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Below ground microbial diversity as influenced by coffee agroforestry systems in the Western Ghats, India

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ABSTRACT

Soil microorganisms *viz.* bacteria, fungi, actinomycetes and arbuscular mycorrhizal (AM) fungi present in different typologies of coffee production systems were compared. In this study, two types of coffee plants, namely Arabica and Robusta, were grown under different agroforestry management such as coffee under one specialized shade species, multi-story coffee systems with 2 shade tree species, and coffee with 3 or more tree species under moist deciduous and evergreen ecological conditions. Samples were collected from 36 points to include different coffee ecosystems. The highest number of infective propagules of AM fungi was encountered in Arabica coffee under evergreen conditions. Population of bacteria, fungi and actinomycetes were higher under evergreen ecosystem compared to that of deciduous conditions. The population of nitrogen fixing bacteria was more than double in evergreen conditions compared to deciduous ecosystem. Number of lignin decomposing bacteria was higher in evergreen compared to deciduous conditions, but starch hydrolyzing bacteria and pectin-utilizing bacteria were more in deciduous ecosystem. Actinomycetes DAT2-1 isolated from deciduous ecosystem showed antagonistic activity against the root pathogen *Fusarium chlamydosporum*. It can be concluded that evergreen coffee system supports higher population of microorganisms. Of the two species of coffee, Arabica harboured more AM fungi, bacterial population, N fixers, P solubilizers and cellulose decomposing organisms while Robusta harboured higher number of fungi and actinomycetes. Of the three typologies, coffee grown under two shade tree species supported higher population of all microorganisms.

1. Introduction

Coffee is an important commercial crop grown in 50 developing countries around the world. Each day nearly 2.5 billion cups of coffee are consumed. It is the 5th most widely traded commodity in the world and millions of people depend directly or indirectly on the production and sale of coffee for their livelihoods. The coffee plant is a woody evergreen perennial that belongs to the Rubiaceae family. While there are several different coffee species, two main species of coffee are cultivated today. *Coffea arabica*, known as Arabica coffee, accounts for 75–80% of the world's production. *Coffea canephora*, known as Robusta coffee, accounts for about 20–25% and differs from the Arabica coffee in terms of taste. The requirement of shade for coffee cultivation is dictated by the ecological conditions prevailing in that country/region. Coffee in India is grown under natural and highly diverse shade canopy of trees (Garcia et al., 2010). This is because of wide fluctuations in light intensity, air temperature and humidity along the year. High light intensity and day temperatures during summer months would affect the photosynthesis efficiency of coffee plants and lead to water and heat stresses. The optimum light intensity for maximum photosynthetic efficiency of coffee plants is reported to be in the range of 900–1300 einsteins (Franck and Vaast, 2009). By providing shade canopy through trees and regulating growth of their canopy, it is possible to ensure desired light intensities and microclimate conditions to the coffee bushes during different times of the year (Vaast et al., 2008).

Kodagu district has 81% of its landscape under tree cover, and is one of the densely forested districts in India (FSI, 2009). Kodagu is dominated by agricultural land, essentially coffee agroforestry

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systems (AFS) that cover around 30% of the total area of the district. Kodagu district is the largest coffee growing region in India producing about 38% of India's coffee with the production area concentrated in the Western Ghats, one of the world's hot spot of biodiversity (Garcia et al., 2007; Garcia et al., 2007). These hotspots are areas, identified by scholars, where exceptional concentrations of endemic species are undergoing exceptional loss of habitat. As such, the stakes are high to ensure the remaining habitats and species do not disappear due to human pressure (Myers et al., 2000). Shade trees, legumes in particular, have an ecological function such as litter fall production, N fixation, reducing soil erosion, utilizing nutrients from deep soil and improving soil biological processes (Young, 1990).

Mycorrhizae are the most common symbiotic association on earth, with arbuscular mycorrhizae (AM) being the most frequent type, occurring in about 80 per cent of plant species and in almost all ecosystems (Strack et al., 2003). There are nearly 230 species of arbuscular mycorrhizal fungi (AMF), which belong to the phylum Glomeromycota (Sturmer, 2012). AM fungi, which are partly within and partly outside the root, take part in the nutrient cycles and transfer of mineral elements in the soil and into the host roots (George et al., 1992). Some minerals such as phosphorus, iron, zinc and copper are of very limited mobility in the soil and are found in extremely low concentrations in soil solution. Their uptake and use by plants is increased by the presence of symbiotic microflora, notably mycorrhizal fungi, which assist their nutrition and growth (Jeffries et al., 2003; Duponnois et al., 2005; Abdul et al., 2013). Like many crops, coffee associates symbiotically with AM fungi (Vaast and Zasoski, 1992). The occurrence of different AM genera and species in coffee soils and roots varies depending on several factors, such as edapho-climatic conditions and cultural practices. In tropical and subtropical regions, climatic seasons are divided into rainy and dry seasons, as the amount of precipitation may vary dramatically. These variations in rainfall regimes may influence mycorrhizal colonization (Andrade et al., 2009).

Bacterial community is very diverse and predominant in soil. Moreover, soil bacteria play an important role in ecosystem services such as nutrient cycling and biological N fixation (Rao, 2007). Microorganisms in the soil have a major impact on soil properties and processes. The microbial population in the soil is controlled by its nutrient supply, moisture, energy source and other physico-chemical factors in the ecosystem. The coffee ecosystem is characterized by a complex and diverse interrelationship among and between microorganisms. Coffee as a perennial crop is supposed to harbour large number of beneficial microorganisms including phosphate solubilizing bacteria in its rhizosphere which may contribute to nutritional requirement of the plant (Mulaw et al., 2010). Nevertheless, many rhizospheric bacterial species remain unknown and more studies are needed to reveal the high biodiversity of these bacteria. Although the study of rhizospheric bacteria is difficult, due to the high number of bacteria present in soil, characterization and identification of these bacteria are necessary for wide ecological studies of the plant rhizosphere (Baon et al., 2012). Coffee farmers worldwide knowingly or unknowingly, have been totally dependent on microbes for various transformations and life processes. The present study was conducted to evaluate the effects of associate shade trees on the population of different microorganisms and their activities in different coffee agroforestry systems.

2. Material and methods

2.1. Study area

This work was carried out in different coffee estates in the villages like Siddapura, Kudluchettalli and Kabbin Kadu in Kodagu district of Karnataka state, India. The soil samples were collected from each experimental field and population of bacteria, actinomycetes, fungi and AM fungi in particular were enumerated. Soil auger was used to collect the soil samples from 0–17 cm depth at each sampling point. At each location, samples were collected from 3 spots. Total of 36 samples covering all the 3 shade typologies (coffee plants grown under one shade tree species, 2 shade tree species and 3 or >3 shade tree species), 2 types of coffee plants (Arabica and Robusta) and 2 ecological conditions (evergreen and deciduous) were collected and used for microbiological analysis.

2.2. Studies on arbuscular mycorrhizal fungi

The root fragments collected in the soil samples were stained with trypan blue as per the standard procedure to study the percent mycorrhizal root colonization (Philips and Hayman, 1970; Kormanik et al., 1980). As the coffee roots were highly pigmented, ammonical hydrogen peroxide treatment was included in the trypan blue staining procedure. The stained root samples were used for determining the percentage mycorrhizal root colonization using the formula,

$$\text{Percent AM colonization} = \frac{\text{Total no. of root fragements positive for AM colonization}}{\text{Total no. of root fragements observed}} \times 100$$

The extraradical spores of AM fungi in the root zone soil was estimated by wet sieving and decantation method (Gerdemann and Nicolson, 1963). The number of infective propagules of AM fungi in the soil samples was estimated by the most probable number method (MPN) outlined by Porter (1979).

2.3. Quantitative analysis of the total microflora

The serial dilution and plating method was used for enumerating total population of bacteria, fungi and actinomycetes in the soil. A dilution of 10^{-4} for bacteria, 10^{-3} for fungi and 10^{-4} for actinomycetes was used. Modified nutrient glucose agar (Baskaran et al., 2011), Martin's Rose Bengal Agar (Martin, 1950) and Ken Knight's agar (Balakrishnan-Nair and Wilson, 1976) were used for enumerating bacteria, fungi and actinomycetes respectively. Enumeration of the population on agar plates was done after 2, 5 and 7 days incubation for bacteria, fungi and actinomycetes, respectively. The representative colonies were purified by streaking on the respective agar plates and preserved in slants for further studies.

2.4. Qualitative analysis of soil microflora

The population of all groups of nitrogen fixing bacteria was enumerated by standard dilution plating technique using combined carbon medium (Rennie, 1981). The soil samples collected from different sampling points were used for enumeration of phosphate solubilizing microorganisms as they show halo around the colonies on Sperber's hydroxy apatite medium (Sperber, 1957) by the standard plating technique. The number of cellulose decomposing microorganisms in the soil samples collected was determined by the standard plating technique using minimal nutrient agar medium containing carboxy methyl cellulose. After incubation, the plates were flooded with Congo red and 1 M sodium chloride solution. Cellulose decomposers showed a clear halo around their colonies (Apun et al., 2000).

The 36 different types of bacteria which were isolated from coffee root zone soil from different ecosystems and maintained on