Keystone microbial taxa regulate the invasion of a fungal pathogen in agro-ecosystems

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ABSTRACT

Uncovering potential soil drivers of soils pathogen suppression represent an essential step in order to develop alternative and sustainable management strategies for disease control and increased soil health. In this study, we tested the potential role of keystone microbial taxa and chemical/physical properties in the suppression (referred to as soil suppressiveness) of the soil-borne model pathogen *Fusarium oxysporum* using soil samples from various crop producing agro-ecosystems in Australia. Using random forest, we identified bacteria belonging to the phyla *Actinobacteria*, *Firmicutes* and *Acidobacteria* as the major microbial predictors for soil suppressiveness at a continental scale. Structural equation modeling approach revealed strong relationship between the relative abundance of phylum *Actinobacteria* and soil functions carried out by soil microbial communities (soil functioning) with pathogen inhibition. Overall our study provided a mechanistic framework showing how microbial communities, soil functionality, and abiotic properties being antagonistic to soil pathogens are linked and interactively shape the suppressive potential of soils at continental scale. This information, upon further validation can be incorporated in risk management tools for developing novel concepts such as "*Know before you Sow*" leading to increased farm productivity and profitability.

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Soil-borne plant pathogens, in particular fungi, are a serious threat to agricultural production worldwide and can cause losses up-to 20–35% in some production systems (Weller et al., 2002; Strange and Scott, 2005; Raaijmakers et al., 2009). However, farmers are being challenged to use less pesticides and fumigants and to apply more sustainable cropping practices (Weller et al., 2002). The intrinsic ability of soils to suppress pathogens is an important yet underutilized resource that can be exploited for controlling soil-borne pathogens (Schroth and Hancock, 1982; Mendes et al., 2011; Cha et al., 2016; Latz et al., 2016). Soil suppressiveness is interactively shaped by various factors such as abiotic properties, management practices, and the structure and activities of indigenous microbial communities (including their direct antagonistic effect on the pathogen or indirect effect mediated through maintenance of several key ecosystem functions) (Garbeva et al., 2004; Berg and Smalla, 2009; Philippot et al., 2013; van Elsas et al., 2012; Santhanam et al., 2015; Latz et al., 2016; Raaijmakers and Mazzola, 2016). A mechanistic framework for predicting the suppressive (or stimulatory) potential of soils is required to provide management options and measures to reduce the impact of soil-borne pathogens. However, the existing knowledge gap on the major drivers of soil suppressiveness both at local and regional scales limits our ability to develop such management tools (Dignam et al., 2016). Despite extensive research on the molecular mechanisms involved in disease suppression by bacteria (Haas and Défago, 2005; Berg and Smalla, 2009; Mendes et al., 2011; van Elsas et al., 2012; Chapelle et al., 2016), there is still a lack of knowledge on the identity of keystone microbial species that regulate the rates of pathogen invasion in soils (Philippot et al., 2013; Latz et al., 2016). This information is crucial for sustaining both plant health and soil fertility and could provide a potential target for novel agricultural management strategies.

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Here, we aim to: (a) identify the most significant bacterial groups involved in soil suppressiveness; and (b) disentangle pathways to identify drivers of soil suppressiveness at a continental scale. We hypothesized that: (a) specific members of the keystone bacterial taxa will have a direct impact on suppressiveness (measured in terms of pathogen inhibition); and that (b) abiotic factors and microbial diversity will affect soil suppressiveness indirectly via regulation of the ability of soils to support soil functioning.

To test our hypotheses, we collected soil samples from major crop producing agro-ecosystems in Australia (n = 24; Supplementary Fig. 1). We characterized the structure (diversity) and composition (relative abundance) of the soil bacterial community using MiSeq and qPCR analysis (abundance only). We analyzed soil bacteria as they constitute the most dominant microbial communities in agricultural ecosystems (van Der Heijden et al., 2008) and have a very strong direct and indirect effect on pathogen establishment (Wei et al., 2015; Latz et al., 2016). Soil properties (including total C, pH) and functions (soil enzymatic activities) were determined by methods described in Trivedi et al. (2016). We calculated a soil functioning index by using the activity of seven enzymes involved in nutrient turnover as described by Delgado-Baquerizo et al. (2016). We then set up microcosm experiments and introduced *Fusarium oxysporum* as a model pathogen in different soil samples. We chose *F. oxysporum* as this a complex of host-specific forma specialis causing root-rots and vascular wilts in many crops around the world (Lockwood, 1986). In our study, we used *F. oxysporum* f. sp. *cucumerinum* J.H Owen that is responsible for vascular wilt in cucumber plants (*Cucumis* sp.; Owen 1955). Pathogen abundance over time was determined by pathogen specific qPCR analysis (Scarlett et al., 2013) and percent inhibition of the pathogen in different soils was calculated. In the microcosms, we noticed a sharp decrease in the pathogen numbers for most of the soil samples within 14 days. However, for most soil samples, the numbers stabilized after this time period as there were no significant differences in the pathogen populations between 14 and 21 days and therefore percent reduction in the pathogen populations was determined by using the data obtained on 21st day after initial inoculation (de Boer et al., 2003). We further used statistical modeling approaches viz. Random Forest (RF) and Structure Equation Modeling (SEqM, for *a priory* modeling see Supplementary Fig. 2) to assess the major drivers of soil suppressiveness across a continental scale. Details of the experimental set up and analysis are provided in Supplementary Material and Methods section.

Overall our sampling sites varied considerably in their soil properties (Supplementary Fig. 3a) and soil bacterial community structure and composition (Supplementary Fig. 3b) and thus provide an excellent framework to test our hypothesis. Due to the high taxonomic diversity in the soil microbial communities there can be large numbers of potential predictors for soil suppressiveness. We therefore conducted random forest (RF) analysis based on the relative abundance of major phyla (or classes for *Proteobacteria*) to identify the most important microbial groups involved in soil suppressiveness. Our RF models indicated that bacteria belonging to the phyla *Actinobacteria, Firmicutes* and *Acidobacteria* were the major predictors for soil suppressiveness in crop producing agro-ecosystems of Australia (Fig. 1a). Our analysis further showed that bacteria belonging to phyla *Actinobacteria* ($R^2 = 91.26$; $P < 0.001$) and *Firmicutes* ($R^2 = 45.01$; $P < 0.001$) were positively correlated with pathogen inhibition. Many culture based studies have shown that different members of these bacterial groups can directly antagonize pathogens through various mechanisms including production of antibiotics; volatiles; and siderophores (Chaurasia et al., 2005; Cha et al., 2016). Recent metagenomic based studies

have also provided strong linkages between the relative abundance of these groups and disease suppression (Mendes et al., 2011; Cha et al., 2016). These studies were however based on observations at local scales that limit our ability to make generalizations for predicting and manipulating the disease suppressive potential of soils. In this context our continental scale results represent an important step forward in evaluating the importance of soil taxa for soil suppressiveness.

Interestingly our RF models did not implicate bacteria belonging to *Gammaproteobacteria* and *Alphaproteobacteria* as significant drivers of soil suppressiveness in our soil samples (Fig. 1a). Various members of these groups have been studied in detail for their role in pathogen inhibition and development of disease suppression in soils in the past (Pandey et al., 2006; Weller, 2007; Trivedi et al., 2008, 2011). This apparent discrepancy may be due to two reasons: (a) bacteria belonging to *Gamma-* and *Alphaproteobacteria* are more abundant in the rhizosphere whereas we analyzed bulk soil samples as we were interested in determining the intrinsic potential of soils to suppress disease without the influence of plant systems; (b) these groups of bacteria might be involved in local soil suppressiveness and therefore were not a major predictor when a variety of soils collected across a continental scale were analyzed. In line with our results, after considering various criteria related to disease suppression, Cha et al. (2016) reported that *Actinobacteria* was the single phylum directly involved in disease suppression of vascular wilt. Recently, Latz et al. (2016) have also reported that the abundance of members of *Gammaproteobacteria* was not directly linked to soil suppressiveness, but their positive correlation with *Actinomyces* density make them appear significantly linked to disease suppression in a linear regression. Our analysis further revealed that bacteria belonging to phylum *Acidobacteria* were negatively correlated with the inhibition of pathogen in soils (Fig. 1d). To the best of our knowledge, members of *Acidobacteria* have not yet been reported for their role in disease suppression. In general, both fungi and *Acidobacteria* favors acid soils (Jones et al., 2009; Rousk et al., 2009) and therefore it is not unexpected that members of *Acidobacteria* are more numerous in soils where the pathogen is also abundant. Further, our RF analysis at lower taxonomic levels of *Actinobacteria* or *Firmicutes* showed that different families within these groups are important predictors of soil suppressiveness (Supplementary Fig. 4). This information can be used to increase the abundance of members of these groups through artificial inoculation or change in management practices that can lead to an increase in disease suppression potential.

We used structural equation modeling to build a system-level understanding of the main drivers of soil pathogen invasion accounting for direct and indirect effects of abiotic (soil C and pH); biotic [including (a) relative abundance based on MiSeq analysis of bacterial phyla that were important predictors for soil suppressiveness as determined by RF; (b) Shannon diversity calculated using MiSeq dataset; and (c) the abundance of total bacteria measured by qPCR analysis]; and soil functioning (i.e. enzyme activities) on soil suppressiveness (see Fig. 2). Our SEqM explained 94% of the variance found in the disease suppression ability of soil from Australian agro-ecosystems (Fig. 2a). We found that the relative abundance of *Actinobacteria* had the most significant and direct effect on pathogen inhibition. This effect was independent and was maintained even after considering the role of other biotic and abiotic components on pathogen inhibition.

Soil microbial groups can either directly impact pathogen inhibition by antagonizing the pathogens or indirectly by maintaining soil health via mediating processes involved in nutrient dynamics (Pandey et al., 2006; Lugtenberg and Kamilova, 2009; Trivedi et al., 2011). In accordance with our initial hypothesis, SEqM analysis revealed that soil functioning had a direct positive and significant