**Response to reviewers**

Dear Dra. Natalie Olifiers,

Editor of the Oecologia Australis journal,

We are submitting, for your consideration, the revised version of our manuscript **“Gasohol effects on microbial community of a soil originated from the Atlantic forest”** for publication in Oecologia Australis journal.

We would like to thank you and the anonymous Referees for their time expended in carefully reading the article and for their constructive suggestions.

All suggested corrections were carefully considered, and a detailed point-by-point response is provided below. Also, all corrections made are highlighted in “red” in the second revised version of the manuscript and the file containing the table.

We declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. There are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. The manuscript has been read and approved by all named authors.

We hope that you find this manuscript version acceptable for publication and thank you in advance for considering our submission.

Sincerely,

Reviewer A:

1) Does the title adequately reflect the content of the manuscript?:

    Yes it does.

2) Is the manuscript a relevant scientific contribution to ecology?:

    Yes it is. Although it overstates some results and offers speculative explanation of some phenomenons, it is still relevant research.

3) Does the summary present the main idea of the manuscript and its objectives and main results and conclusions?:

    Yes. However I would recommend to tone down the description of some results and conclusions.

For instance:

In the first sentence, I would suggest the authors to remove the word "change" by "the effect", once they didn't detect a change in the functional diversity of the bacterial community.

To state that the genetic profile of the bacterial community was exactly the same, using the DGGE approach is quite strong, considering the soil being one of the most diverse environments harboring thousands of bacterial species and the method being limited to precisely differentiate not more than a hundred of bands.

Therefore, I would suggest the authors to state that "the DGGE profile was similar to the one detected on the control." of course, using their own words.

Moreover there should be some mention to the experimental setup like the addition of nutrients to the soil, before addition of the gasohol.

These points were modified in the text.

4) Are the keywords pertinent and different from the words used in the manuscript title?:

    Yes.

5) Does the introduction present the theoretical/empirical content in which the manuscript topic is inserted?:

    The introduction could be improved with citation of works that evidence the lose of "crucial ecological functions" under hydrocarbon contamination, like Jung et al, 2016. On the other hand, the argumentation could be enforced by citing classical support for the expected loss of function linked to the loss of biodiversity, like Wardle and Giller, 1996 or Gittay and Wilson

1996. These changes are suggested for the second paragraph.

Jung J, Philippot L, Park W. Metagenomic and functional analyses of the consequences of reduction of bacterial diversity on soil functions and bioremediation in diesel-contaminated microcosms. Scientific reports. 2016 Mar 14;6:23012.

Wardle DA Giller KE (1996 )The quest for a contemporary ecological dimension to soil biology . Soil Biol Biochem  28 :1549 --1554.

Gitay H Wilson JBB (1996 )Species redundancy:a redundant concept? J Ecol  84 :121 --124.

These references were added and the introduction was modified. More content was added to introduce bioremediation and the hydrocarbons soil contamination effects in the soil.

6) Are the methods adequate and clearly presented?   There should be some improvements in the description of the methods. The sampling was made in a single spot or multiple spots were sampled to represent an area? How much soil was sampled?

20 samples were collected and joined, obtaining around 2 dm3 of soil volume. This was added in the text.

What are the characteristics of this soil, pH, OM, P-REM?

We didn’t soil analysis.

How many experimental replicates were used to draw the statistics? Maybe creating a figure illustrating the experiment helps the reader to understand the details.

Three replicates were used. This was added in the text.

Why the temperature of incubation for the genetic diversity assessment was different than the incubation temperature for the metabolic response (22° and 25° C)?

For genetic profile analysis by DGGE, the samples were incubated at 25 ºC in a BOD type incubator. For analysis of the metabolic response, the samples were at laboratory laboratory temperature, since the respirometer can not be placed in an incubator (because of its dimensions). Nevertheless, the incubation temperature in the latter case was around 22 ºC, that is very close to the one used for the analysis of the genetic profile.

7) Are the results, discussion and conclusion clearly presented and do they correctly address the objectives of the study?:

In the first paragraph, why hydrocarbons and ethanol are separated as substrates? I find it a bit confusing. Maybe the phrase "... newly added substrate (gasohol)."  or "... newly added substrates (petroleum hydrocarbons and ethanol)." would make more sense. Moreover, in the end of the section 3.3, the authors again mention the biodegradation of hydrocarbons and ethanol. Which in my opinion is a mistake as although the gasohol have ethanol in its composition, there was no experimental set up evaluating petroleum hydrocarbons and ethanol separately. I suggest exchanging this nomenclature to "gasohol components".

The modification was made in the text.

One surprising result is that, in Figure 2, the time 0 for the contaminated treatment shows low DNA extraction yields but the same DGGE profile as the control. Does it make sense? Why? Also, on time zero for the contaminated sample, the DNA was extracted how long after the contamination?

Sentences were added on the 2.3 and 4. sections to explain this point. The similar DGGE profile on the zero time was a surprise for us. It’s possible due to solvency potential of the gasohol, affecting all the microbial community. This was added in the text.

Still regarding the Figure 2, would you have a numerical quantification of the DNA extraction, taken with Qubit or a spectrometer? A table with this information would also be informative.

The DNA was verified only in agarose gel.

Regarding the discussion session, it is quite speculative regarding how much volatile hydrocarbons were lost through evaporation and how much was really biodegraded. Maybe some references would help to have an idea. In addition, the whole discussion would benefit from references  regarding ecological theory that could backup the explanations for phenomenons. Otherwise the reader gets only the authors opinions on the results. For example, the citation of Mendes, 2015 on paragraph 4 enriches the discussion, but in the previous paragraph (n. 3) the authors make a big statement about how "the microbial populations in the Atlantic forest have high substrate utilization redundancy" based only in one sample from a small patch of forest in the state of Minas Gerais. This makes the text highly speculative. Citing more evidence helps to back up the arguments specially with small sampling design. Or you should either tone down the discussion for something more locally focused, which is also not bad.

This point was modified in the text.

8) Are all the figures and tables essential and self-explanatory?:

 Yes they are essential. But I couldn't find the  Figure 3B should have a better resolution of the scale numbers and also a

text informing that the scale represents the percentage of similarity between the band profiles.

The figure and its legend were modified.

9)  Are the references pertinent and up-to-date?:

    Yes, but the whole text would benefit from citation of classical microbial ecology theories to sustain their claims, or other studies from the Atlantic Forest considering the authors only assessed one site in a biome of more than 100 000 hectares.

Some references were added.

10) Final Considerations::

    This research is very important for assessing an highly bio diverse ecosystem and for evaluating the effects of contaminant spillage on the microbial community. It is weak regarding its comprehensiveness, but is technically sound and supplies important results. I am sure the readers of Oecologia Australis and all the scientific community will benefit from this research in case of all the issues pointed here being addressed. The authors must be specially careful not to overstate their findings expanding the results to the whole forest, keeping in mind (and making clear in the text) that this was made in laboratory conditions and the sampling design was limited. Therefore I recommend it to be published with major reviews, considering the high tone of their conclusions.

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Reviewer C:

1) Does the title adequately reflect the content of the manuscript?:

    Sim

2) Is the manuscript a relevant scientific contribution to ecology?:

    Sim

3) Does the summary present the main idea of the manuscript and its objectives and main results and conclusions?:

    Sim

4) Are the keywords pertinent and different from the words used in the manuscript title?:

    Sim

5) Does the introduction present the theoretical/empirical content in which the manuscript topic is inserted?:

    Não. A introdução apresenta pouca informação, esta deve ser ampliada com mais informação referente a biorremediação e impacto de hidrocarbonetos sobre a comunidade microbiana do solo.

A introdução foi modificada para adicionar tais informações.

6) Are the methods adequate and clearly presented? :

    Sim. Recomendaria apenas fazer uma melhor descrição da área de coleta da amostra de solo, mencionando as principais características do Bioma Mata Atlántica.

Informações adicionais sobre a coleta foi adicionada no texto. Nos desculpe, mas não achamos viável mencionar as principais características do bioma na seção de metodologia. Isso seria inadequado, portanto, adicionamos mais informações na Introdução.

7) Are the results, discussion and conclusion clearly presented and do they correctly address the objectives of the study?:

    Resultados: Dados de diversidade catabólica foram sitados na conclusão mas não apresentados, sugiro adicionar estes resultados.  Discussão: procurar melhorar e adicionar referencias mais atuais.

Os dados de diversidade catabólica foram apenas citados, visto que o objetivo principal deste trabalha é mostrar a resiliência da comunidade microbiana do solo da Mata Atlántica. Um futuro trabalho a ser submetido abordará como foco as características catabólicas. A discussão foi melhorada e novas referências e novos conteúdos foram adicionados de forma a melhorar o entendimento por parte dos leitores do trabalho.

8) Are all the figures and tables essential and self-explanatory?:

    Sim

9)  Are the references pertinent and up-to-date?:

    Não.

Novas referências foram adicionadas de forma a atualizar o trabalho.

10) Final Considerations::

    O artigo apresenta informação relevante porem precisa de alguns ajustes na introdução e melhorar a discussão.