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Changes in microbial communities in an apple orchard and its adjacent bush soil in response to season, land-use, and violet root rot infestation

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Abstract

Soil microbial communities in an apple orchard and its adjacent boundary bush with or without infestation by violet root rot were investigated for 2 years. Effects of season (spring, summer, and fall), land-use (apple orchard and boundary bush), and violet root rot (infested and healthy) on soil microbial populations, microbial activity, and microbial community structures were determined using physiological, cytochemical, and molecular (PCR-DGGE) approaches. Seasonal fluctuations were significant ($P<0.05$) in viable bacteria and fungal populations, bacterial FAME, fluorescein diacetate (FDA) hydrolysis, and diversity (H') and evenness (J') of community-level physiological profile (CLPP) in both years. However, seasonal differences of soil microbial guilds that utilize carbon substrate groups observed in the first year were not reproduced in the second year. The land-use factor differentiated the apple orchard from the boundary bush where viable bacterial population, bacterial FAME and FDA hydrolysis were significantly greater in both years. Infestation status of violet root rot, on the other hand, significantly increased bacterial FAME and FDA hydrolysis in both years. In addition, neither the land-use nor the disease infestation factor significantly influenced the utilization patterns of individual substrate guilds for the 2 years. In both years, saturated fatty acids were significantly more abundant in the orchard than in the bush soil, and monosaturated fatty acids vice versa. Principal component analyses for CLPP, FAME, and denaturing gradient gel electrophoresis (DGGE) consistently exhibited that, although the violet root rot influenced the soil microbial community structures both in the apple orchard and the boundary bush, overall magnitude of the difference in communities between the violet root rot infested and non-infested sites in the bush were greater than in the orchard, irrespective of the season. These results suggested that the seasonal and the land-use factors affected soil microbial community both quantitatively and qualitatively, whereas the impact of the violet root rot on the soil microbial community was mainly qualitative and more pronounced in the adjacent bush than in the orchard.

Keywords: Soil microbial community; Apple orchard; Boundary bush; Violet root rot; Season

1. Introduction

Violet root rot is a major root disease caused by a soilborne basidiomycete, *Helicobasidium mompa*. The fungus may be pathogenic to various plants extending 45 families, 96 genera, and 1104 species. Many of the hosts are perennial, including apple, pear, peach, tea, and forest trees, causing severe economic damages every year in Japan (Suzuki et al., 1957). Violet root rot has been known to be more commonly found in newly pioneered orchards of these tree crops near forests where the fungus is supposedly originated than in old fields with a long cultivation history (Fujita, 1992). Therefore, boundary lands surrounding orchards may play an important role for ecology of the pathogen as well as development of the disease.

Boundary lands at the margins of arable fields have significant impacts on farmland biodiversity (Marshall et al., 2006). Various interactions of a broad range of organisms between fields and adjacent boundary lands

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have been described. For example, fertilizer and pesticide application affect the growth of boundary flora (Kleijn and Snoeijing, 1997), and some boundary flora spread into crop fields as weeds (Sparkes et al., 1998). Boundary lands also exhibit a series of associated fauna, some of which might be pest species, whereas others are beneficial including crop pollinators (Lagerlöf et al., 1992) and pest predators (Marshall et al., 2006).

Soil microbial communities are increasingly valued for their role in agro-ecosystem sustainability (Brussaard et al., 2007). Soil microorganisms serve various roles in decomposition of organic matter, nutrient cycling, and plant nutrient availability, and are in turn influenced by plant species, crop management, and abiotic conditions (Zaady et al., 1996; Jones, 1998; Calderón et al., 2000). In addition, field boundaries can provide habitats for plant pathogens and/or beneficial microorganisms to control those pathogens. Nevertheless, little is known about the status of soil microbial communities in boundary lands adjacent to agricultural fields.

It is a challenging task to find appropriate methodologies for fully characterizing and evaluating soil microbial communities. Since only a small fraction of soil microorganisms is able to be cultured (Ward et al., 1990), culture-dependent methods for microbial populations provide limited information about soil microbial communities. However, some useful methods that are independent from culture assays have been developed recently for investigating microbial communities. Such methods include molecular analyses of nucleic acids extracted from soil, such as denaturing gradient gel electrophoresis (DGGE) after PCR (e.g., Niemi et al., 2001; Smalla et al., 2001; Dierksen et al., 2002; Ibekwe et al., 2002), and community profiling based on fatty acid methyl esters (FAME) (e.g., Cavigelli et al., 1995; Ibekwe and Kennedy, 1999; Dierksen et al., 2002; Larkin, 2003). In addition, carbon-based substrate utilization patterns have also been widely utilized for describing community-level physiological profiles (CLPP) using Biolog plates (e.g., Garland and Mills, 1991; Zak et al., 1994; Widmer et al., 2001; Larkin, 2003). Although the CLPP method still depends on culturing microorganisms, it can provide valuable information on the functional aspects of the targeted communities. Since no single approach can describe an entire microbial community, a combination of the polyphasic data sets generally yields a better understanding of the community (Widmer et al., 2001).

To determine practical ways of managing the soilborne diseases and microbial environment, it is of paramount importance to understand effects of crop fields and their boundary areas on soil microbial communities. In this study, we aimed to characterize soil microbial communities of an apple orchard and its adjacent boundary bush under the influence of violet root rot in three seasons for two consecutive years. We used multiple applications of CLPP, FAME, and PCR-DGGE combined with conventional microbial population assessment by dilution plating and fluorescein diacetate (FDA) hydrolysis. Our specific objective of this study was to investigate the impact of season, land-use, and root disease infestation on the microbial community indigenous to the soil.

2. Materials and methods

2.1. Field sites and soil sampling

The research sites were established in an apple orchard and its adjacent boundary bush at the Chiba University experimental field at Numata-shi, Japan (36°38′N, 139°02′E, and 750 m altitude). The climate of this region is a temperate monsoon with an average annual air temperature and precipitation of 10.4 °C and 1100 mm, respectively. The average air temperature and precipitation during the sampling months were April, 10.9 °C and 78.7 mm; August, 24.1 °C and 184.1 mm; September, 19.9 °C and 214.8 mm; and November, 8.5 °C and 42.7 mm, respectively. The southeastern area of the orchard (*ca.* 0.2 ha) has been infested for more than 10 years with *H. mompa*, a casual agent of violet root rot.

The root disease sites were established at the infested area of the orchard and the adjacent bush. Orchard soil samples were collected near the bases of three randomly selected apple trees (*Malus domestica*, cv. ''Youko''). These trees were spaced 9–18 m apart. Dark violet rhizomorphs that are typical of *H. mompa* infection were observed at the basal trunk and roots near the ground surface in November 2003 and thereafter every year. The adjacent boundary bush site corresponding to the orchard site was established approximately 5 m from the edge of the orchard near the bases of oak trees (*Quercus serrata*). For the healthy control, three orchard and three bush sites were similarly established in areas with no evidence of *H. mompa* infestation. The healthy sites were located approximately 30 m from the closest diseased tree. Vegetation of the bush sites was similar between the diseased and healthy sites, consisting of *Q. serrata*, *Cornus controversa*, *Castanea crenata*, *Lindera praecox*, *Corylus sieboldiana*, *Rhododendron kaempferi*, and *Kerria japonica*. Triplicate soil subsamples (*ca.* 100 ml) were collected from the ground near each tree, <1 m from the trunk and *ca.* 20 cm depth, in the spring (April), the summer (August or September), and the fall (November) of 2004 and 2005. Soil subsamples from each tree were mixed, sieved (<2 mm) and stored at 4 °C for 1–3 days until analyses.

2.2. Soil chemical analyses

Soil classification of the sites is Umbric Andosol, and the soil texture is loam. Soil pH was measured in deionized water (1:5, soil:water) and in KCl (0.01 M) solution. The total nitrogen and carbon contents of the samples were determined using a NC-analyzer (Sumigraph NC-900; Sumika Bunseki Center Co., Tokyo, Japan). Total P was measured colorimetrically using the molybdate method