Tillage and Fertilization Influences on Autotrophic Nitrifiers in Agricultural Soil

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INTRODUCTION

In aerobic environments, autotrophic nitrification ($NH_3 \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^-$) occurs in two steps: ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) oxidize NH_3 to NO_2^-; nitrite oxidizing bacteria (NOB) oxidize NO_2^- to NO_3^-. The activity and composition of nitrifiers are dynamic and influenced directly or indirectly by changing soil characteristics, such as available ammonia, soil pH, temperature, etc. Even though AOA appear to be numerically dominant ammonia oxidizers in soil, AOB are probable the functionally dominant group for ammonia oxidation in agricultural soil. Nitrification rate may be linked to community diversity, not just population density, because not all nitrifying populations make an equal contribution.

Generally, nitrogen fertilization stimulates nitrification by providing substrate, but there is no agreement about the influence of tillage, which affects many soil physical and chemical properties. In this study we utilized a stable agricultural system (> 40 years, continuous maize monoculture soil) to investigate how fertilizer and tillage influenced population and activity of autotrophic nitrifiers. Our objectives, as part of a larger community ecology study, were to determine if fertilization and tillage changed the population and community structure of nitrifier groups and the nitrification rate. We hypothesized that: (1) nitrifier numbers and potential nitrification rates increase with increasing nitrogen fertilizer; (2) there is greater nitrifier population in plow-tillage soil; (3) with increasing N fertilizer, the ratio of AOB to NOB decreases, and compared to plow tillage, no-till soil has a greater ratio of AOB to NOB; (4) the diversity of AOA will be lower in fertilized soils relative to unfertilized soils and in no-till treatments relative to plow-tillage treatments.

SAMPLE SITE & METHODS

The study site is at the University of Kentucky Agriculture Experiment Station, near Lexington KY (N 38° 07'24", W 84° 29'50"). The site (44.0 m by 48.8 m) is a long-term (> 40 years) field study evaluating tillage management on continuous corn (*Zea mays L.*). The soil is a deep and well-drained Bluegrass Maury silt loam (mesic Typic Paleudalf) with 0-4% slope, which formed in the residuum of phosphatic limestone. N fertilization (NH_4NO_3, broadcast) ranges from 0 to 336 kg ha^{-1} and tillage is either no-tillage (NT) or plow tillage (PT). N fertilization treatments are randomly-distributed among paired tillage plots (n=4) (Fig. 1).

We used Most Probable Number (MPN) analysis to evaluate the number of ammonia-oxidizing (AOB) and nitrite-oxidizing bacteria (NOB) and performed ammonia-oxidation assays to determine potential nitrification rates (PNR).

To evaluate the community structure of AOA, composite soil samples were collected from the top 15 cm in each plot. Total DNA was extracted with MACHEREY-NAGEL NuceloSpin® soil genomic DNA isolation kits (Düren, Germany). Archaeal *amoA* genes were amplified with primers Amo19F (forward, 5'-ATG GTC TGG CTW AGA CG) and Amo634R (reverse, 5'-TCC CAC TTW GAC CAR GCG GCC ATC CA), and a GC clamp (5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G) was attached to forward primer. Amplicons within the PCR products were separated by Denaturing Gradient Gel Electrophoresis (DGGE).

NT 168	PT 168	PT 336	NT 336	NT 168	PT 168	NT 336	PT 336
NT 0	PT 0	PT 84	NT 84	NT 0	PT 0	NT 84	PT 84
NT 336	PT 336	PT 168	NT 168	NT 84	PT 84	NT 0	PT 0
NT 84	PT 84	PT 0	NT 0	NT 336	PT 336	NT 168	PT 168
Block 1		Block 2		Block 3		Block 4	

Figure 1. Experiment design. NT = no till, PT = plow tillage; Numbers refer to fertilization rate in kg ha^{-1}. The 84 kg ha^{-1} rate was not utilized for this study.

RESULTS – MPN

Nitrogen fertilization had a significant effect on the MPN of AOB ($p<0.01$) and NOB ($p<0.01$); more fertilized soils had higher nitrifier MPN (Fig.2).

Tillage treatment had a significant influence on the MPN of NOB ($p<0.01$), but not on the MPN of AOB ($p=0.20$).

The ratio of AOB to NOB MPN decreased significantly when N fertilizer was added in both PT ($p<0.01$) and NT ($p=0.02$) soils (Fig. 4). There were more AOB than NOB in unfertilized treatments (AOB/NOB equals 3.7 in plow tillage and 10.8 in no-till soils), whereas there were fewer AOB than NOB after N fertilization (AOB/NOB < 1).

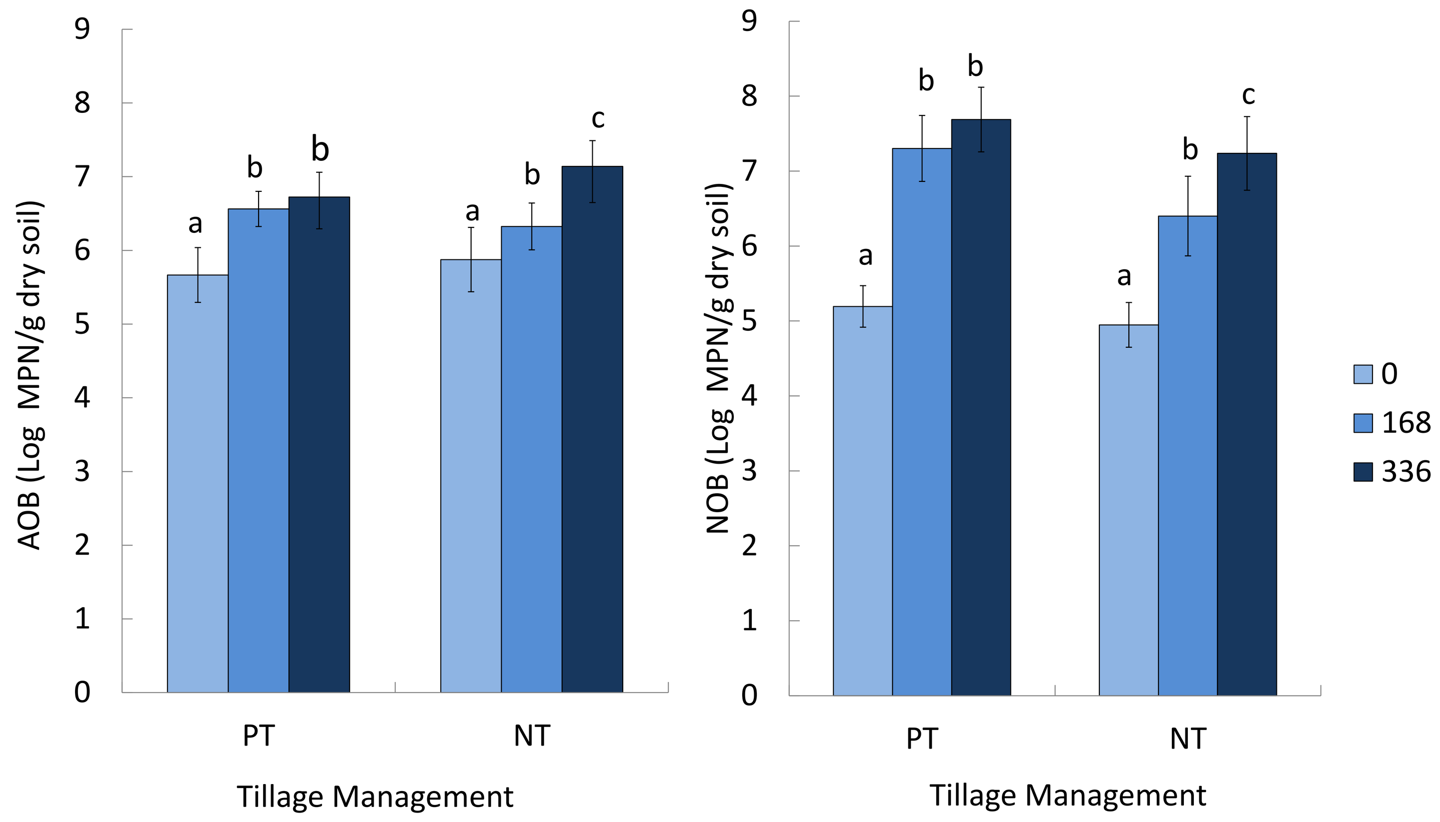

Figure 2. MPN of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) in soil from different N fertilization treatments. PT and NT represent plow tillage and no-till; 0, 168, and 336 represent the fertilization rate (kg N ha^{-1}). Standard deviation is shown by error bars. Columns with the same letter are not significantly different (*p > 0.10*).

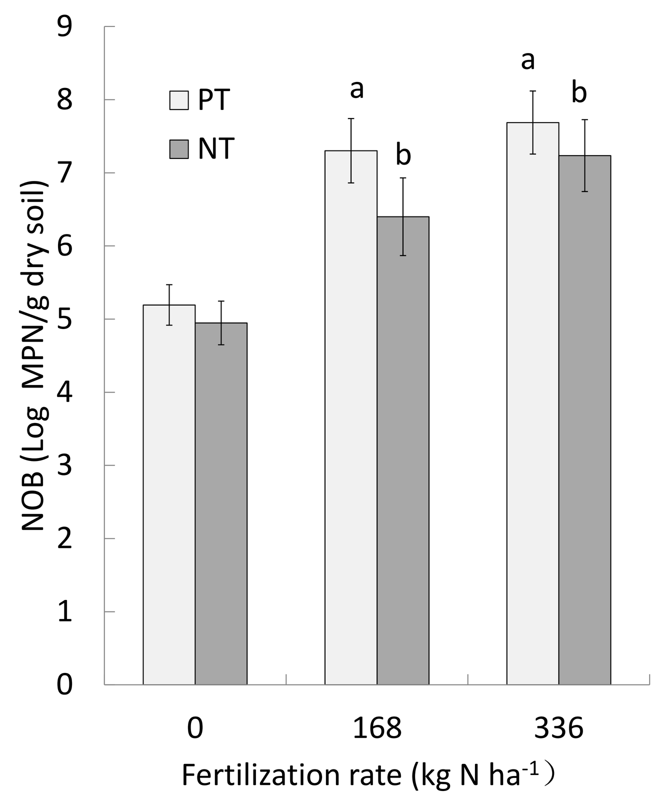

Figure 3. MPN of nitrite-oxidizing bacteria (NOB) in soil of different tillage treatment. PT and NT represent plow tillage and no-till. Standard deviation is shown by error bars. Columns with the same letter are not significantly different (*p>0.10*).

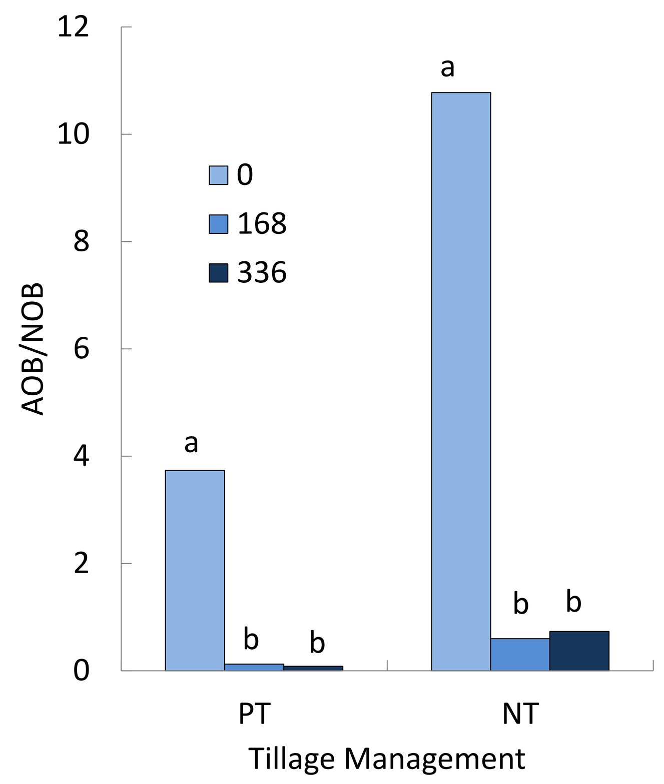

Figure 4. Ratio of AOB to NOB MPN in soil samples of different N fertilization treatment. PT and NT represent plow tillage and no-till; 0, 168, and 336 represent the fertilization rate (kg N ha^{-1}). Columns with the same letter are not significantly different (*p>0.10*).

RESULTS – PNR

Nitrogen fertilization had a significant effect on the PNR ($p<0.01$) in plow tillage and no-till soils ($p<0.01$); more fertilized soils had higher PNR (Fig. 5). The greatest PNR differences were between unfertilized and fertilized treatments.

Tillage treatment had a significant influence on the PNR ($p=0.01$), but only in the highest N fertilizer treatments (336 kg N ha^{-1}) was the PNR significantly higher in no-till soils than in plow tillage soils ($p=0.02$) (Fig. 6).

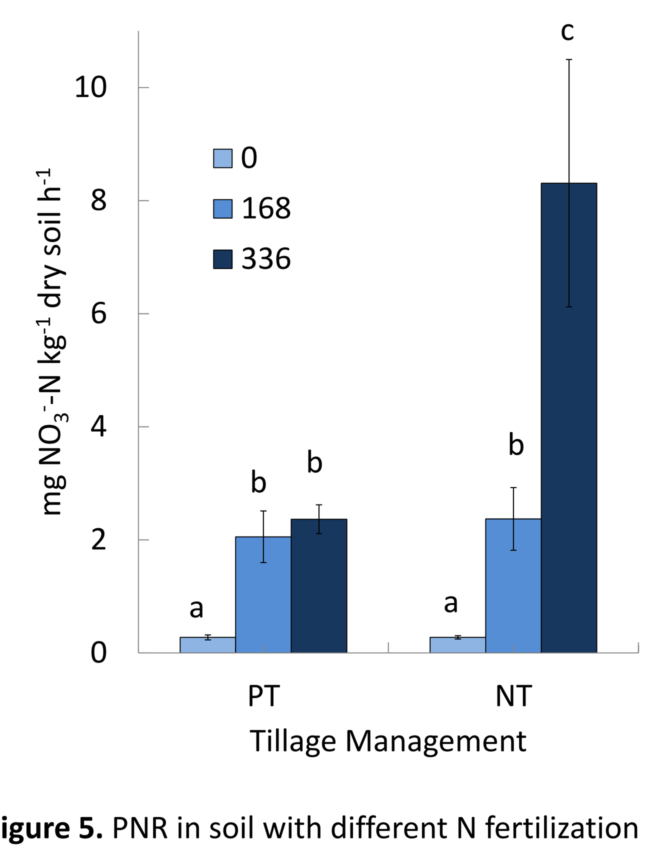

Figure 5. PNR in soil with different N fertilization treatments. PT and NT represent plow tillage and no-till, respectively; 0, 168, and 336 represent the fertilization rate (kg N ha^{-1}). Standard error is shown by error bars. Columns with the same letter are not significantly different within the same tillage (*p>0.10*).

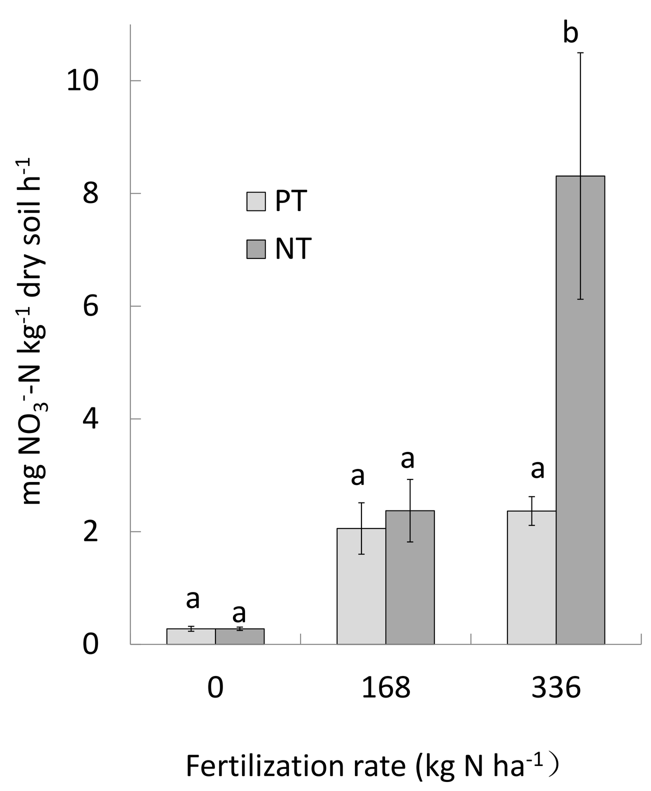

Figure 6. PNR in soil of different tillage treatments. PT and NT represent plow tillage and no-till, respectively. Standard error is shown by error bars. Columns with the same letter are not significantly different within the same fertilization rate (*p>0.10*).

RESULTS – DGGE

Each soil treatment had a distinct AOA-DGGE band pattern implying a unique community structure. Generally, fertilized soils had more bands than unfertilized soils. Between the two fertilization levels, there were more bands in 168 kg ha^{-1} treatments than 336 kg ha^{-1} treatments. Samples from summer (June) had more bands than samples from winter (December). For winter samples, there were more bands in no-till than plow tillage (Fig.7 and Fig.8).

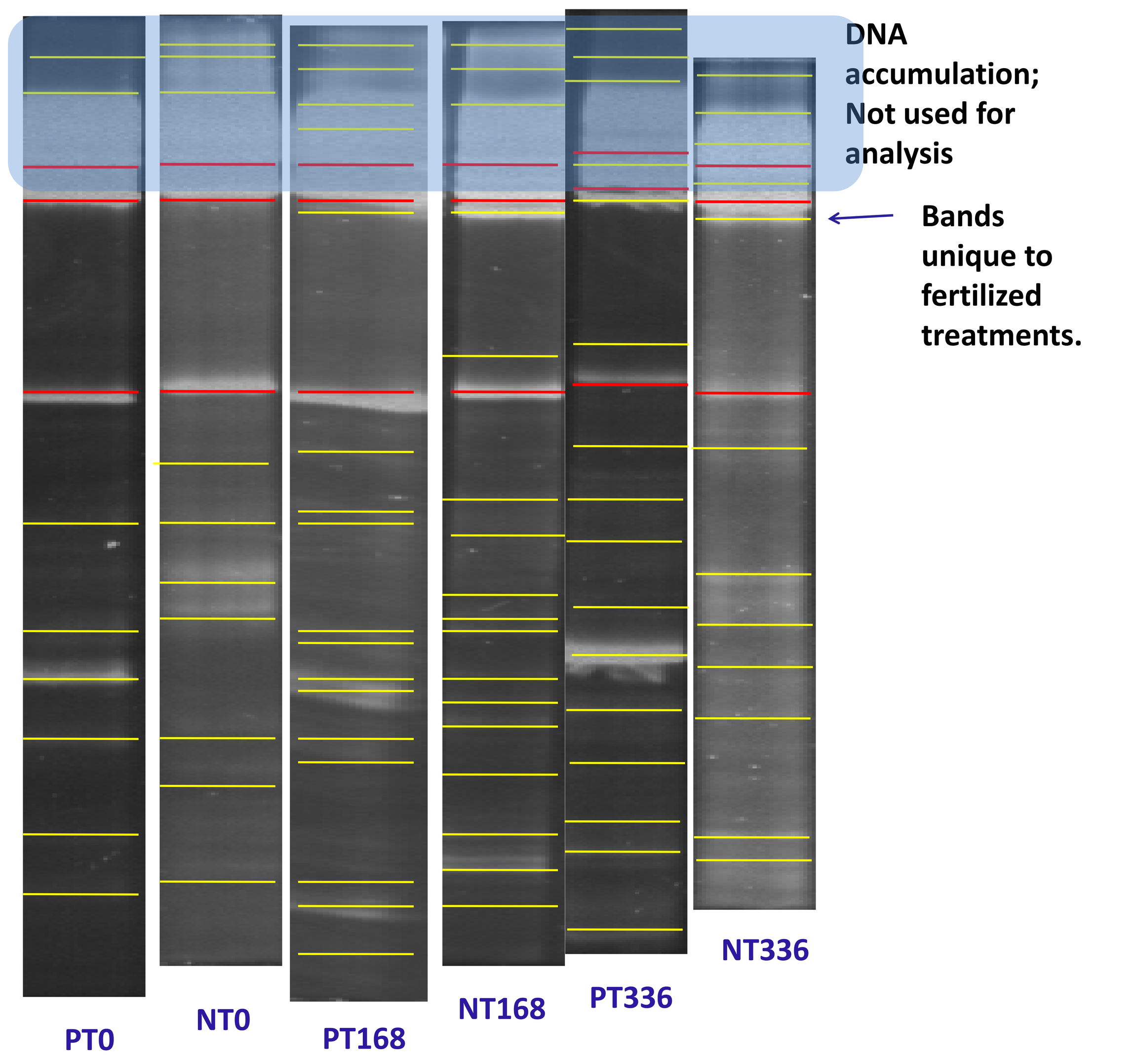

Figure 7. Comparison of DGGE band patterns in different treatments of summer samples. PT and NT represent plow tillage and no-till, respectively. 0, 168, and 336 represent the fertilization rate (kg N ha^{-1}). Lines approximate identified bands.

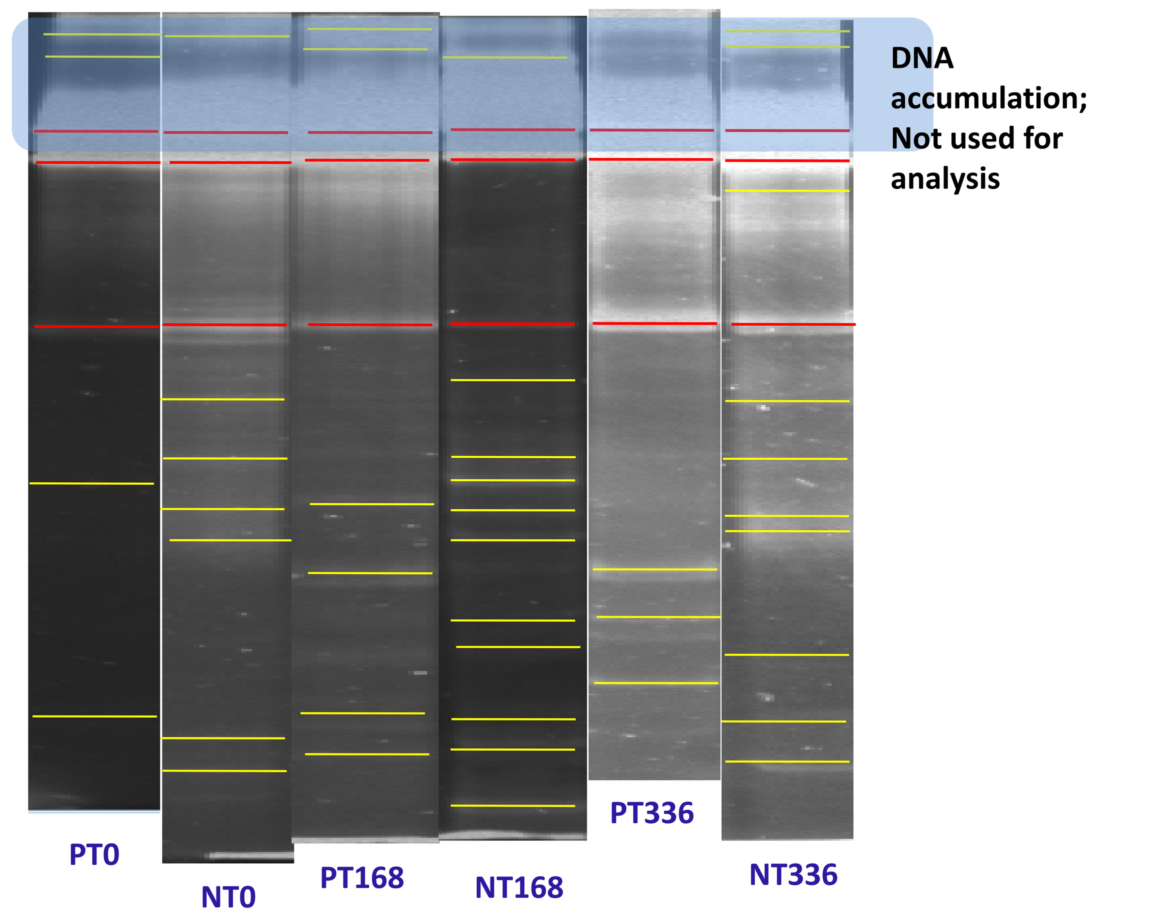

Figure 8. Comparison of DGGE band patterns in different treatments of winter samples. PT and NT represent plow tillage and no-till, respectively. 0, 168, and 336 represent the fertilization rate (kg N ha^{-1}). Lines approximate identified bands.

CONCLUSIONS

Both fertilization and tillage influenced the population and activity of AOB and NOB, and the community structure of AOA. Compared to fertilization, tillage had a minor influence on nitrifier population and nitrification rates. Nitrogen input could be a key stimulus for nitrifier abundance and nitrification rate in long-term arable soils, which indicated that available ammonia limited both nitrification and nitrifier population. The decrease in the ratio of AOB to NOB with increasing fertilizer means NOB benefited more from fertilization than AOB. Nitrogen fertilization and different seasons resulted in significant differences in DGGE patterns, indicating – as previous studies have suggested - that the diversity of the AOA community is responsive to changes in its physical and chemical environment.

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