Supporting Text

Methods

PCR Amplification and Sequencing of *amoA*. Each reaction mixture contained 12.5 μl of MasterAmp PCR premix F (Epicentre Technologies, Madison, WI), 0.5 μM of each primer (Qiagen, Valencia, CA), 2.5 units of Ampli*Taq* DNA polymerase LD (Applied Biosystems), and 0.25 μl of template DNA. Amplification was performed in a total volume of 25 μl in 0.2-ml reaction tubes with a DNA Engine thermal cycler (MJ Research, Cambridge, MA). All PCR was performed in triplicate. PCR products were cloned by using the TOPO TA Cloning Kit (Invitrogen), following the manufacturer's protocol.

Quantitative PCR. The first-round PCR was performed in the same volumes, tubes, and PCR cycler as the first-round PCR for terminal restriction fragment length polymorphism (T-RFLP) analysis. The second-round PCR was performed in 20-μl volumes in Hard-Shell ThinWall 96-Well Microplates (MJ Research) by using Ultra Clear Caps (MJ Research) and the DNA Engine Option 2 System (MJ Research). The melting curve analysis performed after each PCR showed a clear single peak at 90°C corresponding to the 491-bp *amoA* fragment. The size of this fragment was verified by means of agarose gel electrophoresis. Anomalies (due to contamination, primer dimer, false priming, etc.) were not evident in the negative first-derivative plots, as indicated by the lack of additional peaks. However, to avoid any possible primer dimer interference, the temperature at which the fluorescence was read during each cycle was adjusted to 86°C, a temperature above the melting point of the primer dimers. The amount of initial template DNA was estimated by determining the threshold cycle (Ct), the number of PCR cycles required for the fluorescence to exceed a threshold value higher than the background fluorescence. We assumed a threshold value of 25 times the background fluorescence (which we defined as the mean fluorescence values of the first three to eight PCR cycles).

PCR Efficiency Controls. A defined amount of plasmid DNA (pUC-plasmid, Invitrogen) was mixed with the same volume of soil DNA that was used for the regular real-time PCR. An ≈200-bp-long region of the plasmid was then amplified by using the primers M13F and M13R under the same PCR conditions described for the amplification of the *amoA* target (Table 1). Because the amount of the control gene was constant in all samples, any difference in Ct values was a direct measure of the different amplification efficiencies of the soil samples. We corrected for differences in PCR efficiencies by subtracting the Ct of the spiked control gene from the Ct of the target gene for each soil sample, as described by Meijerinck *et al.* (1). All control PCRs were performed in triplicate.

1. Meijerink, J., Mandigers, C., van de Locht, L., Tonnissen, E., Goodsaid, F. & Raemaekers, J. (2001) *J. Mol. Diagn.* **3,** 55-61.