimum Detectable Flux (MDF),*  
192 *Eosense Inc Dartm. N. S. Can., 2016.*

193 2. You write about high-frequency measurements only as sampling measurement plots more  
194 frequently over time. However, you could also address the high-frequency sampling during a  
195 chamber closure since you use high-frequency gas analysers and work with MDF in your data  
196 analysis. There are several automated chamber systems which do not employ high frequency  
197 analysers, but still collect discrete gas samples which have to be analysed with a GC. Especially  
198 for N<sub>2</sub>O it is very interesting to see what fluxes we can capture with CRDS in comparison to GC  
199 analysis.

200  
201 *Response: In a previous study in the same environment (Courtois et al., 2018), we estimated that*  
202 *the minimum detectable fluxes using Gas Chromatography analysis of four discrete gas samples*  
203 *over 30 minutes for N<sub>2</sub>O was ± 8.3 µg N m<sup>-2</sup> h<sup>-1</sup>. MDF estimated in the present study using high*  
204 *frequency measurement was 0.002 nmol m<sup>-2</sup> s<sup>-1</sup> or 0.2 µg N m<sup>-2</sup> h<sup>-1</sup> for N<sub>2</sub>O which is therefore ~*  
205 *40 times lower. We added a sentence in the manuscript to highlight this interesting fact.*

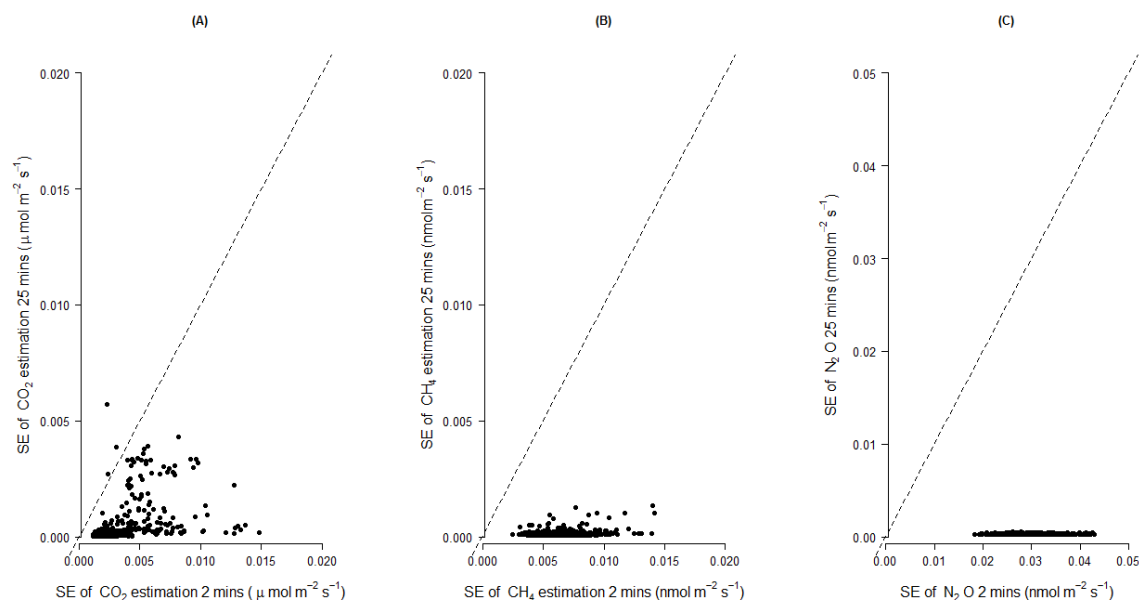
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210

*Courtois, E. A., Stahl, C., Van den Berge, J., Bréchet, L., Van Langenhove, L., Richter, A., Urbina, I., Soong, J. L., Peñuelas, J. and Janssens, I. A.: Spatial Variation of Soil CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O Fluxes Across Topographical Positions in Tropical Forests of the Guiana Shield, Ecosystems, 1–14, 2018.*

211 3. You only write how the SHORT and LONG measurements affected the flux estimates. But how  
212 did they affect the uncertainty of the single flux estimates? How large/small were the error bars  
213 for the flux estimates?

214  
215 **Response:** Comparison of standard error of single flux estimates using 2 minutes or 25 minutes  
216 estimations for two weeks (from August 2nd for August 9th and from August 16th for August 25th)  
217 shows that standard errors are always higher for 2 minutes than for 25 minutes estimation for all  
218 three gases (Figure 2 below). Nonetheless, we decided not to integrate this figure in the manuscript  
219 because it does not add much to the study.

220



221  
222 **Figure 2:** Comparison of standard error of single flux estimates using 2 minutes or 25 minutes  
223 estimations for two weeks (from August 2nd for August 9th and from August 16th for August 25th)

224  
225  
226 4. Could you have just used one, namely the LONG, closure time for all chambers and only choose  
227 for the flux calculation between SHORT and LONG calculation times? This would be more  
228 practical than rotating closure times between chambers.

229  
230 **Response:** Setting all chambers as LONG measurements would have led to a maximum of ~ 3  
231 measurements only per chamber and per day. Mixing LONG and SHORT measurements allows to  
232 maximise the number of measurements per chamber and per days while ensuring a reliable  
233 estimation of the low N<sub>2</sub>O fluxes and to capture transient peaks of CH<sub>4</sub> and N<sub>2</sub>O.

234

235 5. In section 3, the results are clearly presented, but the discussion part is very limited.  
236

237 ***Response:** The main aim of our study was not to identify controls and mechanisms of the soil GHG  
238 fluxes but rather to test novel soil GHG systems for continuous high-frequency measurements. We  
239 think that this manuscript could be used as technical support to set up new soil systems and  
240 contribute to record comparable soil GHG data in other regions around the world. Nonetheless,  
241 the discussion has been revised in the new version of the manuscript to integrate discussion on  
242 spatio-temporal variability of fluxes based on our study.*

243  
244

245 Specific comments:

246 Page 2, line 26: numbers instead of author names for references

247

248 ***Response:** This has been corrected.*

249

250 You are not always consistent in how you write company names (capital versus small letters). Also  
251 often you write 'minute' when you could just use 'min'.

252

253 ***Response:** We corrected this in the new version of the manuscript.*

254

255 Page 3, line 23: What are the pump specifications? Was it the pump supplied by Picarro with the  
256 instrument or did you use another pump?

257

258 ***Response:** We have included more information in the manuscript about the external pump  
259 provided by PICARRO: recirculation pump A0702*

260

261 Page 4, lines 9.: The soil temperature and soil moisture probes, were those the ones which can be  
262 directly attached to the chambers?

263

264 ***Response:** The soil temperature and soil moisture probes were those provided by Li-COR, which  
265 are directly attached to the chambers. The probes measured soil temperature and soil moisture  
266 around the PVC collars. We have added more details in the text.*

267

268 Page 5, line 5.: Did you use the analytical accuracy specified on the technical data sheets of the  
269 analysers or did you perform measurements yourself?

270

271 ***Response:** We used the analytical accuracy specified on the technical data sheets of the analysers.  
272 This is now specified in the new version of the manuscript.*

273

274 Where there significant air pressure and ambient temperature changes at your site over the four  
275 months? If yes, did you test how different temperature and pressure values could change the MDF  
276 estimate? Is it incl. or excl. the deadband?

277

278 ***Response:** Please, see our response to reviewer 1 above (2.). In our study site, because all the  
279 systems were operating under dense understory vegetation and canopy cover, air temperature*

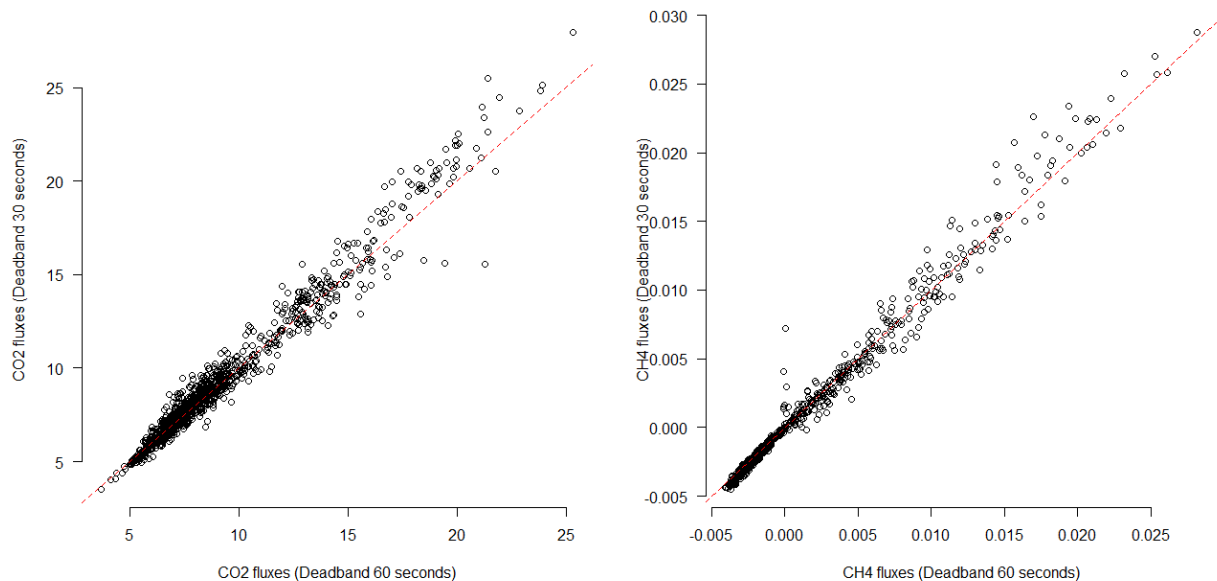
280 *remained relatively constant over the year (i.e. at daily and seasonally time scale) near the soil*  
281 *surface. This is also true for air pressure.*

282  
283 Page 6, line 6: What CO<sub>2</sub> concentrations were reached during LONG closure times (and for CH<sub>4</sub>)?  
284

285 **Response:** *CO<sub>2</sub> concentration can reach 2000 ppm and CH<sub>4</sub> concentration 4 ppm.*

286  
287 Page 6, line 7: I find that confusing in comparison to section 2.5. So considering the deadband, the  
288 chambers were closed for 3 and 26 minutes, respectively?  
289

290 **Response:** *No, the chamber stayed close for 2 minutes and 25 minutes and the first minute was not*  
291 *used for the flux estimation. As we have a sampling frequency of 1 second, it still represents 60*  
292 *points for curve fitting. Nonetheless, we agree that this could be considered as a too short period*  
293 *for CH<sub>4</sub> and CO<sub>2</sub> estimation using the SHORT (2 minutes) closure time. We therefore compared*  
294 *the CO<sub>2</sub> and CH<sub>4</sub> estimations with a deadband of 60 seconds (fluxes estimation with 60 seconds)*  
295 *with a deadband of 30 seconds (fluxes estimation with 90 seconds) for the week from August 16th*  
296 *to August 25th. These two estimations were very well correlated (see figure 3 below) so we decided*  
297 *to keep our 60 s deadband results.*  
298



299  
300 **Figure 3:** *Comparison of CO<sub>2</sub> and CH<sub>4</sub> fluxes with a 30 seconds and a 60 seconds deadband for*  
301 *the week from August 16th to August 25<sup>th</sup>. The red dotted lines represents the 1:1 line.*  
302

303 Page 6, line 20: Why did you consider these fluxes as unreliable when the chamber quality check  
304 using the R<sup>2</sup> for CO<sub>2</sub> was passed? Are you not unnecessarily filtering out fluxes which are not  
305 significantly different from zero, and thus introducing a bias in your data? Because this often  
306 happens when using R<sup>2</sup> as a filter criterion for low fluxes.  
307



308 **Response:** *Because of the high soil respiration activity, low soil CO<sub>2</sub> fluxes do not really occur in*  
309 *this tropical rainforest, not even during the dry season. When the R<sup>2</sup> criterion for CO<sub>2</sub> was not*  
310 *passed, it always corresponded to situations of imperfect closure of the chamber, due to leaves or*  
311 *small branches lying on the soil collars (381 measurements over 17796, i.e. 2.1%). In these cases,*  
312 *it was therefore necessary to remove flux data for the three gases.*

313

314 Page 6, first paragraph of section 3: You had no problems with humidity and the automated  
315 chamber system at your site?

316

317 **Response:** *Please, see our response to reviewer 1 above (1.); at the given flow rate and the small*  
318 *diel cooling, we had no problems with water condensation inside the tubing lines of our system.*

319

320 Page 7, line 3: The conclusion about the 2 min sampling time sounds absolute, but it is only valid  
321 for your small chambers. Except for the necessary descriptions in the method section, you  
322 completely disregard the role of chamber volume for choosing the right chamber closure time.

323

324 **Response:** *We added a sentence to precise that our result is valid for small chambers only.*

325

326 Page 7, lines 20/21: That sentence does not make any sense to me. 85.6 % of the fluxes were above  
327 or below?

328

329 **Response:** *This sentence has been considerably modified.*

330

331 Page 8, line 4: You didn't show the diurnal variation in your data. This is more a point for the  
332 discussion than a conclusion from your presented data.

333

334 **Response:** *We agree with the reviewer; however, ecological interpretation of our data will require*  
335 *more long-term data and will be published in a future paper. Here, we wanted to provide a*  
336 *technical report with information on how to get robust results on GHG flux estimations rather*  
337 *than on how these fluxes are produced and vary.*

338

339 The references are not well formatted.

340

341 **Response:** *References were reformatted*

342

343 Table 1: Use superscript for the units.

344

345 **Response:** *This has been corrected*

346

347 Table 2: Include n for each chamber.

348

349 **Response:** *This information has been added.*

350

351 Check how the units are written on the y-axis of the figures.

352

353 **Response:** *This has been checked..*

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**RC3, Referee #3 (Remarks to the Author):**

In this manuscript, the authors detail a field-deployed and field-tested system for measuring soil greenhouse gas (GHG) emissions (CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O) from a tropical wet forest; the system leverages a commercially-available automated flux chamber system with a commercially-available CRDS analyzer. More specifically, the authors (a) outline the technical protocol for implementing such a system, (b) report the mean fluxes and variability of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O observed over the four-month deployment period and (c) test two chamber closure lengths to determine the most effective experimental design for capturing fluxes above minimum detectable levels. Successfully implementing such a system in the tropics is both difficult and has only been done rarely, so a technical note detailing how to do so is absolutely a contribution to the literature. I have two general/more broad comments about the manuscript, which I detail below, and also include several more specific comments at the end of this review

*Response: We thank the reviewer for the positive comments and constructive inputs.*

1. Concerns regarding flux calculation protocols

In these automated, high-frequency GHG systems, one important set of experimental design and protocol decisions govern how to calculate flux rates and screen for acceptable data points. The authors lay out fairly transparent information about how they calculated their flux rates for each gas, but I wonder if more discussion of the implications of their calculation choices is warranted. I have a few specific questions. Am I correct in understanding that the authors only calculated flux rates for all three gases based on a linear model (Pg 4, Ln 27)? If so, I wonder why they didn't consider also fitting exponential models to the CH<sub>4</sub> and N<sub>2</sub>O fluxes, if not the CO<sub>2</sub> fluxes.

*Response: We first decided to use linear flux calculations only because we thought that the saturation effects characterised by a plateau after a certain time would be low. However, following your comment and comment from other reviewers, we changed flux calculations in the new version of the manuscript to use exponential estimations.*

The authors themselves note on Pg 5, Ln 17 that using certain chamber closure times (which, of course, this paper is very interested in) in combination with a linear flux fit can lead to flux underestimation. Couldn't the "optimal" chamber closure time that the authors attempt to find also include some experimental designs with different closure lengths but non-linear flux fits?

*Response: The standard error approach that we used (Nickerson, 2016) is a first order approximation for the MDF from high-frequency measurements and the "true" MDF is a function of the chamber timeseries fit type as well (i.e. Linear, exponential, quadratic). Nonetheless, while the use of linear regression resulted in systematically smaller fluxes as compared to exponential regression. It is therefore recommended to initially calculate fluxes with linear regression to determine the threshold for "low" fluxes and to recalculate them using exponential regression (Korkiakoski et al., 2017).*

399 *Korkiakoski, M., Tuovinen, J.-P., Aurela, M., Koskinen, M., Minkkinen, K., Ojanen, P., Penttilä,*  
400 *T., Rainne, J., Laurila, T. and Lohila, A.: Methane exchange at the peatland forest floor—automatic*  
401 *chamber system exposes the dynamics of small fluxes, 2017.*

402  
403 *Nickerson, N.: Evaluating gas emission measurements using Minimum Detectable Flux (MDF),*  
404 *Eosense Inc Dartm. N. S. Can., 2016.*

405  
406 Additionally, the Picarro G2308 records numerous diagnostic variables alongside GHG  
407 concentrations, including measures of moisture, temperature, pressure, etc. From methods section  
408 2.6, I am under the impression that fluxes were only struck from the dataset if they were (a) below  
409 the MDF, (b) had an  $R^2$  for  $\text{CO}_2 < 0.9$ , or (c) only struck  $\text{N}_2\text{O}$  data if the SHORT  $R^2 < 0.8$ . First,  
410 the authors might consider including a supplemental figure that justifies their decision not to have  
411 data quality rules around  $\text{CH}_4$ , as they do for  $\text{N}_2\text{O}$ .

412  
413 *Response: We decided to use the same quality check for  $\text{CH}_4$  than for  $\text{N}_2\text{O}$  and to only struck  $\text{CH}_4$*   
414 *data if the SHORT  $R^2 < 0.8$ . As you can see in the  $\text{CH}_4$  figure, the main emission or consumption*  
415 *peak during the 2 minutes measurements are still present.*

416  
417 Second, like the other reviewers, I was curious as to how humidity was dealt with, since it appears  
418 that the moisture-related diagnostics weren't used to evaluate data quality. Was there a water trap  
419 that isn't marked in the instrument set-up diagram?

420  
421 *Response: We regularly checked that no liquid water accumulation occurred in the sampling*  
422 *tubes. Moreover, both analyzers measure also water vapor and its effect on concentration of  $\text{CO}_2$ ,*  
423  *$\text{CH}_4$  and  $\text{N}_2\text{O}$  was accounted for. We also monitored Licor and Picarro inside operating*  
424 *temperature: Picarro temperature remained between 44.99 and 45.01 C and Licor temperature at*  
425 *51.7 C.*

426  
427 More broadly, as this paper aims to outline best practices for setting up this kind of experiment in  
428 the tropics, fleshing out the data management aspect of things would improve the paper, in my  
429 opinion.

430  
431 *Response: We think that we already included many aspect of quality check and data management*  
432 *and more information can be found in the new version of the manuscript. All the subsequent figure*  
433 *and analysis were done using R software and we would be happy to share the codes upon request.*

434  
435 2. Technical note vs. data exploration paper  
436 The aspects of this paper that serve as a technical note are novel and helpful. That said, the results  
437 and discussion section, in which the observed GHG fluxes are analyzed, is perfunctory and is  
438 relatively focused on a methodological question: what chamber closure time should be used in this  
439 system, and how can others determine what chamber closure time to use in their analogous system?  
440 I found myself wishing that there was a more robust analysis of the GHG data itself and the  
441 ecological implications of their various findings.  
442 See also my comment below about Table 2.

443  
444 *Response: See RC2 (5)*

445

446 Specific comments:

447 \* Pg 7, Ln 30: The authors ultimately recommend a sampling protocol that rotates between short  
448 and long closure times. What is preventing them from recommending always doing LONG  
449 chamber closures and only using the first two minutes of chamber closure time to calculate the  
450 CO<sub>2</sub> flux, thus decreasing the amount of human labor needed to swap out the system program once  
451 a week?

452

453 *Response: Setting all chambers as LONG measurements would have led to a maximum of ~ 3*  
454 *measurements only per chamber and per day. Mixing LONG and SHORT measurements allows to*  
455 *maximise the number of measurements per chamber and per days while ensuring a reliable*  
456 *estimation of the low N<sub>2</sub>O fluxes and to capture transient peaks of CH<sub>4</sub> and N<sub>2</sub>O. Moreover, under*  
457 *tropical conditions, a visit once a week is absolutely necessary to ensure a proper maintenance of*  
458 *the setting (removing fallen leaves, branches, etc...).*

459

460 \* Pg 7, Ln 23 / Figure A2: This figure is used to justify why LONG chamber readings weren't  
461 reliable for N<sub>2</sub>O flux estimation, but these data don't indicate that the variable fluxes are unreliable,  
462 only that they are variable and considerably larger in magnitude than the SHORT N<sub>2</sub>O flux  
463 estimates. Can an additional supplemental figure be added showing the R<sup>2</sup> values for the LONG  
464 vs. SHORT N<sub>2</sub>O fluxes? Or some similar figure that shows why the LONG fluxes are considered  
465 unacceptable?

466

467 *Response: If we understood well your question, you are asking why we considered SHORT (not*  
468 *LONG) N<sub>2</sub>O fluxes as unacceptable. In order to compare the fluxes from SHORT and LONG*  
469 *closure time, we have added a third graph to the Figure 2 (previously supplementary Figure 1). It*  
470 *shows that N<sub>2</sub>O fluxes estimated using the SHORT closure time are not correlated with fluxes*  
471 *estimated with the LONG closure time (R<sup>2</sup> of 0.02) and should not be considered.*

472

473 \* Pg 7, Ln 31: "Our unique system..." and Pg 8, Ln 12: "this is the first time that this experimental  
474 set up is described and tested under tropical field conditions." I believe an analogous system was  
475 described in Puerto Rico (O'Connell et al 2018, Nature Communications,  
476 <https://doi.org/10.1038/s41467-018-03352-3>), though not as a technical or methods paper.

477

478 *Response: We realised that, although the suggested reference was in the list of references, it was*  
479 *not mentioned in the text and we now cite this reference in the introduction. In this reference, the*  
480 *authors are measuring CO<sub>2</sub> and CH<sub>4</sub> but not N<sub>2</sub>O fluxes. Moreover, the description of the*  
481 *experimental design of the automated soil fluxes measurement system in this paper is very short*  
482 *and does not give the specification of the multiplexer not the exact model of the CRDS Picarro*  
483 *analyser that they used. We therefore still believe that our study is the first one describing in detail*  
484 *the simultaneous measurement of the three GHGs under tropical field conditions.*

485

486 \* Appendix Figure A1: Might be worth including the N<sub>2</sub>O comparison just as the CO<sub>2</sub> comparison  
487 is included even though the authors discard the LONG CO<sub>2</sub> flux estimates

488 .

489 *Response: In the revised manuscript, this figure is now presented in the main text (Figure 2) and*  
490 *also includes the N<sub>2</sub>O.*

491  
492 \* Table 2: A number of the authors' chambers reported mean N<sub>2</sub>O fluxes below 0. This seems  
493 worth mentioning in the results and/or discussion.

494  
495 *Response: We agree with the reviewer and, as suggested, we have added some comments about*  
496 *the respective parts of the N<sub>2</sub>O and also CH<sub>4</sub> emissions and absorptions. We have estimated that*  
497 *28% of our fluxes indicated a sink for N<sub>2</sub>O and 72% a source for N<sub>2</sub>O and that 59% of our fluxes*  
498 *indicated a sink for CH<sub>4</sub> and 41% a source for CH<sub>4</sub>.*

499  
500 **SC1, Referee #4 (Remarks to the Author):**  
501

502 In the recent years, several studies highlighted the need for continuous measurements of soil GHG  
503 other than CO<sub>2</sub>, which has been technically challenging for long time. However, combination of  
504 different new instrumentation allows addressing this challenge nowadays. I think this manuscript  
505 a timely technical note addressing one of the most important issues regarding continuous  
506 measurements: which is the balance between frequency and reliability of measurements? Despite  
507 some of the points discussed in the paper are instrument specific considerations (Li8100 and  
508 G2308), I think that most of them apply for high-frequency studies using other instrumentation. In  
509 my opinion, two points could be covered more in depth in order to make the manuscript more  
510 strong and inspiring for the community:

511  
512 (i) suitability of linear or exponential fits for estimating GHG fluxes, especially under high  
513 emissions and long chamber closure time

514  
515 *Response: We first decided to use linear flux calculations only because we thought that the*  
516 *saturation effects characterised by a plateau after a certain time would be low. However, following*  
517 *your comment and comment from other reviewers, we changed flux calculations in the new version*  
518 *of the manuscript to use exponential estimations.*

519  
520 (ii) which threshold criteria do we have to apply for low rate fluxes and which are the  
521 consequences of using different criteria on temporal patterns (both short and long term scales)  
522 and on accumulated emission estimates.

523  
524 *Response: Some information regarding this issue were added in the new version of the manuscript.*  
525

526 Finally, I want to recognize the challenge of running this complex instrument setup in a tropical  
527 forest. Dealing with high moisture when using IRGAS and CRDS is not easy, but the authors  
528 succeeded.

529  
530 *Response: Thank you for this positive comment.*  
531

532 I am looking forward to see the data in the full experiment context with their ecological  
533 implications. Here you could find some specific comments, suggestions and open discussion  
534 points:

535 Pg3 L19-28. Li-8100 can detect really small fluxes of CO<sub>2</sub> as well. I guess that the main reason  
536 for using both Li-8100 and Picarro G2308 is that one instrument controls the chambers and the

537 other measures the three gases. Additionally, measuring simultaneously CO<sub>2</sub> with two independent  
538 systems is a good control to validate the proper performance of the instruments. I wonder which  
539 was the agreement in CO<sub>2</sub> between Li8100 and G2308.

540  
541 *Response: In our system, the automated chambers were controlled by the Li-8150, which was*  
542 *controlled by the Li-8100A. The gas analysers were Li-8100A for the CO<sub>2</sub> and PICARRO G2308*  
543 *for the CH<sub>4</sub> and N<sub>2</sub>O. We recommend using the Li-8100A to determine the soil CO<sub>2</sub> effluxes but*  
544 *recognise that CO<sub>2</sub> information provided by the PICARRO can still be used to check for potential*  
545 *leaks in the analysers / tubing.*

546  
547 P4 L24-28. I don't know if I understand this statement, but SoilFluxPro (Li-COR software) allows  
548 to directly upload hundreds of Picarro files simultaneously (up to 2 months). You can choose to  
549 open all the files in one single file, and directly merge it with the Li-COR data.

550  
551 *Response: When we first used this import function in SoilFluxPro, we realised that there were*  
552 *problems when importing Picarro files (that are split in one file per hour) by suing the function*  
553 *IMPORT. When a measurement was overlapping two distinct hours (for example a flux estimation*  
554 *from 8:50 am to 9:15 am, the function RECOMPUTE in SoilFluxPro only take into account the*  
555 *first Picarro file. We contacted Licor for this issue and they agree that this was a weakness of the*  
556 *software. They are currently working on it to implement this in a new version of the software. In*  
557 *the meantime, the use of the R function that we developed and that can be found in the*  
558 *supplementary material to merge all Picarro files in one file allows to overcome this issue.*

559  
560 P4 L28-30. One of the best things of using SoilFluxPro is that calculates the fluxes using both  
561 linear and exponential fits, which could result in substantial differences in fluxes (see the attached  
562 example from my own data). My experience is that exponential equations usually fits better than  
563 linear ones (in terms of R<sup>2</sup>), especially for high flux rates under long chamber closure times.

564  
565 *Response: See responses to previous comments, exponential fits were now used for all flux*  
566 *computations.*

567  
568 P4 L29. Which was the actual length of each measurement without including the deadband?

569  
570 *Response: See comment from RC2*

571  
572 P5 L5. Why are you not using CO<sub>2</sub> measured with Picarro?

573  
574 *Response: Please, see our response to reviewer 1 above (6.).*

575  
576 P5 L7-8. I guess that it has to be the volume of the system (chamber, Li8100, Picarro, multiplexer  
577 and tubing). This is really important since the volume of the system is a parameter controlling the  
578 minimum detectable flux, so Table 1 might substantially change depending on this "detail".

579  
580 *Response: Yes, it is the volume of the whole system.*

581  
582 P5 L16-17. Again, this can be solved using exponential fits.

583  
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*Response: See responses below*

P6 L7, L13 and L17. I guess these are not the correct figures.

*Response: This has been corrected*

P6 L14-20. As far as I understood, you kept values higher than MDF (for emissions) and lower than  $-MDF$  (for sinks), but what happen with values close in between ( $-MDF < x < MDF$ )? What did you do with values close to 0 flux? And what happen if a flux was higher than MDF but had low  $R^2$ ? The same applies for  $N_2O$ . Which criterion we have to use when measuring gas emissions at low rates? Is it a 0 flux, NA, should we keep the calculated flux regardless of the  $R^2$ ? Choosing one or other criterion might have several implications in order to estimate cumulative or mean fluxes, especially if the data does not have normal distribution and it's not 0 centered. In L11 you describe an  $R^2$  criterion for considering stable micrometeorological and chamber conditions based on  $CO_2$ . Then, why we should apply other criterion for the other gases if the conditions are stable? I understand that for this might not be super relevant for a technical note, but this is a key question if you want to quantify emissions in natural conditions. In my opinion, this is the core of the study, and one of the most challenging issues we need to address when measuring  $CH_4$ ,  $N_2O$  and other trace gases. When we have high fluxes, everything is clear, but when we have low fluxes, it turns more complicated. We were discussing this issue in Petrakis et al. 2017, but I still don't have the answers. I guess there is not a silver bullet.

*Response: We agree with the reviewer that this is one of the main challenge of  $CH_4$  and  $N_2O$  soil fluxes estimation. For  $CO_2$ , fluxes from tropical soils are always high so the  $R^2$  criterion allows to easily detect measurement issues such as imperfect chamber closure. In this case, it is logical to remove fluxes estimation for the three gases. For  $CH_4$  and  $N_2O$ , we decided (1) to consider fluxes below MDF as null fluxes (i.e. fluxes so small that they are below detection limit) (2) to consider fluxes above MDF but with a low  $R^2$  were considered as NA (Not available, fluxes estimation impeded by unknown measurement issues).*

Figure 1A. There is something in this panel it's not completely clear. As far as I understand, the air goes from the chamber to the multiplexer, to Li8100, to G2308, to the external pump, to the multiplexer and again into the chamber. However, in the schematic view there is a black circuit (T piece sub-sampling loop) that connects the multiplexer, Li8100, G2308 and the external pump. Since the air composition does not change between these four elements, why the subsampling tub was not inserted in serial at one point of the circuit?

*Response: Inserting the subsampling loop in parallel rather in in serial was a proposition from the manufacturer that we followed here.*

Table 2. I wonder which closure time did you use in this table (2 or 25min). It would be interesting a comparison between 2 and 25 closure times. I'm not sure you will find differences in the means. This would suggest that short closure times might not affect the annual balance but deviation of the data (as we can see in Ap Figure 2).

629 *Response: In this table, we used all fluxes estimation available after quality checking. Your*  
630 *proposition is interesting but as 2 and 25 minutes estimation were not made on the same weeks, it*  
631 *is difficult to compare them. We therefore propose to keep this table as it is but we stated in the*  
632 *table caption that this estimation was made using all the data available and we also added the*  
633 *number of data points that were used for this estimation.*

634  
635 Appendix Figure A1. In my opinion, this is one of the most interesting figures in the manuscript  
636 and I think it should be place in the main manuscript.

637  
638 *Response: The figure was placed in the main text*

639 Some suggestions:

641 a) Regressions will have better fit if you use exponential equations for estimating the flux. For  
642 each flux you can choose linear or exponential depending on the  $R^2$ .

643  
644 *Response: Exponential fits were now used for all fluxes estimation.*

645  
646 b) Could you display  $R^2$  and the coefficients of the regressions between 2 and 25min? Regression  
647 B shows a good fit, but it seems that 2 min fluxes tends to overestimate fluxes compared to 25min  
648 estimates. Again, this could be an artifact of using linear regressions and not exponential.

649  
650 *Response: This figure has been modified by using exponential fits and the  $R^2$  were added*

651  
652 c) It would be interesting plotting the regression for  $N_2O$  including all values (without removing  
653 data using  $R^2$  or MDF criterions)? This is related to my comment on Table 2.

654 Appendix Figure A2. Please, edit the figure caption. Petrakis, S., Barba, J., Bond-Lamberty, B. and  
655 Vargas, R.: Using greenhouse gas fluxes to define soil functional types, Plant Soil, 1–10, 2017.

656  
657 **SC2, Referee #5 (Remarks to the Author):**

658  
659 This manuscript focused on a very important topic about soil  $CO_2/CH_4/N_2O$  fluxes  
660 in tropical rainforest. The experiment was well designed. Particularly, this may be the  
661 world's first report about in situ and simultaneously measurement of soil  $CO_2/CH_4/N_2O$   
662 fluxes at low latitude (between  $10^\circ N$  and  $10^\circ S$ ). I would like to give the authors my  
663 comments.

664  
665 *Response: Thank you for this positive comment.*

666  
667 1. Important references:

668 To date, through the “Web of Science”, I could not find any publication about continuous  
669 measurement of soil  $CO_2$  efflux ( $R_s$ ) using the automated chambers in the low latitude tropical  
670 forests that between  $10^\circ N$  and  $10^\circ S$ . Though two campaign studies in very humid forests ( $\sim 3500$   
671 mm of annual precipitation) using automated chambers each in northeastern Australia  
672 ( $17^\circ S$ ) (Kiese and ButterbachBahl, 2002) and northeastern Puerto Rico ( $18^\circ N$ ) (Wood et al.,  
673 2013) were conducted only less than 6-month period, they observed similar phenomenon with  $R_s$   
674 was higher during the dry season but lower during the wet season. Kiese and ButterbachBahl



675 (2002) also measured N<sub>2</sub>O flux. Conversely, a 4-year continuous measurement of R<sub>s</sub> in a  
676 seasonal dry (1,250 mm of annual precipitation) tropical forest in western Thailand (14\_N)  
677 showed higher R<sub>s</sub> in wet season than that of dry season (Hanpattanakit et al., 2015).

678

679 *Response: In fact, a very recent paper reported continuous monitoring of R<sub>s</sub> during three years*  
680 *in the tropical forest of Panama (Rubio and Detto, 2017). Moreover, a previous study conducted*  
681 *at the same site as ours (Paracou site, near the Guyaflux tower) also reported 577 days of R<sub>s</sub>*  
682 *measurement (Rowland et al., 2014). Both references highlighted a significant effect of soil*  
683 *moisture on seasonal and diurnal cycles of R<sub>s</sub>. Together with the two other references from*  
684 *tropical that you cited, there provide evidences that R<sub>s</sub> in tropical forest soils are typically*  
685 *higher in the wet than in the dry season. The other study that you cited (Hanpattanakit et al.,*  
686 *2015) was conducted in a seasonally dry forest which are apparently reacting differently than*  
687 *typical tropical wet forest (precipitations > 2000m/year). Nonetheless, the results that we are*  
688 *presenting in our study were conducted from June to September 2016 which corresponds in our*  
689 *site to the end of the wet season and the onset of the dry season. With these data, we cannot*  
690 *discuss seasonal effects, at least one full year, or more, of measurements would be necessary for*  
691 *this.*

692

693 *Rowland, L., Hill, T. C., Stahl, C., Siebicke, L., Burban, B., Zaragoza-Castells, J., Ponton, S.,*  
694 *Bonal, D., Meir, P. and Williams, M.: Evidence for strong seasonality in the carbon storage and*  
695 *carbon use efficiency of an Amazonian forest, Glob. Change Biol., 20(3), 979–991, 2014.*

696

697 *Rubio, V. E. and Detto, M.: Spatiotemporal variability of soil respiration in a seasonal tropical*  
698 *forest, Ecol. Evol., 7(17), 7104–7116, 2017.*

699

## 700 2. CO<sub>2</sub> flux:

701 Empirically, also see the above references, CO<sub>2</sub> flux is largely controlled by soil moisture (rain  
702 events) at tropical forests. However, based on Fig 3, during 4-month experiment (June-  
703 September 2016), most of the chambers did not show temporal variation in CO<sub>2</sub> flux. Thus, the  
704 authors are suggested to add soil moisture (and temperature) data to Fig 3 and provide some  
705 discussion about the (lack of) relationships between R<sub>s</sub> and soil moisture and temperature.

706

707 *Response: As discussed in above, a four months period is limited to go deep into such*  
708 *relationships, especially in tropical forest where temporal and spatial variability of fluxes are*  
709 *high. You can find below a figure that can now be found in the supplementary material of the*  
710 *manuscript displaying the relationship of the three gases with soil moisture. Nonetheless, going*  
711 *deeper in the discussion of the effect of rain event, soil moisture and the relative importance of*  
712 *spatial, seasonal and diurnal variability of fluxes cannot be done with these dataset that was*  
713 *specifically constructed to demonstrate the feasibility of running the system under tropical*  
714 *conditions.*

715

## 716 3. CH<sub>4</sub> flux:

717 Generally speaking, upland forest soil is a CH<sub>4</sub> sink, even lowland tropical forest soil. Compared  
718 to R<sub>s</sub>, however, CH<sub>4</sub> flux is more complex and generally has large spatial variation, because the  
719 termite activity can emit CH<sub>4</sub> thus offset a partial of the soil CH<sub>4</sub> sink. I am confused with Table  
720 2, because ten of the sixteen chambers showed CH<sub>4</sub> source. Li-Cor soil chamber (8100-104) can

721 be considered to block most activity of the termite, because the chamber base (collar; 7 cm in  
722 height) was inserted 7 cm into the soil and left another 4 cm above the soil; in addition, the  
723 chamber has relative additional big metal base surround the collar. On the other hand, inserted  
724 chamber base (collar) into the tropical (clay) soil can (sometimes) cause waterlogging inside the  
725 Li-Cor soil chamber (8100-104), which might convert the CH<sub>4</sub> sink to CH<sub>4</sub> source. Same with  
726 CO<sub>2</sub> flux, temporal variations in CH<sub>4</sub> fluxes also could not be detected in Fig. 4. Also,  
727 megascopically, the chambers did not show the common pattern of temporal variation in CH<sub>4</sub>  
728 fluxes (Fig 4). Sure, this forest has plentiful precipitation (about 3000 mm) and very low  
729 elevation, both of these abiotic factors may cause the site as CH<sub>4</sub> source. Thus, the authors are  
730 suggested to provide some more discussion about (the lack of) spatio-temporal variation in CH<sub>4</sub>  
731 flux.

732  
733 *Response: Again here, this result can be easily explained by the time frame of the study. Tropical*  
734 *soils are generally considered as sink at a yearly basis but much study show that there are*  
735 *seasonal variation in CH<sub>4</sub> fluxes and that tropical soils tend to shift from a sink in the dry season*  
736 *to a sources during the wet season. Here, a four months period is limited to go deep into such*  
737 *relationships, especially in tropical forest where temporal and spatial variability of fluxes are*  
738 *high. You can find below a figure that can now be found in the supplementary material of the*  
739 *manuscript displaying the relationship of the three gases with soil moisture. Nonetheless, going*  
740 *deeper in the discussion of the effect of rain event, soil moisture and the relative importance of*  
741 *spatial, seasonal and diurnal variability of fluxes cannot be done with these dataset that was*  
742 *specifically constructed to demonstrate the feasibility of running the system under tropical*  
743 *conditions.*

744  
745 4. Appendix Figure A1:

746 This figure shows a very general (basic) chamber-problem for measurement of soil GHGs fluxes.  
747 Long closure time will cause higher GHGs concentration (if the soil is GHGs source) or lower  
748 GHGs concentration (if the soil is GHGs sink) inside the chamber, which will induce  
749 underestimation of GHGs flux (saturation effect). Saturation effect is generally positively  
750 associated with both flux rate and ratio of the effective chamber volume to the measured soil  
751 surface area. Empirically, I believe the 2-minute  
752 closure time is enough for measurement of both CO<sub>2</sub> and CH<sub>4</sub> flux in tropical forests, even for  
753 most temperate and boreal forests. For Li-Cor soil chamber (8100-104), the ratio is  $(0.0040761 /$   
754  $0.03178 = 0.12826 \text{ m}) = 12.3 \text{ cm}$ . However, for many of the custommade soil chambers, the ratio  
755 is generally higher than 12.3 cm, thus this is might be the specific problem (issue) only for Li-  
756 Cor soil chamber (8100-104). I suggest the authors feedback this problem to Li-Cor and suggest  
757 Li-Cor to draw this problem to their instrument user manual.

758  
759 *Response: Thank you for this feedback. Following comments from the other reviewers, we used*  
760 *exponential fit for estimating all fluxes which improved this saturation issue. Also, as stated in*  
761 *the manuscript, we always used 2 minutes estimation for CO<sub>2</sub> fluxes to overcome this issue.*

762  
763 5. Also for Appendix Figure A1:

764 The authors are suggested to re-draw the Appendix Figure A1 indicating different symbols  
765 (or color) for each of the four chambers.

766

767 **Response:** Following comments from the other reviewers, this figure has been moved to the main  
768 text and now also include N<sub>2</sub>O. We decided to use different colours (black and grey) for the two  
769 distinct weeks that were used for this comparison instead that different colours for the different  
770 chambers because it allows a better view of the fact that these two weeks are covering almost the  
771 whole range of fluxes that can be encountered in the site.

772

773 6. Closure time:

774 When compared Table 1 with Table 2, the closure time of 10 minutes for measurement of N<sub>2</sub>O  
775 flux was enough. Thus, the Table 1 is suggested to be deleted.

776

777 **Response:** We disagree with this comment. A closure time of 10 minutes would have led to a  
778 MDF of 0.009 instead of 0.002. In this case, only 82% instead of 96% of the fluxes would have  
779 been considered of reliable. We therefore decided to maintain Table 1 in the manuscript as it  
780 allows to show that a MDF of 0.002 can only be achieved with a 25 minutes closure time.

781

782 7. Additional suggestion 1:

783 To prove the data quality or measurement precision, the authors are suggested to  
784 add a plot showing changes in CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O concentrations in the chambers.

785 Following is a sample plot (Sample Fig).

786

787 **Response:** We think that this information is not adding much to the comprehension of the study  
788 and it was not included in the new version of the manuscript.

789

790 8. Additional suggestion 2:

791 As I mentioned in the above, this may be the world's first report about in situ and simultaneously  
792 measurement of soil CO<sub>2</sub>/CH<sub>4</sub>/N<sub>2</sub>O fluxes at low latitude (between 10°N and 10°S). I believe  
793 this paper will be a potential high citation rate if the authors can give some more discussion  
794 about spatio-temporal variation in CO<sub>2</sub>/CH<sub>4</sub>/N<sub>2</sub>O fluxes and their control factors. For example,  
795 the coefficient of variation (CV) was used to represent the spatial variation. CV of R<sub>s</sub> can be  
796 calculated by  $CV = (SD / (\text{mean } R_s)) \cdot 100$ .

797

798 **Response:** Mean and SD per chambers are available in Table 2 and we added a figure with mean  
799 value of each chamber per days for the three gases allowing to visualize the spatio-temporal  
800 variability of fluxes.

801

802 **Automatic high-frequency measurements of full soil greenhouse gas**  
803 **fluxes in a tropical forest**

804  
805 **Courtois Elodie A.** <sup>1,2,\*,#</sup>, **Stahl Clément** <sup>3,#</sup>, **Burban Benoit** <sup>3</sup>, **Van den Berge Joke** <sup>1</sup>, **Berveiller**  
806 **Daniel** <sup>4</sup>, **Bréchet Laëtitia** <sup>1,3</sup>, **Soong Jennifer L.** <sup>1,5</sup>, **Arriga Nicola** <sup>1</sup>, **Peñuelas Josep** <sup>6,7</sup>,  
807 **Janssens Ivan A.** <sup>1</sup>

808  
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820

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822 # the two first authors contributed equally to the manuscript.

823

824 **Abstract.** Measuring *in situ* soil fluxes of carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide  
825 (N<sub>2</sub>O) continuously at high frequency requires appropriate technology. We tested the combination  
826 of a commercial automated soil CO<sub>2</sub> flux chamber system (LI-8100A) with a CH<sub>4</sub> and N<sub>2</sub>O  
827 analyzer (Picarro G2308) in a tropical rainforest for 4 months. A chamber closure time of 2 minutes  
828 was sufficient for a reliable estimation of CO<sub>2</sub> and CH<sub>4</sub> fluxes (100% and 98.5% of fluxes were  
829 above Minimum Detectable Flux – MDF, respectively). This closure time was generally not  
830 suitable for a reliable estimation of the low N<sub>2</sub>O fluxes in this ecosystem but was sufficient for  
831 detecting rare major peak events. A closure time of 25 minutes was more appropriate for reliable  
832 estimation of most N<sub>2</sub>O fluxes (85.6% of measured fluxes are above  $\text{MDF} \pm 0.002 \text{ nmol m}^{-2} \text{ s}^{-1}$ ).  
833 Our study highlights the importance of adjusted closure time for each gas.

834

835

836 **Keywords:** Soil fluxes, French Guiana, methods, optimizing, Minimum Detectable Fluxes

837

838

839 **1. Introduction**

840 Carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) are the three main  
841 greenhouse gases (GHGs) in terms of radiative forcing. Increases in these GHG concentrations in  
842 the atmosphere is driving anthropogenic global warming. Understanding the magnitude of GHG  
843 fluxes in natural ecosystems has recently become a priority in the study of GHG balances (Merbold  
844 et al., 2015). Tropical intact forests cover 1392 Mha globally and represent about 70% of the total  
845 tropical forest area (1949 Mha), which accounts for the largest area of global forest biomes  
846 (~50%). Very few reliable long term datasets on full GHG balances are available from tropical  
847 ecosystems, despite their known importance for the global cycles of these three GHGs (Dutaur and  
848 Verchot, 2007). This is in part due to the challenges of designing and operating continuous, multi-  
849 gas flux analysis systems in tropical forests. Soil processes in particular are responsible for an  
850 important part of GHGs that are produced or consumed in tropical ecosystems (Oertel et al., 2016).  
851 Soil physical, chemical, and biological characteristics are linked to variation in GHG emissions  
852 from soils, which in turn can display very high spatial and temporal variability (Arias-Navarro et  
853 al., 2017; Silver et al., 1999).

854 Historically, soil GHG fluxes (emission or consumption) have been measured using the  
855 static chamber method. This involves closing chambers manually for a known period of time,  
856 usually 30-60 minutes, and repeated collection of air samples for further analysis via gas  
857 chromatography (Verchot et al., 1999, 2000). Fluxes are then computed from the increase (or  
858 decrease) in gas concentration per unit time, per surface area enclosed by the chamber, and  
859 corrected by the volume of the chamber. While these labor-intensive and time-consuming manual  
860 measurements are well adapted to capture high spatial flux variability (Arias-Navarro et al., 2017;  
861 Pumpanen et al., 2004), they do not capture high temporal variation, which is necessary for the

862 accurate estimation of annual GHG budgets. Moreover, short term, transient spikes in the emission  
863 or consumption of these GHGs likely remains undetected with static chamber methods, imposing  
864 a lost opportunity to fully understand the production or consumption processes of GHGs and their  
865 response to rapidly changing environmental conditions. One of the key challenges of contemporary  
866 GHG flux research is to close these knowledge gaps in order to improve the quantitative prediction  
867 of GHG fluxes (Merbold et al., 2015).

868 The use of automatic chambers is one approach to obtain continuous estimation of soil  
869 GHG flux data at high temporal frequency (several measurements per days) at various sampling  
870 points. Since the 1970s (Denmead, 1979), a variety of technical solutions for automated flux  
871 sampling have been developed ([Ambus et al., 2010](#); [Breuer et al., 2000](#); [Görres et al., 2016](#);  
872 [Kostyanovsky et al., 2018](#); [O'Connell et al., 2018](#); [Petrakis et al., 2017a](#); [Savage et al.,](#)  
873 [2014](#))(~~[Ambus et al., 2010](#); [Breuer et al., 2000](#); [Görres et al., 2016](#); [Kostyanovsky et al., 2018](#);~~  
874 ~~[Petrakis et al., 2017a](#); [Savage et al., 2014](#)~~), particularly for soil CO<sub>2</sub> fluxes. However, accurate  
875 detection of CH<sub>4</sub> and N<sub>2</sub>O fluxes from soils via flow through systems is more difficult than CO<sub>2</sub>  
876 due to significantly lower background concentrations and lower flux rates (Kostyanovsky et al.,  
877 2018). The budgetary requirements for large infrastructure and intensive maintenance as compared  
878 to manual chamber measurements have prevented the widespread application of automated  
879 systems. The use of automated and continuous methods to estimate full GHG budgets *in situ*  
880 remains scarce, especially in complex biomes with extreme climate such as tropical forests.  
881 Therefore, only a few studies actually address the difficulties and challenges associated with  
882 operating these systems under field conditions (Görres et al., 2016; Koskinen et al., 2014).

883 Recent technological advances have now made more automated chamber systems  
884 commercially available, and an increasing number of custom-made systems are being designed

885 and deployed for soil GHG flux measurements [\(De Klein and Harvey, 2012\)](#)~~[17,18]~~. Here, we  
886 present a detailed field deployment of a custom built, automated soil GHG flux system – the LI-  
887 8100A Soil CO<sub>2</sub> Flux System (LI-COR Biosciences Inc., Lincoln, NE, USA) running in line with  
888 a Picarro G2308 (Picarro Inc., Santa Clara, CA, USA).—Using a 4 month dataset of continuous  
889 measurements of CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O fluxes simultaneously under tropical forest conditions, we  
890 present an optimized sampling protocol for the estimation of the full GHG budget in this  
891 ecosystem.

892

## 893 **2. Methods**

### 894 **2.1. Measurement site**

895 This study was conducted in the Paracou research station (5°15'N, 52°55'W), located in the coastal  
896 area of French Guiana, South America. The automated soil GHG flux system was deployed in the  
897 footprint of the Guyaflux site, which holds a 55 m-tall tower upon which canopy CO<sub>2</sub>, H<sub>2</sub>O and  
898 energy fluxes have been monitored since 2004 using the eddy covariance technique (Aguilos et  
899 al., 2018; Bonal et al., 2008). The site is covered with tropical pristine forest and located in the  
900 northernmost part of the Guiana shield. It is characterized by a succession of small, elliptical hills  
901 rising to 10–40 m a.s.l., sometimes associated with plateaus of similar altitude.

902 The soils are mostly nutrient-poor acrisols (FAO-ISRICISSS, 1998) with pockets of sandy  
903 ultisols developed over a Precambrian metamorphic formation called the ‘Bonidoro series’, and  
904 composed of schist and sandstone, sporadically traversed by veins of pegmatite, aplite and quartz  
905 (Bonal et al., 2008). The forest around the tower is characteristic of a tropical pristine forest with  
906 both high tree density (~ 620 trees with a dbh>10 cm ha<sup>-1</sup>) and species richness (~ 140 species  
907 ha<sup>-1</sup>). The climate is highly seasonal due to the north/south movement of the Inter-Tropical

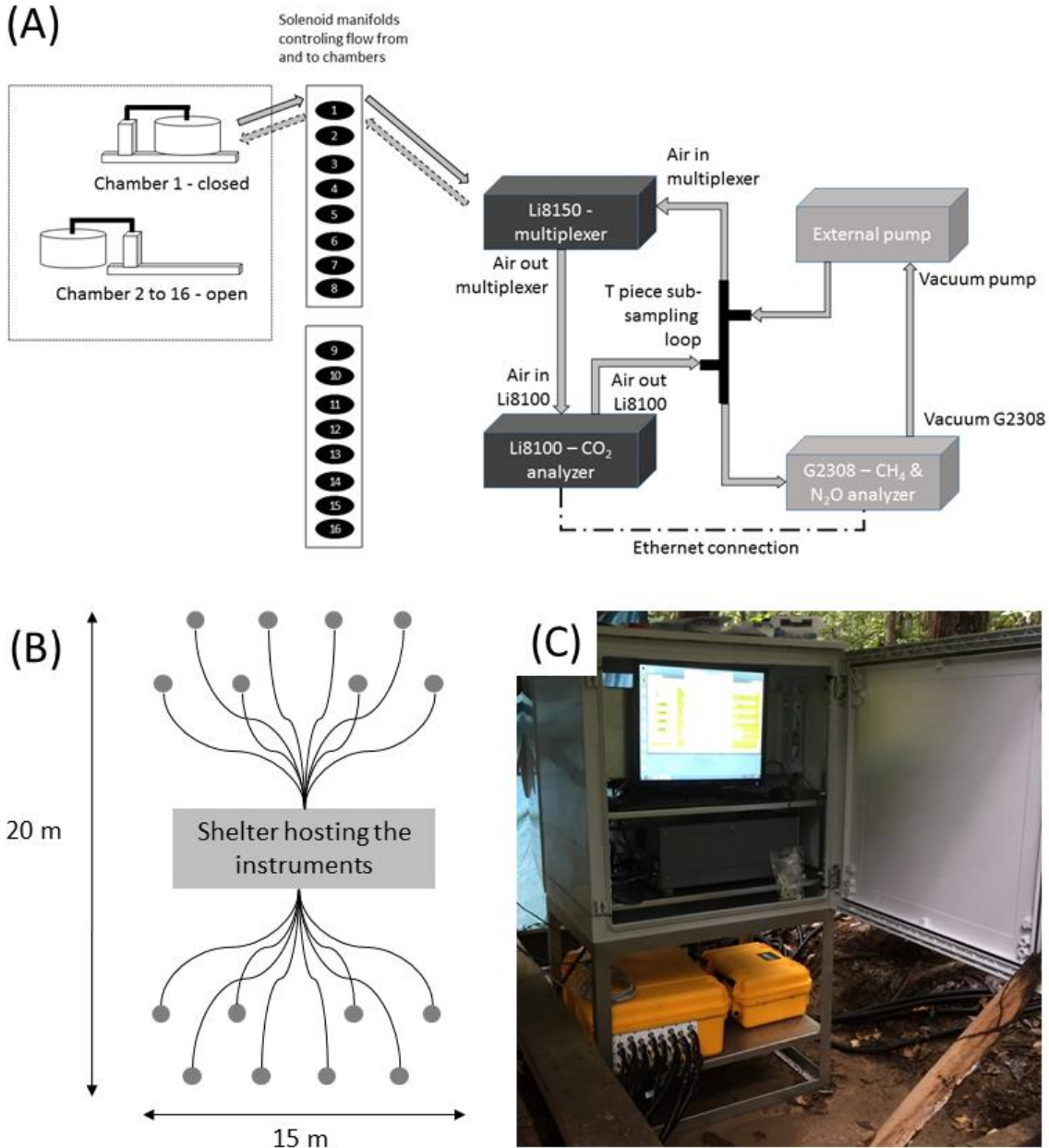


908 Convergence Zone. The wet season, characterized by heavy rain events, lasts for 8 months  
909 (December–July) and alternates with a 4 month dry period (August–November) during which  
910 precipitation is typically lower than 100 mm per month. For the period 2004-2015, annual rainfall  
911 quantities were on average 3103 mm year<sup>-1</sup>, relative extractable water (an index of soil water  
912 availability (Wagner et al., 2011)) varied from 0.93 in the wet season to 0.46 in the dry season and  
913 soil temperature was on average 25.1 with little seasonal nor diurnal variation (Aguilos et al.,  
914 2018).

## 915 **2.2. Automated sampling system**

916 A schematic view of the automatic sampling system is shown in Figure 1(A). The system consisted  
917 of four main components: sixteen automated long-term chambers (8100-104, LI-COR  
918 Biosciences), a multiplexer to link one chamber at a time to the gas analyzers (LI-8150, LI-COR  
919 Biosciences), an infrared gas analyzer (IRGA) to measure CO<sub>2</sub> concentrations (LI-8100A, LI-COR  
920 Biosciences), and a cavity ring down spectroscopy (CRDS) instrument to measure CH<sub>4</sub> and N<sub>2</sub>O  
921 concentrations (G2308, Picarro) that was fitted with an external recirculation pump (A0702,  
922 Picarro). Both the IRGA and CRDS systems were necessary to measure all three GHG  
923 concentrations due to the different abundances and flux rates of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O. The IRGA  
924 methodology is accurate and precise enough to detect small CO<sub>2</sub> concentration changes at high  
925 background concentrations (approximately 400 ppmv; parts per million in volume units).  
926 However, the detection of small changes in CH<sub>4</sub> and N<sub>2</sub>O concentrations, even at their low  
927 background atmospheric concentrations in the order of 2000 ppbv (ppbv=parts per billion in  
928 volume units) and 300 ppbv, respectively, requires higher accuracy and precision levels that can  
929 ~~only~~ be detected with the CRDS.

930



932 **Figure 1: Experimental Design:** (A) Schematic view of the installation composed of four main  
 933 components: sixteen automated long-term chambers (8100-104, LI-COR Biosciences), a  
 934 multiplexer to link one of these chambers to the gas analyzers (LI-8150, LI-COR Biosciences), an  
 935 infrared gas analyzer (IRGA) to measure CO<sub>2</sub> concentrations (LI-8100A, LI-COR Biosciences),  
 936 and a cavity ring down spectroscopy (CRDS) instrument to measure CH<sub>4</sub> and N<sub>2</sub>O concentrations  
 937 (G2308, Picarro) that was fitted with an external pump. (B) Schematic representation of the grid  
 938 with the shelter housing the equipment in the middle and the 16 chambers (grey dots) linked to the  
 939 Li-8150 multiplexer with 15 meters cables (black lines). (C) Picture of the instruments in the field.  
 940

941 Power supply was delivered through a 12 kVa generator (Perkins STORM15) fitted with  
942 batteries located 400 m away from the instruments. Both the CO<sub>2</sub> analyzer control unit and the  
943 multiplexer (LI-COR) had their own weather-proof casing, requiring no additional protection in  
944 the field. Nonetheless, in consideration of the high precipitation at the site, these devices were  
945 placed under a wooden shelter for added protection. The CH<sub>4</sub> and N<sub>2</sub>O analyzer (Picarro), its  
946 external pump and a computer monitor were housed in a waterproof shelter that was specifically  
947 designed to host them (Figure 1(C)). The Li-8100 and the G2308 computers were connected  
948 through ethernet connection to ensure time synchronization. The sixteen automated soil chambers  
949 (8100-104, LI-COR Biosciences) were installed in a grid in the forest (Figure 1(B)) covering in  
950 total an area of approximately 300 m<sup>2</sup> (15 m x 20 m). Each chamber was only closed during  
951 individual chamber measurement periods, and was fully open when not sampling. The PVC collars  
952 that were provided with the 8100-104 automatic chambers were inserted in the soil one month  
953 prior to the first measurement (20.3 cm inner diameter/21.3 cm outer diameter; enclosed soil area  
954 ~ 318 cm<sup>2</sup>; insertion depth ~ 7cm; offset ~ 4cm; green PVC). When the chambers close, they are  
955 automatically lowered so that they cover each soil collar and ensure a fully sealed chamber. The  
956 chamber lid does not directly rest on the collar rim, but on a metal plate surrounding the collar,  
957 leaving the collar undisturbed and minimizing lateral leaks (Hupp et al., 2009).

958 The 16 chambers were connected via 15 m Bev-a-line tubing (8 mm inner diameter) with  
959 the multiplexer (LI-8150), which allows for switching between each of the 16 chambers in any  
960 given sequence. Soil temperature (at a depth of 10 cm) was monitored with 8100-201  $\Omega$  thermistor  
961 probes (Omega Engineering Inc., Stamford, CT, USA), and soil volumetric water content (0-10  
962 cm) was monitored with 8100-202 ECH<sub>2</sub>O Model EC-5 soil moisture sensors (Decagon Devices

963 Inc., Pullman, WA, USA). Soil temperature and soil volumetric water content were directly  
964 connected to the chambers and recorded by the Licor system using the same time step.

965 Each chamber was purged for 15 sec prior to each measurement and 45 sec after each  
966 measurement in order to flush the lines and restore background gas levels in the system. The flow  
967 rate during the purging and the measurements was  $\sim 2.8 \text{ L min}^{-1}$  between the Li-8150 and the  
968 chambers, which ensures sufficient air mixing in the chamber headspace during the measurements  
969 (Görres et al., 2016). Flow rates in the subsampling lines (Li8100 and Picarro) were lower and set  
970 between 1.5 and 1.7 L min<sup>-1</sup> as recommended by the manufacturers. The LI-8100 software  
971 provided the rate of CO<sub>2</sub> concentration increase in the chamber which was used to quantify the  
972 flux of CO<sub>2</sub> from the soil surface into the atmosphere (taking into account the enclosed soil surface  
973 area and the total system volume). A subsampling loop was inserted after the analyzer (LI-8100A)  
974 and before the multiplexer (LI-8150), to pull the air sample through the Picarro G2308 CRDS  
975 analyzer for the determination of CH<sub>4</sub> and N<sub>2</sub>O concentrations and flux estimations, before going  
976 back to the chamber (Figure 1(A)). All three gas concentrations were recorded every second over  
977 the sampling periods.

### 978 **2.3. Flux calculations**

979 All fluxes estimation were done by using commercially available Soil Flux pro software (LI-COR  
980 Biosciences). An R script (Supplementary file 1) was created to merge all the Picarro files from a  
981 given week in order to import them into the Soil flux Pro software. The Picarro creates one file per  
982 hour and when Picarro files are not merged, Soil flux Pro software is not able to deal with  
983 measurements overlapping between two distinct Picarro files (e.g. when a single measurement is  
984 done from 9:50 am to 10:15 am) leading to incorrect estimation of CH<sub>4</sub> and N<sub>2</sub>O fluxes. CO<sub>2</sub>, CH<sub>4</sub>  
985 and N<sub>2</sub>O fluxes were measured as linear-changes-inexponential fit of gas concentration with time

986 using Soil flux Pro software and include a 60 sec dead band to account for soil surface pressure  
 987 disturbances due to the closing of the chamber.

988 **Table 1:** Minimum Detectable Fluxes (MDF) for each gas and for closure times from 2 to 30  
 989 minutes. The two closure times that were used in this study (2 minutes and 25 minutes) are  
 990 highlighted in bold.

Closure time (minutes)	N <sub>2</sub> O (nmol m <sup>-2</sup> s <sup>-1</sup> )	CH <sub>4</sub> (nmol m <sup>-2</sup> s <sup>-1</sup> )	CO <sub>2</sub> (nmol m <sup>-2</sup> s <sup>-1</sup> )
<b>2</b>	0.100	<b>0.040</b>	<b>2.393</b>
5	0.025	0.010	0.605
10	0.009	0.004	0.214
15	0.005	0.002	0.117
20	0.003	0.001	0.076
<b>25</b>	<b>0.002</b>	<b>0.001</b>	0.054
30	0.002	0.001	0.041

991

## 992 2.4. Minimum Detectable Fluxes

993 The minimum detectable flux (MDF) for each gas was estimated by using a metric originally  
 994 developed by Christiansen et al. (2015), which was modified by Nickerson (2016) to make it more  
 995 suitable for high-frequency measurements (Christiansen et al., 2015; Nickerson, 2016):

$$996 \quad MDF = \left( \frac{A_a}{t_c \sqrt{n}} \right) \left( \frac{VP}{SRT} \right)$$

997 Where A<sub>a</sub> is the analytical accuracy of the analyzer (25 ppb for N<sub>2</sub>O and 10 ppb for CH<sub>4</sub> with the  
 998 Picarro G2308 and 600 ppb for CO<sub>2</sub> with the Li8100, recorded from the technical data sheets of  
 999 the analyzers), t<sub>c</sub> is the closure time of the chamber in seconds, n is the number of points that are  
 1000 available to compute the flux (i.e. t<sub>c</sub> divided by the sampling periodicity, every 1 second in this  
 1001 study), V is the chamber volume (0.0040761 m<sup>3</sup>), P is the atmospheric pressure (101325 Pa), S is  
 1002 the chamber surface area (0.03178 m<sup>2</sup>), R is the ideal gas constant (8.314 m<sup>3</sup> Pa K<sup>-1</sup> mol<sup>-1</sup>) and T

1003 is the ambient temperature (298.15 K). We computed the MDF of each gas for closure times from  
1004 2 minutes to 30 minutes in order to select the optimal chamber closure time for each gas in our  
1005 integrated system (Table 1).

## 1006 **2.5. Closure time**

1007 Selecting the best length of time for soil GHG measurements and accurate flux calculation in an  
1008 integrated CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O automated measurement system requires careful consideration. At  
1009 low fluxes, longer measurement periods are needed to reach reliable measurements of real  
1010 concentration changes, while at high fluxes possible storage and saturation effects in the chamber  
1011 headspace might result in non-linear concentration increases and thereby underestimated fluxes.  
1012 In order to maximize the detectable percentage of fluxes for N<sub>2</sub>O and CH<sub>4</sub> without impeding spatial  
1013 coverage and temporal resolution, we built a combined program with two different closure times.  
1014 Each week, four out of sixteen chambers were programmed to stay closed for a longer  
1015 measurement period to ensure a reliable estimation of low fluxes while the other twelve chambers  
1016 were programmed to stay closed for a shorter period to capture diel variation and detect high  
1017 fluxes. For the short closure time (SHORT hereafter), we used a 2 minute measurement period  
1018 because (1) this is a standard closure time for soil CO<sub>2</sub> flux calculations (Janssens et al., 2000),  
1019 even in tropical forests (Epron et al., 2006; Sayer et al., 2007) of this region where MDF for CO<sub>2</sub>  
1020 flux is typically low (Bonal et al., 2008; Bréchet et al., 2009; Courtois et al., 2018), (2)  
1021 corresponding MDFs of CH<sub>4</sub> (0.04 nmol m<sup>-2</sup> s<sup>-1</sup> or ) and N<sub>2</sub>O (0.1 nmol m<sup>-2</sup> s<sup>-1</sup>) are compatible  
1022 with the detection of emission or consumption peaks of these two gases in this region (Courtois et  
1023 al., 2018; Petitjean et al., 2015). For the long closure time (LONG hereafter), we decided to use a  
1024 25 minute measurement period in order to optimize the trade-off between a reliable estimation of

1025 low N<sub>2</sub>O fluxes (Table 1) and a program length that allows for a sufficient number of flux  
1026 measurements per chamber and per day.

1027 We therefore programmed the multiplexer for 2.5-h cycles (9-10 measurements per chamber  
1028 per day), which included four chambers with LONG measurements and twelve chambers with  
1029 SHORT measurements. Each week, the program was modified manually so that the four LONG  
1030 measurements were rotated across the chambers. Each chamber was therefore measured with the  
1031 LONG closure time for one 7 consecutive day period per month (4 weeks).

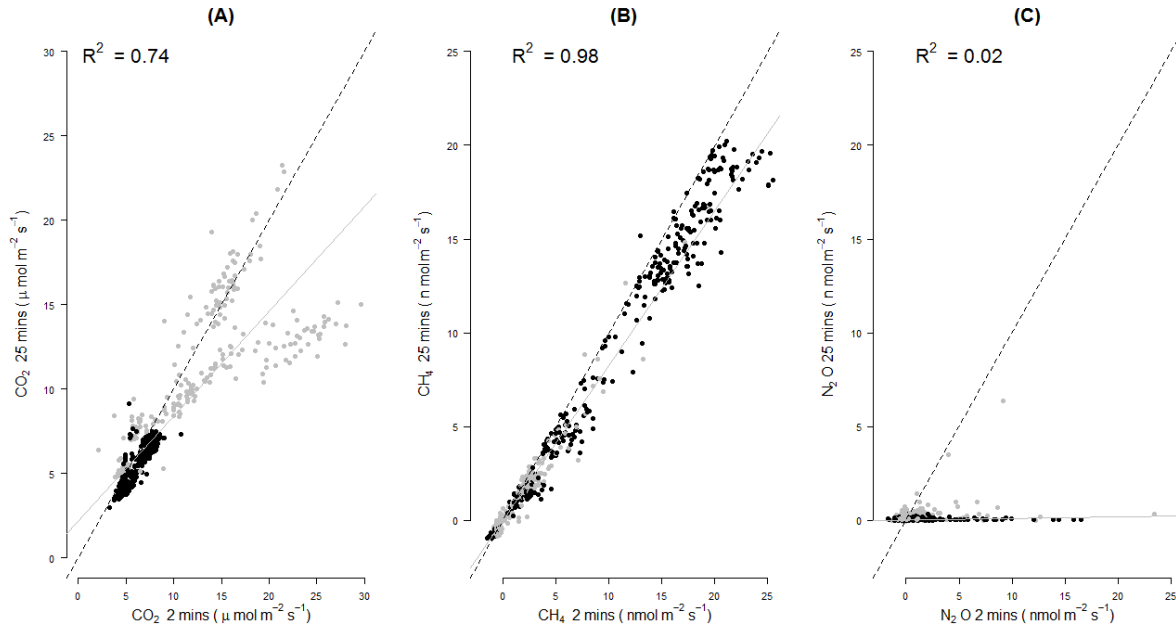
## 1032 **2.6. System maintenance and data processing**

1033 The automated sampling system was installed on June 1<sup>st</sup> 2016 and operated until September 29<sup>th</sup>  
1034 2016 (4 months), totaling ~~17652-17592~~ individual measurements for each gas (~~4326-4098~~ with  
1035 LONG closure time and ~~13326-13494~~ with SHORT closure time). Coarse wood debris ~~was-were~~  
1036 removed weekly but small litter, such as leaves, fruits, and twigs, was left in the collar area. Every  
1037 week, living plants growing inside the collars, and the dead leaves on the chambers, were carefully  
1038 removed by hand. The R<sup>2</sup> value of the exponential increase of -CO<sub>2</sub> over 2 minutes was used as an  
1039 indicator that the system was functioning correctly and not impeded by debris (Görres et al., 2016;  
1040 Savage et al., 2014). When the R<sup>2</sup> of the regression between time and CO<sub>2</sub> concentration was lower  
1041 than 0.9, we considered this as an indication that there may have been an issue with the chamber  
1042 closing and sealing correctly and removed the flux measurement for all three gases from our  
1043 analysis.

1044 For CO<sub>2</sub>, we observed a strong concentration saturation effect when using the LONG  
1045 closure time (25 minutes), leading to an underestimation of fluxes (~~Supplementary Figure 1~~Figure  
1046 2). All CO<sub>2</sub> flux estimates were therefore based on 2 minute regressions only, using either full  
1047 concentration measurements of the SHORT closure time or the 2 first minutes of the LONG

1048 closure time. ~~The  $R^2$  value of the linearly increasing  $\text{CO}_2$  over 2 minutes was used as an indicator~~  
1049 ~~that the system was functioning correctly and not impeded by debris (Görres et al., 2016; Savage~~  
1050 ~~et al., 2014). When the  $R^2$  of the regression between time and  $\text{CO}_2$  concentration was lower than~~  
1051 ~~0.9, we considered this as an indication that there may have been an issue with the chamber closing~~  
1052 ~~and sealing correctly and removed the flux measurement for all three gases from our analysis.~~  
1053 Following recommendations (Rubio and Detto, 2017), we removed anomalous values, i.e.  $\text{CO}_2$   
1054 fluxes estimation with a difference greater than  $5 \mu\text{mol m}^{-2} \text{s}^{-1}$  between with previous or following  
1055 measurements or lower than  $0 \mu\text{mol m}^{-2} \text{s}^{-1}$ . For  $\text{CH}_4$ , we observed only a slight saturation effect  
1056 when using the LONG closure time (Supplementary-Figure 21). Variation in the flux calculations  
1057 did not differ between the SHORT and LONG chamber closure measurements. ~~All fluxes above~~  
1058 ~~or below (for negative fluxes) MDF were considered as reliable and were calculated using the full~~  
1059 ~~data available (2 minutes measurement for the SHORT closure time and 25 minutes for the LONG~~  
1060 ~~closure time).~~  $\text{N}_2\text{O}$  flux calculations were much more variable when measuring with the SHORT  
1061 closure time compared to the LONG closure time (Supplementary-Figure 22). Even if fluxes were  
1062 above the detection limit, the low fluxes estimated with the SHORT closure time were not reliable  
1063 as shown by the low correlation in Figure 2. For both  $\text{CH}_4$  and  $\text{N}_2\text{O}$ , we therefore decided to  
1064 apply the following quality check procedure (1) to keep all ~~All the~~ fluxes estimated with the LONG  
1065 closure time that were not complying with MDF criterion even with low  $R^2$  for the regression  
1066 between time and  $\text{N}_2\text{O}$  concentrations and were discarded. (2) ~~All to consider all~~ fluxes estimated  
1067 with the SHORT closure time with a  $R^2$  lower than 0.8 as unreliable were discarded (Savage et al.,  
1068 2014). (3) We applied the same procedure than for  $\text{CO}_2$  regarding anomalous values (difference  
1069 greater than  $5 \text{ nmol m}^{-2} \text{ s}^{-1}$  between consecutive measurements).





1070 **Figure 2: Comparison of 2 minutes and 25 minutes estimations for (A) CO<sub>2</sub> (B) CH<sub>4</sub> and (C)**  
 1071 **N<sub>2</sub>O fluxes. For this, we used measurements made over 25 minutes and recomputed the flux with**  
 1072 **the two firsts minutes for two weeks (from August 2<sup>nd</sup> for August 9<sup>th</sup> in black and from August**  
 1073 **16<sup>th</sup> for August 25<sup>th</sup> in grey) covering the whole range of fluxes during the study period. All fluxes**  
 1074 **were computed using exponential fit. The dashed line represent the 1:1 line while the solid grey**  
 1075 **line represents the linear regression between 2 minutes and 25 minutes estimations (R<sup>2</sup> of these**  
 1076 **regressions are indicated on each panel).**  
 1077

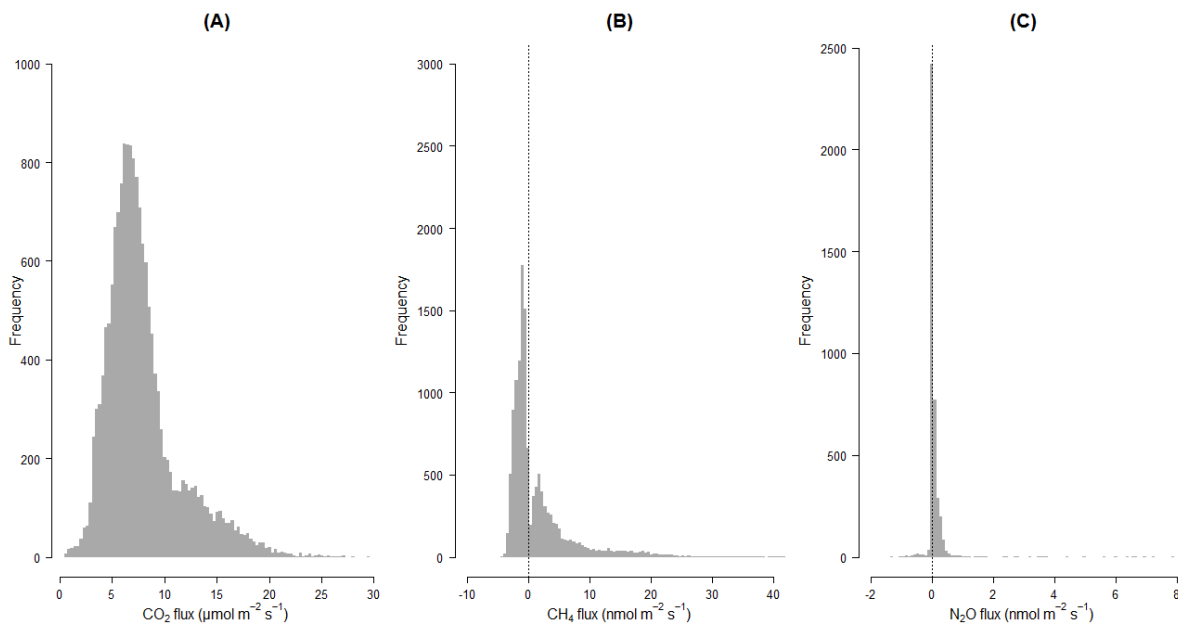
1078 **3. Results and discussions**

1079 A cleaning frequency of once a week was necessary and sufficient to remove falling leaves and  
 1080 branches from the automatic chamber system, prevent leaks and generate a continuous dataset of  
 1081 soil GHG fluxes from this tropical forest. Temperature variations are typically small below the  
 1082 canopy due to the shadowing by dense canopy crown and microclimatic conditions. During the  
 1083 study period, temperature at 2m height varies during the day from 22 °C in the night to 28 °C  
 1084 during the day. The presence of water condensation inside the tubing lines was carefully checked  
 1085 every week and never occurred during the study period. The automatic chamber system worked  
 1086 well most of the time, but some data gaps did exist. Over the 17724–17592 individual flux  
 1087 estimations, 276–343 (1.95 %) had to be discarded because of (1) problems in the connection

between the chamber and the multiplexer (~~196–154~~ measurements, 0.91% of data points); (2) imperfect chamber closing, which was detected by an insufficient increase of CO<sub>2</sub> (~~103–189~~ measurements, 10.6% of data points).

**Table 2:** Mean, standard deviation (SD), minimum (Min) and maximum (Max) values of each gas and each chamber over the study period. These values are computed using all fluxes estimation (either with SHORT or LONG closure time) remaining after quality check. The number (N) of fluxes that were used is also indicated for each chamber. The last line of the table is the mean of all fluxes by chambers by gas and the min and max for all chambers by gas.

	CO <sub>2</sub> (μmol m <sup>-2</sup> s <sup>-1</sup> )					CH <sub>4</sub> (nmol m <sup>-2</sup> s <sup>-1</sup> )					N <sub>2</sub> O (nmol m <sup>-2</sup> s <sup>-1</sup> )				
	Mean	Sd	Min	Max	N	Mean	Sd	Min	Max	N	Mean	Sd	Min	Max	N
Chamber 1	7.19	0.93	2.14	10.81	940	10.97	7.73	-2.08	28.79	840	0.10	0.12	-0.48	0.70	284
Chamber 2	7.60	1.11	4.00	12.21	1166	-1.62	1.75	-4.09	11.68	899	0.00	0.14	-1.03	0.75	285
Chamber 3	5.58	0.99	2.11	11.12	1135	0.35	2.95	-2.48	22.94	745	0.03	0.23	-0.61	2.85	208
Chamber 4	7.94	1.37	4.36	12.13	1154	-1.85	1.23	-3.63	6.09	1105	0.04	0.10	-0.66	0.60	224
Chamber 5	4.14	0.92	0.53	10.05	1139	1.37	3.26	-2.20	12.61	752	0.15	0.33	-1.04	3.23	382
Chamber 6	8.87	1.70	3.36	17.68	1070	-1.38	1.78	-3.20	8.04	801	-0.02	0.12	-1.04	0.63	272
Chamber 7	13.47	2.78	0.89	22.12	988	1.37	3.60	-2.63	19.56	749	0.64	1.37	-0.85	7.93	216
Chamber 8	7.44	1.19	2.03	11.02	1099	0.03	2.96	-3.37	18.47	785	0.02	0.15	-1.36	0.84	202
Chamber 9	4.25	1.20	0.44	11.37	1002	2.06	3.13	-2.14	11.53	879	0.02	0.11	-0.62	0.58	332
Chamber 10	5.60	1.30	0.69	13.13	1037	1.21	2.46	-1.91	10.34	657	0.04	0.13	-0.64	0.77	252
Chamber 11	11.97	2.19	6.84	18.78	1004	6.72	7.61	-1.06	41.49	855	0.03	0.17	-1.01	1.04	199
Chamber 12	9.42	2.70	3.45	21.54	968	1.40	6.68	-3.29	41.94	891	0.02	0.09	-0.75	0.30	204
Chamber 13	5.85	1.34	0.42	8.49	944	5.29	5.92	-4.60	26.64	654	0.10	0.19	-0.84	1.71	335
Chamber 14	5.66	1.15	0.72	10.72	987	2.78	6.22	-2.48	35.15	691	0.09	0.17	-0.63	0.93	231
Chamber 15	16.63	3.27	9.42	29.64	850	-0.46	2.05	-3.25	8.26	839	-0.02	0.16	-0.96	0.72	185
Chamber 16	7.35	1.13	3.98	11.37	994	-1.34	1.48	-3.60	6.11	843	0.00	0.11	-1.00	0.83	187
	<b>8.06</b>	<b>1.58</b>	<b>0.42</b>	<b>29.64</b>	<b>16477</b>	<b>1.68</b>	<b>3.80</b>	<b>-4.60</b>	<b>41.94</b>	<b>12985</b>	<b>0.08</b>	<b>0.23</b>	<b>-1.36</b>	<b>7.93</b>	<b>3998</b>



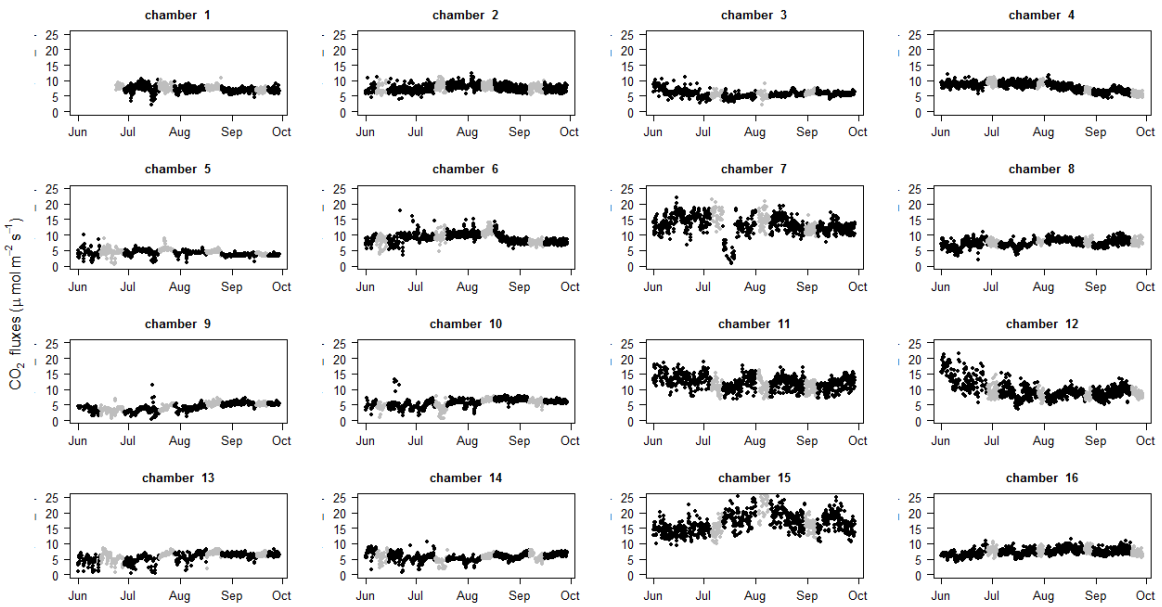
**Figure 32: Distribution of fluxes:** Histogram of (A) CO<sub>2</sub>, (B) CH<sub>4</sub> and (C) N<sub>2</sub>O fluxes over the study period. For (B) and (C), the dotted line represents null fluxes.

### 3.1. CO<sub>2</sub> fluxes

Additionally to the 343 fluxes that were removed after the first steps of quality check procedure, 758 CO<sub>2</sub> fluxes estimations were also considered as anomalous, either because the difference with previous or following measurements were greater than 5 μmol m<sup>-2</sup> s<sup>-1</sup> (758 measurements, i.e. 4.3%) or because they were lower than 0 μmol m<sup>-2</sup> s<sup>-1</sup> (14 measurements). In total, 16477 CO<sub>2</sub> fluxes over 17592 (93.6%) can be used over the four month period. CO<sub>2</sub> fluxes were on average  $87.10634 \pm 13.621$  μmol m<sup>-2</sup> s<sup>-1</sup> (Table 2) which would correspond to a mean annual soil CO<sub>2</sub> efflux of 3050 gC m<sup>-2</sup> year<sup>-1</sup> which falls into the upper range of the extensive review of mean annual soil CO<sub>2</sub> effluxes estimations in tropical forest provided recently by Rubio and Detto (2017). Nonetheless, our study period (June-September) only covered the end of the wet season and more data are needed to precise this estimation, with a high variability among chambers (Table 2). The minimum flux measured during the study period was 0.19 μmol m<sup>-2</sup> s<sup>-1</sup> (Table 2) and the

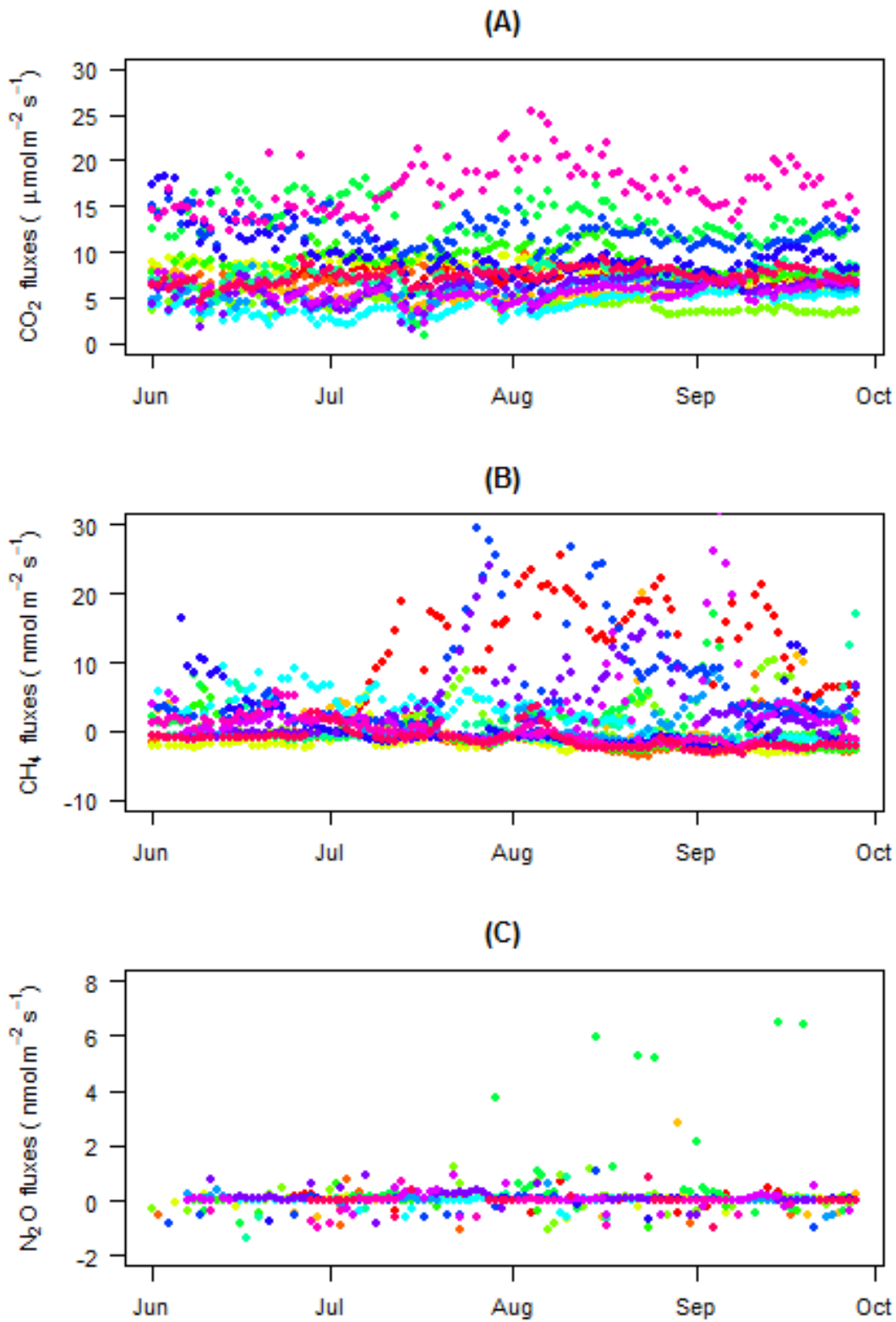
1115 ~~majority of fluxes were between 3 to 10  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Figure 2).~~ All two-minute measurements of  
1116  $\text{CO}_2$  fluxes from the four-month study period were ~~therefore~~ above the MDF of  $2.39 \text{ nmol m}^{-2} \text{ s}^{-1}$   
1117 for the LI8100 analyzer (Table 1). No saturation effect was detected using the SHORT closure  
1118 time and estimation of  $\text{CO}_2$  over a shorter time period is not recommended (Davidson et al., 2002).  
1119  $\text{CO}_2$  fluxes using the LONG closure time would be underestimated due to the buildup of high  $\text{CO}_2$   
1120 concentrations due to large fluxes over this long time period (~~Supplementary Figure 1~~Figure 2),  
1121 and are not recommended. For small chambers as the one that were used in this study, w  
1122 therefore conclude that a 2 minute sampling time should be used for  $\text{CO}_2$  flux calculations since  
1123 the MDF of this short measurement period allowed for the retention of 100% of the data. When  
1124 the chambers stay closed longer for accurate detection of  $\text{N}_2\text{O}$  fluxes, only the first two minutes of  
1125 data should be used for  $\text{CO}_2$  flux calculations.

1126 The use of 16 automated flux chambers allowed for the capture of spatial and temporal variability  
1127 of soil respiration. O~~ver~~ this four month period, corresponding to the end of the wet season in  
1128 French Guiana, -temporal variability remained low (Figure 4). This dataset is therefore not long  
1129 enough to detect seasonal variation of soil respiration that were highlighted in previous study  
1130 (Rowland et al., 2014; Rubio and Detto, 2017). We did found that soil respiration tended to  
1131 decrease in very humid soils (Supplementary Figure 1) as highlighted previously at the same site  
1132 (Rowland et al., 2014) but more data are needed to disentangle precisely the importance of seasonal  
1133 and diurnal variability from the responses to environmental triggers on soil respiration.  
1134 Nonetheless, even during this relatively short period, our data clearly demonstrated a strong spatial  
1135 variability of soil respiration, even at a low spatial scale (Figure 5, Table 2), some local spots  
1136 clearly displaying stronger values of soil respiration during the study period. which is needed to  
1137 constrain ecosystem carbon budgets (Figure 3).



1138

1139 **Figure 43: CO<sub>2</sub> fluxes through time:** CO<sub>2</sub> fluxes for each chamber (1 to 16) over the study period  
 1140 with fluxes estimated with SHORT (2 minutes) closure time in black and fluxes estimated with  
 1141 the 2 first minutes of the LONG (25 minutes) closure time in grey. All panels have the same limits  
 1142 on the y axis (from 0 to 25 μmol m<sup>-2</sup> s<sup>-1</sup>)  
 1143



1144

1145 **Figure 5:** Mean values per days for (A) CO<sub>2</sub>, (B) CH<sub>4</sub> and (C) N<sub>2</sub>O fluxes over the study period.  
 1146 Each chamber is represented by a distinct color.

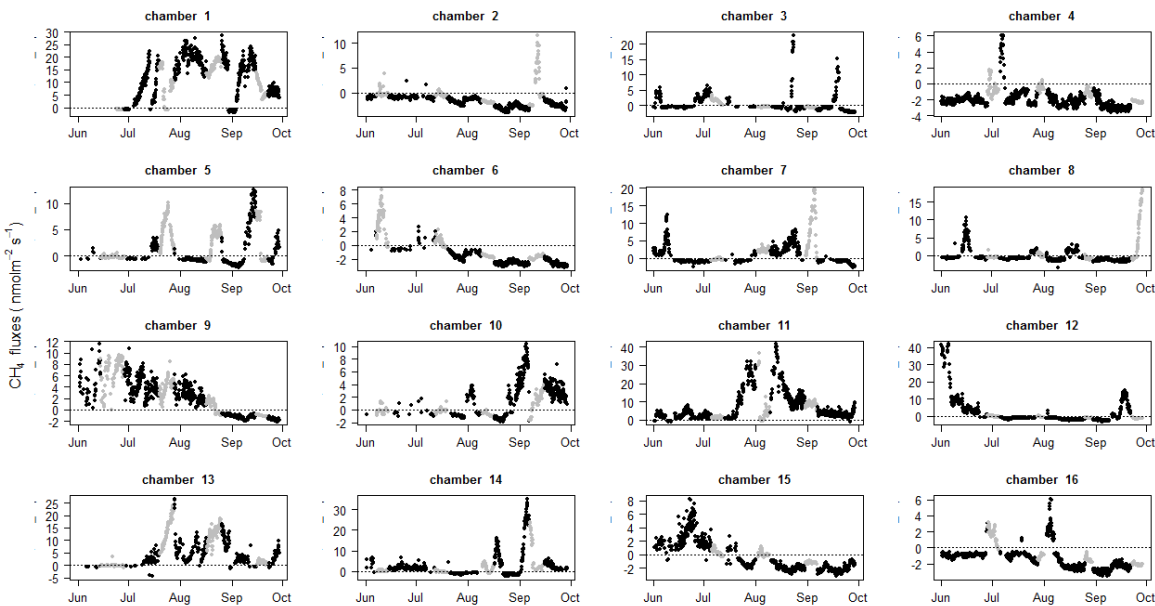
1147

### 1148 3.2.CH<sub>4</sub> fluxes

1149 Additionally to the 343 fluxes than were removed after the firsts steps of quality check procedure,  
1150 CH<sub>4</sub> fluxes estimations were also discarded because of (1) problems with Picarro files (12  
1151 measurements), (2) application of the MDF criterion (137 measurements), (3) application of the  
1152 R<sup>2</sup> criterion for SHORT closure time (3751 measurements, i.e. 28% of the SHORT measurements)  
1153 (4) detection of anomalous values (364 measurements). In total, 12985 CH<sub>4</sub> fluxes over 17592  
1154 (73.8%) can be used over the four month period. No saturation effect was detected using the LONG  
1155 closure time and fluxes estimated with the SHORT closure time were very well correlated to fluxes  
1156 using the LONG closure time, even for small fluxes (Figure 2). ~~CH<sub>4</sub> fluxes were on average 1.06~~  
1157 ~~± 4.52 nmol m<sup>-2</sup> s<sup>-1</sup> with a high variability among chambers (Table 2). Minimum detectable fluxes~~  
1158 ~~for CH<sub>4</sub> were ± 0.04 nmol m<sup>-2</sup> s<sup>-1</sup> using the SHORT closure time and ± 0.001 nmol m<sup>-2</sup> s<sup>-1</sup> using the~~  
1159 ~~LONG closure time (Table 1). 98.568.4 % and 9998.29% of fluxes measured with the SHORT and~~  
1160 ~~LONG closure times, respectively, were retained in our quality control data processing over the~~  
1161 ~~four-month study period. These measurement periods, therefore, allowed for the retention of a~~  
1162 ~~large majority of CH<sub>4</sub> emission or consumption fluxes in our data analysis. ▫~~

1163 CH<sub>4</sub> fluxes were on average 1.706 ± 34.852 nmol m<sup>-2</sup> s<sup>-1</sup> with a high variability among chambers  
1164 (Table 2) but ~~▫~~the frequency of negative CH<sub>4</sub> fluxes (consumption, 59% of fluxes) was greater  
1165 than positive fluxes (emission, 41% of fluxes) during this period (Figure 32). Most of the time,  
1166 soils were either consuming or emitting small amounts of CH<sub>4</sub>, but transient, large emission peaks  
1167 were periodically detected at individual chamber locations during the study period (Figure 64).  
1168 Tropical soils are generally considered as sink at a yearly basis (Dutaur and Verchot, 2007) but it  
1169 is known that these soils can shift from a source in the wet to a sink in the dry season (Courtois et

al., 2018; Davidson et al., 2008; Teh et al., 2014). No clear temporal trend could be detected during the study period and no clear pattern linked CH<sub>4</sub> fluxes with surface soil humidity (Supplementary Figure 1), but as for CO<sub>2</sub>, longer time series covering at least a full year are needed to explore the seasonal and diurnal variability of fluxes. As highlighted previously in French Guiana (Courtois et al., 2018), spatial variability of CH<sub>4</sub> emission was high, even at a small spatial scale (Figure 5, Figure 6). Interestingly, some spots clearly display high CH<sub>4</sub> emission during all the study period (Figure 5, Figure 6).



**Figure 64: CH<sub>4</sub> fluxes through time:** CH<sub>4</sub> fluxes for each chamber (1 to 16) over the study period with fluxes estimated with SHORT (2 minutes) closure time in black and fluxes estimated with LONG (25 minutes) closure time in grey. The dotted line displays the zero flux line. All panels have the same limits on the y axis (from -5 to 30 nmol m<sup>-2</sup> s<sup>-1</sup>)

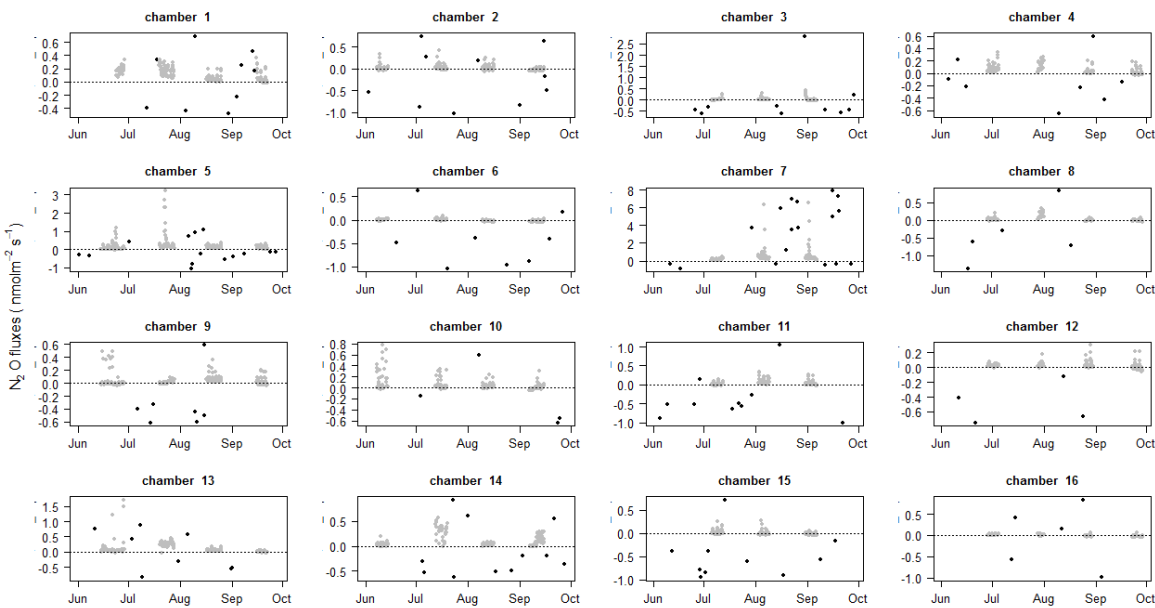
### 3.3. N<sub>2</sub>O fluxes

Additionally to the 343 fluxes that were removed after the first steps of quality check procedure, N<sub>2</sub>O fluxes estimations were also discarded because of (1) problems with Picarro files (12 measurements), (2) application of the MDF criterion (1594 measurements), (3) application of the



1187 R<sup>2</sup> criterion for SHORT closure time (11643 measurements, i.e. 28% of the SHORT  
1188 measurements) (4) detection of anomalous values (364 measurements). In total, 3998 N<sub>2</sub>O fluxes  
1189 over 17592 (22.7%, 140 measurements with the SHORT and 3858 measurements with the LONG  
1190 closure time) can be used over the four month period. 94.1% of fluxes measured with the LONG  
1191 closure times were retained after our quality control data processing over the four-month study  
1192 period. N<sub>2</sub>O fluxes were on average  $0.038 \pm 0.537 \text{ nmol m}^{-2} \text{ s}^{-1}$  with a high variability among  
1193 chambers (Table 2). Most N<sub>2</sub>O fluxes (85.6%) with the LONG closure time were above or below  
1194 MDF ( $\pm 0.002 \text{ nmol m}^{-2} \text{ s}^{-1}$ , Table 1) and varied between  $-2$  to  $2 \text{ nmol m}^{-2} \text{ s}^{-1}$  (Figure 2). When  
1195 measured over 25 minutes, N<sub>2</sub>O fluxes in our site could therefore be considered as reliable. Using  
1196 the SHORT closure time, most flux estimations had to be discarded because they led to unreliable  
1197 flux estimations (Supplementary FFigure 2). ~~Only 7.9% of the measurements using the SHORT~~  
1198 ~~closure time were retained after our quality control checks for the N<sub>2</sub>O flux data.~~ Nonetheless, the  
1199 ~~high frequency of the SHORT closure times~~ still allowed the detection of ~~one~~ high N<sub>2</sub>O emission  
1200 ~~or consumption events (up to  $15 \text{ nmol m}^{-2} \text{ s}^{-1}$ ) that were~~ detected during the study period (Figure  
1201 ~~5, chamber 75 and 7).~~  
1202 N<sub>2</sub>O fluxes were on average  $0.1 \pm 0.2 \text{ nmol m}^{-2} \text{ s}^{-1}$  with a high variability among chambers (Table  
1203 2). At the same chamber, N<sub>2</sub>O flux can shift from consumption to emission with 28% of fluxes  
1204 indicating a sink and 72% a source for N<sub>2</sub>O (Figure 3). The high variability in N<sub>2</sub>O fluxes that we  
1205 detected over four months with our automated system are in agreement with the typical high  
1206 variability in N<sub>2</sub>O fluxes measured from tropical soils over space and time using static chambers  
1207 (Arias-Navarro et al., 2017; Courtois et al., 2018). Moreover, N<sub>2</sub>O fluxes didn't show any  
1208 relationship with surface soil humidity (Supplementary Figure 1), which underline the complexity  
1209 of the biological process underlying these fluxes. In a previous study in the same environment

1210 (Courtois et al., 2018), we estimated that the minimum detectable fluxes using Gas  
 1211 Chromatography analysis of four discrete gas samples over 30 minutes for N<sub>2</sub>O was ± 8.3 μg N  
 1212 m<sup>-2</sup> h<sup>-1</sup>. MDF estimated in the present study using high frequency measurement was 0.002 nmol  
 1213 m<sup>-2</sup> s<sup>-1</sup> or 0.2 μg N m<sup>-2</sup> h<sup>-1</sup> for N<sub>2</sub>O which is therefore ~ 40 times lower. Such result indicates that  
 1214 this long-term system is well-adapted to capture and estimate the low N<sub>2</sub>O fluxes occurring in this  
 1215 ecosystem.



1216  
 1217 **Figure 57: N<sub>2</sub>O fluxes through time:** N<sub>2</sub>O fluxes for each chamber (1 to 16) over the study period  
 1218 with fluxes estimated with the SHORT (2 minutes) closure time in black and fluxes estimated with  
 1219 the LONG (25 minutes) closure time in grey. The dotted line displays the zero flux line. Due to  
 1220 the high differences among chambers, each panel has specific limit on the y axis.  
 1221

1222 **4. Conclusions**

1223 Our **unique**-system coupled a Li8100 CO<sub>2</sub> analyzer and multiplexor with a Picarro G2308 CH<sub>4</sub> and  
 1224 N<sub>2</sub>O analyzer to sample 16 automated soil flux chambers with a rotation of SHORT and LONG  
 1225 closure times for the accurate monitoring of three GHG fluxes over four months with high spatial  
 1226 and temporal resolution. The sampling system of SHORT and LONG closure times with a weekly

1227 rotation presented here has three major advantages, which ultimately can provide high confidence  
1228 in the estimation of annual the full GHG budgets of tropical soils: (1) the LONG closure time  
1229 allows a reliable estimation of the low N<sub>2</sub>O fluxes in this ecosystem, which was clearly not  
1230 achieved using a shorter closure time, (2) the number of data points per day are sufficiently high  
1231 (9 to 10 measurements per day) to capture potential diurnal variation (Nicolini et al., 2013; Rubio  
1232 and Detto, 2017) of the three gases with good spatial replication (16 chambers), (3) periodic  
1233 extreme events of high N<sub>2</sub>O fluxes can still be detected with the SHORT closure time period,  
1234 which occurs at higher frequency than the LONG closure measurements. Our study underlines the  
1235 importance of appropriate closure time for each GHG gas for accurate estimation of GHG budgets.  
1236 We demonstrate here that the combination of a commercial soil GHG chamber system – the LI-  
1237 8100A Automated Soil CO<sub>2</sub> Flux System – running in line with a Picarro G2308, enables the  
1238 continuous, long-term measurement of CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O simultaneously under tropical  
1239 conditions. Similar configurations have been recently implemented in temperate climate (Petraakis  
1240 et al., 2017b, 2017a), but to our knowledge, this is the first time that this experimental set up is  
1241 fully described and tested under tropical field conditions for the measurement of the three soil  
1242 GHG fluxes simultaneously. Additionally, our study determined the optimal chamber closure time  
1243 for each GHG. This information is crucial for the calculation of accurate soil fluxes at diurnal  
1244 timesteps and for the estimation of annual GHG budgets. This combination of automated closed  
1245 dynamic chambers and advanced GHG analyzers allows for, (1) accounting of short-term  
1246 variability in GHG fluxes while taking into account spatial variability, (2) estimating annual GHG  
1247 budgets at these locations, (3) tracking the variability in GHG fluxes along hours, days, seasons  
1248 and years, and (4) studying the impact of climatic change on soil GHG budgets.

1249

1250 **Author contribution.** JVB and NA designed the experiments and EAC, CS, BB and DB carried  
1251 them out. EAC and CS prepared the manuscript with contributions from all co-authors.

1252 **Competing interests.** The authors declare that they have no conflict of interest.

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1265 AC02-05CH11231.

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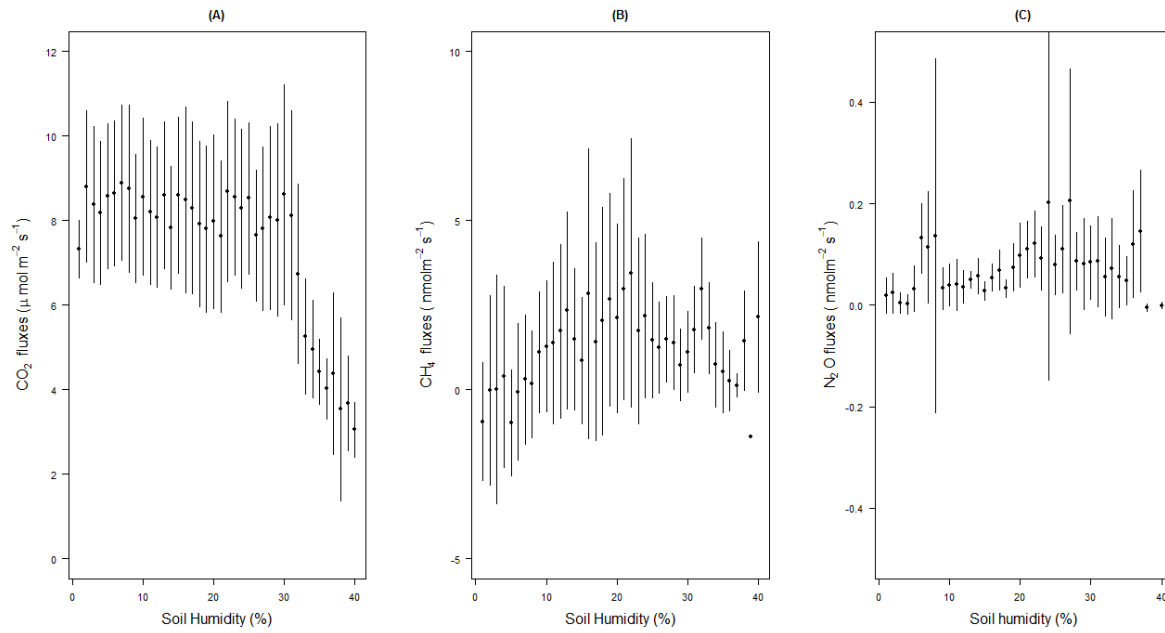
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1268 Supplementary File 1: R code for merging Picarro files to include them in Soil Flux pro
1269
1270 ## to list all the days in a given directory (Picarro makes one directory per day)
1271 ListDay<-list.files()
1272 Pfile<-list()
1273 ## to concatenate all the hourly file in one file per day
1274 for (j in 1:length(ListDay))
1275 {
1276 print(j)
1277 ListFilesPicarro<-list.files(ListDay[j])
1278 Data<-read.table(paste(ListDay[j],"/",ListFilesPicarro[1],sep=""))
1279 for (i in 2:length(ListFilesPicarro))
1280 {
1281 temp<-read.table(paste(ListDay[j],"/",ListFilesPicarro[i],sep=""))
1282 Data<-rbind(Data, temp)
1283 print(i)
1284 }
1285 Pfile[[j]]<-Data
1286 }
1287 ## to concatenante all days and make just one file will all data
1288 MasterData<-Pfile[[1]]
1289 for (k in 2:length(Pfile))
1290 {
1291 MasterData<-rbind(MasterData,Pfile[[k]])
1292 print(k)
1293 }
1294 ## to write the table in a way that SFP can read it
1295 write.table(MasterData, "MasterData.dat", quote=F)
1296
1297

```

1298  
1299  
1300  
1301

**Supplementary Figure 1: Relationship between soil surface humidity and (A) CO<sub>2</sub>, (B) CH<sub>4</sub> and (C) N<sub>2</sub>O fluxes over the study period.**



1302  
1303  
1304

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