Dear Associate Editor and Reviewers,

please find below a detailed response to all Reviewers comments and questions regarding our manuscript. Since the Reviewers gave us a lot of useful hints and comments, we decided to completely rewrite the Discussion and Conclusions. We also significantly revised method, results (including figures) and supplementary materials to improve our manuscript. We hope that the changes we made will increase the quality of our manuscript in order to fulfill the requirements for publication in Biogeosciences.

Sincerely,

Petr Kotas and co-authors

Reviewer 1

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 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 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 mineralize 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?

> Author response: We admit that it was inappropriate to call the measured respiration "basal". The characteristic we measured is rather the "potential respiratory activity". We agree with the reviewer that our methodological approach should be explained better. We also did measure the CO2 emissions during the incubation. We included the explanation in the Methods section (L130-140) and commented on this issue in the results (L238-244, Fig. 2) and discussed the results on L317-325.

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

> Author response: We agree with the reviewer´s concern that discussion was too long and sometimes not organized and confusing. We completely rewrote the whole discussion. We also added information about soil crust, mosses and plant cover in the supplements and comment o it more specifically throughout the manuscript.

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.

> Author response: We extensively searched the WOS again and included new sources in the manuscript. The discussion was completely rewritten.

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?

> Author response: We don´t fully understand these remarks. Isn´t the decreasing plant abundance due to the altitude effect? Please consider our sampling strategy (L 88-92). What connection is there with rareness of high altitude soils? We don´t think that the high altitude soils doesn´t deserve scientific interest (despite of their low C stocks and shallow soil profile), especially in context of proceeding global warming and future development of ecosystems in the Arctic. We would like to point out that the high elevation habitats form significant part of the non-glaciated landscape not only in Svalbard, but also in other parts of the northern circumpolar region. However, we completely revised our conclusions and discussion to make it more straightforward.

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.

> Author response: We agree with reviewer opinion. This issue was revised throughout the manuscript including result (section 3.3) and discussion.

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.

> Author response: We thank the reviewer for pointing out this problem. Soils on Svalbard are neutral or alkaline. The soil pH in other parts of the northern circumpolar region is more variable, ranging from acidic pH (mainly on granite and gneiss bedrock) to highly alkaline pH. We are aware of pH effect on microorganisms. This issue is discussed more thoroughly in the current manuscript version.

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.

Author response: We agree with the reviewer that we should emphasize the spatial variability in the microbial biomass and activity data. The normalized data were removed from the manuscript.

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 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.

Author response: We agree with the reviewer comment. We clarified this throughout the whole manuscript. We clearly distinguished between the effects of altitude (vertical aspect) and transect (horizontal aspect) in the revised manuscript.

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.

Author response: Normalized microbial characteristics were removed from the manuscript.

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.

Author response: We agree with this comment. Abstract was rewritten.

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

Author response: Assumptions were removed from the abstract.

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).

Author response: We removed such conclusions from abstract.

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).

Author response: We completely agree with the reviewer that the high altitude soils are more important in distribution and volume compared to high elevation soils. However, we wanted to point out in these lines that the soil microbial properties were not thoroughly studied yet along the altitudinal gradients in the Arctic. We consider the information given in these lines relevant.

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.

Author response: The sentence was removed.

L47: are the ranges of altitude comparable and are the ecosystems comparable?

> Author response: No, they are not. We are writing here about general altitudinal trends. The referred studies investigated microbial communities in different latitudes and across different altitudinal ranges (please see our next response).

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.

> Author response: We are aware of that. General latitudinal, but also altitudinal trends in diversity of animals and plants are one of the most widely recognized patterns in ecology. However, they are not valid for the microbial diversity as we wanted to show here. The number of references about microbial diversity or community structure along altitudinal gradients from the Arctic is strongly limited.

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 different from that found in other biomes)

> Author response: We completely agree with the reviewer opinion – our study is not better than others and the effect is site specific, which does not allow generalization – as we have written here. What is not true then? We didn´t want to highlight our study here. However, we rewrote the sentence for clarity (L62-64).

L60: “Fundamental” this is a strong word, please rephrase.

> Author response: Sentence was removed..

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.

> Author response: Regarding the primary producers, we have data about plant and lichenized soil crust percentage cover at the sampling sites. Mosses were relatively scarce at these locations. These data were added to the result section (L228-231, Fig. S5, Table S1) and discussed. However, we didn´t assessed the plant diversity and biomass at the sampling sites.

L85: you speak about the bedrock in the entire article but you never define/describe it. Could you give some information on it.

> Author response: We would like to thank the reviewer for pointing out this deficit. We have detailed information about geology of the Petunia Bay. The information was added to the site description (L83-86, see also L379-380).

L86: was an organic horizon present in the low altitude soils?

> Author response: No, the soil profile is poorly developed. Based on our experience there is rather litter layer on the soil surface and then relatively homogeneous mineral soil layer overlaying coarse gravel.

L93: “kept frozen” at which temperature?

Author response: At -20 °C (L95)

L122: section 2.4 there are no references and no justification of your measurements choices: temperature, duration of incubation…?

Author response: The information was added (section 2.4) and the methodological approach was commented in results (L238-244, Fig. 2) and discussion (L317-325).

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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?

Author response: We optimized the PLFA extraction protocol in our laboratory to suit wide range of different soil types (with respect to the amount of lipids and size of the SPE cartridge for lipid fractionation, analytical procedure and stock sample aliquots for eventual reanalysis). As we use 0.7-1g of soil with TOC content around 5% and the microbial biomass is usually proportional to TOC content, we adjust the sample size for C "poor" soils. This modification could not bias our results since the extraction efficiency is not affected and the PLFA yield is thus comparable for all samples. Rather the opposite is true – not accounting for low TOC content could lead to concentrations of particular PLFAs below the detection limit.

L154, 156: change "mL" to "ml"

Author response: The abbreviation mL was used in all recent articles published in BGS.

L161: I guess you checked also for homoscedasticity?

Author response: Yes, we checked the data also for homoscedasticity (L174).

L161: state clearly if you transformed or not the PLFA data.

Author response: The relative data were long-transformed (L174-175).

L163-164: the horizontal and vertical transect are both gradient (one vertical one horizontal). See comment at the beginning.

Author response: We agree with the reviewer. We clearly distinguished between the effects of altitude and transect in the revised manuscript.

L166: how was the forward selection done?

Author response: It was performed using CANOCO 5.0 software. The soil geochemical parameters were used as explanatory variables while the relative abundances of microbial groups (MCS) were used as the dependent variables (RDA). The test offer list of candidate variables sorted according to their contribution to total explained variation in the dependent variables, together with their significance. After selection of the best candidate, the contribution and significance of remaining candidates is recalculated to explain the remaining variability in the dependent data. Then the next candidate can be selected (of course only if significant).

L167: why did you use only P values adjusted by Holms corrections. Any reference for that?

Author response: We used the significant values adjustment to reflect the multiple tests performed on the same dataset. The Holm´s correction follows the approach described *in* Holm (1979): A simple sequentially rejective multiple test procedure. Scand. J. Stat. **6**: 65-70. This procedure is slightly less conservative compared to the often recommended Bonferroni correction. On the other hand, it is a sequential procedure and takes into account that the candidate predictors with stronger effect were selected first. Thus it suits better for the forward selection procedure (please see above our comment on the forward selection). Reference given in L183.

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.

Author response: As we mentioned in lines 185-187, we could assume that the characteristics of each sample will be auto-correlated with characteristics from other two samples taken from the same site (otherwise we had 9 independent transects and not three, which is not the case). In other words, the samples from the triplicate cannot be considered as independent samples due to relatively low inter-sample distance. The sampling design was in this context hierarchical with repeated measurements for each sampling site. We clarified this on L 185-187.

L176: what type of correlation did you use? Why there is no direct reference to correlation in the previous sentence?

Author response: We used the Pearson correlations to find out how tightly were two variables related to each other (L190-191).

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.

Author response: The results were largely rewritten and the duplications were removed (see sections 3.1 and 3.2).

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".

Author response: The changes were done according to reviewers comments throughout the manuscript.

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.

Author response: The normalization per TOC content was removed from the results.

L233: what is the "whole PLFA profile"? You did not use all the biomarkers in previous tests, it is not the same than MCS?

Author response: Comments on PLFA profile were removed.

L229-237: should cite figure 5? For example, L230 which figure you refer to?

Author response: There is no figure showing these results (L 249-251 in the current manuscript version). We think that figure showing the relation between selected environmental variables and MCS (Fig. 3 in the current manuscript version) are more important.

L250: change "typical" by "characterized"

Author response: Sentence was revised.

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: 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 you 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.

> Author response: We agreed with most of the above-mentioned remarks. Based on these numerous comments, we decided to completely rewrite the whole discussion.

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 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.

Author response: The conclusions were completely revised.

Reviewer 2

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. We clarified this throughout the whole manuscript and clearly distinguished between the effects of altitude (vertical aspect) and transect (horizontal aspect). 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. We measured the CO2 emissions during the incubation and included these data in the manuscript. We also included justification of our methodological choices in the Methods section (L130-140) and commented on this in result section (L238-244, Fig. 2) and discussed the results on L317-325.

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: We agree with these comments. Discussion was revised, shortened, and previous sections were merged into two main parts. We also provided the information about plant and lichenized soil crust percentage cover as these data are recently available for the sampling sites (please see L228-231, Fig. S5, Table S1).

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). Justification is given in section 2.4.

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: Our methodological choices and more detailed description of our incubation experiment are given in section 2.4. We further commented implications of our measurement in L238-245 and L317-326.

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: We excluded the specific respiration rate from results. However, we don't think that conversion of soil PLFA content to microbial biomass carbon (or per cell) could add any value. 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: Sentence was rewritten.

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: Sentence was rewritten.

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). 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. Moreover, the studies of Webb (Webb, 1949; reference in the manuscript) support the assumption, that parent material with very high Mg2+ content could have such negative effect on microbes.

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

> Author response: Done

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. The use of horizontal (transect) and vertical (altitude) aspects was emphasized 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 was 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). 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). The MCS used here and in general throughout the manuscript are relative abundances of microbial groups (not scores, see L175-176 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: These results were removed from the manuscript.

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: Section was rewritten and clarified.

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

Author response: Was 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: Sentence was rewritten.

L255: What does this sentence mean?

Author response: The whole section was shortened and clarified.

L265: “positive surface energy balance had a strong..”

Author response: Corrected

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. The whole section was rewritten and clarified.

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

Author response: Clarified.

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

Author response: Discussion was completely rewritten.

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 was completely revised.

L284: “vascular plants also influenced”

L286: Please provide a citation for this.

Author response: citation provided (L337).

L288 – L290: Is this important for your findings?

Author response: Rewritten

L290: Lots of grammar issues.

Author response: Rewritten

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 discussed the implications of our pre-incubation step. The whole paragraph was revised (see our comments to incubation experiment above).

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 Mg^{2+} in solution were above 5 p.p.m and 50 p.p.m. for G- and G+ bacterial species, respectively (Webb 1949, Microbiology 3, 410–424). 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: The normalized microbial characteristics were removed from the manuscript.

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: Rewritten

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

L337 – L347: Very speculative.

Author response: We believe that Mg^{2+} 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 Mg^{2+} concentration levels (difference of one order of magnitude, see our response to comments on L304-308). The Mg^{2+} availability in the investigated soils exceeded these limiting concentrations, especially for G- bacteria (considering all available Mg^{2+} in soil solution and average soil moisture content 30%, the Mg^{2+} concentrations ranged approximately from 50-420 p.p.m.). We thus consider the given interpretation of observed shifts in MCS due to Mg^{2+} availability (Mg^{2+} 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 section was completely revised.

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

Author response: Rewritten

L387 – L388: A confusing sentence, consider revising.

Author response: Rewritten

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 was 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. New version of the figure showing the variability between altitude replicates and transects (Fig. 3 in the current¨version of our manuscript).

Soil microbial biomass, activity and community composition along altitudinal gradients in the High Arctic (Billefjorden, Svalbard)

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Abstract The unique and fragile High Arctic ecosystems are vulnerable to proceeding global climate warming. Elucidation of factors driving microbial distribution and activity in Arctic soils is essential for comprehensive understanding of the ecosystem functioning and its response to environmental change. The goals of this study were to investigate the microbial biomass, activity, microbial community structure (MCS) and their environmental controls in soils along three elevational transects in coastal mountains of Billefjorden, Central Svalbard. Soils from four different altitudes (25, 275, 525, and 765 m above sea level) were analysed for a suite of characteristics including temperature regimes, organic matter content, base cation availability, moisture, pH, basal respiration, and microbial biomass and community structure using phospholipid fatty acids (PLFA). We observed significant spatial heterogeneity of edaphic properties among transects, resulting in transect-specific effect of altitude on most soil parameters. We did not observed any clear elevation pattern in the microbial biomass and the microbial activity revealed contrasting elevational patterns between transects. We found relatively large horizontal variability in MCS, mainly due to different composition of bacterial PLFAs, but also systematic altitudinal shift in MCS related with different habitat preferences of fungi and bacteria, resulting in high fungi to bacteria ratios at the most elevated sites. Our data further showed that the biological soil crusts on these most elevated, unvegetated sites can host microbial assemblages of the size and activity comparable with the arctic tundra ecosystem. The key environmental factors determining horizontal and vertical changes in soil microbial properties were soil pH, organic carbon content, soil moisture and Mg^{2+} availability.

1 Introduction

Knowledge about the spatial distribution and activity patterns of soil microbial communities is essential to understand ecosystem functioning as the soil microbes play fundamental role in biogeochemical cycling and drive productivity in terrestrial ecosystems (van de Heijden et al., 2008). The soil microbial diversity in the Arctic is comparable to that in other biomes (Chu et al., 2010) and the spatiotemporal variability in microbial community composition is large (Lipson, 2007; Blaud et al., 2015; Ferrari et al., 2016). However, it is still uncertain which environmental factors drive the heterogeneity of soil microbial properties in the Arctic.

Altitudinal transects offer great opportunity to study a distribution of microbial communities adapted to local habitats and explain the patterns by natural gradients of soil conditions, vegetation occurrence and climate regimes over short spatial distances (Ma et al., 2004; Körner et al., 2007). The proceeding climate change will further affect environmental conditions in the Arctic (Collins et al., 2013) including expected upward migration of the vegetation and increasing plant cover (Vuorinen et al., 2017; Yu et al. 2017). Therefore, the knowledge of current microbial distribution and activity patterns along the altitudinal gradients together with identifying their controlling factors can help to predict future development of ecosystems in this region. However, such studies are scarce despite the fact that Arctic tundra comprises 5% of the land on Earth (Nemergut et al., 2005) and most coastal areas in the northern circumpolar region have mountainous character. So far, only few studies assessing altitudinal trends in soil microbial properties were conducted in the Scandinavian Arctic (Löffler et al., 2008; Männistö et al., 2007). The research on spatial variation in microbial community composition and activity in polar regions was conducted mainly at narrow elevation range (Oberbauer et al., 2007; Trevors et al., 2007; Björk et al., 2008; Chu et al., 2010; Van Horn et al., 2013; Blaud et al., 2015; Tytgat et al., 2016) or was focused on initial soil development following glacier retreat (Bekku et al., 2004; Yoshitake et al., 2007; Schütte et al., 2010). Majority of studies on the elevational patterns in microbial community structure (MCS) and activity has been done in mountain regions of lower latitudes from tropics to temperate zone. The studies commonly show that the microbial activity decreases with increasing elevation (Schinner, 1982; Niklińska and Klimek, 2007; Margesin et al., 2009), while there are no general altitudinal patterns in soil microbial diversity and community structure. For example, the microbial community composition did not change along elevational gradients in Swiss Alps (Lazzaro et al., 2015), while other studies have documented decreasing bacterial (Ma et al., 2004; Lipson, 2007; Shen et al., 2013) and fungal (Schinner and Gstraunthaler, 1981) diversity with an increasing altitude, and several studies reported the mid–altitudinal peak in microbial diversity (Fierer et al., 2011; Singh et al., 2012; Meng et al., 2013). Beside the fungal and bacterial diversity, the relative abundance of these main microbial functional groups is also variable. For example, Djukic et al. (2010), Xu et al. (2014) and Hu et al. (2016) found decreasing fungi to bacteria (F/B) ratio with an increasing elevation, while Margesin et al. (2009) reported opposite trend in Central Alps.

The research focusing on environmental controls over microbial communities in polar and alpine regions recognized many significant factors, including vegetation, litter C : N stoichiometry, organic carbon content, soil pH, nutrient availability, microclimatic conditions, and bedrock chemistry. However, the effect of these variables was site- and scale-specific (Van Horn et al., 2013; Blaud et al., 2015; Ferrari et al., 2016), which highlights the need for further research on environmental controls of microbial community size, activity and structure at local and regional scales. To extend our knowledge about microbial ecology and soil functioning in the arctic alpine ecosystems, we conducted study aiming to assess the activity, biomass and structure of soil microbial communities and to determine their controlling environmental factors along three altitudinal transects located in Central Svalbard. These transects spanned from the vegetated tundra habitats at the narrow areas at the sea level to unvegetated soils at the top of the coastal mountains. The specific objectives of our study were (i) to describe gradients of microclimatic and geochemical soil properties; (ii) to assess microbial activity (soil respiration) and abundance of main microbial groups (fungi, Gram–negative and Gram–positive bacteria, Actinobacteria, phototrophic microorganisms) using phospholipid fatty acid (PLFA) analysis; and (iii) to identify environmental factors explaining the trends in soil microbial parameters along these altitudinal gradients.

2 Materials and methods

2.1 Study area and soil sampling

The Petunia bay (Billefjorden; 78° 40´ N, 16° 35´ E) is located in the center of Svalbard archipelago and represents typical High Arctic ecosystem in the northern circumpolar region. The mean, minimum and maximum air temperatures recorded in the area at 25 m above the sea level (a.s.l.) were –3.7, –28.3 and 17 °C in the period of 2013–2015, respectively, and stayed permanently below 0 °C for eight months a year (Ambrožová and Láska, 2017). The mean annual precipitation in the Central Svalbard area is only 191 mm (Svalbard Airport, Longyearbyen, 1981–2010) and is equally distributed throughout the year (Førland et al., 2010).

In August 2012, we collected soils from three altitudinal transects (Tr1–3) on the east coast of Petunia bay. Each transect was characterized by four sampling sites at altitudes 25, 275, 525 and 765 m a.s.l. (± 5 m). Transects were located on slopes with similar exposition (Tr1 W–E, Tr2 WNW–ESE, Tr3 WSW–ENE; Fig. 1) and lithostratigraphy. Soils at the lowest elevations developed from Holocene slope (Tr1 and Tr3) and marine shore deposits (Tr2), while the bedrock at more elevated sites is formed by dolomite and limestone with units of basal calcareous sandstone (Dallmann et al., 2004). The soils were classified as Leptic Cryosols (Jones et al., 2010) with loamy texture and clay content increasing with altitude (Table 2), and were from 0.15–0.2 m to only few cm deep at 25 and 765 m a.s.l., respectively. The poorly developed organic horizon was present only at the lowest elevation. The sampling locations were selected in geomorphologically stable areas with a similar slope (20±5°). On each sampling site, nine soil cores (4 cm deep, 5.6 cm diameter) were collected and mixed into three representative samples. Each representative sample was mixed from one soil core taken from the edge of the vegetation tussocks (if vegetation was present) and two other cores taken in increasing distance from the vegetation to maintain the consistency with respect to heterogeneity of vegetation cover and soil surface. The triplicates were collected approximately 5 m apart from each other. Immediately after sampling, the soil was sieved (2 mm) to remove larger rocks and roots, sealed in plastic bags and kept frozen at –20 °C till further processing. Soil subsamples for biomarker analysis were as soon as possible freeze–dried and stored at –80 °C until extraction.

Transects represented climosequences from high Arctic tundra to unvegetated bare soil. Vegetation of two lowest sites was dominated by *Dryas octopetala*, with significant contribution of *Saxifraga oppositifolia*, and variable contribution of *Cassiope tetragona, Salix polaris* and grasses (*Carex nardina, C. rupestris, C. misandra*; Prach et al., 2012; personal observations). The vascular plants species formed scattered vegetation patches at the altitude of 525 m a.s.l. with *Salix polaris* and *Saxifraga oppositifolia* being the most abundant species. The soils at the most elevated sites were covered mainly by soil crusts with scarcely occurring *Saxifraga oppositifolia* and *Papaver dahlianum* (personal observations). The percentage cover of main surface types (i.e. stones, bare soil, vegetation, crusts and mosses) was estimated on each sampling site from approximately 1m^2 area in a close vicinity of coring sites (Table S1, Fig. S6).

2.2 Monitoring of microclimatic characteristics

To describe the soil microclimatic conditions along the altitudinal transects, we continuously measured soil temperature at –5 cm from 2012–2013 directly at the sampling sites of Tr1 using dataloggers (Minikin Ti Slim, EMS Brno, CZ). The soil water content at the time of sampling was determined in soil subsamples by drying to constant weight at 105 °C. The temperature regimes at particular altitudinal levels were characterized by 10 climatic variables (Table 1). The period of above–zero daily mean ground temperatures is referred to as summer season throughout the text. We also considered number of days with daily mean ground temperatures above 5 °C, characterizing a period with conditions suitable for vascular plant growth (Kleidon and Mooney, 2000). The positive soil surface energy balance was calculated as a sum of daily mean summer temperatures. The records from three years (2011–2013) continuous measurements at two automated weather stations located at 25 and 455 m a.s.l. approximately 3 km apart from the observed transects (hereafter referred as AWS_{25}

and AWS_{455}, respectively; Fig. 1; see Ambrožová and Láska, 2017 for detailed description) were used to evaluate seasonal variation of soil temperature and moisture regimes (Figs. S2, S3, respectively), and coupling of soil and atmospheric temperatures (measured at -5 cm and 2 m above terrain, respectively; Fig. S2). Even though we were not able to continuously measure soil moisture directly at the sampling sites, we regarded data from both AWS locations as representative for the evaluation of seasonal moisture regimes.

2.3 Soil characteristics

Komentář [P.K.1]: NO3- and reactive phosphorus (SRP) were excluded from the results

The particle size distribution was assessed using aerometric method (Lovelland and Whalley, 2001), the soil type was classified according to U.S. Department of Agriculture. The soil pH was determined in soil–water mixture (1:5, w/v) using glass electrode. The cation exchange capacity (CEC) was considered to be equal to the sum of soil exchangeable base cations Mg^{2+}, Ca^{2+}, Na^{+}, K^{+} extracted with 1M NH_4Cl (Richter et al., 1992). The amount of H^{+} and Al^{3+} ions was neglected due to the high soil pH. Base cations accessible for plant and microbial uptake (Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}) were extracted by the Mehlich 3 reagent (Zbíral and Němec, 2000). Cations were measured by atomic absorption spectroscopy (AA240FS instrument, Agilent Technologies, USA). Total soil organic carbon (TOC) and nitrogen (TN) contents were measured in HCl fumigated samples (Harris et al., 2001) using elemental analyser (vario MICRO cube, Elementar, Germany).

2.4 Microbial respiration

Since we were not able to measure soil respiration on site or immediately after soil collection, we measured the potential respiratory activity (soil CO_2 production) in the laboratory incubation experiment. We stored and transported the soils frozen because it was previously demonstrated that freezing-thawing has a weaker effect on microbial activity than long-term refrigeration (Stenberg et al., 1998) and comparable effect as drying-rewetting (Clein and Schimel, 1994). We then measured microbial respiration in slowly melted field-moist soils twice during the adaptation period (day 4 and 12), which allowed stabilization of the microbial activity after a respiratory flush following freeze-thaw events (Schimel and Clein, 1996), and at day 13, when we expected a stabilized microbial activity. Briefly, soil subsamples (10 g) were incubated in 100 mL flasks at 6 °C, which corresponds to the mean summer soil temperature of all sites along Tr1. At days 4, 12 and 13, a cumulative CO_2 production from the soils was measured using Agilent 6850 GC system (Agilent technologies, CA, USA). The flasks were then thoroughly ventilated and sealed again. Due to high soil pH, the total amount of produced CO_2 was corrected for its dissolution and dissociation in soil solution according to Henderson-Hasselbach equation (Sparling and West, 1990) and expressed as the microbial respiration rate per day. The daily microbial respiration rates measured between days 4-12 and after stabilization (day 13) were not significantly different in any soil samples, therefore, we present only the later one.

2.5 Microbial biomass and community structure

The soil microbial community structure was defined using PLFA analysis according to modified protocol of Frostegård et al. (1993). Briefly, 1-3 g (according to TOC content) of freeze–dried soil samples was extracted twice with a single–phase extraction mixture consisting of chloroform, methanol and citrate buffer. After overnight phase separation achieved by adding more chloroform and buffer, the organic phase was purified on silica columns (SPE–SI Supelclean 250mg/3 mL; Supelco®, PA, USA) using chloroform, acetone and methanol. The polar fraction was trans–esterified to the fatty acid methyl esters (FAME) (Bossio and Scow, 1998). All FAMEs were quantified by an internal standard calibration procedure using methyl-nonadecanoate (19:0) as an internal standard. To identify the FAMEs, retention times and mass spectra were compared with those obtained from standards (Bacterial Acid Methyl Esters standard, the 37–component FAME Mix, PUFA–2, and PUFA–3; Supelco, USA). The ISQ mass spectrometer (MS) equipped with Focus gas chromatograph (GC) (Thermo Fisher Scientific, USA) was used for chromatographic separation and detection.

Only specific PLFAs were used to assess the microbial community structure: a14:0, i15:0, a15:0, i16:0, i17:0, a17:0 were used as markers of Gram–positive bacteria (G+); 16:1ω9, 16:1ω5, cy17:0, 18:1ω11, 18:1ω7, cy19:0 as markers of Gram–negative bacteria (G–); 10Me16:0 and 10Me18:0 as markers of Actinobacteria (Kroppenstedt, 1985), 18:1ω9, 18:2ω6,9 as fungal markers (Frostegård and Bååth, 1996) and polyunsaturated fatty acids 18:4ω3, 20:5ω3 were used as markers of phototrophic microorganisms (Hardison et al., 2013; Khotimchenko et al., 2002). A sum of Actinobacterial markers, PLFAs specific to G+ and G– bacteria and general bacterial markers 15:0, 17:0 and 18:1ω5 was used to calculate bacterial biomass and fungi to bacteria (F/B) ratio. The sum of all lipid markers mentioned above and nonspecific PLFAs 14:0, 16:0, 18:0 and 16:1ω7 was used as proxy for microbial biomass ($PLFA_{tot}$).

2.6 Sterol analyses

Komentář [P.K.2]: Ergosterol was excluded from the results

The β–sitosterol and brassicasterol were used as biomarkers of plant (Sinsabaugh et al., 1997) and microalgal (Volkman, 1986; 2003) residues in organic matter (OM), respectively. Sterols were simultaneously determined using microwave assisted extraction adapted from Montgomery et al. (2000) and GC/MS (ISQ MS equipped with Focus GC, Thermo Fisher Scientific, USA) analysis. Briefly, 0.5 g of freeze–dried soil was treated with 6 mL of methanol and 2 mL of 2 M NaOH. Vials were heated twice at the centre of a microwave oven (2450 MHz and 540 W output) for 25 s. After cooling, the contents were neutralized with 1 M HCl, treated with 3 mL of methanol and extracted with hexane (3×4 mL). Extracts were spiked by an internal standard (cholesterol), evaporated and derivatized by adding of pyridine and 1 % BSTFA at 60 °C for 30 min prior analysis. Sterols were quantified by an internal standard calibration procedure.

2.7 Statistical analyses

All data were checked for normality and homoscedasticity, and log–transformed if necessary. The relative PLFA data (mol%) were log-transformed in all statistical tests. The significance of environmental gradients and corresponding shifts in MCS (mol% of summed PLFA specific for fungi, G– and G+ bacteria, Actinobacteria and soil phototrophic microorganisms) in horizontal direction (ie. effect of transect) and vertical direction (ie. effect of altitude) were tested using the partial redundancy analyses (RDA) with covariates. Variation partitioning was subsequently performed to quantify the unique and shared effects of transect and altitude on variability of MCS. Forward selection procedure was used to identify the soil geochemical parameters best explaining the shifts in MCS. During the forward selection procedure, only *P* values adjusted by Holms correction were considered. This procedure is slightly less conservative compared to the often recommended Bonferroni correction, but it is a sequential procedure and takes into account that the candidate predictors with stronger effect were selected first (Holm, 1979). The multivariate tests were performed without standardization by samples, but with centering and standardization by variables (because the variables were not always measured at the same scale, see Šmilauer and Lepš 2014) and Monte Carlo test with 1999 permutations. Only adjusted explained variation is referred throughout the text. Since the samples from each triplicate cannot be considered as independent observations due to relatively low inter-sample distance (otherwise we had 9 independent transects), only the sampling sites were freely permuted while the individual samples were exchangeable only within the sampling sites. The differences in particular soil and microbial parameters between respective transects and altitudes were addressed by ANOVA complemented with Tukey–HSD post hoc test. To find out how tightly were variables related to each other, Pearson correlation coefficient was used. All statistical tests were considered significant at $P < 0.05$. Multivariate statistical analyses were performed with CANOCO for Windows version 5.0 (Ter Braak and Šmilauer 2012), for ANOVA, Tukey-HSD test and correlations between soil and/or microbial parameters, Statistica 13 was used (StatSoft, USA).

3 Results

3.1 Altitudinal changes in soil microclimate

The soil microclimate at the studied sites was characterized by two distinct periods respecting the air temperature dynamics (compare Fig. S2a with S2b). The winter period lasted typically from the middle of September to early June. The winter soil temperatures were stratified according to the elevation and the temperature means decreased from –4 °C at 25 m a.s.l. to –10 °C at 765 m a.s.l. (Table 1, Fig. S2). In contrast, a short summer period was characterized by a significant diurnal fluctuation of soil temperatures and weak altitudinal temperature stratification (Fig. S2). The length of the summer season more than doubled at the lowest elevations compared to the most elevated study sites, while the period with daily mean soil temperatures above 5 °C shortened almost four times. Correspondingly, the positive surface energy balance gradually decreased with an increasing altitude (Table 1). The maximum daily mean temperatures and diurnal temperature fluctuation were highest at the mid–elevated sites, with the highest mean summer soil temperature reached at 275 m a.s.l. In contrast, the least and most elevated sites experienced lower summer maximum daily means and soil temperature amplitudes (Table 1). The effect of altitude on soil moisture was significant along Tr1 and Tr3 (P < 0.001 and 0.01, F = 22.76 and 7.39, respectively) with soil moisture content decreasing along with increasing elevation, but nonsignificant along Tr2. Continual volumetric measurements of soil water content at AWS_{25} and AWS_{455} showed that the soil moisture was relatively stable during the summer season and desiccation events did not occur during the summer periods 2011–2013 (for more information, see Fig. S3).

3.2 Gradients of soil geochemical properties and surface vegetation cover

Both factors, transect and altitude, significantly affected soil geochemical properties (partial RDA, pseudo–F = 8.3, P < 0.001) and explained 61% of the total variation in soil characteristics. The RDA ascribed most of the explained variability (73%) to vertical zonation. Accordingly, the effect of altitude was significantly reflected in all soil parameters (Table 2, 3, Fig. S4), but the significant interactive effect between transect and altitude indicated that the elevational trends were in most cases specific for particular transects (Tables 2, 3). Especially the CEC and availabilities of Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} were spatially variable, reflecting complicated geology of the Petunia bay area. The soils along Tr1 were significantly richer in available Mg^{2+} and K^{+} than soils from other two transects (Table 2). The Mg^{2+} availability also significantly increased with increasing elevation along the Tr1 (Table 2). Other soil properties showed more systematic altitudinal patterns. The mean soil pH ranged from 7.8 to 9.0 and increased with altitude along all transects (Table 2, Fig. S4). Oppositely, the soil TOC and TN contents declined towards higher elevations along all transects; the exception was the lowest site along Tr2 with lower soil OM content compared to the respective sites from Tr1 and Tr3. The OM poorest soil occurred at the highest site of Tr1 (Table 3). The soil C/N ratio, sitosterol content in TOC and the ratio between plant–derived sitosterol and brassicasterol of algal origin were solely affected by the altitude. Their values systematically decreased with an increasing elevation irrespective of the soil OM content (Table 3), indicating an altitudinal shift in the OM quality and origin. The percentage of plant cover also continuously decreased with an increasing elevation along Tr1 and Tr3 but was comparable on the three lower sites along Tr2 (Fig. S5), which significantly resembled the trends in soil OM content (r = 0.53; P = 0.001). The lichenized soil crusts were predominant type of soil surface cover at all sites, while mosses covered very small proportion of surface area. The bare surface without any vegetation (bare soil) occurred only at the two most elevated sites (Fig. S5, Table S1).

3.3 Soil microbial biomass and activity

The soil PLFA content, used here as a measure of soil microbial biomass, was significantly correlated with soil TOC and TN contents (r = 0.773 and 0.719, respectively; both P < 0.0001) and soil moisture (r = 0.772; P < 0.0001), and negatively affected by Mg^{2+} availability (r = -0.775; P < 0.0001). Despite these relations, the soil PLFA content did not show any altitudinal pattern. The soil PLFA amounts were comparable among differently elevated sites along particular transect (Fig. 2a). Only the most elevated site of Tr1 had significantly lower soil PLFA content than other sites, which corresponded with its very low stock of OM (Table 3). Similarly, neither the flush of microbial respiration measured after soil thawing (day 4 of incubation) nor the respiration measured after stabilization (day 12, not shown, and day13) showed any systematic altitudinal pattern (Fig. 3b, c). Generally, the flush respiration rate was closely related (r = 0.74, P < 0.0001, n = 36) to microbial respiration after stabilization and ca 2.3 ± 0.3 times faster, showing similar freezing-thawing effect on the whole set of samples independently of altitude and transect. Along each transect, the three lower sites (from 25 to 525 m a.s.l.) had after stabilization comparable microbial respiration rates, but the most elevated sites always differed - along Tr1 had the most elevated site significantly lower microbial respiration rate, whilst the most elevated sites along Tr2 and Tr3 produced markedly more CO_2 compared to remaining sites along these transects (Fig. 2b). The respiration rate was related neither to PLFA nor to TOC contents, but significant positive correlation with soil Ca^{2+} availability and F/B ratio, and negative correlation with Mg^{2+} availability (r = 0.489, 0.661and -0.545; P = 0.003, < 0.001 and 0.001, respectively) was observed.

3.4 Microbial community structure

The partial RDA revealed significant interactive effect of altitude and transect on MCS (pseudo–F = 4.8, P < 0.001). Both factors explained 51% of the total variation in the MCS, with 66 % of explained variability ascribed to altitude, 26% to transect, and 8% of explained variability shared by both factors. The soil geochemical variables explained 72% of the variation in the MCS (pseudo–F = 7.1; P < 0.001) indicating that the interactive effect of altitude and transect on MCS was largely driven by vertical and horizontal variability in soil properties. The forward selection of explanatory variables retained four geochemical parameters: Mg^{2+} availability, pH, moisture and TOC content, all together accounting for 55% of variation in the data (pseudo–F = 11.6, P < 0.001). The most pronounced shift in the MCS was given by different altitudinal preferences of bacteria and fungi. The bacteria were consistently more abundant in the soils from lower elevations, having lower pH and higher TOC and moisture contents (Fig. 3). In general, PLFAs specific to G– bacteria were more abundant than PLFAs of G+ bacteria (Fig. 4a; mean G–/G+ ratio ± SD = 1.76 ± 0.17; n = 36). Oppositely, the fungal contribution to microbial community increased with an increasing altitude, at the sites having TOC poorer soils and higher pH (Fig. 3). Therefore, the F/B ratio gradually increased with an increasing altitude along all three transects (Fig. 4b). The significant interactive effect of altitude and transect on MCS was mainly connected with a strong effect of soil Mg^{2+} availability, which was higher along the whole Tr1 and differentiated its microbial communities from sites located along Tr2 and Tr3, where microbial communities of respective sites were more similar. The differences in MCS among the respective sites along Tr1 and other two transects further increased towards higher elevations in coincidence with an increasing soil Mg^{2+} availability along Tr1 (Fig. 3). In result, the TOC poorest and Mg^{2+} richest soil at the highest site on Tr1 had the most distinct MCS from all the sites. Its microbial community was characterized by higher abundance of Actinobacteria and PLFAs of phototrophic microorganisms and much lower contribution of G– bacteria compared to communities of all other sampling sites (Fig. 3, 4a).

4 Discussion

4.1 Climatic and soil edaphic conditions along altitudinal transects

The coastal area of the Petunia Bay in Svalbard is characteristic by ca four months lasting summer, long winter period (Ambrožová and Láska, 2017) and very low precipitations (Førland et al., 2010). Our measurements in this area further showed that soils along an elevation gradient from 25 to 765 m a.s.l. face significantly different microclimatic regimes. During winter, when the air temperatures varied a lot in time but less with elevation (Fig. S2b, data from AWS_{25} and AWS_{455}), the soil temperatures were relatively stable but significantly stratified with altitude (Fig. S1, S2a). The mean winter soil temperatures decreased from –4 to –10 °C along the elevation gradient from 25 to 765 m a.s.l. (Table 1), which can strongly reduce winter soil microbial activity at high altitudes (Drotz et al., 2010; Nikrad et al., 2016). In contrast, the mean summer soil temperatures did not reflect the site elevation (Table 1) and the comparison of temperature fluctuations, mean and maximum daily mean temperatures showed that the lowest and highest sites experienced during summer on average colder, but more stable soil microclimate compared to the mid–elevated sites (Table 1). However, the summer season prolonged with decreasing elevation and the increasing number of days with mean temperature above 5 °C and a rising positive surface energy balance (Table 1) positively affected the occurrence and spreading of vascular plants (Kleidon and Mooney, 2000; Klimeš and Doležal, 2010), which had strong implications for a transition of edaphic conditions along studied elevation transects. Together with increased litter inputs and stocks of soil OM with lower C/N ratio (Table 3) was the plant growth associated with root respiration, cation uptake, and release of H^+ and organic acids from roots, all together accounting for decreased soil pH (van Breemen at al., 1984). The increasing soil OM content was further positively related to soil moisture (Fig. 3). Interestingly, the soils in general did not suffer from desiccation (Fig. S3), commonly identified among the most stressing factors in polar and alpine ecosystems (Ley et al., 2004; Van Horn et al., 2013; Tytgat et al., 2016), probably due to high cloudiness and fog occurrence (Sawaske and Freyberg, 2015) in the maritime climate.

The alkaline bedrock material resulted in high soil pH (7.8–9) and high availabilities of basic cations, which were, however, spatially variable due to diverse geology of the studied area (Dallmann et al., 2004; Table 2). Beside clear altitudinal trends in soil edaphic conditions connected mostly with the soil OM content, the Mg^{2+} availability was recognized as main factor driving differences in soil microbial properties between transects (Fig. 3). In result, the character of the parent substrate mostly controlled soil microbial properties at the most elevated sites, which had generally low OM content and the most divergent MCS compared to lower located sites (Fig. 3). The highest site along Tr1 was the most extreme habitat among all the chosen sites, with the highest proportion of bare unvegetated soil surface (Fig. S5), the lowest OM and moisture contents, highest Mg^{2+} availability and soil pH, and consequently also the most distinct microbial characteristics (Fig. 2, 3). Towards lower elevations, the soil OM content became increasingly important and the microbial characteristics of the sites on different transects were more similar.

4.2 Soil microbial properties along altitudinal transects

The altitudinal shifts in soil edaphic properties were not significantly reflected in the soil microbial biomass and potential microbial respiration. Generally, the soil PLFA contents were comparable between all the sites along particular elevation transects, with the exception of very low soil PLFA concentration on the highest site of the Tr1 (Fig. 2a). There are no other studies from the High Arctic ecosystems reporting about altitude effect on soil microbial biomass. However, other studies conducted on alpine gradients in the temperate and boreal zones documented weak or absent altitudinal trends in the microbial biomass (Djukic et al. 2010, and Xu et al., 2014 using PLFA; Löffler et al., 2008 using cell counts) but also a negative effect of elevation in the Alps (Margesin et al., 2009) and northwestern Finland (Väre et al., 1997). Importantly, none of the studies considered unvegetated habitats and all of them were conducted in soils with acidic or neutral soil pH.

Microbial respiration also did not change systematically with increasing elevation. The three lowest sites along each transect always had comparable soil microbial respiration rates (Fig. 2b), while soil microbial activities of the highest sites differed. The most elevated site on the Tr1 showed significantly lower respiration rates than the lower sites on this transect, which was in line with the lowest OM content as well as soil PLFA content. However, the soils from the highest sites on both Tr2 and Tr3 respired significantly more than the soils from lower sites on these transects, irrespective of relatively stable microbial biomass. This is in contrast to other studies, which reported decreasing microbial activity with increasing elevation (Schinner, 1982; Väre et al., 1997; Niklińska and Klimek, 2007). However, these studies were conducted in lower latitudes and the studied altitudinal gradients did not include unvegetated habitats. To comment on and justify our results, we are aware that microbial activities were measured in freeze-stored and not fresh samples (see section 2.3 for details) and, therefore, the respiration rates measured after thawing show the potential activity of soil microbial communities in the soils. However, the respiration rates in three subsequent measurements (after flush, during adaptation and after stabilization) were positively correlated ($r = 0.93$ and 0.74, both $P < 0.0001$, $n = 36$), the ratios between the flush and stabilized respiration rates were comparable across all the soils (compare Fig. 2b and c) and the above-described differences in microbial activities among the sites were consistent. Our data are in accord with the study of Larsen et al., (2002), who found comparable response to freeze-thaw events between two different arctic ecosystem types. We thus suggest that the soils responded similarly to the storage treatment independently of site location and that observed differences in soil microbial activities are representative for the studied transects. Therefore, the higher soil microbial respiration at the most elevated sites point to a higher lability of the present OM (Lipson et al., 2000; Uhlířová et al., 2007) and/or to a shift in microbial communities towards groups with higher potential to mineralize the OM (Gavazov, 2010; Djukic et al., 2013). Previous studies, considering either bare soil or vegetated habitats, reported rather increasing complexity of soil OM with elevation (Ley et al., 2004; Xu et al., 2014). However, in this study was majority of OM and microbial biomass at the most elevated sites associated with biological soil crusts with high algal and cyanobacterial abundance (Table S1, Fig. S5), known for their high microbial activity (Pushkareva et al., 2017; Bastida et al., 2014). The high microbial activity in the most elevated sites could be ascribed to prevalence of compounds of algal/cyanobacterial origin with very low portion of complex and slowly decomposable lignin and lignified compounds and protective waxes (like cutin and suberin) mainly derived from vascular plants. In accord, the sitosterol to brassicasterol ratio gradually decreasing with increasing elevation (Table 3) and increasing sitosterol content in the TOC pool at lower elevations pointed to growing importance of microalgal sources of OM in high elevation habitats (Sinsabaugh et al., 1997; Rontani et al., 2012). Even though both sterols can be found in higher plants and microalgae, the changing ratio indicates shift in the origin of OM (reviewed by Volkman, 1986, see also Volkman, 2003). Changes within microbial communities, which can also help to explain higher soil microbial respiration at the most elevated sites are discussed below.

Although the soil PLFA content did not change along the studied elevation transects, we have found a systematic altitudinal shift in the PLFA composition, resulting in significantly increasing F/B ratio towards higher elevations. This shift was best explained by a decreasing soil OM content and soil moisture and increasing pH (Fig. 3). Reports about soil F/B ratios and their altitudinal changes from the High Arctic are missing, but studies from lower latitudes showed either a similar trend of increasing F/B ratio with an altitude in the Alps (Margesin et al., 2009) or the opposite altitudinal effect in the Alps (Djukic et al., 2010) and Himalayas (Xu et al., 2014; Hu et al., 2016). Such divergent results indicate that altitude alone is not the key driving factor of the soil F/B ratio. In contrast to our observation, these studies reported very low soil F/B ratios of 0.05-0.2, which may indicate important role of fungi in functioning of the Arctic habitats. Soil pH was previously identified as the main driver of fungal-bacterial dominance in the soil (Baath and Anderson, 2003; Högberg et al., 2007; Rousk et al., 2009; Siles and Margesin, 2016). Fungi have been found more acid tolerant than bacteria, leading to higher F/B ratio in acidic soils (Högberg et al., 2007; Rousk et al., 2009; reviewed by Strickland and Rousk, 2010). However, here we report high F/B ratios in the alkaline soils (pH 7.8-9.0) and increasing F/B ratios with an increasing soil pH. Similar trend was reported also by Hu et al., (2016), but the authors found F/B ratios one order of magnitude lower compared to our study. The possible explanation of generally high fungal abundance and increasing F/B ratio at more elevated sites, which are typical by unfavourable edaphic conditions and severe winter microclimate, could be higher competitiveness of fungi compared to bacteria in suboptimal conditions due to their wider pH (Wheeler et al., 1991) and lower temperature (Margesin et al., 2003) growth optima. We further found that the increasing F/B ratio was significantly coupled with an increasing soil respiration ($r = 0.649$; $P < 0.001$). Indeed, such relationship can be related to higher fungal ability either to prosper in the soil conditions at the most elevated sites, or to utilize more efficiently available C sources (Ley et al., 2004; Bardgett et al., 2005; Nemergut et al., 2005; van der Heijden et al., 2008). In turn, the higher bacterial contribution at lower elevations may be associated with more benign soil conditions and bacterial preference for utilization of labile root exudates released by vascular plants (Lipson et al., 1999; Lipson et al., 2002). Since the projected warming in the Arctic (Collins et al., 2013) will likely cause an upward migration of the vegetation and increasing plant cover in detriment of lichens and biological soil crusts (Vuorinen et al., 2017; Yu et al. 2017; de Mesquita et al., 2017), the soil microbial communities will likely respond by decreasing F/B ratios at higher elevations.

Apart from the systematic altitudinal shift in the F/B ratio, we observed a strong shift in the bacterial composition, which differentiated the altitudinal trends in the soil MCS along Tr1 from trends along Tr2 and Tr3. This difference between transects increased towards higher elevations and was best explained by Mg^{2+} availability (Fig. 3). The soils from Tr1, except the lowest site, had a lower G– to G+ bacterial ratios within microbial communities than soils from other two transects. Further, the microbial community of the most elevated site along Tr1 was significantly more contributed by actinobacteria and phototrophic microorganisms compared to all other sites (Fig. 3, 4a). It is known that the high Mg^{2+} availability inhibits growth of many soil bacterial species. The observed inhibitive Mg^{2+} levels were 5 and 50 p.p.m for G– and G+ bacteria, respectively (Webb 1949), indicating that these bacterial groups significantly differ in their tolerance for enhanced Mg^{2+} levels. Considering half of available Mg^{2+} in soil solution and average soil moisture content 20%, the Mg^{2+} concentrations ranged approximately from 16-140 p.p.m,, which could explain decreased abundance of G- bacteria in sites with high Mg^{2+} availability. This inhibitive Mg^{2+} effect further corresponds with the negative correlations between Mg^{2+} availability and soil microbial biomass and respiration found in our study, and could explain the lower microbial biomass and respiration in the soils from Tr1. Our data thus indicate that beside the traditionally identified drivers of microbial activity and MCS such as soil OM content, moisture and pH, Mg^{2+}availability is in important factor shaping the microbial environment along the arctic altitudinal transects on dolomitic parent materials.

5 Conslusions

The results obtained in this study have shown significant altitudinal zonation of most edaphic properties, but also significant spatial heterogeneity in horizontal direction, resulting in transect-specific effect of altitude on abiotic soil properties. Our data demonstrated that soils on the most elevated, unvegetated sites around the Petunia Bay can host microbial assemblages comparable in size and activity with the tundra ecosystem. The high microbial biomass and activity at the most elevated sites were almost exclusively associated with biological soil crusts, largely contributed by fungi. However, their development was retarded on some sites by high pH, low moisture and high Mg availability, resulting in pronouncedly low OM content, microbial biomass and distinct MCS. Despite the ubiquitous occurrence of soil crusts, the gradually increasing plant productivity and litter inputs down along transects were associated with decreasing soil pH, increasing OM content and soil moisture. Concurrently, the soil edaphic and microbial properties become more uniform. As the rise in temperatures and humidity predicted by climatic models will likely cause an upward migration of the vegetation and increasing plant cover, the higher plant litter inputs will overreach the influence of parent material and entail an increasing abundance of bacteria and decreasing F/B ratio in the summer microbial assemblages.

Author contribution

P. Kotas and E. Kaštovská analysed the data and wrote the manuscript with assistance of all coauthors. P. Kotas and J. Elster designed the study and performed sampling. The microbial community structure and environmental parameters were assessed by P. Kotas, E. Kaštovská and H. Šantrůčková.

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Competing interests

The authors declare that they have no conflict of interest.

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Tables

Table 1. Climatic variables; temperatures given in °C

Sites [m a.s.l.]	Means Summer	Means Winter	Means Year	Min daily means Winter	Max daily means Summer	Mean daily amplitude Summer	Max daily amplitude Summer	Number of days with daily mean > 0 °C	Number of days with daily mean > 5 °C	Positive soil surface energy balance
25	5.8	-3.6	-0.8	-7.0	11.2	5.2	10.9	110	62	615
280	7.1	-5.7	-2.7	-10.3	14.5	8.5	18.2	96	54	571
520	5.8	-8.9	-4.9	-15.8	14.7	8.1	17.7	91	40	480
765	5.3	-9.5	-6.6	-17.1	11.6	5.5	14.0	51	11	290

Table 2. Geochemical characteristics of soils along the studied altitudinal transects (Tr1-Tr3). Means ± SD (n = 3) are given in the upper part of the table. Results of two–way ANOVAs (F–values) of the effects of transect (Tr), altitude (Alt) and their interaction (Tr x Alt) are presented in the lower part of the table.

transect	altitude [m a.s.l]	soil type	soil moisture [%]	pH	CEC [meq/100g^{-1}]	Ca^{2+} [mg g^{-1}]	Mg^{2+} [mg g^{-1}]	K^{+} [µg g^{-1}]	Na^{+} [µg g^{-1}]
Tr1	25	sandy loam	^{a} 28.4 ± 2.5	^{b} 7.8 ± 0.1	^{a} 35.8 ± 0.4	^{b} 4.9 ± 0.2	^{c} 0.50 ± 0.03	^{b} 104 ± 2.3	^{a} 16.0 ± 1.4
	275	sandy loam-loam	^{b} 18.0 ± 0.5	^{b} 7.9 ± 0.2	^{b} 27.4 ± 2.3	^{b} 5.2 ± 0.6	^{c} 0.55 ± 0.08	^{b} 81 ± 8.8	^{bc} 8.4 ± 1.3
	525	loam	^{b} 18.6 ± 2.5	^{b} 8.1 ± 0.1	^{b} 30.3 ± 0.7	^{b} 4.3 ± 0.4	^{b} 0.85 ± 0.04	^{a} 160 ± 18.1	^{b} 11.3 ± 1.1
	765	clay-loam	^{c} 12.1 ± 1.8	^{a} 9 ± 0.0	^{b} 26.8 ± 2.3	^{a} 19.8 ± 1.0	^{a} 1.25 ± 0.06	^{c} 11 ± 2.7	^{c} 7.3 ± 0.0
Tr2	25	sandy loam	^{a} 21.1 ± 2.4	^{c} 7.8 ± 0.1	^{b} 25.6 ± 2.7	^{b} 14.7 ± 2.6	^{c} 0.19 ± 0.01	^{ab} 52 ± 4.0	^{a} 13.2 ± 1.7
	275	sandy loam-loam	^{a} 21.1 ± 2.4	^{c} 7.9 ± 0.1	^{b} 30.3 ± 1.7	^{ab} 16.5 ± 1.1	^{b} 0.26 ± 0.01	^{a} 59 ± 4.3	^{ab} 10.1 ± 1.7
	525	sandy loam-loam	^{a} 21.7 ± 5.3	^{b} 8.4 ± 0.1	^{b} 30.8 ± 1.1	^{c} 7.8 ± 1.6	^{a} 0.34 ± 0.01	^{a} 69 ± 3.3	^{ab} 9.6 ± 1.8
	765	loam	^{a} 22.5 ± 1.7	^{a} 8.8 ± 0.1	^{a} 45.1 ± 0.5	^{a} 27.9 ± 9.3	^{b} 0.25 ± 0.01	^{b} 41 ± 8.8	^{b} 8.1 ± 1.4
Tr3	25	sandy loam	^{a} 39.5 ± 1.4	^{b} 8.1 ± 0.1	^{a} 49.4 ± 2.1	^{c} 7.7 ± 0.3	^{a} 0.20 ± 0.03	^{b} 52 ± 5.3	^{a} 17.1 ± 1.1
	275	sandy loam-loam	^{ab} 31.9 ± 2.9	^{b} 8.1 ± 0.1	^{b} 39.2 ± 5.4	^{b} 10.8 ± 0.6	^{a} 0.21 ± 0.01	^{ab} 59 ± 1.9	^{a} 18.5 ± 0.5
	525	loam	^{ab} 28.2 ± 6.5	^{b} 8 ± 0.1	^{b} 34.9 ± 3.0	^{ab} 13.0 ± 4.6	^{a} 0.22 ± 0.00	^{a} 66 ± 6.6	^{a} 18.4 ± 3.1
	765	loam	^{b} 22.5 ± 1.7	^{a} 8.8 ± 0.1	^{b} 30.6 ± 3.9	^{a} 14.2 ± 0.1	^{b} 0.16 ± 0.00	^{b} 52 ± 1.6	^{b} 9.9 ± 0.2
		d.f.							
Tr		2	**31.4 *****	0.10	**22.1 *****	**6.43 ****	**634 *****	**51.7 *****	**36.2 *****
Alt		3	**11.1 *****	**98 *****	**4.61 ***	**14.1 *****	**66.9 *****	**74.9 *****	**18.7 *****
Tr x Alt		6	**5.07 ****	**5.6 *****	**20.5 *****	0.83	**60.6 *****	**31.6 *****	**3.94 ****

Different letters indicate significant differences between sampling sites along particular transects (P < 0.05; upper part of the table). Statistically significant differences are indicated by: * P < 0.05, ** P < 0.01, *** P < 0.001 (lower part of the table).

Table 3. Total soil carbon (TOC) and nitrogen (TN) contents, their molar ratios, contents of sitosterol in TOC and sitosterol / brassicasterol ratios and soil PLFA contents in soils along the altitudinal transects (Tr1-Tr3). Means ± SD (n = 3) are given in the upper part of the table. Results of two–way ANOVAs (F–values) of the effects of transect (Tr), altitude (Alt) and their interaction (Tr x Alt) are presented in the lower part of the table.

transect	altitude [m a.s.l.]	TOC [mg g^{-1}]	TN [mg g^{-1}]	TOC/TN	Sitosterol [µg g^{-1} TOC]	Sitosterol / Brassicasterol
Tr1	25	^{c} 70.6 ± 13.4	^{b} 5.0 ± 1.01	^{b} 12.1 ± 0.2	^{c} 534 ± 62.8	^{b} 5.5 ± 0.4
	275	^{b} 21.1 ± 1.9	^{a} 2.0 ± 0.29	^{ab} 9.0 ± 0.7	^{bc} 521 ± 140	^{b} 5.3 ± 0.8
	525	^{b} 18.5 ± 4.2	^{a} 1.8 ± 0.31	^{ab} 8.8 ± 0.7	^{ab} 293 ± 66.5	^{b} 4.7 ± 1.0
	765	^{a} 4.4 ± 1.5	^{a} 0.5 ± 0.07	^{a} 7.9 ± 2.6	^{a} 81.1 ± 2.7	^{a} 2.3 ± 0.4
Tr2	25	^{ab} 30.6 ± 4.8	^{a} 1.9 ± 0.40	^{c} 13.7 ± 0.9	^{bc} 515 ± 44.9	^{b} 6.7 ± 0.7
	275	^{b} 37.2 ± 5.0	^{a} 3.0 ± 0.26	^{b} 10.7 ± 0.7	^{c} 616 ± 143	^{b} 5.6 ± 1.2
	525	^{a} 24.4 ± 7.8	^{a} 1.9 ± 0.64	^{b} 9.8 ± 1.2	^{ab} 299 ± 73.3	^{a} 2.9 ± 0.4
	765	^{a} 21.6 ± 3.6	^{a} 2.8 ± 0.20	^{a} 6.7 ± 0.6	^{a} 161 ± 36.9	^{a} 2.7 ± 0.7
Tr3	25	^{c} 81.1 ± 8.7	^{b} 6.1 ± 0.38	^{b} 11.5 ± 0.7	^{b} 587 ± 144	^{b} 6.4 ± 2.1
	275	^{b} 62.2 ± 9.1	^{ab} 4.8 ± 0.32	^{b} 11 ± 0.7	^{ab} 370 ± 42.9	^{a} 4.2 ± 0.7
	525	^{ab} 39.6 ± 11.4	^{a} 4.8 ± 0.32	^{b} 10.6 ± 0.6	^{a} 270 ± 112	^{a} 3.3 ± 1.0
	765	^{a} 23.1 ± 3.9	^{a} 2.5 ± 0.37	^{a} 7.9 ± 0.2	^{a} 151 ± 37.8	^{a} 3.1 ± 0.9
	d.f.					
Tr	2	**27.8 *****	**31.5 *****	1.57	0.79	1.04
Alt	3	**42.4 *****	**26.4 *****	**23.6 *****	**28.4 *****	**14.4 *****
Tr x Alt	6	**8.33 *****	**11.3 *****	1.96	1.34	2.17

Different letters indicate significant differences between sampling sites along particular transects ($P < 0.05$; upper part of the table). Statistically significant differences are indicated by: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (lower part of the table).

Figures

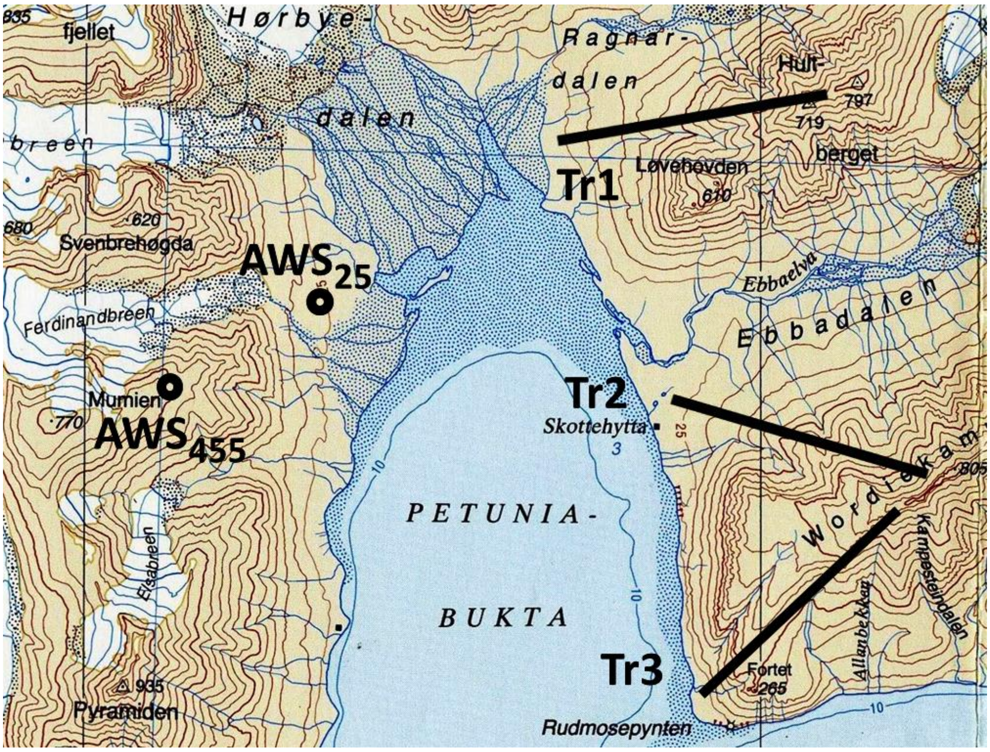

Figure 1. Location of the three investigated transects Tr1–Tr3 and automated weather stations (AWS) in Petunia bay, Billefjorden, Central Spitsbergen. Map source: map sheet C7, Svalbard 1:100 000, Norwegian Polar Institute 2008.

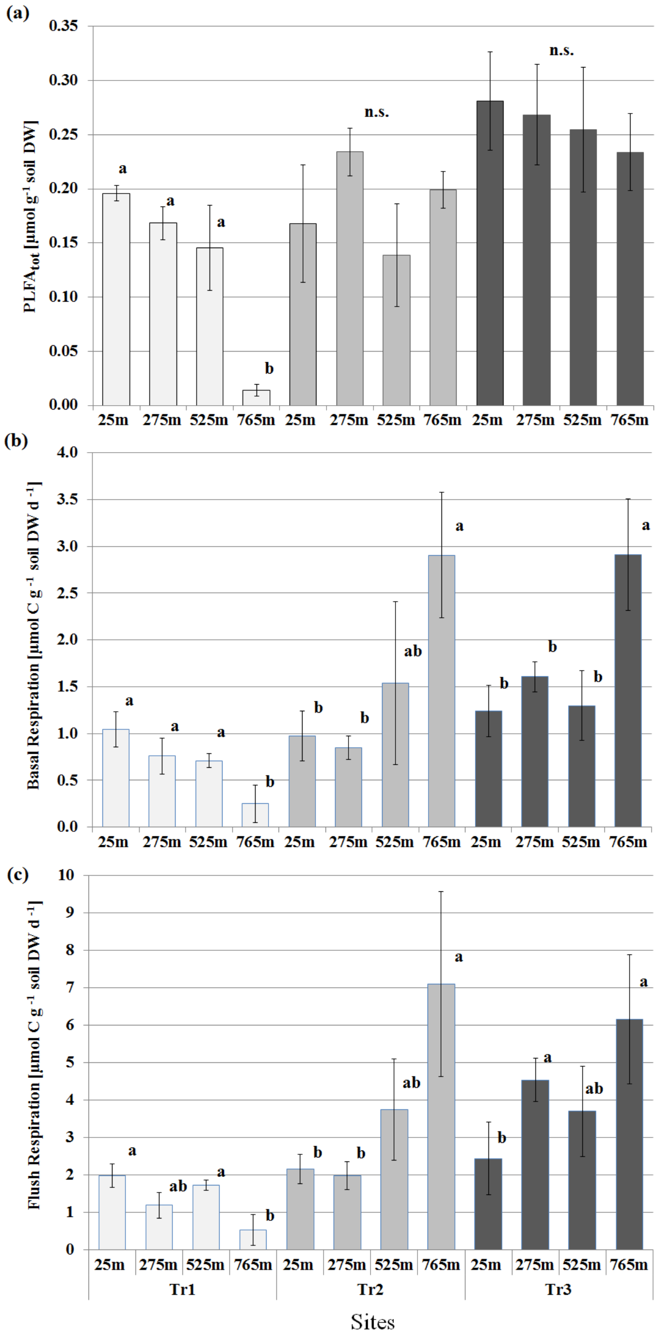

Figure 2. The soil PLFA contents (a), the potential respiration rates (b) and the flush respiration rates (c) in the soils along altitudinal transects (Tr1-Tr3). Error bars indicate mean ± SD (n = 3). Small case letters denote significant differences among altitudes within particular transects (*P* < 0.05; One–way ANOVA combined with Tukey post hoc test).

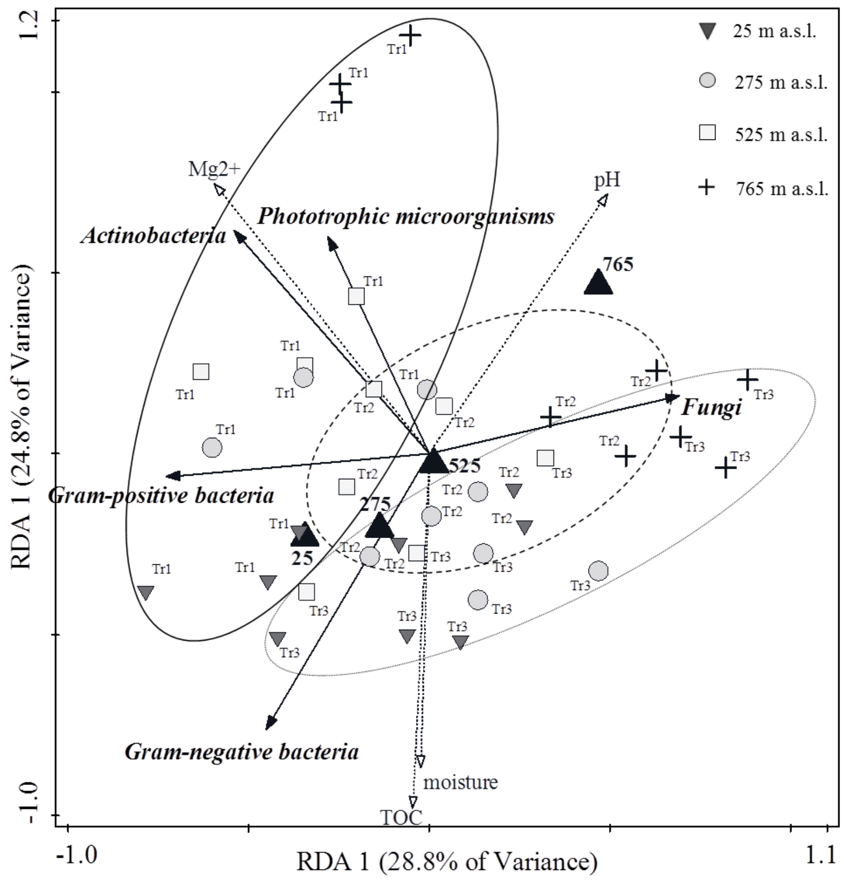

Figure 3. The correlation between abundance of main microbial groups (bold italic) and soil geochemical parameters retained by forward selection of explanatory variables. Results of RDA. Altitude of sampling sites was used as supplementary variable. *Arrows* indicate the direction in which the respective parameter value increases, *solid* lines indicate microbial groups, *dotted* lines indicate selected environmental variables. *Up triangles* are centroids of sites with corresponding elevation (n = 9), numbers indicate elevation (m a.s.l.). The thin solid line encases sites along the Transect 1 (Tr1), the dashed line encases sites along the Transect 2 (Tr2), and the dotted line encases sites along the Transect 3 (Tr3). The numbers in parentheses are the portions of the variation explained by each axis.

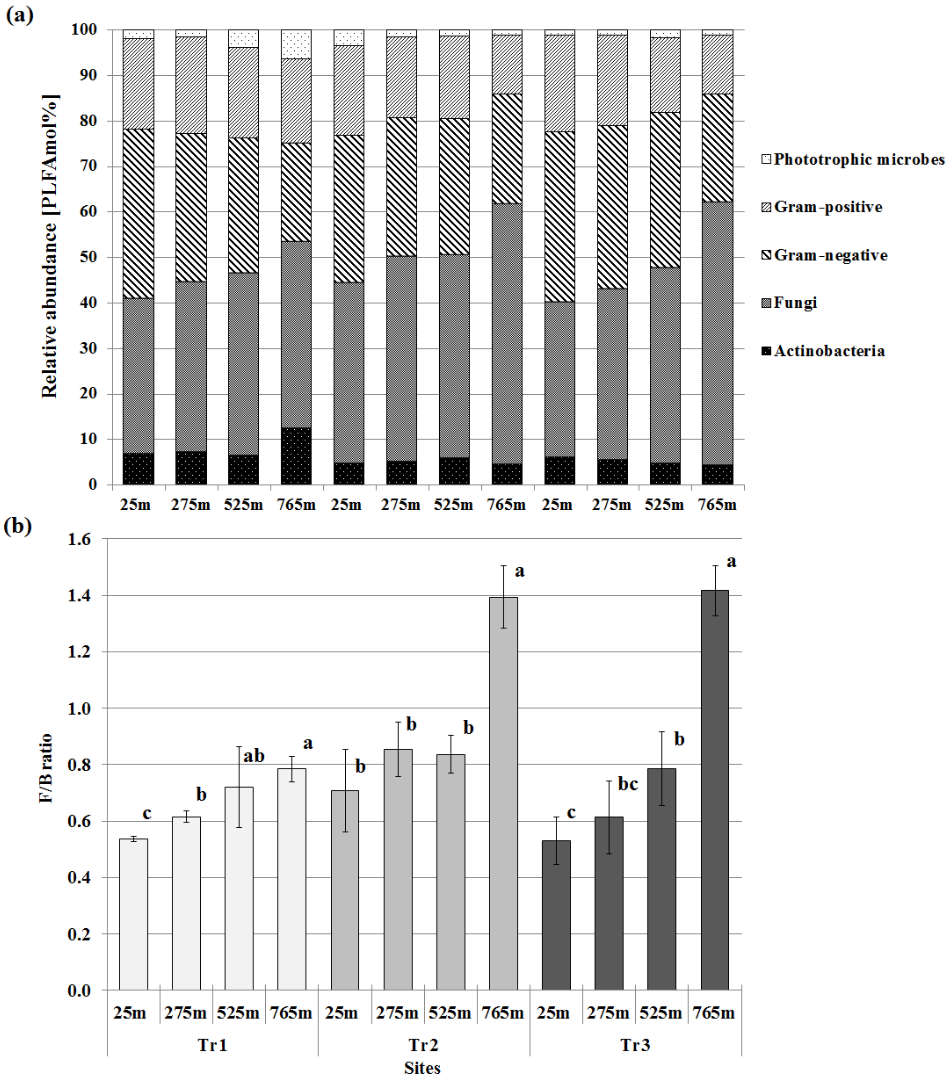

Figure 4. Relative abundance of specific PLFAs within the microbial community (a), and fungi to bacteria (F/B) ratios (b) along altitudinal transects (Tr1-Tr3). Error bars indicate mean ± SD (n = 3). Small case letters denote significant differences between altitudes within particular transects (*P* < 0.05; One–way ANOVA combined with Tukey post hoc test).