

Fungal communities in Scandinavian lakes along a longitudinal gradient

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Supplementary Information

Fig. S1. Correlation matrix of spatial (longitude, latitude and altitude) and environmental (TOC, TP and conductivity, all log transformed) predictors.

Fig. S2. NMDS ordination of Hellinger transformed relative abundance data for 10 samples that were sequenced twice. Each replicate is connected by a line with a respective sample (dark blue is actual sample, light blue is its replicate).

Fig. S3. The relationship between sequencing depth and OTU richness (both log transformed; $P < 0.001$, Pearson correlation coefficient = 0.40) for the whole set of Scandinavian lakes ($n = 77$).

Fig. S4. Assessment of similarity of NMDS ordinations based on different metrics (i.e. Raup-Crick, Jaccard, Bray-Curtis and Gower) on 8 data sets: all lakes (77 lakes); excluding one lake with min total reads (76 lakes); excluding one lake with min total reads and one lake with max total reads (75 lakes); excluding two lakes with both min and max total reads (73 lakes); only lakes with total reads > 50 (64 lakes); only lakes with total reads > 100 (43 lakes); only lakes with total reads > 150 and coverage $> 10X$ (30 lakes); only lakes with total reads > 200 (27 lakes). Procrustes tests are based on 999 permutations.

Fig. S5. The relationship of minimum sequencing depth (reads/site) and Procrustes correlations with reference > 150 total reads/site.

Table S1. Location and environmental characteristics of sampling sites.

Table S2. Information on the fungal phyla and orders presented as % of total reads and total OTUs in n lakes of the whole set of Scandinavian lakes ($n = 77$).

Table S3. Taxonomic assignment of representative OTUs against NCBI v.2.2.29 and UNITE v.7. *Available online*

Table S4. Fitted environmental (TOC, TP, conductivity, all log transformed) and spatial (longitude, latitude, altitude) factors onto NMDS ordination ($k = 2$) for the subset of 30 lakes (with > 150 total reads and $> 10X$ coverage; coverage = total reads/OTU richness per lake). Significant correlation between axes ($P < 0.05$) is indicated by bold font. Number of permutations for assessing significance of factors is 999. Stress = 0.24.

Appendix 1. Classification of the representative OTUs with RDP Bayesian Classifier against Warcup Fungal ITS trainset 2. *Available online*

Appendix 2. The representative sequences of OTUs. *Available online*

Appendix 3. OTU-table with read abundance for aquatic fungal communities. *Available online*

Fig. S1. Correlation matrix of spatial (longitude, latitude and altitude) and environmental (TOC, TP and conductivity, all log transformed) predictors.

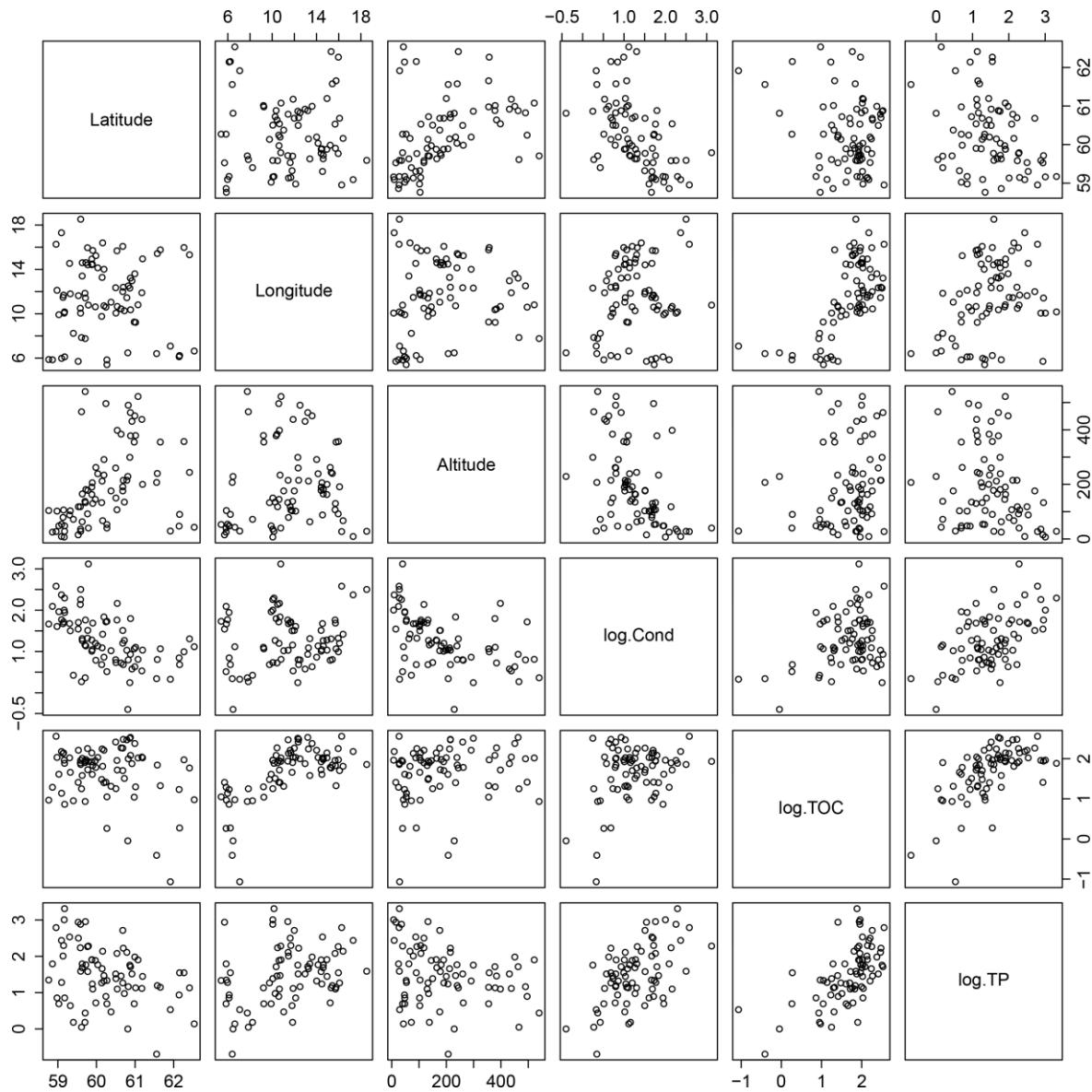


Fig. S2. NMDS ordination of Hellinger transformed relative abundance data for 10 samples that were sequenced twice. Each replicate is connected by a line with a respective sample (dark blue is actual sample, light blue is its replicate).

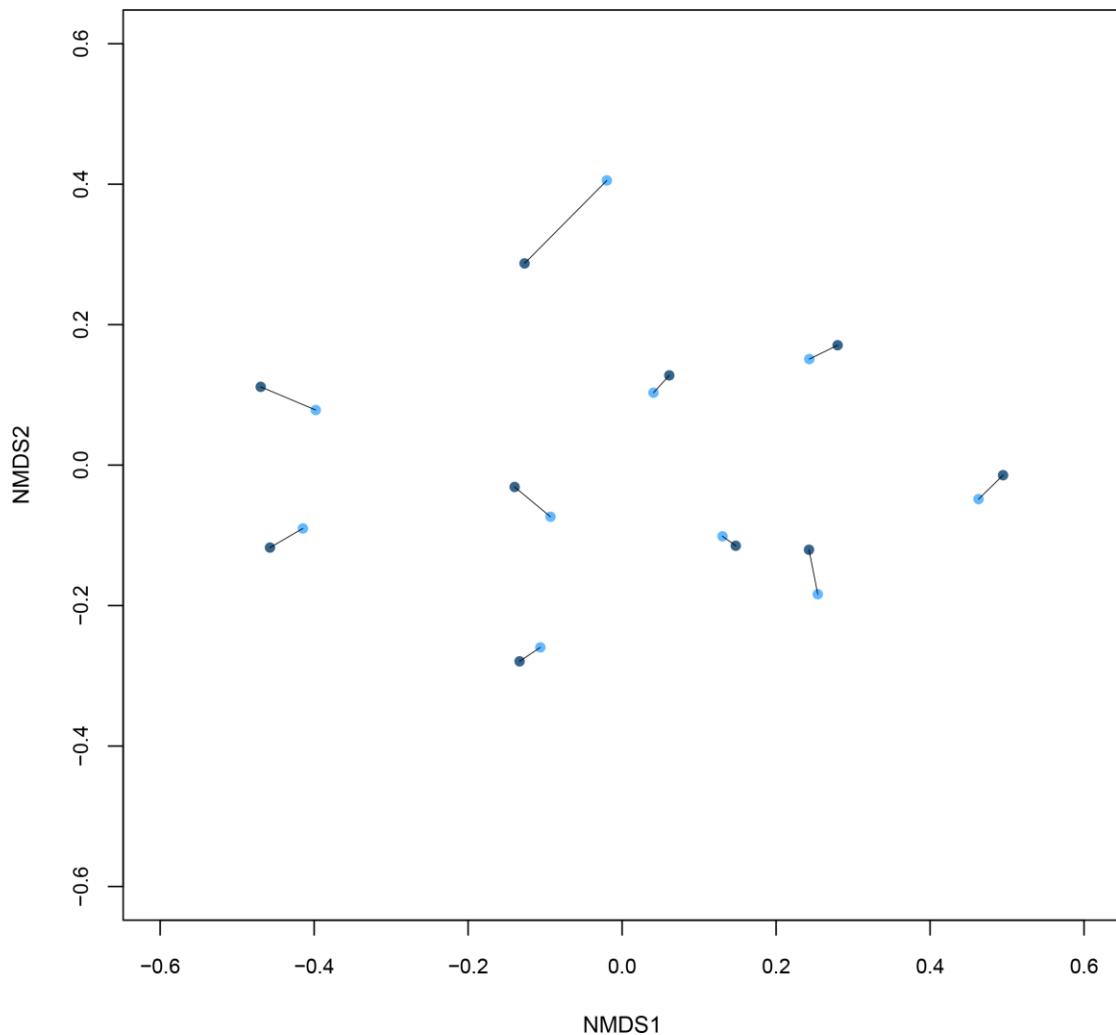


Fig. S3. The relationship between sequencing depth and OTU richness (both log transformed; P < 0.001, Pearson correlation coefficient = 0.40) for the whole set of Scandinavian lakes ($n = 77$).

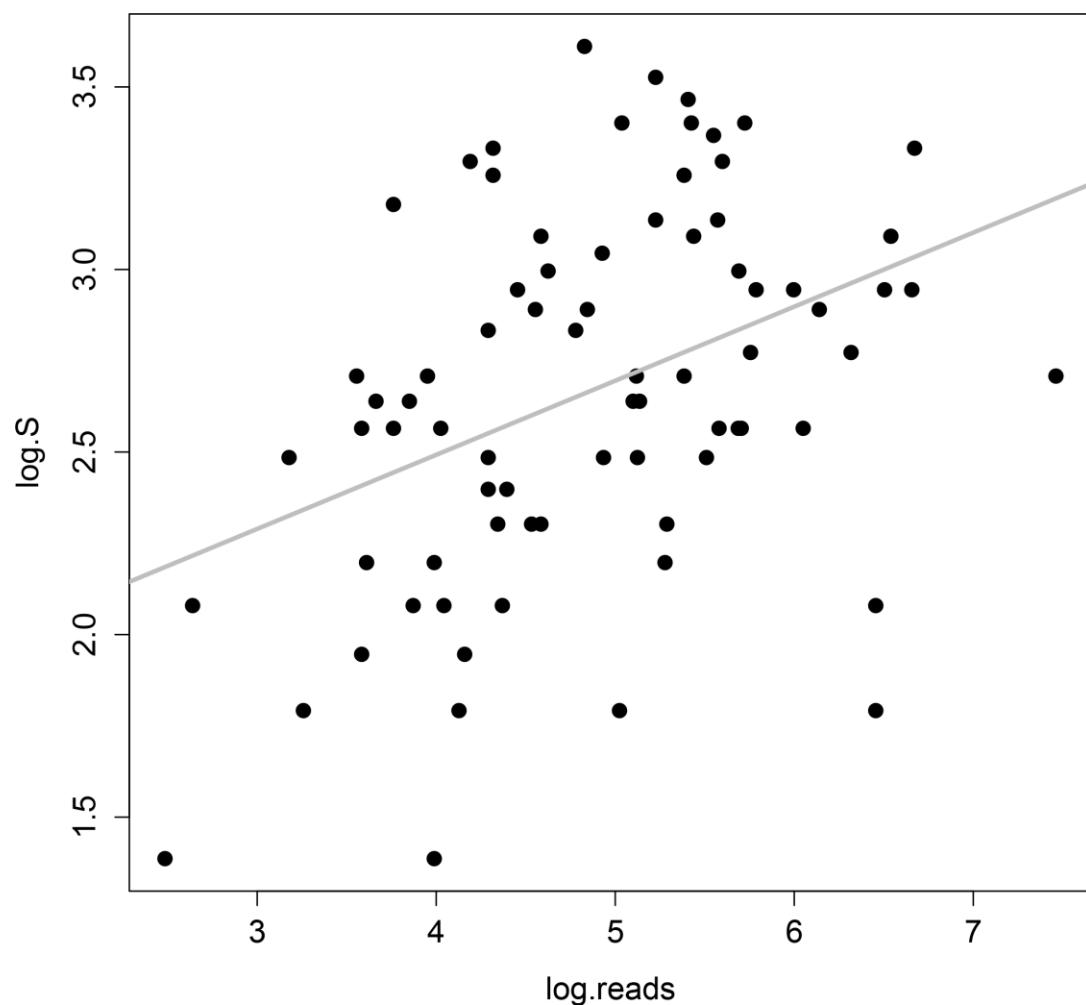


Fig. S4. Assessment of similarity of NMDS ordinations based on different metrics (i.e. Raup-Crick, Jaccard, Bray-Curtis and Gower) on 8 data sets: all lakes (77 lakes); excluding one lake with min total reads (76 lakes); excluding one lake with min total reads and one lake with max total reads (75 lakes); excluding two lakes with both min and max total reads (73 lakes); only lakes with total reads > 50 (64 lakes); only lakes with total reads > 100 (43 lakes); only lakes with total reads > 150 and coverage > 10X (30 lakes); only lakes with total reads > 200 (27 lakes). Procrustes tests are based on 999 permutations.

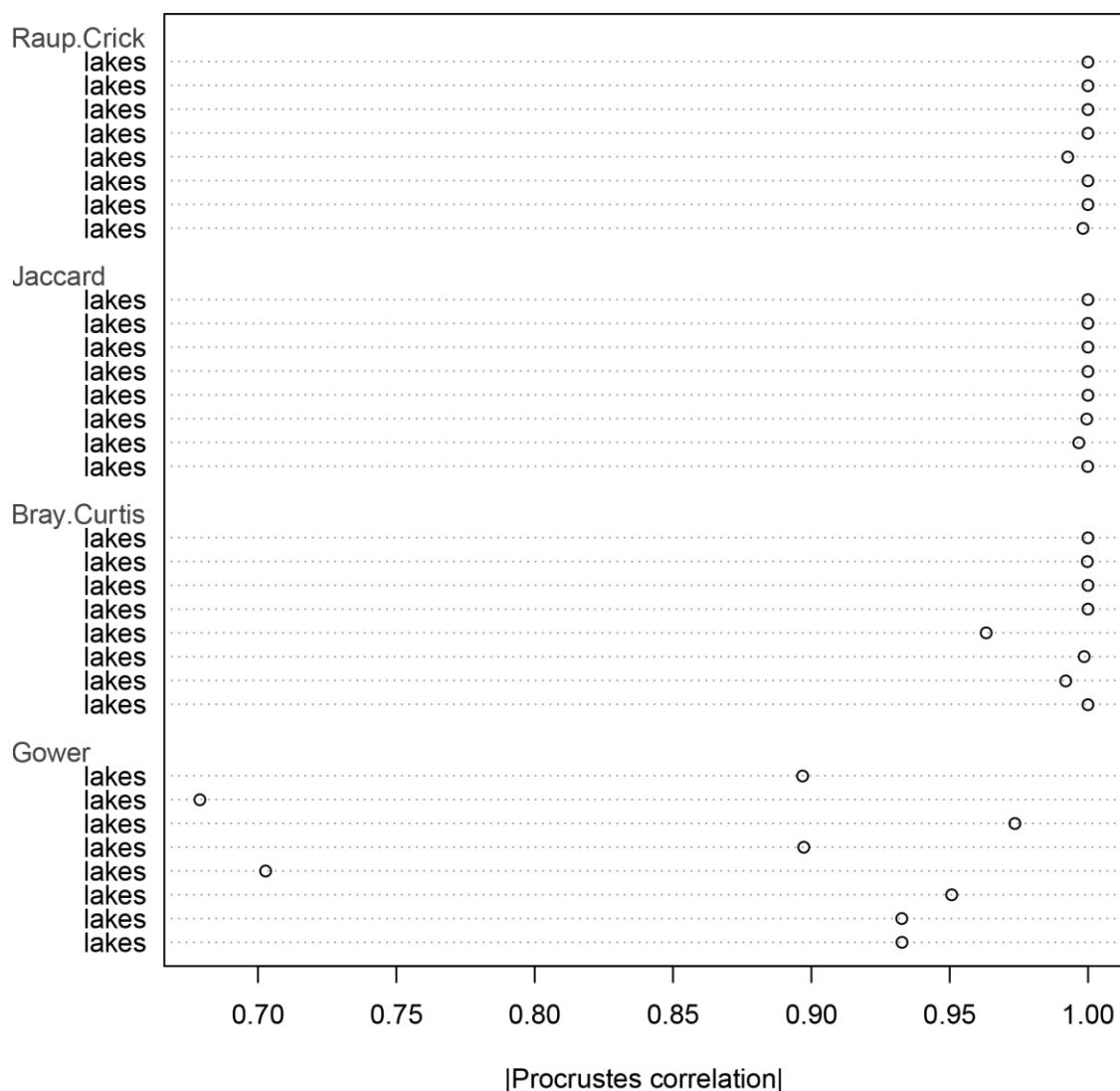


Fig. S5. The relationship of minimum sequencing depth (reads/site) and Procrustes correlations with reference > 150 total reads/site.

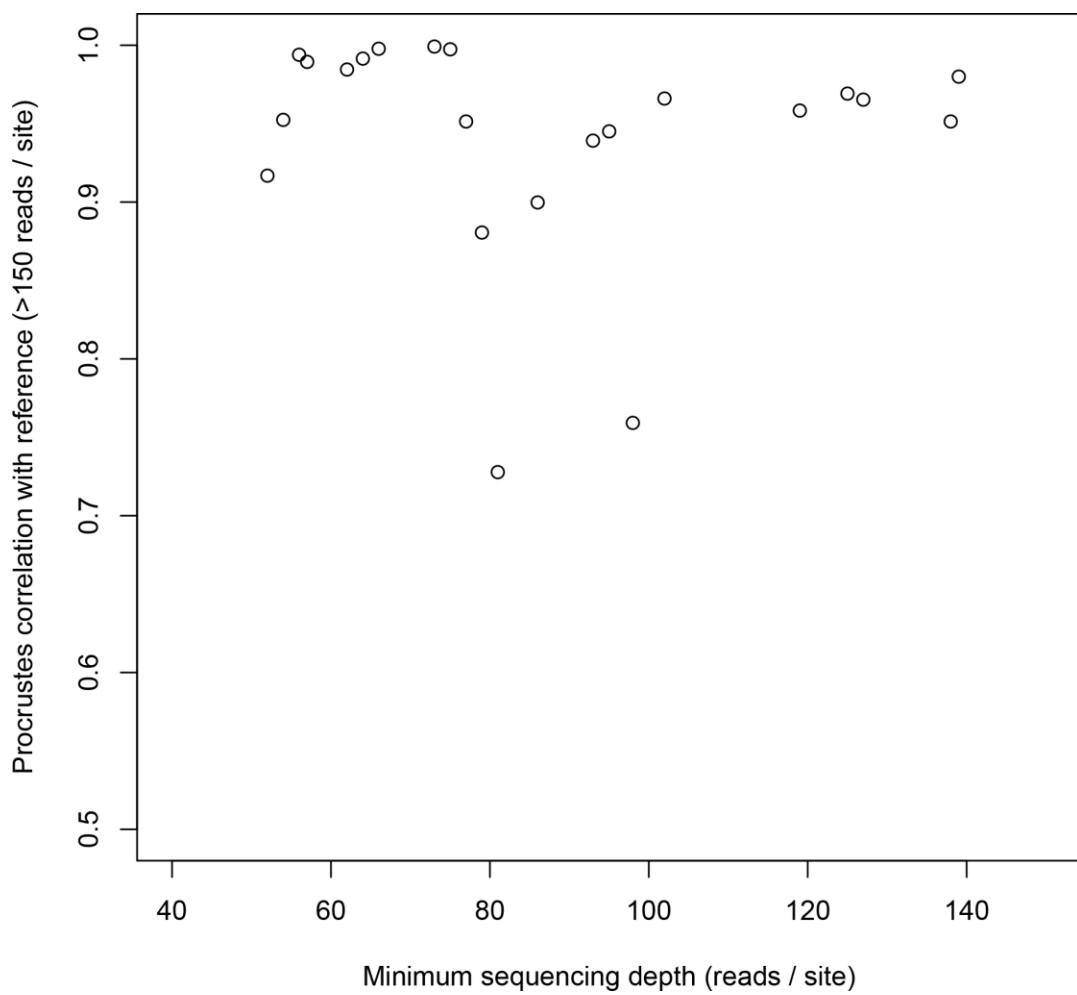


Table S1. Location and environmental characteristics of sampling sites.

| Sample ID | Lake ID | Lake | Country | Latitude | Longitude | Altitude | TOC | TP | Cond | Area.km ² |
|---------------|---------|------------------|---------|----------|-----------|----------|--------|-------|------|----------------------|
| ITS.1 | 170 | Gjersjøen | N | 59,7897 | 10,7749 | 40 | 6,898 | 9,80 | 22,6 | 2,64 |
| ITS.2 | 180 | Øgderen | N | 59,7139 | 11,4130 | 133 | 6,944 | 19,20 | 5,82 | 12,66 |
| ITS.3 | 189 | Krøderen | N | 60,1349 | 9,7586 | 133 | 4,395 | 3,55 | 1,98 | 43,91 |
| ITS.4 | 191 | Rødbyvatnet | N | 59,5818 | 10,4872 | 118 | 6,652 | 9,35 | 8,48 | 1,16 |
| ITS.5 | 194 | Sperillen | N | 60,5040 | 10,0584 | 150 | 3,500 | 4,00 | 2,08 | 37,28 |
| ITS.6 | 214 | Gjesåssjøen | N | 60,6817 | 11,9924 | 176 | 9,613 | 15,10 | 4,52 | 3,98 |
| ITS.7 | 233 | Osensjøen | N | 61,1758 | 11,8877 | 438 | 7,689 | 3,05 | 1,7 | 43,64 |
| ITS.8 | 236 | Rokossjøen | N | 60,7869 | 11,4407 | 215 | 11,810 | 8,25 | 2,8 | 3,77 |
| ITS.9 | 242 | Sør Mesna | N | 61,0764 | 10,8000 | 522 | 7,514 | 6,70 | 2,25 | 6,93 |
| ITS.10 | 252 | Vermundsjøen | N | 60,6951 | 12,3870 | 215 | 11,945 | 9,25 | 1,98 | 3,34 |
| ITS.11 | 258 | Gjønvatnet | N | 60,2697 | 5,8410 | 40 | 1,300 | 2,00 | 1,67 | 2,89 |
| ITS.12 | 261 | Kalandsvatnet | N | 60,2709 | 5,4022 | 53 | 2,854 | 3,80 | 5,64 | 3,37 |
| ITS.13 | 264 | Myrkdalsvatnet | N | 60,8117 | 6,4709 | 229 | 0,954 | 1,00 | 0,67 | 1,56 |
| ITS.14 | 277 | Engsetdalsvatnet | N | 62,5333 | 6,6328 | 43 | 2,656 | 1,15 | 3,05 | 4,40 |
| ITS.15 | 285 | Rotevatnet | N | 62,1405 | 6,1185 | 47 | 3,427 | 2,55 | 2,33 | 1,40 |
| ITS.16 | 288 | Vatnevatnet | N | 62,1531 | 6,2286 | 90 | 1,314 | 4,70 | 1,98 | 2,05 |
| ITS.17 | 326 | Einavatnet | N | 60,5381 | 10,6530 | 398 | 5,572 | 3,00 | 8,7 | 13,74 |
| ITS.18 | 328 | Randsfjorden | N | 60,7230 | 10,2677 | 135 | 4,514 | 2,65 | 3,55 | 140,09 |
| ITS.19 | 339 | Ringsjøen | N | 60,8824 | 10,3547 | 378 | 8,022 | 5,55 | 6,03 | 1,22 |
| ITS.20 | 340 | Sæbufjorden | N | 61,0126 | 9,2151 | 379 | 3,659 | 3,10 | 3,01 | 1,53 |
| ITS.21 | 344 | Strondafjorden | N | 60,9775 | 9,2486 | 355 | 2,833 | 2,00 | 2,9 | 13,36 |
| ITS.22 | 345 | Trevatna | N | 60,6388 | 10,4353 | 384 | 9,680 | 4,30 | 2,06 | 4,80 |
| ITS.23 | 349 | Bogstadvannet | N | 59,9705 | 10,6185 | 145 | 7,026 | 6,60 | 3,31 | 1,09 |

| Sample ID | Lake ID | Lake | Country | Latitude | Longitude | Altitude | TOC | TP | Cond | Area.km² |
|------------------|----------------|----------------|----------------|-----------------|------------------|-----------------|------------|-----------|-------------|----------------------------|
| ITS.24 | 353 | Aspern | N | 59,1471 | 11,6844 | 106 | 8,539 | 9,95 | 5,56 | 6,59 |
| ITS.25 | 361 | Rødenessjøen | N | 59,5320 | 11,6260 | 118 | 9,115 | 12,20 | 5,49 | 15,95 |
| ITS.26 | 363 | Rømsjøen | N | 59,7011 | 11,8438 | 138 | 6,695 | 1,20 | 3,18 | 13,66 |
| ITS.27 | 374 | Edlandsvatnet | N | 58,7626 | 5,8705 | 104 | 2,631 | 3,85 | 5,28 | 2,11 |
| ITS.28 | 378 | Hetlandsvatn | N | 59,1752 | 6,1092 | 48 | 2,370 | 2,35 | 6,99 | 2,06 |
| ITS.29 | 380 | Lutsivatn | N | 58,8601 | 5,8478 | 25 | 3,622 | 6,00 | 8,1 | 2,83 |
| ITS.30 | 394 | Vatsvatnet | N | 59,5267 | 5,7042 | 15 | 4,092 | 18,90 | 4,68 | 2,17 |
| ITS.31 | 395 | Vostervatnet | N | 59,0958 | 5,9754 | 54 | 3,122 | 3,60 | 5,87 | 2,66 |
| ITS.32 | 404 | Jølstravatnet | N | 61,5579 | 6,4005 | 207 | 0,665 | 0,50 | 1,41 | 39,25 |
| ITS.33 | 405 | Oppstrynvatnet | N | 61,9146 | 7,0767 | 29 | 0,344 | 1,70 | 1,39 | 22,97 |
| ITS.34 | 433 | Bandak | N | 59,4016 | 8,2300 | 72 | 2,588 | 1,20 | 1,53 | 26,67 |
| ITS.35 | 436 | Grungevatnet | N | 59,7071 | 7,7592 | 540 | 2,542 | 1,55 | 1,44 | 1,62 |
| ITS.36 | 453 | Vinjevatn | N | 59,6123 | 7,8522 | 466 | 3,490 | 1,05 | 1,31 | 3,23 |
| ITS.37 | 481 | Åsrumvatnet | N | 59,1633 | 10,0578 | 7 | 7,111 | 20,20 | 7,4 | 1,14 |
| ITS.38 | 482 | Bergsvannet | N | 59,5884 | 10,0527 | 36 | 7,070 | 17,85 | 9,55 | 3,01 |
| ITS.39 | 486 | Goksjø | N | 59,1725 | 10,1650 | 28 | 6,576 | 27,45 | 9,91 | 3,40 |
| ITS.40 | 487 | Hallevatnet | N | 59,0253 | 9,9092 | 48 | 5,013 | 2,00 | 7,12 | 3,66 |
| ITS.41 | 498 | Dagarn | S | 59,9037 | 15,7029 | 130 | 6,217 | 3,00 | 5,4 | 1,67 |
| ITS.42 | 519 | Langen | S | 59,5911 | 18,5215 | 28 | 6,392 | 4,90 | 12,2 | 1,38 |
| ITS.43 | 2252 | Rotnessjøen | N | 60,4969 | 12,3412 | 260 | 11,175 | 4,55 | 2,23 | 1,09 |
| ITS.44 | 2268 | Mylla | N | 60,2443 | 10,5911 | 496 | 4,114 | 2,45 | 5,56 | 1,69 |
| ITS.45 | 2312 | Femsjøen | N | 59,1332 | 11,4707 | 79 | 8,420 | 7,45 | 5,32 | 10,66 |
| ITS.46 | 2374 | Klämmingen | S | 59,0920 | 17,2983 | 9 | 8,832 | 11,45 | 10,7 | 9,99 |
| ITS.47 | 2678 | Torrssjøn | S | 58,9783 | 12,1139 | 102 | 7,619 | 2,35 | 4,98 | 1,76 |
| ITS.48 | 2870 | Visten | S | 59,6299 | 13,3968 | 63 | 4,859 | 6,00 | 3,71 | 32,03 |

| Sample ID | Lake ID | Lake | Country | Latitude | Longitude | Altitude | TOC | TP | Cond | Area.km ² |
|---------------|---------|------------------|---------|----------|-----------|----------|--------|-------|------|----------------------|
| ITS.49 | 2872 | Stora Le | S | 59,3222 | 11,7941 | 103 | 5,253 | 1,90 | 4,49 | 84,88 |
| ITS.50 | 2875 | Näsrämnen | S | 60,0339 | 14,1374 | 239 | 7,602 | 5,85 | 3,11 | 2,73 |
| ITS.51 | 2878 | Rangsjön | S | 60,8236 | 12,5075 | 490 | 7,365 | 3,20 | 2,22 | 2,74 |
| ITS.52 | 2887 | Tisjön | S | 60,9188 | 12,9657 | 431 | 6,525 | 4,55 | 1,78 | 27,05 |
| ITS.53 | 2888 | Halsjøen | S | 60,8640 | 12,3111 | 299 | 12,275 | 5,80 | 1,28 | 5,18 |
| ITS.54 | 2899 | Jangen | S | 60,1463 | 13,2715 | 212 | 9,409 | 5,25 | 2,97 | 4,51 |
| ITS.55 | 3017 | Sör-älgen | S | 59,7243 | 14,6065 | 181 | 7,219 | 5,85 | 3,11 | 15,54 |
| ITS.56 | 3019 | Möckeln | S | 59,3039 | 14,5383 | 88 | 7,538 | 12,60 | 5,34 | 17,99 |
| ITS.57 | 3020 | Ljusnaren | S | 59,8792 | 14,9342 | 163 | 8,522 | 6,75 | 3,59 | 9,60 |
| ITS.58 | 3025 | Halvarsnoren | S | 59,6318 | 14,5964 | 165 | 7,441 | 5,40 | 3,54 | 16,88 |
| ITS.59 | 3027 | Nätsjön | S | 59,8913 | 14,4809 | 206 | 4,993 | 3,45 | 2,84 | 2,92 |
| ITS.60 | 3029 | Örlingen | S | 59,8790 | 14,4200 | 190 | 6,836 | 6,70 | 2,74 | 1,40 |
| ITS.61 | 3031 | Saxen | S | 59,7738 | 14,4097 | 178 | 5,612 | 4,85 | 4,53 | 7,02 |
| ITS.62 | 3106 | Långbjörken | S | 59,7680 | 15,9521 | 92 | 9,503 | 9,65 | 3,75 | 1,67 |
| ITS.63 | 3160 | Skattungen | S | 61,1941 | 14,9452 | 200 | 7,553 | 4,25 | 2,81 | 19,50 |
| ITS.64 | 3165 | Bäsingen | S | 60,1606 | 16,3895 | 67 | 6,107 | 8,50 | 4,13 | 12,74 |
| ITS.65 | 3167 | Tisken | S | 60,5309 | 15,6727 | 107 | 7,063 | 8,00 | 5,82 | 63,50 |
| ITS.66 | 3185 | Stora Almsjön | S | 60,8784 | 13,2081 | 463 | 12,545 | 5,55 | 2,54 | 2,04 |
| ITS.67 | 3189 | Dragsjön | S | 60,9927 | 13,5987 | 451 | 10,780 | 7,25 | 1,88 | 1,44 |
| ITS.68 | 3201 | Milsjön | S | 60,1946 | 14,0086 | 291 | 9,118 | 3,75 | 2,37 | 3,29 |
| ITS.69 | 3220 | Stora Korslängen | S | 59,9829 | 15,2304 | 262 | 5,943 | 2,05 | 2,2 | 3,45 |
| ITS.70 | 3384 | Hinsen | S | 60,6821 | 16,0743 | 191 | 5,492 | 3,55 | 3,32 | 11,90 |
| ITS.71 | 3397 | Storsjön | S | 61,6539 | 15,7592 | 355 | 3,768 | 3,15 | 2,9 | 1,89 |
| ITS.72 | 3399 | Grycken | S | 61,5760 | 15,4245 | 240 | 6,299 | 3,30 | 2,24 | 3,17 |
| ITS.73 | 3516 | Holmsjön | S | 62,4103 | 15,3132 | 244 | 5,856 | 3,10 | 3,69 | 50,73 |

| Sample ID | Lake ID | Lake | Country | Latitude | Longitude | Altitude | TOC | TP | Cond | Area.km ² |
|-----------|---------|---------------|---------|----------|-----------|----------|--------|-------|------|----------------------|
| ITS.74 | 3541 | Stornaggen | S | 62,2666 | 15,9710 | 357 | 7,174 | 4,70 | 2,7 | 3,04 |
| ITS.75 | 5000 | Forsjösjön | S | 58,9535 | 16,2635 | 27 | 12,895 | 16,30 | 13,2 | 1,87 |
| ITS.76 | 10000 | Hurdalsjøen | N | 60,3765 | 11,0408 | 176 | 3,711 | 1,60 | 2,77 | 32,81 |
| ITS.77 | 10001 | Harestuvatnet | N | 60,1932 | 10,7121 | 234 | 4,060 | 4,35 | 6,27 | 1,98 |

Basic characteristics of lakes sampled

| | |
|----------------------------|--|
| Lake ID | Project lake identifier |
| Lake | Lake name |
| Country | Country of lake's location (N = Norway, S = Sweden) |
| Latitude | Latitude of sampling site (decimal degrees, WGS84, from airplane GPS) |
| Longitude | Longitude of sampling site (decimal degrees, WGS84, from airplane GPS) |
| Altitude | Lake altitude (m) |
| TOC | Total Organic Carbon (mg/L; NIVA/UiO), Shimadzu TOC analyzer |
| TP | Total Phosphorus (µg/L: below detection limit (= <1) coded as -1; NIVA/UiO), molybdate-blue method (auto-analyzer) on persulfate digested sample |
| Cond | Specific conductivity (mS/m) |
| Area.km² | Lake surface area (km ²) |

Table S2. Information on the fungal phyla and orders presented as % of total reads and total OTUs in n lakes of the whole set of Scandinavian lakes ($n = 77$).

| Phylum | Order | % of total reads | % of total OTUs | Occurrence, n lakes |
|----------------------|-----------------------|------------------|-----------------|--------------------------|
| Ascomycota | | 5.43 | 25.86 | |
| | Capnodiales | 1.27 | 0.86 | 54 |
| | Chaetothyriales | 0.15 | 2.16 | 9 |
| | Dothideales | 0.31 | 0.43 | 19 |
| | Erysiphales | 0.08 | 0.86 | 8 |
| | Helotiales | 0.53 | 3.45 | 35 |
| | Hypocreales | 0.13 | 2.59 | 11 |
| | <i>Incertae sedis</i> | 0.02 | 0.86 | 4 |
| | Lecanorales | 0.07 | 0.86 | 8 |
| | Pezizales | 0.08 | 0.43 | 6 |
| | Pleosporales | 2.22 | 8.62 | 61 |
| | Saccharomycetales | 0.21 | 1.29 | 12 |
| | Taphrinales | 0.25 | 1.72 | 9 |
| | Xylariales | 0.13 | 1.72 | 12 |
| Basidiomycota | | 15.21 | 44.83 | |
| | Agaricales | 1.22 | 9.91 | 35 |
| | Atheliales | 0.02 | 0.43 | 2 |
| | Auriculariales | 0.28 | 1.72 | 11 |
| | Boletales | 0.15 | 2.16 | 11 |
| | Cantharellales | 0.20 | 2.59 | 13 |
| | Cystofilobasidiales | 0.29 | 1.29 | 16 |
| | Entylomatales | 0.03 | 0.43 | 2 |
| | Filobasidiales | 0.07 | 0.43 | 4 |
| | Hymenochaetales | 0.22 | 2.59 | 14 |
| | <i>Incertae sedis</i> | 0.20 | 0.86 | 14 |
| | Leucosporidiales | 0.12 | 1.29 | 9 |

| Phylum | Order | % of total reads | % of total OTUs | Occurrence, n lakes |
|------------------------|-------------------|------------------|-----------------|------------------------|
| | Malasseziales | 0.62 | 0.86 | 31 |
| | Phallales | 0.02 | 0.43 | 2 |
| | Polyporales | 1.02 | 3.88 | 40 |
| | Pucciniales | 0.58 | 1.72 | 23 |
| | Russulales | 0.59 | 3.88 | 24 |
| | Sporidiobolales | 1.21 | 3.02 | 34 |
| | Thelephorales | 0.22 | 0.86 | 18 |
| | Tremellales | 7.52 | 4.31 | 49 |
| | Trichosporonales | 0.55 | 0.86 | 6 |
| | Unassigned | 0.05 | 0.86 | 5 |
| | Ustilaginales | 0.03 | 0.43 | 3 |
| Chytridiomycota | | 63.37 | 20.26 | |
| | Chytridiales | 0.05 | 0.86 | 4 |
| | Rhizophydiales | 1.94 | 1.29 | 9 |
| | Spizellomycetales | 0.06 | 0.43 | 2 |
| | Unassigned | 61.32 | 17.67 | 72 |
| Zygomycota | | 0.19 | 2.16 | |
| | Mortierellales | 0.19 | 2.16 | 9 |
| Unassigned | | 15.81 | 6.90 | 42 |

Table S4. Fitted environmental (TOC, TP, conductivity, all log transformed) and spatial (longitude, latitude, altitude) factors onto NMDS ordination ($k = 2$) for the subset of 30 lakes (with > 150 total reads and $> 10X$ coverage; coverage = total reads/OTU richness per lake). Significant correlation between axes ($P < 0.05$) is indicated by bold font. Number of permutations for assessing significance of factors is 999. Stress = 0.24.

| Parameter | NMDS1 | NMDS2 | R ² | Pr(>r) |
|---------------------|----------|----------|----------------|--------|
| Latitude | -0.28394 | 0.95884 | 0.1486 | 0.117 |
| Longitude | 0.72591 | -0.68778 | 0.495 | 0.001 |
| Altitude | -0.98358 | -0.18047 | 0.099 | 0.234 |
| Conductivity | 0.97807 | -0.20826 | 0.3625 | 0.002 |
| TOC | 0.50257 | -0.86454 | 0.3281 | 0.005 |
| TP | 0.64451 | -0.76459 | 0.4128 | 0.001 |