Fig. a. Phylogenetic relationships among bacterial amoA sequences retrieved from the soil. GenBank accession numbers of the reference sequences are indicated. Sequences with names starting with CK were obtained from the control soil samples. The in silico T-RF sizes are given following the sequence names. Clones with > 95% sequences similarity were considered to be the same OTU. A number in parentheses represents the total number of clones in each OTU. The scale bar represents 5%
sequence divergence. The *amoA* sequences of the reference ammonia-oxidation bacteria are indicated by each accession number. Values indicate the percentage of 100 replicate trees supporting the branching order. *Nitrosomonas* sp. Nm 58 (accession no. AY123820) was used as outgroup to root the phylogram.

The *in silico* digestion of *amoA* sequences cut by MspI revealed similar T-RFs to those detected in T-RFLP profiles. Fifteen sequences that grouped within the *Nitrosospira* cluster 3b were characterized by the 62 bp T-RFs. All *Nitrosospira* cluster 3c-like sequences showed T-RFs of 257 bp or 236 bp and only one 60 bp T-RF characterized the clone within *Nitrosospira* cluster 3a.
Fig. b. Phylogenetic relationships among bacterial nirK sequences retrieved from the soil. GenBank accession numbers of the reference sequences are indicated. Sequences with names starting with CK were obtained from the control soil samples. The *in silico* T-RF sizes are given following the sequence names. Clones with > 95%
sequence similarity were considered to be the same OTU. A number in parentheses represents the total number of clones in each OTU. The scale bar represents 5% sequence divergence. The nirK sequences of the reference denitrifiers are indicated by each accession number. Values indicate the percentage of 100 replicate trees supporting the branching order. The aniA gene from *Neisseria gonorrhoeae* (accession no. M97926) encodes a putative nitrite reductase with homologies to copper containing nitrite reductase from denitrifying bacteria (Mellies et al., 1997) and was used as an outgroup for phylogenetic distance analysis of the nirK sequences.

Sequences from clones were analyzed *in silico* with respect to HaeIII restriction sites and found to correspond to T-RFs of 62, 71, 157, 174, and 191 bp, respectively, as detected in T-RFLP profiles, also considering a possible deviation of up to 2 bp in the sizes of the T-RFs due to the nature of gel separation. *In silico* analyses of 56 sequences indicated that 96% of T-RFs could be assigned to different denitrifier lineages. The 174 bp T-RF was closely related to *Sinorhizobium*. Other T-RFs (62 bp, 71 bp, 157 bp and 191 bp) were associated with more than one lineage, the 62 bp to mainly Bradyrhizobiaceae and occasionally Rhizobiaceae, the 71 bp T-RF to predominantly Bradyrhizobiaceae and to uncultured bacterium clones also, 157 bp T-RF to Rhizobiaceae mostly, and also to Bradyrhizobiaceae and Rhizobiales occasionally, and 191 bp to mainly Bradyrhizobiaceae and occasionally Rhizobiaceae.
Fig. c. Phylogenetic relationships among bacterial nirS sequences retrieved from the soil. GenBank accession numbers of the reference sequences are indicated. Sequences with names starting with CK were obtained from the control soil samples. The in silico T-RF sizes are given following the sequence names. Clones with > 95% sequence similarity were considered to be the same OTU. A number in parentheses
represent the total number of clones in each OTU. The scale bar represents 5% sequence divergence. The nirS sequences of the reference denitrifiers are indicated by each accession number. Values indicate the percentage of 100 replicate trees supporting the branching order. *Pseudomonas stutzeri* (accession no. AY957388) was used as an outgroup to root the phylogram.

The nirS sequences from 41 clones were analyzed *in silico* with respect to HhaI restriction sites, with all of them corresponding to T-RFs of 37 bp, 70 bp, 113 bp and 240 bp. Due to the nature of gel separation, we considered a possible deviation of up to 2 bp in the sizes of the T-RFs.