Characterization of microbial community structure and composition in constructed wetland sediment contaminated by effluent from concentrated poultry feeding operations

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INTRODUCTION

The primary concern of concentrated poultry feeding operations is the manure waste, which may be mixed with floor material depending on the type of poultry production (broilers, layers, etc.). Virtually all broiler and layer operations are conducted under confined conditions. The result of this confinement requires active mitigation of high nutrient concentration from the runoff before it drains out to the watersheds. Excessive nutrients from poultry feeding operations have been linked to downstream eutrophication of surface waters. Additionally, soil runoff and leaching due to exorbitant land application of poultry litter can lead to eutrophication and hypoxia. Constructed wetlands have been proposed as one of the most attractive options available to producers to reduce excessive nutrient discharge from animal production systems including poultry feeding operations without adversely altering production practices. The most common design of constructed wetlands is conjunct with poultry feeding operation houses to intercept and filter runoff before it is discharged into surface water bodies. In constructed wetlands, information about microbial community structure and diversity has been recognized as playing a significant role in understanding wetland functions, such as supporting elemental cycling and biodegradation of organic contaminants. Particularly, the long term exposure of poultry litters in wetland systems may alter the composition of the microbial community structure and diversity. The currently available molecular approaches have been recognized as having great potential for comprehensive environmental assessments of microbial communities in wetlands. This environmental DNA sequencing method allows the rapid analysis and more thorough assessments of microbial communities than non-molecular methods. This molecular assay provides greater sensitivity and specificity through studies that employ techniques like PCR and real-time PCR in environmental samples. PCR primer pairs have been provided with the ability to amplify and analyze all the functional genes in the denitrification pathway of the environmental samples (Braker et al., 2003; Rich et al., 2003).

OBJECTIVES

The objective of the study was to generate information about the bacterial community and the denitrifier population changes under a high level of long term impacted wetland systems from the concentrated poultry feeding operation house. Providing an in-depth knowledge of the nutrient cycle in constructed wetlands under stressed environmental conditions was also a major interest of our study.

STUDY SITE DESCRIPTION

The two ponds for sediment sampling sites are located in the Hill Farm Research Station, LSU AgCenter, near Homer, Louisiana (A: Big pond, contaminated by effluent from poultry house; B: Conner pond, control)

MATERIAL AND METHODS

DNA extraction

DNA was extracted from 1 g of fresh sediment samples using the PowerSoilR DNA Isolation Kit according to the manufacture's manual (Mobio Laboratories, Inc., USA). All the extracted total DNA samples were stored at -80 °C before further analysis.

Cloning and sequencing

After DGGE, prominent bands were excised from the gel. DNA sample from each band was extracted using FastDNA SPIN Kit (Bio101 Inc., USA). The DNA samples were re-amplified with the primer set without GC clamp. The suitable PCR products were sent to Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA for sequencing.

RESULTS

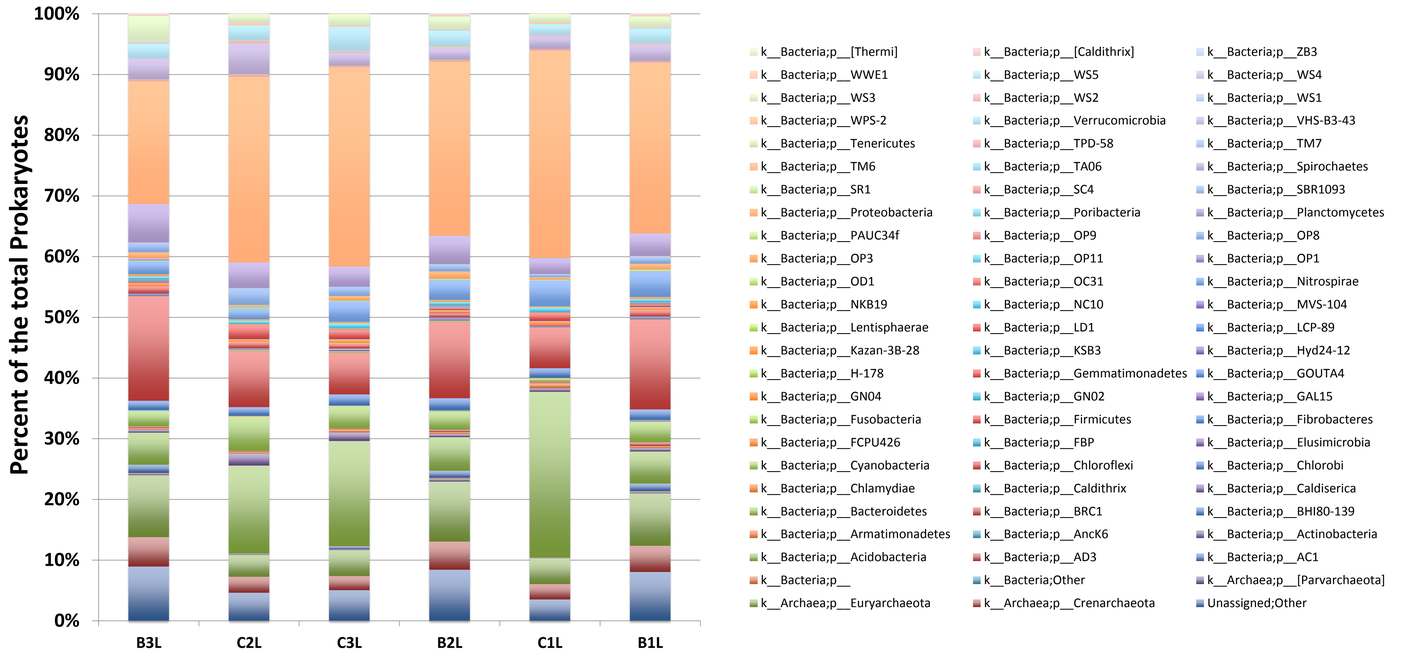

Fig. 2. Distribution of major phylogenetic groups of Bacteria and Archaea at the Big pond (B1L, B2L, B3L) and the Conner pond (C1L, C2L, C3L).

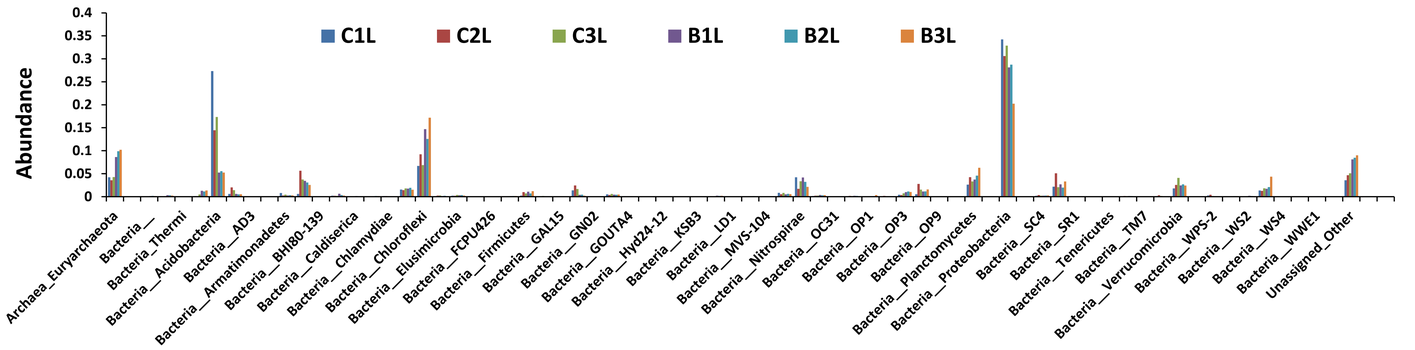

Fig.3. The overall distribution of phylotypes and congruent ranking of abundant at two different sampling sites for both the Bacteria and Archaea.

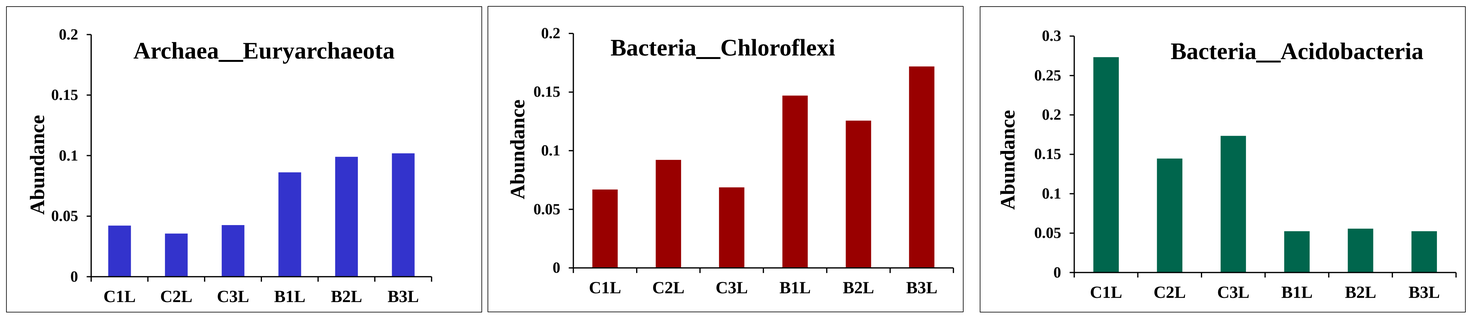

Fig. 4. Comparison of Archaea and Bacterial abundance in selected groups.

CONCLUSIONS

The abundance of Acidobacterial taxa composition was 3.7 times lower at the Big pond compared to the composition at the Conner Pond. It is indicate that the bottom sediment at the Big pond was impacted by the effluent of poultry house because Acidobacteria are particularly abundant within uncontaminated soil environments.

The abundance of Euryarchaeota Archaea and Chloroflexi bacterial communities was shown to be 2.4 and 2.0 times higher, respectively, at the Big pond samples compare to the composition at the Conner pond samples. Euryarchaeota Archaea mostly evolved to produce methane and is often found in intestines. It also includes the halobacteria, which can survive extreme concentrations of salt, and some extremely thermophilic aerobes and anaerobes. In addition, Chloroflexi bacteria are aerobic thermophiles, which use oxygen and grow well in high temperatures, anoxygenic phototrophs, which use light for photosynthesis, and anaerobic halorespirers, which uses halogenated organics (such as the toxic chlorinated ethenes andpolychlorinated biphenyls) as energy sources.