



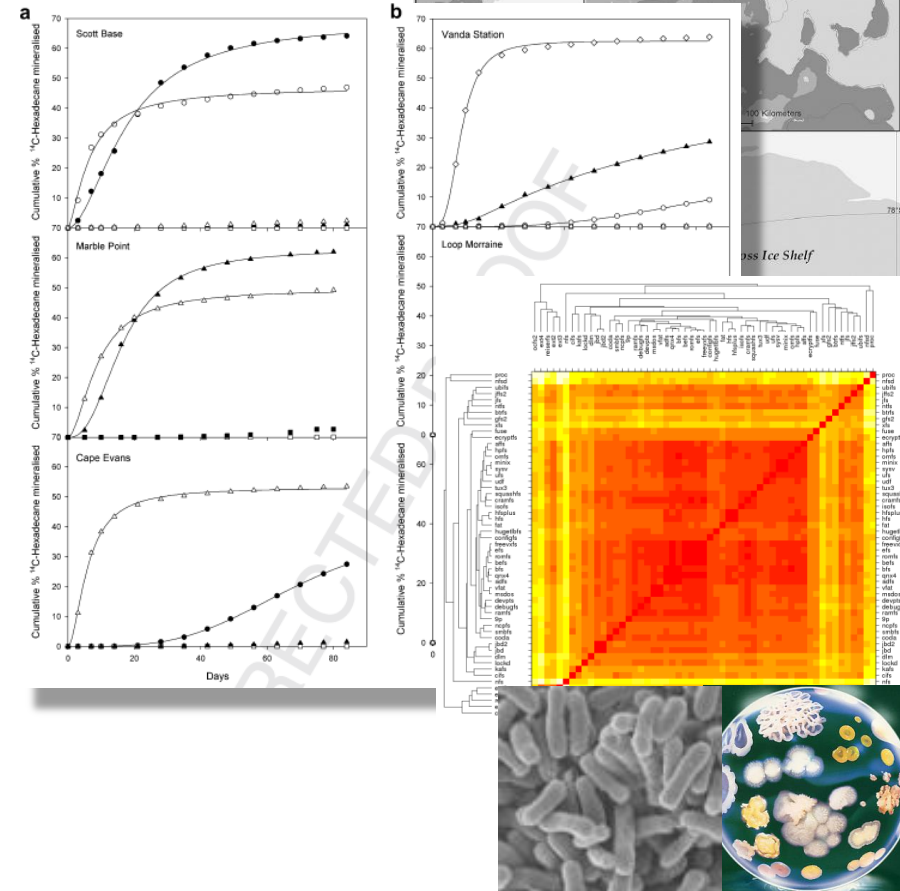
Comprehensive Analysis of Soil Bacterial Diversity and Structure in Terra Nova Bay of Victoria Land

Ahnna Cho, Hyoun Soo Lim, Jeongsu Oh, Ji Hee Kim,
Soon Gyu Hong and Ok-Sun Kim

Korea Polar Research Institute (KOPRI)

Microbes in Antarctic terrestrial environments

- Past: Terrestrial environments in Antarctica had been believed as sterilized habitats without any life forms because of the extreme conditions.
- Recent: Expansions of molecular biological methods to study microbial communities have detected unexpected **high diversity and complexity of bacteria community** in the harsh environment.
- Environmental conditions, such as temperature and freeze-thaw cycles, appear to have profound effects on soil microbial communities (Bokhorst et al., 2007; Yergeau and Kowalchuk, 2008).



Latitudinal Gradient Project

➤ Framework within which interdisciplinary and international collaborations

- Antarctica New Zealand, McMurdo Dry Valleys LTER (Long Term Ecological Research), Italian Antarctic Research Programme (PNRA)
- ITASE (International Trans-Antarctic Scientific Expedition)
- New Zealand's Ministry of Fisheries(MFish)
- Evolution and Biodiversity in the Antarctic: The Response of Life to Change (EBA)

➤ Goals

- Understanding the complex ecosystems that exist along the Victoria Land coast (72°S ~ 86°S)
- Determining the effects of environmental change on these ecosystems

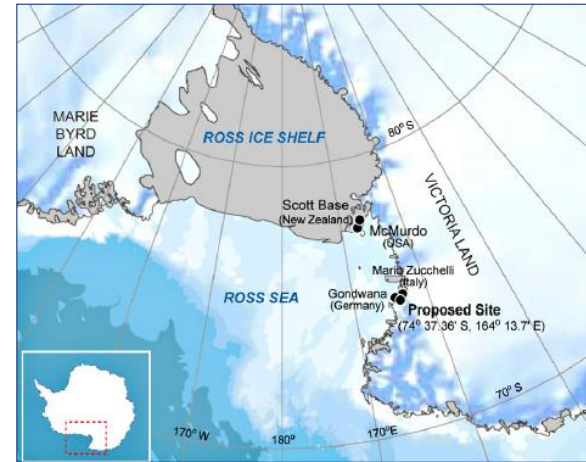
Latitudinal Gradient Project

The screenshot displays the website for the Latitudinal Gradient Project (LGP). The header features the LGP logo and the text "LGP LATITUDINAL GRADIENT PROJECT". Below the header is a navigation menu with "Home" selected. The main content area includes a welcome message, a description of the project's focus on ecosystems research, and information about the project's goals and contributions to the SCAR biology programme. A map of Antarctica is shown on the right, highlighting three research zones: Zone 1 (Cape Adare to Cape Hallett), Zone 2 (Terra Nova Bay to Granite Harbour), and Zone 3 (Darwin Glacier to Beardmore Glacier). The map also shows various geographical features and stations.

Jang Bogo Station in Terra Nova Bay

➤ Terra Nova Bay in Victoria Land

- The boundaries of East Antarctica and West Antarctica
- The good place for ecosystems and biological reactions research, due to human activities and environmental characteristics



➤ Jang Bogo Research Station

- Cape Möbius of Terra Nova Bay
- Coastal northern Victoria Land
- Heavy marine influence
- Long-term monitoring on the effects of climate change and/or human activities



Jang Bogo Station in Terra Nova Bay

- This study can give the chance to compare the change of soil microbial diversity before and after the base construction.
- T_0 values

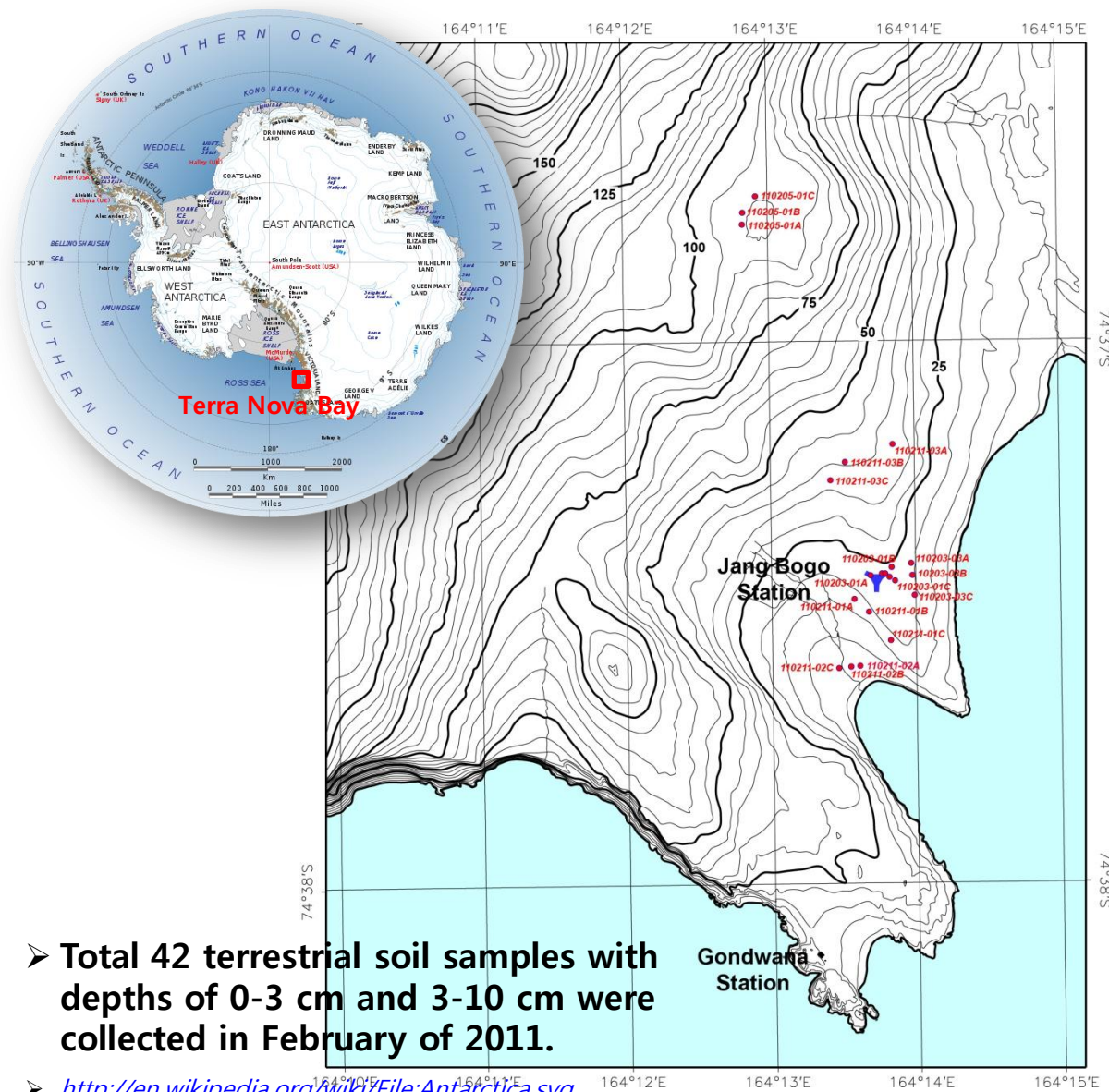


Questions



- 1. What is the dominant bacteria in soil habitats of Terra Nova Bay?**
- 2. How diverse bacteria are present within a habitat?**
- 3. How similar are the bacterial community compositions between habitats?**
- 4. What are the important physicochemical factors for shaping bacterial communities?**

Samples



| Collection date | Sample No. | Elevation (m) | Temperature (°C) |
|----------------------------------|------------|---------------|------------------|
| February 3 rd , 2011 | 0203-01AU | 21 | 8.4 |
| | 0203-01AL | 21 | 8.4 |
| | 0203-01BU | 22 | 6.2 |
| | 0203-01BL | 22 | 6.2 |
| | 0203-01CU | 21 | 3.2 |
| | 0203-01CL | 21 | 3.2 |
| | 0203-02AU | 23 | 2.1 |
| | 0203-02AL | 23 | 2.1 |
| | 0203-02BU | 23 | 0.9 |
| | 0203-02BL | 23 | 0.9 |
| | 0203-02CU | 23 | 1 |
| | 0203-02CL | 23 | 1 |
| | 0203-03AU | 22 | 2 |
| | 0203-03AL | 22 | 2 |
| February 5 th , 2011 | 0203-03BU | 20 | 2.6 |
| | 0203-03BL | 20 | 2.6 |
| | 0203-03CU | 19 | 1.5 |
| | 0203-03CL | 19 | 1.5 |
| | 0205-01AU | 89 | 3.5 |
| | 0205-01AL | 89 | 3.5 |
| | 0205-01BU | 90 | 5.4 |
| | 0205-01BL | 90 | 5.4 |
| | 0205-01CU | 90 | 6.9 |
| | 0205-01CL | 90 | 6.9 |
| February 11 th , 2011 | 0211-01AU | 13 | 5.5 |
| | 0211-01AL | 13 | 5.5 |
| | 0211-01BU | 13 | 5.5 |
| | 0211-01BL | 13 | 5.5 |
| | 0211-01CU | 13 | 5.5 |
| | 0211-01CL | 13 | 5.5 |
| | 0211-02AU | 7 | -3.1 |
| | 0211-02AL | 7 | -3.1 |
| | 0211-02BU | 10 | 0.9 |
| | 0211-02BL | 10 | 0.9 |
| | 0211-02CU | 8 | 0.9 |
| | 0211-02CL | 8 | 0.9 |
| | 0211-03AU | 30 | -1.9 |
| | 0211-03AL | 30 | -1.9 |
| 0211-03BU | 34 | -1.4 | |
| 0211-03BL | 34 | -1.4 | |
| 0211-03CU | 30 | -0.5 | |
| 0211-03CL | 30 | -0.5 | |

- Total 42 terrestrial soil samples with depths of 0-3 cm and 3-10 cm were collected in February of 2011.

➤ <http://en.wikipedia.org/wiki/File:Antarctica.svg>

Analysis of Soil Bacterial Diversity and Structure

DNA extraction and PCR with barcode primers (27f-519r)



Pyrosequencing : 454 GS FLX titanium



Sorting by barcode



Prescreen by quality



Removing non-target sequences



Trimming primer sequences



Assembly of sequences

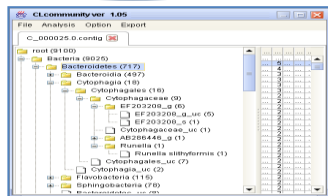


Taxonomic assignment using similarity

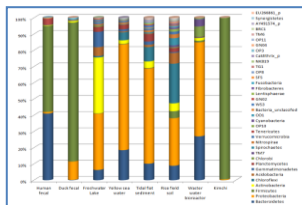


Statistical analysis:
Species richness/Diversity indices (mouther)
Community clustering (primer 6)

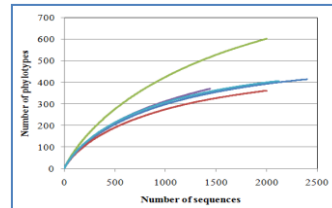
❖ 67,007 sequence reads were obtained and 7,007 OTUs defined by 97% similarity cutoff were detected



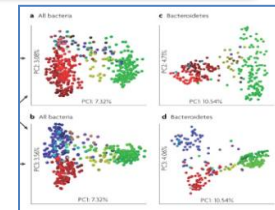
Browsing community structure



Comparing community structures



Estimating diversity



Clustering communities

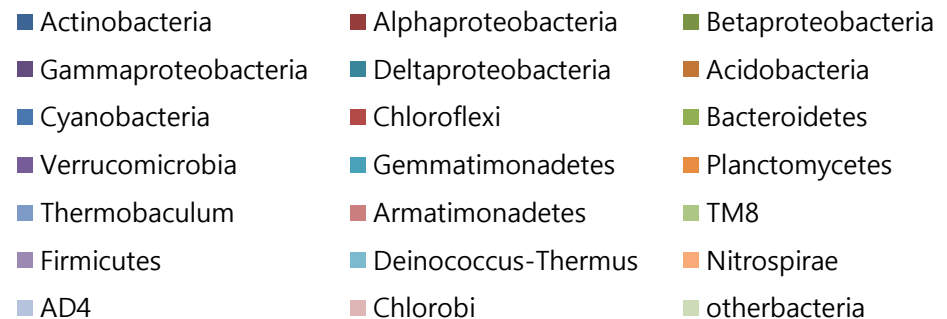
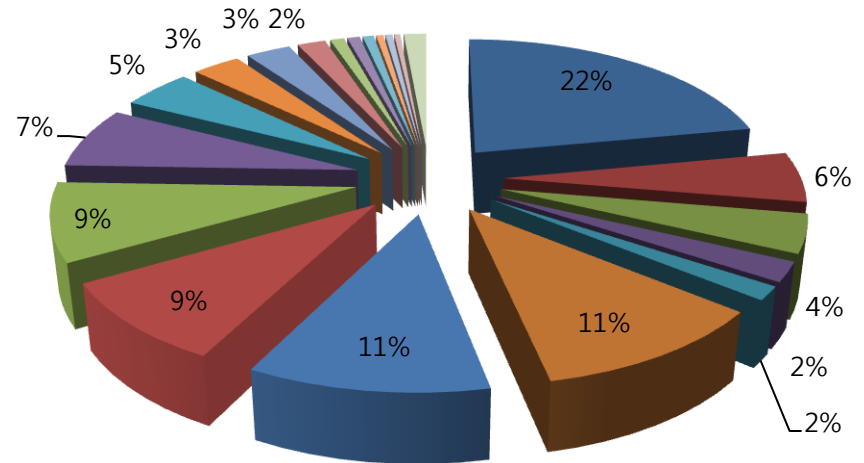


1

What is the dominant bacteria in soil habitats of Terra Nova Bay?

Number of total sequences and proportion

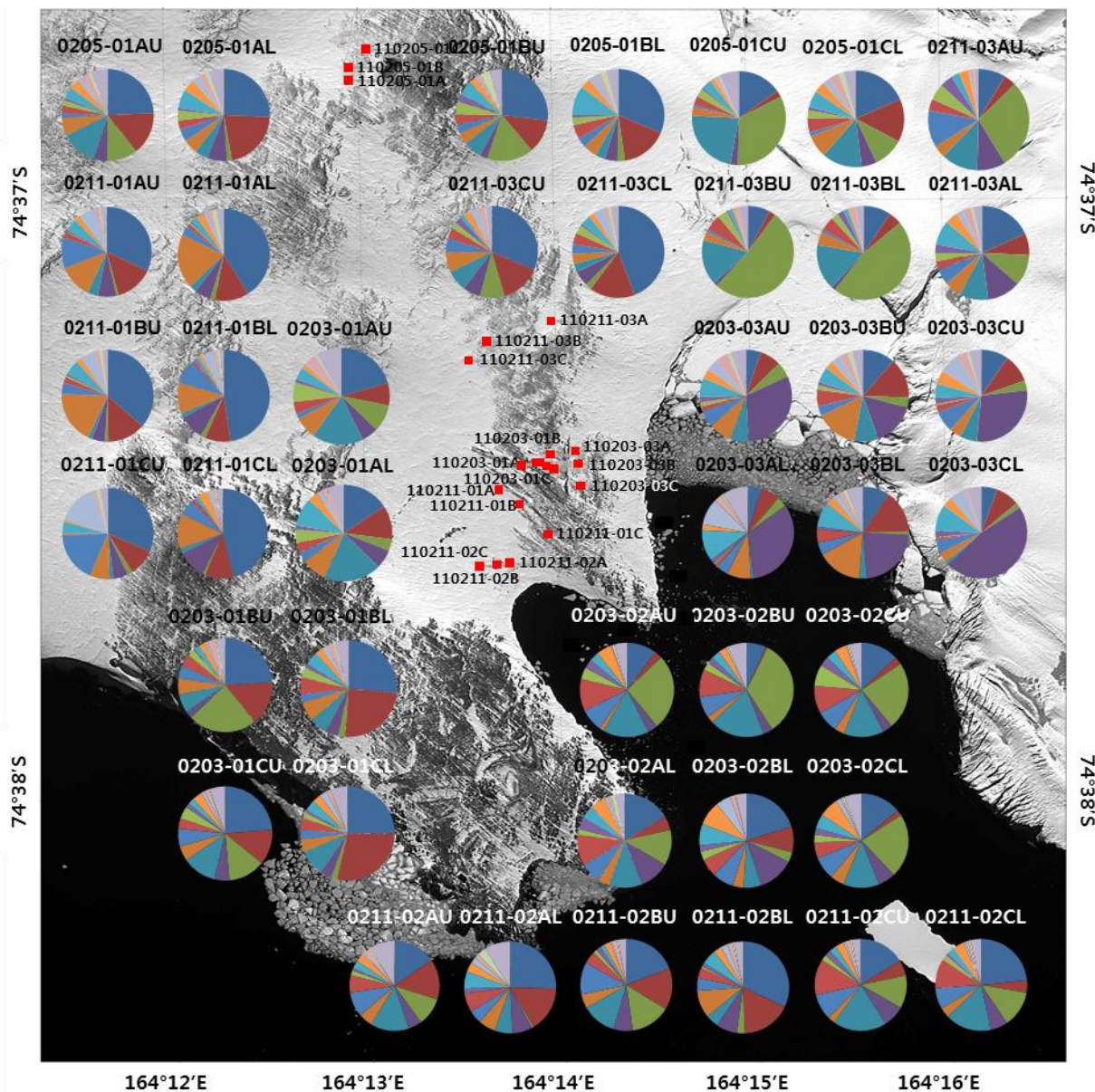
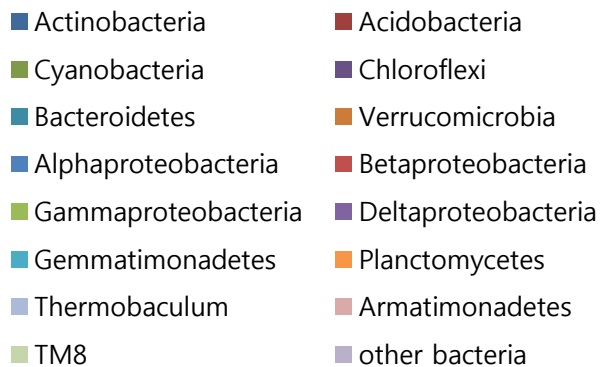
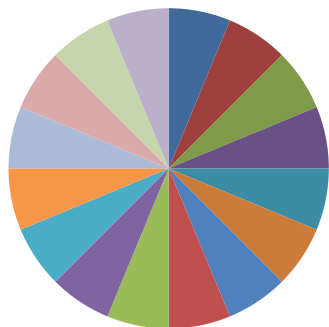
| Phylum | Sequence | Proportion (%) |
|---------------------|--------------|----------------|
| Actinobacteria | 14533 | 22.0 |
| Alphaproteobacteria | 3768 | 5.7 |
| Betaproteobacteria | 2869 | 4.3 |
| Gammaproteobacteria | 1465 | 2.2 |
| Deltaproteobacteria | 1013 | 1.5 |
| Acidobacteria | 7259 | 11.0 |
| Cyanobacteria | 7163 | 10.8 |
| Chloroflexi | 6025 | 9.1 |
| Bacteroidetes | 5852 | 8.9 |
| Verrucomicrobia | 4608 | 7.0 |
| Gemmatimonadetes | 3003 | 4.5 |
| Planctomycetes | 2038 | 3.1 |
| Thermobaculum | 1889 | 2.9 |
| Armatimonadetes | 1192 | 1.8 |
| TM8 | 595 | 0.9 |
| Firmicutes | 535 | 0.8 |
| Deinococcus-Thermus | 472 | 0.7 |
| Nitrospirae | 315 | 0.5 |
| AD4 | 306 | 0.5 |
| Chlorobi | 254 | 0.4 |
| Other bacteria | 951 | 1.4 |
| Total | 66105 | 100 |



➤ The nineteen known divisions recovered corresponded to four phyla, the Actinobacteria, Proteobacteria, Acidobacteria and Cyanobacteria.

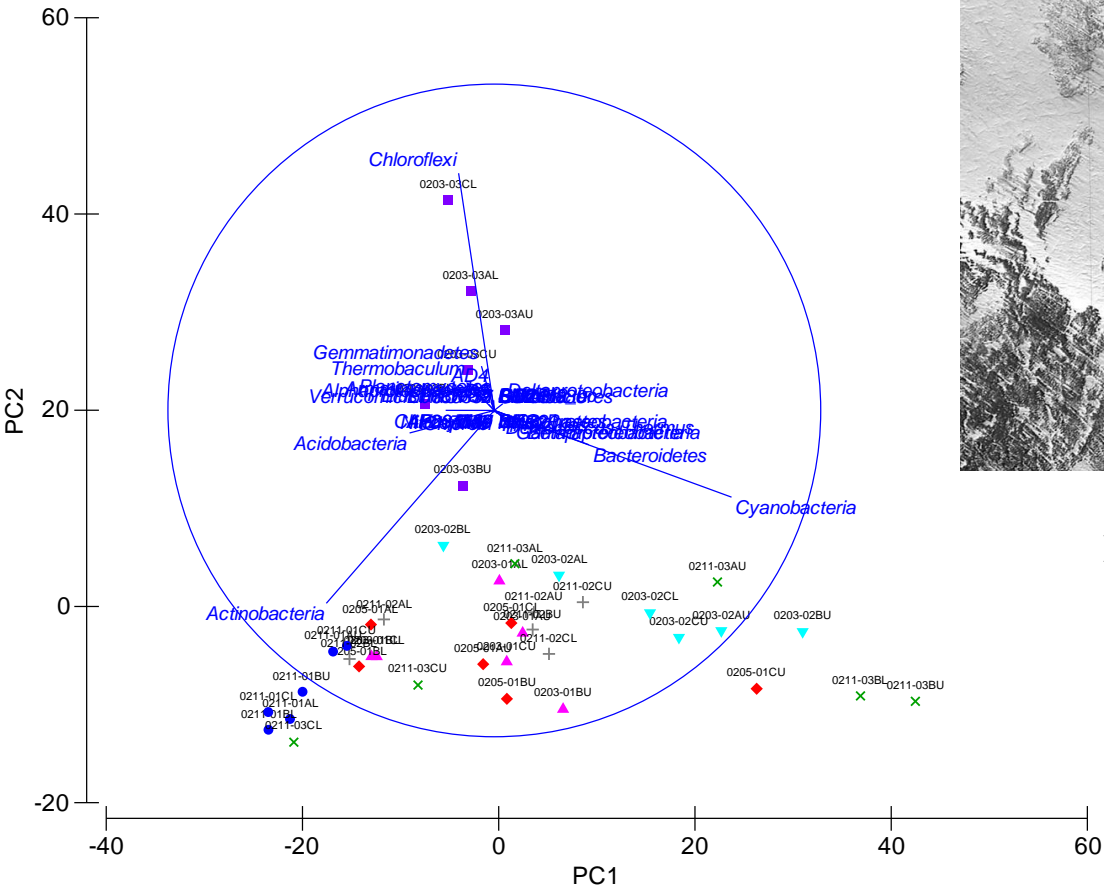
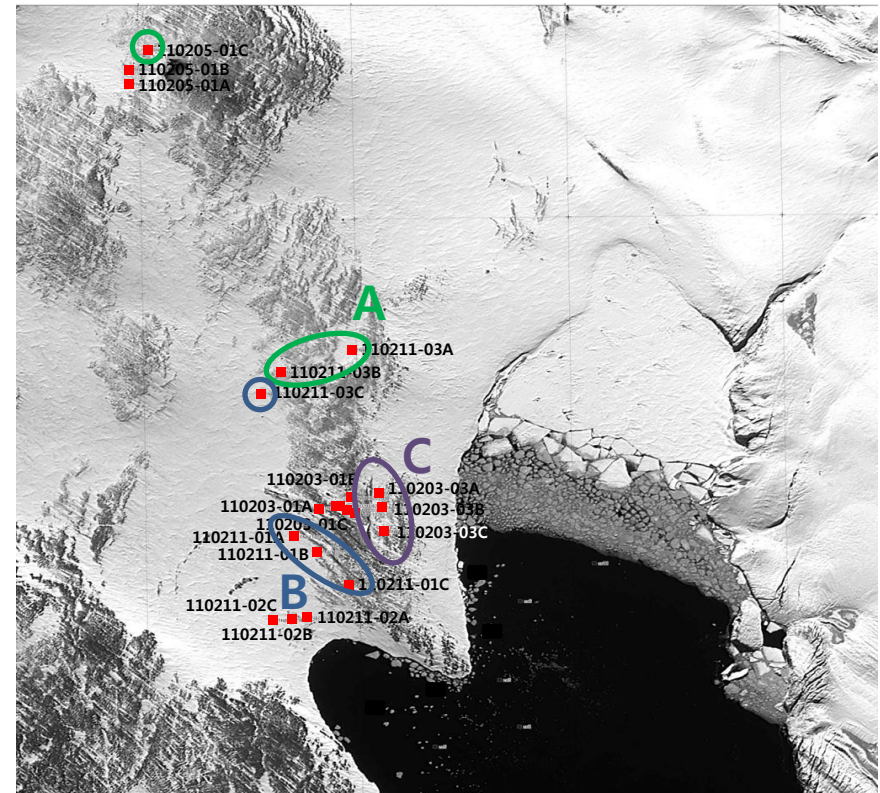
Community structure in phylum level

➤ Bacterial community structures were corresponded to the habitat locality.



Community structure in phylum level

- The most dominant phylum in the area of A, B and C was *Cyanobacteria*, *Actinobacteria* and *Chloroflexi*, respectively.



- PCA plot of investigated phylum.
- Mainly three groups

An aerial photograph of a snowy, rocky landscape. A dark blue lake is visible in the center, with a small boat on its surface. The surrounding terrain is covered in snow and rocks, with some tracks visible. The sky is clear and blue.

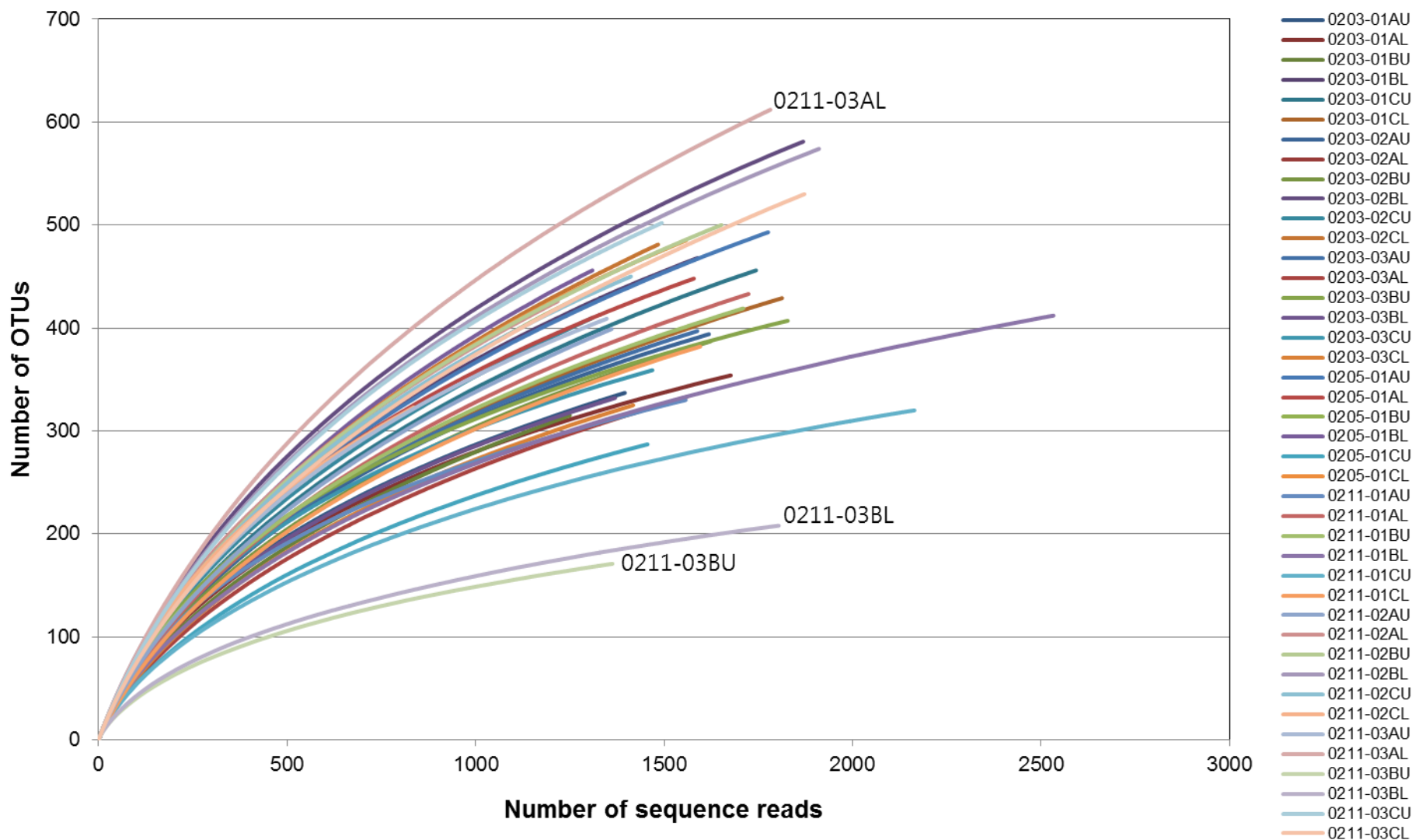
2

How diverse bacteria are present within a habitat?

Summary of sequence results and diversity indices

| Sample name | No. of total reads | Reads of Bac | Reads of Euk | No. of OTUs | Ace | Chao | Shannon | Np-shannon | Simpson | Shannoneven | Simpsonseven | Coverage | |
|-------------|--------------------|--------------|--------------|-------------|-------|-------|---------|------------|---------|-------------|--------------|----------|------|
| 0203-01 | AU | 1,458 | 1,396 | 45 | 337 | 552 | 557 | 5.06 | 5.25 | 0.01 | 0.87 | 0.26 | 0.89 |
| | AL | 1,688 | 1,677 | 10 | 354 | 534 | 528 | 4.93 | 5.12 | 0.02 | 0.84 | 0.16 | 0.91 |
| | BU | 1,276 | 1,250 | 13 | 315 | 720 | 566 | 4.66 | 4.91 | 0.03 | 0.81 | 0.09 | 0.87 |
| | BL | 1,614 | 1,589 | 7 | 468 | 765 | 711 | 5.39 | 5.64 | 0.01 | 0.88 | 0.15 | 0.86 |
| | CU | 1,819 | 1,744 | 47 | 456 | 752 | 693 | 5.27 | 5.51 | 0.01 | 0.86 | 0.19 | 0.88 |
| | CL | 1,838 | 1,813 | 8 | 429 | 700 | 661 | 4.99 | 5.22 | 0.03 | 0.82 | 0.08 | 0.89 |
| 0203-02 | AU | 1,695 | 1,619 | 63 | 394 | 613 | 592 | 5.1 | 5.32 | 0.02 | 0.85 | 0.16 | 0.89 |
| | AL | 1,206 | 1,188 | 13 | 422 | 752 | 673 | 5.43 | 5.74 | 0.01 | 0.9 | 0.28 | 0.82 |
| | BU | 1,667 | 1,623 | 28 | 387 | 787 | 639 | 5.05 | 5.26 | 0.02 | 0.85 | 0.17 | 0.89 |
| | BL | 1,910 | 1,869 | 30 | 581 | 971 | 921 | 5.8 | 6.02 | 0 | 0.91 | 0.35 | 0.85 |
| | CU | 1,351 | 1,311 | 25 | 410 | 1,023 | 797 | 5.31 | 5.54 | 0.01 | 0.88 | 0.22 | 0.83 |
| | CL | 1,510 | 1,484 | 22 | 481 | 1,095 | 862 | 5.46 | 5.72 | 0.01 | 0.88 | 0.19 | 0.83 |
| 0203-03 | AU | 1,631 | 1,588 | 38 | 397 | 789 | 637 | 5.09 | 5.31 | 0.02 | 0.85 | 0.14 | 0.88 |
| | AL | 1,564 | 1,560 | 3 | 333 | 748 | 604 | 4.57 | 4.8 | 0.03 | 0.79 | 0.09 | 0.89 |
| | BU | 1,841 | 1,828 | 10 | 407 | 604 | 578 | 5.33 | 5.5 | 0.01 | 0.89 | 0.3 | 0.91 |
| | BL | 1,375 | 1,370 | 4 | 332 | 680 | 501 | 4.97 | 5.18 | 0.02 | 0.86 | 0.19 | 0.89 |
| | CU | 1,484 | 1,469 | 12 | 359 | 540 | 530 | 5.18 | 5.36 | 0.01 | 0.88 | 0.23 | 0.9 |
| | CL | 1,418 | 1,418 | 0 | 325 | 666 | 519 | 4.65 | 4.89 | 0.03 | 0.8 | 0.1 | 0.89 |
| 0205-01 | AU | 1,823 | 1,776 | 29 | 493 | 1,007 | 773 | 5.49 | 5.71 | 0.01 | 0.88 | 0.25 | 0.87 |
| | AL | 1,603 | 1,579 | 9 | 448 | 886 | 716 | 5.46 | 5.67 | 0.01 | 0.9 | 0.27 | 0.87 |
| | BU | 1,332 | 1,293 | 33 | 433 | 1,056 | 780 | 5.4 | 5.66 | 0.01 | 0.89 | 0.22 | 0.82 |
| | BL | 1,332 | 1,310 | 10 | 456 | 1,042 | 766 | 5.39 | 5.7 | 0.01 | 0.88 | 0.18 | 0.82 |
| | CU | 1,489 | 1,456 | 2 | 287 | 601 | 475 | 4.44 | 4.65 | 0.03 | 0.78 | 0.11 | 0.9 |
| | CL | 1,592 | 1,558 | 11 | 485 | 1,010 | 724 | 5.55 | 5.81 | 0.01 | 0.9 | 0.3 | 0.85 |
| 0211-01 | AU | 1,583 | 1,557 | 22 | 330 | 506 | 503 | 5 | 5.16 | 0.01 | 0.86 | 0.2 | 0.91 |
| | AL | 1,736 | 1,724 | 11 | 433 | 695 | 654 | 5.1 | 5.34 | 0.02 | 0.84 | 0.12 | 0.88 |
| | BU | 1,729 | 1,710 | 11 | 419 | 681 | 675 | 5.23 | 5.43 | 0.01 | 0.87 | 0.2 | 0.89 |
| | BL | 2,558 | 2,533 | 24 | 412 | 616 | 629 | 4.77 | 4.93 | 0.03 | 0.79 | 0.08 | 0.93 |
| | CU | 2,187 | 2,164 | 7 | 320 | 480 | 436 | 4.4 | 4.57 | 0.04 | 0.76 | 0.08 | 0.94 |
| | CL | 1,662 | 1,596 | 46 | 382 | 735 | 576 | 4.72 | 4.98 | 0.05 | 0.79 | 0.05 | 0.89 |
| 0211-02 | AU | 1,385 | 1,361 | 14 | 399 | 881 | 676 | 5.12 | 5.39 | 0.02 | 0.86 | 0.15 | 0.85 |
| | AL | 1,227 | 1,218 | 0 | 426 | 1,030 | 768 | 5.51 | 5.76 | 0.01 | 0.91 | 0.37 | 0.81 |
| | BU | 1,799 | 1,652 | 128 | 500 | 1,067 | 833 | 5.5 | 5.74 | 0.01 | 0.88 | 0.21 | 0.85 |
| | BL | 1,951 | 1,911 | 29 | 574 | 1,150 | 937 | 5.73 | 5.95 | 0.01 | 0.9 | 0.3 | 0.86 |
| | CU | 1,438 | 1,412 | 13 | 450 | 763 | 704 | 5.51 | 5.76 | 0.01 | 0.9 | 0.32 | 0.84 |
| | CL | 848 | 836 | 4 | 323 | 728 | 560 | 5.36 | 5.63 | 0.01 | 0.93 | 0.5 | 0.8 |
| 0211-03 | AU | 1,412 | 1,348 | 59 | 409 | 643 | 630 | 5.35 | 5.6 | 0.01 | 0.89 | 0.24 | 0.86 |
| | AL | 1,831 | 1,782 | 24 | 612 | 1,296 | 1,060 | 5.86 | 6.1 | 0.01 | 0.91 | 0.32 | 0.82 |
| | BU | 1,367 | 1,363 | 2 | 171 | 269 | 290 | 3.55 | 3.71 | 0.09 | 0.69 | 0.07 | 0.94 |
| | BL | 1,812 | 1,804 | 7 | 208 | 412 | 306 | 3.65 | 3.8 | 0.1 | 0.68 | 0.05 | 0.95 |
| | CU | 1,521 | 1,494 | 18 | 502 | 839 | 797 | 5.63 | 5.89 | 0.01 | 0.9 | 0.29 | 0.83 |
| | CL | 1,894 | 1,872 | 11 | 530 | 1,303 | 953 | 5.45 | 5.68 | 0.01 | 0.87 | 0.17 | 0.85 |
| Average | 1,606 | 1,574 | 21 | 409 | 785 | 662 | 5.13 | 5.35 | 0.02 | 0.85 | 0.2 | 0.87 | |
| Min | 848 | 836 | 0 | 171 | 269 | 290 | 3.55 | 3.71 | 0 | 0.68 | 0.05 | 0.8 | |
| Max | 2,558 | 2,533 | 128 | 612 | 1,303 | 1,060 | 5.86 | 6.1 | 0.1 | 0.93 | 0.5 | 0.95 | |

Rarefaction analysis



➤ Rarefaction curves of 16S gene OTUs at 97% cutoff at the sequence level

An aerial photograph of a coastal area. The foreground is a mix of snow and dark rocks. A dark, narrow inlet or bay is visible, with a small red and white boat in the water. The background shows a vast expanse of blue water under a clear sky.

3

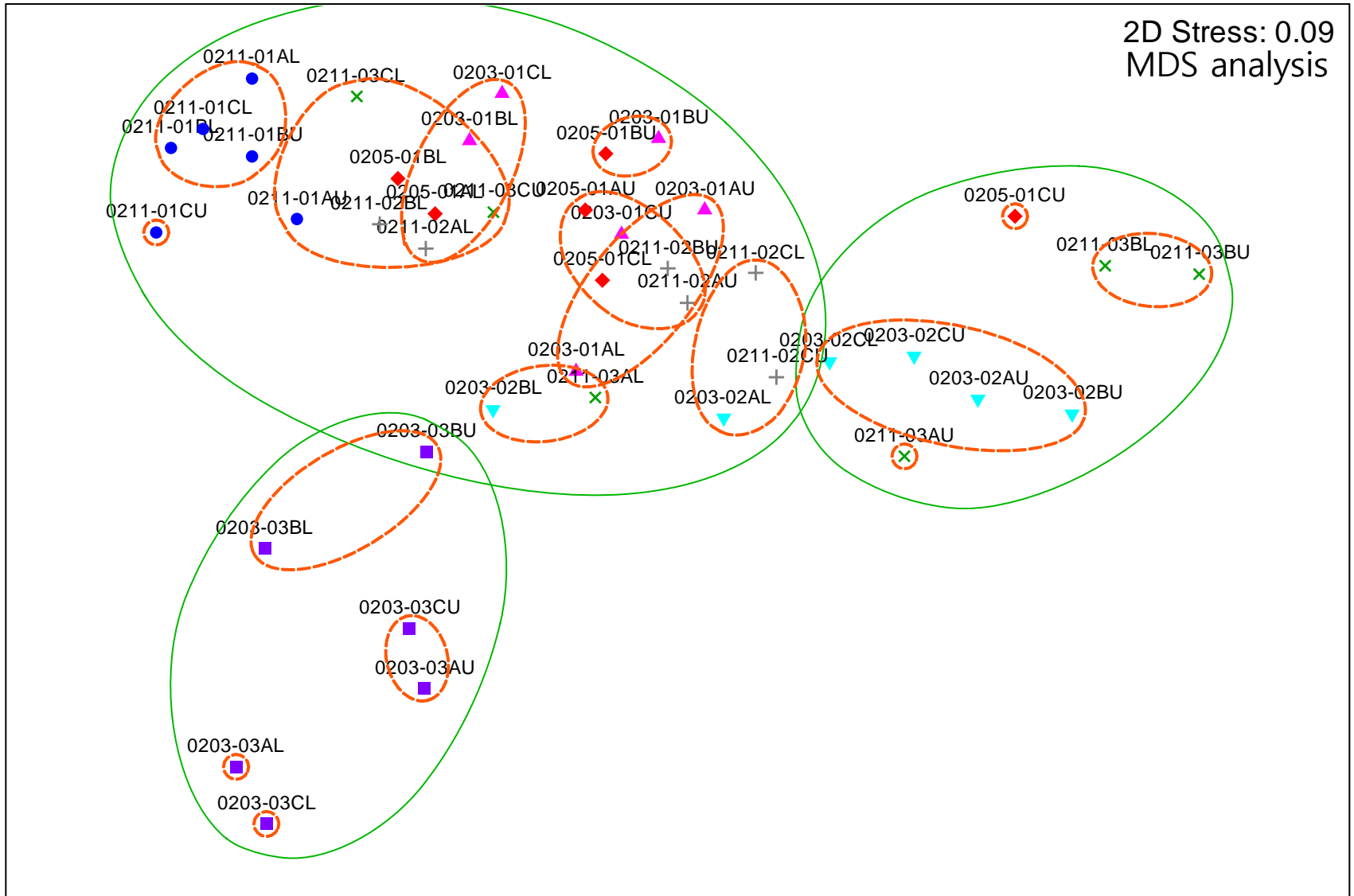
How similar are the bacterial community compositions between habitats?

Similarity between samples_Phylum



Standardise Samples by Total
Resemblance: S17 Bray Curtis similarity

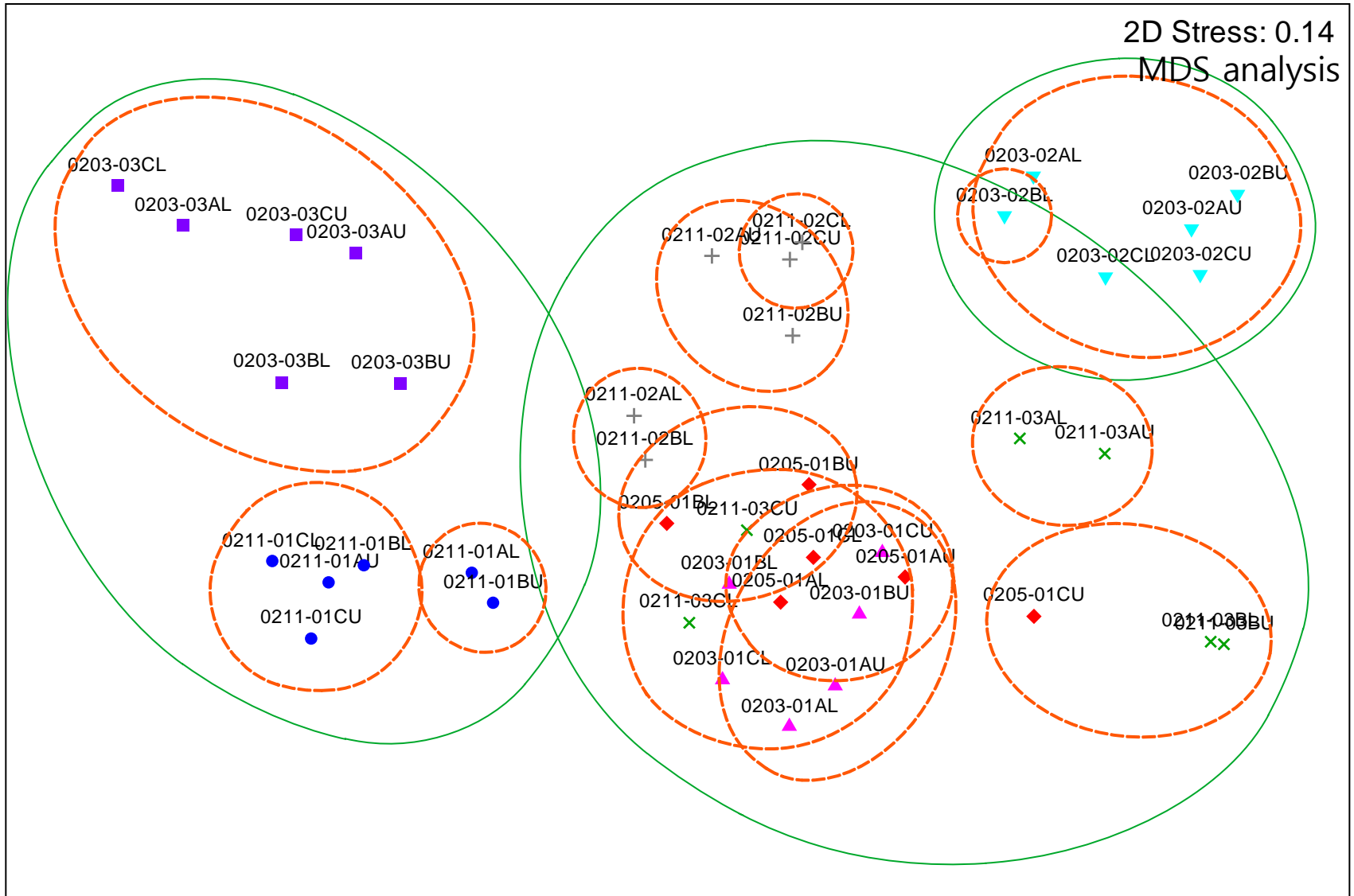
2D Stress: 0.09
MDS analysis



Similarity between samples_OUT (97% cut-off)

- 10
- - - - 30

Standardise Samples by Total
 Resemblance: S17 Bray Curtis similarity



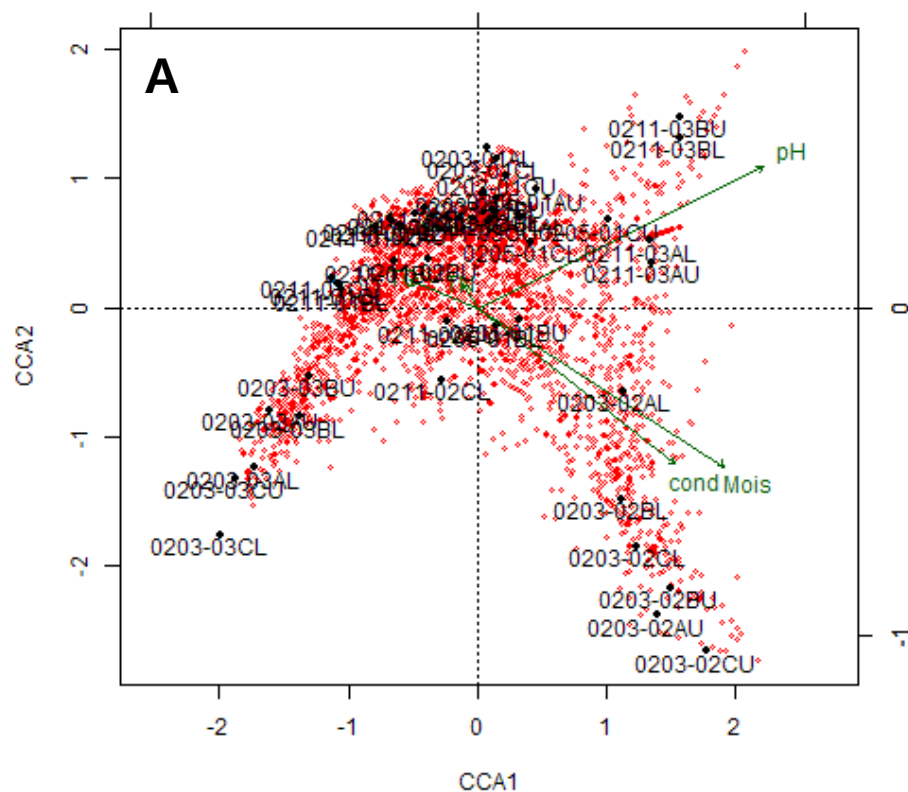
An aerial photograph of a coastal area. The foreground and middle ground are dominated by a rugged, snow-covered shoreline with patches of dark rocks and sand. A small, dark boat is visible in the water near the shore. The background shows a vast expanse of blue water meeting a clear blue sky at the horizon.

4

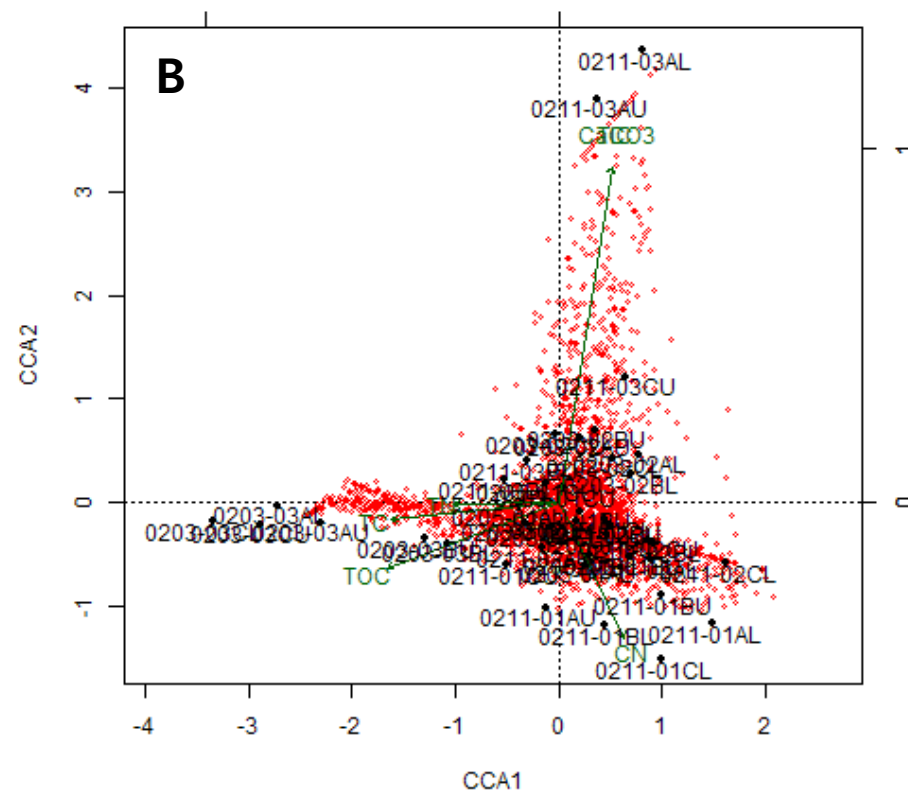
What are the important physicochemical factors for shaping bacterial communities?

Relationship between bacteria and factors

species level CCA analysis



- Environment factors: Moisture, pH, conductivity, TN, TC



- Environment factors: TN, TC, TOC, TIC, C/N ratio, CaCO₃

Summary

1. The similar composition of bacteria was observed between upper and lower layer, but different proportion was shown.
2. Bacterial community structures were significantly corresponded to the habitat locality. In some sites, *Cyanobacteria*, *Actinobacteria* or *Chloroflexi* was dominated with high proportion.
3. The highest bacteria diversity was shown in 2011-03AL, whereas the lowest one in 2011-03BU.
4. Collected at the same point have shown high similarity of bacterial community between samples.
5. Significant relationship between bacterial communities and environmental factors such as moisture contents, pH, conductivity and CaCO_3 was detected.

Thank you for your attention😊

