*The following supplement accompanies the article*

# Interlake variation and environmental controls of denitrification across different geographical scales

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## **Supplement.** Additional information

Table S1. Number of observations and mean (±1 SD) of the data used in correlation and regression analyses on variation in denitrification at the regional (boreal area) and continental (boreal and temperate area) scales. DEN: denitrification; *D*w: denitrification of the nitrate of the water overlying the sediment; *D*n: coupled nitrification-denitrification; [$NO_3^-$], [$O_2$] and *T*: nitrate and oxygen concentrations and temperature of the water overlying the sediment; N load: average, area-specific N load

| Lake | Country | n | Latitude (°N) | Zone | Area (km²) | Mean depth (m) | [$NO_3^-$] (µmol l$^{-1}$) | SD | *T* (°C) | SD | [$O_2$] (µmol l$^{-1}$) | SD | DEN (µmol N m$^{-2}$ d$^{-1}$) | SD | *D*n (µmol N m$^{-2}$ d$^{-1}$) | SD | *D*w (µmol N m$^{-2}$ d$^{-1}$) | SD | N load (µmol N m$^{-2}$ d$^{-1}$) | Source |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lehee | Finland | 2 | 61.17 | Boreal | 1.1 | 1 | 2.2 | 0.6 | 14.7 | 4.1 | 290.6 | 39.8 | 53.8 | 12.7 | 29.1 | 2.4 | 24.7 | 15.1 | | Present study |
| Suolijärvi | Finland | 2 | 61.13 | Boreal | 2.1 | 5 | 15.8 | 2.8 | 10.7 | 1.1 | 185.9 | 174.6 | 215.9 | 0.2 | 64.5 | 25.6 | 151.4 | 25.4 | | Present study |
| Ormajärvi | Finland | 12 | 61.10 | Boreal | 6.5 | 9 | 19.7 | 14.1 | 10.9 | 6.8 | 328.6 | 69.4 | 220.2 | 119.7 | 112.5 | 53.9 | 107.7 | 99.9 | | Rissanen et al. 2011 |
| Pääjärvi | Finland | 2 | 61.07 | Boreal | 13.4 | 15 | 64.3 | 0.5 | 11.2 | 0.8 | 328.1 | 17.7 | 269.2 | 12.3 | 183.6 | 6.0 | 85.6 | 6.3 | | Present study |
| Kirkkojärvi | Finland | 3 | 60.40 | Boreal | 1.6 | 1.1 | 30.0 | 26.1 | 11.1 | 9.9 | 310.4 | 65.7 | 282.6 | 114.6 | 140.2 | 81.2 | 142.4 | 127..3 | | Holmroos et al. 2012 |
| Vallentuna | Sweden | 20 | 59.53 | Boreal | 6.1 | 2.5 | 6.3 | 6.2 | 8.8 | 6.2 | 305.9 | 99.1 | 57.0 | 23.7 | 28.1 | 28.3 | 28.9 | 34.4 | 548.0 | Ahlgren et al. 1994 |
| Norrviken | Sweden | 9 | 59.45 | Boreal | 2.7 | 5.4 | 9.5 | 11.1 | 8.5 | 5.7 | 336.4 | 88.5 | 53.9 | 46.0 | 35.3 | 53.7 | 18.6 | 23.1 | 1878.7 | Ahlgren et al. 1994 |
| Sobygaard | Denmark | 8 | 56.25 | Temperate | 0.4 | 1 | 132.1 | 139.4 | | | | | 1382.4 | 889.9 | | | | | 11135.0 | Risgaard-Petersen et al. 1999 |
| Baldegg | Switzerland | 2 | 47.20 | Temperate | 5.2 | 33 | 130.0 | 0.0 | 5.0 | 0.0 | 290.0 | 14.1 | 3750.0 | 212.1 | 0.0 | 0.0 | 3750.0 | 212.1 | 8199.6 | Mengis et al. 1997 |
| Matita | Romania | 2 | 45.30 | Temperate | 6.4 | 2.5 | 5.7 | 8.0 | 22.5 | 3.5 | 215.0 | 113.1 | 195.0 | 35.4 | | | | | | Friedrich et al. 2003 |
| Uzlina | Romania | 2 | 45.08 | Temperate | 4.7 | 2.8 | 66.8 | 14.0 | 22.5 | 3.5 | 265.0 | 35.4 | 11150.0 | 2474.9 | | | | | | Friedrich et al. 2003 |
| Rosu | Romania | 2 | 45.05 | Temperate | 17.8 | 3.2 | 0.8 | 1.0 | 22.5 | 3.5 | 260.0 | 99.0 | 620.0 | 820.2 | | | | | | Friedrich et al. 2003 |
| Verde | Italy | 2 | 45.06 | Temperate | 0.03 | 5.6 | 80.0 | 103.2 | 9.0 | 5.7 | 200.0 | 240.4 | 1872.0 | 1595.2 | 612.0 | 831.6 | 1260.0 | 763.7 | | Nizzoli et al. 2010 |
| Ca´Stanga | Italy | 2 | 45.05 | Temperate | 0.1 | 9 | 177.5 | 43.1 | 6.0 | 1.4 | 239.5 | 184.6 | 3708.0 | 560.0 | 540.0 | 322.4 | 3168.0 | 882.5 | | Nizzoli et al. 2010 |

Table S2. Mantel's test on relationships between *nirK*-community structure and characteristics of profundal sediment of the boreal study lakes. $[NO_3^-]$, $[NH_4^+]$, $[PO_4^{3-}]$, $[O_2]$, $O_2$ sat., and $T$: concentrations of nitrate, ammonium, phosphate, oxygen, oxygen saturation, and temperature, respectively, of the water overlying the sediment; LOI: sediment organic matter content (% of dry mass); $s[NO_3^-]$, $s[NH_4^+]$, $s[PO_4^{3-}]$: concentrations of nitrate, ammonium and phosphate in sediment porewater (0 to 2 cm surface layer). n = 29 except for analyses of sediment porewater concentrations where n = 7. Significant values in **bold**

| | r | p |
|---|---|---|
| **$[NO_3^-]$** | **0.67** | **0.0001** |
| **$s[NO_3^-]$** | **0.60** | **0.0071** |
| **LOI** | **0.55** | **0.0001** |
| **Porosity** | **0.41** | **0.0001** |
| **$[PO_4^{3-}]$** | **0.31** | **0.0007** |
| $s[PO_4^{3-}]$ | 0.48 | 0.0522 |
| $s[NH_4^+]$ | 0.26 | 0.1189 |
| $[O_2]$ | –0.01 | 0.5037 |
| $O_2$ sat | –0.04 | 0.3814 |
| *T* | –0.04 | 0.3866 |
| $[NH_4^+]$ | –0.11 | 0.0763 |

Table S3. Number of 16S rRNA gene sequences in each OTU (97% sequence similarity cutoff) in each clone library and results of BLASTn search of representative OTU sequences against databases. OJ: Ormajärvi; PJ: Pääjärvi; Acc. No.: accession number; ID%: percent identity of representative OTU sequence to closest BLAST hit; ": same information as in previous row

| OTU | No. of sequences in each OTUs | | | Closest known anammox organism | | | Closest environmental clone | | |
|---|---|---|---|---|---|---|---|---|---|
| | OJ (A) | PJ ($A_a$) | PJ (C) | Species | Acc. No. | ID% | Acc. No. | ID% | Environment |
| 1 | 1 | 0 | 2 | *Candidatus* 'Anammoxoglobus propionicus' | EU478694 | 93 | AB522717 | 99 | lake sediment |
| 2 | 0 | 1 | 0 | *Ca.* 'Brocadia anammoxidans' | AF375994 | 91 | AB522718 | 97 | lake sediment |
| 3 | 13 | 0 | 3 | *Ca.* 'Brocadia fulgida' | EU478693 | 96 | AB602747.1 | 99 | soil |
| 4 | 6 | 0 | 0 | ,, | ,, | 96 | AB602738 | 100 | soil |
| 5 | 3 | 0 | 0 | ,, | ,, | 95 | HM851598 | 99 | river sediment |
| 6 | 1 | 0 | 0 | ,, | ,, | 95 | FN691945 | 98 | soil |
| 7 | 0 | 5 | 0 | ,, | ,, | 94 | AB602748.1 | 98 | soil |
| 8 | 0 | 1 | 3 | ,, | ,, | 94 | AB522734 | 99 | |
| 9 | 2 | 10 | 0 | ,, | ,, | 94 | HQ637487.1 | 100 | soil |
| 10 | 1 | 0 | 0 | ,, | ,, | 94 | AB602645 | 97 | soil |
| 11 | 1 | 0 | 0 | ,, | ,, | 93 | AB602703 | 96 | soil |
| 12 | 0 | 1 | 0 | ,, | ,, | 92 | HQ637487.1 | 97 | soil |
| 13 | 0 | 0 | 1 | ,, | ,, | 92 | AB509334 | 97 | lake sediment |
| 14 | 1 | 0 | 0 | ,, | ,, | 91 | AB602738 | 94 | soil |
| 15 | 1 | 0 | 0 | ,, | ,, | 90 | AB602746 | 93 | soil |
| 16 | 1 | 0 | 0 | ,, | ,, | 89 | AB602639 | 91 | soil |
| 17 | 2 | 4 | 8 | *Ca*. 'Jettenia asiatica' | DQ301513 | 94 | AB522718 | 100 | lake sediment |
| 18 | 0 | 0 | 3 | *Ca.* 'Kuenenia stuttgartiensis' | AF375995 | 94 | FJ490112 | 96 | river sediment |
| 19 | 1 | 0 | 0 | ,, | ,, | 92 | GU084043 | 99 | wetland |

Table S4. Nitrogen retention (N-ret.), mean depth and hydraulic residence (Res.) time of various lakes used in comparison of boreal and temperate lakes (see Fig. 6B). Data were chosen to represent a similar range in hydraulic residence time in both areas

| Lake | Country | Area | Res. time (year) | Mean depth (m) | N–ret. (%) | Source |
|---|---|---|---|---|---|---|
| 1 | Finland | boreal | 0.1 | 1 | 0 | Kuosa et al. 2006 |
| 2 | Finland | boreal | 0.18 | 3.2 | 19 | Knuuttila et al. 1992[a] |
| 3 | Finland | boreal | 0.42 | 5 | 10 | Kuosa et al. 2006 |
| 4 | Finland | boreal | 0.44 | 2.5 | 40 | Knuuttila et al. 1992[a] |
| 5 | Finland | boreal | 0.6 | 3.1 | 48 | Ojanen 1979[a] |
| 6 | Sweden | boreal | 0.85 | 5.4 | 18 | Ahlgren et al. 1994 |
| 7 | Estonia | boreal | 1 | 7 | 14 | Stålnacke et al. 2001[a] |
| 8 | Finland | boreal | 1 | 8 | 43 | Oravainen 2005 |
| 9 | Estonia | boreal | 1 | 2.8 | 53 | Nõges et al. 1998 |
| 10 | Estonia | boreal | 1.3 | 3.6 | 69 | Nõges 2005 |
| 11 | Finland | boreal | 1.5 | | 30 | Kuosa et al. 2006 |
| 12 | Finland | boreal | 1.5 | 2.8 | 60 | Kuosa et al. 2006 |
| 13 | Sweden | boreal | 1.81 | 2.5 | 40 | Ahlgren et al. 1994 |
| 14 | Finland | boreal | 2 | 21 | 25 | Knuuttila et al. 1992[a] |
| 15 | Finland | boreal | 3 | 5.4 | 82 | Ekholm et al. 1997 |
| 16 | Finland | boreal | 3.3 | 15 | 30 | Kuosa et al. 2006 |
| 17 | Denmark | temperate | 0.1 | 2.8 | 53.2 | Andersen 1974 |
| 18 | Netherlands | temperate | 0.12 | 1.5 | 42.6 | Kronvang et al. 2008 |
| 19 | Germany | temperate | 0.13 | 4.9 | 16.6 | Dudel & Kohl 1992[b] |
| 20 | Netherlands | temperate | 0.13 | 1.3 | 51.4 | Kronvang et al. 2008 |
| 21 | Denmark | temperate | 0.15 | 0.9 | 23.7 | Kronvang et al. 2008 |
| 22 | Denmark | temperate | 0.16 | 4.1 | 23.6 | Andersen 1974 |
| 23 | Denmark | temperate | 0.17 | 2 | 49.4 | Kronvang et al. 2008 |
| 24 | Denmark | temperate | 0.21 | 2.6 | 26 | Jeppesen et al. 1998 |
| 25 | Denmark | temperate | 0.23 | 4.6 | 48.1 | Kronvang et al. 2008 |
| 26 | Denmark | temperate | 0.23 | 2.7 | 25.2 | Kronvang et al. 2008 |
| 27 | Canada | temperate | 0.3 | 3.3 | 24 | Dillon & Molot 1990 |
| 28 | Netherlands | temperate | 0.32 | 1.5 | 37.1 | Kronvang et al. 2008 |
| 29 | Canada | temperate | 0.4 | 8.2 | 61 | Leavitt et al. 2006 |
| 30 | Denmark | temperate | 0.46 | 5.6 | 57.7 | Kronvang et al. 2008 |
| 31 | Denmark | temperate | 0.55 | 9.9 | 40.5 | Kronvang et al. 2008 |
| 32 | Denmark | temperate | 0.58 | 3.1 | 49.7 | Kronvang et al. 2008 |
| 33 | Canada | temperate | 0.7 | 9.8 | 50 | Leavitt et al. 2006 |
| 34 | Canada | temperate | 0.7 | 6 | 41 | Leavitt et al. 2006 |
| 35 | Denmark | temperate | 0.77 | 2.9 | 71.4 | Kronvang et al. 2008 |
| 36 | Canada | temperate | 0.9 | 12.2 | 29 | Dillon & Molot 1990 |
| 37 | Canada | temperate | 1 | 6.2 | 46 | Dillon & Molot 1990 |
| 38 | Denmark | temperate | 1.06 | 8.2 | 68.1 | Kronvang et al. 2008 |
| 39 | Canada | temperate | 1.3 | 14.4 | 80 | Leavitt et al. 2006 |
| 40 | Canada | temperate | 1.49 | 9.2 | 36 | Molot & Dillon 1993 |
| 41 | Canada | temperate | 1.5 | 5 | 51 | Molot & Dillon 1993 |
| 42 | Denmark | temperate | 1.75 | 1.9 | 42.6 | Jeppesen et al. 1998 |
| 43 | Denmark | temperate | 1.78 | 6.5 | 57.7 | Kronvang et al. 2008 |
| 44 | Canada | temperate | 1.8 | 8.9 | 40 | Molot & Dillon 1993 |
| 45 | Denmark | temperate | 2.33 | 15 | 60.1 | Kronvang et al. 2008 |
| 46 | Canada | temperate | 2.52 | 14.2 | 40 | Molot & Dillon 1993 |
| 47 | Canada | temperate | 3.16 | 13.3 | 48 | Molot & Dillon 1993 |

[a]As reported in Pietiläinen 2008
[b]As reported in Harrison et al. 2009

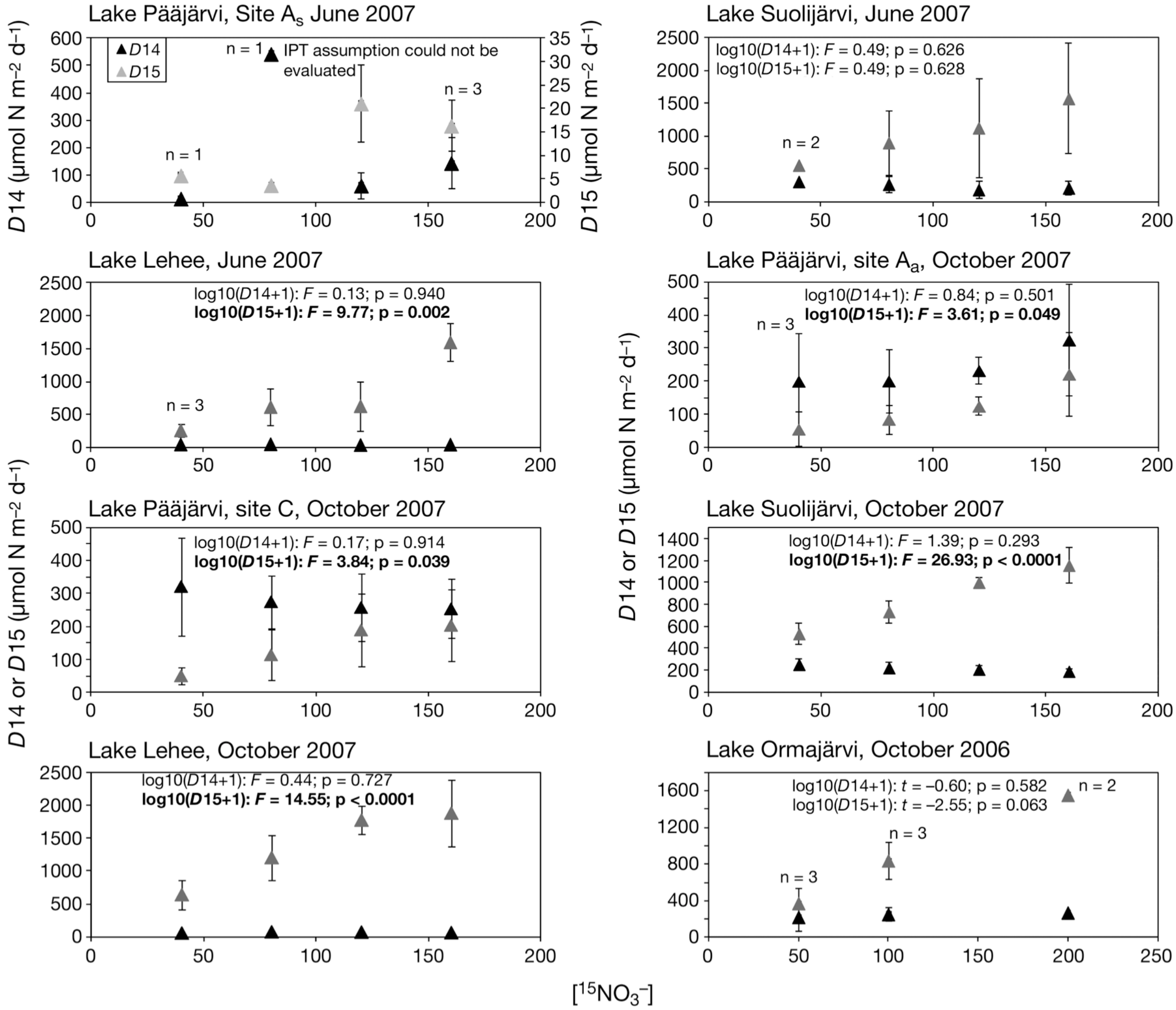


Fig. S1. $^{15}NO_3^-$ concentration tests (mean ± SD, n = 4 unless otherwise indicated). Significant results in ANOVA and *t*-tests ($p < 0.05$) are in **bold**. *D*14: denitrification of the $^{14}NO_3^-$; *D*15: denitrification of the added $^{15}NO_3^-$. Note that tests were conducted using $log_{10}$-transformed variables, but non-transformed data are displayed in the figure

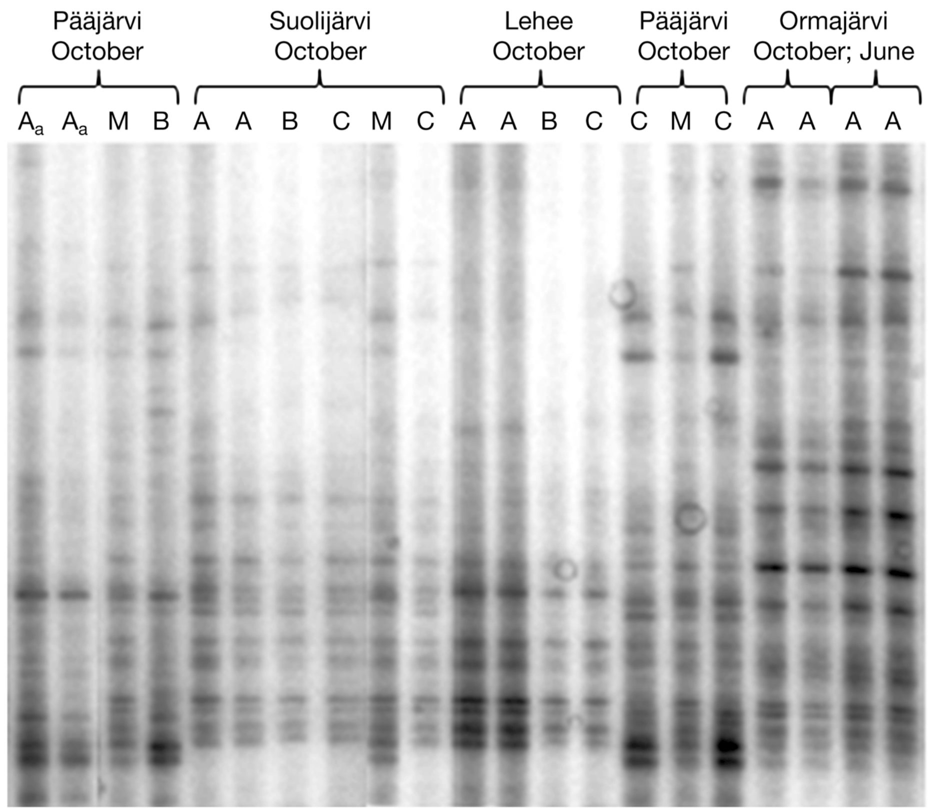


Fig. S2. An example of the results on examination of *nirK* by denaturing-gradient gel electrophoresis (DGGE). A, B and C: study sites (see Fig 1.); M: DGGE standard

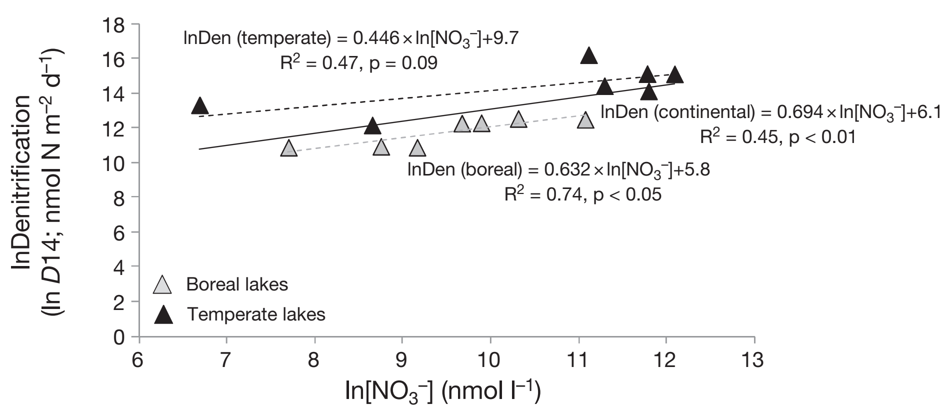


Fig. S3. Relationship between denitrification (Den) and concentration of nitrate in the water overlying the sediment in the combined data set of the 4 boreal study lakes and 10 other European lakes using logarithmically transformed data (same data as in Fig. 5.). Denitrification and nitrate concentrations were converted to nmol N $m^{-2}$ $d^{-1}$ and nmol $l^{-1}$, respectively, toavoid values below 1 in ln-transformation