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Jihočeská univerzita  
v Českých Budějovicích  
University of South Bohemia  
in České Budějovice

## OPPONENT'S REVIEW ON BACHELOR/DIPLOMA\* THESIS

**Name of the student:** Deborah Walter

**Thesis title:** Quantification of specific microbial functional guilds in Arctic soil

**Supervisor:** Ing. Jiří Bárta, Ph.D.

**Referee:** RNDr. Alica Chroňáková, PhD.

**Referee's affiliation:** Biology Centre of Academy of Sciences of the Czech Republic, v. v. i. - Institute of Soil Biology & SoWa Research Infrastructure, Na Sádkách 7, 370 05 České Budějovice

Point scale<sup>1</sup> Points

### (1) FORMAL REQUIREMENTS

<b>Extent of the thesis</b> (for bachelor theses min. 18 pages, for masters theses min. 25 pages), <b>balanced length of the thesis parts</b> (recommended length of the theoretical part is max. 1/3 of the total length), <b>logical structure of the thesis</b>	0-3	3
<b>Quality of the theoretical part (review)</b> (number and relevancy of the references, recency of the references)	0-3	1
<b>Accuracy in citing of the references</b> (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
<b>Graphic layout of the text and of the figures/tables</b>	0-3	2
<b>Quality of the annotation</b>	0-3	2
<b>Language and stylistics, complying with the valid terminology</b>	0-3	1
<b>Accuracy and completeness of figures/tables legends</b> (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	1
<b>Formal requirements – points in total</b>		12

### (2) PRACTICAL REQUIREMENTS

<b>Clarity and fulfillment of the aims</b>	0-3	2
<b>Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions</b>	0-3	1
<b>Discussion quality – interpretation of the results and their discussion with the literature</b> (absence of discussion with the literature is not acceptable)	0-3	2
<b>Logic in the course of the experimental work</b>	0-3	2

\* Choose one

<sup>1</sup> Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Completeness of the description of the used techniques	0-3	1
Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	1
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements – points in total		16

POINTS IN TOTAL (MAX/AWARDED)	48	28 (0-48) <sup>2</sup>
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**Comments of the reviewer on the student and the thesis:**

Generally, the topic of the thesis is actual and interesting, and thus it has a potential to bring new information to the field of microbial ecology in Arctic soils. The results maybe probably used as background for publication after standards in data handling will be applied. Major concern is related to lack of hypothesis (-es; even one may judge them from the description of results, but they were not provided), so it is hard to tell if the aims of the study were fulfilled. Nevertheless, I have concerns to all parts of the thesis. In Introduction and further on, the something like “red line” is missing, some information is provided, however randomly appeared, but many important ones are missing and they are not well interlinked, which decrease the quality of the thesis.

The positive point is that the student experienced in useful methodology and approach, which is still valuable in soil microbial ecology. Student measured large amount of samples by quantitative PCR and probably significantly contributed to the project related to the study. However, I did not have a feeling, that student even understood the methodology used and the way how data were treated and processed, because there was no (or very little) word spent about it. In quantitative PCR, data processing, evaluation and interpretation need to reach high standards and should be described in Materials and Methods in detail, otherwise it is difficult to judge the proper handling of raw data (For example, there is no information about the efficiency of assays used for gene quantification, what DNA type was used as standards for qPCR, information about complete PCR conditions and data capture, how exactly were data treated according to obtained Ct values for internal standards (subtracted?), how raw data were processed to get final dataset). I think it will be very useful.

There is also the issue with the use of proper statistical analyses. It is noted that Pearson correlation test has been performed, but the tables given in the Results do not present correct outputs of the statistic test (see f. e. <https://statistics.laerd.com/statistical-guides/pearson-correlation-coefficient-statistical-guide.php>). Soil parameters used for correlation tests were not provided. It seems that the abundancies of genes are somehow dependent on the efficiency of DNA extraction or soil microbial biomass, because all of them follow the same trend and somehow positively correlate with total C and N content. Moreover, they even strongly correlate with soil moisture. It is apparent, that it is related to the way how data were treated and normalised (f. e. if gene copy numbers obtained per µl of DNA extracted, then recalculated per gram of dry soil – both DNA yields and water content affect the final data). So, it would be helpful to show what were the DNA yields (not provided) and to look if they were not dependent on soil layer.

<sup>2</sup> Enter the number of points awarded.

The interpretation of the results is sometimes misleading, mainly because the misinterpretation of the results of correlation test. In Discussion part, one should be careful to say something about the numbers of microorganisms observed, because this parameter was not measured and it is tricky to infer those numbers from observed abundances of studied genes. Apparently, lack of hypothesis make it difficult for student to discuss observed results and to compare them with conclusions of other studies.

I do not know the reason, however sometimes it is quite difficult to follow the text and get the point, it might be issues with terminology or it might be incorrect English language, or both). In particular, in Discussion there is first time used the term "classifying genes" as an analogue to "functional genes" and they were not anytime defined, do they mean the same? Next, I did not understand what do you mean by "positive carbon feedback", can you please explain to me?

To conclude, the student made a lot of work in the lab, which I appreciate, however there are drawbacks in the literature review, data analyses, interpretation of results and discussion. I would suggest to streamline the aims for the student (f. e. to choose only N cycle or only methane cycle) to have chance to better understand the processes behind, factors which may influence the respective microbial guilds and the ways how to interpret observed results.

### **Suggestions and questions, to which the student has to answer during the defense.**

#### **Mistakes, which the students should avoid in the future:**

I would suggest the student to present the clear hypothesis (or hypotheses), which can be further accepted or rejected.

I would suggest to include to Supplementary material the tables with soil properties instead of numerous figures with regression, which are not mentioned in the text of the thesis. As well, there are graphs showing the abundancies of genes split by localities (sampling sites), why? Did the sampling site have an effect or differences in gene abundancies among soil layers can be generalized to all? It would be helpful to test.

I would suggest to give more information on soil N and methane cycle in Introduction part, especially with focus to studied processes – N<sub>2</sub> fixation, denitrification, and methanogenesis (f. e. schemes are not too much informative – N<sub>2</sub> fixation is even missing in Figure 3). Please, describe briefly all pathways for methanogenesis and all known electron donors and C sources for methanogens. In the second paragraph, p. 5, there are some information missing. There is also citation, which I did not find in the references (Angle, 2017) and I think it should be study of Angel et al., 2017.

Can you please explain, why those processes and respective genes have been selected? I did not found any information about. One can select from large set of the genes involved in N cycle or methane cycle, so can you explain this selection? So, my suggestion is to include this information into Introduction.

In the last paragraph in the p. 9. is written: "After checking the distribution of the data with a histogram, the data was normalized by log<sub>10</sub> transformation." So, does it means that you have been testing the normality/normal distribution? If yes, by which test and did the log<sub>10</sub> transformation helped to reach normal distribution of your data? Is that the same for data used for ANOVA?

Can you please explain the theory behind the correlation test you used? Maybe than you will consider more accurate interpretation of your results and different conclusions (weak or strong, positive or negative correlation between two independent variables – according to results observed). There is misleading title in Tables 2-4-5 related to the results of correlation test - the results of correlation test are shown not the regression (again the issues with terminology).

Can you please describe me what the information is provided by letters given above the bars in Figures 4-8? Is that a results of statistical test? Please explain. There is a mistake in

description of results for *nosZ* gene (discrepancy between information in the text and Figure 7).

The philosophical question: (p.5 Nitrogen cycle) – Can you explain me how fits together the idea of sustainable agriculture with the must to supplement the soil with artificial N?

I would recommend the student to focus to one process of nutrient cycling in soils, which help you to make a deep literature review and understand more the theory behind.

**Conclusion:**

In conclusion, I

**r e c o m m e n d / ~~d o n o t r e c o m m e n d~~\***

**the thesis for the defense and I suggest the grade Good (3) .<sup>3</sup>**

In České Budějovice date 12<sup>th</sup> June, 2019



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signature

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<sup>3</sup> You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).