Fungal networks in yield-invigorating and -debilitating soils induced by prolonged potato monoculture

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Abstract

Most previous studies on soil microbial communities have been focused on species abundance and diversity, but not the interactions among species. In present study, the Molecular Ecological Network Analysis tool was used to study the interactions and network organizations of fungal communities in yield-invigorating (healthy) and -debilitating (diseased) soils induced by prolonged potato monoculture, based on the relative abundances of internal transcribed spacer sequences derived using pyrosequencing. An emphasis was placed on the differences between the healthy and diseased networks. The constructed healthy and diseased networks both showed scale-free, small world and modular properties. The key topological properties and phylogenetic composition of the two networks were similar. However, major differences included: a) the healthy network had more number of functionally interrelated operational taxonomic units (OTUs) than the diseased one; b) healthy network contained 6 (4%) generalist OTUs whereas the diseased contained only 1 (0.6%) marginal generalist OTU; and c) majority (55%) of OTUs in healthy soils were stimulated by a certain set of soil variables but the majorities (63%) in diseased soils were inhibited. Based on these data, a conceptual picture was synthesized: a healthy community was a better organized or a better operated community than the diseased one; a healthy soil was a soil with variables that encouraged majority of fungi whereas a diseased soil discouraged. By comparing the topological roles of different sets of shared OTUs between healthy and diseased networks, it was found that role-shifts prevailed among the network members such as generalists/specialists, significant module memberships and the OTU sets irresponsive to soil variables in one network but responsive in the counterpart network. Soil organic matter was the key variable associated with healthy community, whereas ammonium nitrogen (NH4+ −N) and Electrical conductivity (EC) were the key variables associated with diseased community. Major affected phylogenetic groups were Sordariales and Hypocreales.

1. Introduction

In most natural environments such as soils, individual organisms do not live in isolation but rather form a complex system of inter-species interactions that, to a large extent, determine the structure of an ecological community (Freilich et al., 2010), and consequently the function of the ecosystem (Fuhrman, 2009). However, interactions and the resulting ecological functions are usually difficult to elucidate, especially for soils. Furthermore, most previous analytical techniques can be used to describe community composition, diversity and their changes across space, time, or experimental treatments, but cannot be used to reveal interactions among community members, which could be more important to ecosystem functioning than abundance and diversity, especially in complex ecosystems (Deng et al., 2012).

Network analysis is proven to be a powerful tool in revealing the interactions among entities in a system, such as individuals in a school (Moody, 2001), species in food webs (Krause et al., 2003; Woodward et al., 2012), nodes on a computer network (Pastor-Satorras and Vespignani, 2001; Volchenkov et al., 2002), proteins
in metabolic pathways (Brohée et al., 2008; Guimerà and Amaral, 2005), and genes in regulatory networks (Crombach and Hogeweg, 2008). Yet, until recently, researchers began to use this tool to study complex microbial ecological systems such as marine bacterioplankton (Gilbert et al., 2012), global environments in 16S rRNA dataset (Chaffron et al., 2010), fully sequenced bacterial species (Freilich et al., 2010), dental biofilm (Duran-Pinedo et al., 2011), human microbiome (Faust et al., 2012; Greenblum et al., 2012), bacterial communities in variety of soil samples (Barberan et al., 2012), and the communities in soils influenced by elevated CO₂ (Deng et al., 2012; Zhou et al., 2010, 2011). Despite its pitfalls (Faust and Raes, 2012), the power and usefulness of network analysis in revealing new information on community member interactions, community organizations, keystone organisms, and their responses to environmental factors that cannot be revealed by routine analytical techniques is unequivocally demonstrated. For example, Zhou et al. (2011) demonstrated that Actinobacteria were the keystone bacteria connecting different co-expressed OTUs and were significantly correlated with selected soil variables. Similarly, Faust et al. (2012) predicted novel interactions involving members of under-characterized phyla, providing valuable information on further co-culturing of these organisms. Further, Duran-Pinedo et al. (2011) were even able to identify a helper bacterium successfully helping an uncultured bacterium to grow in petri dishes. Network analysis probably represents a new direction in microbial ecology research (Zhou et al., 2011).

Crop monoculture has long been considered un-sustainable as it often leads to yield decline (Shipton, 1977). The yield decline usually occurs after two or three years of monoculture (as in this study), depending on crops, number of years and soil, and is usually attributed to the increase of yield-debilitating populations and switches of underground microbial communities (van Elsas et al., 2002). However, to date, the questions, such as what species compose yield-debilitating soil microbial community, how a yield-invigorating community is shifted to a yield-debilitating one, and what are the key soil factors responsible for the shift remain unclear. By farmers’ term, the soils under limited length of monoculture (2–3 years) still producing sound yields are called “healthy” soils whereas those under prolonged monoculture producing unacceptably low yields are called “diseased” soils. Because the farmers’ terms “healthy” and “diseased” are simpler than yield-invigorating and yield-debilitating respectively, they are adopted hereafter for concise purpose.

The purpose of present study is to address these questions by a network analysis approach, using the “healthy” and “diseased” soils induced by prolonged potato monoculture as model soils. It has long been recognized that the yield decline under prolonged monoculture is associated with soilborne pathogens (Shipton, 1977), many of which are fungi (Fiers et al., 2012). In present study, major potato diseases found in field included fusarium dry rots, late bright and black scurf/stem canker that are associated with Fusarium sps., Phytophthora infestans, Rhizoctonia solani respectively, all of which are soilborne fungal pathogens (Fiers et al., 2012). Soil fungal community is thus the focus of present study. We hypothesized that a healthy community is likely to be better organized or better operated than a diseased community with respect to network organization and keystone organisms.

2. Materials and methods

2.1. Field experiment description

The experimental sites were located in Taoshan Farm (103°33′–104°43′E; 36°43′–37°38′N), Gansu Province, China. It is a warm terrestrial arid area, with a mean annual temperature 9.1 °C, a mean annual precipitation 185.6 mm, and a mean annual evaporation capacity 1722.8 mm. Mean annual frost-free days are 141 days, sustaining only a single crop (corn or potato) per year. The soil contains 10.1 kg⁻¹ organic matter, 0.71 kg g⁻¹ total N, 66 mg kg⁻¹ alkaline hydrolyzable N, 14 mg kg⁻¹ Olsen-P, and 193 mg kg⁻¹ extractable K, with pH 8.08 (5:1 water to soil ratio).

Field experiment began in 2005 on fields under corn-potato rotations and was designed to collect year-series soil samples in the year 2011. For this, the field was divided into 21 plots, each being 9 × 6.1 m in size. Three plots were randomly selected each year for potato monoculture, leaving other plots to continue corn-potato rotation. The selection was done in such a way that the previous crop of selected plots was always corn. After 7 years (by year 2011), 21 plots in total were used up (3 replicates × 7 years). This experiment design provides opportunity to collect soil samples after culturing mono-crop from 1 to 7 years simultaneously.

Potato was typically seeded on April 25 every year with a few days variation. Seed pieces (Atlantic cultivar, provided by Taoshan Farm) were buried on the top of raised paths (~40 cm in height and 135 cm in bottom width) at 17 cm in between-plant space. Two lines were planted on each raised path with 70 cm in between-line space, resulting in a plant density at 84,075 plants ha⁻¹. Blended fertilizer (15-15-15) additionally supplemented with urea and K₂SO₄ was used at the rate of 210 kg N ha⁻¹, with the ratio N:P₂O₅:K₂O at 1:4:1.0:2.0. Nitrogen form in blended fertilizer is (NH₄)₂SO₄. All fertilizers were applied at the time of seeding by machine. Once seeded and fertilized, the raised paths were covered with plastic film. The field was irrigated three times during growth period, typically on June 1 (seedling stage), July 1 (early flowering stage) and July 20 (tuber enlargement stage), at the rate of 2700 t ha⁻¹ each time. Potato was harvested in late August.

2.2. Soil sampling, variable measurements and grouping

Soil samples were collected in 2011 from 18 plots. The plots set up for potato monoculture in 2007 (5 years) were not sampled. From each plot, 15 sites were randomly sampled for 0–20 cm layer soils and well mixed. Totally 18 samples were obtained. Samples were put into sterile plastic bags, placed into ice box, transferred to laboratory and used as soon as possible, or stored in a refrigerator at ~80 °C if not immediately used. Selected soil variables included organic matter (OM, by dichromate oxidation), total nitrogen (TN, by total Kjeldahl N), NH₄⁺–N, NO₃⁻–N (by 1 M KCl extraction), pH and electrical conductivity (EC) (both at 5:1 water soil ratio).

The yield decline typically started at the fourth year and the yield records of recent two years are shown in Fig. S1. The yields in first three years were more or less the same (with yearly variations) and are within the yield range of local farmers who practice rotations (30–40 t ha⁻¹). A sudden decline occurred at the fourth year and thereafter, which is far below the yield range of local farmers. Based on these results, soil samples were put into two groups, one including the first year, the second year and the third year samples (9 in total), which was herein termed as “healthy” group, and another including the fourth year, the sixth year and the seventh year samples (9 in total) termed as “diseased” group. This grouping allowed us to construct and compare networks between healthy and diseased fungal communities. Soil variables and their statistics based on such grouping are shown in Table S1.

2.3. DNA extraction, amplification, sequencing and sequence treatment

For each soil sample, bulk DNA was extracted in triplicate from 0.5 g of soils (dry weight basis) with a FastDNA SPIN Kit for
