ELSEVIER

Soil Biology & Biochemistry 39 (2007) 1139–1155

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Discriminating the effects of agricultural land management practices on soil fungal communities

Tiehang Wu[a,b,*], Dan O. Chellemi[a], Kendall J. Martin[c], Jim H. Graham[b], Erin N. Rosskopf[a]

[a]*USDA-ARS, US Horticulture Research Laboratory, Fort Pierce, FL 34945, USA*
[b]*University of Florida, Citrus Research and Education Center, Lake Alfred, FL 33850, USA*
[c]*Department of Biology, William Paterson University, Wayne, NJ 07470, USA*

Received 21 September 2006; received in revised form 25 November 2006; accepted 30 November 2006
Available online 12 January 2007

Abstract

The structure of fungal communities was examined in soil subjected to 5 years of different agricultural land management and tomato production practices. Length heterogeneity polymerase chain reaction (LH-PCR) of fungal rDNA internal transcribed spacer-1 (ITS-1) regions was used to create genomic fingerprints of the soil fungal communities. Three years after initiation of land management practices, univariate analysis of genetic diversity failed to detect differences among soil fungal communities in plots managed organically, conventionally or maintained free of vegetation by continuous tillage (disk fallow). Genetic diversity was significantly higher in plots maintained as a perennial pasture grass (*Paspalum notatum* var Argentine bahiagrass) or as an undisturbed weed fallow. The composition of soil fungal communities within organic, pasture grass or disk fallow plots were separated into unique clusters by non-parametric multivariate analysis of their Bray-Curtis similarity matrices, computed from the relative abundance of ITS-1 amplicons, while the composition of communities within disk fallow and conventional plots could not be distinguished from each other. Diversity of soil fungal communities was significantly reduced following the cultivation of tomato in year four when compared to the diversity in plots where tomato was not cultivated. Divergence in the composition of soil fungal communities was observed following the cultivation of tomato under all land management regimes except organic, where communities continued to remained clustered based upon similarities among their ITS-1 amplicons. Divergence in the composition of fungal communities became more pronounced following two major hurricanes (Francis and Jeanne, September 2004) except for communities in the organic and pasture grass plots. Following the completion of a second tomato crop in year 5, genetic diversity and richness was similar under all land management regimes except the pasture grass, where it remained significantly higher. By contrast, following two consecutive years of tomato production, unique but mutually similar compositions of fungal communities were detected only in plots subjected to the organic land management regime. This was supported by observations that fungal communities were dominated by a 341 bp rDNA amplicon fragment in all land management regimes except the organic. Cloning and sequencing indicated that the 341 bp fragment generated by LH-PCR had a sequencing size of 343 bp, which was most closely related to *Fusarium oxysporum*. Thus, land management practices that disturb or disrupt soil fungal communities will significantly reduce their diversity. However, the composition of soil fungal communities is more strongly influenced by land management practices and communities within an organically management system were more resistant to anthropogenic and meteorological disturbances.

Keywords: Fungal community; ITS of rRNA gene (rDNA); Length heterogeneity polymerase chain reaction (LH-PCR); Land management practices; Univariate analysis; Multivariate analysis

1. Introduction

Soil fungi play a critical role in regulating terrestrial ecosystems. Carbon flow is modulated through their effects on organic matter decomposition and nutrient cycling (Went and Stark, 1968; Toal et al., 2000). Plant population

*Corresponding author. USDA-ARS, US Horticulture Research Laboratory, 2001 South Rock Road, Fort Pierce, FL 34945, USA. Tel.: +1 772 462 5849, fax: +1 772 462 5986.
E-mail address: twu@ushrl.ars.usda.gov (T. Wu).

0038-0717/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2006.11.024

dynamics are regulated through symbiotic and parasitic associations between roots and fungi (Bever, 2003). Physical environments of soils are influenced through the effects of fungi on soil aggregation (Beare et al., 1997; Tisdall et al., 1997). In agroecosytems, attention is often focused on plant pathogenic soil fungi due to their immediate economic impacts. In high-value crop production systems, plant pathogenic soil fungi have become an increasing concern due to the impending phase-out of the soil fumigant methyl bromide (Ajwa et al., 2003; Martin, 2003; Rosskopf et al., 2005). Considerable research effort has been focused on the short-term goal of identifying alternative chemical fumigants for soil disinfestation. However, this approach does not address the root cause for epidemics of plant diseases caused by soil fungi and negates the contribution of soil fungi to the regulation of soil ecosystems. A more sustainable, long-term approach is to develop crop production systems that mitigate outbreaks of plant disease, thus reducing the need for intervention and disruption of the agroecosystem.

Agricultural land management practices can greatly influence outbreaks of plant disease caused by soilborne fungi. Organic soil amendments, tillage practices and rotational crops have all been used by farmers to reduce the incidence of plant disease caused by soil fungi (Abawi and Widmer, 2000; Bockus and Shroyer, 1998; Chellemi, 2000; Lazarovits, 2001). While their effects on populations of the plant pathogens have been adequately described, information regarding their impacts on soil fungal communities is paramount to the creation of crop production systems that minimize disruption of the agroecosystem in addition to mitigating pest outbreaks.

The density of fungal propagules in soil subjected to various land management programs has been examined. Total fungal propagules and fungal hyphal biomass were found to be higher in soil collected from organically managed crop production systems when compared to soil from conventionally managed systems (Elmholt and Kjøller, 1989; Fließbach and Mäder, 2000; Shannon et al., 2002; Sivapalan et al., 1993). Crop rotation, soil tillage and animal grazing were all found to affect the density of fungi in soil (Beare et al., 1992; Frey et al., 1999; Hedlund, 2002; Martyniuk and Wagner., 1978; Mazzola, 1999; Ploetz et al., 1985; Singh and Rai, 2004; Wicklow, 1973). Soil fumigation was shown to decrease the density of soil fungi but the effect was temporary (English and Mitchell, 1988; Marois and Mitchell, 1981; Sivasithamparam et al., 1987; Tanaka et al., 2003). Information generated by these studies was limited by the methods used to detect soil fungi and describe their community attributes.

Isolation of soil fungi in the previous studies was based upon culture-dependent methods. However, it has been estimated that only a small amount of known fungal species can be successfully grown in culture (Bridge and Spooner, 2001; Hawksworth, 1991). This excludes the identification of many important soil fungi including arbuscular mycorrhizal fungi and some saprophytic basidiomycetes (Thorn et al., 1996; Warcup, 1955). Also, the propensity for fast-growing fungi to outgrow more fastidious species, due to the selection of the culture medium (Crosby and Criddle, 2003), can mask the identification of some species. Finally, the culture medium and incubation conditions may not represent the growth conditions of fungi in their native habitat, leading to elevated population numbers (Warcup, 1955).

In recent years, culture-independent, molecular methods have revealed an extraordinary diversity of soil microorganisms (Anderson and Cairney, 2004; Kirk et al., 2004; Ward et al., 1990). Among the molecular techniques, polymerase chain reaction (PCR)-based approaches are widely used as culture-independent methods and include PCR-denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP) and length heterogeneity PCR (LH-PCR). PCR-DGGE does not require expensive instrumentation such as access to a genetic sequence analyzer and it offers an opportunity to directly excise and sequence DNA bands. However, migration of DNA fragments in PCR-DGGE is influenced by the selection of gels and it is less successful than T-RFLP at detecting and tracking specific ribotypes (Brodie et al., 2003; Kirk et al., 2004; Singh et al., 2006; Tiedje et al., 1999). T-RFLP has been widely used in soil bacterial community analyses and has proven to be a reproducible and robust method for microbial community analysis (Osborn et al., 2000). Restriction analysis of PCR products produces pseudo-terminal restriction fragments in TRFLP (Egert and Friedrich, 2003). LH-PCR analysis depends on natural variations in the length of selected sequences of ribosomal DNA (rDNA) without the restriction enzyme digestion (Ranjard et al., 2001; Ritchie et al., 2000; Suzuki et al., 1998) and thereby circumvents the problem of pseudo-terminal restriction fragments formed in T-RFLP. LH-PCR was able to better describe soil bacterial communities than T-RFLP in a recent study (Mills et al., 2003). While PCR-DGGE, T-RFLP and LH-PCR methods have been widely used in studies investigating the impacts of agricultural land management practices on bacterial communities, their application to studies on fungal community analysis has been limited. Fungal diversity in soil subjected to conventional and precision farming systems was studied using PCR-DGGE (Hagn et al., 2003b). The community structure of basidiomycete and ectomycorrhizal communities in soil subjected to different silvicultural practices was studied using PCR-DGGE (Edwards et al., 2004; Smit et al., 2003). Changes in soil fungal community structure from various soils were distinguished by T-RFLP and LH-PCR (Edel-Hermann et al., 2004; Ranjard et al., 2001). Anderson and Cairney (2004) reviewed recent advances and potential limitation of molecular techniques used to advance the understanding of soil fungal communities. While no single set of fungal primers or profiling techniques can be universally applied for assessing fungal communities in complex environmental samples, they may still contribute to understanding the