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Interactive comment on "Soil microbial biomass, activity and community composition along altitudinal gradients in the High Arctic (Billefjorden, Svalbard)" *by* Petr Kotas et al.

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Dear Associate Editor and Reviewer, please find below a detailed response to all Reviewer #1 comments and questions regarding our manuscript.

Best regards,

Petr Kotas and co-authors

General comments: The study by Kotas et al. was focused on changes in microbial biomass, activity, and broad community structure (based on PFLA) along altitudinal gradients in the Artic. This question has great significance concerning the implications

of global warming on these ecosystems. The study consists of 3 different transects represented by 4 different elevations, and for each sample the authors collected substantial amounts of data representing soil type, soil chemistry (pH, ion content and concentrations, TOC, TN, moisture content, and temperature ranges), and very briefly mention vegetation coverage. The authors try to disentangle the impacts of all these along with elevation on microbes using partial redundancy analysis as well as several other statistical approaches. They have a robust sample design with good replication to try and address this question.

I did have several issues with the manuscript. First, I found it very confusing that the authors kept referring to two different gradients, altitudinal (the main gradient of interest), and horizontal. However, this horizontal aspect is never discussed in the methods section and I assume it is referring to the south to north orientation of the 3 transects along the Petunia Bay. This needs to be clarified explicitly and its significance needs to be discussed. Is it expected there is a strong S-N effect? I assumed these 3 gradients were expected to be replicates of each other, but they have strong differences in soil characteristics and microbial community (particularly Gr1). This becomes more apparent in the Discussion, but the author's need to make this clear early on.

Author response: We agree with the reviewer opinion. Even though both the horizontal and altitudinal aspects were mentioned in the methods (L 163) and presented in the results (L197-199, L229-231), we agree that this needs to be clarified throughout the text with stronger attention paid also to horizontal variability. We will clearly distinguish between the effects of altitude (vertical aspect) and transect (horizontal aspect) in the revised manuscript. The 3 transects were expected to be replicates of each other. We didn't expect any variability in soil geochemical or microbial characteristics which could be ascribed to the differences in orientation of the selected transects. Opposite was true - we did our best to select similarly oriented transects (slopes on the western coast of Petunia Bay) in order to minimize the effect of distinct slope orientation.

I also had concerns with their microbial respiration data and the authors need to justify their choice of a 2 week pre-incubation at 6 C. The pre-incubation will burn off all the labile carbon and drastically alters this respiration rate. This needs discussed as it can substantially alter the conclusions of a large portion of the paper.

Author response: We agree with the reviewer that we have to justify and discuss our methodological approach. The methodology was chosen according to available knowledge and our experiences with similar experiments. We insist that the presented respiration data corresponds to in situ microbial activity. First of all, we measured the respiration also at day 4 and 12 during the incubation period. Our measurements have shown two important things: i) the daily production of CO_2 during the first four days of incubation was on average 2.6 times higher compared to daily CO_2 production between days 4 and 12 (Fig. 1). This is accordance with strongly enhanced respiratory burst after soil thawing reported previously (Skogland et al. 1998, Soil Ecol. 11, 147-160) due to the flush of easily available substrates from lysed microbial cells. It was estimated that up to 50% of the microbial biomass is killed following a single freeze-thaw cycle (Soulides and Allison, 1961, Soil Sci. 91, 291-298), leading to 10-40 fold increase in dissolved sugars and amino acids (Ivarson and Sowden 1966, 1970, Soil Sci. 46, 115-120 and 50, 191-198, respectively). After this CO_2 flush, the CO_2 production rate decreased and the mean respiration rates measured between days 4-12 and 12-14 did not already differ from each other. This pointed to a stabilization of microbial activities in the soils, as reported by Schimel and Clein (1996, SBB 28, 1061-1066). Therefore we chose relatively long pre-incubation period to ensure that we measured the stabilized respiration not biased by the respiratory burst; ii) The respiration burst between days 0-4 were positively correlated with the respiration rates measured later ($r=0.93$ and 0.74, both $P<0.0001$, $n=36$). Therefore, there was a consistent difference among soil microbial activities along vertical gradient during the whole incubation. Even though the final respiration measurement could be conducted earlier, we are confident that measurement at day 14 did not significantly affect the trends or the absolute values of microbial respiration presented in the manuscript.

The discussion is too long and wordy. I found it difficult to understand the main points the authors were trying to convey. It seemed to be rushed relative to the excellent writing of the rest of the manuscript and has multiple grammar issues. I also think that there was too much superfluous material that distracts from the main message. The authors spend a great deal of time discussing impacts due to plant biomass, but have no data presented quantitatively examining plant communities, biomass, root biomass, etc. A lot of this can be safely removed, especially in sections 4.1 and 4.4, as the degree of detail discussed doesn't add too much to the broader implications of the study.

Author response: Again, we agree with these comments. Discussion will be revised and shortened, especially in section 4.1. Regarding section 4.4., we will provide the information about plant and lichenized soil crust percentage cover as these data are recently available for the sampling sites.

With some mostly editorial changes focusing on clarifying the findings I think this paper represents a significant contribution towards Arctic research and understanding the environmental parameters shaping microbial communities in this sensitive area.

Specific Comments L124: I was interested in why the authors decided to pre-incubate the soils at 6 C (far above the mean of -3.8C, and below the max of 16.2, as well as different from the 5 C cut-off used in L186])?

Author response: The incubation temperature of 6 °C was chosen as it represents mean summer soil temperature along the whole elevational gradient (mean summer temperatures for particular elevational levels ranged from 5.3 to 7.1, see Table 1; the mean summer temperature across the whole gradient is 6 °C).

Also, why did the authors choose to pre-incubate for 2 weeks at this temperature? Is this typical for these kinds of measurements? I would think you want to minimize the pre-incubation time to prevent a strong bottle effect, as well as removing all your labile carbon.

Author response: Please see our response to general comments about our respiration measurements above.

L126: Is the specific respiration ratio typical to compare with the field? Is it possible to convert PLFA to a more generalizable unit (such as per cell, per g biomass etc.) using conversion factors?

Author response: The specific respiration was used primarily to reveal the variability and general patterns in the microbial activity per unit of microbial biomass. In our view, the observed trends are the most important massage. Soil PLFA content is generally accepted as quantitative measure of microbial biomass. We don't think that conversion of soil PLFA content to microbial biomass carbon (or per cell) could add any value to the information presented in the manuscript. The conversion factors vary in the literature sources and are inevitably affected by cell morphology (membrane area versus cell biovolume). There is different PLFA to microbial biomass ratio not only for fungi and bacteria, but also for bacterial cells differing in size and shape. As the fungi to bacteria ratios varied significantly between sites, we consider any recalculation using a single conversion factor as speculative and hardly employable for comparison with other studies based on measurements of soil microbial carbon content (e.g. by chloroform fumigation method).

L144: Is there a reference to support this sum? Are you not overcounting the bacterial contribution by summing general bacterial biomarkers with specific bacterial group biomarkers (Actinos, G-, G+)? Would it not be preferable to us general fungal : general bacterial only?

Author response: The bacterial abundance is in majority (if not all) of papers using PLFA as quantitative measure of microbial biomass calculated as a sum of all markers specific to bacteria. The specific bacterial groups (Actinobacteria, G-, G+) belongs to bacteria and they need to be considered when calculating the F/B ratio. Considering only general bacterial markers, which are specific to bacteria but cannot be ascribed to

one of the above mentioned bacterial groups, would lead to significant overestimation of fungal presence in the soil (references e.g. Frostegård and Bååth 1996, Biol. Fert. Soils 22, 59-65; Bååth and Anderson 2003 SBB 35, 955-965; Kaiser et al. 2010, New Phytologist 187, 843-858).

L189: Maybe change "In contrary" to "In contrast".

Author response: We agree

L214: Maybe add at the end "and was instead transect specific". I realize this is implied, but I feel it makes it clearer.

Author response: We agree

L213 – L227: This section is confusing to me. It is very surprising that microbial activity (as you assayed it) is not related to carbon or nitrogen content and is instead related to positively with Ca and negatively with Mg. I worry the trend in increasing respiration with altitude is due to the pre-incubation.

Author response: This relationship between respiration and base cation availabilities was surprising also for us. However, the microbial activity (respiration in this case) doesn′t have to correspond with biomass as was shown previously (Šantrůčková and Straškraba, 1991, SBB 23, 525-532). Moreover, available nutrients rather than total C and N stocks affect microbial activity (unfortunately, we were not able to extract the available nutrients in the field and this information is missing in our dataset). Based on the background data from our respiration measurements (please see above our response to general comments), we insist that the presented respiration data are not a result of our pre-incubation step and can be used as potential respiratory activity of soil microbes. We thus believe that soil geochemical properties such as high magnesium availability can be very important drivers of microbial activity and abundance in these arctic soils.

L228: Write out "Microbial Community Structure" in the header of this section.

Author response: We agree

L229: Gradient here is the transect? Does this mean there is a continuous change along the S-N transects or that each is different?

Author response: Yes, gradient is transect here. The results mean that there is a significant shift in the MCS not only between elevations, but also significant differences between transects in horizontal direction. We admit that the horizontal/vertical aspects must be commented more clearly throughout the manuscript.

L230: Nice to see so much explained due to altitude! L231: Which gradients? Elevation or between the transects? Please fix or clarify this terminology!

Author response: Terminology will be clarified throughout the manuscript.

L229 – L233: These few sentences are quite confusing and I think readers would be helped if you clarify. If I understand, the microbial community structure is impacted by elevation, but even more so by how the soils change with elevation? You ran multiple different tests to parse out these effects at different levels? Also, is microbial community structure here a relative score or absolute values?

Author response: We will clarify these statements. Let us to offer brief explanation: the microbial community structure significantly changed along the elevational gradients and between transects (ie. both factors, transect and elevation, were significant; L229-231). The significant effects of transect and elevation can be well explained by spatial variability in the soil geochemical properties which were determined (ie. horizontal and altitudinal variability in the soil properties, L 231-233). The MCS used here (and in general throughout the manuscript) are relative abundances of microbial groups (not scores, see L161-164 in Method section).

L237: Re-running the analysis with the selected variables was non-significant? Can you clarify this statement? Why do you want to run the forward selection if the variables selected do not significantly explain the microbial community composition? Is the main

message of this part, that these variables are not significant while altitude is?

Author response: As we mentioned in the previous comment, the effect of transect and elevation on microbial community structure could be explained by the variability in soil properties retained by forward selection (see also section 3.2. in the manuscript). However, we wanted to find out whether the retained soil properties sufficiently explain the elevation and transect effect (the explanatory variables never explain 100% of variability in the community composition). Thus we used the variables retained by forward selection (ie. variables with the highest power to explain variability in the MCS, see L165-166) as covariates (ie. we tested just the remaining variability in the MCS not associated with these variables), assuming that if there are missing important environmental variables that control the spatial variability in MCS, the test on transect and/or elevation effect will remain significant (L168-169). Only the elevation effect remained significant, meaning that the retained soil properties satisfactorily explained the differences between transects, but not the elevational trends. In other words, there are still missing some environmental variables in our dataset which shape the MCS along the elevational gradients. We consider this information interesting and important.

L240 – L251: Nice results! I think this is more interesting that the previous paragraph. However, there are a lot of grammar mistakes here, some listed below. Maybe re-write this section for clarity.

Author response: Will be clarified.

L243: missing a space L247: “A similarly significant trend” L248: Change to PFLAs. L249: change discrepant to disparate

Author response: Will be corrected.

L249: Consider re-writing, this is a very long sentence that can be shortened, maybe “The most disparate site in terms of MCS was the highest elevation sampled along Gr1. It was typified by a high abundance of PLFAs specific to Actinobacteria and a lower abundance of fungal PFLAs compared to analogous sites along Gr2 and Gr3."

Author response: Will be rewritten.

L255: What does this sentence mean? Author response: The whole section 4.1. will be shortened, especially first two paragraphs. The sentence will be removed. L265: "positive surface energy balance had a strong.."

Author response: Will be rewritten.

L273: This is an incredibly important but difficult to decipher sentence. I think a lot of the sentences above it can be shortened or removed, but this should be clarified. Do you mean that "Mean temperatures and temperature stability did not change with altitude in this study"? [Therefore, variations in your parameters due to altitude are not simply due to temperature differences?] Here I would start off with a stronger statement of what you mean, and then offer your support.

Author response: We mean that mean temperatures and temperature stability (diurnal temperature fluctuation) does not change with elevation as we expected – ie. temperature will decrease with increasing elevation and the microclimate will be less stable in higher altitudes. We also expected generally higher fluctuation of soil moisture. However, we found very similar temperature conditions in the lowest and highest elevations, while the mid-elevated sites experienced warmer but less stable summer soil microclimate. The most important microclimatic parameter thus seemed to be the length of vegetation season and its effect on vegetation. We will rewrite this section in order to keep it concise.

L277: Extremely important to clarify what gradient you are talking about here.

Author response: This will be clarified throughout the manuscript. Please see also our response to your comment regarding L237.

L277: Are you missing a "not". This is a confusing sentence.

Author response: Corrected sentence: "...while the effect of transect was not significant".

L281 – L296: Simplify this! It is too wordy and difficult to follow. E.G. "We explain this discrepancy by the proximity of glacier stream, which could wash away the upper soil organic layer during abnormal spring-melt events in the past", can be changed to "The only exception was the lowest site of Gr2 which had similar OM content to higher elevation sites along the other transects. This is likely due to the proximity of a glacier stream, which would wash away the topsoil during a flood."

Author response: We agree that the paragraph is too wordy. Paragraph will be shortened.

L284: "vascular plants also influenced"

Author response: Sentence will be reworded.

L286: Please provide a citation for this.

Author response: Sinsabaugh et al. (1997). Reference given in Materials and Methods and will be provided in discussion.

L288 – L290: Is this important for your findings?

Author response: We explained the occurrence of β-sitosterol as indicator of plant derived organic matter transported from lower elevations.

L290: Lots of grammar issues. L292: Or high lichen components at high elevation?

Author response: We agree that the importance of lichens must be thoroughly discussed. However, lichens contain algal and cyanobacterial photobionts so there is not a conflict with our statement.

L298 – L314: You need to discuss the implications of your pre-incubation step in this section. It can also be clarified or simplified for the readers.

Author response: We will discuss the implications of our pre-incubation step. The whole paragraph will be revised.

L304-L308: Please include relevant concentrations of the Mg inhibitory effect here.

Author response: We would like to thank the reviewer for this comment. The inhibitory concentrations of Mg2+ in solution were above 5 p.p.m and 50 p.p.m. for G- and G+ bacterial species, respectively (Webb 1949, Microbiology 3, 410âĂŠ424). The limiting concentrations will be mentioned in the discussion.

L309 – L314: This is a nice summary. However, the normalized characteristics are inherently dependent on the soil OM, so isn't their increase directly due to the OM decrease?

Author response: Not completely. The altitudinal trends in microbial biomass and respiration did not always follow the altitudinal trends in TOC content (compare data in Table 3 with Fig. 3). There was high variability in OC content along the particular transects. For instance Gr1 shown the most pronounced decrease of OC content with elevation, but the microbial characteristics normalized per OC content did not correspond to this trend. In contrast, the TOC content from the lowest and highest sites along Gr2 did not differ, but the altitudinal trend in microbial characteristics was significant. We thus don′t agree with the opinion that use of microbial characteristics normalized per TOC content doesn′t add any other information beside that there is a natural gradient in TOC content. Many papers were published based on microbial data normalized per TOC content only. We consider this information as important characteristic of particular sampling sites and indication of differences in a lability of organic matter and soil C sequestration.

L323 – L324: Please clarify this statement. What shift in resources lead to the slow accumulation of low quality OM? What are the ramifications of your pre-incubation when you are suggesting some samples are enriched in more recalcitrant OM?

Author response: We agree that this statement is dubious and confusing. The meaning is that high elevation habitats have higher proportion of active microbial biomass per OM content, including microbial primary producers (ie. microalgae; we admit that their presence in lichens must be discussed). Their necromass is much more vulnerable for decomposition compared to the plant litter. The higher productivity of plants and slow decomposition of their litter lead to TOC accumulation in the lower elevated soils, while the predominantly microbial primary production at the most elevated sites offer more available substrate for microbial growth. We consider this as the main reason for the observed pattern of high microbial activity per TOC content in the most elevated sites

L327 – L336: A lot of speculation. Is all this necessary?

Author response: We agree that this paragraph could be shortened. However, the F-B ratio is important indicator of microbial community composition and functioning. In our view is the interpretation of observed changes in F-B ratio and comparison with published data important.

L337 – L347: Very speculative.

Author response: We believe that Mg2+ availability is very important factor shaping MCS along the transects. It largely explained the trends in G-/G+ bacteria ratios (compare Table 3 and Fig. 6c, d in the manuscript). It was shown that growth of G- and G+ bacteria is limited at very different Mg2+ concentration levels (difference of one order of magnitude, see our response to comments on L304-308). The Mg2+ availability in the investigated soils exceeded these limiting concentrations, especially for G- bacteria (considering all available Mg2+ in soil solution and average soil moisture content 30%, the Mg2+ concentrations ranged approximately from 50-420 p.p.m.). We thus consider the given interpretation of observed shifts in MCS due to Mg2+ availability (Mg2+ availability was retained by RDA with forward selection of explanatory variables) as critical evaluation of relevant literature. However, we admit that statements about substitution of fungi by Actinobacteria are speculative and will be removed. The paragraph will be shortened.

L384: “bedrock chemistry were recognized as the main factors”

Author response: Sentence will be reworded.

L387 – L388: A confusing sentence, consider revising.

Author response: Sentence will be revised.

Figure2: Consider moving either this figure, or Table1 to the supplemental information to shorten the main paper.

Author response: We would like to keep Table 1 in the main text. Figure 2 will be moved to supplements.

Figure 4: How much variation is there between altitude replicates? Maybe add a supplementary figure showing ellipsoids or individual sample points.

Author response: We agree with reviewer comment on Fig. 4. We attached new version of this figure and propose that it could be used in the main text instead of previous version. Please note that different length of the arrows (relative to centroid position) compared to previous version of this figure is due to different scaling.

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2017-184, 2017.

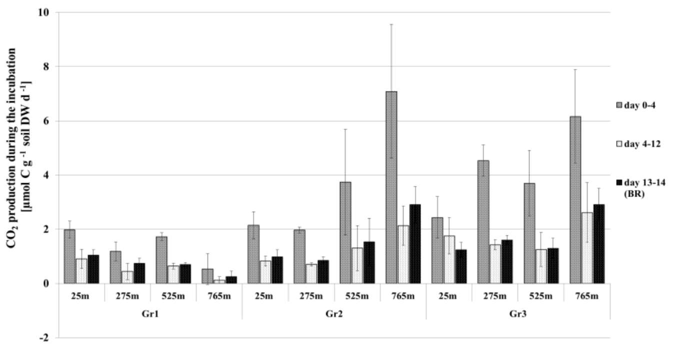
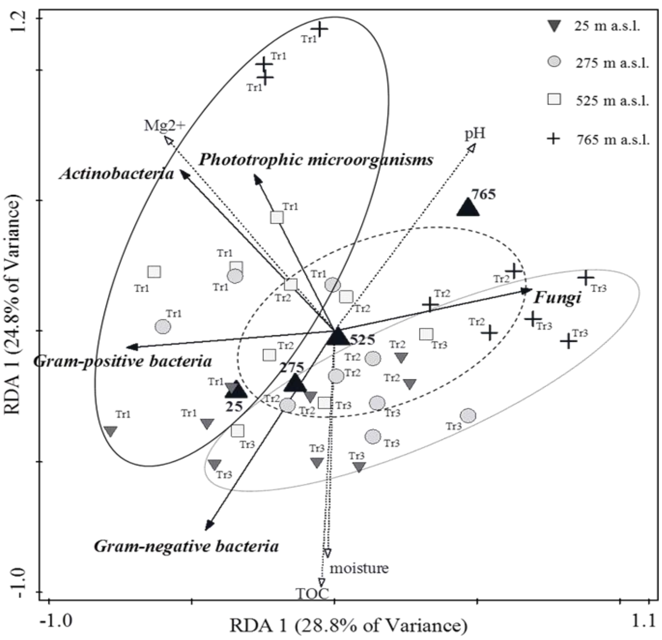


Fig. 1 Comparison of mean daily CO_2 production at days 0-4, 4-12 and 13-14 (respiration presented in the manuscript).



Revised Fig. 4