# **Response to Reviewers and Editor**

We thank the editor and both reviewers for the constructive and thorough reviews of our manuscript. Here we present our responses to reviewer comments and the revised manuscript. We sincerely hope the changes satisfy both reviewers. We believe the quality of the manuscript has improved substantially thanks to these reviews. We also improved the calculations of the  $CO_2$  fluxes and changed the values in the manuscript. To keep it clear for both the editor and the reviewers, we put the original comments in grey, our response in black.

## Anonymous Referee #1

The topic is very important because little is known about the carbon balance of the Congo basin forests. However, the manuscript fails at bringing this missing information because of an unsuitable measurement technique and a very poor number of replicates (3 chambers only were used). Overall, the sampling strategy in poorly described; the site also. In addition, the manuscript is poorly written, and the discussion related to the stable isotope is too much speculative.

# We thank the Referee for their review. We acknowledge that the referee is critical and identified three main issues raised by the referee. We will respond to each of these issues below and present our changes in the manuscript.

Page 1 A scientific article is different to a competitive grant proposal: let's try to stay humble. Remove "enormous" (line 3), "for the first time" (line 4)

## We removed "enormous" and "for the first time" from the manuscript (p.1, L. 2-3)

Line 6: Respiration in montane forest soils => Soil respiration in a montane forest. To avoid confusion, use either soil respiration or soil CO2 efflux but avoid mixing the two.

# We thank the reviewer for this observation and we amended the manuscript where necessary to get a more coherent text throughout the manuscript.

Line 8-10: be more precise. What are the differences that lead to this suggestion? And this suggestion is quite speculative because you compare soil respiration and soil C, but soil respiration also includes root and rhizopheric respiration that are less connected with the isotope composition of the soil C. It was only a suggestion line 10 but it becomes a firm conclusion line 14. This is annoying.

#### We made the sentence in Line 9 clearer and toned down the conclusion in line 14.

Page 2 Line 4: Ruehr 2010 is not the correct citation. In addition, there are lots of much older papers to cite here

# The reviewer is right. We corrected the citation to "Ruehr et al., 2010", and added "Rustad et al., 2000" (p. 2, L. 4).

line 5: "respiration of organic matter" has no meaning. Soil organic matter is not a living organism.

# We adjusted the sentence to: "... whereas soil moisture affects the diffusion of C substrate, atmospheric oxygen and respired $CO_2$ through soil pores." (p.2, L. 5).

Overall, the paragraph in lines 3 to 9 is poorly written and lacks logical structure

## This paragraph has been edited now by a native speaker (p.2, L. 3-9).

line 12-13: What is the reason why a high flux of CO2 and a high production would indicate a rapid turnover of C. Turnover (or mean residence time, the inverse) are flux divided by stock

### We changed this sentence and avoided the term "C turnover" (p. 2, L. 13 - 15).

line 27: What do you mean by soil CO2 consumption

# By the term " $CO_2$ consumption" we meant photosynthesis, however, we removed the term from the manuscript as it does not fit (p. 2, L 28).

What is the link between an increase in air temperature (line 31) and the length of the dry season (line 34). To my knowledge, the dry season in the Congo is cooler than the rainy season. At least, it does not indicate change in temperature (line 35)

# We thank the reviewer for identifying this point of confusion. In this paragraph, we want to emphasize that changes in temperature and precipitation affect soil respiration. We have now adjusted the whole paragraph to make this clear (p. 2, L 30 to 32).

Page 3 Isotopic signatures of leaf-litter, soil organic carbon, soil respired CO2 and dissolved stream water CO2 are not enough to determine sources of soil respired CO2. And what are the sinks? A bit wordy here. of soil respired CO2.

#### We rephrased this sentence (p. 3, L. 21).

Information on the stand structure are missing (at least tree density and basal area), as well as dominant species. Fine root biomass would also bring valuable information for comparing the two sites

We thank the reviewer for identifying these parameters as important missing data in the manuscript. We have now added information on basal area and dominant species where it was available (p. 3, L. 28-29; p.4, 1-4). Unfortunately, we do not have information on fine root biomass.

Page 4 line 3-4: The duration of the measurement is therefore not three years in contrast to what is claimed in the abstract

# We thank the reviewer for pointing this out. In the abstract we meant measurements during three calendar years, we adjusted the wording in the abstract (p. 1, L 5).

line 7: three chambers for one site! That is definitely not enough to cover spatial variability. If there is something that is well documented in tropical forests, this is the large spatial variability.

Regarding the number of replications, we used a minimum of three chambers per site as replicates which we described in the methods section. During the short-term sampling campaigns, seven chambers were used. The results from these short-term campaigns with the additional chambers showed extremely low variability between chambers at the same site. We only conducted the first year of the long-term measurements in the montane forest with only three chambers in the same locations because the sampling material and time of our field assistants were limited given the logistics with doing research in the DRC. Moreover, three of our sites were located in the lowland forests (Yangambi, Djolu and Yoko) and separated by more than 100 km. These sites exhibited both low intra- and inter-site variability, further confirming that our number of replicates was sufficient for measuring soil CO<sub>2</sub> fluxes from these forests. We have added more information about the numbers

# of chambers used in the subsection "Soil $CO_2$ flux measurements" of the Methods section (p. 5, L. 7-14) and present CV values for inter- and intra-site variability in the result section (p. 8, L. 6-9).

line 10: Static chambers installed for 12 hours! Does the chamber remain in the same place for two years? Measurements last one hour. Were the chambers opened before and after? 1 hour is already very long. How much CO2 accumulate during this time? Based on the keeling plots, concentration seems to vary from 500 to 2000ppm. It is far from the state of the art in terms of measurement of soil CO2 efflux.

The static manual chamber method used in this study is a well-established method used to measure soil GHG fluxes (e.g. Garcia-Montiel et al., 2004; Imer et al., 2013; Werner et al., 2014; Courtois et al., 2018). While we acknowledge that more advanced methods to measure GHG fluxes exist (i.e. portable gas analyzers, automated chambers, etc.), logistical constraints of working at four remote sites in the Democratic Republic of the Congo for extended periods of time prohibited methods that required multiple expensive instruments and reliable access to electricity. We chose the well-established methodology of evacuated gas sampling of static chambers because they 1.) were cost effective for sampling multiple sites for 2.5 years, 2.) were simple to perform for our field assistants, 3.) did not require electricity, and 4.) did not require materials that could be stolen or easily damaged. In addition to conducting scientific research, our group is interested in teaching and building capacity within our local student collaborators. The static chamber method, as opposed to automated chambers, allowed our students to play more significant role in the research. In conclusion, to conduct a long-term survey in the Congo Basin, we decided the exetainer sampling of permanently installed static chambers was the most suitable technique for our particular study.

Regarding the concerns over the chamber installation and measurement procedure. The chambers (PVC collars without lid) remained installed after initial placement. We waited with the first measurements for at least 12h after initial placement. The chambers were only closed for the duration of measurement (1h). In response to the concern regarding the 1 hour sampling duration, none of the total 1108 individual soil CO<sub>2</sub> flux measurements showed saturation of CO<sub>2</sub> concentration in the chambers (i.e. by reaching a plateau, see examples for each site in Figure 1). All of the measured fluxes exhibited linear increases with very high r<sup>2</sup> (see Figure 2) and only fluxes with a r<sup>2</sup> > 0.9 were considered in our analyses. Furthermore, we would like to point out that, in the absence of chamber saturation effects, longer flux durations result in more accurate flux calculations, since the  $\Delta$ CO<sub>2</sub> is larger for each time interval relative to the measurement accuracy. Nevertheless, we thank the reviewer for requesting more detailed method descriptions and we edited the manuscript accordingly (p. 5, L. 19, 24-25).



Figure 1: Examples of CO2 concentration over time during a single chamber measurement for each site



Figure 2: Histogram of the r<sup>2</sup> values for each linear fit

page 5 line 10: this equation was used by many before Imer 2013

We thank the reviewer for pointing out that the equation was used prior to Imer 2013 and have replaced this reference with "Hutchinson and Mosier (1981)", which is, to our knowledge, the earliest usage (p. 6, L. 1).

Line 31: how many litter traps? Line 33: how many soil samples?

Eight litter traps were installed per site and arranged in two rows of four. There was a distance of eight meters between traps. Soil samples were collected at three random positions at each site. We thank the reviewer noting this unclarity and we now added this information to the manuscript (p. 6, L. 27-30).

page 11, line 31: Universally????

# Indeed, this was too strong of an adverb to use. We replaced the word "universally" with "generally" (p.13, L. 11).

Page 12, line 13-16: it's totally speculative. Root respiration may contribute differently in the type of forest. Nothing allows the authors to test their hypothesis that decomposition is faster and soil microbes carbon-limited.

While we feel the inclusion of stable isotopes is relevant and of interest to the reader, we agree that certain points of the discussion are maybe somewhat speculative. We have toned down the language of this section in the revised draft and offer carbon limitation as merely a possible explanation for the observed trends (p. 13, L. 33-35). Moreover, we offer the caveat that contributions of  $CO_2$  from root respiration can vary with forest type, which may confound inter-site comparison (p. 14, L. 1-3).

#### Anonymous Referee #2

#### General comments:

The paper by Baumgartner et al on soil CO2 emissions from tropical rainforests in the Congo Basin is relevant and mostly well written. It addresses the knowledge gap on GHG fluxes from the African continent, which is still critically under-researched and represents one of the main causes of uncertainty in global GHG budgets. The paper is generally well structured, and the results mostly support the drawn conclusions. There are a few areas, however, that could to with a little revision and rewriting, and some of the conclusions based on the isotopic signature of different ecosystem C compartments might be a little speculative and could do with some rephrasing. Furthermore, some details on the experimental setup are missing and should be added to the materials and methods section. The statistics are sound but could be presented in a more attractive format. But if the authors address these concerns in an adequate manner, I am convinced that this paper can be a valuable contribution to Biogeosciences.

We thank the Referee for the positive review and constructive comments. We take their point that some conclusions based on the isotopic signatures are somewhat speculative and have now toned them down in the revised manuscript. We added a line into the discussion that our interpretation is speculative and that further research is needed to test our hypothesis (p. 14, L. 2-3).

We also edited the materials and methods section so that the experimental set up is clearer to the readers (p. 5, L. 7-14 and p. 6, L. 27-30).

To avoid confusion, we split up the analysis of the  $CO_2$  fluxes in two models: one for the lowland and one for the montane forest. Both results are now presented in a new Table 1 (p. 8). We also split up

# the statistical analysis of the $\delta^{13}$ C values into the two forest sites and present the values and significances in a new Figure 3 (p. 10). The seasonality of the $\delta^{13}$ C values are now presented in a new supplementary figure (Figure A4, p. 19).

Specific comments:

Introduction p.1 L17: fungi are also considered to be microorganisms. Therefore it is enough to say microbial respiration, or alternatively, fungal and bacterial respiration.

## Thank you for the specificity, we changed it to "... bacterial and fungal respiration" (p. 1, L. 17).

L19: the reference for global C flux via soil photosynthesis is a bit old, I suggest using the numbers from the latest IPCC report.

# Unfortunately, in the latest IPCC report, soil respiration rates were only presented together with the C loss via fires. In order to report more recent numbers, as the reviewer suggests, we decided to use the numbers of the most recent global carbon project report (p. 1, L. 19).

p. 2 L15-34: The authors highlight why it is crucial to understand soil respiration especially in ecosystems that are less well researched (i.e. tropical African rainforests). This paragraph is a bit lengthy because I think the reader of Biogeosciences is aware of that fact. Please shorten this paragraph, and instead add some information on 13C partitioning throughout the C cascade of tropical rainforests, and what different d13C values can mean, as this will guide the reader towards the research questions.

# We agree that the ideas can be condensed and have now shorted this paragraph. Additionally, we moved the beginning of the paragraph 4.4 to the introduction, to give some information about <sup>13</sup>C partitioning (p.3, L. 5-14).

#### p. 3 L4-9: what were your hypotheses?

Since our objectives were to quantify annual soil  $CO_2$  fluxes from forests of the Congo Basin and to assess differences between forest types, this was primarily a descriptive study. However, we added now that we expected higher soil  $CO_2$  fluxes in the lowland forest compared to the montane forest, as we expected higher soil temperature and WFPS conditions (p. 1, L. 17-19).

Material and methods: p. 3 L15, L19-20, and throughout the manuscript: please don't confuse the terms "average" and "mean". The (geometric) mean is a form of the average, in addition to the median and the modus. It should, therefore, be "mean annual rainfall" and "mean annual temperature". This should also be addressed throughout the results and discussion section (e.g. mean flux, etc).

#### We replaced the term "average" with "mean" throughout the manuscript.

p. 4: the section on soil CO2 flux measurements lacks some important details: how big were the study areas and plots? How many plots were installed per site? What was the vegetation composition (dominant tree species, presence or absence of dense understorey, basal area of trees, etc)? Did you use 3 flux chambers per site or per plot (i.e. more per site)? How were the chambers arranged in plots (e.g. distance from large trees, understorey vegetation, depressions/mounds, etc)?

We thank the reviewer for requesting more details regarding the flux measurements. We installed one plot in a mixed forest site in the montane forest. There we used seven chambers for the shortterm campaigns and three chambers in the first year of long-term measurements. In the second year of the long-term measurements, we increased the number of chambers to five. In the lowland forests of Yoko and Yangambi, we installed two plots in each site, one in a mixed forest and one in a mono-dominant forest, where more than 60% of the basal area consists of the species *Gilbertiodendron dewevrei*. For the short-term campaigns, we used four chambers in the mixed forest and three chambers in the mono-dominant forest. We started the long-term campaign with four chambers in the mixed forest and two chambers in the mono-dominant forest and after a year, we proceeded with five chambers in the mixed forest and stopped sampling in the mono-dominant forest. The chambers were randomly placed between trees and we avoided hills and depressions. We have now added more information about the plot distribution per site in the paragraph of the study site description (p. 4, L. 11-13) and specified the numbers and placement of soil flux chambers in the subsection of "Soil CO<sub>2</sub> flux measurements" (p. 5, L. 7-14). Moreover, we included all information available (basal area, dominant species) in the study site description (p. 3, L. 28-29; p. 4, L. 1-4).

One more note on the number of replicates for CO2 flux measurements: This is not 100% clear from the authors' description, but if I understand correctly, only 3 flux chambers were installed per study site. This is critical because spatial heterogeneity of soil respiration has been described in numerous previous studies, and this could lead to under- or overestimation of soil flux estimates. However, there are a couple of points that the authors could use to address this shortcoming: first, they have measured soil CO2 flux not only in one but in 3 lowland rainforests, and they could look at the difference between sites to describe spatial heterogeneity in the region. Second, if the flux chambers were always installed following a similar scheme, e.g. always at a fixed distance from trees, they would still be comparable even if not 100% representing absolute fluxes. Third, data on GHG fluxes from Africa are very scarce, and one of the reasons is the difficulty in getting research material into or out of the respective countries. I know from personal experience that it can be very difficult to buy or import even simple building material to construct flux chambers, and shipping of environmental samples can be complicated and often requires a lot of paperwork. I can imagine that the situation in DRC might have been similar. Therefore, for future studies on GHG fluxes in regions that are not easily accessible, I recommend the use of the gas-pooling technique by which gas samples from multiple (usually 3-6) chambers are put into the same GC vial, which can help to cover spatial heterogeneity while at the same time reducing the total number of samples. Nevertheless, even if the number of replicates is low and this probably introduces some uncertainty, this information on the magnitude of fluxes and their dynamics is still highly valuable, and I therefore still recommend the study for publication in BG.

We thank the reviewer for acknowledging the difficulties working in remote places and the related compromises sometimes to be made. We realize that our description of replication is lacking important details. As stated above, we used a minimum of three chambers per site. However, for the short-term campaigns, we used up to seven chambers per site. The results from these short-term campaigns showed relatively low variability between chambers at the same site (C.V. of 23% in the lowland forest and 18% in the montane forest) and thus we decided to reduce the number of chambers due to the limited number of evacuated vials for gas sampling (four were used per chamber per sampling for this study). Additionally, the reviewer correctly acknowledged that we measured CO<sub>2</sub> fluxes at three different lowland forest sites, which are separated by more than 100 km. The average fluxes of these three sites were also similar (inter-site C.V. of 29%) which gave us further confidence that even those periods where only three chambers were used are representative. We have added these variability statistics to the manuscript (p. 8, L. 6-9). As the reviewer points out already, it was not easy to get materials into the DRC. Therefore, we were only able to increase replication with additional visits to the sites, taking more material to the DRC. Ideally, this would have been done from the start but was not possible due to logistical constraints. Moreover, we appreciate the suggestion to use gas-pooling and will consider adopting this technique in the future.

p. 5 d13C measurements L24-25: wouldn't drawing 3 analytical samples of 20 ml each from the headspace of a 110 ml vial create an underpressure? How did the authors address this?

When withdrawing the samples from the 110 mL vials, a luer-stopcock between syringe and needle was used to avoid underpressure problems when removing the needle from the vial headspace during subsampling. That is, after withdrawal of 25 mL of sample, the luer-stopcock valve between needle and syringe was closed and the syringe was removed from the headspace. After, the plunger was pushed to 20 mL before opening the valve and injecting the subsample to 20 mL Labco vials. This procedure was repeated 3 times. The precision of three analytical replicates was excellent, with a maximum standard deviation of 0.25‰. We have now added a detailed description to the manuscript (p. 6, L. 17-20).

L31: how many litter traps were installed per site, and how were they arranged? L33: how many soil samples were collected per site? What was their arrangement (e.g. distance to chambers, distance to trees, etc)?

Eight litter traps were installed per site and arranged in two rows of four. There was a distance of eight meters between traps. Soil samples were collected at three random positions at each site. We thank the reviewer for their attention to detail and have added this information to the manuscript (p. 6, L. 27-30).

Statistics L6: you assumed little year-to-year variability of your data, but did you actually check if the climatic conditions (rainfall, temperature, moisture) varied between years?

We compiled the flux, temperature and WFPS data in weekly bins for easier presentation of the seasonality of the fluxes. However, for statistical analysis (influence of soil temperature and soil moisture on soil  $CO_2$  fluxes) we used the individual fluxes with the actual soil temperature and soil moisture conditions during each flux measurement. As a result, the weekly bins did not affect the results of the statistical analysis. We clarified this issue in the description of the statistical analyses (p. 7, L. 6-8).

p. 6 Figure 2: there are two dips in WFPS in March and October in the lowland forest, where WFPS dropped rapidly from c. 30 to 20%, and then recovered within a week or so – do you have an explanation for this?

# The two observed dips are located right before the peak rainy season, therefore, speedy recovery of soil moisture can be expected.

p. 7 Table 1: I know that the R output of Imer looks like this, but it's not very convenient for the reader to understand the results of the statistical analysis. For example, for d13C there is a significant effect of "Montane forest – stream CO2". This is ambiguous: does it mean that the d13C of stream-CO2 is different in montane and lowland forests? Or that the d13C of stream-CO2 is different from the d13C of the other compartments (soil CO2, litterfall, SOC) only in the montane but not the lowland forest? Please use a different way to present these results as they are critical. For example, as a start you could add letters/starts to Figure 2, presenting sign. differences between compartments via different letters, and differences between forests via stars (or something like that).

We agree with the reviewer that we can improve this presentation, to ensure unambiguous interpretation. The p-values (as well as R<sup>2</sup>s) in the case of linear mixed effect models are only estimations of p-values, and should be interpreted with caution either way, hence the interpretation of the table was mainly meant for the effect sizes. We have remade Figure 3 and included the model information in a new Figure 3 (p. 10). For the ease of interpretation, we split the modeling up in two parallel analyses (one for lowland, one for montane). This now avoids confusion with the

interpretation of too many interaction effects, but the effect sizes of both models will still allow the reader to interpret both inter and intra-forest type effects on  $\delta^{13}$ C. We also added a new supplementary figure (Figure A4), where the effect sizes of the different  $\delta^{13}$ C compartments during the seasons are presented. We thank the reviewer for making this clear, it is in our best interest that the readership of the paper can easily interpret the data we show.

L4-6: You state that stream-CO2 was significantly depleted in the wet season in lowland forests but not montane forests. However, in Table 1 you state "montane forest – wet season – stream CO2" to be significant. Isn't this contradictory (or just another example of how Table 1 could be misinterpreted)?

We thank the reviewer for their attention to detail. In fact, the new parallel analysis of both forest types separately showed that both biomes show a significant depletion of  $\delta^{13}C$  – Stream CO<sub>2</sub> during the wet season. We amended this in the result section (p. 9, L. 5-8) and added a new Figure A4.

Discussion p. 8 L5-7: Move this to the results section.

## Thank you for the suggestion, we have now moved this sentence to the result section (p. 8, L. 6-9).

p. 10 L9-12: Careful, while it is true that with increasing dry season length soil CO2 fluxes might decrease, but it is not clear how future more erratic rainfall patterns and the corresponding more extreme drying-rewetting events will affect respiration, and whether potential CO2 pulses after rewetting compensate or outweigh reduced soil respiration.

# We thank the reviewer for providing this qualification and have rewritten this statement to reflect a larger degree of uncertainty, for example, that there could also be $CO_2$ pulses that compensate for lower respiration as a result of these extreme drying-rewetting events (p. 11, L. 18–20).

L25-30: Good call! I agree that the correlations between soil CO2 flux and temperature in tropical systems that show very little annual variation should be handled with care. In your case, they might be significant simply because your sample size is large enough, but I would not over-interpret them. As you correctly state, moisture and C availability are likely the bigger players here.

#### We are glad that the reviewer agrees with our conservative interpretation.

p.11 L1-4: soil moisture not only controls O2 diffusion but also the diffusion of C substrates to soil microorganisms. Therefore, the response of respiration to moisture is more often an effect of C limitation (at low moisture) than an effect of O2 limitation (which really only becomes critical at very high moisture contents). Please add this to the discussion, and I recommend these papers on the mechanisms underlying this: Manzoni S, Moyano F, Kätterer T, Schimel J (2016) Modeling coupled enzymatic and solute transport controls on ecomposition in drying soils. Soil Biol Biochem 95:275–287. doi: 10.1016/j.soilbio.2016.01.006 Moyano FE, Manzoni S, Chenu C (2013) Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. Soil Biol Biochem 59:72–85. doi: 10.1016/j.soilbio.2013.01.002 Moyano FE, Vasilyeva N, Bouckaert L, et al (2012) The moisture response of soil heterotrophic respiration: Interaction with soil properties. Biogeosciences 9:1173–1182. doi: 10.5194/bg-9-1173-2012

We thank the reviewer for this nuanced perspective and have integrated the point along with the mentioned references into the discussion. The sentence now reads: "Soil moisture can influence soil respiration physically and biologically. Physically, soil moisture can limit the transport of C substrate to soil microorganisms (at low soil moisture conditions) and the diffusion of gases through soils,

including both oxygen required for aerobic respiration and respiratory  $CO_2$  (in high soil moisture conditions)." (p.12, L. 10-13).

L20: you mention photosynthesis, yet this was not measured and is therefore a bit speculative.

We agree with the reviewer that using the term "photosynthesis" here is a bit misleading in the sense that it indeed does sound this parameter had been measured. We have now re-written this section to avoid the term photosynthesis: "In this study, the link between C assimilation and soil  $CO_2$  is evident through [...]" (p. 12, L. 30).

p. 12 L4: which canopy processes other than photosynthesis could those be? Furthermore, how do you think that vegetation composition might affect d13C, and could this explain differences between lowland and montane forests? Can different trees have different leaf d13C signatures, which could be reflected throughout the C cascade?

These are two very good points raised by the reviewer. We were mainly thinking of stomatal conductance as the other important canopy process determining <sup>13</sup>C discrimination. Since there is an interplay between photosynthesis and stomatal conductance on <sup>13</sup>C discrimination, we lumped these two processes together (as canopy processes). This definition of canopy processes has been added (p. 13, L. 22-23).

The effect of altitude on  $\delta^{13}$ C of canopy leaves is well known (Körner et al., 1988, Hultine & Marshall, 2000; Chen et al., 2015) and can be explained by a combination of factors and the two consistent patterns associated with increasing elevation are a decrease in atmospheric pressure and in temperature. The decrease in O<sub>2</sub> partial pressure and temperature supposedly promotes a decline in ci/ca and the direct implication of this decline is that  $\delta^{13}$ C values become less negative (Wang et al., 2017). We have now added this information to the manuscript (p. 13, L. 9-16).

L6: what are the mechanisms underlying the enrichment of 13C at lower temperatures?

In the subsequent sentence, we explain that temperature changes can result in shifts in microbial communities, which can impact fractionation during heterotrophic soil respiration (Andrews et al., 2000). We have now also added more information about the underlying mechanisms to the manuscript (p. 13, L. 23-24).

Conclusions This is mostly a repetition of the results. Please instead give the "message of the story" – what are the implications of the results you found? What are questions that remain open? And what have we learned?

We agree with the reviewer and rewrote the conclusion section to focus more on the implications of the study results and the remaining research questions.

L24: how were the sites different in vegetation composition? Please describe in the M&M section and also address in discussion

We added information on the vegetation composition to the site description (p. 3, L. 28-29 and p. 4, L. 1-3). Although the vegetation composition may have an effect on rooting density and carbon uptake (net ecosystem uptake) we refrain from elaborating in the discussion as we lack detailed information and would prefer to avoid speculative statements.

L27: what does this indicate, that there was no temperature dependency of soil respiration between sites?

# As our results suggests that respiration in the lowland forests is substrate limited, we reason that the higher temperatures, compared to the montane forest, will not result in an increased soil CO2 flux. We amended this sentence and clarified it in the manuscript (p. 14, L. 15-19).

p. 13 L4: you conclude the paper with the statement that these forests might become C sources under a warming climate, yet you did not find a strong effect of temperature! Instead, you could state that changes of C balance might happen in response to more erratic rainfalls and weather extremes.

# We thank the reviewer for pointing us to this important contradiction. We have rephrased the final statement in our conclusion in a manner that it better fits our observations (p. 14, L. 28-30).

Appendix A: Method supplement L6-25: please use the past tense throughout this section.

## We modified the method supplement to the past tense (p. 15, L. 3-21).

p. 16 Figure 3A: change x-axis labels of panel d to the format HH:MM (e.g. 10:00, 15:00,...) to make it clear that those are hours.

## We changed the axis label of the d panel (Fig A3, p. 18).

Technical corrections: p. 5 L30: please correct "...during the wet season from October to May"

## This has been changed. (p. 8, L. 4)

p. 6 L9: please correct "values were found" (use past tense throughout the results section)

## The results section have been modified to the past tense. (p. 8, L. 17)

L8 and elsewhere: You very often use the term "respectively"; however, I'm not a big fan of it, for two reasons: first, sentences become very complex and sometimes hard to understand when using this term, and second, it forces the reader to jump back and forth between the end and the start of the sentence, which disrupts the flow of reading. Very often, you'll find that your sentence won't actually become any longer if instead of using "respectively", you describe the results one after the other, in this case, this could be "The mean [instead of "average", see my earlier comment] annual values we measured in this study in the Congo basin, which are 3.83 \_mol m-2 s-1 for the montane forest and 3.69 \_mol m-2 s-1 for the lowland forest, are within the range of reported values from other tropical forests." I propose that you revise the MS and try to reduce the use of "respectively". This will make the paper easier to follow.

# We very much agree with this comment and have rephrase those sentences throughout the manuscript.

L14: please rearrange "...and they were rather low compared to our flux rates"

## The sentence was rearranged. (p. 10, L. 15)

p.9 L2: "...showed marked seasonality [comma] with a 34 % decrease during the dry season [comma] whereas.."

## We have added the commas. (p. 11, L. 8)

L4: please rephrase "however, the decrease they found was not as pronounced as..."

## This sentence has been rephrased (p. 11, L. 10).

p. 10 L22: "statistically significant correlation"

## This has been corrected (p. 11, L. 30).

L18: please rephrase "play a crucial role in controlling soil respiration"

## We added "controlling" (p.11, L. 26)

p. 11: L4: please add a comma here, otherwise the phrase is misleading: "stress soil microbial communities, and autotrophic respiration"

## A comma has been added. (p. 12, L. 14)

L10: please rephrase as this is otherwise misleading "While soil respiration in lowland forests is most likely C-limited, respiration in montane forests seems to be more sensitive to environmental conditions and could represent a potentially large C source with climate change."

## This sentence has been rephrased (p. 12, L. 21-22).

p. 12 L17: enrichment does not occur in the "location" but in the movement from one compartment to another. Please rephrase "the highest enrichment occurs in the last step from soil to streamdissolved CO2".

#### This sentence has been rephrased (p.14, L. 4).

L25: please rephrase: "However, in contrast to the lowland forest, the montane forest site exhibited strong seasonality of soil respiration, primarily driven by WFPS during the dry season."

#### We have rephrased these sentence (p. 14, L. 15-19).

#### References

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# Seasonality, drivers, and isotopic composition of soil CO<sub>2</sub> fluxes from tropical forests of the Congo Basin

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#### Abstract.

Soil respiration is an important carbon flux and key process determining the net ecosystem production of terrestrial ecosystems. To address the enormous lack of quantification and understanding of seasonality in soil respiration of tropical forests in the Congo Basin, soil  $CO_2$  fluxes and potential controlling factors were measured for the first time annually in two dominant

- 5 forest types (lowland and montane) of the Congo Basin during three-over two years at varying temporal resolution. Soil CO<sub>2</sub> fluxes from the Congo Basin resulted in  $3.69 \cdot 3.45 \pm 1.22 \cdot 1.14$  and  $3.82 \cdot 3.13 \pm 1.15 \cdot 1.22 \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  for lowland and montane forests, respectively. Respiration Soil CO<sub>2</sub> fluxes in montane forest soils showed a clear seasonality with decreasing flux rates during the dry season. Montane forest soil CO<sub>2</sub> fluxes were positively correlated with soil moisture while CO<sub>2</sub> fluxes in the lowland forest were not. Paired-Smaller differences of  $\delta^{13}$ C values of leaf litter, soil organic carbon (SOC)
- 10 and soil CO<sub>2</sub> indicated that SOC in lowland forests is more decomposed than in montane forests, suggesting that respiration is controlled by C availability rather than environmental factors. In general, C in montane forests was more enriched in  $^{13}$ C throughout the whole cascade of carbon intake via photosynthesis, litterfall, SOC, and soil CO<sub>2</sub> compared to lowland forests, pointing to a more open system. Even though soil CO<sub>2</sub> fluxes are similarly high in lowland and montane forests of the Congo Basin, the drivers of them were seem to be different, i.e. soil moisture for montane forest and C availability for lowland forest.

#### 15 1 Introduction

Soil basal respiration, the sum of carbon dioxide ( $CO_2$ ) produced both autotrophically by roots and heterotrophically by microbial bacterial and fungal respiration, represents the biggest natural transfer of carbon (C) from the terrestrial biosphere to the atmosphere (Raich and Schlesinger, 1992). Globally, soil respiration is the second-largest terrestrial C flux after photosynthesis, emitting <u>98–120</u> Pg of C per year as CO<sub>2</sub> (Bond-Lamberty and Thomson, 2010)(Friedlingstein et al., 2019). As such,

the flux of  $CO_2$  from soils represents a significant component of net ecosystem production (NEP). Research into the abiotic and biotic controls of this flux are thus critical for understanding the overall C balance of ecosystems.

There are a number of different parameters that can influence soil  $CO_2$  efflux, with soil temperature and soil moisture being the most important drivers (Ruchr et al., 2010) (Rustad et al., 2000; Ruchr et al., 2010). Soil temperature affects biological

- 5 activity whereas soil moisture affects both the respiration of organic matter and the diffusion of the diffusion of C substrate, atmospheric oxygen and respired CO<sub>2</sub> through soil pores (Janssens et al., 1998; Doff Sotta et al., 2004; Sousa Neto et al., 2011; Courtois et al., 2018). In addition to temperature and moistureFurthermore, soil pH (Courtois et al., 2018), through its effects on microbial communities, and root density can also affect soil CO<sub>2</sub> production (Janssens et al., 1998). Another particularly important driver is photosynthetic activity, as it describes the rate of carbohydrate supply from leaves to roots, where both root
- 10 and rhizo-microbial respiration occur (Ekblad, 2001). While the magnitude of soil respiration CO<sub>2</sub> fluxes can vary significantly between and within different ecosystems, soil CO<sub>2</sub> fluxes from tropical forests are generally higher than from any other vegetation type, due to high soil temperature, high soil moisture (Raich et al., 2002), and weak C stabilization (Doetterl et al., 2018). This Although tropical forests experience high soil CO<sub>2</sub> fluxes and high production, as as a whole, the tropical terrestrial biosphere acts as a
- 15 net C sink by production outbalancing the high soil respiration (Melillo et al., 1993; Pan et al., 2011; Palmer et al., 2019). Despite the importance of tropical forests for the global C cycle, there is a lack of research into CO<sub>2</sub> fluxes from soils in these ecosystems. The Congo Basin in central Africa, hosts the second largest tropical forest on Earth, but has been particularly neglected in biogeochemical research . A recent paper on the greenhouse gas budget of Africa by Valentini et al. (2014)-identified current key uncertainties and research gaps, especially on data availability of respiratory fluxes from tropical forests.
- 20 This holds especially for the Congo basin, since there is and only one study from 1962 reporting soil respiration rates CO<sub>2</sub> fluxes from these forests (Maldague and Hilger, 1962) are available. Hence, while many studies model soil and ecosystem respiration in the Congo, there are almost no empirical data on soil CO<sub>2</sub> fluxes to validate the models. To model C fluxes in tropical Africa, these studies either upscale from a few FLUXNET stations or apply rates from other tropical forests (Jung et al., 2011) . However, differences in species composition (Slik et al., 2015), forest structure (Lewis et al., 2013), nutrient atmospheric
- 25 supply (Bauters et al., 2018), and elimate patterns call for cross continental and spatially exhaustive monitoring across the tropics to fill up this important data gap (Corlett, 2006). Eddy-covariance towers are the most common methods to measure  $CO_2$  fluxes over different ecosystems and larger areas. However, continuous measurements of soil respiration <u>CO<sub>2</sub> fluxes</u> close to the surface are needed to assess temporal trends of processes controlling soil  $CO_2$  production and consumption (Ogle, 2018). This is particular important in light of recent data that show that the ratio of soil respiration to primary production has increased
- 30 over time (Bond-Lamperty et al., 2018). In particular, heterotrophic respiration has increased as soil microbes became more active in response to increasing temperatures (Bond-Lamperty et al., 2018). Bond-Lamberty and Thomson (2010) estimated that global soil respiration increased by 0.1 Pg C yr<sup>-1</sup> between 1989 and 2008, mostly due to an increase in air temperature. If this process proceeds such that ecosystem respiration exceeds primary production, terrestrial ecosystems could be transformed from sinks to sources of C. Thus, understanding baseline rates of soil respiration and the role of environmental drivers is crucial
- 35 to assess future responses to climate change. This is especially important in the Congo Basin, as a recent study showed that the

length of dry seasons have increased by 6.4-10.4 days per decade since 1988 (Jiang et al., 2019). These changes in precipitation and temperature could trigger an ecosystem response, including shifts in soil respiration. Furthermore, short-term events, such as extreme rain or prolonged dry periods, are predicted to occur more frequently with climate change and will most likely impact soil respiration rates (Hopkins and Del Prado, 2007; Borken and Matzner, 2009).

- 5 It is well known that soil respiration and canopy processes are linked in forests (Ekblad, 2001). Carbohydrates produced by photosynthesis are subsequently transported to the roots and rhizosphere, where they are respired by root or microbial respiration (Ruehr et al., 2009). Thus, the isotopic signature of soil-derived CO<sub>2</sub> is mostly governed by isotope fractionation processes that occur at the leaf scale, since a significant portion of soil respired CO<sub>2</sub> is supplied by recent photoassimilates (Högberg et al., 2001; Brüggemann et al., 2011; Barthel et al., 2011). Generally, all environmental parameters affecting photosynthesis
- and thus CO<sub>2</sub> discrimination are likely to influence the δ<sup>13</sup>C signal of soil respiration (e.g. precipitation, vapor pressure deficit) (Bowling et al., 2002). Furthermore, the differences of the stable isotope signatures between different C compartments (litter, soil C, soil CO<sub>2</sub>, stream dissolved CO<sub>2</sub>) can give information about the opennesss' of a system. To an 'open system' we refer if the system experiences a continuous supply of substrate, while products are lost from the system, whereas a 'closed system' lacks new inputs of substrate. The fractionation between compartements is higher in a more open system (Fry, 2006).
- In light of these issues, the objectives of this study were to provide 1) the first empirical quantification of annual soil  $CO_2$ fluxes from forests of the Congo Basin, 2) gauge variability between two dominant forest types within the basin, and 3) assess whether and to what extent soil temperature and moisture influence  $CO_2$  fluxes. Soil respiration was We hypothesized higher soil  $CO_2$  fluxes in the lowland forest due to higher temperature and soil moisture regimes compared to the montane forest. Soil  $CO_2$  fluxes were measured weekly to assess the role of seasonality and environmental drivers of soil  $CO_2$  fluxes. Additionally,
- stable C isotopic signatures ( $\delta^{13}$ C) of leaf-litter, soil organic carbon (SOC), soil-respired CO<sub>2</sub> and dissolved stream water CO<sub>2</sub>, were measured to determine sources and sinks give us more information about sources and fate of soil respired CO<sub>2</sub>.

#### 2 Methods

#### 2.1 Study sites

Old-growth forest sites in the Democratic Republic of Congo (DRC), contrasting in altitude, were selected to conduct long-term
static manual chamber CO<sub>2</sub> flux measurements. The first site (KB) is situated in the Kahuzi-Biéga National Park (S 02.215°, E 28.759°) northwest of the city of Bukavu in the South-Kivu province and represents a montane tropical <u>mixed</u> forest at an altitude of 2120 m a.s.l with an <u>annual mean mean annual</u> temperature of 15 °C and an <u>average mean</u> annual rainfall of 1500 mm (Bauters et al., 2019). Species composition of this forest is well described in Imani et al. (2016) and main species are <u>Maesa</u> lanceolata, Lindackeria kivuensis and Allophyllus kiwuensis. Rainfall peaks in both April and October, with a dry season from

June to September in between (Alsdorf et al., 2016). The soils in KB are broadly classified as Umbric Ferralsols (Jones et al., 2013) with a sandy loam (upper 15 cm) to silt loam (15-30 cm) texture. The second site (YO) is situated in the Yoko Forest Reserve, south of the city of Kisangani in the Tshopo province (N 0.294°, E 25.302°). The YO site is a lowland tropical mixed forest with an annual average with a mean annual temperature of 24.2 °C and an average a mean annual rainfall of 1800 mm



**Figure 1.** Map of part of the Congo Basin with the different vegetation types. Red dots indicate sampling locations. Lowland: Djolu, Yangambi, and Yoko. Montane: Kahuzi-Biéga. Map modified based on Verhegghen et al. (2012).

(Bauters et al., 2019). The mixed forest YO site consists of two dominant forest types, a lowland tropical mixed forest and a mono-dominant forest, where more than 60 % of the basal area consists of the species *Gilbertiodendron dewevrei*. The mixed forest plot is a classic African lowland rainforest with about 70 species per hectare and a canopy height up to 40 m and a basal area of  $34 \text{ m}^2 \text{ ha}^{-1}$  (Doetterl et al., 2015; Kearsley et al., 2017). Like KB, there are two wet seasons, a short one from March

- 5 to May and a longer one from August to November. The soils in YO are deeply weathered and nutrient poor Xanthic Ferralsols (Jones et al., 2013) with a loamy sand texture (0-30 cm). Because lowland forests are the main forest type within the Congo Basin, two additional lowland forest sites (Djolu and Yangambi) were selected to conduct short term campaigns, assessing spatial robustness of the results (Figure 1). Yangambi is a UNESCO biosphere reserve and lies at the river bank of the Congo river about 100 km west of the city of Kisangani (Figure 1). Djolu is a territory just north of the equator roughly 300 km west
- 10 of the city of Kisangani, in the north-east of Tshuapa province where measurements were conducted in protected forest areas (Figure 1). In Yangambi and KB, one plot (40 by 40 m) in a mixed forest was installed. In Yoko and Yangambi, two plots for each site were installed, one in a mixed forest and one in a mono-dominant forest. In Djolu measurements were conducted in one plot in an old-growth mixed forest sites.

#### 2.2 Soil CO<sub>2</sub> flux measurements

Flux chamber measurements were carried out at different time intervals during 2016-2019. Weekly to fortnightly sampling in YO was conducted from November 2016 to March 2019 and in KB from April 2017 to March 2019. In addition, several 2-week sampling campaigns with daily and sub-daily sampling were conducted to rule out diurnal soil respiration cycles (Figure

- 5 A3). These short-term sampling campaigns were conducted in KB (September 2016, April 2017), YO (October 2016, Mai 2017), Yangambi (September 2016) and in Djolu (May 2016). Sampling was done using the static manual chamber method, as described in Hutchinson and Mosier (1981). Briefly, at each site, a minimum of three In the montane sampling plot, seven chambers were installed for the short-term campaigns. Due to material and logistical constrains, we started the first year of long-term measurements with three chambers and increased the number of chambers to five in the second year of long-term
- 10 sampling in the montane forest. In YO, short-term campaigns were conducted with four chambers in the mixed forest plot and three chambers in the mono-dominant plot. We then started the long-term campaign in YO with four chambers in the mixed forest and two chambers in the mono-dominant forest and after one year, we proceeded with five chambers in the mixed forest while we stopped the sampling in the mono-dominant plot. Fluxes in Djolu were measured with four chambers. In every plot the chambers were randomly placed between trees and we avoided hills and depressions. PVC chambers with a diameter of
- 15 0.3 m, a height of 0.3 m, an airtight lid, and a vent tube to avoid pressure disturbances were installed. A thermocouple (Type T, Omega Engineering Deckenpfronn, Germany) was inserted through a gas tight cable gland to measure temperature in the chambers at each sampling time point. Following established methods, the chambers were inserted into the forest floor at least 12 hours prior to taking the first sample to avoid altered results due to soil disturbance. The chambers remained in place throughout the measurement campaign. For each flux measurement, the lids of the chambers were closed for one hour and 20
- 20 mL headspace gas samples were withdrawn every 20 minutes (t0, t20, t40, and t60) with a 20 mL syringe. Gas samples were stored in pre-evacuated 12 mL vials with airtight septa (Exetainer; Labco Ltd, High Wycombe, UK). To avoid gas leakage issues as described by Knohl et al. (2004), the septa were additionally sealed with a thin layer of silicon. To ensure that the headspace was well-mixed and that there was no static concentration gradient inside the chamber, the syringe was flushed with air from the chamber headspace and reinjected into the chamber prior to sample withdrawal. The chambers were only
- 25 closed for the duration of the measurements. Soil moisture probes (ECH<sub>2</sub>O-5, Meter Environment, Pullman, U.S.) and air temperature data loggers (iButton, Maxim Integrated, San Jose, U.S.) were installed at each chamber cluster. Soil temperature was measured during each sampling event at 20 cm depth using a thermocouple (Type T, Omega Engineering, Deckenpfronn, Germany). To standardize soil moisture data between sites and soil types, the water filled pore space (WFPS) was calculated for each volumetric water content measurement using bulk soil density data provided from Bauters et al. (2019) and particle
- 30 density of soil minerals of 2.65 g cm<sup>-2</sup>.

Gas samples were analyzed for concentrations of  $CO_2$  at ETH Zurich (Zurich, Switzerland) using gas chromatography (Bruker, 456-GC, Scion Instruments, Livingstone, U.K.). Soil gas fluxes were calculated using the linear increase of the gas concentration in the head space of the chambers over time, corrected for pressure and temperature according to the ideal gas law, divided by chamber area (Imer et al., 2013)(Hutchinson and Mosier, 1981). Using the micrometeorological convention, a flux from the soil to the atmosphere is denoted as positive flux.

### 2.3 $\delta^{13}$ C-CO<sub>2</sub> of streams and soil respiration

- After concentration measurements, the remaining gas was analyzed for δ<sup>13</sup>C of CO<sub>2</sub> for one week of each month and site
  to derive a representative δ<sup>13</sup>C signature of the monthly soil-derived CO<sub>2</sub> via the Keeling plot approach (Keeling, 1958). All Keeling plots yielded an r<sup>2</sup> > 0.99 (Fig A1). Post-run off-line calculation and drift correction for assigning the final δ<sup>13</sup>C values on the V-PDB scale were done following the "IT principle" as described by Werner and Brand (2001). The δ<sup>13</sup>C-values of the laboratory air standards were determined at Max-Planck-Institute for Biogeochemistry (Jena, Germany) according to Werner et al. (2001). Briefly, linking measured δ<sup>13</sup>C values of CO<sub>2</sub> gas isolated from standard air samples relative to the carbonate
  V-PDB scale was done via the Jena Reference Air Standard (JRAS), perfectly suited to serve as a primary scale anchor for CO<sub>2</sub>-in-air measurements. The measurement of the aliguots of the laboratory standards is routineously better than 0.15%. In
- addition to soil CO<sub>2</sub>, dissolved CO<sub>2</sub> samples of six pristine headwater streams near the chamber sites were taken in April (wet season) and September 2018 (dry season) using the headspace equilibration technique. At each stream site, 20 mL of unfiltered water sampled from the thalweg was injected into  $\frac{110 \text{ mL}}{10 \text{ mL}}$ , N<sub>2</sub>-flushed (Alphagaz 2, Carbagas, Gümlingen,
- 15 Switzerland) serum crimp vials containing 50 μL of 50 % ZnCl. From the headspace of the crimp vials, three analytical replicates were subsampled into evacuated 12mL exetainers (Labco Limited, High Wycombe, UK) following Bastviken et al. (2008). To avoid underpressure problems when withdrawing samples from the 110 mL vials, a luer-stopcock between syringe and needle was used. After withdrawl of 25 mL of sample-headspace, the luer-stopcock valve was closed and the syringe was removed from the headspace. After, the plunger was pushed to 20 mL before opening the valve and injecting the subsample
- 20 to the new vial. The precision of the three analytical replicates was excellent, with a maximum standard deviation of 0.25 %. According to Szaran (1998) only 1.03 permille % fractionation occurs between dissolved and gas phase, thus  $\delta^{13}$ C of headspace CO<sub>2</sub> can be used as a representative measure for dissolved  $\delta^{13}$ C of CO<sub>2</sub>. All CO<sub>2</sub> samples were analyzed for  $\delta^{13}$ C of CO<sub>2</sub> with a modified Gasbench II periphery (Finnigan MAT, Bremen, D) coupled to an isotope ratio mass spectrometer (IRMS; Delta<sup>plus</sup>XP; Finnigan MAT; modification as described by Zeeman et al. (2008)) (see Supplemental Information).

#### 25 2.4 $\delta^{13}$ C of litter and soil

Litterfall collected fortnightly between 2015 and 2016 from traps installed at the same sites was used to determine  $\delta^{13}$ C of leaves. Eight litterfall traps were installed per plot and arranged in two rows of four with a distance of eight meters between traps. At each site, the leaves were combined into monthly samples which were subsequently dried, homogenized, and ground (Bauters et al., 2019). Soil samples were taken at the montane and the lowland forest plots at 0-30 cm depth and three random

30 positions and subsequently air dried, sieved and milled. Litter and soil samples were analyzed using elemental analyzer (Automated Nitrogen Carbon Analyser; ANCA-SL, SerCon, UK), interfaced with an Isotope Ratios Mass Spectrometer (IRMS; 20-20, SerCon, UK).

#### 2.5 Statistical Analyses

In total 1108 single flux measurements have been conducted (398 in the montane forest and 710 in the lowland forests, respectively). As the campaigns from the different sites were spread over several years, all data were compiled and averaged into weekly bins prior to plotting time series of the data assuming little year-to-year variability. In that way, yearly site averages

- 5 were not weighed by periods of intensive sampling as each single week had an assigned median value regardless of measurement frequency. Effects of forest type, This compiling was only conducted for easier representation of the data. For statistical analysis of the effects of soil temperature and WFPS on the soil CO<sub>2</sub> flux, we used the individual fluxes with the actual soil temperature and WFPS conditions during each flux measurement. Effects of soil temperature and WFPS on the soil CO<sub>2</sub> flux from each forest type were quantified using a linear mixed effects modelmodels, including all fluxes that were measured, and
- 10 controlling for the soil chamber via a random intercept. Because a full model was not converging for soil CO<sub>2</sub> flux, including all interaction terms between the three predictors, interaction between WFPS and soil temperature were omitted. Likewise, **a** model was run two models, one for the wet season and one for the dry season, were fitted to explain effects of forest type, ecosystem compartment (litter, soil CO<sub>2</sub> flux and stream CO<sub>2</sub>) and season (wet/dry season) on  $\delta^{13}$ C values in the lowland and montane forests, including sample spot (litterfall trap, soil flux chamber and sampled stream) as a random effect. Models were
- 15 fitted using maximum likelihood methods via the lmerTest package (Kuznetsova et al., 2017). P-values of the fixed effects elevation, transect and their interaction - were determined based on the denominator degrees of freedom calculated with the Satterhwaite Satterthwaite approximation. Marginal (m) and conditional (c)  $R_{adj}^2$  are proxies for the variation explained by the fixed effects, and both the random and fixed effects, respectively, were calculated following Nakagawa and Schielzeth (2013), via the MuMIn package (Barton, 2019). For all statistical analyses, the R-software was used (R Development Core Team,
- 20 2019). All model fits were validated by checking normality and homoscedasticity of the residuals. QGIS version 2.18 was used to compile the map of the Congo Basin.

#### **3** Results

#### 3.1 Temperatures and Soil Moisture

Weekly mean soil and air temperature were both stable throughout the year in both forest types (Figure 2a). Average Mean soil
temperatures were 24.0 in the lowland forest sites and 15.3 °C in the lowland and montane forestsites, respectivelymontane
forest. Air temperatures were slightly lower in both lowland and montane sites, averaging 23.5 and 14.7 °C, respectively. The
WFPS at 30 cm depth in the lowland forest was quite constant. However, a decrease in WFPS was observed during dry season
in the montane forest (Figure 2b). Annual average Mean annual WFPS in the montane forest was higher (51.4 %) than in the
lowland (29.6 %).

#### 30 3.2 Soil CO<sub>2</sub> fluxes

**Table 1.** Fixed effects estimates for both the CO<sub>2</sub> flux and fluxes in the  $\delta^{13}$ C response variables, including 1) forest type (lowland – and montane) forests, including water-filled pore space (WFPS in %), soil temperature (in °C) and their interactions as predictors for the soil CO<sub>2</sub> efflux (in  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>), and 2) forest type (lowland – montane), ecosystem compartment (litter – soil CO<sub>2</sub> – stream CO<sub>2</sub>) and season (wet – dry season) and their interaction as predictors for the  $\delta^{13}$ C values (in). For each effect, estimated standard error and estimated P-values is given, along with the estimated marginal (m) and conditional (c) R<sup>2</sup><sub>adi</sub> (Nakagawa and Schielzeth, 2013)

Response	Effect	Estimate	SE	P-value	$\mathbf{R}^2_{adj,m}$	$R^2_{adj,c}$
CO <sub>2</sub> flux - Lowland	Intercept	9.202	3.14	<0.01	0.07	0.48
	WFPS	0.024	0.01	0.05		
	Soil temperature	-0.279	0.13	0.03		
CO <sub>2</sub> flux - Montane	Intercept	-10.888	1.87	<0.001	0.69	0.69
	WFPS	0.106	0.01	< 0.001		
	Soil temperature	0.561	0.13	< 0.001		

Average Mean annual soil CO<sub>2</sub> fluxes were (average mean  $_{minimum}^{maximum} \pm$  SD)  $3.69 \frac{9.64}{1.30} 3.45 \frac{9.20}{1.22} \pm 1.22 1.14 \ \mu mol m^{-2}$ s<sup>-1</sup> and  $3.82 \frac{6.33}{0.42}$  for the lowland forests and  $3.13 \frac{10.57}{0.33} \pm 1.15 1.22 \ \mu mol m^{-2} s^{-1}$  for the lowland and montane forests, respectivelymontane forest. Soil CO<sub>2</sub> fluxes in the montane forests were lowest during the dry season from June to September ( $2.95 \frac{5.44}{1.25} 2.42 \frac{5.40}{0.97} \pm \frac{0.95}{0.87} \ \mu mol m^{-2} s^{-1}$ ) and highest during the wet season during from October to May ( $4.49 \frac{6.33}{0.42} \frac{0.33}{0.42} \pm \frac{0.79}{0.33} \pm \frac{0.79}{0.33} \pm \frac{0.79}{0.33} \pm \frac{0.79}{0.33} = \frac{0.79}{0.79} 1.16 \ \mu mol m^{-2} s^{-1}$ ) (Figure 2c). Lowland fluxes were more stable throughout the year, with only a small

- 5  $3.71_{0.33}^{10.57} \pm 0.79 + 1.16$  µmol m<sup>-2</sup> s<sup>-1</sup>) (Figure 2c). Lowland fluxes were more stable throughout the year, with only a small increase at the end of the wet season in June ( $4.67_{2.04}^{6.12} + 3.50_{1.22}^{9.20} \pm 1.31_{1.15}^{1.15}$  µmol m<sup>-2</sup> s<sup>-1</sup>) (Figure 2c). The results from the intense sampling campaigns showed that there is not a big variability in soil CO<sub>2</sub> fluxes between chambers within a site (lowland: CV = 24%, montane: CV = 18%) and also between the different lowland forest sites (Figure A3 b, CV = 28%) and that fluxes are stable within a site and throughout a day (Figure A3 d). The linear mixed-effect model for soil CO<sub>2</sub> fluxes between fluxes are stable within a site and throughout a day (Figure A3 d).
- 10 explained 68 in the lowland explained 48 % of the overall variability, with 40 % whereas the model for the montane soil CO<sub>2</sub> fluxes explained 69 %, all allocated to fixed effects (forest type, soil temperature and WFPS) (Table 1). The linear mixed effect model showed a negative effect of soil temperature on soil CO<sub>2</sub> flux in the lowlands (P-value <0.01=0.03) but a positive effect in the montane forest (P-value <0.001). In montane the montane and lowland forest, a positive effect of WFPS on soil CO<sub>2</sub> flux (P-value <0.0010.05) was observed (Table 1).

# 15 3.3 $\delta^{13}$ C values of leaf, litter, soil respired CO<sub>2</sub>, and dissolved CO<sub>2</sub> in headwater streams

For each category (litter, soil, soil CO<sub>2</sub>, stream CO<sub>2</sub>), the  $\delta^{13}$ C values in the lowland sites were always more negative than in the montane forest (Figure 3). The most negative values are were found in leaf litter (-29.91 ± 0.94 for the lowland forest and -28.56 ± 0.85% for lowland and montane, respectively the montane forest). The highest values were found in stream dissolved CO<sub>2</sub>, with -22.74 ± 2.34 in lowland streams and -16.68 ± 0.95% in lowland and montane streams, respectivelymontane

20 streams. In both forest types, the  $\delta^{13}$ C values increased from litter via SOC and soil respired CO<sub>2</sub> to dissolved CO<sub>2</sub> in streams.



Figure 2. (a) Weekly averaged Mean weekly air (solid line) and soil temperature at 20 cm depth (dashed line) in the lowland (dark green) and montane (light green) forest sites. (b) Yearly median air- and soil temperature. (c) Weekly average Mean weekly water-filled pore space (WFPS) [%] in the lowland and montane forest soils at 30 cm depth. (d) Median WFPS in the lowland and montane forest. (e) Weekly median soil CO<sub>2</sub> fluxes (*F*) with error bars indicating the standard deviation. Green horizontal bars on top of panel C indicate the dry seasons in the lowland and in the montane forests, respectively. (f) Median soil CO<sub>2</sub> fluxes i the lowland and montane forests.

Only soil  $\delta^{13}$ C-CO<sub>2</sub> in the montane forest showed a small decrease relative to soil C (Figure 3). Monthly leaf litter  $\delta^{13}$ C did not show temporal variability (Figure A2d). The <u>average mean  $\delta^{13}$ C value of soil respired CO<sub>2</sub> was -28.35 ± 0.58 in the lowland forest and -26.39 ± 1.03% in the lowlands and montane forests, respectivelymontane forests. The linear mixed model showed a statistical difference in the  $\delta^{13}$ C values of soil CO<sub>2</sub> in the montane forest between the wet and dry season, however,</u>

5 no difference was found in the lowland forest. Whereas there was no significant difference Both, lowland and montane forests, showed significant differences in <sup>13</sup>C values of dissolved CO<sub>2</sub> between wet and dry season in the montane streams, a significant depletion in <sup>13</sup>C in streams, where the  $\delta^{13}$ C-CO<sub>2</sub> signature is more depleted in the wet season in lowland streams was found (Table 1 compared to the dry season (Figure A4).



Figure 3.  $\delta^{13}$ C values of litter, soil organic carbon, soil respired CO<sub>2</sub> and dissolved CO<sub>2</sub> in headwater streams adjacent to the monitoring sites in the lowland and montane forests. Numbers on top indicating the effect sizes of the two separate (lowland and montane) linear mixed effects models. Left numbers are intercept, all subsequent numbers - soil, soil CO<sub>2</sub> and stream CO<sub>2</sub> - are effect estimates relative to the litter.

#### 4 Discussion

#### 4.1 Soil CO<sub>2</sub> Fluxes

Long term studies of soil  $CO_2$  fluxes in tropical forests are scarce, especially in the Congo Basin. The Here we present high temporal resolution data presented here, with 1108 individual soil  $CO_2$  flux measurements over a period of more than 2 years,

- 5 represents the most exhaustive dataset for the Congo Basin. The results from the intense sampling campaigns showed that there is not a big variability in soil CO<sub>2</sub> fluxes between different lowland forest sites (Figure A3 b) and that fluxes are stable within a site and throughout a day (Figure A3 d). The average annual values. The mean annual values from forests of the Congo Basin reported in this study of 3.82, which are  $3.13 \pm 1.15 \cdot 1.22$  µmol m<sup>-2</sup> s<sup>-1</sup> and for the montane forest and 3.45  $3.69 \pm 1.22 \cdot 1.14$  µmol m<sup>-2</sup> s<sup>-1</sup> for the montane and lowland forests in the Congo Basin (Figure 2f), respectively, lowland forests,
- 10 are within the range of reported values from other tropical forests. It is, reported average mean soil CO<sub>2</sub> fluxes from South and Central American tropical forests were for French Guiana (2.30 to 5.30  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, (Buchmann et al., 1997; Janssens et al., 1998; Bréchet et al., 2011; Epron et al., 2013; Courtois et al., 2018)), Brazil (2.64 to 4.30  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, (Davidson et al., 2004; Doff Sotta et al., 2004; Sousa Neto et al., 2011; Sotta et al., 2007; Garcia-Montiel et al., 2004)), and Panama (5.20  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, (Pendall et al., 2010)). To our knowledge, the only reported soil CO<sub>2</sub> fluxes from a tropical forest in Africa in recent
- 15 years are from Kenya (Arias-Navarro et al., 2017; Werner et al., 2007) and they were rather low compared to our flux rates

rather low (i.e., between 1.04 and 1.66  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>). Higher fluxes were reported in tropical forests in Hawaii (6.96  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, (Townsend et al., 1995)) and Thailand (9.76  $\mu$ mol m<sup>-2</sup> s<sup>-2</sup>, (Hashimoto et al., 2004)). The data presented here are the first from tropical forests within the Congo Basin since Maldague and Hilger (1962) reported soil respiration values CO<sub>2</sub> fluxes from lowland forests in the DR Congo of 3 to 4  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. These values from the year 1962 lie exactly within the

5 range of the values measured in this study, although it is important to note that the 1962 fluxes were derived from only four observations.

#### 4.2 Seasonality of CO<sub>2</sub> Fluxes

Fluxes in the montane forest showed marked seasonality, with a 34 % decrease during the dry season, whereas fluxes in the lowland forests did not show any seasonality (Figure 2c). Courtois et al. (2018) have shown a similar trend of decreased fluxes

- 10 during the dry season (15.7 % decrease) in tropical forests in French Guiana, however<del>they were, the decrease they found was</del> not as pronounced as in the montane forests we sampled. One possible reason for the seasonal difference between montane and lowland forests is that the lowland dry season is not as distinct as in the montane regions. Rainfall events during the "dry season" in the lowlands are not uncommon (Figure A2). A model study by Raich et al. (2002) concluded that in seasonally dry biomes, soil CO<sub>2</sub> emissions positively correlate with precipitation. Precipitation was also identified as the main driver of
- 15 maximum C assimilation rates in 11 Sub-Saharan ecosystems, which in turn was an ultimate driver of soil  $CO_2$  fluxes (Merbold et al., 2009). Thus, given the results of the present study and the projected increase in dry season length in the Congo Basin, as recently reported by Jiang et al. (2019), one would expect a future decrease of C fluxes in the montane forests while little to no effect might be expected in the lowland forests. However, a change in rainfall patterns can also lead to more extreme drying-rewetting events and this might affect soil  $CO_2$  fluxes, as potential  $CO_2$  pulses after rewetting compensate for the
- 20 possible reduced soil respiration (Waring and Powers, 2016).

#### 4.3 Temperature and Soil Moisture Controls

Despite the markedly different temperature regimes between the lowland and montane forests, yearly averaged soil  $CO_2$  fluxes were almost identical (Figure 2 f). Such inter-site temperature independence of soil  $CO_2$  flux is unique compared to other tropical (e.g. Brazil (Doff Sotta et al., 2004; Sousa Neto et al., 2011) or temperate forests (e.g. Switzerland (Ruehr et al., 2010)),

- where strong correlations between soil  $CO_2$  fluxes and soil temperature were found. However, in addition to temperature, soil geochemistry can play a crucial role in soil respiration controlling soil  $CO_2$  fluxes, particularly via soil C stabilization processes and their rates (Doetterl et al., 2018). Short-term changes in C fluxes are mostly related to respiration of nonprotected soil C (plant residues, root exudates, rhizodeposition) while the majority of stored C in soils is stabilized within the mineral matrix (Doetterl et al., 2018). Thus, a potential increase in soil respiration  $CO_2$  efflux due to change in soil temperatures
- 30 in the lowland might be counteracted by higher C protection due to soil geochemistry. Within sites, a statistical statistically significant correlation between soil temperature and  $CO_2$  flux was found, however, montane and lowland forest displayed opposite relationships with soil temperature (Table 1). The negative relationship with soil temperature in the lowland forest indicates that soil temperatures are already too high for an optimal microbial activity. Nevertheless, it is important to note that

the soil temperatures within different forest types of the Congo Basin are relatively stable throughout the year (Figure 2 a), with standard deviations of  $0.34^{\circ}$ C and  $0.42^{\circ}$ C for montane and lowland soil temperature, respectively. Thus, given the lack of variability in soil temperatures, the accuracy of detected relationships could be questioned and extrapolations of the here found CO<sub>2</sub> flux responses beyond the soil temperature ranges observed in this study should be handled with care. For better

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understanding of temperature dependencies of soil respiration in these forest soils, warming experiments (incubation or field) are needed.

Despite the higher total annual rainfall in the lowlands (Figure A2), the montane forest soils exhibited higher WFPS (Figure 2 b). The lower relative WFPS in the lowlands was likely due to the sandier soil texture leading to faster drainage. Moreover, the montane forest site showed a clear positive relationship between soil  $CO_2$  and WFPS, whereas in the lowland site the effect

- 10 size of WFPS on soil CO<sub>2</sub> flux was uncorrelated with WFPS a lot smaller (Table 1). Soil moisture can influence soil respiration physically and biologically. Physically, soil moisture can limit the transport of C substrate to soil microorganisms (at low soil moisture conditions) and the diffusion of gases through soils, including both oxygen required for aerobic respiration and respiratory CO<sub>2</sub> (in high soil moisture conditions) (Manzoni et al., 2016; Moyano et al., 2012, 2013). Biologically, soil moisture can affect the activity of heterotropic respiration, where low soil moisture conditions stress soil microbial communities, and
- 15 autotrophic respiration (Xu and Qi, 2001; Rey et al., 2002). The latter is linked to canopy processes, where water limitation can lead to stomatal closure, limiting plant photosynthesis and thus also belowground respiratory processes (see also 4.4). One possible explanation for why the lowland soils CO<sub>2</sub> flux did not vary with WFPS is that the soil respiration is <u>potentially</u> limited by soil C availability, indicated by the similar isotope composition of litter, SOC and soil emitted CO<sub>2</sub> in the lowland forests (see also 4.4). Therefore, if soil respiration in lowland forests is indeed likely substrate limited, then environmental factors such
- 20 as soil moisture or temperature may have less control on soil respiration (Davidson and Janssens, 2006). While lowland forests are soil respiration in lowland forests is most likely C-limited, montane forests are respiration in montane forests seems to be more sensitive to environmental conditions and could represent a potentially large C source with climate warmingchange.

#### 4.4 Isotopic source indicators

It is well known that soil respiration and canopy processes are linked in forests (Ekblad, 2001). Carbohydrates produced

- 25 by photosynthesis are subsequently transported to the roots and rhizosphere, where they are respired by root or microbial respiration (Ruehr et al., 2009). Thus, the isotopic signature of soil-derived CO<sub>2</sub> is mostly governed by isotope fractionation processes that occur at the leaf scale, since a significant portion of soil respired CO<sub>2</sub> is supplied by recent photoassimilates (Högberg et al., 2001; Brüggemann et al., 2011; Barthel et al., 2011). Generally, all environmental parameters affecting photosynthesis and thus CO<sub>2</sub> discrimination are likely to influence the  $\delta^{13}$ C signal of soil respiration (e.g. precipitation, vapor pressure deficit)
- 30 (Bowling et al., 2002). In this study, the link between photosynthesis C assimilation and soil CO<sub>2</sub> is evident through the distinctively different  $\delta^{13}$ C signatures between lowland and montane forests along the whole cascade of carbon intake via photosynthesis, litterfall, SOC, soil CO<sub>2</sub> and stream dissolved CO<sub>2</sub>. This difference holds throughout most of the year for leaf litter and soil respired CO<sub>2</sub> between the lowland and the montane site (Figure A2). The strongest <sup>13</sup>C enrichment of soil CO<sub>2</sub> was observed at the end of the dry season (September) in the montane site, likely caused by lower photosynthetic <sup>13</sup>C-CO<sub>2</sub> discrim-

ination conveyed to soil respiration (Figure A2 c). Indeed, the enrichment of  $^{13}$ C of autotrophic soil respiration resulting from stomatal closure during periods of drought has been widely documented (Ekblad, 2001; McDowell et al., 2004; Blessing et al., 2016; Salmon et al., 2019). Such distinct enrichment was presumably not detected in the lowland sites due to the absence of a prolonged dry season (Figure A2 a). A study by Ometto et al. (2002) found similar seasonal dependencies of two tropical forest

5 of the Amazon Basin, with one site (Santarem forest) showing a distinct seasonality of  $\delta^{13}$ C signal of <del>cosystem respiration</del> <u>soil CO<sub>2</sub> fluxes</u> in response to large variation in rainfall whereas the other (Manaus forest) had only little variation in rainfall and thus also little variation in  $\delta^{13}$ C signal of <del>cosystem respirationsoil CO<sub>2</sub> fluxes</del>.

The  $\delta^{13}$ C value of various ecosystem components (leaf litter, SOC, soil respired CO<sub>2</sub>, and riverine CO<sub>2</sub>) were <del>universally</del> generally enriched in the montane compared to lowland forests (Figure 3). Increased foliar  $\delta^{13}$ C values at higher altitudes are

- 10 a commonly reported tendency (Körner et al., 1988; Hultine and Marshall; Chen et al., 2014). This observation is generally explained by the decrease in atmospheric pressure (and thus decreasing partial pressure of  $O_2$  and  $CO_2$ ) and decreasing temperature and its effect on partial stomatal closure and lower  $c_i/c_a$  (ratio of intercellular to ambient  $CO_2$ ) (Roderick and Berry, 2001) . While the decrease of  $O_2$  partial pressure and temperature are increasing the carboxylation efficiency of the Rubisco molecule and thus declining  $c_i/c_a$  (), declining temperature also effects the viscosity of water and alter the flux of water into the plants,
- 15 resulting in stomatal closure and decreasing  $c_i/c_a$  (Roderick and Berry, 2001). A decline in  $c_i/c_a$  will increase the foliar  $\delta^{13}$ C value (Farquhar and Richards, 1984). Similar isotopic enrichment with altitude has been shown even within small-scale gradients in Amazonian forests (de Araújo et al., 2008). In the Amazonian study, the relatively enriched values of leaf and ecosystem respiration in the high elevation sites was explained by increased leaf-level photosynthetic capacity (higher leaf nitrogen content and leaf mass per unit area (LMA)), which is decreasing intercellular CO<sub>2</sub> concentrations and reducing leaf discrimination,
- 20 resulting in increasing <sup>13</sup>C concentrations in the leaves (de Araújo et al., 2008). However, Bauters et al. (2017) reported decreasing leaf nitrogen content and LMA with higher elevations in tropical forests of the Congo Basin. It is more likely, that the higher  $\delta^{13}$ C values in the montane forest are linked to canopy processes and lower temperatures. A (photosynthesis and stomatal conductance) resulted from lower atmospheric pressure and lower temperatures. Furthermore, a shift in microbial communities due to temperature changes has been found to impact fractionation of the C isotope in heterotrophic soil respira-
- 25 tion (enrichment of <sup>13</sup>C at lower temperatures) (Andrews et al., 2000). Overall, different  $\delta^{13}$ C values in the studied ecosystem components between the two forest types might be due to a combination of different effects including temperature, canopy processes, and open vs. closed system isotope dynamics.

As C is respired and transferred down the cascade from photosynthesis to stream dissolved  $CO_2$ , it becomes more enriched with heavier isotopes when transiting from one pool to the next due to isotope fractionation (as <sup>12</sup>C tends to be preferentially

- 30 consumed). This is generally a feature of 'open systems' in which reactions occur with a continuous supply of substrate, while the residual substrate and products are lost from the system. In contrast, a 'closed system' is characterized by the absence of new inputs and results in less fractionation between substrate and product (Fry, 2006). The different enrichment gradients observed between lowland and montane tropical forests indicate typical-more closed vs. more open system dynamics, respectively. In particular, the similar isotopic signatures of litter, SOC, and emitted soil CO<sub>2</sub> at the lowland site indicated a more complete
- 35 decomposition of the C input into the different compartments and thus relatively closed system isotope dynamics (Figure 3).

However, it needs to be stated that this interpretation is somewhat speculative, as contributions of  $CO_2$  from root respiration can vary with forest types which may confound inter-site comparison. Additional research would be needed to test for the hypothesis of the lowland forests being closed C systems. Moreover, in both systems, the highest enrichment occurs in the terminal location as last step from soil to stream dissolved  $CO_2$ . A similar enrichment of stream  $CO_2$  relative to soil respired

5  $CO_2$  has also been found in the Amazon by Quay et al. (1989). However, since stream  $CO_2$  is governed by a multitude of factors (Enrichment factors: aquatic photosynthesis, equilibration with atmosphere, outgassing, and weathering of carbonate/silicate minerals (depends on  $CO_2$  source for  $SiO_2$ ); Depletion factors: respiration of organic C, possibly photodegradation) it remains difficult to isolate a single factor causing the different isotope effects between soil  $CO_2$  and stream dissolved  $CO_2$  for lowland and montane forest.

#### 10 5 Conclusions

Although the lowland and montane forests of the Congo Basin differed in terms of vegetation composition, climate, and edaphic conditions, there was no significant difference in annually averaged annual mean soil  $CO_2$  flux observed in this study. The montane forest site exhibited strong seasonality, primarily driven by water filled pore space. It was not possible to assess temperature dependency within a site, as the temperature range was too small. Furthermore, no temperature dependency

- 15 between sites was found.  $\delta^{13}$ C signatures exhibited a relative enrichment in montane site compared However, in contrast to the lowland across various ecosystem components (leaf litter, SOC, soil respired CO<sub>2</sub>, and riverine CO<sub>2</sub>). The montane forest also uniquely displayed seasonal variations of  $\delta^{13}$ C signal of soil respired forest, the montane forest site exhibited strong seasonality of soil CO<sub>2</sub>, likely driven by changing discrimination at the canopy scale. In contrast, the efflux, primarily driven by WFPS during the dry season. The nearly identical C isotopic signatures of soil derived CO<sub>2</sub>, litter, and SOC in the lowland
- 20 forest indicate that respiration is likely substrate limited. Substrate limitation in the lowlands would also limit the influence of environmental factors such as WFPS on the  $CO_2$  flux rate, which corresponds well to the observed lack of correlation between soil moisture or temperature with soil  $CO_2$  fluxes. However, this hypothesis of substrate limitation in the lowlands is highly speculative and further research would be needed to test it. Furthermore, we cannot rule out changes in soil  $CO_2$ fluxes with changing environmental conditions given the low range in variation observed over the study period. Overall, these
- 25 results fill a critical knowledge gap for soil respiration rates of major tropical forests, provide baseline flux magnitudes to better parameterize earth system models, and highlight how soil respiration in montane tropical forest soils of the Congo Basin are relatively sensitive to environmental changes and may become significant source of C to the atmosphere under a warming climate regimethat changes in the C balance might happen in response to more erratic rainfalls and weather extremes. Further monitoring in the Congo Basin is necessary (for example eddy covariance flux towers), to set this results in context of total
- 30 NEP in these ecosystems.

Data availability. All data used in this study were published at Zenodo and are available under http://doi.org/10.5281/zenodo.3757768.

#### **Appendix A: Method supplement**

#### A1 $\delta^{13}$ C measurement of air samples with the Gasbench

Carbon isotopic composition of  $CO_2$  in gaseous samples were measured with a modified Gasbench II periphery (Finnigan MAT, Bremen, D) coupled to an isotope ratio mass spectrometer (Delta<sup>*plus*</sup>XP; Finnigan MAT; modification as described by

- 5 Zeeman et al. (2008)). In short, the modification of the Gasbench comprises comprised the replacement of the GC-type split by a ConFlo III-like split and the addition of a home-built cold trap (1/10" SS capillary filled with Ni-wire, Goodfellow GmbH, Bad Nauheim, D) instead of the standard sample loop of the 8-port valve inside the Gasbench II. The gas mixture in the exetainer is-was transferred to the cold trap after piercing the septum with a vendor-supplied double-holed needle connected to two capillaries (fused silica and 1/32" steel capillaries). The feed capillary delivers delivered pure He allowing a pressure
- 10 build-up in the exetainer which flushes-flushed the sample gas at a rate of about 0.5 mL/min over Nafion dryers to the cold trap where condensible gases (mainly CO<sub>2</sub> and N<sub>2</sub>O) are were frozen out with liquid nitrogen. The cold trap is was connected to a pressurized Dewar vessel and equipped with a computer-controlled automatic refill unit (Zeeman et al., 2008) allowing automatically refilling of the cold-trap. After diverting the non-consensible gases to a vent, the cold trap is was thawed and the content of the cold trap is was automatically injected on a GC column (Poraplot Q 25 m x 320 mm i.d. (Varian, Walnut Creek,
- 15 USA) held at 24°C) to allow separation of the isobar gases CO<sub>2</sub> and N<sub>2</sub>O. Post-run off-line calculation and drift correction for assigning the final  $\frac{13C}{\delta}$  values on the V-PDB scale were done following the "IT principle" as described by Werner and Brand (2001). The  $\delta^{13}$ C- (and  $\delta^{18}$ O-) values of the laboratory air standards were determined at the Max-Plack-Institute for Biogeochemstry (Jena, D) according to Werner et al. (2001). The linking of the measured  $\delta^{13}$ C (and  $\delta^{18}$ O) values of CO<sub>2</sub> gas isolated from air samples relative to the carbonate V-PDB scale was done via the Jena Reference Air Standard (JRAS),
- 20 perfectly suited to serve as a primary scale anchor for  $CO_2$ -in-air measurements. The measurement of the aliquots of the laboratory standards is was routineously better than 0.15 %.

#### Appendix B: Results supplement



**Figure A1.** Shown are all individual Keeling plots for each chamber replicate (eolourcoloured, n = 3) per site and per month of Kahuzi-Biéga (circles, solid lines) and Yoko (triangles, dashed lines) forest sites.

Appendix B: Results supplement



**Figure A2.** a) Monthly rainfall in mm at a lowland site in Yangambi and in Bukavu near the montane site. b) Monthly median  $CO_2$  fluxes in the lowland and montane forests. c) Monthly median  $\delta^{13}C$  values of the soil respired  $CO_2$ . d) Monthly  $\delta^{13}C$  of litter in montane and lowland forests. Error bars indicating standard deviation.



**Figure A3.** Median  $CO_2$  fluxes with errorbars indicating standard deviation. a) Sampling campaign in a lowland forest in Djolu between May and August 2016. b) Sampling campaign in Kahuzi-Biéga (montane forest) Yoko and Yangambi (lowland forests) in September and October 2016. c) Sampling campaign in Yoko in May 2017. d) Sub-daily sampling in Kahuzi-Biéga and Yoko. x-Axis shows the hour of the day.



Figure A4. Seasonality of  $\delta^{13}$ C values from different compartements in the lowland forest (a) and montane (b) forest. Plot showing mean values with errorbars indicating the standard deviation. Soil  $\delta^{13}$ C values are not included, as they were not sampled in different seasons. Numbers on top indicate the effect sizes of the two separate (wet and dry season) linear mixed effects models. Left numbers are intercept, all subsequent numbers - soil CO<sub>2</sub> and stream CO<sub>2</sub> - are effect estimates relative to the litter.

*Author contributions.* SB, MBarthel and JS were responsible for study design. Fieldwork was conducted by SB, MBarthel, IAM, JKM, LS, and NG. Lab work was conducted by MBarthel and RAW. Data interpretation was performed by SB, MBarthel, TWD, MBauters supported by KVO, PB and JS. The manuscript was written by SB with contributions from all co-authors.

Competing interests. The authors declare no conflict of interest.

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