

RPF - III
(PROFORMA FOR SUBMISSION OF FINAL
REPORT OF RESEARCH PROJECTS)

Part- I: General Information

800 Project Code :

8001 Institute Project Code No. : SSC III (813)

8002 ICAR Project Code No.

801 Name of the Institute and Division

8011 Name and address of Institute : Indian Institute of Spices Research (ICAR)
P.O. Box 1701, Marikunnu P.O., Calicut

8012 Name of Division / Section : Division of Crop Production and PHT

8013 Location of the Project : IISR, Calicut

802 Project Title : Assessment of quality of soils under spice
based cropping systems

803 Priority Area :

8031 Research Approach: Applied Res. / Basic Res. / Process / Transfer of
or Technolo. of Tech.
Develop.

01 02 03 04

804 Specific Area : Spice based cropping systems

805 Duration of Project : 4 years

8051 Date of start : December 2004

8052 Date of Completion : December 2008

806 Total cost /Expenditure Incurred: 18.45 lakhs
(Give reasons for variation, if any from original estimated cost)

807 Executive Summary

Studies on the chemical, biochemical and biological indices of soils under spices based cropping systems are very few and scarce. The focus of this study was to generate information on various soil quality parameters involving physico-chemical, biochemical and microbial indices of soils under various spice based cropping systems. For the study, soils were collected from various spices under pepper, ginger, and turmeric with contrasting nutrient management regimes [chemical, fully organic, integrated nutrient management (INM)]. Soils were also collected from various cardamom based cropping systems The soils were then analyzed for

physico-chemical (pH, OC, N, P, K, Ca, Mg, Zn, Cu, Fe, Mn), biochemical (Acid-phosphatase, alkaline-phosphatase, inorganic pyrophosphatase, CM-cellulase and BAA-protease) and microbial parameters [Microbial biomass- C (C_{MIC}), -N (N_{MIC}), -P (P_{MIC}), soil respiration (measured as CO_2 evolution), metabolic quotient (qCO_2)] etc. The microbial community structure in soils under black pepper, ginger and turmeric were also determined. The inter-relationships between these parameters under various sites were analyzed using appropriate statistical tools.

Briefly, the results revealed that in case of organic sites under black pepper, organic C levels were medium to high, levels of available N low, Bray P very low and available K low to medium. DTPA extractable Zn, Cu, Fe and Mn and enzyme activities did not vary markedly between various organic sites. Among the organic, chemical and INM sites under black pepper organic C, available N, Bray P, DTPA Zn, Cu and Fe levels and the enzyme activities were relatively higher in the organic and INM sites compared to chemically managed sites.

In case of organic, chemical and INM sites under ginger, organic C was highest in the organic site, while available N, Bray P and available K did not differ significantly between the sites. DTPA- Zn and -Cu levels were lower at all the sites, with organic sites registering relatively higher levels, followed by chemical and INM sites. DTPA-Mn levels were relatively greater under INM and organic sites compared to chemically managed site. The enzyme activities were relatively higher in the organic and INM sites compared to chemically managed sites. In soils under turmeric, organic C was highest in the INM sites followed by organic sites. Available N did not vary significantly between sites. Contrarily, Bray P levels were significantly higher in chemical treatment followed by organic sites. DTPA -Zn, -Cu, -Fe and -Mn levels, however, did not vary significantly between sites. Among the biochemical parameters, most of the enzyme activities were higher in organic sites. In soils under cardamom based cropping systems, the component crops had little influence on the various physico-chemical and biochemical parameters studied. However, available Zn and P levels in soils were very low in all the cardamom based cropping systems.

Soil microbial community structure was studied using molecular methods (RFLP). In soils under ginger and turmeric with contrasting nutrient management regimes, the diversity of microorganisms was studied using seven different primer sets for multi and single copy genes. Fungal population was found to be maximum under chemical and control treatments, while fully organic and INM treatments registered maximum population of prokaryotes and nematodes. Maximum eukaryotic population was also observed under INM and fully organic treatments. The control registered the lowest eukaryote and prokaryote population.

808 Key words: Soil quality, Spice based cropping systems, Soil physico-chemical parameters, Soil biochemical parameters, Soil microbial community structure

Part-II: Investigator Profile

(Please identify clearly changes, if any in Project personnel)

810 Principal Investigator

8101 Name : Dr R. Dinesh
8102 Designation : Sr. Scientist (Soil Fert./ Soil Chem./ Soil Microbiol.)
8103 Division/ Section : Division of Crop Production & PHT
8104 Location : IISR, Calicut
8105 Institute Address : IISR, Marikunnu PO, Calicut-673012

811 Co- Investigator:

8111 Name : Dr. V. Srinivasan
8112 Designation : Sr. Scientist (Soil Fert./ Soil Chem./ Soil Microbiol.)
8113 Division/ Section : Division of Crop Production & PHT
8114 Location : IISR, Calicut
8115 Institute Address : IISR, Marikunnu PO, Calicut-673012

812 Co- Investigator:

8121 Name : Dr S. Hamza
8122 Designation : Technical Officer (T7)
8123 Division/ Section : Division of Crop Production & PHT
8124 Location : IISR, Calicut
8125 Institute Address : IISR, Marikunnu PO, Calicut-673012

Changes in project personnel:

Dr B. Chempakam, Head, Crop Production was involved in the project during the first year (2004-05) only.

Dr T.E. Sheeja was involved in the project for the first three years (Dec 2004 -05, 2005-06, 2006-07). During 2007-08 she provided external support and during 2008-09 she disassociated from the project.

Part-III: Technical Details

820 Introduction and objectives

8201 Project Objectives :

- To determine the physico-chemical, biochemical and microbial characteristics of soils associated with spices based cropping systems
- To examine the inter-relationships among the physico-chemical, biochemical and molecular indices in these soils

8202 Background information and importance of the project

Growing awareness of the progressive degradation of soils has tended to hasten the search for reliable indicators of soil quality. Traditionally, the quality of soil has been associated solely with its productivity. However, more recently soil quality has been defined in terms of sustainability i.e. the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation. Soil quality depends on a large number of physical, chemical, biological and biochemical soil properties, and its characterization requires the selection of properties most sensitive to changes in management practices. Biological and biochemical soil properties fulfill this requirement and are also sensitive to environmental stress. Apparently, comprehensive assessment of soil quality of soils under spices based cropping systems through biochemical and microbial indices would be precise and reliable. Hence, based on the hypothesis that a combination of properties need to be studied for an in-

depth insight into major soil processes influencing soil quality, the project was formulated encompassing physico-chemical, biochemical and microbial approaches. This would also pinpoint the exact changes occurring due to anthropogenic disturbances. Besides, the assessment of the impact of manmade intrusions, a typical situation in the tropics, on soil quality shall be highly useful for formulating appropriate conservation strategies.

Individual soil biological and biochemical properties, though sensitive to environmental changes, are not useful measures of soil quality because of their seasonal and spatial variation. The project aims to integrate both conventional techniques of soil quality assessment *viz.*, physico-chemical, with biochemical and microbial methods.

821 Project Technical Profile

8211 Technical programme

(Indicate briefly plan of procedure, techniques, instruments and special materials, organisms, special environments etc.)

A. Soil sampling and analyses

(i) Collection of soil samples from spice based cropping systems which will include

- Black Pepper: Soils from rhizosphere of various tree support-black pepper systems, organic, chemical and integrated nutrient management sites.
- Ginger: Sites under fully organic, chemical and integrated nutrient management.
- Turmeric: Sites under fully organic, chemical and integrated nutrient management.
- Cardamom: Sites under cardamom-black pepper, cardamom-nutmeg, cardamom-all spice, cardamom-coffee, cardamom—cinnamon, cardamom-arecanut, cardamom-clove, cardamom alone etc.

(ii) Analysis of soil samples for biochemical and microbial indices

- General parameters: Biomass C and N, soil respiration, N mineralization capacity, dehydrogenase activity, catalase activity
- Specific parameters: Urease, BAA protease, Casein protease, Phosphomonoesterase, Phosphodiesterase, Aryl sulfatase, Invertase, CM-Cellulase

(iii) Analysis for basic physico-chemical properties like pH, CEC, organic C, major nutrients (N, P, K), secondary nutrients (Ca, Mg), micronutrients (Zn, Cu, Fe, Mn)

(iv) Determination of soil microbial community structure: Isolation of DNA from soils and DNA fingerprinting by PCR and RFLP/ denaturing gel. Prior to this PCR conditions will be optimized for amplification of single and multicopy genes and its detection through RFLP, silver staining techniques and denaturing gel will also be standardized.

B. Plan of procedure/ techniques:

(i) Soil sampling

Soil samples (quadruplicate) were collected using a steel augur from various spice based sites. In the laboratory, the living plant material and coarse roots were removed prior to estimation of moisture content in the samples. The soil samples were then air-dried and ground to pass a 2mm sieve. A portion of each sample required for estimating the biological parameters was stored at 4°C.

(ii) Determination of soil physico-chemical parameters

Soil pH was determined in a 1:2.5 soil: water suspension, organic carbon by the dichromate oxidation method (Nelson and Sommers, 1996) and total N by the Kjeldahl method (Bremner 1996). Bray P in soils was determined by the method of described by Kuo (1996) and exchangeable K by the NH₄OAc method described by Helmke and Sparks (1996). Exchangeable Ca & Mg was estimated by the method described by Suarez (1996) and DTPA extractable Zn, Cu, Mn and Fe by the method described by Lindsay and Norvell (1978).

(iii) Determination of soil biochemical/ microbial parameters

Dissolved organic carbon (DOC) and dissolved organic N (DON) were determined by incubating samples at constant temperature (20°C) and moisture content (50% WHC) for 3 weeks. Water extractions were made initially and after the incubation. The soil samples were shaken in 1.5 l ultra-pure water for 2 h (200 rpm), and the suspensions were filtered through a millipore filter (0.45µm) using a vacuum system (Smolander and Kitunen 2002). In the extract total N was determined by alkaline persulfate oxidation (Cabrera and Beare 1993) and organic C as mentioned earlier.

The parameters indicating microbial activity in soils *viz.*, microbial biomass carbon (C_{MIC}), Basal respiration (CO₂ evolution) and activities of enzymes involved in C, N, P and S cycles in soils including dehydrogenase were determined in fresh soil samples. For measuring basal respiration, 100 g (oven-dry basis) of moist soil was adjusted to 55% of its water holding capacity and pre-incubated for 7 days at 20°C in the dark. The CO₂ production was measured for another 7 days. The CO₂ produced was trapped in 20 mL 0.05 M NaOH, precipitated with BaCl₂, and then measured by titration of the excess NaOH with 0.05 N HCl (Salamanca et al. 2002). The metabolic quotient was calculated as follows: $[(\mu\text{g CO}_2\text{-C evolved in 7 days (g soil}^{-1}) / (\mu\text{g biomass C (g soil}^{-1}) / (7 \text{ days} \times 1000))] = [\mu\text{g CO}_2\text{-C (mg biomass C)}^{-1} \text{ day}^{-1}]$. The results are expressed in mg CO₂- C (g biomass C)⁻¹ day⁻¹.

The microbial biomass C was estimated by fumigation-extraction. (Vance et al., 1987) using a K_{EC} = 0.45 (Joergensen 1995). Soil dehydrogenase was assayed by the method of Casida et al. (1964) using 2, 3, 5-triphenyltetrazolium chloride as the substrate. Urease was assayed by using urea as the substrate (Kandeler and Gerber 1988), BAA-protease using α-benzoyl-N-arginamide as the substrate (Gil-Sotres et al. 1992), acid phosphomonoesterase using *p*-nitrophenyl phosphate as the substrate (Tabatabai and Bremner 1969), arylsulfatase using *p*-nitrophenyl sulfate as the substrate (Tabatabai and Bremner 1970) and carboxymethylcellulase (CM-cellulase) using carboxymethylcellulose as the substrate (Schinner and von Mersi 1990).

(iv) *Determination of soil microbial community structure*

In rhizosphere soils of black pepper-tree supports

This was done using molecular methods. Briefly, DNA isolation was done as per standard procedures of Yeates et al. (1998) and Parham et al. (2003). The PCR amplification was performed using sets of primers mentioned in Table 1a.

The PCR amplified DNA was subjected to restriction digestion using restriction enzymes Hae III and Msp 1 (Genei, Bangalore). RFLP profiles were analysed on 8% non-denaturing polyacrylamide gels using standard protocols. The gels were stained by silver staining (Caetano-Anollés and Gresshoff, 1994) with slight modifications. The gels were photographed under white fluorescent light in an Alpha Imager Gel Documentation System. The bands were scored as 1 for presence and 0 for absence and the binary matrix was analysed using NTSYS ver. 2.1 to generate dendrogram by unweighted pair group mathematical average (UPGMA) method for depicting the relationship between soils of the various systems based on their microbial community structure.

In soils under ginger and turmeric

DNA isolation was done as per the procedures of Yeates et al. (1998) and Parham et al. (2003). The PCR amplification was performed using sets of primers mentioned in Table 1a & b and the composition of the reaction mixture used for PCR is described in Table 2a. For gel electrophoresis, an aliquot of each amplification reaction was analysed on 2% w/v agarose gels cast and run in TBE buffer (pH 8.3). Gels were stained with ethidium bromide and photographed using transmitted UV light and Polaroid film. A 100 base pair marker (Genei, Bangalore) was included on every gel.

The PCR amplification was done following the standard protocol *viz.*, Initial denaturation at 92°C for 3min, Denaturation at 92°C for 1 min 10sec, Annealing at 55° C for 30 sec (34 cycles repeat), Extension at 72°C for 2 minute, Final extension at 72°C for 10 min. The soil DNA was subjected to restriction digestion for testing the quality and purity of the DNA. Two enzymes were used namely ECOR 1 and Hind III. Both single and double digestions were performed as per the manufactures instructions. The composition of the reaction mixture is mentioned in Table 3a. The amplification products were separated on 2.0 % agarose (Biogene Reagents Inc.) on 1% TAE buffer (40 mM Tris acetate, pH 8.2, 1mM EDTA). The amplification product after gel separation was visualized using ethidium bromide staining and photographed in a Gel documentation system (Biorad). Raw gel images were recorded through molecular Analyst Software/PC version. The PCR amplified DNA was subjected to restriction digestion using restriction enzymes Hae III and Msp 1 as per the manufactures instructions for analyzing the RFLP profiles on 8% polyacrylamide gels. The composition of the reaction mixture used is mentioned in Table 4a and chemical constituents of 8% polyacrylamide gel is given in Table 5a.. The polyacrylamide gel after electrophoresis was subjected to silver staining as per Caetano-Anollés and Gresshoff (1994) with slight modifications. The gels were photographed under white fluorescent light in an Alpha Imager Gel Documentation System. The bands were scored as 1 for presence and 0 for absence and the binary matrix was analysed using NTSYS ver. 2.1 to generate dendrograms by unweighted pair group mathematical average (UPGMA) method for depicting the relationship between soils based on their microbial community structure.

C. Statistics

Analyses for physico-chemical and biochemical/ microbial parameters were performed on all soil samples, mean values determined, and the values are expressed on an oven-dry soil basis (24 h at 105⁰C). The significance of treatment effects was determined by one-way analysis of variance. Where the F values were significant, post hoc comparisons were made using the least significant difference test at the 0.05 probability level.

The relationships between the different chemical and biological properties of the soils were determined by principal component analysis (PCA) using the varimax rotation to achieve either small or large component loading and an Eigen value of 0.1 as the lower limit. All statistical analyses were performed using Statistica 5.1.

8212 Total man months involvement of component project workers

PI: 16 months

CoPI: 10 man months (Dr V. Srinivasan)

Co-PI: 8 man months (Dr T.E. Sheeja)

Co-PI: 1 man month (Dr B. Chempakam)

Co-PI: 12 man months (Dr S. Hamza)

822 Final Report on the Project

Detailed report containing all relevant data with a summary of results
(not exceeding 2-5 pages)

8221 Achievements in terms of targets fixed for each activity

1. Sites under black pepper

Soils were collected from the following black pepper sites

- INM/ Organic/ Chemically managed sites from Calicut, Panniyur, P'muzhi
- TMBP sites: Kasaragod, Calicut
- Rhizosphere of various tree support-black pepper systems (RARS, Ambalavayal)

Soil samples were collected from Peruvannamuzhi, Madikeri, Kannur, Kasaragod. In case of organic sites, soil pH exhibited minimum variation between sites and varied between 5.12-5.81 (Table 1). Organic C varied from 1.5-3.7%, available N levels were low to medium (129-203 mg kg⁻¹), Bray P low (0.12-4.6 mg kg⁻¹), and available K low to medium (109-238 mg kg⁻¹). Among the micronutrients, available Zn ranged between 0.4-3.4 mg kg⁻¹, Cu between 1.7-8.7 mg kg⁻¹, Fe between 7.9-44.6 mg kg⁻¹ and Mn between 2.8-36.4 mg kg⁻¹.

Among the biochemical parameters (Table 2), acid phosphatase activity ranged between 24.3-26.7 mmol pnp/g/h, phosphodiesterase between 3.6-4.8 mmol pnp/g/h, invertase between 6.2-7.9 mmol glucose/g/h, cellulase between 0.26-0.43 mmol glucose/g/h, urease between 5.36-7.52 mmol NH₃/g/h, BAA-protease between 3.7-4.4 mmol NH₃/g/h, arylsulfatase between 0.39-0.44 mmol pnp /g/h and dehydrogenase between 132-147 nmol TPF/ g/ h.

Samples were also taken from sites under contrasting management under black pepper *viz.*, organic, chemical and INM sites. Soil pH was slightly acidic (5.27-5.46), organic C levels were

relatively higher in the organic and INM sites (2.27 and 2.05% respectively). Similar observations were made with respect to available N and P levels (Table 3). Contrarily, available K levels were lowest in the organic sites (146 mg kg⁻¹) compared to chemical (245 mg kg⁻¹) and INM (264 mg kg⁻¹) sites. Among the micronutrients, DTPA- Zn, -Cu and -Fe levels were consistently greater in the organic (6.2, 9.2 and 42 mg kg⁻¹ respectively) and INM (3.2, 4.2, 42 mg kg⁻¹ respectively) sites compared to the chemically managed sites (1.8, 1.1, 38 mg kg⁻¹ respectively). DTPA Mn levels did not vary considerably between sites and ranged from 4.5-6.0 mg kg⁻¹. With regard to enzyme activities all the enzymes showed relatively greater activities in the organic and INM sites compared to chemically managed sites (Table 4).

A. Sites under various standards of black pepper (BP)

Black pepper sites under commonly used tree supports like *Ailanthus malabarica* DC, *Erythrina indica* Lamk., *Gliricidia sepium* (Jacq.) Steud. and *Garuga pinnata* Roxb., were selected for the study. A site under dead standard (RCC pole) was also selected for comparison. Soil samples were collected using a steel augur from the rhizosphere of the respective tree support-BP sites. The sites selected had no recent history of being applied with any chemical or organic fertilizers.

(i) Soil pH, SOC and DOC

The data on soil physico-chemical parameters is given in Table 5. In general, soil pH in the rhizosphere of all tree species was acidic (range 4.16-4.62), while the soil in the control had slightly higher pH (5.86). Contrary to soil pH, the rhizosphere of all tree species showed markedly higher SOC levels than the control soil. In fact, SOC levels were greater by 30.5-43.4% in the tree rhizospheres. Similar to SOC, the DOC levels were also markedly higher in the tree rhizosphere compared to the control (Table 2). Among the tree species, DOC levels were highest for *G. pinnata* and *G. sepium*, while the lowest level was registered by *E. indica*. In the tree rhizosphere, the DOC level was greater by 51.3-55.5% compared to the control. This indicated that SOC differed in quantity as well as quality in the tree rhizosphere and control.

(ii) Soil mineral N, DON and total N mineralized

Higher mineral N and DON levels (Tables 5 and 6) also corresponded to the rhizosphere of *G. sepium* and *G. pinnata*. The rhizosphere of *E. indica* and *A. triphysa* registered lower levels of mineral N and DON, although in both cases they were still higher than the control. In general, in the rhizosphere of tree species, mineral N levels were greater by 54.0-66.6% and DON levels by 36.0- 50.2% in comparison to the control. The inorganic N was predominantly ammoniacal (Table 6) and the tree species registered greater total inorganic N (range 74.5-112.3 mg N kg⁻¹ per 10 days) compared to the control (46.8 mg N kg⁻¹ per 10 days). The accumulation of mineral N, DON and N mineralized in the rhizosphere was, however, related to tree species. Greater levels were observed in the *G. sepium* rhizosphere possibly because it is a leguminous tree capable of fixing N.

(iii) Bray P and exchangeable cations (K, Ca and Mg)

Bray P levels were significantly higher in the tree rhizospheres especially the *G. pinnata* rhizosphere (Table 5) suggesting an increase by 57.7-73.8% compared to the control. In case of exchangeable cations (Table 5), exchangeable K level was greater in the *A. triphysa*

rhizosphere, exchangeable Ca in the *E. indica* rhizosphere and exchangeable Mg in the *G. sepium* rhizosphere. The levels of these exchangeable cations in the tree rhizosphere were, however, not always higher than the control. For instance, exchangeable K levels in the rhizosphere of *G. sepium* and *G. pinnata* were at par with the level in the control. Also, the levels of exchangeable Ca and Mg were markedly lower in the *A. triphysa* rhizosphere compared to the control soil. The tree rhizosphere, therefore, had a negative influence on the levels of exchangeable -Ca and -Mg. This is possibly due to leaching caused by acid deposition or exudation of low-molecular-weight organic acids by the roots (Schroth et al., 2007) and uptake and sequestering in woody biomass (Huntington et al., 2000).

(iv) Available micronutrients (Zn, Cu, Fe and Mn)

Higher available Zn, Mn and Cu levels corresponded to the rhizosphere of *G. pinnata* and *G. sepium* and higher Fe levels corresponded to the rhizosphere of *E. indica* and *A. triphysa* (Table 5). Since our study site was identical in macro-environmental conditions, it seems likely that the tree species were primarily driving the differences that we detected in the soil. The rhizosphere of *G. Sepium*, a fast growing pioneer species probably exuded more C and therefore, showed greater accumulation of SOC and organic substrates followed by *G. Pinnata*, *E. Indica* and *A. Triphysa* in that order.

(v) Microbial biomass -C (C_{MIC}), -N (N_{MIC}) and -P (P_{MIC})

C_{MIC} and N_{MIC} varied appreciably among the tree species, and the levels were highest for *G. sepium* and *G. pinnata* and lowest for *E. indica* (Table 7a). In contrast, P_{MIC} levels showed little variation among the tree species. However, the C_{MIC} , N_{MIC} and P_{MIC} levels under the tree species were greater than the respective levels in the control. In the rhizosphere, C_{MIC} levels were greater by 50.6-56.7%, N_{MIC} by 60.7-65.6% and P_{MIC} by 47.0-51.0% compared to the respective levels in the control. From the results it is evident that decreased levels of biologically available substrates and organic matter lead to simultaneous decrease in microbial activity in the control. This was well reflected by the C_{MIC} : SOC ratios, which ranged from 1.37-1.96% (Table 7a). The C_{MIC} : OC ratios were greatest for tree species (range 1.96-1.79 %) and least for the control (1.37%). In contrast to C_{MIC} : OC ratio, C_{MIC} : N_{MIC} ratio was greatest for control (18.6) and least for the tree species (14.3-15.1). The C_{MIC} : P_{MIC} ratios were greatest for *G. sepium* (34.8) and *G. pinnata* (32.0) and almost identical for *A. triphysa*, *E. indica* and control (28.8, 29.6 and 28.4 respectively).

(vi) Soil respiration and metabolic quotient (qCO_2)

Unlike microbial biomass, soil respiration rates did not vary significantly among the tree species and lowest soil respiration was registered in the control (Table 7a). Respiration rates were, in general, greater by 23-32% in the tree rhizospheres compared to the control. Unlike soil respiration, metabolic quotient (qCO_2) was markedly higher in the control and least in the rhizosphere of tree species especially *G. sepium*, *G. pinnata* and *E. indica* (Table 7a). Among the tree species, higher qCO_2 level in *A. triphysa* probably indicated its relatively poor rhizosphere health.

(vii) Soil enzyme activities

Dehydrogenase activity was highest for *G. sepium*, and *G. pinnata* and the activity under the tree species were greater by 43.0-49% in comparison to the control. The activities of hydrolases

involved in C (β -glucosidase), N (urease), P (acid phosphatase) and S (arylsulfatase) cycles in soils were also markedly higher in the tree rhizosphere compared to the respective levels in the control. In the rhizosphere, the per cent increase in activity of acid phosphatase ranged between 37.6-43.0% and β -glucosidase between 24.8-41.3%. Urease activity was greatest in *G. sepium* and *G. pinnata*, but was almost identical under *A. triphysa*, *E. indica* and control. However, not all the tree species affected the enzyme activities to the same degree. For instance, the rhizosphere of *G. sepium* registered the highest urease activity and *G. pinnata* rhizosphere the highest β -glucosidase and acid phosphatase activity. Arylsulfatase activity was greatest in the *G. pinnata* and *A. triphysa* rhizosphere and was almost identical in the *E. indica* rhizosphere and control.

(viii) *Inter-relationships between various soil parameters*

This was studied using PCA (Table 7b) wherein, the Factor I (PC1) explaining 67.7% of the total variance was defined mainly by SOC, mineral N and N mineralized, DOC, DON, dehydrogenase, acid phosphatase, β -glucosidase, C_{MIC} , N_{MIC} , P_{MIC} and soil respiration. This reflected the size and activity of the microbial biomass and possibly reflected the strong relationship between the availability of labile and easily mineralisable organic matter and the activity of microbial populations. The inclusion of soil respiration, P_{MIC} , phosphomonoesterase and arylsulphatase in this factor indicated the strong relationship between microbial activity and N/ S cycles in soils. The negative loading of qCO_2 in this factor suggested a decrease in substrate use efficiency in soils with low organic matter especially the control. Factor II (PC2) explaining 20.5% of the total variance was defined mainly by urease and somewhat lower positive loadings of SOC, mineral N and N mineralized, reflecting the strong relationship between urease activity, N dynamics and organic matter mineralization in soils.

(ix) *Microbial community structure*

The study included soil DNA extraction and optimization of PCR conditions for detection through RFLP and determination of the microbial community structure in these soils. High molecular weight DNA (23 Kb) was recovered from all the soil samples (Fig.1). The isolated DNA was of good quality with A260/A280 of 1.75. The soil DNA recovered was most abundant in gliricidia- BP system (15.2 μ g/g soil), followed by ailanthus- BP system (11.8 μ g/g soil) and least in RCC pole- BP system (6.8 μ g/g soil). There was no humic acid contamination and the DNA formed a clear solution in water. The undiluted DNA was subjected to restriction digestion using two enzymes ECOR I and Hind III singly and in combination. Characteristic restriction patterns were obtained.

To determine the diversity of microorganisms in soils of various systems different primer sets (Table 8) of varying phylogenetic specificity, including both multi and single copy genes were employed (Yeates et al., 1998). With appropriate dilution of DNA, successful PCR amplification was achieved with all primers tested. The 16S rDNA primers amplified almost full-length 16S rDNA close to 1.6 Kb (Fig.2). Fungal ITS amplified one fragment of ~600 bp in all the samples. In case of eukaryotic ITS, the amplification resulted in three bands appearing as smears (>100 bp) and for prokaryotic IGS, bands greater than 800 bp was visible. The amplified products were subjected to restriction. However, the restricted products did not give clear resolution on 2.5 and 3% agarose. Hence, PAGE was conducted at 8% concentration of polyacrylamide. Ethidium bromide and silver staining methods were used for visualizing bands on PAGE, but silver staining was found to give clear bands. Given the fact that numerous discrete bands were visualized on the gel, there were many species of bacteria detected. All the amplified products possessed Msp1, Hae III sites. On an average, we could detect 7-22 bands

per primer with 16S rDNA/ Msp1 and Prokaryotic IGS/Msp1 combinations. Maximum bands detected for a single primer was 17 in the case of gliricidia-BP system for Prokaryotic IGS-Msp1 combination. Prokaryotes were found to dominate the bacterial population under all the systems. The number of common bands detected was maximum of 7 for 16S rDNA/Msp1 combination. Duplicate PCR yielded nearly indistinguishable results, as did multiple DNA isolations from a single rhizosphere sample, suggesting a high level of reproducibility in the DNA isolation, PCR and RFLP procedures (Figs 3 to 6).

The technique used was sensitive enough to detect changes in soil microbial community in the rhizosphere of various BP systems. Additional bands were observed in all the soils, unique to it. In case of Prokaryotic IGS/Msp1 combination 6 unique bands could be detected. In case of Prokaryotic IGS, maximum bands identified were using Msp1, while in Prokaryotic 16 S rDNA maximum bands were identified in Hae III digestion. Restriction with Msp1 detected maximum bands using Eukaryotic rRNA ITS primers. In case of Fungal ITS, Msp1 detected maximum bands. Among the systems, maximum number of bands was identified in gliricidia-BP system (68), followed by garuga- (66) and ailanthus- BP systems (65). Erythrina- and RCC pole- BP systems registered low levels of diversity of 48 and 50 bands respectively (Table 9). The rhizosphere soils of RCC pole-BP system showed a reduced microbial population compared to tree standards-BP systems. Relatively higher population of bacteria was found under BP systems of garuga (54), followed by gliricidia (43); erythrina (35), ailanthus (33) and RCC pole (32) registered the lowest. Fungal population was greatest under ailanthus-Bp system (19), while the other systems registered significantly lower fungal diversity (6-8) as evident from the different distances the PCR product migrated on the gel.

The microbial structure the rhizosphere of each system was distinctly characterized with unique populations. Rhizosphere soils of ailanthus- BP system showed 15 unique isolates followed by gliricidia-BP (11), garuga-BP (6), erythrina-BP (5) and RCC pole-BP (1). Some of the bands were found to be sensitive to a particular standard and was found to disappear, for eg. garuga-BP (1), gliricidia-BP (3) and ailanthus-BP (2). The dendrogram (Fig 7) generated from the banding pattern formed 2 major clusters. The rhizosphere soils of RCC pole- and erythrina-BP systems showed maximum similarity of 80%. The rhizosphere soils of ailanthus-BP system were found to be distinct from the rest and showed only 50% similarity.

B. Sites under cardamom based cropping systems

Soil samples were collected from cardamom based cropping systems which will included

(i) Cardamom-Pepper (ii) Cardamom-Coffee (iii) Coconut-Pepper (iv) Arecanut-Pepper (v) Cardamom-Coffee-Pepper (vi) Cardamom alone (vii) Pepper alone (viii) Arecanut-Tree spices (ix) Arecanut-Vanilla

Soil samples (0-30 cm) were collected from various cardamom based cropping systems. Soil pH varied between 4.8-5.2 and organic C between 1.9-2.7%. In general, the component crops had little influence on the various physico-chemical and biochemical parameters studied (Table 10 & 11). Available N levels ranged between 121-148 mg kg⁻¹, Bray P between 2.1-4.1 mg kg⁻¹, and available K between 212-338 mg kg⁻¹. Among the micronutrients, DTPA Zn was significantly lower at all sites (0.72-1.04 mg kg⁻¹), while DTPA Cu ranged between 4.1-13.0 mg kg⁻¹, DTPA Fe between 17.5-48.4 mg kg⁻¹ and DTPA Mn between 2.9-8.9 mg kg⁻¹.

With regard to biochemical parameters (Table 10), acid phosphatase ranged between 17.5 (cardamom alone) to 26.5 mmol pnp/g/h (cardamom + clove), phosphodiesterase between 1.3 (cardamom + allspice) to 2.9 mmol pnp/g/h (cardamom + clove) and invertase between 5.0

(cardamom + cinnamon) to 6.4 mmol glucose/g/h (cardamom + allspice). Likewise, levels of cellulase ranged between 1.8 (cardamom + allspice) to 3.3 mmol glucose/g/h (cardamom + clove), urease between 3.3 (cardamom + allspice) to 4.2 mmol NH₃/g/h (cardamom + allspice) and BAA protease between 1.8 (cardamom alone) to 2.8 mmol NH₃/g/h (cardamom + coffee). Arylsulphatase (0.38-0.46 mmol pnp /g/h) and dehydrogenase (132-184 nmol TPF/ g/ h) activities also differed little between the sites.

C. Sites under turmeric

Soils were collected for three consecutive years from turmeric sites under various nutrient management regimes consisting of the following treatments:

- (i) Chemical nutrient management (CNM): 60-50-120 kg ha⁻¹ NPK applied as urea, rock phosphate (RP) and muriate of potash (MOP) respectively. RP was applied as basal, urea in two splits and MOP in two splits (45th and 90th day after planting (DAP)).
- (ii) Integrated nutrient management (INM): 60-50-120 kg ha⁻¹ NPK applied exactly as above+ (10 kg FYM + 1.0 kg neem cake applied to one bed).
- (iii) Organic nutrient management (ONM): 20 kg FYM + 1.0 kg neem cake + 0.5 kg ash + 2.0 kg vermicompost (applied at 45 DAP) + Azospirillum (10⁸ CFU g⁻¹ soil) + Phosphobacteria (10⁸ CFU g⁻¹ soil), all applied to one bed

The study also consisted of an absolute control where no inputs, whatsoever, were applied. Soil samples (0-15 cm) were taken from the beds after clearing the litter layer. The soils samples (four per bed) were taken from the inner two-thirds of each bed, bulked to obtain a composite sample, cleared of any organic debris and transferred for storage in sealed plastic bags. The data presented (Tables 12-14) are means averaged across three years (2005-2007).

(i) Physico-chemical parameters, DOC and DON

The data on soil physico-chemical parameters is given in Table 12. In general, soil pH was acidic (range 4.54-5.30) and did not vary significantly among the treatments. However, the levels of SOC and mineral N varied markedly between the treatments. Greater levels of SOC, mineral N, DOC, DON, Bray P and exchangeable K were registered by either ONM or INM. Compared to CNM, SOC and mineral N levels in ONM were greater by 17% and 12% respectively. Similarly, SOC and mineral N levels in INM was greater by 13% and 11% respectively compared to the CNM. Bray P level was greatest in INM, exchangeable K and DOC in ONM and DON in the INM treatment. DOC level in ONM was greater by 25% and DON level in INM was greater by 34% compared to CNM.

(ii) Soil microbial biomass- C (C_{MIC}), -N (N_{MIC}), -P (P_{MIC}) and soil respiration

Appreciable variations in C_{MIC} levels were observed among the treatments, with maximum levels in ONM and INM (Table 13). In fact, C_{MIC} levels in ONM and INM were greater by 24% and 23% compared to the CNM. Contrary to C_{MIC} , P_{MIC} levels did not vary markedly between the treatments (range 13-15 $\mu\text{g g}^{-1}$). However, the levels of N_{MIC} were lower in ONM and INM (30 and 29 $\mu\text{g g}^{-1}$ respectively) but relatively higher in CNM (36 $\mu\text{g g}^{-1}$). Similar to C_{MIC} levels, soil respiration (measured as CO₂ evolution) was greater in INM and organic

treatments (29 and 28 $\mu\text{g CO}_2\text{-C g}^{-1}\text{day}^{-1}$ respectively). The chemical treatment registered a slightly lower rate of 25 $\mu\text{g CO}_2\text{-C g}^{-1}\text{day}^{-1}$, while the control registered the lowest (Table 13).

(iii) Total N mineralized/ Dehydrogenase (DHA) and catalase activity

Our results indicated that manure application to the soil created conditions that promoted an increase in the easily mineralizable inorganic-N pool, which was predominantly ammoniacal (Table 13). The total inorganic N mineralized was 32-35% higher in INM and ONM compared to CNM.

The oxyreductase enzyme (DHA) has been proposed as a measure of overall microbial activity and in our study, the activity of DHA in ONM and INM was greater by 22% and 20% compared to CNM (Table 14). The results also revealed that catalase activity in INM and CNM was greater by 50% compared to CNM. In particular, a significant correlation ($r = 0.79$, $P < 0.01$; $n=16$) was found between DHA and DOC suggesting a relationship between the availability of labile and easily mineralisable organic matter and the activity of microbial populations. In addition, DHA activity was positively correlated ($P < 0.01$, $n = 16$) with catalase activity ($r = 0.84$) and both enzyme activities with SOC ($r = 0.79$ and 0.81 , respectively) and SR ($r = 0.80$ and 0.76 , respectively) suggesting that addition of mineralizable organic residues provided substrates for these enzymes and enhanced microbial growth.

(iv) Soil hydrolytic enzyme activities

Similar to DHA, all the other enzymes involved in C, N, P and S cycles were activated to different degrees in the treated beds (Table 14). However, not all the treatments affected the enzyme activities to the same degree. For instance the activities of acid phosphatase, phosphodiesterase, β -glucosidase, BAA-protease and CM-cellulase were greater in ONM, while the activities of arylsulphatase, casein-protease, urease and invertase were greater in INM. In ONM, activity of acid phosphatase was greater by 28%, phosphodiesterase by 37%, β -glucosidase by 19%, BAA-protease by 5% and CM-cellulase by 30% compared to CNM. Similarly in INM, activity of arylsulphatase was greater by 35%, casein- protease by 33%, urease by 26% and invertase by 29% compared to CNM. Nevertheless, CNM registered relatively greater activities of all the enzymes compared to the control.

(v) Ratios of various microbial parameters

From the results it is evident that decreased levels of biologically available substrates and organic matter led to simultaneous decrease in microbial activity in the control. This was well reflected by the ratio of C_{MIC} : SOC, which was greatest in INM (2.68%) and ONM (2.59%) and least in CNM (2.36%). However, the C_{MIC} : N_{MIC} ratio did not vary appreciably between control, INM and CNM, though the ratio was greatest in the ONM (14.2).

The metabolic quotient ($q\text{CO}_2$) provides a measure of the specific metabolic activity and has been proposed as a bioindicator for environmental stress and reflects the efficiency of the use of SOC by microorganisms. In our study, $q\text{CO}_2$ was greater under control and CNM than under INM/ ONM. In fact, the levels in the control were greater by 17 and 21% compared to the INM and ONM.

(vi) Inter-relationships between various soil properties

To study the relationships among the soil properties, principal component analysis (PCA) was employed. The analysis revealed that 83% of the variance was explained by three factors (Table 15). The first (PC1), which accounted for 37% of the total variance, was defined by mineral N, DON and the measured biochemical parameters like C_{MIC} , DHA, BAA-protease,

casein-protease and phosphodiesterase. This reflects the size and activity of the microbial biomass and possibly reflected the strong relationship between the availability of labile and easily mineralisable organic matter and the activity of microbial populations. The negative part of the first factor was defined by N_{MIC} , reflecting increasing mineralization of N by bacteria in the organic and INM treatments. The positive part of the second factor (PC2) explaining 31% of the variance was defined by the soil respiration, P_{MIC} , phosphatase, β -glucosidase, urease, carboxymethylcellulase and invertase. This indicated the strong relationship between microbial activity and N/ P cycles in soils. Also the higher loadings of β -glucosidase, invertase and CM-cellulase suggested that in soils relying solely on chemical fertilizers the potential to mineralise organic matter, and so the activity of the C-cycle, is reduced. The negative loading of qCO_2 in this factor suggests a decrease in substrate use efficiency in beds with low organic matter especially the CNM and control treatments. The third factor (PC3) with 15% of the variance was defined by Bray P and exchangeable K. This reflected the logical dependence of microbial biomass on available P and K in the soil. The fact that DOC and soil C contents are included with high loadings in more than one factor indicates the effect of the different nutrient management regimes on the composition of soil organic matter and the typical interactions among the properties involved in the C, N, P and S cycles.

D. Sites under ginger

Soils were collected for 3 consecutive years from a study on ginger consisting of the following nutrient management regimes.

- i. Chemical: 75-50-50 kg ha⁻¹ NPK applied as urea, rock phosphate (RP) and muriate of potash (MOP) respectively. RP was applied as basal, urea in two splits (45th and 90th day after planting) and MOP in two splits (45th and 90th DAP).
- ii. Integrated Nutrient Management (INM): 10 kg FYM + 1.0 kg neem cake + urea (2 splits as above) + MOP (2 splits as above) + Biofertilizers (Phosphobacteria) applied to one bed.
- iii. Organic: 20 kg FYM + 1.0 kg neem cake + 0.5 kg ash + 2.0 kg vermicompost (applied at 45 DAP) + Biofertilizers (Azospirillum + phosphobacteria) applied to one bed.

The study also consisted of an absolute control where no inputs, whatsoever, were applied. Soil samples (0-15 cm) were taken from the beds after clearing the litter layer. The soils samples (four per bed) were taken from the inner two-thirds of each bed, bulked to obtain a composite sample, cleared of any organic debris and transferred for storage in sealed plastic bags.

(i) Physico-chemical parameters, DOC and DON

The data on soil physico-chemical parameters are given in Tables 16 & 17. In general, soil pH was acidic (range 4.77-5.32) and did not vary markedly among the treatments. However, the levels of organic C, available N, Bray P and exchangeable K varied markedly between the treatments. Significantly higher level of organic C level was registered by the organic treatment (1.84%), while the lowest level was recorded by the chemical and control treatments (1.68%).

Available N varied between 106-126 mg kg⁻¹, Bray P between 15-30 mg kg⁻¹ and exchangeable K between 172-221 mg kg⁻¹. Available N level was markedly higher in the chemical and INM treatments (126 mg kg⁻¹), Bray P in the organic treatment (30 mg kg⁻¹) and

exchangeable K in the INM treatment (221 mg kg⁻¹). The control treatment consistently registered lower levels of organic C, available N, Bray P and exchangeable K.

Exchangeable Ca and Mg levels were markedly higher in the INM treatment (458 and 84 mg kg⁻¹ respectively) and least in the chemical treatment (306 and 48 mg kg⁻¹ respectively; Table 17). Among the micronutrients, soil DTPA-Zn and -Cu levels were significantly higher INM treatment (0.99 and 2.7 mg kg⁻¹ respectively) and DTPA-Fe and DTPA-Mn were greater in the chemical treatment. Lower levels of DTPA-Cu, -Fe and -Mn were registered by the organic treatment and DTPA-Zn by the control treatment.

(ii) Soil biochemical/ microbial parameters

Soil microbial biomass carbon (C_{MIC}) varied significantly among the treatments, with the maximum levels in INM treatment (480 µg g⁻¹) followed by organic treatment (460 µg g⁻¹; Table 18). In general, C_{MIC} accumulated at markedly lower level in the control treatment (355 µg g⁻¹) followed by the chemical treatment (383 µg g⁻¹). The C_{MIC}: organic C ratio also varied among the treatments and ranged from 2.5-4.0%. The soils of the INM and organic treatments registered markedly greater C_{MIC}: organic C ratios (3.7 and 4.0% respectively) compared to the chemical and control treatments (2.28 and 2.11 respectively). Contrary to C_{MIC} levels, microbial biomass N (N_{MIC}) and microbial biomass P (P_{MIC}) did not vary markedly among the INM, chemical and organically managed beds (31-33 and 11-14 µg g⁻¹ respectively). However, the control treatment registered significantly lower N_{MIC} and P_{MIC} levels (24 and 7 µg g⁻¹) compared to the treated soils.

Soil basal respiration (measured as CO₂ evolution) ranged from 25-29 µg CO₂-C g⁻¹day⁻¹ and was greatest in organic and INM treatments and least in the chemical treatment (Table 18). Similar to basal respiration, considerable variation in metabolic quotient (qCO₂) was observed among the treatments. In general, qCO₂ levels were relatively lower in the organic and INM treatments compared to the chemical and control treatments.

The soils were also assayed for enzymes involved in C, N, P and S cycles (Table 18). Though there was no consistent variation among the sites, the organic and INM treatments registered greater activities of most of the enzymes assayed. For instance, the activities of dehydrogenase, phosphodiesterase, arylsulphatase, β-glucosidase, casein -protease and urease were greater in either the INM treatment or the organic treatment, while the activities of phosphomonoesterase, BAA-protease and CM cellulase were greater in the chemical treatment. Nevertheless, the control treatment registered relatively lower activities of almost all enzymes compared to the other treatments.

The activities of most enzymes (in both ginger and turmeric) did not show consistent variation among the treatments. In the short-term, addition of organic matter often leads to increased enzyme activities and long-term organic amendments can also stimulate enzyme activities and microbial biomass. Thus, despite changes in microbial community structure, changes in enzyme activities were less consistent with the contrasting nutrient management regimes employed in ginger and turmeric. Due to the functional redundancy of soil microorganisms (i.e. one function can be carried out by a range of different microorganisms), changes in microbial community structure do not necessarily lead to changes in enzyme activities. This is particularly true for the breakdown of sugars, proteins and organic P but may be less so for the decomposition of recalcitrant compounds. Two points regarding enzyme activities should be noted. Firstly, any short-term changes in enzyme activity may be masked by enzymes that are adsorbed to soil particles and which can retain their activity for many years. Secondly, enzyme activity tests measure the potential activity of a given enzyme in soil, not the actual activity.

(iii) Soil microbial community structure under ginger and turmeric

The study included establishing a simple and rapid soil DNA extraction protocol and optimization of PCR conditions for detection through RFLP and determination of the microbial community structure in soils under ginger and turmeric through DNA fingerprinting by PCR and gel electrophoresis. The touch down procedure was also tested for better amplification and resolution. The finger prints developed were analysed to deduce the microbial community structure using softwares. The isolated DNA was of good quality with A260/A280 of 1.75 (Fig.8). The DNA recovered was abundant with an average yield of 20 µg/g of soil. There was no humic acid contamination and the DNA was appropriately diluted and 25-50 ng was used/25 µl for PCR using primers listed in Table 1. The PCR amplified DNA was subjected to two methods of analysis *viz.*, PCR RFLP and denaturing PAGE and compared. The former involved digestion with restriction enzymes Hae III, ECOR I, Bam H1, Taq I, Rsa I, Sma I and Hind III. Characteristic restriction patterns were obtained. PCR RFLP was performed using the forward and reverse primers ITS1 F and ITS 1 R. The number of bands obtained using various enzymes is given in Table 7 It was observed that maximum number of bands was found under IPNM in comparison to the rest. Soils under chemical treatment showed least number of bands.

Under denaturing conditions, in presence of urea and heat (55⁰C) greater number of bands was observed than in case of PCR RFLP. This method was found to be better than PCR RFLP in our study. Hence to determine of the diversity of microorganisms from which DNA had been extracted six different primer sets were tested, including both for multi and single copy genes. The multicopy targets included the prokaryotic small subunit rRNA, prokaryotic rRNA intergenic spacer region, the eukaryotic rRNA internal transcribed spacer region (ITS) region, ITS region for fungi, ITS region specific to nematodes, universal primers specific to ITS1 and ITS2. With dilution of DNA from each extraction technique, successful PCR amplification was achieved with all primers tested.

The DNA at appropriate dilutions of 50 ng/25 µl yielded successful amplification with all six primers *viz.*, 16S rDNA, prokaryotic intergenic spacer, eukaryotic internal transcribed sequence, fungal internal transcribed sequences, internal transcribed sequences (ITS1), internal transcribed sequences (ITS 2), nematode specific internal transcribed sequences with all the four rhizosphere samples under ginger (Figs 9, 10). In PCR RFLP the amplified products were subjected to restriction digestion as per manufacturers instruction and it was observed that overnight restriction using PCR products diluted twice gave better DNA profiles. The products were initially run on agarose gels, however, only ITS products gave satisfactory resolution. The restricted products from all PCRs were hence run on 8% PAGE. Ethidium bromide and silver staining methods were used for visualizing bands on PAGE, but silver staining was found to give clear bands. Given the fact that numerous discrete bands were visualized in the gel, there were many species of bacteria detected. All the amplified products possessed restriction sites as evident from the banding patterns. On an average we could detect a maximum of 6 under fully organic condition in case of both Taq 1 and ECOR1 and ITS1 primer and a minimum of 1 in case of BAM H1 and Msp 1 bands per primer with Msp1 and Bam H1. Duplicate PCR yielded nearly indistinguishable results, as did multiple DNA isolations from a single rhizosphere sample, suggesting a high level of reproducibility in the DNA isolation, PCR and RFLP procedures. The technique used was sensitive enough to detect changes in microbial community under various rhizosphere samples.

A comparison of PCR RFLP and denaturing PAGE showed that the resolution and number of bands detected were more in latter. In case of PCR RFLP primer chosen was ITS1 and five restriction enzymes were employed. Maximum number of bands was obtained under fully organic treatment (6) for ECOR1 and Taq enzymes for ITS 1 primer (Table 7). However, the

number of bands detected was very low in comparison to the profile under denaturing condition. Fig 11 (a-b) depicts the number of bands obtained with each primer under denaturing PAGE. All the primers taken together, maximum number of bands were obtained in case of IPNM (60). Maximum bands were obtained in IPNM for ITS1, ITS2, prokaryotic IGS and 16S rDNA primers. Chemical treatment registered maximum for ITS fungal primers. In case of fully organic treatment maximum bands were observed for prokaryotic IGS primers. The control registered the lowest population in all the cases.

Touch Down PCR was performed with seven primers under study. The profiles generated showed better resolution and greater number of bands compared to PCR RFLP (Figs 9, 10). Maximum bands could be identified in case of fully organic treatment (64) when amplification products of all the primers were taken together. Maximum number of nematodes was found in case of IPNM and fully organic treatments. Fungal population was higher in chemical treatment. In case of primer ITS2 chemical treatment showed least number of bands. In general prokaryotic population was highest under IPNM and fungal population was highest under chemical treatment. Maximum eukaryotic population was observed under IPNM and fully organic treatments. Controls invariably showed low number of bands in all the cases. The banding profiles when analysed showed that based on the presence and absence of bands maximum similarity of 66% was observed among chemical and control treatments the banding profiles of fully organic and IPNM treatments showed a close similarity with each other and clustered together showing a similarity in their community composition, separate from the first cluster. The similarity between the two was around 60%.

In general, addition of organic inputs in the fully organic and integrated treatments showed a priming effect i.e. the increase in soil organic matter (SOM) decomposition rate after fresh organic matter input to soil. This is often supposed to result from a global increase in microbial activity due to the higher availability of energy released from the decomposition of fresh organic matter. Indeed, the maintenance or restoration of soil fertility on the one hand, and the need for soil carbon sequestration in the context of global warming on the other, demand an efficient management of the interactions between fresh and stabilized organic matter. To achieve this goal, much more attention should be paid to the 1. Enzymatic activities of SOM feeding microorganisms in relation to nutrient availability and energetic status; 2. The inability of microorganisms to control the dynamics of the extracellular enzymes they produce; 3. The environmental control (biotic and abiotic) of the intensity of the competition for fresh organic matter (FOM) between r-strategists with rapid growth rates and K-strategists with slow growth rates; 4. The mechanisms of the impact of the chemical characteristics of FOM (C/N ratio, polymerization) and soil nutrient content on the intensity of priming effect; and 5. the impact of the temporal course of FOM incorporation to soil on the intensity of priming effect.

8222 Questions- Answered

- Levels of macro-, secondary- and micro-nutrients in soils of black pepper under different nutrient management regimes and in the rhizosphere of various tree support-black pepper systems.
- Levels of biochemical parameters (enzyme activities) and microbial indices in the rhizosphere of various tree support-black pepper systems.
- Shifts in soil microbial community structure in the rhizosphere of various tree support-black pepper systems.

- Levels of macro-, secondary and micro- nutrients in soils under various cardamom based cropping systems.
- Levels of biochemical parameters (enzyme activities) and microbial indices in soils under various cardamom based cropping systems.
- Levels of macro-, secondary and micro- nutrients in soils under ginger and turmeric as influenced by contrasting nutrient management regimes.
- Biochemical and microbial indices of soils ginger, and turmeric under contrasting management regimes.
- Shifts in microbial community structure of soils under contrasting nutrient management regimes of ginger and turmeric.
- Inter-relationships between various soil quality parameters in soils under ginger and turmeric.

8223 Process/ Product/ Technology Developed

- Data on soil quality parameters especially biochemical and microbial parameters has been generated for spices based cropping systems.
- Technique for extraction of microbial DNA from soil was improvised and standardized. Denaturing PAGE and touch down protocol and techniques for better resolution of bands was attempted and standardized.

8224 Practical Utility

(Not more than 150 words)

- The information on the physico-chemical, general and specific biochemical properties and microbial indices of soils under spices would form a part of comparable database on properties of soils of other cropping systems. This would also provide a general estimate on the degree of degradation at the said sites.
- In all the soils, the most limiting nutrients were P and Zn, while N and K levels were low to medium. Organic C levels were, in general medium to high and in some cases low. It is therefore imperative that fertilization through a judicious mix of organic and inorganic fertilizers based on initial soil test values is resorted to in these soils. This is vital to getting sustainable yields considering the fact that both ginger and turmeric are nutrient exhaustive crops. Application of micronutrients especially Zn has by far been neglected in these soils. But the data suggests that application of micronutrients based on critical limits is essential for good yields.
- The results provide clear evidence that the agroforestry tree species employed as supports for BP exert a large control on the rhizosphere microbial community composition, an effect attributed to variation in organic C/ substrate levels, root activities such as root exudation etc. The results imply that among the tree species studied, *G. sepium* and to some extent *G.*

pinnata can be used as live stakes for the restoration of degraded black pepper plantations and overall improvement in soil quality in the plains of the tropics.

- In ginger and turmeric, the results indicated that integrated nutrient management (INM) and exclusive use of biological fertilizers (organic nutrient management) positively affected soil quality due to enhanced organic matter content. Consequently, the nutrient levels and properties reflecting biological activity *viz.*, C_{MIC}, N_{MIC}, P_{MIC}, CO₂ evolution, N mineralization and enzyme activities etc were higher in the organic/ INM treatments.
- The results revealed that except for the activities of β -Glucosidase and CM cellulase, which were greater in the chemical treatment, the activities of dehydrogenase, phosphomonoesterase, phosphodiesterase, BAA-protease, arylsulfatase, casein –protease and urease were markedly higher in the INM or organic treatments. This is apparently a consequence of enhanced substrate and microbial biomass levels in these treatments suggesting that greater levels of labile substrates led to greater microbial proliferation and subsequently to greater enzyme synthesis and accumulation in the organic and INM treatments.
- Apparently, microbial activity was limited by the supply of biologically available substrates in the chemical and control treatments suggesting that even short-term incorporation of organics [singly (fully organic) or in combination with chemical fertilizers (integrated)] promoted microbial and enzyme activities and improved soil nutrient availability. Contrarily, the more direct supply of nutrients from decomposing organic manure and the indirect supply of nutrients from the mineralization of organic matter led to significantly higher microbial activity in the organic and INM treatments.
- These results suggest that the nutrient management regimes resulted in diverging microbial communities with possibly different ecological functions. If the application of manures results in a rhizosphere-like response, then the microbial community in manured soils is likely to be more metabolically active, responding quickly to the input of fresh organic residues. Indeed, the use of organic nutrient sources depends on microbial activities for the mineralization of plant available nutrients. While organic manures are typically applied to supply crop N, P and K needs, the impact on the soil, and consequently, crop growth and microbial activities, goes beyond the application of those nutrients. The use of organic manure (in INM and fully organic treatments) also supplies large amounts of readily available C, resulting in a more diverse and dynamic microbial system than in inorganically fertilized soil.

8225 Constraints, if any: Nil

823 Publications and Material Development

(One copy each to be supplied with this proforma)

8231 Research papers

Dinesh R., Srinivasan V., Hamza S. Soil physico-chemical, biochemical and microbial properties in the rhizosphere of tree species used in black pepper plantations of the tropics. (Communicated to Geoderma, Elsevier), (NAAS impact-8.6).

Dinesh R, Srinivasan V, Hamza S, Manjusha A. (2010) Short-term incorporation of organic manures and biofertilizers influences biochemical and microbial characteristics of soils under an annual crop [Turmeric (*Curcuma longa* L.)] (*Accepted for publication in Bioresource Technology, Elsevier*), (NAAS impact-8.6).

8232 Popular articles

8233 Reports

Book Chapter

Dinesh R, Srinivasan V, Kandiannan K, Hamza S (2008) Organic manures. In: Organic spices (Eds Parthasarathy VA, Kandiannan K, Srinivasan V), New India Publishing Agency, New Delhi, pp 39-100.

Review

Dinesh R, Kandiannan K, Srinivasan V, Hamza S, Parthasarathy V A. 2005. Tree species used as supports for black pepper (*Piper nigrum* L.) cultivation. Focus on Pepper (*Piper nigrum* L.) 2, 39-47.

8234 Seminars, conferences and workshops (relevant to the project) in which the scientists have participated.

- Workshop on Organic farming and Post Harvest Technology, Dimapur, Nagaland, 18-21 October 2005.
- SYMSAC III: Crop and Product Diversification, Lalbagh, Bangalore, 08-10 November 2006.
- Biennial Group Meeting of research workers of AICRPS, IISR, Calicut, 25-27th May 2006.
- National Seminar on Organic Spices and Aromatic Crops, Calicut, 01-02 February 2007
- XIX National group meeting of research workers of AICRP on spices, OUAT, Bhubaneswar, 23-25 November 2007.
- National symposium on spices, medicinal and aromatic crops (SYMSAC IV), 25-26 November 2007 at OUAT, Bhubaneswar.
- National Workshop on Zingiberaceous Spices at IISR, Calicut during 19-20 March 2008.

824 Infrastructural facilities developed

(Details of field, laboratory, note books and final material and their location)

The lab note book & data register are available in the division.

825 Comments / Suggestions of Project Leader regarding possible future line of work that may be taken up arising out of this Project.

- The result clearly indicates the selective influence of plant species (tree supports of black pepper) on the microbial composition of rhizosphere soils, which indicated that the rhizosphere soils under tree supports- BP systems are more active and plant exudates have a profound influence on the microbial content and structure. Based on differences in root exudation and rhizodeposition in different root zones, rhizosphere microbial communities can vary in structure and species composition in different root locations or in relation to soil type, plant species, nutritional status, age, stress, disease and other environmental factors. Hence, future studies should focus on studies that involve identification of allelochemicals in the extracts of such tree supports, the direct effects of these extracts on soil microbial community structure vis-à-vis soil biochemical and physico-chemical properties.
- The study also showed that nutrient management has induced an alteration of soil microbial communities, corresponding to a lower microbial activity in soils with less organic inputs (chemically managed soils and control). Further investigation should focus on establishing which microbial functions are involved in such alteration and their effects on long-term soil fertility. The differences between bacterial and fungal populations indicate that biological carrying capacities of the soils of the various spices based cropping systems varied in magnitude. The future challenge would be to identify the populations and guilds of microorganisms, which have key functional roles in specific soil processes under various systems. Sensitive methods (DGGE, TGGE and FISH) could be employed for phylogenetic identification of microorganisms under various systems. However, the challenge for microbial ecologists will be to understand how microbial communities are organized in soils and how they interact with their physical, chemical and biological environments.
- In general, addition of organic inputs in the fully organic and integrated (INM: chemical + organic) treatments showed a priming effect i.e. the increase in soil organic matter (SOM) decomposition rate after fresh organic matter input to soil. This is often supposed to result from an increase in microbial activity due to the higher availability of energy released from the decomposition of fresh organic matter. The maintenance or restoration of soil fertility on the one hand, and the need for soil carbon sequestration in the context of global warming on the other, demand an efficient management of the interactions between fresh and stabilized organic matter. To achieve this goal, much more attention should be paid to 1. The enzymatic activities of SOM feeding microorganisms in relation to nutrient availability and energetic status; 2. The inability of microorganisms to control the dynamics of the extracellular enzymes they produce; 3. The environmental control (biotic and abiotic) of the intensity of the competition for fresh organic matter (FOM) between r-strategists with rapid growth rates and K-strategists with slow growth rates; 4. The mechanisms of the impact of the chemical characteristics of FOM (C/N ratio, polymerization) and soil nutrient content on the intensity of priming effect; and 5. The impact of the temporal course of FOM incorporation to soil on the intensity of priming effect.

Part-IV: Project Expenditure
(Summary)

830 Total Recurring Expenditure

8301 Salaries: (Designation with pay scale)	<u>Actual (Rs)</u>
i) Scientific	9,85,000
ii) Technical	3,15,000
iii) Supporting	1,05,000
iv) Wages	45,000
Sub-Total	14,50,000
8302 Consumables	
i) Chemicals	2,00,000
ii) Glasswares	80,000
iii) Others	40,000
Sub-Total	3,20,000
8303 Travel	75,000
8304 Miscellaneous (other costs)	-
8305 Sub-Total (Recurring)	18,45,000
831 Total Non – Recurring Expenditure (Equipments and works)	-
823 Total (830 and 831)	18,45,000

Part-V: Declaration

This is to certify that the final report of the Project has been submitted in full consultation with the Project workers as per the approved objectives and technical program and the relevant records, note books and materials are available for the same.

Signature of the Project Investigator: R. Dinesh

Co-Investigators: 1. V. Srinivasan

2. S. Hamza

Signature & Comments of the Head of the Division/ Section:

Signature & Comments of the Joint Director (Research):

Signature & Comments of the Director:

Table.1a. Primers used in the study for PCR amplification of multi-copy genes

Target for amplification	Primer sequence
Prokaryotic 16 S rRNA	FOR B 5' AGAGTTTGATCCTGGCTCAG REV B5' GGTTACCTTGTTACGACTT
Prokaryotic rRNA intergenic spacer (IGS) region	SPRRNAF 5' GAAGTCGTAACAAGG SPRRNAR 5' CAAGGCATCCACCGT
Eukaryotic rRNA internal transcribed spacer (ITS) region	ITS1 5' TCCGTAGGRTGAACCTGCGG ITS 4 5' TCCTCCGCTTATTGATATGC
Fungal specific ITS	LICHITSF5' GCGGAAGGATCATTACTGAG LICHITSR 5' GGGTATCCCTACCTGATCCG

Table 1b. Primers used for PCR amplification

Target for amplification	Primer sequence
Prokaryotic 16 S rRNA	FOR B 5' AGAGTTTGATCCTGGCTCAG REV B5' GGTTACCTTGTTACGACTT
Prokaryotic rRNA intergenic spacer (IGS) region	SPRRNAF 5' GAAGTCGTAACAAGG SPRRNAR 5' CAAGGCATCCACCGT
Eukaryotic rRNA internal transcribed spacer (ITS) region	ITS1 5' TCCGTAGGRTGAACCTGCGG ITS 4 5' TCCTCCGCTTATTGATATGC
Fungal specific ITS	LICHITSF5' GCGGAAGGATCATTACTGAG LICHITSR 5' GGGTATCCCTACCTGATCCG

Table 2a. Composition of the reaction mixture used for PCR

Component	1x (25 μ L)	5 x (25 μ L)
10X buffer	2.5	12.5
DNTP	2.5	12.5
MgCl ₂	3.0	15
Primer	2.0	10
DNA	2.0	10
Water (distilled)	13.75	68.75
Taq polymerase	0.25	1.25

Table 3a. Composition of reaction mixture used for restriction digestion of DNA

Component	Single digestion with ECOR I (μ L)	Single digestion with Hind III (μ L)	Double digestion with ECOR I & Hind III (μ L)
Water	11.2	11.2	9.4
Buffer	2	2	4
B.S.A	0.2	0.2	0.4
Enzyme	0.6	0.6	1.2
DNA	5.0	5.0	5.0

Table 4a. Composition of reaction mixture used for RFLP analysis

Component	Msp I (μ L)	Hae III (μ L)
Water	7.4	7.5
Buffer	2.0	2.0
B.S.A	0.2	0.2
Enzyme	0.4	0.3
PCR product	10.0	10.0

Table 5a. Chemical constituents of 8% polyacrylamide gel used in the study

Chemical	Volume
30% Acrylamide	10.66ml
Water	25ml
5X TBE buffer	4ml
10% APS	400µl
TEMED	20µl

Table 1: Soil physico-chemical properties in organic sites of black pepper

	pH	OC (%)	Available N	Bray P	Available K	Zn	Cu	Fe	Mn
	-----mg kg ⁻¹ -----								
Kasaragod	5.19	3.7a	146c	0.12d	211ab	0.40d	8.7a	7.9d	2.84d
Kannur	5.12	1.7c	156b	4.60a	109d	1.08c	1.7d	20.9c	6.90bc
Pulpally	5.26	1.5cd	129d	1.39c	145c	3.40a	3.3c	38.0b	36.4a
P'muzhi	5.81	2.6b	203a	2.22b	238a	1.90b	7.9ab	44.6a	8.30b

In a column, means followed by the same letter are not significantly different at P<0.05

Table 2: Soil biochemical properties in organic sites of black pepper

	Kasaragod	Kannur	Pulpally	P'muzhi
Phosphatase (µmol pnp/g/h)	26.3ab	24.3cd	24.4c	26.7a
Phosphodiesterase (µmol pnp/g/h)	3.60c	4.30ab	4.80a	4.30ab
Invertase (µmol glucose/g/h)	7.80ab	7.40bc	6.2d	7.90a
Cellulase (µmol glucose/g/h)	0.31c	0.26d	0.42ab	0.43a
Urease (µmol NH ₃ /g/h)	5.36d	6.44bc	7.52a	6.73b
Protease (µmol NH ₃ /g/h)	4.10ab	4.00bc	4.40a	3.70d
Arylsulfatase (µmol pnp /g/h)	0.39bc	0.40ab	0.40ab	0.44a
Dehydrogenase (µmol TPF/ g/ h)	132.0cd	136.0c	145.0ab	147.0a

In a row, means followed by the same letter are not significantly different at P<0.05

Table 3: Soil physico-chemical properties in organic, chemical and INM sites of black pepper

	pH	OC (%)	Available N	Bray P	Available K	Zn	Cu	Fe	Mn
-----mg kg ⁻¹ -----									
Chemical ^a	5.33	2.05ab	137bc	1.0cd	245b	1.8cd	1.1cd	38ab	6.0a
INM ^b	5.27	1.65c	146b	5.6b	264a	3.2b	4.2b	38ab	5.2b
Organic ^c	5.46	2.27a	174a	9.6a	146c	6.2a	9.2a	42a	4.5bc
Control	5.31	1.65c	112d	1.3c	142cd	1.9c	1.9c	27c	3.8cd

^aChemical: 140-50-270 g NPK/ vine; ^bIPNM: 5 kg FYM + Recommended Dose of Fertilizer + PSB;

^cOrganic: 5 kg FYM + 500g Neem cake + 500 g Ash+ 2 kg Vermicompost with biofertilizer – *Azospirillum*, *PSB* and *Pseudomonas sp.*

Table 4: Soil biochemical properties in organic, chemical and INM sites of black pepper

	Chemical ^a	INM ^b	Organic ^c	Control
Phosphatase (μmol pnp/g/h)	33.2c	38.3a	36.2ab	31.3cd
Phosphodiesterase (μmol pnp/g/h)	2.3cd	4.0ab	4.6a	2.6c
Invertase (μmol glucose/g/h)	8.3ab	6.8c	8.7a	6.3cd
Cellulase (μmol glucose/g/h)	0.8b	1.2a	1.2a	0.8b
Glucosidase (μmol pnp/g/h)	6.9c	8.6b	12.7a	5.2d
Urease (μmol NH ₃ /g/h)	8.7a	7.3b	6.2c	5.1d
Protease (μmol NH ₃ /g/h)	2.4c	3.9a	3.2ab	1.9cd
Arylsulfatase (μmol pnp /g/h)	0.41ab	0.45a	0.45a	0.38bc
Dehydrogenase (μmol TPF/ g/ h)	174c	212b	232a	118d

Table.5. Soil pH, organic C and nutrient levels in the rhizosphere of tree species used in black pepper plantations

	Ailanthus ^a	Gliricidia ^b	Garuga ^c	Erythrina ^d	Control
pH (1:2.5 H ₂ O)	4.52bc	4.16de	4.21cd	4.62b	5.86a
Organic C (g kg ⁻¹)	21.6cd	26.5a	24.8b	22.4c	15.0e
Mineral N (mg kg ⁻¹)	56cd	78a	74ab	63c	26e
Bray P (mg kg ⁻¹)	5.6c	6.8b	8.4a	5.2d	2.2e
Exchangeable K (mg kg ⁻¹)	322.0a	233.0cd	236.0de	297.0b	240.0c
Exchangeable Ca (mg kg ⁻¹)	360.0e	540.0bc	573.0b	456.0d	643.0a
Exchangeable Mg (mg kg ⁻¹)	96.0e	118.0c	113.0cd	131.0b	165.0d
Available Zn (mg kg ⁻¹)	1.8c	2.7a	2.6ab	1.8c	1.3d
Available Cu (mg kg ⁻¹)	6.8e	16.0b	18.3a	14.2c	13.6cd
Available Fe (mg kg ⁻¹)	88ab	80cd	82c	92a	76de
Available Mn (mg kg ⁻¹)	4.8de	10.6a	8.9b	8.4bc	5.4d

Means followed by the same letter are not statistically significant at $P < 0.05$

^a*Ailanthus triphysa* (Dennst.) Alston. DC.; ^b*Gliricidia sepium* (Jacq.) Steud.; ^c*Garuga pinnata* Roxb., ^d*Erythrina indica* Lamk.

Table 6. Biochemical properties of soils in the rhizosphere of tree species used in black pepper plantations

	Ailanthus ^a	Gliricidia ^b	Garuga ^c	Erythrina ^d	Control
Dissolved organic C (mg kg ⁻¹)	398.2bc	419.6a	402.5b	384.2c	186.8d
Dissolved organic N (mg kg ⁻¹)	44.6ab	45.8a	43.2ab	35.6c	22.8d
Total inorganic N mineralized (mg N kg ⁻¹ per 10 days)	78.6c	112.3a	103.4ab	74.5cd	46.8e
Dehydrogenase (nmol TPF g ⁻¹ soil h ⁻¹)	234c	244ab	249a	221d	126e
Urease (μmol NH ₃ -N g ⁻¹ h ⁻¹)	7.3de	8.2a	7.9ab	7.4cd	7.6bc
Acid phosphatase (μmol <i>p</i> -nitrophenolg ⁻¹ h ⁻¹)	12.9b	12.7bc	13.9a	12.7bc	7.9d
Arylsulfatase (μmol <i>p</i> -nitrophenol g ⁻¹ h ⁻¹)	0.56ab	0.52c	0.58a	0.45de	0.48d
β-glucosidase (μmol <i>p</i> -nitrophenol g ⁻¹ h ⁻¹)	7.4cd	7.8bc	9.4a	8.3b	5.5e

Table 7a. Microbial biomass- C (C_{MIC}), -N (N_{MIC}), -P (P_{MIC}), soil respiration and ratios of various microbial indices in the rhizosphere of tree species used in black pepper plantations

	Ailanthus ^a	Gliricidia ^b	Garuga ^c	Erythrina ^d	Control
C _{MIC} (μg g ⁻¹)	423c	474a	454ab	415cd	205e
N _{MIC} (μg g ⁻¹)	28b	32a	29ab	29ab	11c
P _{MIC} (μg g ⁻¹)	14.7a	13.6a	14.2a	14.0a	7.2b
Soil respiration (μg CO ₂ -C g ⁻¹ day ⁻¹)	25.0a	24.0ab	24.0ab	22.0bc	17.0d
C _{MIC} : organic C (%)	1.96a	1.79cd	1.83bc	1.85b	1.37e
C _{MIC} : N _{MIC}	15.1b	14.8b	14.6b	14.3b	18.6a
C _{MIC} : P _{MIC}	28.8a	34.8a	32.0a	29.6a	28.4a
qCO ₂ (mg CO ₂ -C (g biomass C) ⁻¹ day ⁻¹)	59b	51cd	53c	53c	83a

Means followed by the same letter are not statistically significant at P < 0.05

Table 7b. Principal component loadings after Varimax rotation. The soil parameters are grouped according to the maximum fittings to principal components (Correlation coefficients > 0.50; n=30)

Measurements	Principal components ^a	
	PC1	PC2
Eigen values	13.55	1.45
Explained variance (%)	67.7	20.5
Rotated loading on two retained components ^b		
pH	-0.87	-0.52
Organic C	0.78	0.56
Mineral N	0.79	0.54
N mineralized	0.66	0.72
Bray P	0.76	0.42
Dissolved organic C	0.86	0.25
Dissolved organic N	0.83	0.35
Dehydrogenase	0.83	0.27
Urease	-0.05	0.87
Acid phosphatase	0.81	0.04
Aryl sulphatase	0.43	0.35
β -glucosidase	0.78	0.33
C _{MIC}	0.84	0.31
N _{MIC}	0.81	0.23
P _{MIC}	0.73	0.08
Soil respiration	0.83	0.15
qCO ₂	-0.84	-0.31

^a Only principal components with eigen values > 1 and those explaining > 10% of the total variance were retained.

^b Correlations with absolute values higher than 0.50 are in bold.

Table.8. Primers used in the study for PCR amplification of multi-copy genes

Target for amplification	Primer sequence
Prokaryotic 16 S rRNA	FOR B 5' AGAGTTTGATCCTGGCTCAG REV B5' GGTTACCTTGTTACGACTT
Prokaryotic rRNA intergenic spacer (IGS) region	SPRRNAF 5' GAAGTCGTAACAAGG SPRRNAR 5' CAAGGCATCCACCGT
Eukaryotic rRNA internal transcribed spacer (ITS) region	ITS1 5' TCCGTAGGRTGAACCTGCGG ITS 4 5' TCCTCCGCTTATTGATATGC
Fungal specific ITS	LICHITSF5' GCGGAAGGATCATTACTGAG LICHITSR 5' GGGTATCCCTACCTGATCCG

Table 9. The number of bands identified in rhizosphere soils under live (tree species) and non-living (RCC pole) supports of black pepper

No.	Primer/ Restriction enzyme	Number of bands identified				
		Erythrina	Garuga	RCC pole	Gliricidia	Ailanthus
1	Pro IGS/Msp1	10	18	10	17	8
2	Euk rRNA ITS/Msp1	2	2	3	9	5
3	Pro IGS/Hae III	9	14	10	15	7
4	Euk rRNA ITS/Hae III	4	4	8	8	8
5	Pro 16S rDNA/Msp1	5	6	4	6	4
6	Pro 16S rDNA/Hae III	11	16	8	5	14
7	Fungal ITS/Msp1	4	4	5	6	7
8	Fungal ITS/Hae III	3	2	2	2	12
	Total	48	66	50	68	65

Table 10: Soil physico-chemical properties in cardamom based systems

	pH	OC (%)	Av N	Bray P	Av K	Zn	Cu	Fe	Mn
	-----mg kg ⁻¹ -----								
Cardamom + Clove	5.1	1.9	135	3.5	338	0.85	7.8	17.5	5.1
Cardamom + Nutmeg	5.2	2.7	148	3.8	319	0.84	5.1	45.0	6.5
Cardamom + Coffee	5.1	2.3	143	2.3	214	1.04	13.0	32.3	2.9
Cardamom + Cinnamon	4.9	2.3	129	2.1	335	0.72	4.5	46.8	8.9
Cardamom + Allspice	4.8	2.1	132	2.2	265	0.75	5.0	48.4	4.9
Cardamom alone	4.9	2.6	121	4.1	212	0.75	5.6	41.2	6.8

Table 11: Soil biochemical properties in cardamom-based systems

	Cardamom +					
	Clove	Nutmeg	Coffee	Cinnamon	Allspice	Cardamom alone
Acid Phosphatase	26.5	22.0	25.4	25.0	21.7	17.5
Phosphodiesterase	2.9	2.2	2.1	2.6	1.3	1.6
Invertase	5.6	6.2	5.2	5.0	6.4	5.1
Cellulase	3.3	2.6	2.3	2.1	1.8	1.9
Urease	4.2	3.4	3.9	3.7	.3	3.3
Protease	2.1	2.5	2.8	2.2	2.0	1.8
Arylsulfatase	0.41	0.41	0.45	0.38	0.46	0.41
Dehydrogenase	184	172	132	156	151	176

Phosphatase/ Phosphodiesterase/ β -glucosidase/ Arylsulfatase - $\mu\text{mol pnp/g/h}$
 Invertase/ Cellulase- $\mu\text{mol glucose/g/h}$; Urease/ Protease- $\mu\text{mol NH}_3/\text{g/h}$
 Dehydrogenase- $\text{nmol TPF}/\text{g/h}$

Table 12. Soil pH, soil organic C (SOC), levels of major nutrients, dissolved organic- C (DOC) and -N (DON) in soils under various nutrient management regimes of turmeric

	Chemical	INM	Organic	Control
pH (1:2.5 H ₂ O)	5.30	4.54	5.05	5.09
SOC (g kg ⁻¹)	16.0c	18.3b	19.2a	15.3cd
Mineral N (mg kg ⁻¹)	114cd	128ab	130a	115c
Bray P (mg kg ⁻¹)	17.0ab	18.0a	13.0c	13.0c
Exchangeable K (mg kg ⁻¹)	234b	248a	214c	177d
Dissolved organic C ($\mu\text{g g}^{-1}$)	346c	462ab	464a	248d
Dissolved organic N ($\mu\text{g g}^{-1}$)	35c	53a	47ab	30cd

In each row, means followed by the same letter are not significantly different at $P < 0.05$

Table 13. Biochemical/ microbial properties of soils under various nutrient management regimes of turmeric

	Chemical	INM	Organic	Control
Microbial biomass C ($\mu\text{g g}^{-1}$)	378c	491ab	498a	367d
Microbial biomass N ($\mu\text{g g}^{-1}$)	36a	29bc	30b	30b
Microbial biomass P ($\mu\text{g g}^{-1}$)	14ab	15a	15a	13c
CO ₂ evolution ($\mu\text{g CO}_2\text{-C g}^{-1}\text{day}^{-1}$)	25bc	29a	28ab	19d
qCO ₂ ($\text{mg CO}_2\text{-C (g biomass C)}^{-1}\text{ day}^{-1}$)	72ab	63c	60cd	76a
Microbial biomass C: SOC (%)	2.36cd	2.68a	2.59ab	2.40c
Microbial biomass C: N	12.6b	12.6b	14.2a	12.2bc
NH ₄ mineralized (mg N kg^{-1} per 10 days)	54b	91a	89a	21c
Total inorganic N mineralized (mg N kg^{-1} per 10 days)	68b	101a	105a	13c

In each row, means followed by the same letter are not significantly different at $P < 0.05$

Table 14. Enzyme activities in soils under various nutrient management regimes of turmeric

	Chemical	INM	Organic	Control
Dehydrogenase ($\text{nmol TPF g}^{-1}\text{soil h}^{-1}$)	187c	233a	241ab	183cd
Catalase ($\text{mmol H}_2\text{O}_2$ consumed $\text{g}^{-1}\text{ h}^{-1}$)	1.6bc	2.4a	1.7b	0.9d
Acid phosphatase ($\mu\text{mol } p\text{-nitrophenol g}^{-1}\text{ h}^{-1}$)	10.3ab	14.3ab	14.4a	7.4c
Phosphodiesterase ($\mu\text{mol } p\text{-nitrophenol g}^{-1}\text{ h}^{-1}$)	2.11c	3.31ab	3.37a	2.07cd
Arylsulphatase ($\mu\text{mol } p\text{-nitrophenol g}^{-1}\text{ h}^{-1}$)	0.32c	0.49a	0.40ab	0.30cd
β -glucosidase ($\mu\text{mol } p\text{-nitrophenol g}^{-1}\text{ h}^{-1}$)	3.69a	4.43bc	4.56ab	3.13d
BAA-protease ($\mu\text{mol NH}_3\text{-N g}^{-1}\text{ h}^{-1}$)	7.24bc	7.51ab	7.63a	6.32d
Casein-protease ($\mu\text{mol tyrosine g}^{-1}\text{ h}^{-1}$)	1.72c	2.56a	2.38ab	1.46cd
Urease ($\mu\text{mol NH}_3\text{-N g}^{-1}\text{ h}^{-1}$)	6.21c	8.43a	7.89ab	5.06d
CM cellulase ($\mu\text{mol glucose g}^{-1}\text{ h}^{-1}$)	0.50a	0.67ab	0.71bc	0.43d
Invertase ($\mu\text{mol glucose g}^{-1}\text{ h}^{-1}$)	8.21bc	11.57a	10.28ab	5.09d

In each row, means followed by the same letter are not significantly different at $P < 0.05$

Table 15. Loading of physico-chemical, biochemical and microbial properties on the factors identified by principal components analysis. The soil parameters are grouped according to the maximum fittings to principal components (Correlation coefficients > 0.50^b; n=16)

Variable	Principal components ^a		
	PC1	PC2	PC3
pH	n.s. ^c	n.s.	n.s.
Organic C	0.55	0.65	n.s.
Mineral N	0.88	n.s.	n.s.
Bray P	n.s.	n.s.	0.83
Exchangeable K	n.s.	n.s.	0.84
DOC	0.72	0.61	n.s.
DON	0.87	n.s.	n.s.
C _{MIC}	0.81	0.51	n.s.
N _{MIC}	-0.87	n.s.	n.s.
P _{MIC}	n.s.	0.81	n.s.
CO ₂ evolution	n.s.	0.90	n.s.
qCO ₂	n.s.	-0.80	n.s.
DHA	0.88	n.s.	n.s.
Acid phosphatase	n.s.	0.82	n.s.
Phosphodiesterase	0.84	n.s.	n.s.
Arylsulphatase	n.s.	n.s.	n.s.
β-glucosidase	0.60	0.72	n.s.
Casein-protease	0.67	n.s.	n.s.
BAA-protease	0.80	n.s.	n.s.
Urease	0.52	0.67	n.s.
Cellulase	n.s.	0.89	n.s.
Invertase	n.s.	0.70	n.s.
Explained variance (%)	36.7	31.3	14.6

^a Only principal components with eigen values > 1 and those explaining > 10% of the total variance were retained.

^b Correlations with absolute values higher than 0.50 are in bold.

^c loadings lower than 0.50

Table 16. pH, Organic C and levels of major nutrients (N, P, K) in soils under various nutrient management regimes of ginger

	pH (1:2.5 H ₂ O)	Organic C (%)	Available N -----	Bray P (mg kg ⁻¹)-----	NH ₄ OAc-K
Chemical ^a	4.80	1.68	126	22.0	173
INM ^b	5.32	1.78	126	26.7	221
Organic ^c	5.19	1.84	120	30.0	211
Control ^d	4.77	1.68	106	15.0	172
lsd (0.05)	NS	0.4	12	6	17

Table 17. Levels of secondary (Ca & Mg) and micronutrients (Zn, Cu, Fe & Mn) in soils under various nutrient management regimes of ginger

	-----Exchangeable-----		-----DTPA-extractable-----			
	Ca	Mg	Zn	Cu	Fe	Mn
	----- (mg kg ⁻¹)-----					
Chemical ^a	306	48	0.58	2.7	78	5.6
INM ^b	458	84	0.99	2.7	62	6.6
Organic ^c	363	71	0.76	1.9	59	2.7
Control ^d	426	69	0.56	2.1	77	3.3
lsd (0.05)	34	15	0.21	0.5	11	1.7

Table 18. Biochemical properties of soils under various nutrient management regimes of ginger

	Chemical	INM	Organic	Control
Dissolved organic C	324c	433b	481a	236d
Dissolved organic N	32c	49a	48ab	31cd
Microbial biomass C ($\mu\text{g g}^{-1}$)	383c	480a	460b	355d
Microbial biomass N ($\mu\text{g g}^{-1}$)	33a	33a	31ab	24c
Microbial biomass N ($\mu\text{g g}^{-1}$)	27c	48ab	51a	26cd
Microbial biomass P ($\mu\text{g g}^{-1}$)	11ab	14a	12ab	7c
CO ₂ evolution ($\mu\text{g CO}_2\text{-C g}^{-1}\text{day}^{-1}$)	25c	29a	28ab	23cd
qCO ₂ ($\text{mg CO}_2\text{-C (g biomass C)}^{-1}\text{day}^{-1}$)	65a	60bc	61b	65a
Microbial biomass C: organic C (%)	2.28c	3.70ab	4.00a	2.11cd
Microbial biomass C: N	14.2a	10.0c	9.0cd	13.7ab
Dehydrogenase ($\text{nmol TPF g}^{-1}\text{soil h}^{-1}$)	178c	239ab	243a	176cd
Phosphomonoesterase ($\mu\text{mol } p\text{-nitrophenol g}^{-1}\text{ h}^{-1}$)	14.6a	14.3ab	14.2ab	9.3c
Phosphodiesterase ($\mu\text{mol } p\text{-nitrophenol g}^{-1}\text{ h}^{-1}$)	1.99cd	3.25a	2.97ab	2.11c
Arylsulphatase ($\mu\text{mol } p\text{-nitrophenol g}^{-1}\text{ h}^{-1}$)	0.41cd	0.51ab	0.56a	0.42c
β -Glucosidase ($\mu\text{mol } p\text{-nitrophenol g}^{-1}\text{ h}^{-1}$)	4.40bc	4.41ab	4.56a	3.62c
BAA-protease ($\mu\text{mol NH}_3\text{-N g}^{-1}\text{ h}^{-1}$)	7.78a	7.78a	6.71bc	6.81b
Casein -protease ($\mu\text{mol tyrosine g}^{-1}\text{ h}^{-1}$)	1.63c	2.36ab	2.52a	1.41cd
Urease ($\mu\text{mol NH}_3\text{-N g}^{-1}\text{ h}^{-1}$)	5.73c	8.24a	8.12ab	5.14cd
CM cellulase ($\mu\text{mol glucose g}^{-1}\text{ h}^{-1}$)	0.62a	0.57ab	0.62a	0.51bc

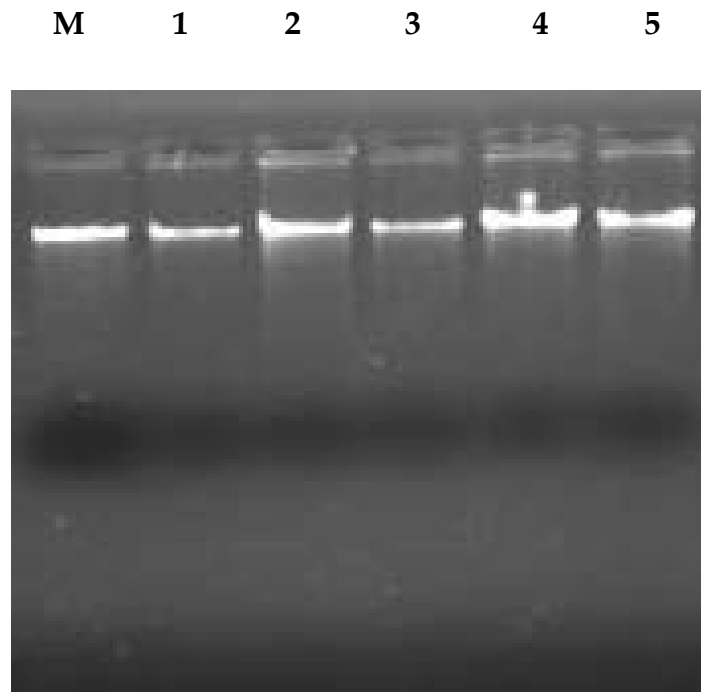


Fig 1. DNA isolation from rhizosphere soils under various BP systems (M- human genomic DNA marker; Erythrina-BP; 2-Garuga-BP; 3- RCC pole-BP, 4-Gliricidia-BP; 5- Ailanthus-BP)

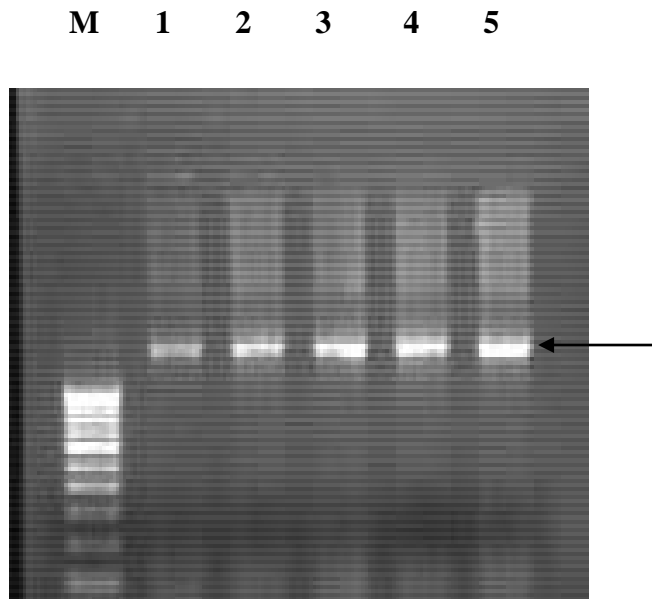


Fig 2. Representative photograph showing PCR amplification of rhizosphere soil DNA using 16 S rDNA primer (M- Molecular weight marker, 1- Erythrina-BP; 2-Garuga-BP; 3- RCC pole-BP, 4-Gliricidia-BP; 5-Ailanthus-BP; arrow indicates amplified product- 1.6 kb)

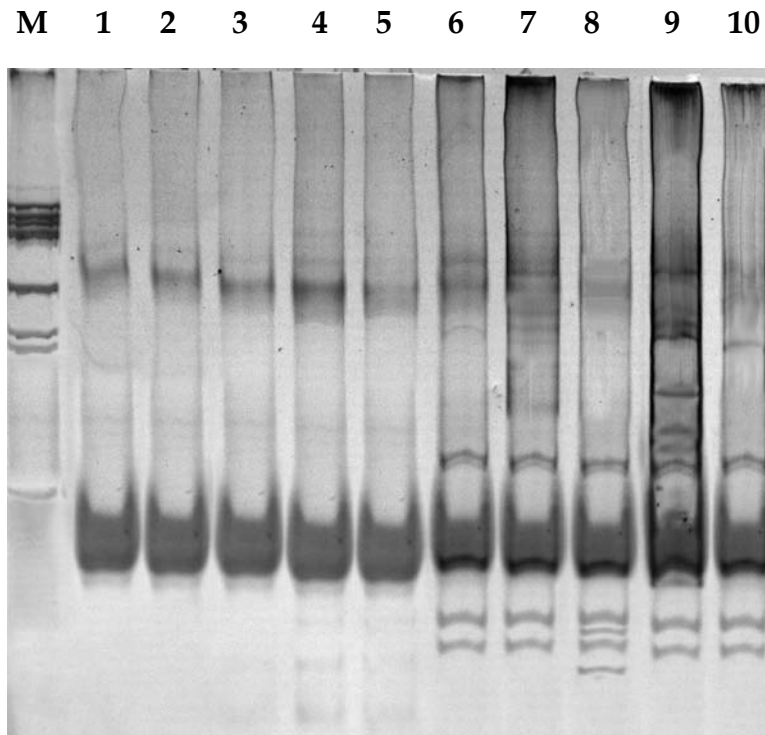


Fig 3. PCR-RFLP profiles obtained from rhizosphere soils under various systems using eukaryotic ITS (1 to 5) and prokaryotic IGS (6-10) specific primers with Msp1 restriction (1 & 6: Erythrina-BP, 2 & 7: Garuga-BP; 3 & 8: RCC pole-BP; 4 & 9: Gliricidia-BP; 5 & 10: Ailanthus-BP)

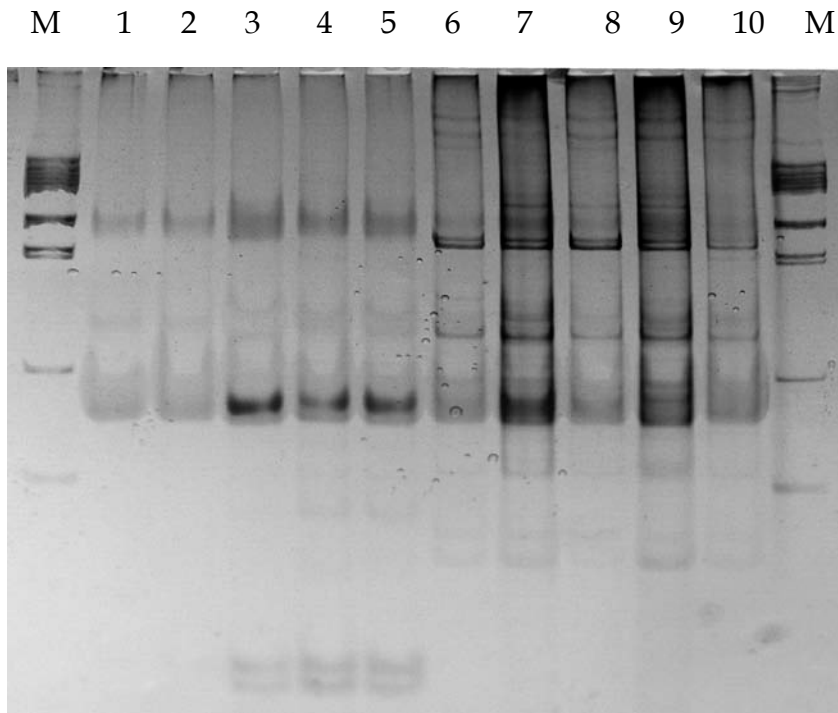


Fig. 4. PCR-RFLP profiles obtained from rhizosphere soils under various systems using eukaryotic ITS (1 to 5) and prokaryotic IGS (6-10) specific primers with Hae III restriction (1 & 6: Erythrina-BP; 2 & 7: Garuga-BP; 3 & 8: RCC pole-BP; 4 & 9: Gliricidia-BP; 5 & 10: Ailanthus-BP)

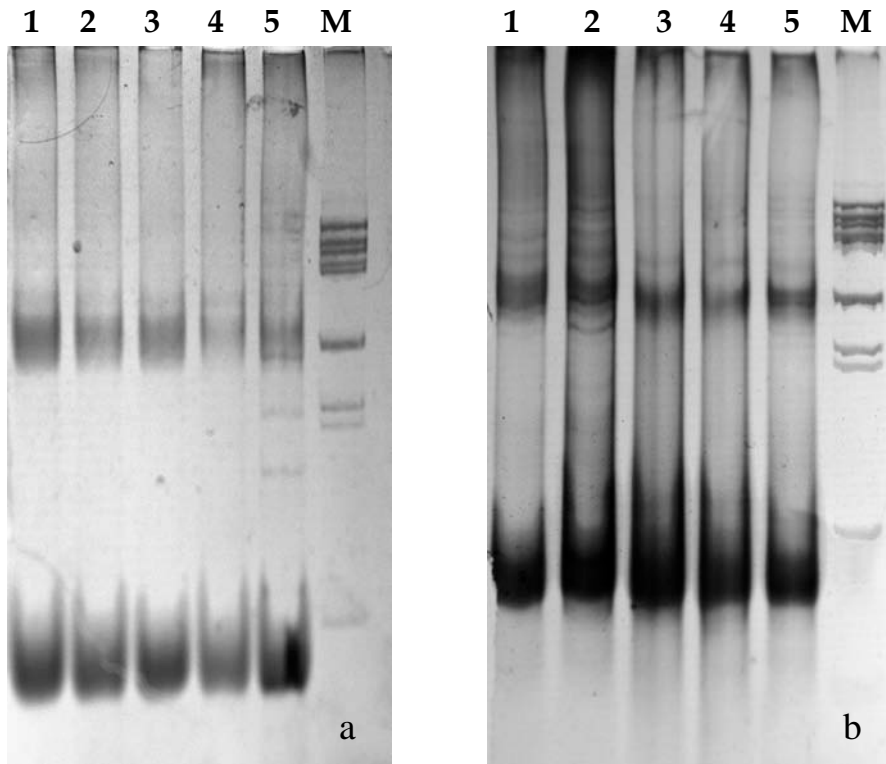


Fig 5. PCR-RFLP profiles obtained from rhizosphere soils under various systems using (a) Fungal ITS and (b) 16 S rDNA specific primers with Hae III restriction (M- Molecular weight marker, 1- Erythrina-BP; 2-Garuga-BP; 3- RCC pole-BP, 4-Gliricidia-BP; 5-Ailanthus-BP)

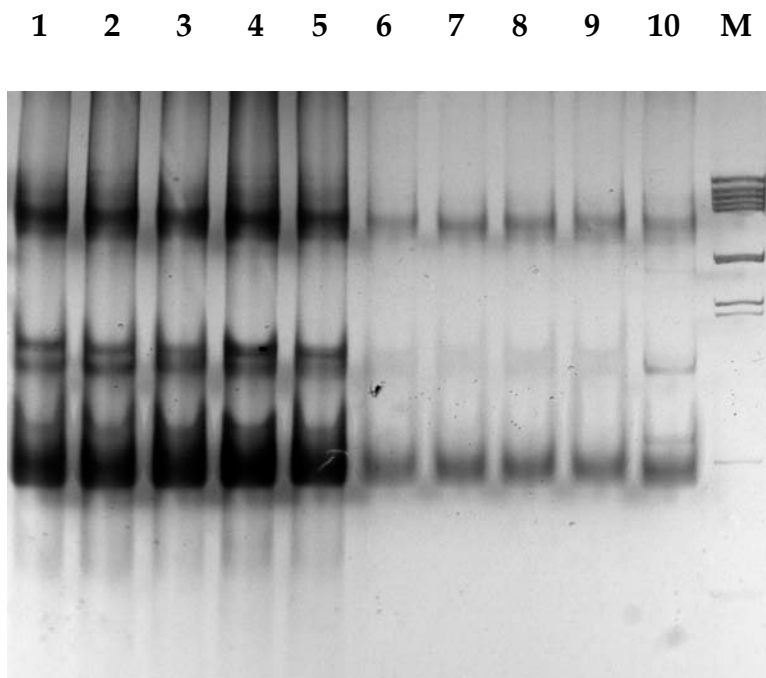


Fig 6. PCR-RFLP profiles obtained from rhizosphere soils under various systems using 16S rDNA (1 to 5) and Fungal ITS (6-10) specific primers with Msp1 restriction

(M- Molecular weight marker; 1 & 6: Erythrina-BP; 2 & 7: Garuga-BP; 3 & 8: RCC pole-BP; 4 & 9: Gliricidia-BP; 5 & 10: Ailanthus-BP)

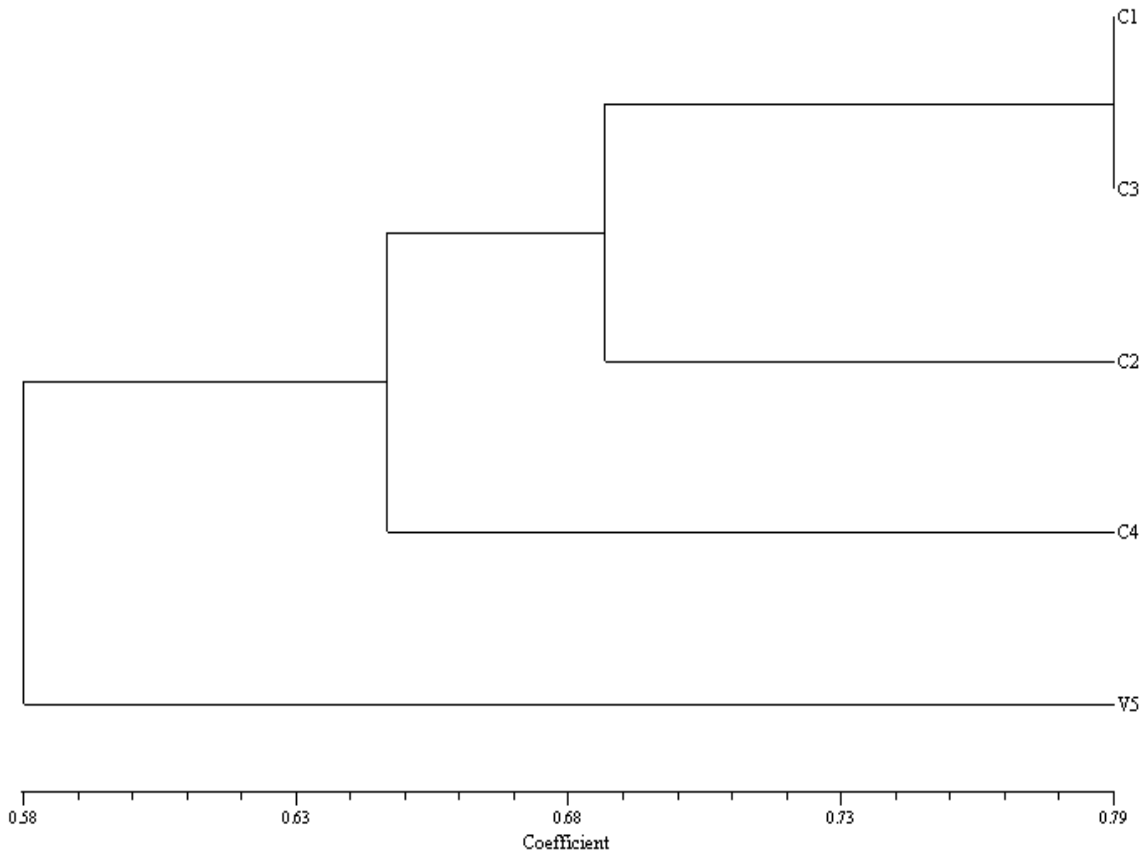
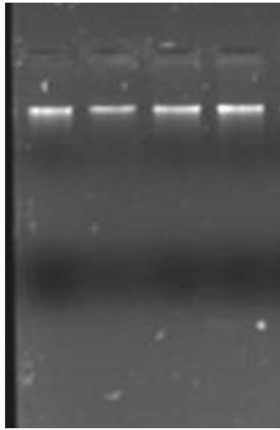


Fig. 7. Dendrogram based on Jaccard's similarity of soils in the rhizosphere depicting community relationships of various standard- black pepper systems (C1- Erythrina-BP; C2- Garuga- BP; C3- RCC pole-BP; C4- Gliricidia- BP; V5- Ailanthus- BP)



Isolation of DNA from ginger soils under different management practices. 1: 100 bp marker; 2: control; 3: fully organic; 4: chemical; 5: IPNM

Fig.8. Isolation of DNA from ginger soils under various nutrient management regimes

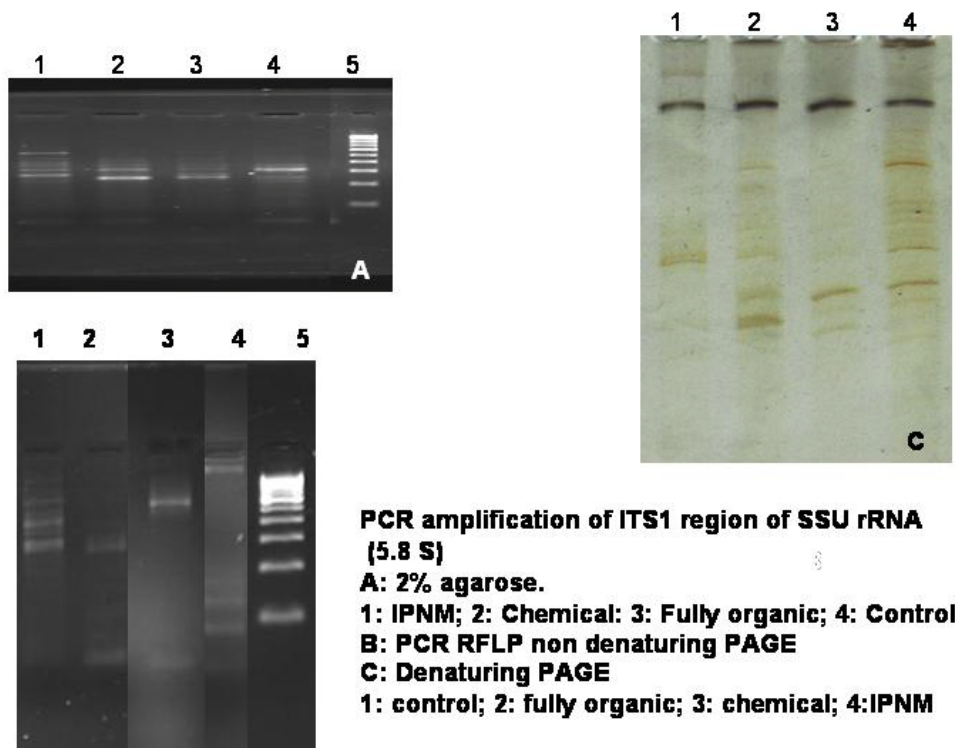
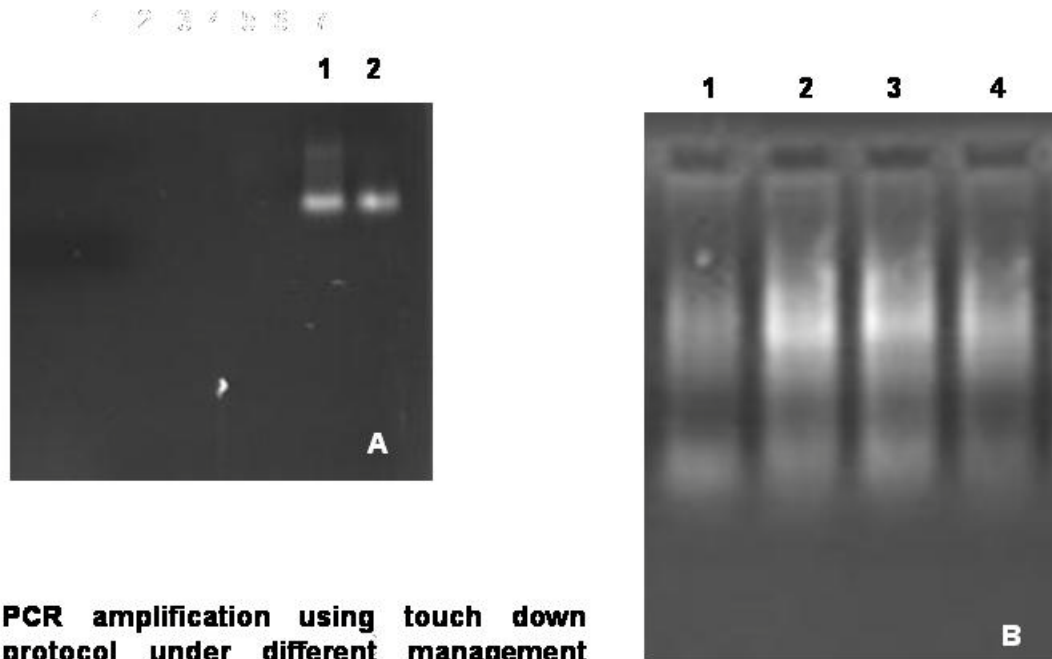


Fig.9. PCR amplification of ITS 1 region of rRNA



PCR amplification using touch down protocol under different management practices of ginger soils. A: nematode specific primer 1: IPNM; 2: chemical. B: Prokaryotic IGS primers 1: IPNM 2: chemical 3: fully organic 4: control

Fig 10.PCR amplification using touch down PCR

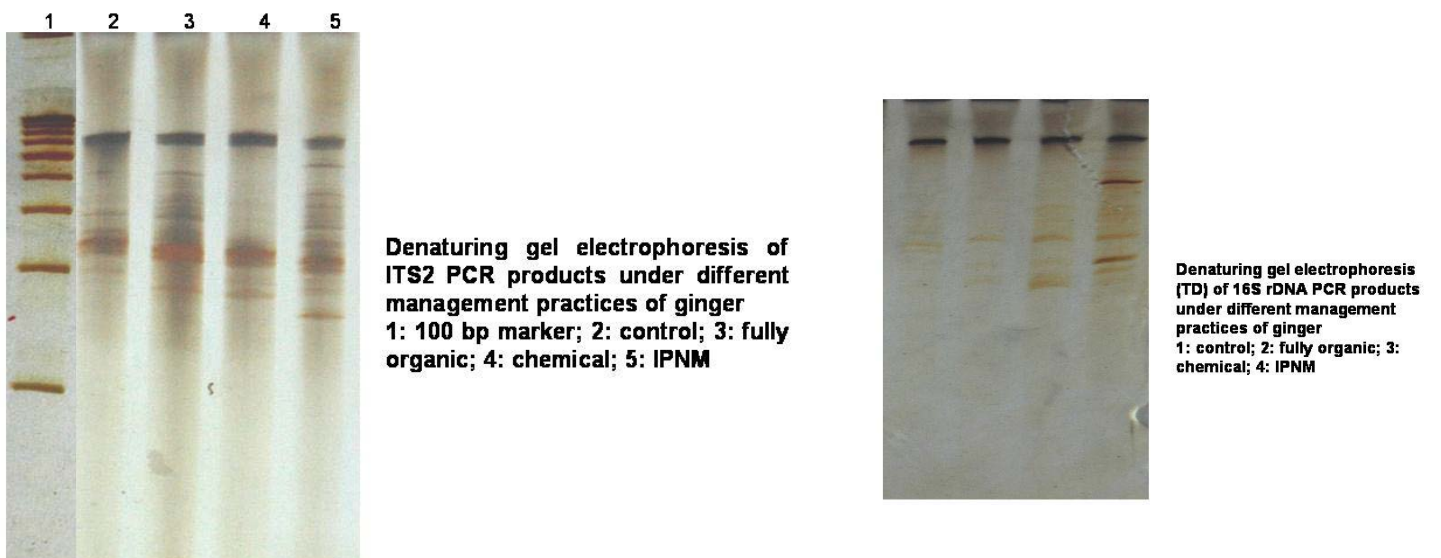
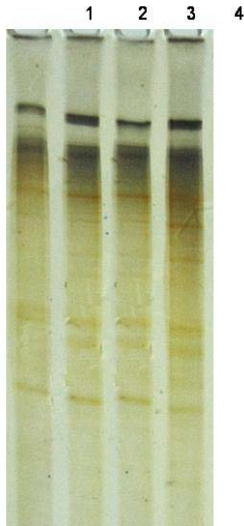


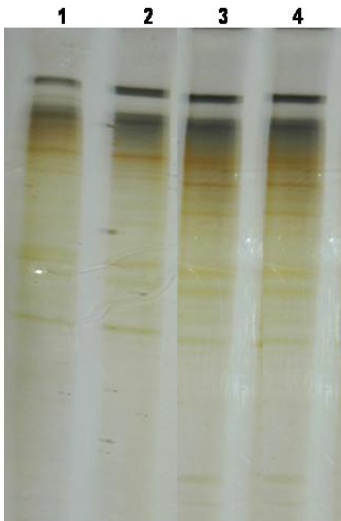
Fig 11a. Denaturing gel electrophoresis of PCR products



Denaturing gel electrophoresis of Euk. ITS PCR products under different management practices of ginger
 1.IPNM; 2:Chemical, 3: Fully organic, 4:Control



Denaturing gel electrophoresis of ITS1 PCR products under different management practices of ginger
 1: Control, 2: Fully organic, 3: Chemical, 4: IPNM



Denaturing gel electrophoresis of Fungal ITS PCR products under different management practices of ginger
 1: control; 3: fully organic; 4: chemical; 5: IPNM



Denaturing gel electrophoresis of Prokaryotic ITS PCR products under different management practices of ginger
 1: 100 bp marker; 2: control; 3: fully organic; 4: chemical; 5: IPNM 1.100bp marker: 2. ipnm: 3. chemical: 4. fully organic: 5 .control

Fig 11b. Denaturing gel electrophoresis of PCR products



Denaturing gel electrophoresis of Nematode ITS PCR products under different management practices of ginger
 1. ipnm, 2. chemical, 3. fully organic, 4. control