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ANAEROBIC SOIL DISINFESTATION IN MICROCOSMS OF TWO SANDY SOILS

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SUMMARY

In recent years, anaerobic soil disinfestation (ASD) has been proposed as an alternative control method of soil-borne plant pathogens. It involves adding a labile carbon source, irrigating the soil to stimulate decomposition of organic material and then covering the soil with air-tight plastic to limit gas exchange. During the ASD process, soil microorganisms switch from aerobic to anaerobic metabolism. As a result, by-products of anaerobic metabolism are released into the soil environment such as various organic acids and gases. These by-products are reported to have a negative effect on survival of soil-borne plant pathogens. However, the efficacy of ASD to reduce soil-borne pathogens in practice may vary significantly. Therefore, we studied the efficacy of the ASD process in two different soils. In addition, it was investigated whether a pre-treatment with an anaerobic bacterial inoculum prior to ASD affected the efficacy of the process.

Two sandy soils (dune sand and glacial sand) were inoculated in 2 L soil microcosms. We tested the efficacy of ASD treatment against the potato cyst nematode *Globodera pallida*. For each soil, three treatments were used: control treatment (no Herbie addition, aerobic incubation), ASD 1 (organic substrate addition, anaerobic incubation) and ASD 2 (organic substrate and anaerobic bacterial inoculum addition, anaerobic incubation). Soil microcosms were incubated in the dark at 20ºC for two weeks.

We observed that anaerobic soil disinfestation treatments were highly effective against Potato Cyst Nematode (PCN), with pathogen being eradicated totally in all but one ASD treatment (glacial sand ASD2) within two weeks. The relative abundance of *Firmicutes* (spore-forming bacteria, often fermentative) in total bacteria increased significantly in ASD treated soils. Numbers of these bacteria correlated positively with increased concentrations of acetic and butyric acids in soil water phase in ASD treatments.

Key words: anaerobic soil disinfestation, *Globodera pallida*, soil-borne plant pathogens, anaerobic bacteria, *Firmicutes*

INTRODUCTION

Soil is the environment in which large number of plant pathogenic organisms such as fungi (e.g. *Verticillium* spp.), oomycetes (e.g. *Pythium* spp.) or nematodes (e.g. *Meloidogyne*) can function. These organisms can cause significant crop losses in monocultural practices in greenhouse horticulture, where susceptible plant species are cultivated almost continuously. Chemical pesticides use is being more restricted in recent years. One of the methods to assure the soil is free of plant pathogens is anaerobic soil disinfestation (ASD) which was suggested by Blok et al. (2000). This method involves incorporating organic substrate into sufficiently moist soil and sealing it off with air-tight foil for a few weeks. ASD relies on activity of

microorganisms naturally occurring in the soil. During ASD (anaerobic) processes in soil a variety of products of microbial metabolism are formed, including fatty acids, N_2O, H_2S, CH_4 and NH_3 (Tiedje et al., 1984). Previous research has shown that some of these products may be responsible for inhibition of plant pathogens in soil (Spaull et al., 1992, Chitwood, 2002, Ricke, 2003, Momma et al., 2006). In this paper we describe investigation of a number of biological and chemical factors which may influence survival of potato cyst nematode (PCN) in two sandy soils during ASD. Moreover, we wanted to investigate if use of anaerobic inoculum (as often done in practice) can influence the speed of ASD process.

MATERIALS AND METHODS

Microcosm experiment set up

Soils used for microcosm experiment were: dune sand and glacial sand. Incubation took place in 2 L glass bottles. For the ASD1 treatment, soils were mixed with balanced carbon source Herbie®7022 (Thatchtec BV, The Netherlands) in a dosage of 2 g of crude protein/L soil (≈ 4,5 g of Herbie). For the ASD2 treatment, soils were prepared as described above and 2mL of anaerobic soil bacterial inoculum (10^{10} cfu/mL) were added per bottle. This dosage of inoculum was chosen to imitate the dosage of anaerobic inoculum for ASD being used in practice. The control treatment had no addition of organic material and remained oxic throughout the incubation. Experiment was conducted in triplicate. Nylon mesh bags containing cysts of the pathogen potato cyst nematode (*Globodera pallida*) were incorporated into the soil. Bottles for treatments ASD1 and ASD2 were then sealed off (air tight). Control treatment remained aerobic throughout the whole incubation period. Destructive sampling was performed after 4 hours, 7 and 14 days of incubation.

Biological and chemical analysis of soils

At each sampling time point three replicates of each treatment were destructively sampled. Concentrations of oxygen in the headspace were measured before each destructive sampling. Survival of Potato Cyst Nematode eggs (contained in cysts) was determined in a hatch test (Been & Schomaker, 1998). Total nematode counts were determined in 100mL volume of studied soils. Total bacterial numbers and functional groups of bacteria were determined with the help of qPCR method according to protocols described by Fierer et al. (2005). Concentrations of some short chain fatty acids (e.a. acetic, propionic, butyric) were determined in soil water phase extract with HPLC. Additionally, chemical parameters such as organic matter content, pH, nutritional status and EC, were measured.

RESULTS AND DISCUSSION

Two sandy soils chosen for the experiment had different chemical characteristics. Organic matter content of dune sand was 2.5% and for glacial sand it was 3.7%. Soil pH was 6.9 and 5.2 for dune and glacial sand, respectively. Two soils did not different significantly in N_{total}, but C:N ratio was significantly lower in dune sand (C:N ratio 14) compared with glacial sand (C:N ratio 20). Oxygen concentration in the headspace of ASD treatments remained below 0.3% O_2 after one week of

incubation. Only in ASD2 treatment of glacial sand O2 concentration increased between day 7 and 14 (to 0,7%). In controls oxygen concentration in de headspace remained at 20.9 % during the whole incubation.

Survival of Potato Cyst Nematode (PCN) and total nematode counts during ASD treatment in two sandy soils

Potato Cyst Nematode was successfully eradicated during ASD process in 2L soil microcosms (Figure 1). For the ASD treatments where anaerobic bacterial inoculum was used more time (2 weeks) was required to complete the process. Total nematode numbers followed roughly the same pattern as PCN numbers in ASD treatments (Figure 1). Total nematode numbers correlated positively with PCN numbers in hatch test (Pearson correlation coefficients: dune sand ASD1- 0.98, dune sand ASD2- 0.95, glacial sand ASD1- 0.91, glacial sand ASD2- 0.88)

Figure 1. Survival of Potato Cyst Nematode and total nematode numbers counts in two sandy soils during Anaerobic Soil Disinfestation

We observed increased accumulation of acetic and butyric acids in the water phase of two sandy soils during ASD process (Figure 2). Increasing concentrations of fatty acids were negatively correlated with numbers of surviving potato cyst nematodes and total nematode counts in the soils. The negative correlation was stronger in dune sand (Pearson coefficient of -0.95) than in glacial sand (Pearson coefficient of -0.71). This could point to an important role of fatty acids in eradication of plant pathogenic nematodes.

Figure 2. Concentrations of two fatty acids (acetic and butyric) in soil water phase during Anaerobic Soil Disinfestation

Concentrations of fatty acids were positively correlated with the increasing numbers of bacterial taxon *Firmicutes* in ASD treatments in both soils (Pearson coefficient >0.75 for ASD1 treatments). Mowlick et al. (2013) also observed higher numbers of Firmicutes in soils subjected to ASD.
Short chain fatty acids were shown to have a nematicidal properties. However they would need to be present in soil in undissociated form to be most effective. According to Katase et al. (2009) more undissociated form of a fatty acid is present in soil in lower pH (between 4 and 5.5), but even at pH above 6 some 10% of acetic acid was present in undissociated form in the soil. Concentrations of as low as 5mM undissociated acetic acid were able to reduce survival rate of *Meloidogyne incognita* to 0% in their experiments. We have not measured undissociated form of fatty acid in our experiments, but we could hypothesize that they were present in abundance at least in glacial sand with pH 5.2. Moreover, in our experiment the nematodes were subjected to high concentrations of fatty acids over longer time (2 weeks) than 72 hours in experiment of Katase et al (2009).

CONCLUSION

Anaerobic soil disinfestation is a viable option for control of plant pathogenic nematodes. Increasingly more evidence points to fatty acids being responsible for reduction of survival rate of these pathogens. Fatty acids are being produced by bacteria in anaerobic soils. It is therefore vital for the success of ASD to monitor bacterial activity during ASD and environmental factors (such as oxygen) which could influence them.

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