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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.

Anonymous Referee #1

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General comments The authors investigate the effect of horizontal (across a valley) and vertical (altitude) gradients on microbial community structure (PLFA), biomass and activity in High Arctic. They found that both gradient affect microbial parameters, with shift in the dominance of bacteria and fungi related to the chemistry of the bedrock. The study target interesting question, is relevant for publication in Biogeosciences and is overall well done. My main criticisms are the method used to measure microbial activity (main issue), and too many assumptions made outside the variables measured, going beyond what the results can show.

The measure of microbial activity seems unrealistic. First, 2 mm soil was used which

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was frozen and thaw prior to incubation in the lab. So, the microbial community and soil endure 1 freeze-thaw cycle + sieving that will affect OM availability and the microbial community. Then you left the samples for 14 days at 6 degrees before measuring the CO2 for 24h, which you define as "basal respiration". Fourteen days represent $\frac{1}{4}$ of the summer (> 5 degrees) in the Arctic (low altitude) or even your entire summer for the high-altitude site (Table 1), this is a significant amount of time in the Artic. There is no justification and references used to explain why you made these choices. Overall, we can doubt that the high altitude produce high CO2 emissions in in-situ conditions and we can ask the values of your results regarding microbial activity. You did not discuss at any time the limitations of such measurement. We can imagine that the microbial community adapted better or took longer to adapt to incubation condition in high altitude soil explaining the higher CO2 emissions at 14 days. We can also imagine that at low altitude, because of the higher TOC, the CO2 emissions are high rapidly after thawing and after 14 there is not much activity, while for high altitude it took longer to mineralise more complex OM. In other words, your results of microbial activity could be just the results of your incubation/sample preparation. You need to fully acknowledge this in the article, and avoid any conclusion stating that high altitude is a hot spot of microbial activity because your data can't fully support this. You need to be much more conscious about the microbial activity result. Have you measure CO2 emissions over time?

The discussion and conclusions are too long and go far beyond what you can say based on your results. There are many sections you discuss about the dynamic of microbial community but you only did one sampling time. You can't make big conclusions about dynamic of the system, such as L362-376. You need to just briefly mention potential dynamic but don't go much further. Similarly, you speak a lot about the effect of plant cover even you did not measure any parameters to characterize the plant cover (you also forgot to mention anything about mosses and lichens despite their importance in the Arctic) such as above ground biomass, root biomass, percentage cover (did you properly assessed it?), diversity. You just described the main vascular plants. So your section 4.4, is simply too long and not fully supported with your data. This entire section

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could be reduced in few sentences and focus on presence/absence of plants and not linked to microbial dynamic.

On the other hand, your discussion lack of putting your results in perspective with the literature, for example other studies investigating microbial community in bare/unvegetated soil (there is several article on this in the Arctic). It would be interesting to see if F/B ratio is similar in unvegetated a low altitude compared to other study. Your main conclusion should be the absence of plant rather than altitude effect, especially if we consider that high altitude soil in the Arctic (especially High Arctic) is likely to be rare, and also extremely shallow (only few cm) and not a massive stock of C. So we can wonder of their importance? Also you should more conclude on the site effect you have (Gr1) as site is clearly an effect on microbial biomass (consistently lower) and structure for the high altitude site.

Another point you miss in your discussion is the fact that the soil you study is always alkaline (are alkaline soils prevalent on Svalbard or acidic?). You need to discuss or conclude if your results would be similar on acidic soil in the Arctic and compare with the relevant literature as pH is a big driver of microbial community including in the Arctic. This is an important point to make and be more critical about your results.

Finally, you find an effect of altitude on microbial biomass mainly when you divide the data by TOC. This bias the data and don't reveal hidden effect of altitude. Yes, you have less C at high altitude but you don't have more microbial biomass. The fact there is more biomass per unit of C is not of a major interest. Dividing your results by TOC is not important and bias your results. Focus on the altitude effect on microbial community structure and ratio, and acknowledge that there is not a major effect of altitude on biomass but rather a site effect.

Specific comments Altitude and transect are both gradient and not only transect. This is really confusing in the text when you speak about gradient, as it is unclear if you speak about vertical or horizontal. You can't refer to the horizontal gradient as "gradient" and

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the vertical one as altitude. Decide if you speak about vertical or horizontal gradient, or altitude and transect. Change in the entire text, but don't go from "gradient" for horizontal and then use gradient also for "vertical". Be consistent.

Introduction L20: true but it is simply related to less C from a plant origin, it does not mean that there is more biomass. This is not true as well for GR1. You are telling only one part of the story here. L21: "the 2 dominant microbial groups" it sounds like it is unusual or a result on its own but in the same time with PLFA you have access to fungi and bacteria only. Just speak about fungi and bacteria. L23: you didn't measure microbial dynamic over time, only the change in soil temperature. So, I would focus on what you measured and not make assumption, especially in the abstract. Keep this for the discussion L25-26: the conclusion is an overstatement. In general, unvegetated area should be considered as previous studies showed. Speaking about high elevation as hotspots of microbial activity based on 1 measurement is an overstatement (see main comment). L36-41: this is normal as high altitude usually have no soil present or are extremely shallow (few cm) and may not be as important in their distribution and volume than low altitude (see main comment) L42: not true, you can assess the effect of changing microclimate not using altitudinal climate as you can be using different sampling time (which you did not do), different exposition, open top chambers etc. So, just focus on altitudinal but you can't say that other studies can't assess change of microclimate. L47: are the rang of altitude comparable and are the ecosystems comparable? L51: you cite articles on complete different ecosystems, such as Fierer et al 2011 (tropical), Meng et al 2013 (forest)... Focus on the Arctic and no other biomes, and same ecosystems (i.e. tundra and not forest) as you will not expect to have the same trends. Clearly state the location and ecosystems the studies you cited are based on. L59: this is not true. Your study is also true at your sites and at other sites the effects will differ. The number of studies help us to determine the common drivers across different sites. Your study is not better than others at that level. For generalisation, you could have cited Chu et al 2010 as global study of microbial diversity across the Arctic (Soil bacterial diversity in the Arctic is not fundamentally

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different from that found in other biomes) L60: “Fundamental” this is a strong word, please rephrase

Materials and Methods L80-84: any idea of the percentage plant cover? Did you measure it? You don’t mention mosses and lichens, but they represent a large part of the plant cover in Arctic tundra and are completely missing from your description. L85: you speak about the bedrock in the entire article but you never define/describe it. Could you give some information on it. L86: was an organic horizon present in the low altitude soils? L93: “kept frozen” at which temperature? L122: section 2.4 there are no references and no justification of your measurements choices: temperature, duration of incubation. . .? L131: why did you adjust the amount of soil based on TOC and in which way? Could this bring a bias if you have more soil for example in high altitude to compare you results? L154, 156: change “mL” to “ml” L161: I guess you checked also for homoscedasticity? L161: state clearly if you transformed or not the PLFA data. L163-164: the horizontal and vertical transect are both gradient (one vertical one horizontal). See comment at the beginning. L166: how was the forward selection done? L167: why did you use only P values adjusted by Holms corrections. Any reference for that? L172-173: it is really confusing when you speak about whole-plots vs splits-plots when you don’t have a plot experiment. I am not sure what you refer to here. L176: what type of correlation did you use? Why there is no direct reference to correlation in the previous sentence?

Results L190-192: this is a repetition of L 204. L192, it is also wrong what you say as the low altitude site show higher soil moisture than high altitude. Delete the sentence. L187, 203: which gradient are you talking about, be clear. L214: cite the Table you refer to L217-218: say if the correlation is positive or negative when you mention correlation even if it is given in brackets. L220: finish the sentence by “while increased in Gr2 and Gr3”. L223-225: this should be given in the materials and methods and justify why you should use it. This problematic for me and can bias your results as mentioned in the main comment. L233: what is the “whole PLFA profile”? You did not use all

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the biomarkers in previous tests, it is not the same than MCS? L229-237: should cite figure 5? For example, L230 which figure you refer to? L250: change “typical” by “characterized”

Discussion L256: do you mean “did not” or “did” correspond. Looking at your plot, you have the same trend between soil and atmospheric, just few degrees’ differences. Nothing surprising here. Your explanation is not logical, snow will insulate the soil from air temperature, so having less snow should make the soil temperature more similar to air temperature but at the beginning you say they don’t correspond. So, what are you trying to say? L255-264: this whole section I am not sure what message you are trying to deliver. Nothing is really new here, and could be condensed in a shorter section L281: OM does not grow but increase. Change “growing” by “increasing” L285: “documented growing contribution of “, rephrase this is difficult English to understand L288-290: ok there is no vascular plant, but what about mosses and lichens? Could they be partly responsible for presence of sitosterol? You have to discuss about mosses and lichens in the article, you completely omitted to mentioned them, and I don’t think they are not present on the soil. Lichens have a distribution up to high-altitude and you often find them on Svalbard on top of mountain even without any soil. L306: this reference is a bit old, is there any more recent references done on a larger number of bacteria? L307: what is the parental material? L309-314: of course if you divide microbial biomass and activity by TOC you will find an altitude effect, because there is less plant input at higher altitude so lower TOC. It does not mean your microbial biomass is higher at high altitude or there is higher microbial activity. Is it really important or interesting to know that there is higher proportion of living microbial biomass per soil TOC content? Your site effect if stronger on microbial biomass (PLFA /g soil) than altitude (only present for GR1) which is an interesting result on its own and show the variability of the suppose vertical gradient. L316-324: This is repetition of L309-324. You need to merge both section and make it shorter, and again that you have higher microbial biomass per TOC is not of a major interest. L330-331: keep in mind that you work on alkaline soil and it might be difficult to compare to other soil which are acidic. . . L348:

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you are not working on “dynamics” because you only have one sampling time. Remove dynamic from the title as you can only make some assumption L349: Why do you think it is low quality litter? Compare to what? The fact you have a high C/N does not mean low quality but just more OM and less degraded. Low OM quality usually have low C/N. This also could contradict what you say L354 “which released easily assimilable”. Is low quality litter easily assimilable? L352: So what? Ok it reduces the microbial biomass per unit of C, it just means that there is more C in the soil. You describe this as an issue but I don’t see why it should be an issue? The microbial biomass does not decrease with increasing altitude! Sorry to repeat myself but you use the results your prefer to support your theory without considering your entire results. L356-357: this is a strange wording which make the sentence difficult to understand. What do you mean by “inverse consequences from soil MCS compared to development of microbial communities”? In which way this is “inverse” and how do you have an inverse microbial community structure (or just different) and why you speak about development or young soil when you don’t measure microbial growth or dynamic and the age of the soil? L358: Are you talking of your study or not? You are not working on a “succession”. You are again making too many assumption and extrapolation based on your results. This is also contradictory to what you say in the previous sentence. Here you say at the “maximal plant biomass” the fungi dominate, while you say bacteria dominate in the previous section and in general in the article. L360: so, any conclusion? Can you really make this comparison based on 1 study? L362-376: your results do not support what you say. You have one sampling time point, don’t make assumption on what you don’t measure: dynamic. Focus on your results. You can say that the presence of ergosterol coincide with continuous dominance of fungi at high altitude sites, but you don’t need an entire section about it. Delete most of this section into one or few sentences

Conclusion: L379: move “were” just before “characterized” L380: this is not true. Unless you divide by TOC, there is no consistent effect of altitude on biomass and activity. L381: can you really say that there is negligible effect of microclimatic conditions over the summer with only one date of sampling? Do you think you have enough resolution

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with your sampling strategy to assess the effect of summer microclimate? L382: again, you use gradient without saying which gradient you are talking about and when you define in the material and methods “gradient” to refer to horizontal not vertical. L383: you need to clearly state the decrease in pH. The decrease is less than a pH unit and the soil remains slightly alkaline. This is important because your results are likely to be completely different on acidic soil. L384-385: again, what is the bedrock at your sampling site? L386-388: well, there is plenty of unvegetated area at low altitude and even when there are plants. Your thinking must be developed to unvegetated area not only at high altitude. Do plants will colonise high altitude soil which are only few cm thick with global warming? Also give a reference for the potential increase in plant cover in the Arctic as several articles were recently published. L389: you can’t really say that it diminishes the variability because it depends on plant species colonizing new area, the bedrock as you say. You don’t measure variability with PLFA, the resolution in the method you use is not high enough. L390: you don’t measure microbial diversity, how do you know this could have a negative effect? L393: again not true, you don’t have a considerable microbial biomass and your measure of microbial activity is questionable. You just can’t make this conclusion L379-394: there is no mention of the site effect even if you clearly have a site effect on microbial biomass and activity. This should be clearly stated as the vertical gradient is directly affect by the horizontal one in relation (in your study) to bedrock

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