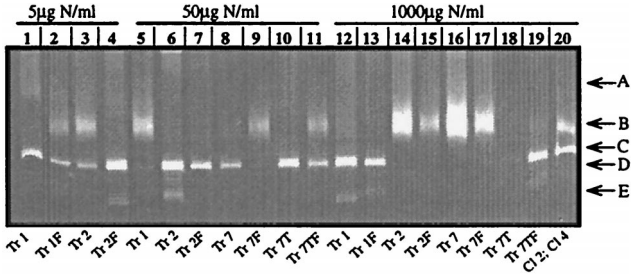

FIG. 5. DGGE analysis following PCR amplification of 16S rDNA of β-subgroup ammonia-oxidizing bacteria from the MPN dilution tubes of LTER soils incubated with 5, 50, and 1,000 μg of NH_4^+-N ml^{-1}. DNA was extracted from the tube with the highest dilution that showed growth in all eight replicates. Lanes 1 to 4, treatments 1, 1F, 2, and 2F, respectively, with 5 μg of NH_4^+-N ml^{-1}; lanes 5 to 11, treatments 1, 2, 2F, 7, 7F, 7T, and 7TF, respectively, with 50 μg of NH_4^+-N ml^{-1}; lanes 12 to 19, treatments 1, 1F, 2, 2F, 7, 7F, 7T, and 7TF, respectively, with 1,000 μg of NH_4^+-N ml^{-1}; lane 20, control for clusters (Cl) 2 (pH4.2A/27) and 4 (pH7B/C3). Band migration distances are noted as A to E and refer to the bands excised for sequence analysis in Fig. 6 and 7. See the legend to Fig. 1 for explanations of designations.

ments and might have resulted from difficulties in removing sufficient material from the wells of microtiter plates, particularly where evaporation was significant. Although no sequence representative of the *Nitrosomonas* clade was detected in the DNA extracted directly from soil samples, banding patterns typical of *Nitrosomonas* were detected in DGGE gels of DNA amplified from the MPN samples after incubation for 1 month. Representative DGGE banding profiles from MPN samples of cultivated and successional soils with 5, 50, and 1,000 μg of NH_4^+-N ml^{-1} are illustrated in Fig. 5. In many samples, banding patterns were similar to those obtained from DNA extracted directly from the soil, but in several samples, a band typical of *Nitrosomonas* was observed (for example, Fig. 5, lanes 2, 3, 5, 9, and 11). This result was particularly evident for samples from cultures obtained with medium containing 1,000 μg of NH_4^+-N ml^{-1}, where a *Nitrosomonas* band frequently appeared to the exclusion of the *Nitrosospira* bands (Fig. 5, lanes 14, 15, 16, and 17).

Sequence analysis. The presence in soil and MPN cultures of particular clusters of β-proteobacterial ammonia-oxidizing bacteria was confirmed by sequencing of bands excised randomly from DGGE gels. Phylogenetic analysis (Fig. 6 and 7)

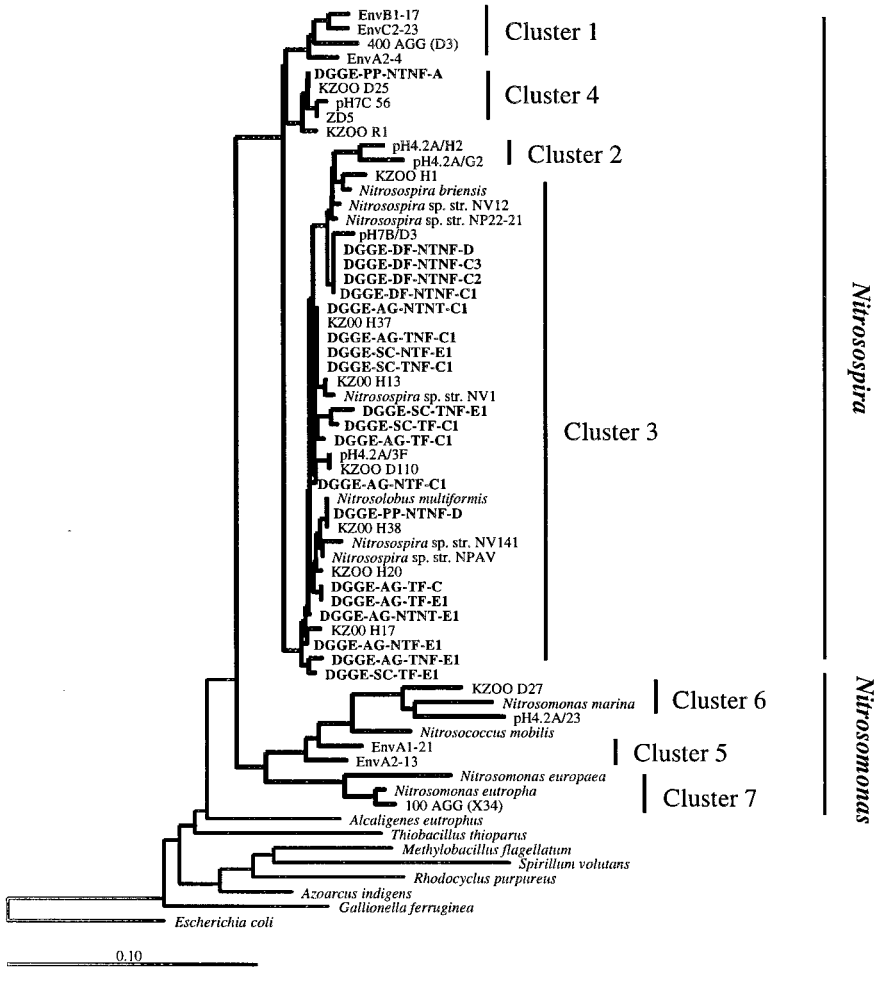

FIG. 6. Neighbor-joining tree showing the relationship of the sequences obtained from bands excised from DGGE gels after PCR amplification of β-subgroup ammonia-oxidizing bacteria from DNA extracted directly from LTER soils. The tree was based on an analysis of 294 bases of aligned 16S rDNA sequences. Bands excised from the gels (shown in bold) have the nomenclature DGGE, to distinguish them from clone sequences and pure-culture sequences, followed by the treatments AG (cultivated), SC (successional grassland), DF (deciduous forest), and PP (*Populus* trees). The treatment variables—tillage (T), no tillage (NT), fertilization (F), and no fertilization (NF)—are followed by the migration distances of the bands (A to E) (see the legends to Fig. 3 and 5). Scale bar, 0.1 substitution per nucleotide.

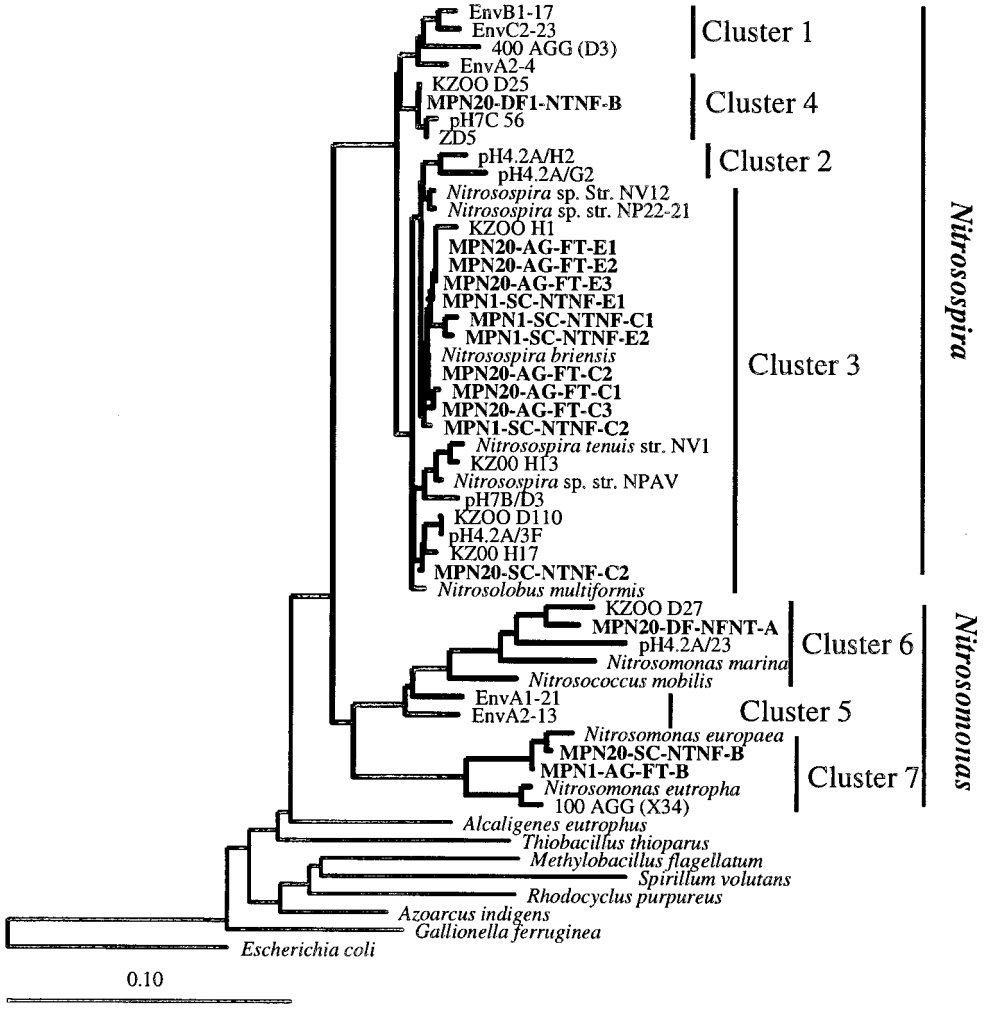

FIG. 7. Neighbor-joining tree showing the relationship of bands excised from DGGE gels after PCR amplification of DNA extracted from MPN dilution tubes of LTER soils incubated with 5, 50, and 1,000 μg of NH_4^+-N ml^{-1}. Sequences excised from the gel (shown in bold) have the nomenclature MPN1 or MPN20 to indicate incubation with 50 or 1,000 μg of NH_4^+-N ml^{-1}, respectively, followed by the treatments DF (deciduous forest), AG (cultivated), SC (successional grassland), and PP (*Populus* trees). The treatment variables—tillage (T), no tillage (NT), fertilization (F), and no fertilization (NF)—are followed by the migration distances of the bands (A to E) (see the legends to Fig. 3 and 5). The scale bar is as described in the legend to Fig. 6.

showed that all of the bands fell within the known β-proteobacterial ammonia oxidizer groupings described by Stephen et al. (40). All bands sequenced that were representatives of the *Nitrosospira* grouping belonged to cluster 3. However, bands excised from unusual banding profiles obtained from MPN cultures of samples from the NDF and from poplar plots (treatment 5) were representative of cluster 4 *Nitrosospira.* The *Nitrosomonas* bands detected in the MPN samples were closely related to *N. europaea*. One sequence from the MPN samples was found to lie within cluster 6 and was closely related to another sequence, KZOO_D27, that was also isolated from this location (5). Within the cluster 3 grouping, sequences from the NDF samples clustered together, suggesting that there might be a treatment effect within cluster 3. Some sequences from the slowly (band C) and quickly (band E) migrating bands were different by only 1 bp over the 290 bp used for phylogenetic analysis; however, despite this fact, they consistently migrated at different rates in gels. This mismatch was in the middle of the sequence, but single mismatches within the primer region due to an ambiguous base led to closely migrating bands (19).

DISCUSSION

Abundance estimates. This study used conventional and molecular techniques to assess the relationship among the abundance, activity, and diversity of ammonia oxidizer populations in soils. The communities reflected treatments ranging from intensive cultivation to NDF. Estimated concentrations of ammonia-oxidizing bacteria were dependent on the enumeration method and protocol. With the exception of the poplar plot, the use of higher concentrations of ammonia in the growth media significantly reduced MPN counts. Similar results have been reported by other workers (3, 41) and may result from growth inhibition of the ammonia oxidizers at high ammonia concentrations (41, 42). Ammonia oxidizer cell concentrations obtained by cPCR were 10- to 1,000-fold higher than MPN counts at these sites, with the exception of the poplar soil. The anomalous results found for this treatment may have been due to differences in the cover crop and potential consequent changes in the activities of different groups of ammonia oxidizers in this soil. DeGrange and Bardin (9) also found that the

numbers of bacteria calculated by MPN-PCR counts were 100 times higher than those calculated by traditional MPN counts in a sandy calcareous soil, whereas the difference was only 10-fold in a sandy loam soil.

Detection limits for cPCR methods were observed to be between 10 and 1,000 times lower than those for standard dilution plating methods when a genetically modified strain of the fungus *Trichoderma virens* in soil was investigated (2). The differences might also reflect limitations of laboratory growth media and incubation conditions, which do not support the growth of all culturable organisms within natural populations and which will not detect nonculturable cells. Populations with lag periods longer than the incubation period also will not be detected, and Matulewich et al. (23) found increasing MPN counts of nitrifying bacteria even after incubation for 90 days. Belser and Schmidt (3) showed that the use of different media for MPN enumeration of ammonia oxidizers produced different results in an actively nitrifying soil. They also found dominance by *Nitrosomonas* in media inoculated with lower sample dilutions and by *Nitrosospira* at higher dilutions. In our study, comparison of 16S rDNA partial sequences amplified from DNA extracted directly from the soil and from positive MPN cultures indicated a similar shift in composition. Samples from the MPN cultures were dominated by sequences representative of *Nitrosomonas*, which were not detected in soil DNA extracts, while *Nitrosospira*-like sequences, which dominated in soil DNA extracts, were less frequent in MPN cultures and sometimes were not detected. Selection for *Nitrosomonas* was greatest in MPN counts when 1,000 μg of NH_4^+-N ml^{-1} was used. Hiorns et al. (14) have detected *Nitrosomonas* DNA in lake water and sediment enrichments but not in extracted DNA, supporting the belief that *Nitrosomonas*-like organisms are better adapted to growth on laboratory media (3).

Compositional differences in ammonia oxidizers. Despite the significant differences in potential nitrification rates among these communities, ammonia oxidizers were found to constitute a relatively small proportion of the total bacterial population detected by microscopic DTAF staining. MPN estimates were 6 to 8 orders of magnitude lower than total cell counts. cPCR may provide a more accurate estimate of total cell counts; in this study, cPCR indicated that β-proteobacterial ammonia oxidizers constituted a maximum of 0.01% of the total population. This low relative abundance in soil may explain the lack of detection of ammonia oxidizer sequences in clone libraries generated by amplification of 16S rDNA using eubacterial primers (17, 21, 25, 28). Borneman et al. (4) found that the majority of the β-proteobacterial clones from a Wisconsin soil showed 80% homology to the ammonia oxidizers. Our data indicate that the characterization of several thousand eubacterial clones would be necessary for the detection of ammonia oxidizers, even in agricultural soils, and that detection by DGGE analysis of eubacterial PCR products would be unlikely.

Potential nitrification rates were higher in cultivated soils than in native soils and successional grassland soils. This result may have been due to increased aeration of these soils through repeated crop regimens. The types of plant community and N fertilization dictate the amount of available ammonia for oxidation by microbes, as was particularly evident in the poplar plots. Soils that were not tilled (treatment 2) had significantly higher nitrification rates than their nontilled equivalents for both fertilized and nonfertilized plots. Soils under no-till practice maintain pore structure and continuity, leading to significantly greater hydraulic conductivity and infiltration rates than are found in conventionally tilled soils (1). This information might mean that ammonia oxidizer communities in nontilled soils would be more stable and therefore more active than the communities in tilled soils. Treatment effects were not detectable by MPN counts, but cPCR data indicated that fertilization led to larger populations. This result might reflect the ability of molecular methods to detect nonculturable organisms in environmental samples. There was no correlation between observed nitrification rates and the numbers of ammonia oxidizers present, calculated by either traditional MPN counts or cPCR.

Although nitrification rates and ammonia oxidizer cell concentrations varied with different treatment regimens, there were no detectable differences in the compositions of the ammonia oxidizer communities, as determined by DGGE analysis of 16S rDNA partial sequences obtained by PCR amplification of extracted DNA using primers specific for the β-proteobacterial ammonia oxidizers. Soils from all sites were dominated by members of *Nitrosospira* cluster 3, which are commonly found in soil (5, 40) and which contain the majority of cultured representatives of the genus *Nitrosospira*. Bruns et al. (5) did not detect *Nitrosospira* cluster 3 in native and unfertilized soils, but we found no effects of fertilization or cultivation on community structure, and *Nitrosospira* cluster 3 dominated in all soils sampled. Sequence analysis of DGGE bands indicated that for different soils, there was a clustering of sequences within cluster 3. This result was particularly evident for deciduous forest soils, although the conclusions drawn must be considered tentative given the small number of sequences analyzed. The stability of other components of the microbial community in these soils has been reported by Buckley et al. (6), who found no differences in *Crenarchaeota* sequences in cultivated and native soils. However, significant differences were seen in a comparison of two Norwegian agricultural soils for total bacterial diversity (29).

Relating structure and function. There are several explanations for the lack of correlation between β-proteobacterial ammonia oxidizer population structure and nitrification rates. The treatments imposed, i.e., tillage and fertilizer, may not drive ammonia oxidizer community structure, which may be more dependent on soil properties, which were initially the same for all treatments. The already established populations survived in systems that lowered the available NH_4^+ substrate levels, and substrate additions would be required to bring in new populations. On a phylogenetic level, it has been suggested that two sequences showing up to a 0.3% difference in sequence homology in the 16S rDNA gene could represent two species with different ecological functions (43). Pankhurst et al. (30) suggested that there does not need to be great taxonomic diversity for there to be functional diversity in soils. In this study, differences seen in the sequences of cluster 3 may mean that, although the organisms are very closely related phylogenetically, they are in fact physiologically different, leading to the differences in the nitrification rates observed between treatments.

The AMO primers are not completely specific for β-proteobacterial ammonia oxidizers but, in combination with CTO primers, amplify all known sequences representative of this group. Although primer bias cannot be dismissed, similar findings have been reported with either set of primers for amplification of 16S rDNA sequences from the same soils and marine sediments (25, 39, 40). The possibility that ammonia oxidizers in natural communities have sequences that are not amplified by these primers cannot be excluded.

This study has demonstrated that the structures of β-proteobacterial ammonia oxidizer populations were quite similar in soils collected from a wide range of communities under different soil cultivation conditions, which resulted in significant changes in potential rates of nitrification and in the sizes of ammonia oxidizer populations. Community structure was

assessed at the level of precision provided by analysis of clusters characterized by 16S rDNA sequences and indicated dominance by *Nitrosospira* cluster 3. Further studies are required to determine whether subtle changes occur within this cluster or whether stability under a variety of environmental conditions is due to physiological and functional diversity within the populations.

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