1. General comments

During the last decade, substantial emissions of methane (CH4) from stem surface of mature trees have been reported in various tree species which are capable of surviving the anoxic soil condition in temperate and tropical wetland forests. Researchers have been trying to clarify the underlying mechanisms and potential rate-controlling factors of tree-mediated CH4 transport/emission, and to evaluate the relative contribution of stem CH4 emission in the total CH4 flux of the ecosystems or global CH4 budget. It requires intensive gas flux measurements at stem surface of canopy trees, in terms of space and time, to clarify the whole nature of tree-mediated CH4 emission, because CH4 emission rates from tree stems have been reported to vary significantly among tree individuals, size and species, and seasonally as well. This technical note deals with the development of a newly-designed semi-rigid gas flux chamber which has vari- ous advantages over conventional rigid chambers in the field measurement of gas ex- change at tree stem surface. Volume accuracy and permeability of the newly-designed chambers were compared to the conventional rigid chamber in the laboratory, and the examples of CH4 flux measurements using the semi-rigid chamber in the fields are also shown in this paper. The aims of the paper are quite clear and relevant, and there seems to be no problem in logical composition and data reliability. I would recommend that this technical note could be acceptable after minor revisions commented below.

2. Specific comments

[P.16026, L.8] There is no description on the definition of "Sstem" appearing in the equation 6. Its definition should be added in the text just above the equation.

Authors: We changed the text at L5-L6 to:"...by considering the sector (K) of the **stem** surface **($S_{stem}$)** covered by the chamber at the circumference of the stem..."

[P.16026, L.8] In the equation 6, I suppose that a term "$\pi$" may be not necessary.

Authors: Indeed, we removed it.

[P.16027, L.9] Information on the trees used in the field test of the chambers, i.e., the number of trees for each tree species, and DBH and height of the trees, should be added here.

Authors: At line L7 we inserted: "on twelve tree-stems (diameter at breast height: 25-45 cm)" at the place of "on various" which we deleted.

[P.16030, L.7-13] The authors attribute the variability in observed volume of the sleeve or chamber to the compaction of the Neoprene form. If so, the observed volumes (V'tot) are supposed to be always smaller than the theoretical ones (Vtot). However, the observed values are sometimes larger than the theoretical ones for the large sleeve and for the rigid chamber (Supplement S1). There might be some other causes for the variability in actual volume of the sleeves (chambers).

Authors: 1) As we state on page 16030, the "This compaction was less than 3% of $V_{tot}$, which was a maximum considering the pulling force of 200N applied on the straps (twice 100 N). In reality, it is much less as we used just a fraction of the full strength. A little tension is enough to seal the chamber.

2) The observed values $V'_{tot}$ are used to calibrate the theoretical $V_{tot}$. The idea of making these comparisons was to provide new chamber users with an idea of the accuracy when volumes are

pragmatically calculated from chamber sizes, and to show that it is alright to do so, although the dilution will calibrate it.

3) We made a small mistake when calculating the theoretical volume $V_{tot}$ of the rigid chamber. The mistake only changes very slightly some values and has absolutely no consequences on the drawn conclusions.

Thereafter:

In Table 1 for the rigid chamber we updated H (30), $S_c$ (1413), $V_c$ (13165), $V_{tot}$ (13581), P (14.62), RSE of P (1.86). At the footnote of Table 1 we changed the volume inaccuracy to 4.1 for the rigid chamber.

In supplement S1 we updated H (30), $S_c$ (1413), $V_c$ (13165), $V_{tot}$ (13581), J ($-7.9*10^{-6}$), P ($1.46*10^{-6}$) and volume Inaccuracy (4.09)

In supplement S2 for the rigid chamber we changed H (30), $V_c$ (2120), EC (1413), CD (154) and CD/EC (0.109), and for the semi-rigid chamber we changed H (30), $V_c$ (13164), EC (1413), CD (333) and CD/EC (0.236)

We further updated the text with:

P16024 L19: "30" instead of "28"

P16030 L9: "13581" instead of "12702"

P16031 L12: "14.1x" instead of "13.2x", and "9.7x" instead of "9.0x"

P16031 L28: "2.17x" instead of "2.19x"

4) For the cause for the smaller $V_{tot}$ of large sleeves at P16034 L4 we added: "The average 33 $cm^3$ greater $V'_{tot}$ values as compared to $V_{tot}$ for the large sleeve can be attributed to the volume of the wedges that were also undergoing a compaction when deployed as the interior periphery gets compressed. This tiny volume correction was not inserted in formula 4 for the sake of simplicity and because the difference with the calibration was still below 5%."

[P.16030, L.21-22] There is no description, in any part of the manuscript including the Table 1, on how "the overall inaccuracy for the permeability" was calculated.

Authors: To make it clearer we replaced the text at P16030 L15-22 with: "By dividing the absolute value of the bias through the predicted value we get an estimate of the inaccuracy of $V_{tot}$ (chamber, tubes and detector's cell). As all terms of the fraction (Eq. 4) are linearly dependent, the inaccuracy of the permeability (P) is the quadratic mean of all other terms (Table 1, footnote). The gas exchange surface ($S_c$) could be precisely determined and we assume that there is no error associated to it. The inaccuracies in the concentration measurements are dependent on the uncertainty of the UGGA, which in our case was <1% for the un-calibrated device."

The error propagation formula is placed in the footnote of Table 1. We updated the inaccuracies for the permeability as those were approximations based on sums, which were always bigger than the propagation calculations. Thereafter, at P16042 Table 1 footnote, we changed "…Summed up overall inaccuracies: " with "Permeability inaccuracies*: " and added one line below: "*Calculated from error propagation formula:

$\frac{dP}{|P|} \leq \sqrt[2]{\left(\frac{dC}{|C|}\right)^2 + \left(\frac{dV}{|V|}\right)^2 + \sqrt[2]{dC^2 + dC_{atm}^2}} \cong \sqrt[2]{\left(\frac{dV}{|V|}\right)^2 + 2}$ "

[P.16031, L.1] "The relative standard error (RSE)" should be followed by "of the initial concentration (Co)" for more explicit explanation.

Authors: We changed it exactly the way you suggested it at L1.

[P.16034, L.9-11] The authors should cite some related articles regarding the minimiza- tion of potential errors in gas exchange measurement by a chamber.

Authors: Good idea. We inserted these references at the end of the sentence: "(Christiansen et al., 2011; Hutchinson and Livingston, 2001; Juszczak, 2013; Pihlatie et al., 2013)".

[P.16036, L.2-3] Surface of tree bark is often rough and has many cracks, especially when some wetland tree species, such as Alnus or Fraxinus spp. are selected for the measurement of stem methane flux. So, the expression of "In very rare case" seems to be not appropriate.

Authors: This is true. What we wrote was more related to our own work and we understand that in the scope of generalisation it is better to change the text into: "In some situations,…"

[Supplement S1] At the end of the table caption, there is the expression of "difference between Vtot (predicted) and V'tot divided by Vtot (observed)". Is this correct? I sup- pose that it may be "difference between Vtot (predicted) and V'tot (observed) devided by Vtot".

Authors: We changed it as reviewer 1 suggested.

[Supplement S4] In this series of tables, two data sets, i.e. Run 4 (#518-#623) and Run 6 (#734-840), are annotated by a word of "Bad" in the column of tree species, which seems to mean those two runs were the flux measurements with gas leakage between inside and outside of a sleeve. In the text (P.16032, L.16-18) and Figure 6, however, the measurements with leakage were the Run 3 and 6. Please recheck the data regarding this discrepancy.

Authors: There is indeed an assignment error in the table S4. The "Bad", which we have changed to "Leaking" has been assigned to Run 3 instead of Run 4. The examples we chose are indeed on *Betula* (Run 3) and *Pinus* (Run 6).

3. Suggestions for technical corrections

[P.16027, L.6] The "Table 1" is referred at the end of this sentence. As the "Table 1" shows the results of laboratory measurements on volume accuracy and permeability of the three types of chambers, it seems a bit strange that the table is referred in the sentence mentioning the field deployment of the chamber. If the authors intend to show the dimensions of the chamber used in the field test, those information should be described in the text.

Authors: In a technical note, it is good practice to keep the paper short. To meet this requirement, we opted to use a table in the method section if the information is relevant. Adding text would be redundant. We prefer to leave it unchanged.

[P.16027, L.8] There is a typing error; "Betula Pendula" should be "Betula pendula".

Authors: We changed the "P" into "p"

Review of Siegenthaler, Welch, Pangala, Peacock, and Gauci 'Technical Note: Semi-rigid chambers for methane gas flux measurements on tree-stems' submitted to Biogeosciences Discussions.

Overview and recommendation.

This paper describes the building and testing of semi-rigid chambers for measuring the flux of gases, in this case methane (CH4), from tree trunks. The authors provide a good rationale for the study; there is, indeed, a need to understand more about the importance of fluxes of CH4 from trees to the overall flux of CH4 from a range of wetland ecosystems. The presentation is mostly clear and easy to follow, and the testing of the new types of chambers appears to have been carried out rigorously. I think the paper will be of interest to a reasonably wide constituency of researchers interested in CH4 emissions from wetlands and climate-change scientists interested in modelling the source strength of different land cover types. Given the above, the paper would be a useful addition to the literature. However, the paper does contain quite a few errors. Most of these are minor grammatical or typographical errors, but in some places the descriptions and explanations could be clearer. Additionally, there may be one or two errors in the way the work was done. I recommend these minor errors are addressed before the paper is published. My detailed comments are appended below.

Page 16020, line 8. Sentence starting "We compared...". The structure of this sentence is a little awkward. I recommend re-wording.

Authors: We re-worded it: "We compared the $CH_4$ permeability of the new semi-rigid chambers with that of the more traditional rigid chamber approach,…"

Page 16020, line 19. Add '(CH4)' after "methane"?

Authors: We inserted "($CH_4$)" just after "methane"

Page 16021, line 8. "flux rates". A flux is a rate; the "rate" here is redundant. I recommend correcting this expression wherever it appears in the document.

Authors: We replaced "flux rates" by "fluxes"

Page 16021, line 11. What does "ventilate" here mean? Open the chamber to ambient air or fit it with a fan (or something else)? The explanation here could be a little clearer.

Authors: We made it clearer by adding, "circulate the air in their headspace" just after "making it important to" instead of "ventilate".

Page 16021, line 16. Can something become "progressively obsolete"?

Authors: We deleted "progressively"

Page 16022, line 4. "and therefore voids underestimations due to non-optimal integrations" is quite awkward; I recommend re-wording in simpler language.

Authors: We re-worded it with: "and therefore it also avoids underestimations due to regressions made over longer periods of time"

Page 16022, line 9. "the use of a smaller stem chamber with a larger gas" Page 16022, line 13. Delete the comma after "challenge".

Authors: done.

Page 16023, line 3. "reduced greenhouse gases" is a rather odd expression. Do you simply mean gases produced in anaerobic conditions?

Authors: Yes, we changed it.

Page 16023, line 5. No capital P needed in "polyethylene".

Authors: done.

Page 16023, line 11. Comma needed after "approach".

Authors: done.

Page 16023, line 18. Which STP was used? There are 'competing' STPs. Did you use that of IUPAC?

Authors: We modified the text: "standard ambient temperature and pressure (SATP from UIPAC) at line 18. We also changed "STP" into "SATP" at P16027 line 22 as well as at P16029 line 18.

Page 16023, line 23. "but is hardly compressible" – under what loading?

Authors: We specified this at page 16024 Line 13. To make easier for the reader we add "with 200 N" after (≤3% " at line 23.

Page 16024, line 2 Here and elsewhere in the document I think this should be "Los Gatos Research Inc., Mountain View, CA, USA".

Authors: Done.

Page 16024, line 4. "Polyvinyl Chloride" – capital letters not needed.

Authors: Done.

Page 16024, line 6. Vent tubes were used. How much did these affect leakage/permeability? I'm not sure if there is an assessment of this effect in the paper. How much gas exchange occurred through the vents compared to the seals?

Authors: this subject has been addressed in detail by Hutchinson and Mosier 1981, and further modelled by Hutchinson et al 2001. As we wrote at page 16034 line 9 we referred to the recommendations made in other studies. In our case, we downscaled the vent by a factor 48 in terms of vent volume, which is greater than the factor 10-20 downscaling from Hutchinson et al 2001 14 L chamber to our semi-rigid sleeve. The diffusion path is also 3 cm longer than the one from Hutchinson et al. 2001. These authors showed that the loss of the "gas by diffusion through the leaking seal of a non-vented chamber was greater in all cases than loss by diffusion from a vented chamber with a perfect seal". In their study a perfectly sealed chamber, the gas losses through the sole vent represented 0.038% of a target gas after 30 minutes of deployment. In our case, with a more than proportional downscaled vent tube, the total losses were between 1.4 and 2.9%, which give a reasonable idea of the negligible losses through the vent tubes.

We made a typing mistake at P16024 L6. The vents were 1.2 mm in internal diameter. The 0.6 was for the radius. We changed it. The factor 48 downscaling includes that change.

To make things more informative, at P16024 line 6, we added: "We downscaled the vent described by Hutchinson and Livingston (2001) by a factor 48 in terms of vent volume whereas the sleeves were a factor 10 to 20 less voluminous as compared to the authors' chamber (14 L). Their study showed that in a perfectly sealed chamber, after 30 minutes of deployment the gas mass loss through the sole vent represented 0.038% of the target gas."

And at line P16033 Line 22 we added: "The average $CH_4$ mass losses (2.2-3.3 %) from the sleeves after 20 minutes of deployment were two orders of magnitude greater as compared to the 0.038% mass loss after 30 minutes of deployment reported by Hutchinson and Livingstone (2001) for a perfectly sealed chamber with a sole vent tube. Thereafter, our downscaled vent tube was proportioned to the $CH_4$ losses from the sleeves."

Page 16025, line 10. How were the chambers deployed when undertaking the empirical estimates of chamber volume? Where they attached to the inert stainless steel cylinders mentioned later in the paper? Also, it is noted here that the dilution tests took seconds, but later in the paper the dead band time is quoted as 90 s. There seems to be a discrepancy here.

Authors: At P16025 L7 we added: "The two semi-rigid sleeves and a rigid chamber were attached to an inert stainless steel cylinder (see chamber deployment). The dilution was done in 90 seconds…".

Page 16025, line 16. "uncompressible" should be "incompressible".

Authors: Done

Page 16026, line 21. "sporadic concentration drawdowns" Why are these typical of a leaking chamber? I would have thought the most common type of leakage was a steady leakage. Was leakage a two-way (iso- thermal and iso-baric) exchange of gases between the chamber and the air outside, or was it pressure driven, due for example to increases in chamber temperature? More explanation here would help. It is interesting to consider what is shown later in Runs 3 and 6 in Figure 6. The sporadic changes in [CH4] comprise both sudden decreases and increases, not just drawdowns as suggested by the authors. Why is this? What mechanism in terms of flow of gas across a leaky seal could explain these? In particular, how are the sudden increases explained?

Authors: Yes, these fluctuations are typical and they can be easily proven in practice when the re-sealing of the chamber changes this fluctuation into a steady increase. They are not steady leakages because of the vibrations coming from the pump circulating air. We think that the pump and pressure valves generates small jolts in the flow (small pressure waves) resulting in expulsion of the gas when ΔP are positive. During those events the stems continue to emit in the background. The resulting concentration is a balance of all these processes. Once ejected the gas diffuses in the less concentrated atmosphere, and when the pressure flow is inverted (ΔP negative) the air taken up by the chamber is less concentrated in that gas as it has diffused. These small pressure fluctuations have no consequence on the overall average pressure when the system is not leaking. That type of leakage is primarily pressure driven (Hagen-Poiseuille law) but it is also diffusive (Ficks laws). Since the concentration changes are globally increasing in the examples reviewer 2 mentions, there must logically be more increases than drawdowns (quantity x time). What reviewer 2 defines as "sudden increases" should rather be seen as the normal gas accumulations, which would appear less impressive if the graphs were rescaled. Anything that restrains that logistical gas build-up can be seen as drawdown.

To make things easier for the reader we change the text at P16026 L21: “A leaking chamber typically displayed fluctuating concentrations with concentration build-up being recurrently drawn down.”

Remark: the vent tube reduces the small pressure fluctuations (around the mean pressure) generated by the pump because the expelled air from the headspace during a positive ΔP is captured within the vent tube and then returned to the headspace when the external pressure rises again.

Page 16027, line 9. "Betula Pendula" should be "Betula pendula".

Authors: Done

Page 16027, line 27. "linear regression of declining concentrations" Above it is suggested that leakage occurred sporadically. It's not clear here that the simple dilution tests used by the authors accurately replicated how leaks occur during field deployments. I think a little more explanation would help. It would also be useful to see the dilution datasets.

Authors: Above at P16026 Line 21 we changed the sentence and the term “sporadic” has been replaced by a more explicative sentence.

As it was wrongly placed it within the lab permeability tests paragraph, the sentence at P16026 L18-21 was placed in the field deployment paragraph at P16027 Line 12 just before “Finally,…”.

Additionally to make the differentiation between permeability and leakage clear we (1) we changed the text in the brackets within the above displaced text bloc to: “(mainly pressure driven bulk flows following Hagen-Poiseuille’s law), and (2) we changed the text in the introduction at P16022 L10 to “…(gas conductance following Fick’s first law of diffusion)…”.

Remark: The concentration decreases (permeability) after dilution of the target gas was very steady as compared to the fluctuating concentrations changes in the leaking chambers of the field examples. The comparison of a leaking chamber with a normally functioning chamber (including its pre-tested gas permeability) in a situation where the partial target gas pressure (or concentration) is building-up from stem-emissions is made in the complement S4.

Page 16028, line 5. A comma is needed before and after "a posteriori".

Authors: Done

Page 16028, line 12. I don't think the temperature and pressure recorded by the UGGA's flow cell represent those in the chamber. Therefore, it is not appropriate to use T and P from the cell for the flux calculations. This is quite important. In work I have been involved with, we have always measured T and P in the flux chamber separately.

Authors: P and T of the cell are analytically essential to determine the gas concentration at the point of analysis in the analyser. P and T of the chamber are physiologically important to monitor changing conditions that may alter the physiology of the gas exchanges between the stem and the atmosphere.

To give more details about our choices we inserted the following text at P16028 L13 just after "…slope calculations": "The advantage of using the cell temperature is the perfect synchronicity of the airflow with the temperature measurement. In previous tests we showed that the cell temperature was strongly correlated (R2 = 0.994) to the chamber temperature measured with a small data logger (ST-171, Clas Ohlson, Insjön, Sweden). Besides, the analytical laser did not significantly increase the temperature of the closed circuit (cell, connection tubes and chamber), as the temperature drift over 20 minutes of enclosure was only +0.7 % under lab conditions (SATP). The chamber pressure was equilibrated to the outside monitored atmospheric pressure (Gas pressure sensor, Vernier, Beaverton, USA) via the vent tube." What is heating up is the internal ambient temperature of the gas analyser (Amb_T) and not the cell temperature (Gas_T).

To group information we moved the sentence at line 13 starting with "In the manual sampling…" to line 9 just after "420 seconds" and changed the "15 minutes" into "900 seconds" in that sentence.

Remark: In the lab, we tested the chamber permeabilities under SATP conditions (UIPAC); and there was not a significant possibility for the chambers to be heated up by the artificial light in the lab. In the field, we shaded the sleeves with a plasticized aluminium foils and the forest in which we worked had no more than 51 µmol $m^{-2}$ $s^{-1}$ of incoming light, which was not enough to change the chamber conditions in such a way that it would affect the gas exchange between the stem and the atmosphere. Additionally, the field measurements were done in the same forest locations (boreal or tropical) within a short period of time where no significant changes in air temperatures took place.

At P16027 L10 we inserted: "We shaded the sleeves with a plasticized aluminium foil to prevent any alteration of the chamber temperature and stem-gas exchange processes as compared to those prevailing without the enclosure. In the lab this measure was unnecessary."

For more precaution with the use of temperature measurements at P16036 L11 we added: "Under changeable conditions such as varying sunlight intensities we recommend to measure the temperature inside and outside of the sleeve, and to shade the sleeve as these variable conditions may alter the gas exchange processes between the stem and the atmosphere as compared to those prevailing without the enclosure."

Page 16028, line 19. Here and elsewhere in the document "Push" should simply be "push" (no cap needed).

Authors: Done

Page 16028, line 20. Here and elsewhere in the document "Off" should be "off". "ICOS" should be given in full – all acronyms should be when first used.

Authors: We replaced it with "Off-Axis Integrated Cavity Output Spectroscopy (OA-ICOS)" and as capitalized it as it is a proper noun (c.f. biogeosciences guidelines)

Page 16029, line 12. "Fick, 1855". Did you consult the original? If not then provide your more recent source.

Authors: Yes we consulted it. It is the original work and should be credited.

Page 16030, line 10. How did bark roughness affect chamber volume? In very rough barks such as on Pinus sylvestris and perhaps some tropical tree species I imagine this could lead to quite big differences to volumes estimated using equation (4).

Authors: During the measurements for this paper, the roughest barks we encountered were those of *Pinus sylvestris* and the variability in the thickness was around 3.4% (based on calibrated image analyses of photos taken on the side of sleeves). Note, the flakes of bark on *Pinus sylvestris* can be somewhat compressed against the stems. In terms of volume "roughness" means that you have bumps and hollows and the stem periphery should be set at half distance between bumps and hollows. The Neoprene foam will absorb the bumps and just cover the hollows so that it will sink into the stem on average by half of the height bump-hollow. In other campaigns we increased the thickness of the chambers to reduce that importance. In extreme cases we had to level the bark with mastic or play dough.

To give more detail at P16036 L6 we added: "In some other situations it was enough to increase the thickness of the sleeves to reduce the percentage of uncertainty in the chamber volume (Vc). The impact of both crevices and bumps could be assessed with distance measurements made on photos taken on one side of the deployed sleeves."

Page 16030, line 15. The sentence starting "By dividing" is difficult to follow. I recommend re-wording it or breaking it into two simpler sentences.

We changed the sentence as follows: "By dividing the absolute value of the bias through the predicted value we get an estimate of the inaccuracy of $V_{tot}$ (chamber, tubes and detector's cell). As all terms of the fraction (Eq. 4) are linearly dependent, the inaccuracy of the permeability (P) is the quadratic mean of all other terms (Table 1, footnote)."

Page 16031, line 7. " chamber, and that the"

Authors: Done

Page 16032, line 2. "concentration developments" is an odd phrase. I prefer "concentration changes" or "concentration increases".

Authors: we changed "concentration development(s)" into "concentration change(s)".

Page 16032, line 5. The r2 increase is actually reasonably large. Page 16032, line 9. "an exponential".

Authors: we changed it to: "…the coefficient of determination increased substantially"

Line 9: Done

Page 16033, line 5. "lightweight, and can be locally sourced" Page 16034, line 8. " associated to with the gas"

Authors: Done

Page 16035, line 17. "or by installing a complementary fan if the sleeves were to be built much larger" – miniature fans as used in larger laptops could be used.

Authors: Here we leave it open to how big the fan may be (whether 3.5'-drive, laptop or tower computer fans). We leave it unchanged.

Page 16036, line 2. "very rare". How rare is "very rare"? Quite a few wetland tree species can have rough bark such as alder (Alnus) and willow (Salix). Tropical forest trees also often have rough bark and those that are smooth may have lianas and other climbers growing up them which serve, in effect, to make the bark rough.

Authors: What we wrote was related to our own work and we understand that in the scope of generalisation it is better to change the text into: "In some situations,…"

Page 16036, line 19. Delete comma after "both".

Authors: Done

Page 16037, line 22. " and an optimal"

Authors: Done

Page 16038, line 16. The authors rightly highlight the portability of the flexible chambers but they don't discuss the problem of using on-line gas analysers like the UGGA manufactured by Los Gatos Research. These analysers are very accurate and give good data, but are actually quite heavy – at 15 kg without batteries and 17+ kg with batteries (excluding the re-inforced backpack needed to carry them). So, while the flexible chambers are highly portable, the recommendation that they be used with a heavy on-line analyser almost seems contradictory.

Authors: In terms of weight, to take a syringe with approx. 2x1000 12 mL glass GC vials together with a whole collection of rigid chambers (10x as heavy) and 4 big steal Handy-grips is actually heavier than a 17 Kg UGGA. The portable gas analysers bring in many advantages (closure time reduction, leakage tests, multiple gases, etc…). The problem with the logistics is not so much the weight but more a problem of volume. The gain in volume is considerable when using multiple semi-rigid chambers. Rigid chambers are difficult to transport on a plane and in the field, and need special care for them not to break or crack, plus cost much more to build. They need to be built in advance not knowing what field conditions to expect. A more extreme, although realistic example; it would need approximately 3000 litres chamber in order to enclose trees of 150 cm of diameter. So, based on our field experience, we suggest that carrying a UGGA is a small issue when compared with the logistics of using rigid chambers and vials for GC analysis.

Figure 4. The letters denoting the variables in the figure itself should be italicised.

Authors: Done

Tables S1 and S2. All letters denoting variables in the caption and the table itself should be italicised. S4 "Bad Pinus sylvestris" should be "Bad Pinus sylvestris". Also, why bad?

Authors: Done. We also changed Table 1 and the figure captions of Fig. 4, 5 and 6.

For the table S4 we cannot see what should be different as what reviewer 2 proposes is strictly the same. We will however change "Bad" into "Leaking".

Associate editor's comments

BG-2015-433

Below are my suggestions (page and line numbers refer to these in the track changes version):

Page 1 line 16: delete "describe"

Authors: Done.

Page 1 line 18: delete "more"

Authors: Done.

Page 1 lines 19-21: "We found that the semi-rigid chambers performed well, and had numerous benefits including reduced gas permeability and optimal stem gas exchange surface to total chamber volume ratio (Sc/Vtot) allowing better headspace mixing": Personally I do not like the hollow words like "the semi-rigid chambers performed well", you either delete them or be specific to let the readers know in what way and by what kind of parameters you claimed that the semi-rigid chambers performed well. Similarly I also do not like "numerous benefits", just directly list the most important advantages. Better just change to "had reduced gas permeability and optimal ratio of stem gas exchange surface to total chamber volume (Sc/Vtot)…".

Authors: Done.

Page 2 line 28: "access logistics are": change to "access logistics is"?

Authors: Done.

Page 14 Lines 376-378: The rigid chamber was 14.1x larger than the small sleeve and 9.7x larger than the large sleeve, and the initial concentration gradient in the rigid chamber was 14.0x smaller compared to the smaller sleeve and 9.01x compared to the bigger sleeve": 9.01 x smaller?(line 378). What does "14.1 x larger" really mean? (Please note that mathematically 6 is 3 times of 2, or 2 times larger than 2). Also "14.0x smaller" is somewhat weird. To be accurate, better change the style "14.1 x" in the whole text. For example, "The rigid chamber was 14.1x larger than the small sleeve" can be rewrite as "The rigid chamber was 14.1 times that of the small sleeve in volume"? And "the initial concentration gradient in the rigid chamber was 14.0x smaller compared to the smaller sleeve" can be rewrite as "the initial concentration gradient in the rigid chamber was only 1/14.0 of that in the smaller sleeve"?

Authors: We changed it the way you suggested.

Authors: As you suggested we re-checked carefully the manuscript and suggest small stylistic changes:

- In the title, there should be no "-" between tree and stems

- To simplify the text we changed the sentence of the track-changed version at L228 P9 to: "We shaded the sleeves with a plasticised aluminum foil to minimise any changes to chamber temperature which might otherwise affect stem-gas exchange processes.

- ➔ To simplify the text we changed the sentence of the track-changed version at L543 P20: “Under changeable conditions such as varying sunlight intensities we recommend that the temperature inside and outside of the sleeve is measured, and to shade the sleeve.  Otherwise, these varying conditions may alter the gas exchange processes between the stem and the atmosphere.”

# Technical Note: Semi-rigid chambers for methane gas flux measurements on tree stems

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## Abstract

There is increasing interest in the measurement of methane ($CH_4$) emissions from tree stems in a wide range of ecosystems so as to determine how they contribute to the total ecosystem flux. To date, tree $CH_4$ fluxes are commonly measured using rigid closed chambers (static or dynamic), which often pose challenges as these are bulky and limit measurement of $CH_4$ fluxes to only a very narrow range of tree stem sizes and shapes. To overcome these challenges we aimed to design and test new semi-rigid stem-flux chambers (or sleeves). We compared the $CH_4$ permeability of the new semi-rigid chambers with that of the traditional rigid chamber approach, in the laboratory and in the field, with continuous flow or syringe injections. We found that the semi-rigid chambers had reduced gas permeability and optimal stem gas exchange surface to total chamber volume ratio (Sc/Vtot) allowing better headspace mixing, especially when connected in a dynamic mode to a continuous flow gas analyser. Semi-rigid sleeves can easily be constructed and transported in multiple sizes, are extremely

Andy Siegenthaler 20/12/y 17:09
**Supprimé:** -

Andy Siegenthaler 8/1/y 14:33
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**Supprimé:** We compared semi-rigid chamber's gas permeability to $CH_4$ against the traditional rigid chamber approach

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light, cheap to build and fast to deploy. This makes them ideal for use in remote ecosystems where access logistics is complicated.

## 1 Introduction

Recent research into ecosystem greenhouse gas fluxes has shown that tree stems emit significant amounts of methane ($CH_4$) (Terazawa et al., 2007; Rusch and Rennenberg, 1998; Gauci et al., 2010; Pangala et al., 2013; Rice et al., 2010; Terazawa et al., 2015) although the transport mechanisms and global importance of tree-mediated emissions remain largely unknown. These past investigations have used a variety of closed chambers adapted to various tree-stem sizes. Presently, the most common chambers used to measure $CH_4$ emissions from tree-stems are closed rigid chambers in the form of either a vertical cylinder, a horizontal cylinder or a cube fitted around tree-stems (e.g. Gauci et al., 2010; Pangala et al., 2013; Terazawa et al., 2007; Hari et al., 1991; Rusch and Rennenberg, 1998). These chambers can be deployed either vertically by enclosing the whole stem or, alternatively when the stems are too large, laterally on the stem, covering only a small fraction of the stem surface (e.g. Levy et al., 1999; Teskey and McGuire, 2005; Ryan, 1990; Hari et al., 1991). These techniques were originally designed to measure $CH_4$ and carbon dioxide ($CO_2$) from samples manually taken with syringes and analysed by gas chromatography. The ratio between the gas exchange surface and the chamber volume ($S_c/V_{tot}$) was transposed from soil chambers and were not necessarily adapted to the lower fluxes found in tree-stems, and are therefore often too high (Hutchinson and Livingston, 2001). In other words, if the chambers are too large for a given exchange surface, mixing problems may occur, making it important to circulate the air in their headspace (Hutchinson and Livingston, 1993; Rusch and Rennenberg, 1998).

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With the advent of continuous flow analytical techniques and increasing precision of instruments (e.g. cavity ring-down spectroscopy, infrared and photo-acoustic gas analysers), the need for longer accumulation periods to detect significant concentration changes has become obsolete. The tendency is to reduce the accumulation period as much as possible in order to be able to use more straightforward linear regressions to determine fluxes closest to the point of chamber closure.  Unlike open chamber techniques which allow steady state measurements (e.g. Bortoluzzi et al., 2006; Norman et al., 1997; Subke et al., 2003; Pumpanen et al., 2004), closed chambers are non-steady state systems; the diffusive laws advocate the use of non-linear regressions of gas concentrations as a function of time to determine rates, as these decrease with increasing gas saturation (Hutchinson and Livingston, 2001; Pihlatie et al., 2013; Pumpanen et al., 2004; Kutzbach et al., 2007).

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With continuous flow gas analysers there are three main advantages: 1) they are non-dispersive as no gas needs to be taken out of the measurement system and irreversibly "consumed", 2) they circulate air between the chamber and the gas detectors, which for small chamber volumes could represent enough mixing to avoid underestimations of fluxes by as much as 36 to 58% in non-mixed soil-atmosphere exchanges (Christiansen et al., 2011), and 3) with measurement frequencies of up to 10 Hertz and precisions of ±2 ppb the closure time needed to get a representative accumulation slope has been dramatically reduced using these devices (excluding the equilibration period) and therefore it also avoids underestimations due to regressions made over longer periods of time (Hutchinson and Livingston, 2001; Pihlatie et al., 2013). In addition, recent work has focused on trace gases (e.g. $CH_4$ and $N_2O$) which have lower accumulation rates compared to the more frequently measured $CO_2$ (IPCC, 2007), moderating  the saturation issue inherent to non-steady state setups (Hutchinson and Livingston, 2001). Altogether, these point towards the use of a smaller stem chamber with larger gas exchange surface per chamber volume proportion (Sc-to-Vtot ratio).

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A further complicating factor is field access.  Stem-methane emissions have recently begun to be investigated in remote areas such as in forested tropical wetlands with often no road access. In those areas it is a logistical challenge to carry large/heavy loads. Moreover, because of the great variety of stem sizes/shapes, a whole collection of rigid chambers is usually needed to cover most of the ecosystem tree species thus creating further logistical and cost issues.

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In order to meet the new challenges presented by the growing interest in measuring greenhouse gas fluxes from tree-stems we aimed to design, describe and test/deploy new semi-rigid stem-emission chambers in the laboratory and in the field, and to compare their permeability to $CH_4$ (gas conductance) with previously described rigid chambers. Thus far, semi-rigid sleeve chambers have been used effectively in several of our measurement campaigns. We therefore consider their detailed reporting to be of interest to a broader constituency of eco-physiologists and biogeochemists. We also examine various methodological benefits and logistical advantages of using this new approach.

## 2 Materials and methods

### 2.1 Chamber designs: semi-rigid sleeve and rigid chamber

Our approach to measure stem $CH_4$ emissions, which could also include other greenhouse gases produced in anaerobic conditions such as $N_2O$, uses a semi-rigid chamber (or sleeve). The preferred material was a pre-shaped and gas impermeable PET (polyethylene terephthalate) or PC (polycarbonate) plastic sheet with a natural tendency to curve induced by 3-4 vertically distributed imprinted rims on the periphery. These rims ensured good stability and helped maintain the desired natural curvature of the sleeve that proved to be very helpful for the deployment of the sleeves on the stems as the sleeve could hold in place without straps.

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To investigate permeability changes due to both the size and the approach, we used two semi-rigid sleeves together with a rigid chamber. As this was straightforward, for the smaller semi-rigid sleeve we sourced the pre-shaped material from a cylindrical 3 L soft drink bottle, which already had the desired imprinted rims. The 0.1 mm thick bottle was truncated above and below the cylindrical section, and opened vertically on the side. For the larger sleeve we sourced the material from 0.2 mm thick not pre-shaped semi-rigid PC sheets. Both types of plastic sheets have very low gas permeabilities under experimental standard ambient temperature and pressure (SATP from UIPAC) conditions and short chamber enclosure times (McKeen, 2012).

The edges of the sheets were framed with 1.5 cm thick and 3 cm wide adhesive backed expanded Neoprene strips (Seals+Direct Ltd, Hamphshire, UK); closed cell neoprene foam that is gas tight and can be bent, but is hardly compressible (≤ 3 % with 200 N). This Neoprene strip was placed as a frame around the rectangular sheet to provide a seal and to ensure a constant volume between the sheet and the tree stem (Fig. 1). The adhesive was provided on one side of the expanded Neoprene strips. Inside this framed volume we placed two Neoprene vertical wedges (1.5 cm thick and 3 cm wide) to keep the sheet equidistant from the stem all along the radial periphery of the sleeve. The sleeve was also equipped with two snap-on rubber caps with inserted three-way Luer-lock stopcocks (BBraun, Bethlehem, USA) that permitted connection to the Ultraportable Greenhouse Gas Analyser (UGGA, Los Gatos Research Inc., Mountain View, USA) via two 4.6 m long and 5 mm inside diameter PTFE (polytetrafluoroethylene) coated PVC (polyvinyl chloride) parallel tubes (Nalgene, Rochester, USA). As venting was recommended (Hutchinson and Livingston, 2001; Christiansen et al., 2011) both sleeves were equipped with a coiled vent tube (18 cm long, 1.2 mm inner diameter). We downscaled the vent described by Hutchinson and Livingston (2001) by a factor 48 in terms of vent volume whereas the sleeves were a factor 10 to 20 less

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voluminous as compared to the authors' chamber (14 L). Their study showed that in a perfectly sealed chamber, after 30 minutes of deployment the gas mass loss through the sole vent represented 0.038% of the target gas.

We tested all the components of the semi-rigid sleeves independently for unwanted background contaminations that could interfere with $CH_4$ emissions from the stems by incubating them for two hours in 500 mL borosilicate glass beakers filled with air and connected in continuous flow with the UGGA. The selected raw material was inert and did not interfere with measurements from the environment. We also tested the compressibility of sleeves by pulling the straps with a 200 N force (twice 100N) and measuring the thickness of the Neoprene frame before and after pulling (Fig. 2, see also chamber deployment section).

We also compared the $CH_4$ losses from our new semi-rigid sleeves with a previously used rigid chamber design, similar to the ones constructed and described in other studies (Rusch and Rennenberg, 1998; Gauci et al., 2010; Pangala et al., 2013). The closed rigid chamber was constructed from cylindrical Perspex® (Perspex, Tamworth, UK) of inner diameter of 28 cm and had an inner height of 30 cm. The cylinder was cut into two halves, which were held together with a metal hinge. The two half-cylinders were framed within a 5 cm wide and 1 cm thick frame made of flat Perspex® that was fitted with Neoprene strips. The cylindrical chamber had a central opening to enclose the tree stem. Two smaller cylinders (18 cm diameter x 5 cm height) were attached on either side of that opening (Fig. 3). The chamber was equipped with a gas sampling port and a small vent tube (12 cm long; 6 mm diameter).

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## 2.2 Enclosed chamber volume and gas exchange surface determinations

The volume of the semi-rigid sleeves could be determined precisely in two different ways. Firstly, we extrapolated the empirical total chamber volume ($V'_{tot}$) from the $CH_4$

concentration dilution factor after having inserted a known volume ($V_{standard}$) of a 2000 ppmv $CH_4$ standard (Air Liquide, Paris, France) into the sleeve's enclosed volume and measuring the end concentration ($C_0$) after dilution, and subtracting the atmospheric $CH_4$ concentration ($C_{atm}$) originally in the chamber. The two semi-rigid sleeves and a rigid chamber were attached to an inert stainless steel cylinder (see chamber deployment). The dilution was done in 90 seconds so that the losses through gas permeability of the chambers remained negligible. This extrapolation was formalised as:

$$V'_{tot} = V_{standard} * \frac{(C_{standard})}{(C_0 - C_{atm})} \quad (1)$$

Secondly, we also calculated the theoretical volume of the sleeves ($V_c$) by subtracting a sector (K) of both, a smaller cylinder volume ($V_{stem}$) from a larger cylinder volume ($V_{ext}$), minus the volume taken by the vertical wedges ($V_{wedges}$) (Fig. 4). The sector (K) was determined from a ratio between the sleeve length (L) and the circumference at the external edge of the sleeve ($\pi D_{ext}$). The sleeve length (L) is the length of the incompressible external edge of the chamber and represents a fraction of the total circumference given by $\pi D_{ext}$. The diameter of the smaller cylinder (the compressible internal foamy edge) is given by the diameter of the stem ($D_{stem}$). The larger cylinder diameter ($D_{ext}$) is the diameter given by the stem ($D_{stem}$) plus the thickness (T) of the sleeve. Both cylinders have the same height (H). Thereafter, we have:

$$D_{ext} = D_{stem} + 2T \quad (2)$$

$$K = \frac{L}{\pi D_{ext}} = \frac{L}{\pi(D_{stem} + 2T)} \quad (3)$$

$$V_C = K(V_{ext} - V_{stem}) - V_{wedges} = \frac{HL}{(D_{stem}+2T)} * \left[\left(\frac{D_{stem}+2T}{2}\right)^2 - \left(\frac{D_{stem}}{2}\right)^2\right] - V_{wedges} \quad (4)$$

However, the total volume ($V_{tot}$) is the sum of the chamber volume ($V_c$) plus the dead volume enclosed in the gas analyser and the tubes ($V_{dead}$):

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$$V_{tot} = V_C + V_{dead} \quad (5)$$

Similarly, the gas exchange surface of the sleeves ($S_c$) was calculated by considering the sector (K) of the stem surface ($S_{stem}$) covered by the chamber at the circumference of the stem ($\pi D_{stem}$) and the height of the sleeve (H), minus the small surface covered by the vertical wedges ($S_{wedges}$):

$$S_c = K * S_{stem} - S_{wedges} = \frac{HL}{(D_{stem}+2T)} * D_{stem} - S_{wedges} \quad (6)$$

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### 2.3 Chamber deployment

The three types of chambers (two semi-rigid sleeves and one rigid chamber) were deployed on a gas-inert stainless steel cylinder of diameter 15 cm. The semi-rigid chambers were flattened around the cylinder and subsequently attached and tightened with two metal cam straps at the top and bottom of the frame (Figure 2). The straps were 1.5 m long and 3 cm wide. An additional strap was necessary at mid-height of the bigger sleeve to ensure a good cohesion of the vertical Neoprene frames and vertical wedges with the stem (steel cylinder in this case).

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**Supprimé:** To ensure optimal gas tightness it was important to distribute the pressure of each strap all around the surface of the sleeve. We visually checked for gaps between the stem and the Neoprene strips. Monitoring the $CH_4$ concentration development (increase or decrease, as a cohort of dependent concentrations) in a continuous flow mode made an optimal gas tightness test.

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Before installing the rigid acrylic chamber, closed cell Neoprene foam bands (7 cm wide and 4 cm thick) were attached at the bottom of the inert stainless steel cylinder and also at 35 cm height using double-sided Scotch tape (3M, St-Paul, USA) to append the extremities of the band as well as packing brown tape (5 cm wide) to tighten the band firmly against the metallic cylinder. The two mobile panels of the chamber were opened and the upper and lower half-necks of one panel were lodged around the two foam bands by compressing the foam so as to ensure gas tightness. Finally, all open-end flanges surrounding the cylindrical volume were progressively closed with Handy-grips (Irwin, Vernier, Switzerland).

We used the larger semi-rigid chamber to exemplify the field deployment (Table 1). We deployed it on twelve tree-stems (diameter at breast height: 25-45 cm) located in the northern boreal zone (*Pinus sylvestris* and *Betula pendula*, Degerö mire, Sweden) as well as in a tropical lowland forest (*Heisteria concinna,* Barro Colorado Island, Panama). The sleeves were placed at mid-height on the stems at 35 cm of height. We shaded the sleeves with a plasticised aluminum foil to minimise any changes to chamber temperature which might otherwise affect stem-gas exchange processes. In the lab this measure was unnecessary. We tested the sleeve's $CH_4$ concentration change on both, very smooth birch stems and very rough pine-tree stems to contrast the concentration readings as much as possible. To ensure optimal gas tightness it was important to distribute the pressure of each strap all around the surface of the sleeve. We visually checked for gaps between the stem and the Neoprene strips. Monitoring the $CH_4$ concentration change in a continuous flow mode made an optimal gas tightness test. A leaking chamber (mainly pressure-driven bulk flow following Hagen-Poiseuille's law) typically displayed fluctuating concentrations with concentration build-up being recurrently drawn down. Finally, we also used the larger semi-rigid sleeve together with a manual syringe sampling. For that purpose we used a 30 mL plastic syringe fitted with a Luer-lock three-way stopcock (BBraun, Bethlehem, USA) and connected it to one of the two stopcocks on the sleeve. At t=0, t=5, t=10 and t=15 minutes we collected 12 mL of gas sample from the sleeve and transferred it into pre-evacuated glass Exetainers (Labco Ltd, Ceredigion, UK) before analysing $CH_4$ concentrations on a Fast Methane Analyser (Los Gatos Research Inc., Mountain View, USA) equipped with a sampling loop as described in Baird et al. (2010).

## 2.4 Gas Analyses

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For the permeability tests, the $CH_4$ concentration change was analysed in the laboratory under SATP conditions for three types of chamber (Table 1, Supplement S1); a rigid chamber and two semi-rigid sleeves. We injected 50 mL of a 2000 ppmv methane standard (Air Liquide, Paris) into these chambers after which the $CH_4$ concentration decline was measured over 20 minutes in continuous flow mode. Each chamber type was tested in triplicate. For the blanks, we injected ambient air. The slopes were measured from a linear regression of declining concentrations starting after an equilibration time of 90 seconds (dead band) and running for 20 min. This dead band represents a maximum time for the continuous flow circuit to mix the entire headspace ($V_{tot}$).

In the field, the $CH_4$ concentration changes of a larger sleeve were monitored when deployed on various tree-stem species (see chamber deployment). In order to have a set of contrasting responses we selected, a posteriori, measurement runs with both high and low rates, and also included runs where leakages of the sleeve were present (Figs. 5 and 6, Supplements S3 and S4). Methane concentration accumulations were measured as in the laboratory although with shorter runs of approximately 420 seconds. In the manual sampling mode with syringe, the accumulation period was 900 seconds. The slopes were measured from linear, quadratic and exponential regressions of increasing concentrations starting after a dead band of 90 seconds. The gas pressure, temperature and humidity inside the stem sleeve were measured from the circulated gas running through the UGGA's flow-cell and we used temperature, pressure and humidity compensated $CH_4$ concentrations for the slope calculations. The advantage of using the cell temperature is the perfect synchronicity of the airflow with the temperature measurement. In previous tests we showed that the cell temperature was strongly correlated ($R^2$ = 0.994) to the chamber temperature measured with a small data logger (ST-171, Clas Ohlson, Insjön, Sweden). Besides, the analytical laser did not significantly increase the temperature of the closed circuit (cell, connection tubes and chamber), as the temperature drift

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over 20 minutes of enclosure was only +0.7 % under lab conditions (SATP). The chamber pressure was equilibrated to the outside monitored atmospheric pressure (Gas pressure sensor, Vernier, Beaverton, USA) via the vent tube.

All chambers were connected to an UGGA via two flexible tubes (see chamber designs section) set in parallel in a continuous flow mode; one tube bringing air from the gas analyser towards the chambers and the other tube pumping air from the headspaces towards the gas analyser. The tubes were connected to the gas analyser via two ¼ inch push-connect fittings. The UGGA's pump ensured a continuous flow of 2-4 L min$^{-1}$. The UGGA measured $CH_4$ with the Off-Axis Integrated Cavity Output Spectroscopy (OA-ICOS) at a frequency of 0.33 Hz. The analyser's uncertainty in the range of 0.01 ppmv to 100 ppmv of methane is <1% without calibration and the precision is ±0.6 ppb over a period of 100 seconds (LGR, 2013).

## 2.5 Methane permeability calculations

In order to quantify and compare $CH_4$ losses from the three types of chambers (two semi-rigid sleeves and one rigid chamber) attached to an inert stainless steel cylinder we corrected the loss rates by taking into account both the stem exchange surface covered by each sleeve (or chamber) as well as the concentration gradient between inside and outside of each chamber. To express this we calculated the permeability as a function of the effluxes (outgoing fluxes) and the concentration gradient between inside and outside the chambers.

In the first step we multiplied the slope (mg m$^{-3}$ s$^{-1}$) by the total volume of the chamber ($V_{tot}$) to get the loss rates (mg s$^{-1}$). We then divided the loss rates from each sleeve (or chamber) by the stem exchange surface ($S_c$) covered by each sleeve (or chamber) to express the methane flux (J) which can be used for both the permeability experiment on the metallic cylinder and the methane accumulation runs from tree-stems in the field:

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$$Loss\ rate = slope * V_{tot} = \frac{dC}{dt} * V_{tot} \left[\frac{mg}{s}\right] \quad (7)$$

$$Flux\ (J) = \frac{Loss\ rate}{S_c} = \frac{dC}{dt} * \frac{V_{tot}}{S_c} \quad \left[\frac{mg}{m^2 s}\right] \quad (8)$$

In the second step, from Fick's first law (Fick, 1855) we could apply the general equation often used in cell biological or textile fabric applications (Ogulata and Mavruz, 2010) to calculate, for each sleeve (or chamber), the $CH_4$ permeability (P) through a porous medium by dividing the $CH_4$ flux (J) by the $CH_4$ concentration gradient ($\Delta C$) between inside ($C_{chamber}$) and outside of the sleeve ($C_{atm}$). We assume that the diffusive $CH_4$ losses (including dilutions) through the rigid and semi-rigid material are negligible at SATP conditions (McKeen, 2012). Thereafter the equation was:

$$J = -P * \Delta C \rightarrow Permeability\ (P) = -\frac{J}{(C_{chamber} - C_{atm})} \left[\frac{m^3}{m^2 s}\right] \quad (9)$$

## 2.6 Numerical analyses

We used linear, quadratic and exponential regressions to fit the $CH_4$ concentrations as a function of the accumulation time in the chambers. The fitting was based on sum of squares' minimisation of the errors. The frequency distribution, homogeneity and homoscedacity of the residuals were previously checked using normal quartile plots, residual versus predicted plots, and box plots. The coefficient of determination ($R^2$) was used to quantify the level of fit. All the data was analysed with the SAS software (SAS Institute Inc., Toronto, Canada).

# 3 Results

## 3.1 Calibration of the semi-rigid sleeves

The compared predicted (theoretical) and the mean observed empirical $V'_{tot}$ (Eqs. 1 and 4) were respectively: 966 and 933 $cm^3$ for the small sleeve, 1406 and 1439 $cm^3$ for the large sleeve, and 13581 and 13026 $cm^3$ for the rigid chamber (Supplement S1). The observed $V'_{tot}$ values included variability due to the possible but very tiny compaction of the Neoprene foam over the whole frame. This compaction was less than 3% of $V_{tot}$, which was a maximum considering the pulling force of 200 N applied on the straps (twice 100N).

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The difference between the mean observed $V'_{tot}$ and the predicted $V_{tot}$ values gave us an estimate of the bias in size. By dividing the absolute value of the bias through the predicted value we get an estimate of the inaccuracy of $V_{tot}$ (chamber, tubes and detector's cell). As all terms of the fraction (Eq. 4) are linearly dependent, the inaccuracy of the permeability (P) is the quadratic mean of all other terms (Table 1, footnote). The gas exchange surface ($S_c$) could be precisely determined and we assume that there is no error associated with it. The inaccuracies in the concentration measurements are dependent on the uncertainty of the UGGA, which in our case was <1% for the un-calibrated device.

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**Supprimé:** By dividing the absolute value of the bias through the predicted value we get an estimate of the inaccuracy of both, $V_{tot}$ (chamber, tubes and detector's cell) and the volume dependent inaccuracies of fluxes (J) and permeability (P) as they are linearly dependent of each-other (Table 1). The gas exchange surface ($S_c$) could be precisely determined and we assume that there is no error associated to it. These inaccuracies were also dependent on the uncertainty of the UGGA, which in our case was <1% for the un-calibrated device. Thereafter, the overall inaccuracy for the permeability can be summed up (Table 1, footnote).

The precision of our measurement system, related to repeatability, is the level to which repeated measurements show the same results under the same conditions. For each sleeve or chamber we repeatedly injected 50 mL of a 200 ppmv standard and measured the initial concentration ($C_0$, Table 1, Supplement S1) in the enclosed volume. We used the relative standard error (RSE) of the initial concentration ($C_0$) to express the level of precision between different types of chambers. Thereafter, precision is of ±1.82% for the small sleeve, ±1.59% for the large sleeve and ±1.68% for the rigid chamber.

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## 3.2 Chamber permeability comparisons

The comparison of permeability (Table 1, Supplement S1) of the three types of chamber shows that the semi-rigid sleeves are on average less permeable than the rigid chamber, and that the smaller semi-rigid sleeve had a higher permeability compared to the larger one. It was also interesting to note that the $CH_4$ loss (negative slope) is lower for the rigid chamber compared to semi-rigid sleeves. The contrasting higher permeability of the rigid chamber was counterbalanced by the much greater $V_{tot}$ as well as a much lower initial concentration gradient between inside and outside of the chamber ($dC = C_0 - C_{atm}$). The rigid chamber was 14.1 times that of the small sleeve in volume and 9.7 times that of a large sleeve, and the initial concentration gradient in the rigid chamber was only 1/14 of that in the smaller sleeve and 1/9 of that in the bigger sleeve. Moreover, the larger sleeve had a larger $S_c$-to-$V_{tot}$ ratio (0.42) compared to the smaller sleeve (0.34).

In order to understand why the permeability of the semi-rigid sleeves was lower than that of the rigid chamber we compared and calculated the potential contact distances between air from inside and outside of the chamber volumes (Fig. 3, Supplement 2). Those contact zones represented the paths where gas effusion could occur, which were driven by the architecture of the chamber. For that purpose we distinguished two types of contact lines: 1) mobile lines that needed to be sealed properly every time the chambers were deployed and from which most of the losses were likely to occur, and 2) fixed lines that resulted from the manufacture which could be cracked and leak as a result of twisting forces on the rigid joints. The result was that for the same theoretical stem gas exchange surface ($S_c$) between the two chambers (same length and height), the ratio between the length of the mobile lines and the stem gas exchange surface ($S_c$) was 2.17x smaller for the semi-rigid as compared to the rigid approach.

### 3.3 Stem-methane emissions and field deployments

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In the field, the manual sampling by syringe showed steady concentration changes with the sleeve technique (Fig. 5, Supplement S3), and the linear fitting of those concentration changes was always high ($R^2 \geq 0.924$). When applying a quadratic fit the coefficient of determination improved substantially ($R^2 \geq 0.995$). In continuous flow mode the concentration changes were also consistent with the sleeve technique (Fig. 6, Supplement S4), and the linear fitting was very high ($R^2 \geq 0.989$) for all the runs not displaying leakages. Equally to the manual sampling mode, in continuous flow mode, the fitting improved slightly when applying a quadratic function or an exponential function ($R^2 \geq 0.998$).

The two modes also distinguish themselves by the fact that with the continuous flow mode the runs are shorter compared to the manual mode. The runs were set to 15 minutes closure for the manual mode and to 7 minutes closure for the continuous flow mode.  These times included a maximum of 90 seconds equilibration time just after the sleeve was deployed to allow the headspace to mix properly.

Runs 3 and 6 of the continuous flow mode were deliberately presented to display situations where leakages from sleeves were occurring when placed on *Betula pendula* or *Pinus sylestris* tree-stems (Fig. 6). In those cases, the $CH_4$ concentrations developed in a disordered way with periods of increases immediately followed by sudden drops. These analytically monitored leakages were confirmed when checking the chamber fitting on the stems.

The determination of the coefficient of variation of the root-mean-square error CV(RMSE), often used to measure the relative differences between two populations of values, and which was calculated between the linear fitted slopes and the non-linear fitted slopes, was higher in the case of the manual sampling mode (0.69) as compared to the continuous flow mode (0.45). In other words, the difference between the linear and non-linear fittings was 53% higher in the manual mode as compared to the continuous mode. This went in parallel with the differences

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between the average slope in the linear fitting and that from non-linear fitting which was 27% higher with the manual sampling mode as compared to 18% with the continuous mode.

# 4 Discussion

## 4.1 Semi-rigid sleeve construction

The semi-rigid sleeves are easy to assemble, lightweight, and can be locally sourced. The sleeves could easily be assembled on-site following transportation. This allows for minimal luggage or shipping space and low costs, a major asset in terms of logistics where remote fieldwork is concerned. The PET or PC sheets were precisely cut in advance whereas the framing with the Neoprene strips was done on-site. We made sure that all components were not emitting $CH_4$, which might otherwise confound in-situ measurements. Nevertheless, the raw materials are commonly available internationally, could be found on-site and likewise tested. For small sleeves (stem diameters ≤ 15 cm) and middle-sized sleeves (stem diameters ≤ 25 cm) the pre-shaped PET sheet can easily be constructed from soft drink PET bottles or PC water-fountain tanks. Larger sleeves (stem diameters > 25 cm) can be built from flat PC sheets as the curvature and volume stability of the chamber becomes less compromised with larger stem diameters. Most important for the construction of the sleeves are the vertical wedges that keep the sheet equidistant from the stem along the radial periphery of the sleeve. The construction of a sleeve took about one hour and there was no requirement for specific machine tools and no adhesives were needed, as the Neoprene bands used were adhesive backed. For the production of large numbers of sleeve rectangular plastic sheets could be thermoformed using a specially designed mould (Throne, 1996).

The average $CH_4$ mass losses (2.2-3.3 %) from the sleeves after 20 minutes of deployment were two orders of magnitude greater as compared to the 0.038% mass loss after 30 minutes of deployment reported by Hutchinson and Livingstone (2001) for a perfectly sealed chamber with a sole vent tube. Thereafter, our downscaled vent tube was proportioned to the $CH_4$ losses from the sleeves.

### 4.2 Calibration of the semi-rigid sleeves

All the chambers were reasonably precise (repeatable) in terms of total volume and the semi-rigid chambers (sleeves) performed equally compared to the rigid chambers. In terms of total volume inaccuracy, all chambers were below the threshold significance level of 5%. Moreover, the semi-rigid sleeves' total volume accuracy increased with increasing $S_c/V_{tot.}$ Nevertheless, getting good accuracy is a matter of calibration as biases can be subtracted from the original readings.

The average 33 $cm^3$ greater $V'_{tot}$ values as compared to $V_{tot}$ for the large sleeve (Supplement S1) can be attributed to the volume of the wedges that were also undergoing a compaction when deployed as the interior periphery gets compressed. This tiny volume correction was not inserted in formula 4 for the sake of simplicity and because the difference with the calibration was still below 5%.

We added a known amount of $CH_4$ instantaneously to the chambers and followed its decline and associated chamber permeability. Thereafter, we can be aware of how well the chambers are doing in keeping the considered gas but not how well they do in minimizing the errors associated with the gas exchange processes between stems and the chamber. For those errors we referred to recommendations from other studies, such as: ensuring air-mixing, venting, reducing closure times, reducing chamber volume and considering non-linear fitting (Christiansen et al., 2011; Hutchinson and Livingston, 2001; Juszczak, 2013; Pihlatie et al., 2013).

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### 4.3 Chamber permeability comparisons

A reasonable mechanistic explanation to the fact that both semi-rigid sleeves were on average 57% less permeable compared to the rigid chamber (Table 1) could come from the sleeve's smaller proportion of air contact lines between inside and outside the chambers thereby reducing opportunities for gas diffusion to occur. The difference in that proportion is similar in order of magnitude to the difference in permeability (Supplement S2). Moreover, it is possible that with an aging rigid chamber the permeability could increase faster than in the case of an aging semi-rigid sleeve as the proportion of fixed contact lines could be exposed to more cracks and unforeseen reduced air-tightness (Fig. 3, green lines).

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This is also in line with the fact that for the same semi-rigid chamber design with the increasing $S_c$-to-$V_{tot}$ ratio, thus by increasing frame size, there is a concurrent decrease in the proportion of contact lines as well as a concurrent decrease in permeability. The rigid chamber had a much lower $S_c$-to-$V_{tot}$ ratio when compared to the sleeves and showed the greatest permeability. From our observations we can generalise the common trend found for all chamber types by saying that the larger the total volume of a stem chamber is, for a given gas exchange surface, the greater the expected permeability.

With the same logic and by considering the strong leverage effect of the concentration gradient ($\Delta C$) between inside and outside the chamber, the advantage of the larger rigid chamber is that it keeps the concentration gradient more constant during the chamber deployment and therefore minimizes the non-steady-state gas saturation effect of the closed chamber system. However, this advantage loses its importance when semi-rigid sleeves are connected to precise gas analysers with analytical frequencies of up to 10 Hertz as the gradient effect can equally be minimized by reducing the closure times to a few minutes.

Additionally, by increasing the $S_c$-to-$V_{tot}$ ratio by 6 fold compared to rigid chambers and by mixing the enclosed gas through the continuous flow circulation, we also avoided the problems associated with large volume chambers (Hutchinson and Livingston, 2001, 1993).

Nevertheless, the only non-compressible time factor is the sleeve's equilibration period; a 90 second period for the continuous air circulation to mix the entire headspace. This could be shortened by reducing the tube length, increasing the pump's flow-through or by installing a complementary fan if the sleeves were to be built much larger. In any case, the threshold time by which the sleeve headspace is mixed entirely can be monitored graphically while running every sample. Retrospectively, 90 seconds of equilibration, together with 3-minute closure time, conservatively characterised all replicates made for two different sleeve sizes (n=24).

### 4.4 Deployment in the field

As expected, deployment of the semi-rigid sleeve was very straightforward and could be operated by a single person. The fact that the sleeves had a natural tendency to curve (pre-shaped) allowed them to stay in place when initially placed around the stem. This gave the researcher free hands to attach the straps subsequently. The whole setup takes two minutes to install and swapping the sleeves between different stem heights was also done much more efficiently in comparison to the rigid chamber deployment.

In theory all stem sizes could be fitted, the only limitation comes from the stem texture and this is valid for both semi-rigid sleeves as well as rigid chambers. In some situations, the tree bark had large crevices and it was necessary to prepare the stem prior to attachment of the sleeves or rigid chambers. The preparation was made by filling the crevices with mastic or play dough in the shape of a frame before the chamber or sleeve could be sealed to the stem. In some other situations it was enough to increase the thickness of the sleeves to reduce the

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percentage of uncertainty in the chamber volume ($V_c$). The impact of both crevices and bumps could be assessed with distance measurements made on photos taken on one side of the deployed sleeves.

Using five sleeve sizes it was possible to cover stem diameters ranging from 5 cm to 127 cm at breast height (DBH). Moreover, in terms of weight the two sleeves we tested were respectively 156 and 297 grams, compared to 3.3 kg for the rigid chamber. As a consequence, the whole collection of sleeves fitted in a single backpack and was light to carry.

Under changeable conditions such as varying sunlight intensities we recommend that the temperature inside and outside of the sleeve is measured, and to shade the sleeve. Otherwise, these varying conditions may alter the gas exchange processes between the stem and the atmosphere.

### 4.5 Sampling modes and regression fits

In both cases, for manual sampling and continuous flow (Figs. 5 and 6), methane accumulation rates were better fitted with non-linear functions (quadratic or exponential). This confirms that the sleeve's closure system was sealing properly against the stems, as the headspace concentration change of a closed non-steady-state chamber (static chamber) will always remain non-linear and this is driven by the laws of diffusion (Hutchinson and Livingston, 2001). For the semi-rigid sleeve, the difference between both the $R^2$ and the slopes between the linear fitted and the non-linear fitted concentration changes were roughly twice as small compared to those reported in the literature for soil chambers (Christiansen et al., 2011; Hutchinson and Livingston, 2001; Juszczak, 2013; Pihlatie et al., 2013).

Furthermore, the impact of the manual syringe sampling on the pressure fluctuation in the sleeve could be somewhat minimised by the fact that the chamber volume ($V_c$), where the actual air mixing occurred, was increased by the additional dead volume added from the

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analyser and tubing in continuous flow mode. Thus, the total volume ($V_{tot}=V_c+V_{dead}$) was increased as much as 76% with the smaller sleeve. With rigid soil chambers this aspect is often not mentioned as in those cases the dead volume is negligible compared to the large chamber volume. In our case, for the manual sampling, over a 15 minutes period, we drew 1.8% of the total volume from the larger sleeve (4 steps of 0.44%), which in terms of mass loss remains below the significance level of 5% and could be accounted for if more accuracy is needed. Although the repeated gas sampling minimises somewhat the pressure build up, recent studies have recommended avoiding manual sampling as much as possible because of associated pressure fluctuations (Christiansen et al., 2011; Juszczak, 2013).

The coefficient of variation of the root-mean-square error CV (RMSE) gave 53% higher coefficients for the manual sampling mode compared to the continuous flow mode thus indicating that the discrepancy between the linear fitting and the non-linear fitting is higher for the manual sampling mode. Moreover, as reported by some authors, fluxes calculated using linear fitting together with non-steady state chambers could be underestimated by as much as 40% (Christiansen et al., 2011; Pihlatie et al., 2013; Kutzbach et al., 2007). In our case, the underestimation was 27% for manual sampling mode and 18% for the continuous flow mode. As a consequence we would recommend using non-linear fitting (quadratic or exponential) together with manual sampling of the semi-rigid sleeves. In continuous flow mode, it is better to reduce the closure times as much as possible if planning to use linear fitting for greater simplicity. Both measures will contribute to improving line-fitting and estimating $CH_4$ accumulation rates.

# 5 Conclusions

Although all chamber types performed well, the semi-rigid design had numerous benefits including reduced gas permeability and an optimal $S_c$-to-$V_{tot}$ ratio.  Furthermore, they can be easily constructed and transported in multiple sizes, are extremely light, cheap to build and fast to deploy. As an example, in three of our tropical campaigns it was possible to carry a complete collection of semi-rigid sleeves in a single backpack. The collection covered the sampling of all ecosystem stem-sizes. Alternatively, we could also build the chambers on-site after prior testing of the compounds for background emissions. The PET and PC sheets of the sleeves are sturdy and lasted the duration of the campaigns, while the closed-cell Neoprene strips could be used for several weeks in the field before they needed to be replaced.

Connecting the sleeves in continuous flow mode to fast and precise laser-spectroscopic gas analysers (CRD or OA-ICOS technologies) enables the combined analysis and air mixing of the sleeve's enclosed volume, as well as reducing the closure periods to no-more than three minutes, making linear fitting from initial rates less problematic. To ensure optimal accuracy of the concentration measurements, it is best to calibrate each individual sleeve's total volume by diluting a standard gas in the entire setup (chamber, connectors, tubes and analyser) prior to starting a measurement programme.

Finally, to make good estimates of the global importance of tree-stem $CH_4$ emissions, it is essential to make measurements that cover all types of trees (species and morphotypes) present within the often remote ecosystems explored. This necessitates great adaptability in the chamber sizing and transport logistics. The semi-rigid sleeves meet these requirements without compromising the quality of the data collected.

## Acknowledgments

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**Figure captions**

**Figure 1**. Smaller semi-rigid stem sleeve attached to a stem. The plastic PET sheet (a) has three imprinted circular rims (b) that ensured good stability and natural curvature of the sleeve. The circumference of the sheet was framed with a 1.5 cm thick and 3 cm wide expanded Neoprene strip (c) that sealed off the headspace located between the sheet and the stem. Inside this volume there were two vertical wedges (d) that kept the sheet at equidistance from the stem along the radial periphery of the sleeve. In its centre the sleeve was equipped with two

Snap-on rubbers with inserted three-way stopcocks (e) that were further connected to PVC tubes that went from the sleeve to the Ultraportable Greenhouse Gas Analyser. A coiled vent was placed in one corner of the sleeve (f) to regulate the pressure. The chamber was tightened to the stem with the help of two straps that perfectly aligned on top of the horizontal strips.

**Figure 2**. The three steps of the semi-rigid stem sleeve deployment. To ensure a good contact between the frame strips and the stem it was important to distribute the pressure of each strap all around the frames' periphery when tightening the sleeve. Close to the centre two Snap-on rubbers with inserted three-way stopcocks were pressed into the PET or PC plastic sheet. These stopcocks were connected to the two PVC tubes that circulated air in a continuous flow mode when connected to an Ultraportable Greenhouse Gas Analyser (UGGA).

**Figure 3**. Potential air contact path lines (chamber air versus ambient air) where gas diffusion can occur; a comparison between the acrylic rigid cylinder approach and the semi-rigid sleeve approach. The red lines represented the mobile contact lines that needed to be sealed properly every time the chambers were deployed and where most of the losses were likely to occur. The green lines represented the fixed contact lines which could have been leaking as a result of twisting forces on the joints leading to cracks.

**Figure 4**. 2-D Layout for the chamber volume ($V_c$) calculation based on the stem diameter ($D_{stem}$), the thickness of the chamber ($T$), the sector covered by the chamber ($K$) and the volume of the wedges ($V_{wedge}$). Refer to the text for the volume calculations.

**Figure 5**. Contrasting methane concentration changes in the semi-rigid sleeve from enclosed gas samples measured in a manual mode (syringe) from tree-stems. In the first six runs (top quadrants) the concentration changes were regressed with a linear fit, while in the second set of runs they were regressed with a quadratic fit (non-linear). All runs 1-6 were measured on *Heisteria concinna* stems from a tropical lowland forest. The blue line corresponds to 95%

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confidence intervals, *RMSE* = root-mean-square error, $R^2$ = coefficient of determination, *Y* = methane concentration in ppmv.

**Figure 6**. Contrasting methane concentration changes in the semi-rigid sleeve from enclosed gas samples measured in continuous flow mode (UGGA) from tree-stems. In the first six runs (top quadrants) the concentration changes were regressed with a linear fit, while in the second set of runs (bottom quadrants) they were regressed with quadratic fit (non-linear). Runs 1, 2, 3, and 5 were made on *Betula pendula* stems, runs 4 and 6 were made on *Pinus sylvestris* stems, runs 3 and 6 show the concentration responses in situations where the sleeves were leaking. The blue line corresponds to 95% confidence intervals, *RMSE* = root-mean-square error, $R^2$ = coefficient of determination, *Y* = methane concentration in ppmv.

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**Table 1**. Chamber dimensions and mean permeabilities ($P$) determined, for each replicated chamber (n = 3), from the methane decline slope (*Slope*), the total chamber volume ($V_{tot}$), the initial concentration gradient between outside and inside ($C_0$-$C_{atm}$) and the gas exchange surface ($S_c$). $D$ = metallic cylinder diameter, $L$ = peripherical length of the enclosure, $H$ = height, $T$ = thickness, $C_0$ = initial enclosure concentration, $C_{atm}$ = 1.8951 ppmv, $R^2$ = coefficient of determination of the decline regression, $V_c$ = volume of the chamber, $V_{tot} = V_c + V_{dead}$, $V_{dead}$ = dead volume of the analyser plus the tubes = 416 cm$^3$. Values in brackets represent the standard error of the mean (±SEM).

| **Enclosure type** | $D$ **(cm)** | $L$ **(cm)** | $H$ **(cm)** | $T$ **(cm)** | $S_c$ **(cm$^2$)** | $V_c$ **(cm$^3$)** | $V_{tot}$ **(cm$^3$)** | $C_0$ **(ppmv)** | *Slope* **(mg m$^{-3}$ s$^{-1}$) *10$^{-4}$** | $R^2$ | $P$ **(m s$^{-1}$) *10$^{-7}$** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Small sleeve | 15 | 25 | 16 | 1.5 | 330 | 550 | 966[a] | 109.12 (2.00) | -21.40 | 0.930 | 8.30 (0.85)[d] |
| Large sleeve | 15 | 30 | 24 | 1.5 | 594 | 990 | 1406[b] | 71.43 (1.14) | -9.86 | 0.922 | 4.77 (0.64)[e] |
| Rigid chamber | 15 | 28 | 30 | 6.5 | 1413 | 13165 | 13581[c] | 9.58 (0.16) | -0.82 | 0.931 | 14.62 (1.86)[f] |

Volume inaccuracies: [a] ±3.4%, [b] ±2.4%, [c] ±4.1%; Permeability inaccuracies*: [d] ±3.7%, [e] ±2.8%, [f] ±4.3%

*Calculated from the error propagation formula:

$$\frac{dP}{|P|} \leq \sqrt[2]{\left(\frac{dC}{|C|}\right)^2 + \left(\frac{dV}{|V|}\right)^2 + \sqrt[2]{dC^2 + dC_{atm}^2}} \cong \sqrt[2]{\left(\frac{dV}{|V|}\right)^2 + 2}$$



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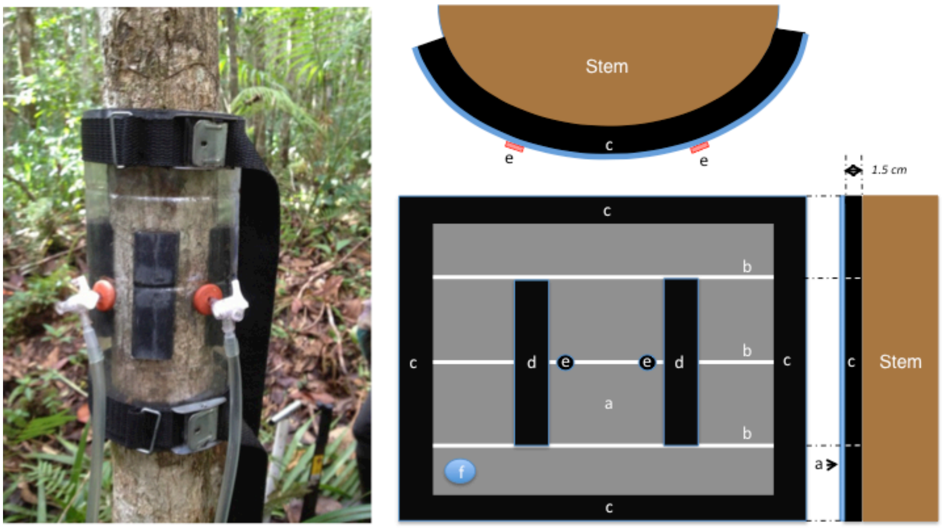


Fig01

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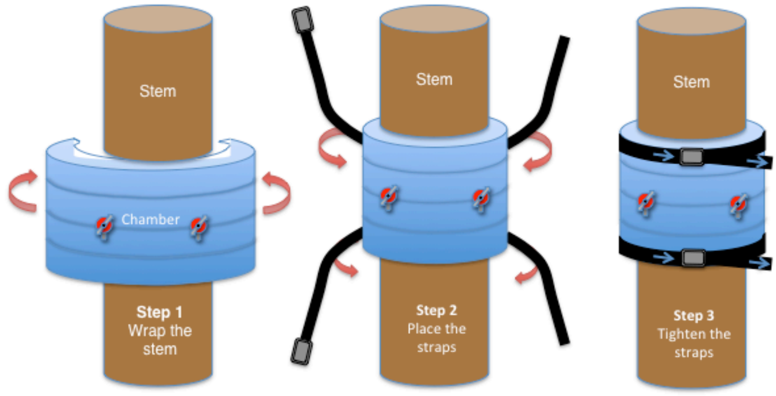


Fig02

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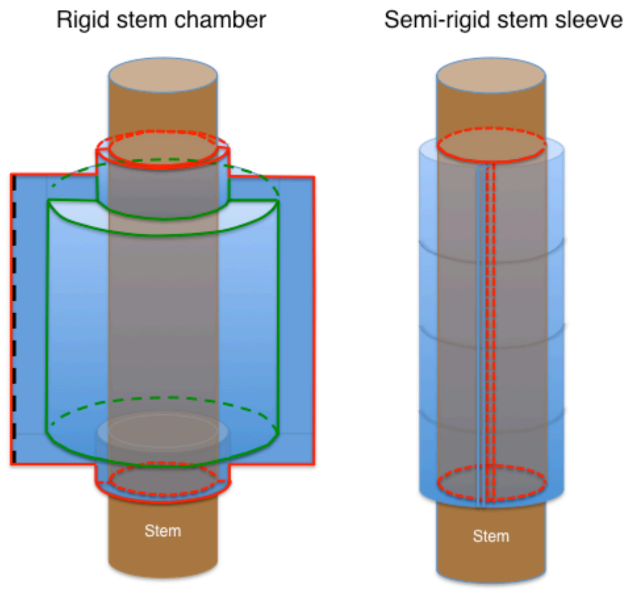


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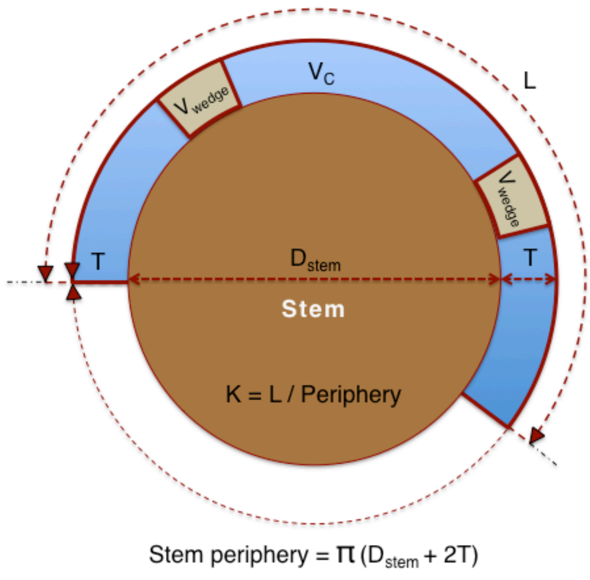


Fig04



Unknown
**Mis en forme:** Police :Times New Roman, Couleur de police : Texte 1

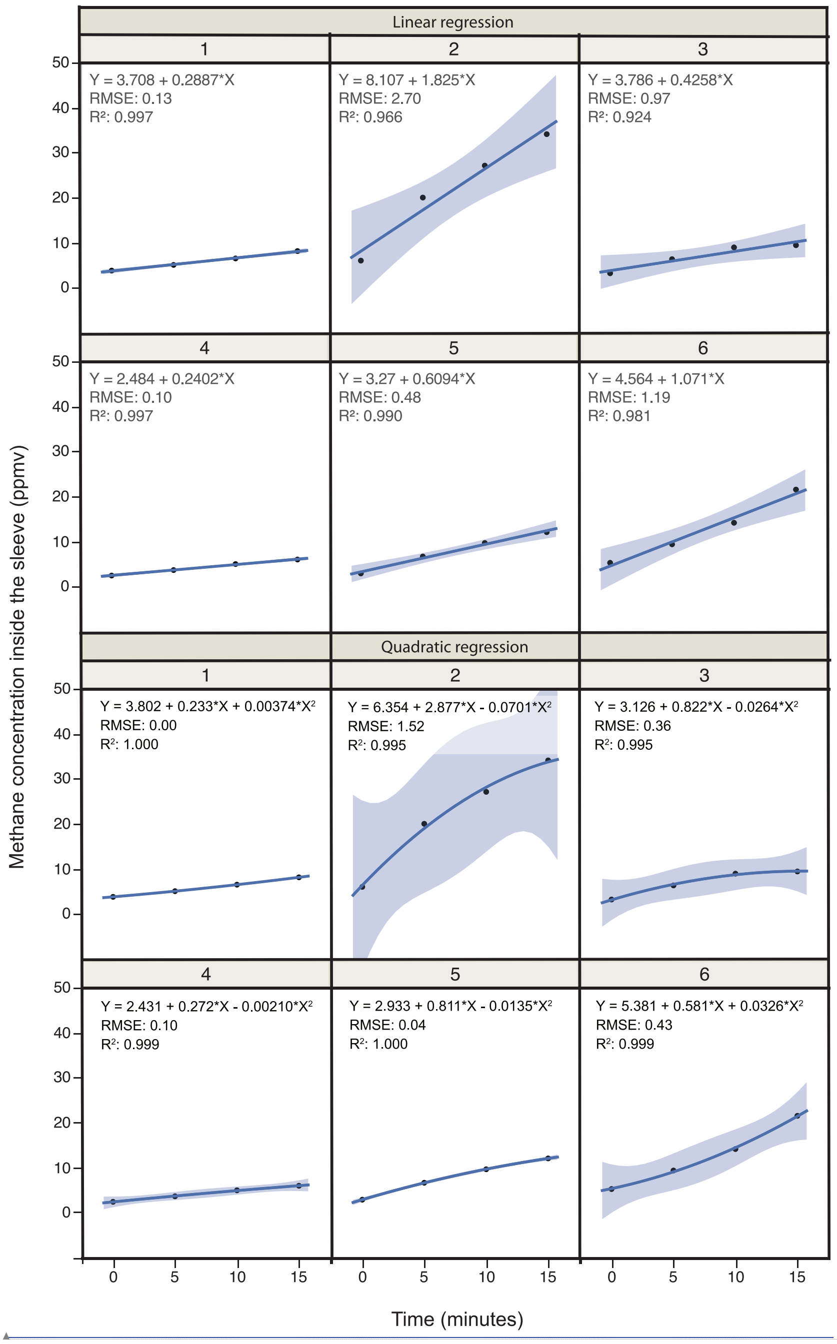


Fig05

Unknown
**Mis en forme:** Police :Times New Roman, Couleur de police : Texte 1

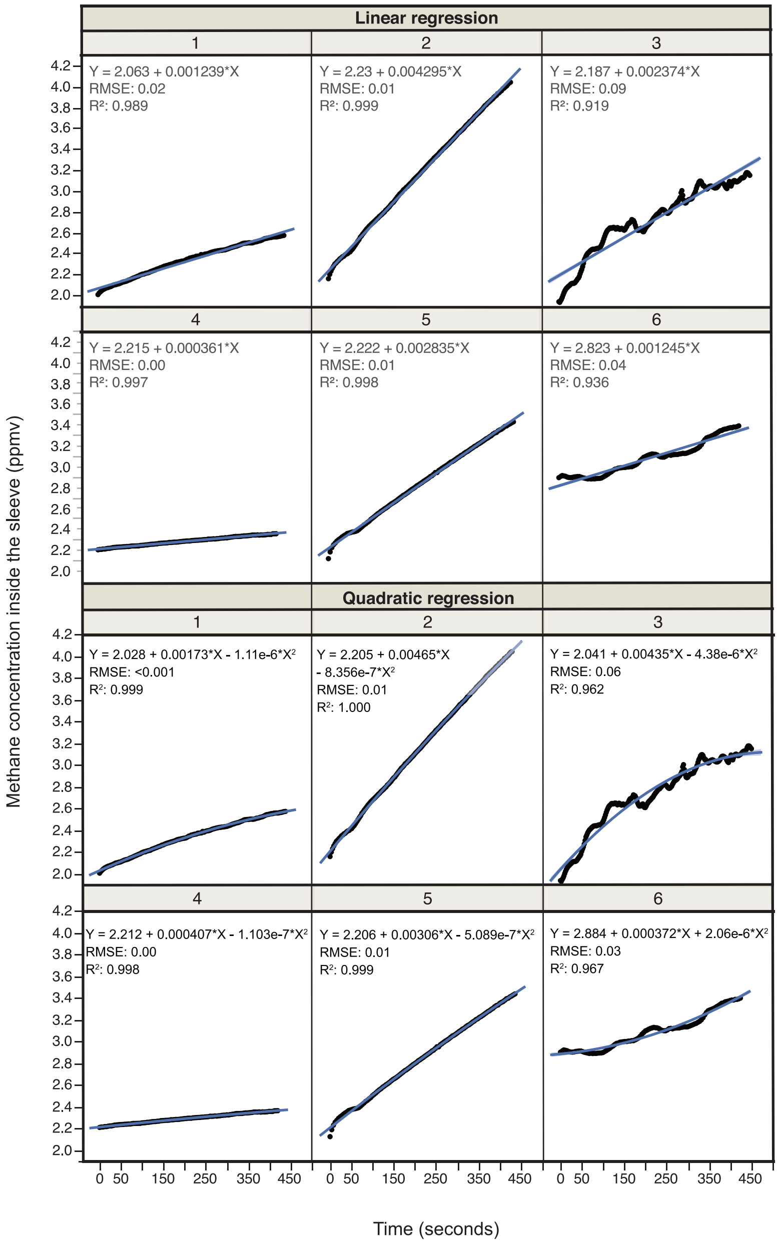


Fig06

Unknown
**Mis en forme:** Police :Times New Roman, Couleur de police : Texte 1