Supplementary Figure 1. Experimental set up for temperature dependent anammox and denitrification rate measurements. A) Full view on incubation bottle with activated sludge. Pressure adjustment syringe is connected to the bottle via a port. Red arrow points to the oxygen sensor spot which is placed at the inner wall of the bottle. B) Side view on incubation bottle in temperature bath. A magnetic stirrer for gentle mixing of the sludge is placed below the temperature-controlled water bath. C) Top view on incubation bottle in temperature bath.
Supplementary Figure 2. Proof of concept for adequate mixing of headspace between incubation bottle and pressure adjustment syringe. A $^{15}$NO$_2^-$-labelling experiment was performed with activate sludge from the biological tanks at 20°C as described in the materials and methods section, but activated sludge was enriched with 250 µM Na-acetate to ensure high microbial activity. Microbial activities were stopped after 10 min by adding 8 mL ZnCl$_2$ (50% w/v) to 400 mL activated sludge and headspace was sampled 1) without any manual mixing of the headspace in the pressure adjustment syringe and the headspace of the incubation bottle, so that gas only diffuses through the port, 2) after gentle mixing of both headspaces by moving the piston of the pressure adjustment syringe several times a few millimeters up and down, as applied during the experiments, and 3) after full mixing of both headspaces by moving the piston of the pressure adjustments syringe fully up and down a few times. One-way ANOVA (multiple comparison of means) shows no significant difference between $^{29}$N values (p=0.133) and no significant difference between $^{30}$N values (p=0.122) after non, gentle or full mixing. Thus, it can be concluded that a sufficient gas-mixing between the incubation bottle and the pressure adjustment syringe is given.
Supplementary Figure 3. Satellite picture (upper left corner; google maps) and scheme of the nitrification/denitrification tanks of Marselisborg WWTP. Squares with red dots show the bottom aeration zones and letters the sampling sites (A) close to, (B) within, and (C) outside aeration zone (personal communication Flemming Husum from Aarhus Vand).
Supplementary Table 1. TSS (total suspended solids) of activated sludge and DW (dry weight; DW equals TSS) of anammox granules from the DEMON and mainstream of Marselisborg WWTP. For the DEMON, approximately 250 mL sludge was vigorously mixed, a subsample of 20 mL was used for TSS determination of the sludge, and 200 mL were used to pick anammox granules for DW determination. For the mainstream anammox granules were picked from 250 L water (n=29). All samples were heated at 100˚C for 3 days for TSS and DW determination, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Activated sludge (TSS g L⁻¹)</th>
<th>Anammox granules (DW g L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEMON</td>
<td>3.7</td>
<td>0.823</td>
</tr>
<tr>
<td>Biological tank</td>
<td>4.0*</td>
<td>0.00018</td>
</tr>
</tbody>
</table>

*average number taken from Marselisborg WWTP