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Effects of metam sodium fumigation on the abundance, activity, and diversity of soil bacterial communities

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ABSTRACT

Metam sodium is a fumigant used as a crop pretreatment in agriculture to control a wide array of pests, and soil borne diseases that may adversely affect plant quantity and quality. This present study utilized control and treated field plots to examine the effects of metam sodium on indigenous soil microbes in terms of numbers, activity, and diversity. Following metam sodium application, culturable heterotrophic counts in all soils increased significantly for 24 h, but quickly returned back to original levels. Application resulted in decreased microbial activity, detected by ATP-based assays, that was significantly lower in treated soils than control plots, but recovered quickly. High-throughput sequencing of the 16S rRNA gene showed that treated plots contained significantly lower numbers of observed OTUs, particularly 14, 21, and 28 days after treatment. Soil bacterial communities were significantly altered by MS-treatment due to increased relative abundances of Actinomycetales, Bacilli, and Chloroflexi, as well as decreased Acidobacteria. These dominant taxa observed in MS-treated plots are major contributors to biological activity in various healthy soils and rhizospheres. Therefore, the increase in relative abundance of these biologically productive phyla coupled with abundant ATP production suggests that soil health recovered following MS-treatment and remained functionally intact.

1. Introduction

Fumigation is a common treatment for pest control in agricultural soils, with metam sodium (MS) being the most commonly used soil fumigant (Zheng et al., 2006), and the third most commonly used agricultural pesticide (Pruett et al., 2001) in the United States. MS is registered by the United States Environmental Protection Agency (US EPA) for agricultural use on food and feed crops as well as turfgrass.

In the presence of water, MS salt hydrolyzes into methyl isothiocyanate (MITC), a highly toxic and volatile gas with broad pesticidal activity (Zheng et al., 2006). Byproducts include carbon disulphate, dihydrogen sulfide, and elemental sulfur (Deguigne et al., 2011). MS is effective against pests, with broad biocidal activity (Macalady et al., 1998), when applied in sufficient concentrations, but is prone to uneven application rates due to soil structure (Candole et al., 2007). During MS application, agricultural farmers cover their soils with tarps or plastic mulch to retain more MITC for longer periods of time, increasing its efficacy and lowering the economic burden through better crop pest management and more conservative pesticide use. The unreacted MITC in the soil eventually diffuses into the atmosphere, where it is broken down through photolysis. The rate of conversion from MS to MITC can vary from one hour to one day, with initial concentrations of MS and soil moisture greatly impacting the process (Turner and Corden, 1963; Smelt et al., 1989; Gerstl et al., 1977; Wales, 2000). The rate of volatization of MITC and subsequent breakdown into its byproducts can vary from one to several days, but does not depend as heavily on soil moisture (Zheng et al., 2006). Still, soils are subject to repeated MITC exposure due to slow hydrolyzing of MS, which can result in long-term influences on soil composition and microbiota (Taylor et al., 1996).

Although MS is often applied as a broad fumigant, pesticide, herbicide, and/or fungicide, such chemicals can have adverse effects on soil bacteria. Unwarranted biocidal effects can cause impactful responses on the numbers, types, and activities of indigenous soil microorganisms with regards to soil fertility and plant growth (Parr, 1974). In particular, MS has been shown to disturb microorganisms responsible for nutrient cycling, especially nitrogen transformation, and pollutant degradation (Sinha et al., 1979; Macalady et al., 1998; Toyota et al., 1999). Mycorrhizae and fungi have shown susceptiblility to MS treatment, while actinomycetes are unaffected or quickly reestablish

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normal population counts (Corden and Young, 1965; Sinha et al., 1979). Yet, the effects on communities is relatively unknown. Previous studies have analyzed changes in bacteria communities following MS fumigation via conventional culturing, substrate-utilization, fatty acid composition, %GC profiles, and DNA fingerprinting techniques (Sinha et al., 1979; Macalady et al., 1998; Toyota et al., 1999). In general, MS treatment caused significant decreases in bacteria populations that require sufficient periods of time for recovery (Macalady et al., 1998; Toyota et al., 1999). However, there was no difference in total bacterial DNA profiles between MS-treated and control plots (Toyota et al., 1999). This implies that total numbers of all bacteria decrease and recover in a similar manner, but this phenomenon is unlikely and difficult to confirm for nonculturable species.

The present study aims to further understand how MS alters soil bacterial communities by utilizing advanced techniques to measure overall activity, as well as culturable and non-culturable bacteria. To provide a thorough and unique evaluation of the effects of MS on soil bacterial communities, microbial assays were conducted on untreated (control) and MS-treated soils over a four-week period. To our knowledge, this is the first study to simultaneously evaluate the biocidal effects of MS on soil indigenous microbial populations in terms of numbers, activity, diversity, and community composition.

2. Material and methods

2.1. Soil treatment with MS

The trial was conducted from Nov. 4–Dec. 2, 2015 utilizing field plots established at the University of Arizona Campus Agricultural Center (Tucson, USA). The sandy loam soil at the study site was a Gila coarse-loamy, mixed, superactive, calcareous, thermic Typic Torrifluvent with good aeration and drainage and low amounts of organic matter and clay. These soil conditions likely resulted in higher rates of gas flux to the atmosphere, which could have affected the ability of the soil to retain MITC.

Triplicate 1 × 1 m control and MS-treated plots were spatially separated by 6 m buffer zones. MS-treated plots received Vapam® HM at a rate of 200–300 L per acre (0.38 g MS per ml Vapam), a rate typically used by farmers. The MS was applied to soil via a drip irrigation system, and immediately covered with a clear tarp to prolong the retention time of the MITC gas. Control plots were treated identically with tap water without the Vapam being added. Overall, each plot received 45.42 L of solution.

2.2. Field sampling & transport

A hand trowel pre-sterilized with 70% ethanol was used to collect soil samples down to a 10 cm depth at three randomly selected locations within each plot. Approximately 5 g of soil from each triplicate trowel was added into a sterile 1-L Nalgene bottle and mixed to create triplicate composite samples. Then, samples were labled, placed in a cooler, and transported to the laboratory for immediate processing. This process was performed at pre-application (0 days), 24 h (1 day), and weekly for 28 days. A total of 108 samples was collected; triplicate samples of each triplicate plot over six time points for each MS-treated and non-treated soils.

2.3. Soil analyses

2.3.1. Heterotrophic plate counts

Heterotrophic plate counts (HPCs) were determined for each soil sample via a culturable dilution and plating method using R2A agar, as previously described (ASTM D5465, 2012; Pepper and Gerba, 2004).

2.3.2. ATP-based activity assays

LuminUltra® Deposit and Surface Analysis (DSA) kit (prod. no. DSA-100, Fredericton, NB, Canada) was used to measure total microbial activity, as described in the manufacturer's protocol. The kit uses a luciferin-luciferase protein-enzyme complex to measure adenosine triphosphate (ATP) concentrations in samples. Briefly, one gram of moist soil was mixed in the kit's UltraLyse 7™ tube containing the tATP extraction (total ATP: intra-cellular and extra-cellular ATP) reagent. Subsequently, one mL of the resulting solution was placed into an UltraLute™ tube, inverted, and 100 µL of the mixture was aliquotted and combined with 2 drops of Luminase™, that contains the protein-enzyme complex. The tube was inserted into a Luminometer™ to detect the amount of light produced and provide a subsequent estimation of ATP concentration. Microbial equivalents (MEs) of tATP per gram of dry soil were calculated via the following calculation:

$$\text{tATP (MEs/g)} = \text{tATP (pg ATP/g)} \times 1\ \text{ME}/0.001\ \text{pg ATP} \quad (1)$$

The concentration of ATP was converted to microbial equivalents, which represent total viable microbial numbers. It was assumed that all free ATP degraded quickly and detected ATP were only from living or recently living cells. All ATP was assumed to be from typical *Escherichia coli*-sized cells (LuminUltra®, 2013); thus, every cell is assumed to contain 0.001 pg of ATP.

2.3.3. DNA extraction

MoBio Laboratories, Inc. PowerSoil® DNA Isolation Kit (cat. no. 12888-50, Carlsbad, CA, USA) was used to extract community DNA from 8 g of moist soil, as described in the manufacturer's protocol. Briefly, bead technology and centrifugation in conjunction with a series of kit solutions were used to lyse cells, extract DNA, and remove inorganic contaminants, humic acid, and other PCR-inhibitory substances. The final extracted DNA was held in 1× Tris buffer (EDTA-free), and stored in a 1.5-mL DNA Lo-Bind Eppendorf tube at −20 °C.

2.3.4. 16S rRNA gene amplification and purification

A custom Illumina 16S rRNA amplicon sequencing protocol was followed using manufacturers instructions (Illumina, San Diego, CA, USA). 16S rRNA gene amplification was performed using specific primers targeting the V3 and V4 regions of the 16S rRNA gene. These primers were ligated to overhang adapter sequences. The full-length primer sequences were adapted from primer pairs previously reported: (i) S-D-Bact-0341-b-*S*-17, 5′-tcgtcggcagcgtcagatgtgtataagagacagcctacgggnggcwgcag-3′, (ii) S-D-Bact-0785-a-A-21, 5′-gtctcgtgggctcggagatgtgtataagagacaggactachvgggtatctaatcc-3′ (Klindworth et al., 2012).

2.3.5. Sequencing and sequence processing

Samples were sequenced at The University of Arizona Genetics Core using a MiSeq sequencer (Illumina, Inc., San Diego, CA, USA). For quality control, the barcode and adapter sequences were trimmed through Trimmomatic software using the following parameters: LEADING:3; TRAILING:3; SLIDINGWINDOW:4:15 (Bolger et al., 2014). A quality check was conducted on MiSeq Reporter software to remove low quality reads (phred score of Q30). Bioinformatics software Quantitative Insights into Microbial Ecology (QIIME) was used to process and analyze reads (Caporaso et al., 2010). First, paired-end reads were combined using the fastq-join method (Aronesty, 2011). Next, the open-reference OTU-picking method (Rideout et al., 2014) was used to cluster sequences based on 97% nucleotide similarity (Edgar, 2010). GreenGenes reference database (DeSantis et al., 2006) provided chimera screening/removal and alignment to sequences in the database. Taxonomic assignment at various levels was given by RDP Classifier (Wang et al., 2007). Sequences representing any OTUs unclassified at the domain taxonomic level or classified as mitochondria or chloroplasts were removed. As different samples yielded different numbers of sequences, samples were rarefied to 25,000 sequences per sample prior to statistical analyses to enable legitimate comparisons to be