
Dear editor,

We are deeply grateful for the efforts of you and reviewers to improve the quality of our manuscript. We have made our efforts to revise the manuscript with clarifications/elaborations as following.

In this version, we have made revisions based on the comments: (1) adjusted some content, incorporate the suggested edits, and carefully edit the entire manuscript for grammar and clarity. (2) adjusting reference list based on manuscript preparation guidelines. Meanwhile, the manuscript has been polished by proof reading service (<https://www.proof-reading-service.com/en/>). A list of all the changes made can be found in the point-by-point response to the reviewers' comments.

Our response is in **normal font** and colored in **blue**, and *the revised text* is in *italic font* and colored in *blue*. Lines number refer to the track changed version.

General Response to Reviewer 2 Comments

The authors did a nice job adequately addressing most of the reviewers' comments. In particular, the methods are much more clear now. However, the writing still needs some work. There are some sentence fragments and run-on sentences, some citations seem incorrect or out of place, and in many places it is difficult to understand the authors' argument. I have made some suggestions below, but I recommend additional editing and revising.

Thanks for your comments and suggestions. We are appreciated with your helpful advice, and we have made our efforts to revise the manuscript with clarifications/elaborations as following.

Our response is in **normal font** and colored in **blue**, and *the revised text* is in *italic font* and colored in *blue*. Lines number refer to the track changed version.

(1) Line 1 (Title) - Suggestion - Diversity and assembly processes of microbial eukaryote communities in Fildes Peninsula Lakes (West Antarctica)

Response: Revised. "community" to "*communities*"

(2) Line 24: ranges should be indicated by an en dash, not a tilde.

Response: Revised. In the manuscript, we revised all descriptions indicating ranges, and "~" was replaced with "-".

(3) Line 25: when “lake” is used as part of a proper name, it is capitalized, e.g., “lake Chang Hu” should be “Lake Chang Hu”.

Response: Revised in the manuscript.

(4) Line 25: ...with higher values in Lake Chang Hu and Lake Kitec and the lowest value in Lake Yue Ya.

Response: Revised. “...with higher values recorded in Lake Chang Hu and Lake Kitec and the lowest value obtained for Lake Yue Ya” Line 25-26

(5) Line 37: The stochastic processes... to Stochastic processes (“the” is unnecessary – check articles throughout the manuscript, “the” is often used when “a” or no article is more appropriate).

Stochastic processes (e.g., homogenizing dispersal and undominated process) dominated community assembly compared to deterministic processes.

Response: Agreed and revised. “*Stochastic processes (e.g., homogenising dispersal and undominated processes) predominated in community assembly over the deterministic processes*” Line 34-36

(6) Line 39: These findings demonstrate the diversity of microbial eukaryotic communities in the freshwater lakes of the Fildes Peninsula and have important implications for understanding community assembly in these ecosystems.

Response: Agreed and revised according to your advice. Line 35-38

(7) Line 45: A suggestion: The Fildes Peninsula--which makes up the southwestern end of King George Island, South Shetland Islands, Antarctica--is home to a relatively high density of scientific research stations. The peninsula is commonly ice-free throughout the austral summer, making it the largest ice-free area (40 km²) on King George Island. Falling within the maritime Antarctic, the peninsula experiences 400-600 mm of precipitation each year and has an average annual(??) temperature of -3°C. Nevertheless, permafrost and periglacial processes can be found in the region. Lakes on the Fildes Peninsula, along with those found in other ice-free areas of Antarctica, represent the year-round liquid water reservoirs on the continent. Water availability and quality are impacted by sea conditions, macro-fauna usage, and anthropogenic influences, such as solid, volatile, and fluid waste production and disposal. Antarctic lake systems are

sentinels for climate change and host globally-relevant microbes and biogeochemical cycles, thus making a more complete understanding of the processes shaping microbial communities there a priority. Moreover, the physical stability observed in these lakes makes them a good model system for interrogating biogeochemical processes within water columns.

Response: Agreed and revised according to your advice. Line 44-70

(8) Line 61: ranges should be indicated by an en dash, not a tilde.

Response: Revised as suggested.

(9) Line 68: A suggestion: However, microbial eukaryotes in Antarctic lakes have been understudied due to their small cell size and lack of conspicuous morphological features. Microbial Eukaryotes have not been neglected in all systems - this sentence needs more specificity to be true. Even in the revised form, I suspect many people would object to the statement. Maybe it is better to say that they are less studied than bacteria or zooplankton.

A better (?) suggestion:

Microbial eukaryotes are generally difficult to study due to their small size and common lack of distinguishing morphological features, especially among the pico- and nanoeukaryotes.

Response: Thanks for a better suggestion. We have revised the manuscript according to your advice. Line 79-81

(10) Line 79: reflection of environmental conditions

Response: Revised.

Methods

(1) How was water collected for Chl a and nutrient analysis? Niskin bottle? What volume was filtered through what kind of filter (GFF?) for acetone extracted Chl a?

Response: We have made a detailed addition in the Method. *“In addition to the in-situ measurements, a water sample obtained 0.5 m below the surface was collected at each monitoring site using a 5 L plexiglass sampler. For measurements of chlorophyll a (Chl a) contents, 1 L water samples were filtered using GF/F filters (0.70 μm,*

Whatman), and Chl a was then extracted with 90% acetone over 24 h and measured spectrophotometrically” Line 170-174

(2) Line 223: the “respectively” is unnecessary

Response: Deleted.

(3) Line 225: OTUs occurring in at least five samples

Response: Revised. “occurred” to “*occurring*” Line 264

(4) Line 258: Bastian M et al., 2009 should just be Bastian et al., 2009 ?

Response: Revised. Line 267

(5) Line 261: neutral should not be capitalized

Response: Revised.

Results

(1) Lines 283-290 should be rewritten for clarity. There are fragments and run-ons. Again, tildes are not appropriate for ranges.

Response: Revised. “*Water temperature ranged from 0.90°C to 7.14°C, and the water temperature in YO was significantly higher than in the other lakes (Table S1, $P < 0.05$). Nutrients in five lakes showed lower values of 0.00-0.15 μM (NO_2^-), 0.05-0.74 μM (NH_4^+) and 0.02-2.29 μM (PO_4^{3-}). Relatively higher and lower levels of nutrients were identified in YY and XH, respectively. The lowest value of SiO_3^{2-} was recorded in YY (1.43 μM) and the highest in CH (51.5 μM). The highest and lowest Chl a were reached in YY and CH (2.11 and 0.25 $\mu\text{g L}^{-1}$, respectively). pH showed minimum and maximum values in YY (7.65) and CH (8.27). Sal values ranged from 0.00-0.14 PSU and were significantly lower in YO than in the other lakes ($P < 0.05$).”* Line 292-300

(2) Line 294: Diveristy and composition of microbial eukaryotic communities

Response: Revised. “Diversity and composition of microbial eukaryotic communities” Line 310

(3) Line 329: over the years [because there is only one sample per year)

Response: Revised. Line 335

(4) Line 332: years

Response: Revised. Line 337

(5) Line 361-2: clustered separately.

Response: Revised. Line 365

(6) Line 371: variability among lakes, but there was still a large amount of unexplained variation.

Response: Revised. Line 375-376

(7) Line 373: made up the microbial eukaryotic community network

Response: Revised. Line 377

Discussion

(1) Line 416: differs

Response: Revised. “*The environmental conditions (e.g., low light and low nutrient contents, etc.) in Antarctic freshwater lakes differ from those of temperate lakes*” Line 415-416

(2) Line 425: the maritime and continental regions of Antarctica? If not, then remove “the”. If yes, say it.

Response: Thanks for your suggestions. Revised as “*the maritime and continental regions of Antarctica*” Line 427

(3) Line 430-437: I think the present tense was correct here

Response: We agreed with your advice. Revised.

(4) Line 465: states that there should be an increased species number as habitat area increases with a specific area

Response: Revised according to your advice. Line 468-469

(5) Line 471: Previous studies have demonstrated

Response: Revised. Line 476

(6) Line 472-473: In this study, we found

Response: Revised. Line 478

(7) Line 477: I would keep water temperature as water temperature throughout the discussion

Response: Thanks for your suggestion. We agreed with you. We keep water temperature as water temperature in discussion and results. But we still use the WT as water temperature in Figures.

(8) Line 492: A substantial amount of variation was unexplained, which could be due to a number of reasons. First, it is not possible to measure all environmental factors that can influence microbial communities and, thus, some significant driving factors may not have been included in the study. Potentially vital abiotic factors in Antarctic lakes include: solar cycle, light availability, ice cover (thickness and duration), physical mixing of snow melt, and other hydrological processes. Second, relationships between microorganisms could not be quantified, and these relationships are potentially essential factors shaping community structure. For example, predation pressure can manifest as a top-down control of microbial eukaryotes. Third, ...

Response: Thanks for your suggestion. We agreed with you and revised the manuscript as suggested. Line 498-514

(9) Line 507: Network analysis can help illuminate complex biological interactions

Response: Revised. “could” to “can”. Line 521

(10) Line 514: we found that positive correlations were much more common (82%) than negative correlations (18%). These results suggest that positive relationships (e.g., due to cross-feeding, niche overlap, mutualism, or commensalism) might play a more important role in Antarctic lake ecosystems than negative relationships (e.g., predator-prey, host-parasite, or competition).

Response: Revised as suggested. *“Our network analysis revealed that the positive correlations were much more common (82%) than negative ones (18%). These results*

suggested that positive relationships (e.g., due to cross-feeding, niche overlap, mutualism, or commensalism) might play a more important role in the lake ecosystems of Antarctica than negative relationships (e.g., predator-prey, host-parasite, or competition)” Line 528-534

(11) 527: the clause “and these might weaken the role of environmental selection in community assembly” does not make sense

Response: Thanks for your comments. We deleted this clause.

(12) 528: The sentence starting with “Previous studies have shown the high response of microbial ... “seems completely unrelated to the rest of the paragraph

Response: Thanks for your comments. We deleted this sentence.

(13) 545 and 549 - I think this should be present tense

Response: Revised. “existed” to “*exist*”, “influenced” to “*can influence*”. Line 550 and 554

(14) 552: from aquatic ecosystems

Response: Revised. “from the aquatic ecosystems” to “for other microbial eukaryotic communities *in aquatic ecosystems*”. Line 557

(15) 552: For example, picoeukaryotic communities in the lower ...

Response: Revised. “The picoeukaryotic communities in the lower ...” to “*For example, picoeukaryotic communities in the lower ...*”. Line 558

(16) 554: Results from our study supported a more prominent role for stochastic processes than deterministic in shaping the assembly of microbial eukaryotic communities.

Response: Revised as suggested. Line 559-561

(17) 557: a small amount of variation in our

Response: Revised. “a small number of variations...” to “*a small amount of variation...*” Line 564-565

(18) 565: It is still unclear what is being referred to and why it is significant - I googled

what the Middle Route Project of the South-to North Water Diversion Project is and it does not seem to be related to lakes in polar ecosystems, why did you choose this citation? There must be something published that is more relevant and can better put your findings in context?

Response: Thanks for your suggestion. The South-North Water Transfer Project represents a special kind of freshwater ecosystem. Previous studies of eukaryotic microorganisms in this region using neutral community model (NCM) have similar results to our study.

Considering that the South-North Water Transfer Project is indeed not a polar ecosystem, we deleted the citation to this literature.

(19) Undominant processes should be better defined? I think a better description would help readers.

Response: Thanks for your suggestion. Undominant processes mostly consist of diversification, drift, weak selection, and weak dispersal, but it is still a challenge to directly divide into different components (Zhou and Ning, 2017; Stegen et al., 2015). For better understanding, we have indeed defined “Undominant processes” in detail in the material and method. “and undominated processes (i.e., weak selection, weak dispersal, diversification, and drift processes) with $|\beta_{NTI}| < 2$ and $|RC_{bray}| < 0.95$.”
Line 286-287

- (1) Stegen, J. C., Lin, X. J., Fredrickson, J. K., and Konopka, A. E.: Estimating and mapping ecological processes influencing microbial community assembly, *Frontiers in Microbiology*, 6, <https://doi.org/10.3389/fmicb.2015.00370>, 2015.
- (2) Zhou, J. Z. and Ning, D. L.: Stochastic Community Assembly: Does It Matter in Microbial Ecology?, *Microbiol Mol Biol R*, 81, <https://doi.org/10.1128/MMBR.00002-17>, 2017.

(20) 617: “were proved” seems too strongly worded. Maybe: Stochastic processes and biotic co-occurrence patterns were shown to be important in shaping microbial eukaryotic communities in the area.

Response: Thanks for your comments. We agreed with you and revised according to your advice. Line 608-609

(21) 621: the sentence starting with “Stochastic processes played a very prominent ...” is repetitive and should be removed or edited

Response: Thanks for your comments. We removed the sentence.