Dynamics of soil microbial community structure and activity during the cropping period of cotton

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Abstract
Healthy soil is a prerequisite for maintaining agricultural productivity and microorganisms are integral part of the soil ecology. We attempted to study the temporal changes in the soil microbial activity as well as community structure by analyzing soil samples collected at different stages of cotton crop growth. Soil physical and chemical properties were analyzed. Enzymes are the direct mediators of soil health; therefore dehydrogenase activity of different soil samples was compared. Application of molecular techniques has revolutionized the soil microbial studies by bypassing the need of culturing the microbes. Community amplified ribsosomal DNA restriction analysis (ARDRA) using universal 16S rDNA primers revealed close similarity of samples collected on 60th day and onwards. Zero day sample was distinct from other samples. Data obtained from the dehydrogenase assay and ARDRA fingerprinting did not correlate. It is advised that multidimensional/polyphasic approach should be adopted to fully understand the soil biological processes.

Key Words
Microbial community, soil properties, dehydrogenase, soil DNA, 16SrDNA, ARDRA.

Introduction

Soil is an integrated system constituting various interdependent physical, chemical as well as biological processes that are markedly influenced by environmental factors. Healthy soil is a prerequisite to a strong agricultural economy. Soil microorganisms providing the biological interface with the soil physical and chemical environment; affect the environment and in turn, get affected by it. The soil microbial community is organized in complex food webs and stabilizes various soil processes including the biogeochemical processes. It is of great practical significance to observe and compare temporal microbial community diversity in an agricultural field throughout the cropping period of a crop in order to identify the factors that influence the temporal microbial community structure and function. The bacterial diversity associated with the agricultural cotton field crop was investigated using culture-independent approaches like ARDRA and DGGE of the soil community DNA. Dehydrogenase activity was measured, as enzymes are also direct mediators of soil mineralization and processes.

Methods

Experimental site, soil sampling and storage
The study site was the experimental field of cotton crop in Indian Agricultural Research Institute, Pusa, New Delhi, India. Eight random subsamples were collected from the field (0-10 cm depth) using rectangular sampler (5 x 5 x 10 cm), pooled, sieved (2mm mesh size) and stored at -80ºC. Soil sampling has been done 7 times corresponding to 0 day, 15th day, 30 th day, 60 th day, 90 th day, 120 th day and 150 th day after the day of sowing (0 day).

Analysis of physical properties of soil
All the analysis were done in triplicates.
1) *Dry matter and water content:* Determined by the weight loss method **(**Schichting and Blume 1966).
2) *Maximum water holding capacity:* 50g moist soil samples were saturated with water. From each cylinder, 25 grams of soil was taken in porcelain dishes, dried to constant weight at 105^{o}C for 3 hours, cooled in desiccator and weighed. It is expressed in terms of gram water x 100. % WHC = (saturated soil-dried soil)/ dried soil x 100
3) *Particle size distribution:* Percentage was calculated using the following formula.
Sand fraction % = Weight (g) of fraction on sieve/25 x 100; Silt fraction % = Weight (g) of fraction under sieve/25 x 100; Clay fraction % = Oven dried soil weight (g)/25 x 100

Analysis of chemical properties of soil
All the analyses were done in triplicates.
1) *pH:* Saturated soil solution (1:2.5::soil: water) was prepared and pH was determined using a glass electrode pH-meter.
2) *Organic carbon:* It was calculated by titration protocol of Walkley and Black (1934).
3) *Exchangeable sodium:* It was calculated by flame photometry.
4) *Exchangeable potassium:* It was calculated by flame photometer.
5) *Available nitrogen:* It is present in the form of NH^{4+}, NO^{3-} and NO^{2-} (nitrite doesn't contribute significantly) in the soil and can be extracted and measured spectrophotometrically (Keeney and Nelson 1982).
6) *Available phosphorus:* It was calculated colorimetrically (Olsen and Sommers 1982).

Dehydrogenase activity of the soil
Dehydrogenase activity was measured in triplicates by adding 2.5 mL of sterile ddw and 1 mL of 3% aqueous solution of triphenyl-tetrazolium chloride to 6 g of soil sample, followed by incubation at 30ºC for 24 hrs in dark and methanol extraction afterwards. After determining the absorbance at 485 nm, the amount of TPF produced was calculated by reference to a calibration graph prepared from TPF standards (100 µg of TPF/mL methanol).

Community DNA extraction and quantification
Soil microbial community DNA was extracted in triplicates using direct lysis based on the method of Zhou et al. 1996 (Williamson, personnel communication) and checked using standard marker by agarose gel electrophoresis (0.8%). The DNA was gel extracted using QiaexII kit (Qiagen, Germany). Nanodrop Spectrophotometer ND-1000 quantified the DNA prior to any further analysis.

Polymerase Chain Reaction Amplification of 16S rDNA
PCR amplification from 50 ng of extracted soil DNA was conducted with a total volume of 50 µl by using universal primers 27f and 1492r in trplicates (Martin-Laurent *et al.* 2001, Suzuki *et al.* 2005) in PTC-200 (MJ Research). Other reagents were 200 mM of each dNTP, 2 U of DNA polymerase and 1× PCR buffer under the following conditions: 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C, plus an additional 15-min cycle at 72°C.

Amplified Ribosomal DNA Restriction Analysis
Pooled 16S rDNA amplicons were concentrated using Microcon-PCR centrifugal devices (Millipore Corp., USA) and subjected to *HaeIII* ARDRA. 10 µl amplicons were restricted overnight with 5U of restriction endonuclease. ARDRA profile was checked on 2.5% Metaphor agarose gel electrophoresis, converted into a 2-dimensional binary matrix and analyzed using MVSP 3.1. UPGMA dendrograms were constructed by calculating Jaccard's coefficient of similarity.

Results
Soil characteristics were determined by analysis of physical and chemical properties of the soil sampled on the 15th day (Table 1). DNA yield/g soil was calculated as microbial biomass (Table 2) that is showing no significant variation among the samples and pure DNA was extracted in nearly all the samples after gel extraction.

Table 1. Analysis of soil physical and chemical properties.

Soil	pH	Organic Carbon (%)	Available Nitrogen (kg N/ha)	Available Phosphorus (kg P/ha)	Available Potassium (kg K/ha	Exchangeable Sodium (me/100g)	Particle size distribution			Soil Type	Water Holding capacity (%)
							Sand	Silt	Clay		
							(--------%-------)				
Cotton field soil	7.7	0.58	221	44.8	467	1.1	58	29	13	Loam soil	52.7

Humic acids and other geochemicals were also got co-extracted with the community DNA (Figure 1), so the necessity of gel elution method can't be ruled out. The PCR amplification was quite difficult in the 90th day soil. Figure 2 showed the pattern of variation of the dehydrogenase activity corresponding to the different phenological stages of crop plant. Dehydrogenase activity is direct indicator of microbial activity. A plateau of highest microbial activity was observed during 30th day (6.56 µg/mL) and 60 day (6.79µg/mL) after

sowing. The 0 day activity was lowest as 4.84µg/mL which rose to 6.79 µg/mL measured on 60th day and again fall down to a value of 5.47 µg/mL before reaching to 6.02 µg/mL on the 150th day of sampling. ARDRA profile of 16SrDNA amplicons revealed marked similarity of 30th, 60th, 90th, 120th and 150th day sample. 15th day sample was also almost 95% similar to the rest. Only 0 day sample was showing deviation of around 75% from the rest of the group revealing the microbial community profile of o day was very much different from the rest of the samples indicative of shifting in the bacterial diversity across the cropping period (Figure 3). Figure 4 depicted the presence of various bands of different sizes in all the samples.

Table 2. Estimates of microbial biomass in terms of DNA yield (ng DNA /g soil), absorbance ratio and PCR results associated with the samples.

Soil sample collected	Microbial Biomass (DNA extracted, ng DNA g/soil)[A]	Absorbance at260 nm/ Absorbance at280 nmc	Absorbance at260 nm/ Absorbance at230 nmd	16S rDNA amplification results b
0 day (16th May 2006)	297.8±16.6	1.38	0.29	40272
15 day	284.1±28.4	1.75	0.13	40272
30 day	255.4±20.1	2.18	2.44	40272
60 day	314.6±16.4	2.03	0.57	40272
90 day	279.4±31.7	2.27	-1.34	40271
120 day	312.6±19.7	1.58	0.26	40272
150 day	300.8±27.0	1.89	0.78	40272

[A]DNA yields represent the averages ± standard deviations of DNA extracted in triplicates. [b]Amplification results are presented as the number of DNA samples (soil samples) yielding the desired size amplified product divided by the number of (DNA samples) soil samples analyzed. [c]High 260/280 ratio (>1.7) indicates pure DNA. [c]Low 260/280 ratio indicates protein contamination. [d]Low 260/230 ratio indicates humic acid contamination.

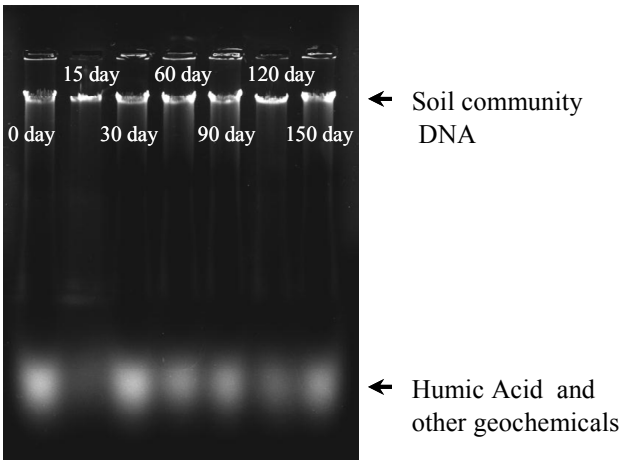

Figure 1. Soil Community DNA extracted from the samples collected at different stages of plant growth.

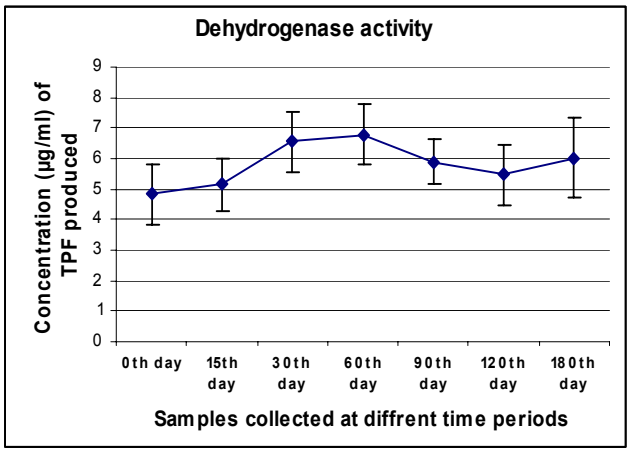

Figure 2. Soil dehydrogenase activity assayed at different stages of plant growth.

370bp, 450bp and 485bp bands were noticed in all the samples except for the first sample which only as showing the presence of 280bp band. Overall comparison of the first and the last sample corresponding to the day of sowing and day of harvesting respectively revealed marked variation the microbial community structure.

Conclusion

Noticeable impact of plant growth stages as well as routine agricultural practices (chemical inputs, irrigation, seasonal variations, etc.) was observable on microbial activity and community structure which are constantly changing throughout the cropping period corresponding to the different phenological stages of the plant. Changes in chemical composition of the plant root exudates with the growing age definitely decide the microbial community structure and diversity around the rhizosphere. No correlation was found between the microbial activity and community structure. Only 16S rDNA approach is not sufficient in describing the dynamics of microbial ecology in an agricultural field. So, multidimensional/ polyphasic approach should be adopted in understanding soil biology aspects.

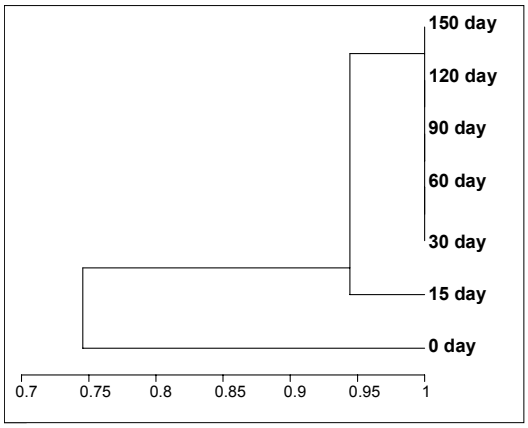

Figure 3. Cluster analysis of community ARDRA band patterns using Jaccard's coefficient of similarity and the UPGMA method of tree construction. The labels represent the time period of the sample collection considering 0 day as the day of sowing and 150 days as the day of harvesting.

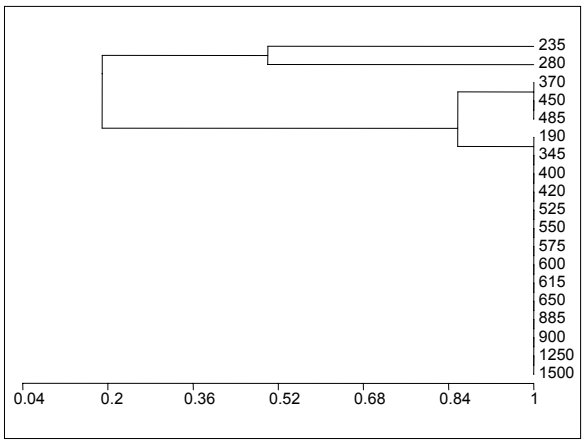

Figure 4. Cluster analysis of community ARDRA band patterns using Jaccard's coefficient of similarity and the UPGMA method of tree construction. The labels represent the approx. band sizes of the restricted ARDRA fragments.

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