***Sphagnum capillifolium* holobiont from a subarctic palsa bog aggravates the potential of nitrous oxide emissions**

**Yanxia Nie1,2,5,\*,** **Sharon Yu Ling** **Lau3,5,** **Xiangping Tan1, Xiankai Lu1, Suping Liu1,****Teemu** **Tahvanainen4,** **Reika Isoda5,** **Qing** **Ye1,2, Yasuyuki** **Hashidoko5**※

1Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China.

2Southern Marine Science and Engineering Guangdong Laboratory, Guangzhou 511458, China.

3Sarawak Tropical Peat Research Institute, Kuching-Samarahan Expressway, Kota Samarahan, 94300, Malaysia.

4Department of Environmental and Biological Sciences, University of Eastern Finland, Joensuu FI-80100, Finland.

5Graduate School of Agriculture, Hokkaido University, Sapporo 060-0808, Japan.

※Deceased

**\* Correspondence:**

Corresponding Author: Dr. Yanxia Nie

Email: [nieyanx@scbg.ac.cn](mailto:nieyanx@scbg.ac.cn)

South China Botanical Garden, Chinese Academy of Sciences, Xingke Road 723, Tianhe District, Guangzhou, 510650, China.



*Sphagnum fuscum*   *Sphagnum capillifolium*

Fig. S1 Vegetation of *Sphagnum* mosses in a plateau of a permafrost mound of a palsa mire near Kilpisjärvi (68° 52’ 45; 21° 4’ 40), Finland. Two characteristic species including *Sphagnum fuscum* and *Sphagnum capillifolium* were collected.

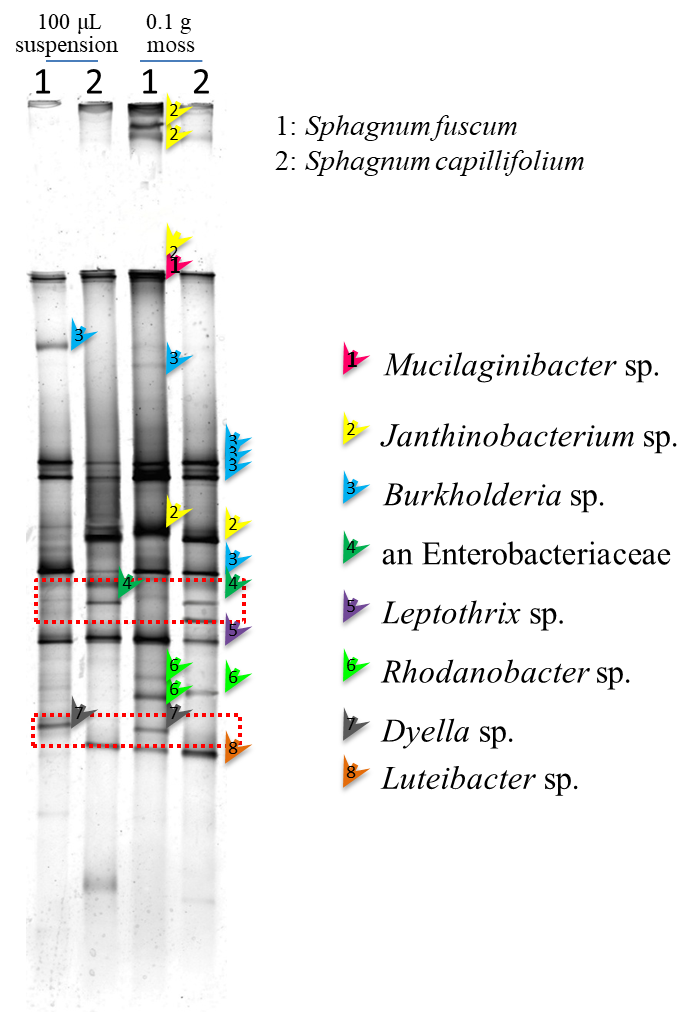


Fig. S2. The profile of DGGE of the two *Sphagnum* species



Fig. S3. Detection of *nar*G, *nir*S, and *nos*Z genes of the three active N2O emitters.

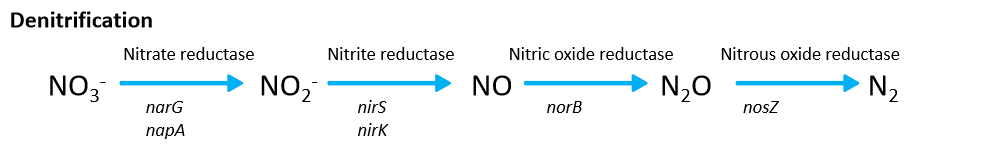


Fig. S4. Functional genes in the process of denitrification.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Target**  **gene** | **Primer set** | **Sequence (5'–3')** | **Thermal profile** | **Reference** |
| *nar*G | 2168F  2391R | 5‘-TCG GGC AAG GGC CAC GAA TAC-3‘  5‘-TTC TCG TAC CAC GTC GCG GTC-3‘ | 95˚C 10 min, 30 cycles of 52˚C 1 min, 72˚C 1 min, 72˚C 10 min | This study |
| *nir*S | cd3AF  R3cd | 5‘-GTSAACGTSAAGGARACSGG-3‘  5‘-GASTTCGGRTGSGTCTTGA-3‘ | 95˚C 10 min, 30 cycles of 52˚C 1 min, 72˚C 1 min, 72˚C 10 min | Throbäck et al., 2004 |
| *nos*Z | 661F  1527R | 5‘-CGG CTG GGG GCT GAC CAA-3‘  5‘-CTG RCT GTC GAD GAA CAG-3‘ | 95˚C 10 min, 30 cycles of 55˚C 1 min, 72˚C 1 min, 72˚C 10 min | Scala and Kerkhof, 1998 |

Table S1 The detail reaction conditions of PCR amplifications of *nar*G, *nir*S, *nos*Z genes.

Table S2 Screening of the N2O emitters isolated from the surface of *Sphagnum* mosses leaves. Incubation conditions of these isolated pure strains: pH=5, incubated at 15˚C, 5 days, n=3, with 0.05% sucrose. (-) indicated inactive N2O emitters, (+) indicated active emitters.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *Sphagnum* species | Bacterium | Most aligned sequences | N2O (ng vial-1 d-1) | N2O emitters |
| SF | SF-B1 | *Burkholderia* sp. | 62.12 | - |
| SF-D2 | *Burkholderia* sp. | 54.02 | - |
| SC | SC-L1 | *Enterobacteriaceae.* | 338.49 | + |
| SC-K1 | *Serratia* sp. | 186.22 | + |
| SC-H2 | *Pseudomonas* sp. | 427.35 | + |
| SC-M1 | *Burkholderia* sp. | 51.90 | - |