Soil Biology and Biochemistry 125 (2018) 275–285

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Yellow Canopy Syndrome in sugarcane is associated with shifts in the rhizosphere soil metagenome but not with overall soil microbial function

Kelly Hamonts[a,*], Pankaj Trivedi[b,**], Jasmine Grinyer[a], Paul Holford[c], Barbara Drigo[d], Ian A. Anderson[a], Brajesh K. Singh[a,d,***]

[a] Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW, Australia
[b] Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, USA
[c] School of Science and Health, Western Sydney University, Penrith, NSW, Australia
[d] Global Centre for Land-based Innovation, Western Sydney University, Penrith, NSW, Australia

ARTICLE INFO

Keywords:
Rhizosphere soil microbiome
Microbial functions
Sugarcane
Yellow canopy syndrome

ABSTRACT

The Australian sugarcane industry is facing a new threat of the currently undiagnosed Yellow Canopy Syndrome (YCS). Here, we investigated if YCS is linked to detrimental shifts in soil microbial function and/or altered physico-chemical soil properties. We examined changes in rhizosphere soil microbial assemblages, functional gene profiles and microbial activity associated with YCS development. Shifts in soil bacterial and fungal community assemblages with YCS appeared variety-specific with limited consistent trends emerging. However, significant, consistent shifts in the rhizosphere soil metagenome with YCS were found, suggesting that YCS incidence might be linked to changes in specific soil microbial functions. Functional gene categories involved in prokaryotic immune response and in metabolism of compounds present in root exudates were consistently detected in higher abundance in the rhizosphere of YCS-affected plants, while gene categories involved in DNA, RNA and protein processing were consistently less abundant. Soil nutrient status (C, N), extracellular enzyme activity and substrate-induced respiration either did not significantly differ between affected and healthy fields of three sugarcane varieties, or showed inconsistent trends with variety. Altogether, our results did not show a direct link between soil microbial richness, overall soil microbial activity, soil nutrient status and YCS incidence. However, rhizosphere microbial communities responded consistently to YCS incidence by enrichment of genes encoding functions involved in defence against pathogens and root exudate metabolism which may have potential implications for the future development of diagnostic tools and an effective management practice to minimise impact of YCS on farm productivity.

1. Introduction

Farm yield decline is a complex issue caused by several factors including loss of soil health as a result of long-term monoculture, soil compaction due to extensive heavy machinery use, excessive tillage and other practices that depleted soil organic matter (Garside et al., 2005; Kibblewhite et al., 2008). Soil health, encompassing soil physical, chemical and biological components, is critical for sustaining plant productivity (Kibblewhite et al., 2008; Trivedi et al., 2016a; Zhu et al., 2016). It is well established that soil microbiota play a key role in plant health and productivity through beneficial plant-microbe interactions such as nutrient supply, growth promotion and disease suppression (e.g., Mendes et al., 2011; Schmalenberger et al., 2008). However, soil microbes are sensitive to land management practices and other environmental changes and intricately linked to soil physico-chemical health. Therefore, it is not surprising that the ultimate expression of sugarcane yield decline in Australia was reported through adverse effects of pathogens on sugarcane root systems (Garside et al., 2005).

The Australian sugarcane industry has experienced declining yields during the past few decades (Garside et al., 2005) and, in addition, is now facing a serious threat of Yellow Canopy Syndrome (YCS). YCS is a largely undiagnosed condition impacting sugarcane crops (Saccharum spp. hybrids) across Queensland, Australia, causing significant yield losses (Marquardt et al., 2016). Key YCS symptoms are a typical leaf

* Corresponding author.
** Corresponding author.
*** Corresponding author. Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW, Australia.
E-mail addresses: K.Hamonts@westernsydney.edu.au (K. Hamonts), Pankaj.Trivedi@colostate.edu (P. Trivedi), b.singh@westernsydney.edu.au (B.K. Singh).

https://doi.org/10.1016/j.soilbio.2018.07.019
Received 8 March 2018; Received in revised form 19 July 2018; Accepted 21 July 2018

yellowing that differs from symptoms due to drought stress, phytotoxicity, insect attack, known diseases, nutrient deficiency or natural maturation. Since the first observation of YCS in 2011, the condition has spread to all major cane growing areas and is a critical issue for the industry. Despite elimination of some potential causes, such as known Australian sugarcane pathogens, the causal agent of YCS remains unknown (Marquardt et al., 2016). This raised the question whether YCS is a consequence of reduced soil health, due to altered physico-chemical soil properties and/or detrimental shifts in soil microbial functions. YCS is associated with a typical yellowing of sugarcane leaves (Supporting Information Fig. S1), instigated by an increase in leaf sucrose levels triggering reduced leaf photosynthesis and stomatal conductance and disruption of the electron transport chain (Marquardt et al., 2016). The condition affects all varieties of sugarcane in Australia and appears to occur periodically from December onwards (Australian summer). A particular field site can thus be affected by YCS multiple times throughout the growing season, resulting in significant crop losses. Although YCS is yet to be reported from other sugarcane producing regions of the world, recent history with other plant diseases (Wang and Trivedi, 2013; Lang et al., 2017) suggests that YCS might quickly spread to other continents. Given the high global demand for sugar and biofuel, finding management strategies is critical to minimise YCS-linked productivity losses.

Healthy microbiomes can protect crops from biotic and abiotic stresses (Busby et al., 2017; Singh and Trivedi, 2017; Mendes et al., 2011). However, the key first steps required to harness microbiomes for reduced plant stress include (1) defining a healthy microbiome and comparative knowledge to distinguish it from a stressed plant microbiome, and (2) identifying the key microbial functional gene categories that drive differences between microbiomes of healthy and stressed plants. Therefore, understanding if and how the rhizosphere functional microbial community responds to YCS could provide important data that in the long-term might result in the development of diagnostic tools and identification of potential management strategies for YCS, including an improved microbiome-assisted breeding program, or a better prediction of management outcomes.

In this study, we hypothesised that YCS incidence promotes a loss of specific microbial mediated functions involved in nutrient cycling and plant disease suppression, and that these shifts result in a decline of overall soil nutritional health. To test our hypothesis, we investigated the response of the rhizosphere soil metagenome and functions to YCS occurrence. Our study had four interlinked objectives: (1) to systematically determine shifts in rhizosphere soil microbial assemblages with YCS incidence in three sugarcane varieties; (2) to examine changes in the rhizosphere soil metagenome and specific functional gene categories associated with YCS development; (3) to determine if soils of YCS symptomatic fields contained consistently lower amounts of essential soil nutrients such as carbon and nitrogen compared to asymptomatic fields; and (4) to investigate if soil microbial activity (as determined by soil extracellular enzyme activities and substrate-induced respiration) was lower in fields impacted by YCS. For this purpose, we collected rhizosphere soil samples from both asymptomatic and YCS symptomatic fields of three sugarcane varieties (Saccharum spp. hybrids KQ228^{A}, MQ239 and Q240^{A}) near Ingham in the Herbert growing region (Queensland, Australia) and assessed their physico-chemical properties, microbial community profiles, functional gene profiles and certain soil functions.

2. Materials and methods

2.1. Sample collection

Rhizosphere soil samples were collected from eight sugarcane fields growing three sugarcane varieties (Saccharum spp. hybrids KQ228^{A}, MQ239 and Q240^{A}) near Ingham, Queensland, Australia in November 2014 (Table S1). The sugarcane crop was in tillering phase at the time. For each sugarcane variety, field sites with typical YCS symptoms were carefully selected, as well as appropriate asymptomatic control fields of the same variety in close proximity, grown in the same soil type and under a similar management regime. In each asymptomatic field, three sugarcane clumps (stools) with healthy, green canopies were randomly selected, while in each YCS symptomatic field three stools with typical YCS symptoms were chosen. To remove potential edge effects, stools were not selected in the outer two sugarcane rows of a field. Stools were excavated from the soil, and rhizosphere soil samples were collected from underneath each stool. Rhizosphere soil was collected in two ways: (1) as the soil still attached to the roots after manually shaking them to remove loosely attached soil aggregates (duplicate samples per stool for molecular analysis); and (2) as the soil immediately underneath each excavated stool (one replicate per stool for molecular analysis, physico-chemical analyses and microbial activity measurements). Rhizosphere soil samples for molecular analysis (8 fields × 3 stools × 3 replicates: 72 in total) were snap-frozen in liquid nitrogen on the field, transported to the laboratory on dry ice and stored at −80 °C until analysis. Rhizosphere soil samples collected for physico-chemical analysis and soil functional measurements (8 fields × 3 stools: 24 in total) were collected in zip-lock bags, cooled on the field and stored at 4 °C in the laboratory until analysis. For this study, we focused on rhizosphere soil microbial communities as these are considered to play the most significant role on the overall fitness of plants (Busby et al., 2017; Singh and Trivedi, 2017).

2.2. DNA extraction and next-generation sequencing, quantitative PCR

DNA was extracted from the 72 rhizosphere soil samples collected for molecular analysis (Table S1) using the MoBIO PowerSoil® DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA), following the manufacturer's instructions.

For all 72 soil DNA extracts, amplicons targeting the bacterial 16S rRNA gene (341F-805R, Herlemann et al., 2011) and the fungal ITS2 region (FITS7-ITS4R, Ihrmark et al., 2012) were sequenced at the Western Sydney University NGS facility (Sydney, Australia) using Illumina® MiSeq 2 × 300 bp paired end sequencing. Raw amplicon sequence data related to this study are available in the NCBI Sequence Read Archive under Bioprojects PRJNA390435 (bacteria) and PRJNA390436 (fungi).

Shotgun metagenomics was performed on 24 selected rhizosphere samples (one sample from each of the three sampled stools per field site) at the Western Sydney University NGS facility (Sydney, Australia). Rhizosphere soil DNA sequencing libraries were prepared using the Illumina® Nextera DNA sample preparation kit, and the libraries were paired-end sequenced on three lanes of an Illumina HiSeq 2500 producing 125 bp read lengths. Raw sequence data were submitted to the European Nucleotide Archive under accession numbers PRJEB9951 and ERP011107.

Bacterial and fungal abundance in the rhizosphere soil samples selected for shotgun sequencing was estimated by qPCR of the bacterial 16S rRNA and fungal 18S rRNA genes. For amplification of bacterial 16S rRNA, primer set Ba519f/Ba907r was used with protocol described earlier (Lueders et al., 2004a). For fungal abundance, the 18S rRNA primer set fun5f/FF390r was used with protocol described by Lueders et al. (2004b). The qPCR products were amplified using a Rotor-Gene Q5plex Platform system (QIAGEN, Hilden, Germany).

2.3. Processing of amplicon sequence data

The quality of all Illumina R1 and R2 reads was assessed using FastQC (Andrews, 2010), low quality regions (Q < 20) were trimmed from the 5' end of the sequences using SEQTK (https://github.com/lh3/seqtk), and the paired ends were subsequently joined using FLASH (Magoc and Salzberg, 2011). Remaining primer sequences were removed from the resulting reads using SEQTK, and a further round of