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Amplicon-based metabarcoding reveals temporal response of soil microbial community to fumigation-derived products

Feng Wei, Thomas Passey, Xiangming Xu*

NIAB East Malling Research, East Malling, Kent ME19 6BJ, UK

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ABSTRACT

The use of soil fumigation products to manage soilborne pathogens raises the question of whether it has undesirable effects on the soil ecosystem. Therefore, the aim of this study was to investigate the impacts of alternative fumigation products on soil microbial population structure. We characterised soil bacterial and fungal communities in response to the soil treatment with microencapsulated terpene, *Brassica* seedmeal (BioFence^{TM}) and chloropicrin in a field trial. The effect of soil treatments on the overall microbial population structure and relative abundance of individual microbial OTUs (operational taxonomic units) was assessed using an amplicon-based metabarcoding approach at three time points. Classifying representative OTU sequences into taxonomic groups was more uncertain for bacteria than for fungi. Chloropicrin dramatically altered both bacterial and fungal populations within four weeks of application. The effect on bacterial population structure is short-lived and became non-significant 16 weeks after treatment; however, fungal population structure was more persistently affected by chloropicrin. Neither terpene nor BioFence^{TM} significantly affected soil microbiota. This study highlights the need for reliable algorithms in classifying sequences into taxonomic units and also the importance of identifying microbes into finer taxonomic groups for understanding soil microbiota and their effects on crop production.

1. Introduction

Soil microbial communities represent the greatest reservoir of biological diversity known (Buee et al., 2009; Curtis et al., 2002; Gans et al., 2005). Microorganisms are vital to the soil ecosystem, being an integral part of important soil processes such as carbon and nitrogen cycles, decomposition of organic residues, formation of humic substance, and pollutant degradation. Microorganisms in soil are crucial for plant health (Berendsen et al., 2012) and crop yield (Xu et al., 2015), and shaped by numerous factors such as plant species and soil type. To maintain soil functions supporting the ecosystem, it is important to understand how soil microorganisms respond to natural or human-mediated disturbance (Griffiths and Philippot, 2013). Sustainable crop production to secure food supply is vital, which depends critically on availability of high quality soils.

Managing soilborne pathogens and pests is indispensable to food production worldwide, particularly for high-value horticulture crops. For instance, Verticillium wilt on strawberry, caused by the soilborne fungal pathogen *Verticillium dahliae* Kleb., is a major disease responsible for significant yield losses in commercial strawberry production (Maas, 1998). Without soil fumigation, it can result in 75% crop failure in susceptible strawberry cultivars (Wilhelm and Paulus, 1980). Pre-planting soil fumigation with broad-spectrum pesticides, e.g. methyl bromide and chloropicrin, have been indispensable for controlling soilborne pathogens on many crops for over 50 years (Martin, 2003). The broad-spectrum activities of these chemofumigants result in significant losses in soil microbial diversity (Dangi et al., 2015; Omirou et al., 2011; Spyrou et al., 2009). Because of the withdrawal of methyl bromide and the uncertain future of remaining chemicals (chloropicrin and dazomet) due to legislations, sustainable management of soilborne diseases has again become a major issue for production of many crops in soil.

During the last two decades, much effort has been directed to search for alternative methods to manage soilborne diseases, including soil amendments by green or animal manures, biofumigation using Brassicaceae plants, disease suppressive crop rotations, anaerobic soil disinfestations and other non-chemical methods (e.g. soil solarisation and high-temperature steam treatment) (Colla et al., 2012; Goicoechea, 2009; Momma et al., 2013). However, use of these techniques to replace methyl bromide

* Corresponding author.
E-mail address: xiangming.xu@emr.ac.uk (X. Xu).

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encountered several problems, including the emergence of new diseases or the re-emergence of old ones (Colla et al., 2012). Control of soilborne pathogens (including *V. dahliae*) through the use of Brassica species plants is believed to result from the toxic isothiocyanates (ITCs), the hydrolysis products of glucosinolate, released into the soil after incorporation of glucosinolate-containing plant tissues (Angus et al., 1994). Biofumigation induces changes in the structure and function of soil microbial communities in microcosm experiments (Hollister et al., 2013; Mazzola et al., 2015; Wang et al., 2014); the magnitude of impact depends on the type of ITCs (allyl, butyl, phenyl, and benzyl ITC) released (Hu et al., 2015).

One promising measure of soil amendment from outside the Brassicaceae family is with fresh and waste lavender or lavandin (López-Escudero et al., 2007; Yohalem and Passey, 2011). Three terpenes were identified as key compounds in lavender waste responsible for the observed biocidal effect against *V. dahliae* (Yohalem and Passey, 2011). Unlike biofumigation based on Brassicaceae family, most studies concerning amendment of other plant material has focused only on its efficacy against soilborne pathogens (Ochiai et al., 2007; Wiggins and Kinkel, 2005); thus their effects on non-target members of the soil microbial community are not well understood. One published study suggested that botanical pesticides did not alter the structure of soil microbial communities (Spyrou et al., 2009).

Most of published studies that profiled microbial populations used denaturing gradient gel electrophoresis (DGGE) and characterised microbial functions using phospholipid fatty acid (PLFA) analysis. Several recent studies investigated microbial population changes in response to treatment under field conditions (Mazzola et al., 2015; Xu et al., 2015) or in microcosm experiments (Hollister et al., 2013; Hu et al., 2015) using next-generation sequencing (NGS) technology. The NGS provides an opportunity to profile soil microbial communities at an improved resolution in terms of the number of distinct microbial groups recovered and their taxonomical information.

One of the key issues in sustainable intensification of agriculture is the resilience of soils in response to agricultural practice, including fumigation and soil amendment. As soil health in terms of crop production is intrinsically related to soil microbial populations, better understanding of temporal changes in soil microbiota in response to soil treatment can contribute to developing management practices to improve crop productivity whilst minimizing negative impact on soil health. Research focus so far has been on characterising microbial population changes at a single time point following fumigation or amendment with (primarily Brassicaceae) plant residues or compost. Only a few studies have dealt with temporal changes, suggesting such effects could be rapid, but short-lived (Hollister et al., 2013), or they could last for more than two years (Mazzola et al., 2015).

The objective of this work was to characterise temporal changes in soil bacterial and fungal communities in response to fumigation with microencapsulated terpenes, *Brassica* seedmeal and chloropicrin in a field trial.

2. Materials and methods

2.1. Field experimental design and treatments

A field experiment was conducted to study several fumigation treatments on strawberry wilt and on soil microbiota over time at NIAB East Malling Research, United Kingdom (UK). A completely randomised block design with four replicates (blocks) was used to assess the effects of six treatments; but only four treatments were sampled for microbial profiling. The four treatments consisted of a microencapsulated terpene product, a *Brassica* seedmeal product (BioFence^{TM}), chloropicrin and untreated control. There has been no known history of fumigant treatment at the experimental field, which has not been used for strawberry production in recent years; the soil is classified as profondic chromic Luvisol (IUSS Working Group WRB, 2015). Each block consisted of six plots of 15 m long raised-beds, established in June 2014, with a 4 m gap between two neighboring plots.

An experimental product of microencapsulated terpenes was manufactured by Eden Research (Witney, Oxfordshire, UK) in May 2014, which contained 9.9% cineole, 3.3% camphor and 3.3% borneol—these terpenes were identified as key compounds in lavender waste responsible for the observed biocidal effect against *V. dahliae* (Yohalem and Passey, 2011). The application dose of this product was recommended by the manufacturer based on other experimental terpene products manufactured by Eden Research (Edmonds, Eden Research—pers. com.), equivalent to 30 L of the terpene product per ha of land; the product was diluted at 1:20 (product: water) for application. A liquid formulation of BioFence^{TM} was obtained from Tozer seeds (Cobham, Surrey, UK), which was only released in 2014 for evaluation in the UK, and was used in order to standardise application method via injection into the soil. The maximum permissible application dose under the UK nitrate vulnerable zones (NVZ) regulations was used for BioFence^{TM} (400 L of the final made-up liquid BioFence^{TM} per ha of land, equivalent to 3 ton of the pellet BioFence^{TM} [assuming 2% N] per ha of land). Chloropicrin was applied at the recommended commercial application dose (200 L per ha of land).

The three products were injected to the soil to a depth of approximately 20 cm with a commercial injector/soil mixer and the plots were immediately covered with black polythene after injection. All plots were irrigated as necessary prior to planting in accordance with best agronomy practice. Cold stored bare-rooted strawberry runners of cultivar Elsanta were planted three weeks after soil treatment.

2.2. Sampling and soil microbial biomass profiling

Soil samples were collected from each plot immediately before treatment (zero wbt), then four and 16 weeks post treatment (four and 16 wpt). For each plot, a composite sample of soils was obtained, consisting of six soil cores obtained with a sampler (2.5 cm in diameter) to a depth of 20 cm at randomly selected locations. Each sample was mixed by sieving (mesh size 2 mm) and a subsample (approximately 2 g) of each composite sample was collected in a 2 mL Eppendorf tube and stored at −80 °C until DNA extraction.

Total DNA was extracted from 0.25 g using PowerSoil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) following manufacturer's instructions with minor modifications for homogenisation of the samples as described previously (Xu et al., 2015); extractions were carried out in triplicate for each soil sample. Triplicate samples were pooled after extraction, purified with GeneClean Turbo Kit (MP Biomedicals) as protocol, and quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Bacterial 16S rRNA genes (V1–V3 region) and the fungal internal transcribed spacer (ITS) region were amplified from the DNA extracts using bacterial primers 27F/534R (Muyzer et al., 1993) and fungal primers ITSI-F (Gardes and Bruns, 1993)/Ek28-R (ITS4G) (Guzmán-Dávalos et al., 2003). The two primer sets were modified at the 5′ end with Illumina adapter overhang nucleotide sequences as per 16S Metabarcoding Sequencing Library Preparation protocol. All PCR reactions were carried out in sextuplicate 13.0 μL reactions using the protocol described previously (Xu et al., 2015) with following modifications: in order to minimise amplification biases, reaction cycles were reduced to 20 cycles for bacterial primers and