Resources but not soil particle size influence ammonia oxidizing communities

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Key words: Soil particles, microbiome, AOA, AOB, X-Ray CT, ammonia oxidation

Abstract

Introduction

Ammonia oxidation first step in nitrification carried out by ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB). Recently, a group of bacteria (i.e. comammox) has been described, known to perform complete nitrification. While little is known about the distribution of comammox organisms in the soil, we have a large number of studies investigating the environmental and evolutionary preferences of AOA and AOB in the soil environment. AOA have been shown to typically outnumber the abundance of AOB in natural soils, however this is not true for managed soils where nitrogen sources are usually added. AOA and AOB commonly compete for same resources, however research has shown that AOA activity is increased under low ammonium concentrations but reverse is tru for AOB. Additionally, ammonium derived through mineralization is thought to be the major source for AOA but organic ammonium for AOB. Along with the ammonium resource distribution, pH seems to play an important role in shaping the abundance and activity of AOA and AOB in soil. Soil particles of different sizes are known to have different chemical composition. Carbon and nitrogen content is thought to increase with _____ and pH drops with size. Here mention the importance of clay and water content.

We hypothesized that the co-occurrence of AOA and AOB in soil environment is due to the soil chemical and size heterogeneity which they preferentially and selectively occupy.

Materials and Methods

Soil collection and rhizotron setup

In September 2017, after harvesting the common beans, field soils were collected from two location in Michigan, Montcalm Research Farm (coordinates) and Saginaw Valley Extension and Research Center (coordinates). Around 50 kg of soils were collected from the top 20 cm and sieved through 4mm sieves on the location. Sieved soil was placed in buckets and transported to the lab where it was stored at 4C until rhizotron assembly. Back in the lab, rhizotrones were constructed, nine for each sampled soil. The soil was gently added to the rhizotrones to avoid over compactedness. Each rhizotron was divided by a barrier made out of multilayered Miracloth into a larger compartment where up to 4 sprouted common bean seeds (*Phaseolus vulgaris* cv. Eclipse) were grown and the smaller compartment on the side of the rhizotrones where no seeds were placed. Rhizotrones were covered with aluminum foil to prevent light accessing to the root system. Plants were grown in growth chambers (14h/10h and 26C/20C day/night cycle) and watered every 4 days until plants reached V4 stage from when the plants were watered every 2 days.

Soil sampling and sieving

When plants reached the senescence stage, soil was sampled by first removing the aboveground biomass. Despite the barrier between the compartments the roots penetrated it and grew into the seedless compartment. Thus, only the large compartment fully grown with roots was collected and is referred to as rhizosphere from now on. Soils were

DNA isolations, PCR and sequencing

Using DNeasy PowerSoil DNA isolation kit (QIAGEN) to isolate DNA from sieved soil fractions. 3 samples of 15 g were taken from each pooled soil size fraction and these were treated as biological replicates (totally 42 samples). From each of the replicate triplicate DNA isolations were performed. each eluted in 25 ul buffer. The isolated DNA from the same sample (n=3) was pooled, checked on agarose gels using gel electrophoresis and quantified by Qubit using the dsDNA BR Assay kit. For 16S rRNA amplicon sequencing, primer pair 515f and 806r was used (Carporaso et al.) and sequencing was done using Illumina MiSeq v250 kit, yielding 250bp paired end reads. Amplification, library preparation and sequencing was done at the Michigan State University Genomics Core Facility. For ammonia oxidizing communities we first performed end point PCR targeting amoA gene using specific primers for AOA, AOB and comammox. While PCR counting AOA

and AOB specific amoA primers were able to produce amplicons in all soil size fractions, there was no amplification of comammox amoA genes in these soils regardless of the soil fraction. Thus, we focused on the AOA and AOB communities only. To prepare amoA genes for sequencing, we first amplified them using primers containing CS overhangs. 50 ul PCR mixtures were prepared for AOA and AOB using primers (final concentration 600 nM), Pfu polymerase (Thermo Scientific, USA) (1.5U) and Pfu Polymerase buffer (1x). The PCR protocol.

Determining the soil particle surface area

Amplicon sequence analysis

Statistical analysis

Results

We found that:

1. Microbial richness is influenced by the soil particle size.

2. Chemical properties of soil particles are very different and are site dependent.
3. Ammonia oxidizing communities are influenced by the ammonium concentrations and not soil article sizes.
4. Richness of AOA>AOB.
5. Soil particle surface area.

Additional results:

- no detection of comammox in the system
- the proportion of sieved fractions is (as % of the whole soil) different between the two soils

Discussion

Acknowledgments

References