

## ***Interactive comment on “The stable carbon isotope signature of methane produced by saprotrophic fungi” by Moritz Schroll et al.***

**Moritz Schroll et al.**

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We thank Referee 2 for the positive evaluation of our work and for the helpful comments to improve the manuscript. All comments and requested changes were taken into account. Please note that comments by the referee are in italics and that in the authors' answer the mentioned line numbers refer to the version of the revised manuscript including track changes.

Referee 2: General comments: Methane is the second important anthropogenic greenhouse gas after carbon dioxide. Recent studies have shown that this gas can be produced under aerobic conditions by plants, algae, fungi and animals. In this manuscript, Schroll et al. cultivated two saprotrophic fungi on three different substrates and mea-

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sured the stable carbon isotope values of methane. This study is the first to report the analysis of stable carbon isotope values of methane emitted from saprotrophic fungi. The authors found that the source values of  $\delta^{13}\text{CH}_4$ , emitted by the fungi, were dependent on the fungal species and the metabolized substrate. Although this paper has some limitations in terms fungal species and substrates, it certainly opens the door for new and exciting work in the area of aerobic methane emissions. Overall, this is a well-written manuscript and deserves to be published in Biogeosciences after minor revisions.

Authors: We thank the referee for the positive evaluation of our manuscript. The reviewer's concerns are addressed below.

Specific comments:

1) L16. eukaryotes,

Authors: Change applied.

2) L17-18. ecosystems via decomposition of plant litter

Authors: Change applied.

3) L18. Although the methane

Authors: Change applied.

4) L19. In this study,

Authors: Change applied.

5) L20-21. The common names of fungi must be mentioned here

Authors: The common names of the fungi have been added to the revised manuscript.

6) L21. , cultivated... (pine...), reflecting

Authors: Change applied.

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7) L21-22. Which grass? It is better to provide the Latin names of pine, grass (species name) and corn

Authors: The Latin names of the pine, grass and corn species have been added to the revised manuscript.

8) L23. Keeling; K must be uppercase here and in other places

Authors: Change applied.

9) L27. 'Whilst' should be replaced; it is mentioned in the previous sentence

Authors: Change applied.

10) L29. We found that the values of  $\delta^{13}\text{C}_{\text{CH}_4}$  emitted

Authors: Change applied.

11) L30. What is 'They' in 'They cover'?

Authors: Change applied.

12) L34. Fossil fuel burning indicates a process but not source; source is fossil fuel, biomass, and...

Authors: Change applied.

13) L37. microorganisms,

Authors: Change applied.

14) L40. discovered,

Authors: Change applied.

15) L45. It is better to delete 'therefore'

Authors: Change applied.

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16) L46-47. White rot fungi (e.g., Latin name)... brown rot fungi (e.g., Latin name)

Authors: Examples for white rot fungi and brown rot fungi are now included in the manuscript.

17) L49. in the synthesis of  $\text{CH}_4$

Authors: Change applied.

18) L51. archaea with essential substrate... in fungus-infected wood stem

Authors: Change applied.

19) L55. might be an underestimated

Authors: Change applied.

20) L56. It is better to delete 'Applications of'; It is better to start the sentence with Stable isotope procedures

Authors: 'Applications of' has been deleted. Please note, that we would like to write 'Stable carbon isotopes', as in this context it refers to stable isotopes in a general meaning.

21) L57-58. 'they' is referred to what?

Authors: Change applied.

22) L64. have been identified

Authors: Change applied.

23) L67-68. plant-derived  $\text{CH}_4$ ..., and UV-induced  $\text{CH}_4$ ...

Authors: Changes applied.

24) L69. In this study, we...

Authors: Change applied.

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25) L76. Pleurotaceae and Polyporaceae are the family names and should not be italicized.

Authors: Changes applied.

26) L81. Both common and Latin names should be provided for pine, grass (specific plant species) and corn

Authors: Both names have been added to the revised manuscript.

27) L97-98. It is better to provide the temperature for autoclave

Authors: A more detailed description of the autoclave method was added to this section.

28) L114. What are those five different gases?

Authors: The five reference gases were certified gas mixtures of CH<sub>4</sub> and CO<sub>2</sub> with five different concentrations by Deuste Steining GmbH. The name of the company was added to the manuscript to clarify the origin of the reference gases.

29) L141-143. Is 'the working reference gas' the standard reference gas?

Authors: We modified 'working reference gas' to read "working standard". We also corrected an error (L142) where the two reference standards are CH<sub>4</sub> and not CO<sub>2</sub>. Those two CH<sub>4</sub> reference standards are calibrated and certified and are used for the normalization of the samples. According to the 'Principle of identical treatment' the CH<sub>4</sub> reference gases were measured exactly in the same way as the samples.

30) L149. substrate was put... the resulting gases were separated...

Authors: Change applied.

31) L151. 27.5 m ... then reached

Authors: Change applied.

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32) L153. Keeling

Authors: Change applied.

33) L159. Keeling

Authors: Change applied.

34) L161. Keeling...Keeling

Authors: Change applied.

35) L163. It is better to delete the first 'grown on pine'

Authors: Change applied.

36) L167. Keeling

Authors: Change applied.

37) L178. Was there a reason for using Fisher test instead of a robust test, such as Tukey's test?

Authors: The statistical evaluation with two way ANOVAs was chosen to conclude if there is a general effect of the fungi and substrates on CH<sub>4</sub> and CO<sub>2</sub> mixing-ratios,  $\delta^{13}\text{C}_{\text{CH}_4}$  and  $\delta^{13}\text{C}_{\text{CO}_2}$  values and the CH<sub>4</sub> : CO<sub>2</sub> emission ratios. The results of the post-hoc tests (Fisher least significance difference and Tukey) are attached in the supplement to this comment. Please note, that the post-hoc tests only have a limited value as there are only three repeated measurements for each parameter (n=3) and post-hoc tests are generally designed for a greater number of repeated measurements. Therefore, we prefer not to show the post-hoc tests in this manuscript and keep the general effects that are expressed by the two-way ANOVAs. Nevertheless, for the  $\delta^{13}\text{C}_{\text{CH}_4}$  and  $\delta^{13}\text{C}_{\text{CO}_2}$  isotope values p-values calculated with the Fisher LSD and Tukey test are similar and produce only minor differences. Please note that p-values (> 0.05) for CH<sub>4</sub> and CO<sub>2</sub> mixing-ratios might occur because either the quantity of

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emitted CH<sub>4</sub>/CO<sub>2</sub> by the fungi is similar and/or the biomass of the fungi within the flasks varies. The manuscript was changed accordingly (L177-178) to clarify that the statistical methods applied in the manuscript refer to the results of two-way ANOVAs.

38) L185. Keeling

Authors: Change applied.

39) L187. The second 'source' can be deleted.

Authors: Change applied.

40) L193. 'the' should be deleted.

Authors: Change applied.

41) L197. The second 'grown' should not be italicized.

Authors: Change applied.

42) L203-205. Most of the controls? It is better to be specific.

Authors: The sentence was modified to be more specific.

43) L205. ... . respectively were observed

Authors: This part of the sentence was replaced because of the changes made to the previous comment 42).

44) L215. was present

Authors: Change applied.

45) L229. thereby. both...; the 'both' after substrate should be deleted.

Authors: Change applied.

46) L230. Is it  $P < 0.001$ ?; a comma should be added after *sapidus*

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Authors: Yes. it is  $p < 0.001$ ! The comma was added after *P. sapidus*.

47) L237. 'in a good accordance' is not clear. it needs to be rewritten.

Authors: 'in a good accordance' was replaced by 'in the same order of magnitude' to make this sentence clearer.

48) L238. It should be noted that CH<sub>4</sub>

Authors: Change applied.

49) L272-274. It is better to rewrite this sentence. like: ...Keeling plot analysis that range ... are presented.

Authors: Change applied.

50) L276 and L280.  $P < 0.001$  (number should not be italicized)

Authors: Change applied.

51) L289. Keeling; one of the 'values' should be deleted.

Authors: Change applied.

52) L295. 'as so far' is not clear

Authors: 'As so far' was replaced by 'as up to the present' to make the sentence clearer.

53) L297-298. The values of... that range from... are presented in Table 2

Authors: Change applied.

54) L300. 'more' should be deleted.

Authors: Change applied.

55) L304-305. 'Although... substrate' is not a sentence and should be rewritten.

Authors: The sentence was rewritten.

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56) L309. 'usually' should be deleted from here and added after 'are'

Authors: Change applied.

57) L311. 'slightly more' should be reworded.

Authors: Change applied.

58) L318. CH<sub>4</sub> and CO<sub>2</sub> are derived

Authors: Change applied.

59) L331. a wide range

Authors: Change applied.

60) L351. sources. such as methanogenic archaea and eukaryotes.

Authors: Thanks for the note. We changed 'abiotic processes' to 'abiotic CH<sub>4</sub> sources' because the term 'abiotic processes' might be misleading. Nevertheless, we would like to keep the 'abiotic CH<sub>4</sub> sources' in this sentence.

61) L351. 'and from abiotic processes' should be deleted or modified in such a way to show sources Authors: Please see response to previous comment 60).

62) L353. processes. resulting

Authors: Change applied.

63) L354-357. The sentence that starts with 'Thus. studying' is not clear and should be rewritten.

Authors: Change applied.

64) L358. research. stable

Authors: Change applied.

65) L374. Grant Numbers

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Authors: Change applied.

66) L398. In CO<sub>2</sub>. 2 should be subscript.

Authors: Change applied.

67) L405. The title of this paper should be written in correct format.

Authors: Change applied.

68) L455. The Latin name should be italicized.

Authors: Change applied.

69) L461. In CH<sub>4</sub>. 4 should be subscript.

Authors: Change applied.

70) L462-L463. CH<sub>4</sub> and 13C/12C should be written in correct format.

Authors: Changes applied.

71) L465-467. In CH<sub>4</sub>. 4 should be subscript; In 13C/12C. 13 and 12 should be superscript.

Authors: Changes applied.

72) L526. The Latin name should be italicized.

Authors: Change applied.

73) L547. Plant Cell Environ.

Authors: Change applied.

Please also note the supplement to this comment:

<https://www.biogeosciences-discuss.net/bg-2020-108/bg-2020-108-AC2-supplement.pdf>

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