The Persistent Effects of Chicken Cultivation on the Community Composition of Soil Ammonia-Oxidizing Bacteria

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The addition of chicken manure, a common nitrate supplement for agricultural plants, has been shown to affect the soil ammonia-oxidizing bacterial community composition. To analyze the persistent effects of chicken cultivation on soil ammonia oxidizing bacteria community composition, denaturing gradient gel electrophoresis analysis of PCR amplified 16S rRNA specific to ammonia-oxidizing bacteria was performed. Ammonium and pH levels were also measured to determine any correlation between shifts in community composition and measured values. Ammonium levels and pH values were found to vary with no specific trend. Furthermore the community diversity of soil ammonia-oxidizing bacteria was shown to be fairly consistent while some differences in relative abundance were observed through the 3 to 20 month time course. Therefore, it was concluded that chicken cultivation had some persistent impact on soil ammonia oxidizing bacteria community composition.

Nitrifying bacteria play an essential role in the global nitrogen cycle (1). Nitrification is the two-step oxidation of ammonia to nitrate and each step is catalyzed by different microbial groups (1). The first and rate limiting step, conversion of ammonia to nitrite, is carried out by the ammonia-oxidizing bacteria (AOB) while the nitrite oxidizing bacteria complete the nitrification process, converting nitrite to nitrate (12). These nitrifying microbial groups are found in a range of ecosystems, including sewage, soils, sediments, fresh water and marine environments (9, 18). In carrying out the rate limiting step of nitrification, the ammonium and nitrate content of such environments is closely linked to AOB population and activity.

Both ammonium and nitrate are essential nutrients in plant growth, acting as primary nitrogen sources for synthesis of building blocks or use as growth signals (4). Fertilizers are often used to supplement plants with these essential nitrogen sources to promote growth, especially in agriculture. However, these fertilizers can have considerable effects on the AOB soil population (6). The soil dwelling AOB are chemolitho-autotrophic bacteria, which belong to the Nitrosomonas and Nitrosospira genera within the β-Proteobacteria subclass (12). Studies have shown that soils with high ammonium concentrations from fertilization can lead to shifts in the AOB community structure, from a diverse population to domination by Nitrosospira cluster 1 or 3 which are two of the four Nitrosospira clusters obtained from the 16S rRNA-based phylogenetic classification analysis (23, 24). As the nitrification activity of the soil dwelling AOB alters the availability of ammonium and nitrate in the soils, shifts in the AOB community structure may in turn significantly effect plant growth (3).

Due to the potential environmental concerns with chemical fertilizers, various alternative biofertilization plans, such as use of unprocessed manure, are currently in practice (7). Currently, plans are underway to incorporate chicken cultivation into the crop rotation cycle at the University of British Columbia Farms. Chicken cultivation on the site will result in the deposition of chicken litter, which is a mix of chicken manure, feathers, and waste feed, providing a rich source of organic matter and nitrogen (25). Evaluation of chicken manure has shown that a majority of the nitrogen (60-70%) is in the form of uric acid and urea, which are converted to ammonium by bacterial ureases and contribute to a lowering of soil pH (16). AOB populations have been shown to be sensitive to ammonium and pH levels (19, 20). Therefore, similar to the past fertilizer studies, chicken cultivation, and subsequent deposition of chicken litter, is likely to affect AOB community structure. To date, there have been no studies performed on the effects, nor on the duration of the effects of chicken cultivation on soil AOB community composition.

The persistent effects of chicken cultivation on agricultural soil AOB community composition over a twenty month span was analyzed by molecular methods. The 16S rRNA of AOB was amplified via nested polymerase chain reactions (PCR) and then subjected to denaturing gradient gel electrophoresis (DGGE). DGGE is a molecular fingerprinting technique performed on a polyacrylamide gel with an increasing gradient of chemical denaturants, and allows for the separation of PCR amplicons of differing gene

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sequence from a mixed microbial population (8). In order to monitor the agricultural soil conditions, the ammonium concentration and pH of the soil were also measured.

MATERIALS AND METHODS

Sample collection. Samples were collected as soil cores using a soil auger (2 cm diameter, 20 cm length). A total of ten cores, taken to approximately 10 cm depth, were collected from each sample plot. Soil samples were pooled, sifted through 2mm wire screen, and frozen at -20°C.

Soil pH and ammonium content measurement. pH measurements were performed by mixing soil samples with distilled H₂O to create a 1:4 soil to water mixture. Mixtures were stirred and allowed to settle, and pH readings were taken by placing a pH meter electrode (Accumet pH Electrode, Fisher Scientific) 1 cm above the top of the settled soil. Ammonium concentration measurements were performed by mixing soil samples with distilled H₂O to create a 1:2 soil to water mixture. Mixtures were stirred, allowed to settle, and solutions above settled soils were transferred to new tubes. Ammonium chloride standard solutions were prepared and measured with an ammonium probe (Ammonium Combination Electrode, Fisher Scientific) and volt meter (Accumet 900 Meter, Fisher Scientific). Sample solutions were diluted 1 in 100 into 10 M NaOH solutions above settled soils were transferred to new tubes. Ammonium chloride standard solutions were prepared and measured with an ammonium probe (Ammonium Combination Electrode, Fisher Scientific). Sample solutions were diluted 1 in 100 into 10 M NaOH and readings were taken. Millivolt readings from standards were used to create a standard curve to determine sample ammonium concentrations.

DNA extraction. Duplicate soil samples (0.5 g) from each treatment were placed in 2 ml screw cap tubes containing glass beads 150 to 600 μm in diameter. Phenol:chloroform:isoamyl alcohol (0.5 ml, 25:24:1) and extraction buffer (0.5 ml, 1:1 mixture 10% CTAB, 0.7M NaCl and 240mM potassium phosphate buffer) was then added to the soil samples in the screw cap tubes. The tubes were then shaken on a vortex adaptor at speed 6 for ten minutes (Vortex Adapter CAT# 13000-V1, MO BIO Inc. Carlsbad, California, US). Following the shaking, the sample was centrifuged at 16,000 g for 5 minutes, and supernatants were transferred to new microfuge tubes. Chloroform:isoamyl alcohol (0.5 ml, 24:1) was added to these supernatants. The tubes were vortexed and centrifuged at 16,000 g for 5 minutes. The new supernatants were transferred to new microfuge tubes following centrifugation. Afterwards, 1 ml 30% polyethylene glycol/1.6M NaCl solution was added to the supernatant for nucleic acid precipitation. The samples were then mixed thoroughly and incubated on ice for two hours. After the incubation, the tubes were centrifuged at 16,000 g for 20 minutes. The supernatant was discarded following the centrifugation. The remaining pellets were washed with 0.5 ml of ice cold 70% ethanol and centrifuged at 16,000 g for 5 minutes. The pellets were dried and resuspended in 50 μl of DNAse/RNase free H₂O. The quantification of the extracted DNA samples were performed with PicoGreen fluorescent dye (Invitrogen, Eugene, Oregon, US), spectrophotometer (CytoFluor II, excitation light 450 nm, detection 530 nm) and respective software (CytoFluor Reader Version 4.2, PerSeptive Biosystems, GMI Inc., Ramsey, Minnesota, USA).

PCR amplification. AOB 16S rRNA was amplified from soil DNA extracts using nested PCR reactions (15). The reagent mixture used for the first reaction consisted of the following reagents at the following final concentrations: 1x PCR buffer, 1.5 mM MgCl₂, 250 μM deoxynucleotide triphosphate mixture (dNTP), 0.2 μM CTO189f primer, 0.2 μM CTO654r primer, 2 mg/ml BSA, 0.4 U/μl DNA Taq polymerase, and 130 ng DNA from soil DNA extract (15). The PCR cycle used: 5 minute 95°C initial denaturation, 30 cycles of 92°C for 0.5 minutes, 55°C for 0.5 minutes, and 72°C for 1 minute; and 72°C for a 10 minute final extension. The reagent mixture for the second round PCR was identical to the first round with the exception of no added BSA, CTO189f primer and CTO654r primers were replaced with 0.2 μM Muyzer356f-GC and Muyzer518r primers, and first round PCR products were used in place of soil DNA extracts (15). The PCR cycle conditions used were identical to the first round PCR. Negative controls were included during each PCR run by adding 1 μl DNAse/RNase free H₂O. Successful PCR amplification was confirmed through running of PCR samples on 1.5% agarose gels, run in 1x TAE buffer (40mM Tris-acetate, 0.4 mM ethylenediaminetetraacetic acid (EDTA)).

Denaturing gradient gel electrophoresis (DGGE). PCR amplicons were analyzed using D-Code Universal Mutation Detection System (Bio-Rad, Mississauga, Ontario, Canada). A 25 well, 8% acrylamide/bis-acrylamide (37.5:1) gel containing a 30-60% v/v (2.1 M urea, 12% formamide to 4.2 M urea, 24% formamide) denaturing gradient, was created using a Model 475 Gradient Delivery System (Bio-Rad, Mississauga, Ontario, Canada). PCR products, mixed with DNA loading buffer, were loaded onto the gel in the central lanes, and run at 75 V, for 16 hours, in 1x TAE buffer at 60°C. Following the completion of electrophoresis, SYBR green stain (Invitrogen, Eugene, Oregon, US, 3:20 v/v SYBR Green: 1x TAE Buffer) was applied to the gel. Visualization of the gel was performed by a UV light detection system (Typhoon Variable Mode Imager, GE Healthcare, Baie d’Urfe, Quebec, Canada) and respective scanner software (Typhoon Scanner Control v5.0, GE Healthcare, Baie d’Urfe, Quebec, Canada).

RESULTS

Site treatment and description. Soil samples were taken from four different treatment plots on UBC Farm for molecular analysis: previously subject to chicken cultivation and left vacant and unused for three, four, eleven and twenty months. Control samples were also collected from each site. The control soils were considered soils that had not been exposed to chicken cultivation but were within a close proximity to the treatment sites. The samples were collected 2 meters

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TABLE 1. PCR primers utilized.

<table>
<thead>
<tr>
<th>PCR Round</th>
<th>Primer Name</th>
<th>Primer Sequence*</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CTO189f, from ref. 15</td>
<td>GGAGGAAAGTAGGGGATCG</td>
<td>~465</td>
</tr>
<tr>
<td></td>
<td>CTO654r, from ref. 15</td>
<td>CTAGCTTTGTAGTTTCAACGC</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Muyzer356f-GC, from ref. 15</td>
<td>CGCCCGCCGCGCGCGCGCGCGCGGGGACGGG</td>
<td>~161</td>
</tr>
<tr>
<td></td>
<td>Muyzer518r, from ref. 15</td>
<td>GCTACTCGGGAGGGACGCAG</td>
<td></td>
</tr>
</tbody>
</table>

*Primer sequences listed from 5’ to 3’.
outside the respective treatment areas. The two meters range was estimated to be sufficiently far away from any potential chicken cultivation effect. The control was collected to confirm that the change in observed AOB population composition is actually due to the effects of chicken cultivation rather than the intrinsic physiochemical properties of the sampled soils. During the sampling process, no sites were then currently occupied by chickens. Therefore, samples were taken from a site that was most recently cultivated by chickens (post 3 months). However, this site differed in both duration of chicken cultivation and chicken species from the other sites. Agasi Cross, a crossbreed of Road Island Red and Plymouth Rock chicken breeds, was raised on the post 3 month site. The post four, eleven, and twenty month sites were co-occupied by two chicken breeds, Bovan and Lohman. The chicken diet consisted of the vegetation present on the land plots during cultivation and was supplemented with metal and antibiotic free organic chicken feed. During winter months, the diets were supplemented with lime to prevent the formation of brittle egg shells.

**Soil pH measurement.** The soil pH values did not exhibit any observable trend with time elapsed following chicken cultivation between the different sampling sites (Table 3). However, it was found that the post 11 and 20 month sites had a lower pH than the sites with recent chicken presence, with pH values of both sites below 5. The pH differed only minutely between the post 4 and 11 month sites and their respective controls, suggesting the pH values may have normalized at or before 4 months following cultivation. The largest difference in pH observed between a site and its respective control was found in the post 3 month site. The pH for the treatment and the control were pH 5.3 and 4.6, respectively, and thus amounts to a 5 fold difference in H⁺ concentration. It was expected that the lower pH would be found in the cultivated site, however the reverse was observed. This difference is likely the result of the effects of chicken cultivation on the soils, while the higher observed pH in the cultivation soils is likely from the recent addition of lime to the chicken diet. The post 20 month site was 2.5 fold more acidic than its relative control. However, this was counter to our expectations of pH stabilizing over the near 2 year recovery period and suggests that geographical variables may be playing a role in soil pH.

**Soil ammonium content measurement.** The difference in ammonium concentration among the soil sites was much more apparent (Table 3). The post 11 and 20 month sites showed much higher ammonium concentrations, with an approximate 36 and 62 fold difference from the post 3 and 4 month sites, respectively. This result was unexpected as we hypothesized that the ammonium concentration would decrease with time following recovery from chicken cultivation. However, this may indicate that there is higher nitrification activity in the more recent chicken cultivated sites, thus utilizing ammonia at a faster rate, leading to the concentrations observed. The post 4 month control site had an ammonium concentration approximately 84 fold higher than its relevant cultivation site, which may be due to an unknown variable present in the soil sample or an outlier measurement. The remaining control sites, with the exception of the post 4 month control, showed 28-48% of the ammonium concentration found in their nearby chicken cultivated sites. This was contrary to our expectations of minimal difference in ammonium concentrations between cultivation sites and the control for the post 11 and 20 month sites. This was predicted as the lack of ammonium input at the cultivation site would be expected to eventually lead to the return of the AOB populations to pre-cultivation levels, and thus a decrease in nitrification activity and corresponding ammonium consumption.

**DGGE Banding Pattern.** The replicates yielded near identical banding patterns with slight variances in certain band intensities which are evident for the post 3 and 4 month controls, as well as the post 20 month site (Fig.1). These slight variances were expected due to

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### TABLE 2. Soil sample site locations and cultivation history.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Date of Chicken Cultivation</th>
<th>Global Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post 3 month</td>
<td>May 2008 - November 2009</td>
<td>49N 15'1.3206&quot;, 123W 14'17.0556&quot;</td>
</tr>
<tr>
<td>Post 4 Month</td>
<td>June 2009 - October 2009</td>
<td>49N 14'58.7328&quot;, 123W 14'7.6200&quot;</td>
</tr>
<tr>
<td>Post 11 Month</td>
<td>November 2008 - March 2009</td>
<td>49N 15'1.8966&quot;, 123W 14'16.6590&quot;</td>
</tr>
<tr>
<td>Post 20 Month</td>
<td>March 2008 - June 2008</td>
<td>49N 15'4.4352&quot;, 123W 14'22.1424&quot;</td>
</tr>
</tbody>
</table>

### TABLE 3. Soil pH and ammonium concentration following recovery from chicken cultivation for varying durations of time.

<table>
<thead>
<tr>
<th>Soil Site</th>
<th>pH</th>
<th>Ammonium (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post 3 month</td>
<td>5.3</td>
<td>2.4</td>
</tr>
<tr>
<td>Post 3 month control</td>
<td>4.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Post 4 month</td>
<td>5.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Post 4 month control</td>
<td>5.7</td>
<td>122.0</td>
</tr>
<tr>
<td>Post 11 month</td>
<td>4.6</td>
<td>87.0</td>
</tr>
<tr>
<td>Post 11 month control</td>
<td>4.7</td>
<td>40.3</td>
</tr>
<tr>
<td>Post 20 month</td>
<td>4.9</td>
<td>88.6</td>
</tr>
<tr>
<td>Post 20 month control</td>
<td>5.3</td>
<td>24.4</td>
</tr>
</tbody>
</table>
differences in soil microenvironments that can exist, even in the pooled soil samples as well as differences in PCR amplification.

It was expected that the patterns of AOB diversity of the recently cultivated sites would vary considerably from their respective controls with these differences decreasing with time elapsed. Differences in banding patterns were detected, with some bands varying greatly in intensity between treatment and control sites, and other bands present in some lanes while absent in others. However, bands of this nature appear to constitute only a small proportion of the overall banding pattern. Generally, the majority of these bands maintain a similar presence throughout the DGGE, with many bands of equal migration distance appearing in every lane. Bands such as C, D, and E, were present in all the samples, albeit with minor differences in intensity detected (Fig. 1). The high number of very similar bands would suggest that similar species are present across the entire soil environment within the sampling plots. This may indicate that a number of species can survive a range of pH and ammonium concentrations and are present across all the soil samples.

Though numerous bands do maintain their presence under the various treatments, the intensity of many bands was found to vary (Fig. 1). For example, band F shows considerable variation in intensity across all samples, while bands A and B generally appear to be more intense in the control sites. This may suggest that although the species diversity is fairly constant in these soils, the AOB population proportions vary over treatments. This may indicate that certain soil conditions favor some species over others, with other soil properties, in addition to ammonia and pH level, possibly effecting the AOB composition.

The labeled bands (Fig. 1) were chosen as examples to illustrate species that have remained relatively consistent across all samples and those that appear to vary in presence or abundance in different sites. These do not represent particular key species nor are they necessarily species of particular importance. Due to the lack of exemplary representative species of *Nitrosomonas* and *Nitrosospira* belonging to particular clusters, and lack of sequencing data for the selected bands, these bands do not serve the purpose of identification. However, these bands were used for the comparisons of general patterns of AOB community composition.
It was expected that there would be higher diversity in the older post 11 and post 20 month samples as these sites had been in the recovery stage for a significant period of time. The existence of many microenvironments would select for many species that can survive in these niches. We predicted that these sites would have similar banding patterns to their respective controls as differences from chicken cultivation would likely disappear by this time. The post 3 month site was expected to have the least species diversity due to a relatively homogenous environment created from constant influxes of ammonium from chicken cultivation, selecting species best able to grow under such conditions.

DISCUSSION

It was expected that the pH of the sites with more recent chicken cultivation would yield low pH values due to the high uric acid levels in poultry manure (16). In addition, with high ammonia deposition into the soils, higher nitrification activity was expected, and as the nitrification reaction generates H⁺, it would contribute to the lowering of pH (12).

However, the higher pH of the post 3 month site compared to its control may be a result of the addition of the lime to the chicken diet during winter months (Table 3). Lime was mixed with the supplemental chicken feed during winter months to ensure egg shell integrity. As the feed was spread, not all of it would have been consumed by the chickens, and the lime may have leached into the soils. The presence of lime in the soils would raise the soil pH and may account for the 0.7 pH difference seen between the post 3 month site and its control (Table 3). Such similar trend was, however, not observed in the post 11 month site although the chickens cultivated on the treatment site were also supplemented with lime in their diet (Table 3). One possible reason for this discrepancy may be that the acidification of soils by rain water may have eliminated the pH raising effects of lime (14).

It has been shown that AOB communities are sensitive to soil pH (11, 20). At pH below 5.8, small irreversible community changes can occur, as low soil pH selects for acidophilic ammonia oxidizers (11, 17, 20). Once the soil pH reaches below 5.8 for a period of time, only acidophilic ammonia oxidizers will be present in the soil even after the pH returns to its original value (11, 17, 20). All of our soil samples had pH values below 5.8 (Table 3). Therefore, an irreversible shift in the AOB community composition towards dominance by acidophilic ammonia oxidizers, such as *Nitrososphaera* cluster 4 species, may have already occurred prior to chicken cultivation (20, 21). As the acidophilic ammonia oxidizers are tolerant of pH ranges from 4 to 6, the shift in soil pH caused by chicken cultivation would fall within this range and thus be insufficient to alone cause significant changes in AOB community composition (11, 20).

Ammonia concentration has been shown to significantly affect AOB community structure (2, 10). The conversion of ammonia to nitrite is catalyzed by the ammonia-monooxygenase enzyme (12). AOB species have been found to possess ammonia-monooxygenase enzymes with varying affinity constants for ammonia, making AOB sensitive to shifts in ammonia concentration as each are adapted to specific ammonia levels (22). This may explain the differences in banding intensity between samples observed in the DGGE (Fig. 1). An intense band indicates that a species makes up a large proportion of the population within the overall AOB community, while a less intense band of equal migration distance indicates that the same species makes up a smaller proportion in that particular sample. A species forming a greater proportion of population may be the result of this species being favored by the particular environmental conditions. In the case of bands such as A and B, the higher intensity of these bands in the control sites indicates that the conditions of the uncultivated soils favor the growth of the species represented by these bands (Fig. 1). Therefore, this would suggest that the effects of chicken cultivation could be detrimental to the growth of these particular species. The variable intensities in bands, such as F, across all sites could be the consequence of the varying affinity for ammonia by the ammonia-monooxygenase enzymes in the different AOB species (Fig. 1). With each AOB species optimized to specific levels of ammonia, the varying concentrations of ammonia in the sampled sites could account for the observed variations in band intensity.

Given the high input of ammonium associated with chicken cultivation, with corresponding increase in ammonia, and the sensitivity of the AOB to ammonia levels, it was predicted that there would be high ammonium levels and a corresponding shift in the AOB community composition in recently cultivated sites. Although the intensity of many bands did vary between samples, contrary to our expectations, low ammonium levels were detected and the overall diversity patterns across all soil samples remained mostly consistent with only minor variations in presence/absence of particular bands. The lack of significant change in AOB diversity may be due to a low degree of soil ammonium accumulation. The levels may be sufficient to cause changes in the relative proportions of the AOB species but insufficient to cause a significant shift in soil AOB diversity.

The low ammonium levels measured could be due to ammonium depletion resulting from high nitrification activity promoted by a high AOB population. Even
though the overall diversity did not change significantly, the AOB population could be changing in overall abundance. This may account for the level of ammonium measured. However, no tests were done to investigate the change in AOB abundance.

Rainwater could also have been a contributing factor in lowering the level of ammonia in soils. It has been noted that the efflux rate of ammonia always increases with the level of precipitation (5). Therefore, the high precipitation in Vancouver could have significantly affected the ammonia levels in soils (5).

The rapid depletion in soil ammonia level may also be due to the uptake of ammonia by organisms other than AOB in the soil environment (26). Organisms such as heterotrophic bacteria and plants can outcompete the AOB for available ammonia and therefore sequester the majority of the environmental ammonia (26). Therefore, the presence of such organisms could result in a lowering of ammonia and corresponding ammonium levels in soils. Additionally, it has been shown that the ammonia-oxidizing archaea are abundant in much greater numbers than the AOB in many environments (13). Therefore, it is possible that the competing ammonia-oxidizing microbes, such as ammonia-oxidizing archaea and eukaryotes, could have consumed the majority of the available ammonia before AOB could utilize it. These low ammonium levels in soils could have yielded patterns of AOB diversity observed in this study.

A study of AOB diversity in environments with varying levels of ammonium found that high ammonia concentrations (600 µM) corresponded to significant differences in AOB diversity between sampling sites (22). Conversely, low ammonia concentrations (300 µM) yielded very similar AOB patterns of diversity from the multiple sites sampled (22). This suggests that ammonia levels below this concentration are primarily dominated by a specific community of AOB, such as *Nitrosospira* cluster 4 species, which have been found to be dominant at low ammonia concentrations (11). Since low soil ammonia concentrations were observed in this study, the lack of difference in AOB diversity between multiple sites is in agreement with these results. Furthermore, with our soil ammonia levels being significantly lower than 300 µM, a community of *Nitrosospira* cluster 4 species, which are adapted to these low ammonia conditions, may have dominated all sites (Table 3).

The treatment to the post 3 month site was significantly different than that of the other sites, as it was cultivated with a single and different chicken species for approximately 4 times the length of the other sites. As we saw the same repeated pattern of soil AOB community composition within this site, this provides further support that these soil AOB populations are very stable.

This high degree of similarity between all sampling sites, may also suggest that the AOB return rapidly, within 3 months, to pre-cultivation community composition, regardless of duration or type of chicken cultivation. Investigation into a sample site that has not had time to recover from chicken cultivation may help to support this claim.

In conclusion, AOB community composition appeared to shift in species proportion but only exhibited minor differences in diversity in the agricultural soils over a 3 to 20 month recovery period following termination of chicken cultivation, despite differences in measured soil pH and ammonium content. This lack of variation may possibly be explained by changes in the abundance of AOB, insufficient duration of treatment, external environmental conditions, competition for available ammonia, pre-selection of condition tolerant AOB or simply rapid return to pre-cultivation community composition. Further studies are needed to confirm these hypotheses. Our results have indicated that chicken cultivation can trigger some prolonged effects in AOB community composition. However, the direct impact of these minor composition changes on plant growth can not be determined without proper correlation to nitrification activities. Therefore, whether chicken cultivation is likely acceptable for integration into the crop rotation cycle at UBC farms requires further study.

**FUTURE EXPERIMENTS**

To verify the proposed causes behind the similar patterns of diversity for the soil AOB and to further investigate the persistent effects of chicken cultivation on the ammonia-oxidizing microbes in the soil, several follow-up experiments and modifications could be made.

During the sample collection stage, it was noted that sites that were then currently subject to chicken cultivation were unavailable. Without data from such sites, it was not possible to confirm a steady AOB community composition during and following chicken cultivation. Therefore, for future experimental attempts, data from land plots under current cultivation by chickens, as part of the sampled treatments, should be performed.

Sequencing of the particular dominant bands present in all lanes of the denaturing gradient gel would allow for possible classification of the AOB species present into clusters within the *Nitrosomonas* and *Nitrosospira* genera. Such data may help confirm or deny our prediction of a *Nitrosospira* cluster 4 domination of these agricultural soils and is thus suggested for future investigations.
Though our data suggests that composition remains relatively stable with time, the AOB populations may shift in abundance in response to chicken cultivation and a shift in nitrification activity. Therefore, it is pertinent to determine the abundance of AOB in the soil samples in future investigations. Such data could be collected through quantitative real time polymerase chain reaction (RT-PCR) amplification of the ammonia-monooxygenase gene (amoA) specific to AOB. In a more general respect, it may also be beneficial to determine overall nitrification levels for the soil samples.

As it was proposed that certain eukaryotes and archaea may outcompete the AOB for available soil ammonia, such organisms may be more influenced by chicken cultivation than the AOB. Investigations into the composition of these groups following the removal of chicken cultivation is suggested and may provide a more complete picture into the effects on chicken cultivation on the soil microbial community.

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