

FUNGAL POPULATION AND DIVERSITY IN ORGANICALLY AMENDED AGRICULTURAL SOILS OF MEGHALAYA, INDIA

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Abstract

The effect of different organic fertilisers (farm yard manure [FYM]; vermicompost [VC]; plant compost [PC] and integrated compost [INT], i.e. a combination of FYM, VC and PC in a 1:1:1 ratio) on the population and diversity of soil fungi was investigated in a maize-French bean trial. Fungal populations were much higher in organically fertilised plots as compared to the control (CTRL) and showed a decreasing trend in the order FYM>PC>INT>VC>CTRL. Altogether, 122 fungal species and two sterile mycelia were isolated from all the plots of which 25 fungal genera belonged to Deuteromycotina, seven to Ascomycotina, four to Zygomycotina and one to Mastigomycotina. The most common genera isolated from all the plots include *Penicillium*, *Aspergillus*, *Acremonium*, *Fusarium*, *Mortierella*, *Mucor*, *Paecilomyces*, *Talaromyces*, *Trichoderma* and *Verticillium*. Significant positive correlations between fungal populations and C_{org} were observed in all the organic amended plots. The organic matter level in the organically managed soil systems can play a pivotal role in fungal growth, sporulation and diversity.

Keywords: organic, compost, microbial, fertility, physical properties, chemical properties.

Introduction

In Meghalaya, agriculture is the main stay of the people and about 70% of the population depends on agriculture for their livelihood. During the 20th century, conventional agricultural management, reliant on mineral fertilisers, has been popularised in this area for increasing the yield of crops. On average, the consumption of chemical fertilisers in the State is 18 kg/ha per annum and is concentrated mainly in potato and other vegetables (Meghalaya Agriculture Profile, 2006). Additionally, a large part of the population in Meghalaya still practises shifting agriculture or *jhuming* (Saleh, 1988; Borah, 1999) which has deleterious effect on forest area and soil fertility and has increased the need for chemical fertilisers application to retain soil nutrients. The intensive use of agrochemicals is known worldwide to reduce biodiversity, increase erosion and deplete soil organic matter (Dick, 1992) and affect surface and ground water quality, especially through leaching (Schiavon *et al.*, 1995; Tate, 1995). Such activities (i.e. *jhuming* and use of chemical fertilisers) in the hilly regions may affect neighbouring regions as well (Ghosh, 2003).

Soil is a most precious natural resource and contains the most diverse assemblages of living organisms. Indigenous microbial populations in soil are of fundamental importance for ecosystem functioning in both natural and managed agricultural soils (O'Donnell *et al.* 1994; Doran and Zeiss 2000) because of their involvement in such key processes as soil structure formation, organic matter decomposition, nutrient cycling and toxic removal (Van Elsas, 1997; Doran and Zeiss, 2000). The community of soil flora and fauna is influenced directly or indirectly by management practices, e.g. cultivation and the use and application of organic and inorganic fertilisers (Bloem *et al.* 1994; Matson *et al.*, 1997). A growing number of studies show that organic farming leads to higher soil quality and more biological activity (microbial populations and microbial respiration rate) in soil than conventional farming (Droogers and Bouma, 1996; Mader *et al.* 2002; Girvan *et al.*, 2004). Microbial population size and community structure are sensitive to changes in chemical properties of the surrounding soil (Pansombat *et al.*, 1997; Tokuda and Hayatsu, 2002). Further, considerable evidence indicates that changes in the composition of a microbial community can be used to predict and dictate alteration in soil quality (Van Brugen and Semenov, 2000; Breure, 2005).

Microbial communities, particularly bacteria and fungi constitute an essential component of biological characteristics in soil ecosystems. It has been estimated that 1.5 million fungal species are present in natural ecosystems, but only 5–10% have been described formally (Hawksworth 2001). Schmit and Mueller (2007) estimated that there is a minimum of 7, 12,000 fungal species worldwide. The actual number of fungi is still unknown; however, only 5-13 % of the total estimated global fungal species have been described (Wang *et al.* 2008). Research on fungal diversity provides a basis for estimating the functional role of fungi in ecosystems. Soil fungal population is favoured largely by organic farming systems (Drinkwater *et al.*, 1995; Girvan *et al.*, 2004) but not much has been published about its population and diversity in these systems

especially in the agricultural lands of Meghalaya. A better understanding of the fungal diversity in soil with different organic amendments may prove crucial in predicting which is the best for application.

A precise study on the fungal communities associated with organic farming systems particularly in this poorly studied area can be crucial in appraising the beneficial and harmful aspects of these soil microbes. As such, this study was carried out with an aim to study the fungal population and diversity of organic systems in a maize-French bean field trial.

Materials and methods

Site description

The field experiment was conducted at a lowland experimental block of Agronomy Division, Indian Council of Agricultural Research (ICAR) for North Eastern Hill (NEH) Region, Meghalaya. The geographical location of the study site is 25°41' 26.7"N latitude and 91°55'26.2"E longitude and is located at an elevation of 956 m (asl). The climate of the area is humid sub-tropical with temperature ranging from 6° C (January) to 30°C (July) and an annual precipitation of 2320 mm. On an average, 90% of the total rainfall is received during April-October. Soil texture of the experimental site is silty loam (Clay - 32.58%; Sand - 12.83%; Silt - 54.58%).

Experimental design and treatments

For the experimental set up two crops grown in rotation were selected viz., maize (*Zea mays* L.) (May – July) and French bean (*Phaseolus vulgaris*) (August – September). The experimental field was divided into five plots with each having three replicates for the different organic amendments. The net plot size was 3m x 4m. A control plot without organic fertiliser (CTRL) was also maintained. The different organic amendments used included farm yard manure (FYM); plant compost (PC); vermicompost (VC) and integrated compost (INT) i.e. combination of FYM, VC and PC (1:1:1). Optimum fertiliser dosage was applied to the field as recommended by ICAR (Table 1). The organic amendments were applied twice in a year i.e., early April and late July before sowing maize and French-bean seeds respectively. Seeds were manually sown in rows 25 cm apart at a depth of 4-5 cm. Hand weeding was done to manage the weeds. Both the crops were cultivated under rainfed conditions.

Table 1. Type of organic amendments and doses in the experiment.

Fertiliser	Source	Dose (tonnes/ha)	Dose (kg/plot)
Farm Yard Manure (FYM)	Dried cow dung	5	48
Plant Compost (PC)	Weeds from the field	5	48
Vermicompost (VC)	Earthworm cast	5	48
Integrated Compost (INT)	FYM:PC:VC (1:1:1)	5	48 (16:16:16)

Soil physico-chemical properties

Soil sampling was done from the upper 0 - 15 cm depth at monthly interval from pre-sowing to post-harvest period for a period of two years (2006 and 2007). The soil samples were collected randomly from five different locations (i.e. from the three replicate plots) during each crop cycle under different organic fertiliser amendments. The soil samples from each organic fertiliser treatment were pooled together and mixed thoroughly so as to get a composite sample.

Collected samples were brought to the laboratory sieved through 2mm sieve at field moist conditions and determination of soil moisture content and pH was done. Air dried ground and sieved (0.25 mm) samples were used for the estimation of organic C, total N, available P and K content. Three replicate samples were used for each analysis. Moisture content (MC) was determined by weight loss after drying 10 g of soil at 105°C for 24 hours and expressed as percentage dry weight. Soil pH was measured in a 1:5 water suspension using a portable digital pH meter. Colorimetric method (Anderson and Ingram, 1993), micro Kjeldahl distillation and titration method (Jackson, 1973), Molybdenum blue method (Allen *et al.*, 1974) and the ammonium acetate flame photometry method (Jackson, 1973) were applied to estimate organic carbon (C_{org}), total nitrogen (N), available phosphorus (P) and exchangeable potassium (K) respectively.

Fungal population count

For fungal population analysis, serial dilution plate method (Johnson and Curl, 1972) was followed using Rose Bengal Agar medium (Martin, 1950) supplemented with streptomycin sulphate. The inoculated Petri plates were incubated in a sterile culture room at $25^{\circ} \pm 1^{\circ}\text{C}$. Colony forming units (CFU) were estimated by counting the number of colonies after five days. Fungal colonies formed were calculated on per gram dry soil basis. Fungi were identified according to their macroscopic and microscopic features. Identification at the species level was carried out according to the morphological characters found principally in publications by Gillman (1957), Barnett and Hunter (1972), Domsch *et al.* (1980), Subramanian (1983), Ellis (1993) and Watanabe (1994). Pure cultures of fungi were maintained in test tubes slants containing Czapek Dox agar medium (Raper and Thom 1949) and preserved in deep freezer at -20°C .

The following indices for fungal species diversity were calculated using the Index of general diversity (H') or Shannon and Weaver (1949) diversity index, $H' = -\sum(ni/N \log_e ni/N)$ and the Index of dominance (C) or Simpson (1949) index of dominance, $C = \sum (ni/N)^2$.

Statistical analysis

Correlation coefficient was done to test the relationship between fungal population and the soil physico-chemical properties. Difference at $p \leq 0.05$, 0.01 and 0.001 levels were considered as statistically significant. Analysis of Variance (ANOVA) (Tukey's test) was carried out to compare the variation of the fungal population means in the different organic amendments. All statistical analyses were performed using Statistica 6.0 software package.

Results

Fungal population and diversity

Fungal population was comparatively higher in organically amended plots as compared to control. Among all the treatments FYM showed significantly higher fungal population. The fungal population showed the trend in decreasing order as $\text{FYM} > \text{PC} > \text{INT} > \text{VC} > \text{CTRL}$. Inconsistent monthly variation in the fungal population was also observed in all the treatments (Figure 1). Significant variation was observed in all the plots (control and organically amended plots) according to Tukey's test (ANOVA) at $p \leq 0.05$ (Figure 2).

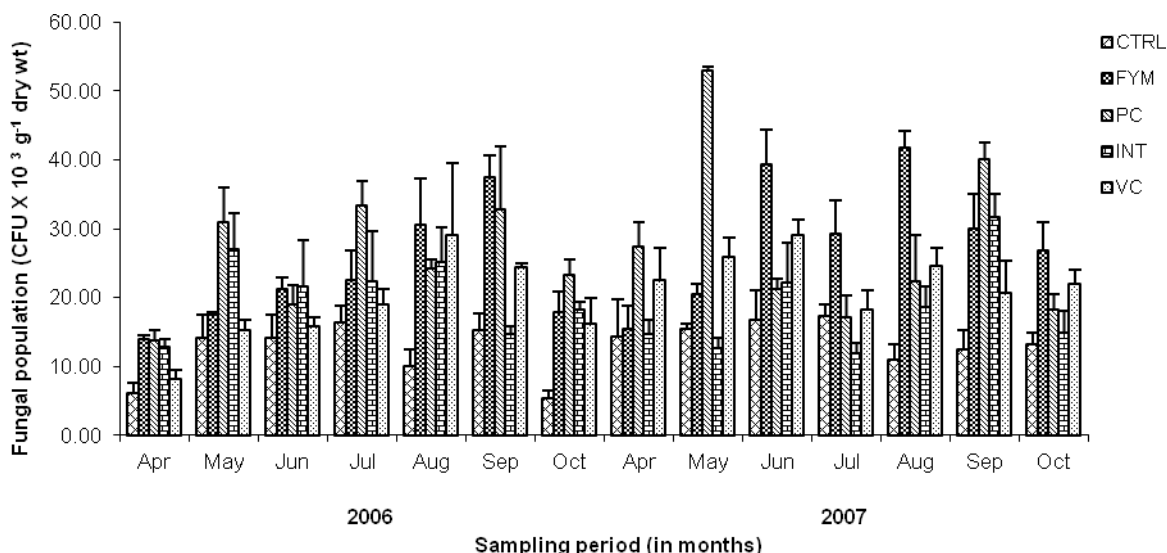


Figure 1. Fungal population in maize/French-bean field soils under different organic treatments. Mean \pm SE shown. (CFU = Colony forming units; CTRL = control; FYM = farm yard manure; PC = plant compost; VC = vermicompost; INT = integrated compost.)

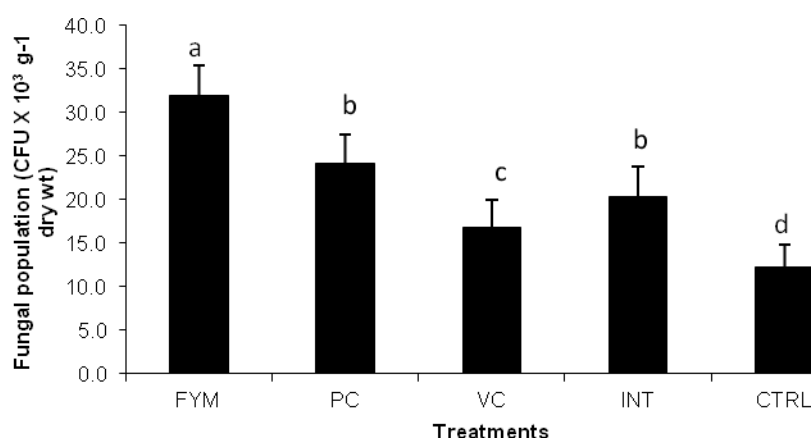


Figure 2. Effect of organic amendments on the fungal population in maize/French bean field soils. Mean \pm SE with the same letter on top do not differ significantly. (CFU = Colony forming units; CTRL = control; FYM = farm yard manure; PC = plant compost; VC = vermicompost; INT = integrated compost.)

A total of 122 fungal species and two sterile mycelia were isolated from all the plots. The list of fungal species isolated from the different plots is depicted in Table 2. During the maize crop cycle, 111 fungal species were isolated; however, 82 fungal species were isolated from the French-bean crop cycle. Comparing the two crop cycles no significant variation in the fungal population was observed. Nevertheless, fungal species was comparatively higher during maize crop cycle than the French-bean crop cycle. Maximum fungal species were isolated from FYM treated plot i.e. 77 species and least from control plot, i.e. 52 species. Shannon diversity index (Figure 3A) showed that PC plot showed a slightly higher species richness value (2.50) than FYM plot (2.45). Simpson index of dominance (Figure 3B) showed a higher value in the CTRL plot and the lowest value in PC treated plot.

The fungal species isolated belonged mostly to Deuteromycotina (25 species) followed by Ascomycotina (7 species), Zygomycotina (4 species) and Mastigomycotina (1 species). Two species belonging to Mycelia Sterilia were also isolated. At the level of genera, *Penicillium* (24 species), *Aspergillus* (8 species), *Acremonium* (6 species), *Fusarium* (6 species), *Mortierella* (6 species), *Mucor* (6 species), *Paecilomyces* (4 species), *Talaromyces* (4 species), *Trichoderma* (4 species) and *Verticillium* (4 species) and were found to be among the most common. At the species level, the dominant species are mainly the cellulose-degrading fungi belonging to Deuteromycotina except for *Pythium irregulare*. These includes *Aspergillus flavus*, *A. niger*, *Fusarium oxysporum*, *Humicola fuscoatra*, *H. grisea*, *Penicillium janthinellum*, *P. lanosum*, *P. rubrum*, *P. simplissimum*, *P. verrucosum*, *Phoma eupyrena*, *Pythium irregulare*, *Trichoderma koningii*, and *T. viride* (Table 2).

Table 2. List of fungal species isolated from soils treated with different organic fertilisers under maize/French-bean rotation. (CTRL = control; FYM = farm yard manure; PC = plant compost; INT = integrated compost, VC = vermicompost.)

Species	Maize					French-bean				
	CTRL	FYM	PC	INT	VC	CTRL	FYM	PC	INT	VC
<i>Absidia corymbifera</i>	-	-	-	+	-	-	-	-	-	+
<i>Absidia cylindrospora</i>	-	+	-	-	-	-	-	-	-	-
<i>Absidia glauca</i>	-	-	+	-	-	-	-	-	-	-
<i>Absidia spinosa</i>	-	-	+	-	-	-	-	-	-	-
<i>Acremonium butyri</i>	-	-	+	-	-	-	-	-	-	-
<i>Acremonium cerealis</i>	+	+	-	+	+	-	+	-	+	+
<i>Acremonium furcatum</i>	-	+	+	+	-	+	+	-	-	-
<i>Acremonium fusidioides</i>	+	+	-	-	+	-	-	-	-	+
<i>Acremonium kiliense</i>	-	-	-	+	+	+	+	+	+	+
<i>Acremonium strictum</i>	-	+	+	+	+	-	-	+	-	-
<i>Alternaria alternata</i>	-	-	+	-	-	-	-	-	-	-
<i>Alternaria citri</i>	+	-	-	-	-	-	-	-	-	-
<i>Alternaria longipes</i>	-	+	+	-	-	-	-	-	-	-
<i>Anthroderma cuniculi</i>	+	-	+	-	-	-	-	-	-	-
<i>Anthroderma insingulare</i>	-	-	+	-	+	-	-	-	-	-
<i>Aspergillus clavatus</i>	-	-	-	+	-	-	-	-	-	-
<i>Aspergillus flavus</i>	+	+	+	+	+	+	+	+	+	+
<i>Aspergillus fumigatus</i>	+	+	+	-	+	+	+	+	+	+
<i>Aspergillus japonicus</i>	+	-	-	-	-	-	-	-	-	-
<i>Aspergillus niger</i>	+	+	+	+	+	+	+	+	+	+

<i>Aspergillus oryzae</i>	-	-	-	+	-	-	-	-	-	-
<i>Aspergillus wentii</i>	-	-	+	-	-	-	-	-	-	-
<i>Aspergillus versicolor</i>	-	-	+	-	-	-	-	-	+	-
<i>Beltrania sp.</i>	-	-	-	-	+	-	-	-	-	-
<i>Chaetomium sp.</i>	-	-	+	-	-	-	-	-	-	-
<i>Cladosporium cladosporioides</i>	-	+	+	+	+	-	+	+	+	+
<i>Cladosporium herbarum</i>	-	-	+	+	+	-	+	+	+	-
<i>Cladosporium macrocarpum</i>	-	-	-	-	-	-	-	+	-	-
<i>Cochliobolus sativus</i>	+	-	-	-	-	-	+	-	-	-
<i>Curvularia pallasensis</i>	-	-	+	-	-	-	-	-	-	-
<i>Cylindrocladium scoparium</i>	-	-	+	-	+	-	-	-	-	-
<i>Fusarium moniliforme</i>	-	-	-	-	+	-	-	-	-	-
<i>Fusarium oxysporum</i>	+	+	+	+	+	+	+	+	+	+
<i>Fusarium redolens</i>	-	-	-	-	+	-	-	-	-	-
<i>Fusarium semitectum</i>	-	+	+	-	+	-	-	+	+	-
<i>Fusarium solani</i>	+	-	-	-	-	-	-	-	+	+
<i>Fusarium sporotrichioides</i>	-	-	+	-	-	-	-	-	-	-
<i>Gliocladium catenulatum</i>	+	-	+	+	+	+	+	+	-	+
<i>Gliocladium roseum</i>	-	+	+	+	-	-	+	+	+	-
<i>Gongronella butleri</i>	+	+	+	+	+	-	+	+	+	+
<i>Gymnoascus ressei</i>	-	-	-	-	-	-	-	-	-	+
<i>Helicosporium sp.</i>	-	-	-	-	+	-	-	-	-	-
<i>Helminthosporium sp.</i>	-	-	-	+	-	-	-	-	-	-
<i>Humicola fuscoatra</i>	+	+	+	+	+	+	+	+	+	+
<i>Humicola grisea</i>	+	+	+	+	+	+	+	+	+	+
<i>Hyphomyces chrysospermus</i>	-	-	+	-	+	-	+	+	+	-
<i>Mammaria echinobotryoides</i>	-	+	-	-	+	+	+	+	-	-
<i>Monilia sitophila</i>	-	+	+	-	+	-	-	-	-	-
<i>Mortierella alpina</i>	-	-	-	+	-	-	-	-	-	-
<i>Mortierella elongata</i>	-	-	+	-	+	-	-	-	-	+
<i>Mortierella gamsii</i>	+	-	+	+	+	+	+	+	+	+
<i>Mortierella hyalina</i>	-	-	-	+	-	+	-	-	-	-
<i>Mortierella nanna</i>	+	-	-	-	-	-	-	-	-	-
<i>Mortierella parvispora</i>	-	+	-	-	+	-	-	-	-	+
<i>Mucor circinelloides f. circinelloides</i>	+	+	+	-	+	+	+	+	-	-
<i>Mucor circinelloides f. griseo cyanus</i>	-	-	-	-	-	+	+	-	+	-
<i>Mucor hiemalis f. hiemalis</i>	-	-	-	-	-	-	-	-	+	-
<i>Mucor hiemalis f. silvaticus</i>	-	-	-	-	-	-	+	+	+	+
<i>Mucor mucedo</i>	+	+	-	+	+	-	+	-	+	-
<i>Mucor racemosus</i>	-	+	+	+	+	-	+	-	-	+
<i>Myrothecium cinctrum</i>	+	+	+	-	-	-	+	-	-	-
<i>Myrothecium verrucaria</i>	-	+	-	-	+	-	-	-	-	-
<i>Nannizzia incurvata</i>	+	-	-	-	-	-	-	-	-	-
<i>Nannizzia grubya</i>	+	+	-	-	-	-	-	-	-	-
<i>Nectria ventricosa</i>	-	-	-	-	-	+	+	-	-	+
<i>Oidiodendron echinulatum</i>	-	+	-	-	-	-	-	-	-	-
<i>Oidiodendron truncatum</i>	-	+	+	-	+	-	+	-	+	+
<i>Paecilomyces carneus</i>	-	+	+	+	+	+	+	+	+	+
<i>Paecilomyces lilacinus</i>	-	-	+	-	-	-	+	-	-	+
<i>Paecilomyces variotii</i>	-	-	-	-	-	-	+	-	-	-
<i>Paecilomyces marquandii</i>	-	-	-	+	+	+	+	+	+	+
<i>Penicillium atroveneretum</i>	+	-	+	-	-	+	-	-	-	-
<i>Penicillium brevicompactum</i>	+	+	+	+	+	-	+	+	+	+
<i>Penicillium canescens</i>	+	-	+	+	+	-	-	+	+	+
<i>Penicillium chrysogenum</i>	-	+	+	-	-	-	-	+	+	-
<i>Penicillium citrinum</i>	+	+	+	-	-	-	-	+	-	-
<i>Penicillium daleae</i>	+	+	+	+	+	+	+	-	+	+
<i>Penicillium decumbens</i>	-	-	-	-	+	+	-	-	-	-
<i>Penicillium digitatum</i>	-	-	-	-	+	-	-	-	-	-
<i>Penicillium fellutanum</i>	-	+	-	-	-	-	+	-	+	+
<i>Penicillium frequentans</i>	+	+	+	+	+	+	+	+	+	-
<i>Penicillium herquei</i>	-	+	+	-	+	+	+	+	+	-
<i>Penicillium implicatum</i>	-	-	-	-	+	-	-	-	-	-
<i>Penicillium janthinellum</i>	+	+	+	+	+	+	+	+	+	+
<i>Penicillium jensenii</i>	+	+	+	+	-	-	+	+	+	+
<i>Penicillium lanosum</i>	+	+	+	+	+	+	+	+	+	+
<i>Penicillium nigricans</i>	-	-	-	-	-	-	-	-	-	+
<i>Penicillium regulosum</i>	-	-	-	-	-	-	+	-	-	-
<i>Penicillium restrictum</i>	+	-	-	+	-	-	+	-	-	-
<i>Penicillium rubrum</i>	+	+	+	+	+	+	+	+	+	+
<i>Penicillium simplissimum</i>	+	+	+	+	+	+	+	+	+	+
<i>Penicillium stoliniferum</i>	+	+	-	+	-	-	-	-	+	-

<i>Penicillium variabile</i>	-	-	-	-	-	-	-	+	-	-
<i>Penicillium verrucosum</i>	+	+	+	+	+	+	+	+	+	+
<i>Penicillium waksmanii</i>	+	-	-	-	-	-	-	-	-	-
<i>Phialophora cinerescens</i>	-	+	-	+	-	-	-	-	-	-
<i>Phialophora festigiata</i>	-	-	-	-	-	-	+	+	-	-
<i>Phoma eupyrena</i>	+	+	+	+	+	+	+	+	+	+
<i>Phoma medicagnis</i>	+	-	-	-	-	+	+	-	-	-
<i>Plectosphaerella cucumeria</i>	-	-	-	-	+	-	-	-	-	-
<i>Pseudoeurotium zonatum</i>	-	-	-	-	+	-	-	+	-	-
<i>Pythium aphanidermatum</i>	-	+	-	-	-	-	+	-	-	-
<i>Pythium intermedium</i>	+	+	+	-	+	+	+	+	-	+
<i>Pythium irregulare</i>	+	+	+	+	+	+	+	+	+	+
<i>Ramichloridium schulzeri</i>	-	+	-	+	-	-	+	-	-	-
<i>Rhizopus stolonifer</i>	+	+	+	+	+	+	-	+	+	+
<i>Scopulariopsis brumptii</i>	-	+	-	+	-	-	-	-	-	-
<i>Scopulariopsis stercoraria</i>	+	-	-	-	-	-	-	-	-	-
<i>Staphylotrichum coccosporum</i>	+	+	-	+	+	-	-	+	-	+
<i>Talaromyces emersonii</i>	-	-	+	-	+	-	-	-	-	-
<i>Talaromyces helicus</i>	-	-	-	+	-	-	-	-	-	-
<i>Talaromyces stachyspermum</i>	-	-	+	-	-	-	-	-	-	-
<i>Talaromyces wortmanii</i>	+	-	+	-	-	+	-	+	-	+
<i>Torula herbarum</i>	-	-	-	+	-	-	-	-	+	-
<i>Trichoderma hamatum</i>	-	+	-	-	-	-	-	-	-	-
<i>Trichoderma koningii</i>	+	+	+	+	+	+	+	+	+	+
<i>Trichoderma polysporum</i>	-	+	+	+	+	-	-	+	+	-
<i>Trichoderma viride</i>	+	+	+	+	+	+	+	+	+	+
<i>Verticillium albo-atrum</i>	-	+	+	+	+	+	-	+	-	+
<i>Verticillium chlamydosporium</i>	-	-	-	-	+	-	-	-	-	-
<i>Verticillium dahliae</i>	-	+	-	-	-	-	-	+	-	-
<i>Verticillium nigrecens</i>	-	-	+	-	-	-	-	+	-	-
White sterile mycelium	-	-	+	+	+	+	+	-	+	+
Yellow sterile mycelium	-	-	-	-	+	+	-	-	-	+

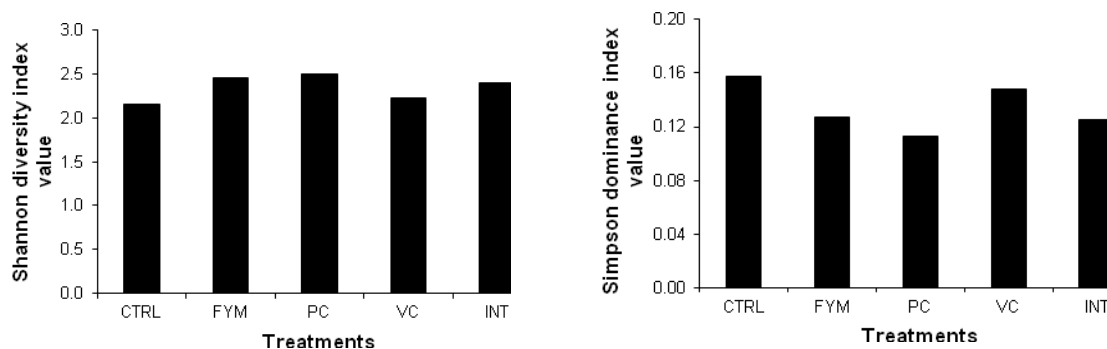


Figure 3. List of fungal species isolated from soils treated with different organic fertilisers under maize/French-bean rotation. (CTRL = control; FYM = farm yard manure; PC = plant compost; INT = integrated compost, VC = vermicompost.)

Soil physico-chemical properties (pH, MC, C_{org}, N, P and K)

The pH of the soil in the present investigation was found to be acidic. The pH was significantly increased by FYM application. Moisture content and C_{org} was significantly increased by PC application. Again, FYM treated plot showed highest total N, available P and exchangeable K contents. The lowest nutrient contents (N,P,K) were observed in the CTRL plot (Table 3). Significant positive correlations of fungal population with C_{org}, total N, available P and exchangeable K were observed in all the organically treated plots (Table 4).

Table 3. Physico-chemical properties of maize/French bean field soils with standard error (SE). The range of the values are given in parenthesis. (CTRL = control; FYM= farm yard manure; VC = vermicompost; INT = integrated compost; PC = plant compost; MC= moisture content, OC= organic carbon, AP= available phosphorus, K= exchangeable potassium, TN= total nitrogen.)

Properties	Treatments				
	CTRL	FYM	PC	INT	VC
pH	4.683 ± 0.031 (5.1- 4.1)	5.470 ± 0.028 (5.9 - 4.7)	5.305 ± 0.031 (5.9 - 4.7)	4.886 ± 0.028 (5.6 - 4.4)	5.155 ± 0.017 (5.8 - 4.5)
MC (%)	22.98 ± 0.091 (28.70 - 20.50)	24.81 ± 0.244 (28.47 - 20.90)	25.74 ± 0.266 (31.33 - 22.20)	23.85 ± 0.102 (26.17 - 21.17)	24.10 ± 0.162 (26.70 - 20.50)
OC (%)	1.205 ± 0.014 (1.477 - 0.847)	1.449 ± 0.015 (1.793 - 0.856)	1.651 ± 0.014 (1.860 - 1.144)	1.400 ± 0.014 (1.644 - 1.113)	1.382 ± 0.018 (1.779 - 0.851)
TN (%)	0.250 ± 0.006 (0.368 - 0.130)	0.431 ± 0.008 (0.658 - 0.280)	0.360 ± 0.008 (0.568 - 0.252)	0.309 ± 0.007 (0.540 - 0.200)	0.388 ± 0.008 (0.625 - 0.253)
AP (µg-1)	16.936 ± 0.028 (21.60 - 13.80)	28.671 ± 0.022 (43.60 - 16.80)	21.279 ± 0.034 (31.60 - 16.20)	24.036 ± 0.029 (38.00 - 15.10)	20.107 ± 0.023 (30.60 - 14.40)
Ex K (%)	0.028 ± 0.000 (0.051 - 0.006)	0.040 ± 0.001 (0.122 - 0.029)	0.039 ± 0.000 (0.098 - 0.014)	0.034 ± 0.000 (0.091 - 0.015)	0.047 ± 0.001 (0.106 - 0.025)

Table 4. Correlation coefficient (r) values among fungal population with soil physico-chemical properties in control (CTRL), farm yard manure (FYM), vermicompost (VC), integrated (INT) and plant compost (PC) under maize/French bean rotation. (FP = Fungal population; MC= moisture content, OC= organic carbon, AP= available phosphorus, K= exchangeable potassium, TN= total nitrogen; AM = ambient temperature; RF = rainfall; a, b, c = significance level within each column at 0.05, 0.01 and 0.001 respectively; NS= not significant.)

Treatments	pH	MC	OC	AP	K	TN	AT	RF
CTRL	NS	0.63 a	0.68 b	NS	NS	NS	NS	0.56 a
FYM	0.54 a	0.77 b	0.73 b	0.66a	0.63a	0.62 a	NS	0.61 a
PC	NS	0.60 a	0.77b	0.64 a	0.65 a	0.55 a	NS	0.57 a
INT	NS	NS	0.65 a	0.58 a	0.63 a	0.57 a	0.60 a	0.57 a
VC	NS	0.62 a	0.79 c	0.60 a	0.70 b	0.60 a	0.55 a	0.60 a

Discussion

Fungal population and diversity

Results from the present investigation showed that the increase in the available nutrients in FYM amended plots resulted in maximum fungal population as indicated by significant correlations ($p \leq 0.05$, 0.01 and 0.001) with soil pH, MC, C_{org} , N, P, K and rainfall. Significant correlations between fungal population with C_{org} and rainfall in all the organically amended plots noticeably indicate that organic carbon level in the soil and precipitation play pivotal role in fungal growth and sporulation. However, in the control plot as well, correlations between fungal population and C_{org} and rainfall suggested that the plant residues returned to the soil and the death and decay of organisms provided the necessary organic carbon necessary for the fungal communities in this particular plot where there is no input of organic amendments. Greater microbial populations in FYM treated plots as compared to chemically amended plots were reported by Venkateswarlu (2000) and Sharma *et al.* (1983). Application of farm yard manure to agricultural fields can be viewed as an excellent way to recycle nutrients, maintain soil quality and in harbouring higher fungal populations.

Inconsistent monthly variation in fungal population in all the plots could be due to the different stages of the crop growth, the type and amount of organic amendment supplemented and the degree of decomposition of the organic amendment. During the crop growing stages nutrient uptake by the plants increases and this resulted in insufficient or depletion of nutrient availability for the fungi. As such, fungal population decreases when the crop growth is at its peak. In our study, cultivation was done from April to October and the field is left fallow during the winter season. Lower fungal population in the pre-harvest (i.e. April) is attributed to lack of vegetation and organic amendment input during the winter months. Even though the treatments were done in the same plots during the study period, the rows were not established in exactly the same location and the timing of fertiliser input was not same in both the years. This could be another factor for the uneven distribution of soil nutrients and hence, inconsistent monthly variation in fungal population and diversity. Song *et al.* (2007) indicated that difference in the establishment of rows during the field preparation leads to alteration of microbial communities.

Higher fungal species during the maize crop cycle is due to the fact that maize cultivation provides adequate plant cover which creates favourable microclimatic conditions for the profuse growth and sporulation of the fungal species. On the other hand, a relatively longer growth period of the maize plant is also another aspect which can influence the fungal species composition as compared to the French bean crop cycle. It can be proposed that incorporation of organic manures directly have an impact on the soil properties, the plant growth which in turn influence the fungal population and species. Hackl *et al.* (2000) indicated that the plant species growing on the soil also equally influence the population and species composition of the soil fungi.

Addition of fresh plant residues and the amount of residue returned from the standing crop increases the organic matter accumulation in the soil. Microbial activity occurs at a faster rate when maximum organic matter and favourable conditions are available. This is reflected by higher fungal species diversity and richness in PC amended plot. As such, utilisation of weeds and other crop residues (in the form of PC) from the field act a good source of organic fertiliser both for the population and diversity of the fungal species. Higher Simpson index value of dominance in the control plot indicates the presence of more dominant species in this plot. Conversely, the result from the control plot helps in estimating and identifying the indigenous fungal population and diversity of the study site.

The fungal genera isolated are active in decomposing and thrive best in decaying organic debris (Domsch *et al.* 1980, Subramanian 1983). Since the fertilisers used were of organic origin, these fungi are therefore commonly isolated. On the other hand, the frequent occurrence of these species in all the plots is also attributed to the fast growing nature of these species, similar soil properties, the same type of rotation and the land use history. As dilution plate method was followed for fungal population estimation *Penicillium* species were detected in higher frequency as compared to other species. This is in agreement with the finding of Domsch *et al.* (1980) who also showed that soil washing technique can however reduce the frequency of *Penicillium* species.

Organic manures when applied to the soil supply readily available substrate to the cellulose decomposing fungi. This could be one explanation for the dominance of Deuteromycotina species in the organically amended plots in this study. Nonetheless, *Pythium irregulare* and *Fusarium oxysporum* known as the most pathogenic species of its genus (Domsch *et al.* 1980) were also the dominant species isolated from all the plots. This finding is consistent with Abawi and Widmer (2000) who showed an increase in pathogenic fungi with organic fertiliser application. However, these pathogenic fungi did not cause any severity to the crop plants throughout the study period. The occurrence and dominance of antagonist fungal species i.e. *Trichoderma viride*, *Penicillium* species and *Aspergillus* species might have aid in antagonising the pathogenic species and reduce the disease severity which these fungi can inflict on the crop plants studied. As these species were largely isolated from the organically managed soils, enhancing the use of organic manures in a long run may perhaps prove crucial for large scale control of many soil-borne fungal diseases. This is of paramount importance in organic farming systems where soil fungus itself acts as a biological agent which can help in excluding the use of synthetic fungicides.

Soil physico-chemical properties (pH, MC, C_{org}, N, P and K)

The acidic nature of the soil was influenced by rainfall ($p \leq 0.05, 0.01$) as heavy rainfall causes leaching out much of the basic forming cations (Ca^{2+} , Mg^{2+} , K^{+} and Na^{+}) leaving mostly H^{+} and Al^{3+} cations which are largely responsible for soil acidity. Conversely, FYM amendment plays an important role in improving the soil quality by buffering the pH of the soil whereby, they increase the basic cations. Parham *et al.* (2002) showed that the soil pH increased significantly in the plots treated with cattle manure while chemical fertiliser application resulted in slightly lower pH values. Maximum moisture content in the PC treated plot is due to higher accumulation of organic matter with this treatment. Accumulation of organic matter in the surface soil and vegetation cover (i.e. plant compost) is highly effective in checking soil evaporation which increases the soil water holding capacity. It can be also be hypothesised that fresh plant compost application accelerates rapid decomposition thereby, increasing microbial respiration (Coyne 1999) which might have led to increase in the soil moisture content level. Coyne (1999) indicated that most of the organic C in soil comes from decomposition of plant residues and as green plant residues consists mostly of water (Brady and Weil 1996) they undergo decomposition at a much faster rate compared to the complex nature of animal manures. Thus, PC treated plot resulted in higher C_{org} in this study. Perrucci *et al.* (1997) also showed that burying crop residues in soil could help limit the gradual depletion of soil organic matter and improve chemical properties of the soil. Farm yard manure is a potentially important source of nitrogen (N), phosphorus (P), potassium (K). As such, significant increase in total N, available P and K contents with FYM addition in our results is directly related to the large content of these nutrients in this compost. This is in agreement with the findings of Motavalli *et al.* (2002), Plaza *et al.* (2004) and Sadej and Przekwas (2008).

Conclusions

From our study it can be concluded that (i) organic fertilisers particularly farm yard manure (FYM) and plant compost (PC) have better impact on the fungal population, its diversity and the physico-chemical properties of the soil than not adding an organic amendment. The study conducted however, have some limitation where the soil sampling was confined only to selected experimental plots. There is need for a wider study area so as a complete representation of the fungal diversity and beneficial aspects of these significant microbes in organic farming systems is acquired. This will enable augmentation and promotion of organic agriculture in the region.

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References

- Abawi, GS and Widmer, TL 2000. Impact of soil health management practices on soil-borne pathogens, nematodes and root diseases of vegetable crops. *Applied Soil Ecology*, 15: 37-47.
- Anderson, JM and Ingram, JSI 1993. *Tropical Soil Biology and Fertility. A Handbook of Methods*. CAB International, Oxford.
- Allen, SE, Grinshaw, HM, Parkinson, JA, Quaramby, C 1974. *Chemical Analysis of Ecological Materials*. Blackwell Scientific Publications, Oxford.
- Barnett, HL and Hunter, BB 1972. *Illustrated Genera of Imperfect Fungi*. Burgess Publishing Company. Minneapolis.
- Bloem, J, Lebbnik, G, Zwart, KB, Bouwman, LA, Burgers, SLEG, de Vos, JA de Ruiter, PC 1994. Dynamic of microorganisms, microbivores and nitrogen mineralisation in winter wheat field under conventional and integrated management. *Agriculture Ecosystem and Environment*, 51: 129-143.
- Borah, KC 1999. Land utilisation and cropping pattern in Mizoram. In Kainth, GS (ed.) *Developing Hill Agriculture*. Regency Publications, New Delhi.
- Brady, NC and Weil, RR 1996. *The Nature and Properties of Soils*. Prentice Hall, Upper Saddle River.
- Breure, AM 2005. Ecological soil monitoring and quality assessment. In: Doelman P., Eijsackers, H.J.P. (eds.) *Vital soil: function, value and properties*. Elsevier, Amsterdam, pp 281-305.
- Coyne, M 1999. *Soil Microbiology: An Explanatory Approach*. Delmer Publisher, New York.
- Dick, RP 1992. A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. *Agricultural, Ecosystems and Environment*, 40: 25-36.
- Domsch, KH, Gams, W, Anderson, TH 1980. *Compendium of Soil Fungi*. Academic Press, London.
- Doran, JW and Zeiss, MR 2000. Soil health and sustainability: managing the biotic component of soil quality. *Applied Soil Ecology*, 15: 3-11.
- Drinkwater, LE, Letourneau, DK, Workneh, F, Van Bruggen, AHC, Shennan, C, 1995. Fundamental differences between conventional and organic tomato agroecosystems in California. *Applied Ecology*, 5: 1098-1112.
- Droogers, P and Bouma, J 1996. Biodynamic versus conventional farming effects on soil structure expressed by stimulated potential productivity. *Soil Science Society of American Journal*, 60: 1552-1558.
- Ellis, MB 1993. *Dematiaceous Hyphomycetes*. CAB International, Wallingford.
- Ghosh, N 2003. Organic farming in north-east hill region in India. In: 3rd Biennial Conference on "Biodiversity and Quality of Life", 18-20 December, 2003, Calcutta, India. pp 1.
- Gillman, JC 1957. *Manual of Soil Fungi*. Oxford and I.B.H Publishing company (Indian reprint).
- Girvan, MS, Bullimore, J, Ball, AS, Pretty, JN, Osborn, AM, 2004. Responses of active bacterial and fungal communities in soils under winter wheat to different fertilizer and pesticide regimens. *Applied Environmental Microbiology*, 70: 2692-2701.
- Hackl, E, Bachmann, G, Boltenstern-Zechmeister, S 2000. Soil microbial biomass and rhizosphere effects in natural forest stands. *Phyton*, 40: 83-90.
- Hawksworth, DL 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research*, 105: 1422-1432.
- Jackson, ML 1973. *Soil Chemical Analysis*. Prentice Hall India, New Delhi.
- Johnson, LF and Curl, AE 1972. *Method for the Research on Ecology of Soil Borne Plant Pathogens*. Burgess Publishing Company, Minneapolis.

- Mader, P, Flißbach, A, Dubois, D, Gunst, L, Fried, P, Niggli, U 2002. Soil fertility and biodiversity in organic farming. *Science*, 296: 1694-1697.
- Martin, JP 1950. Use of acid, rose Bengal and streptomycine in a plate method for estimating soil fungi. *Soil Science*, 69: 215-233.
- Matson, PA, Parton, WJ, Power, AG, Swift, MJ 1997. Agriculture intensification and ecosystem properties. *Science*, 277: 504-509.
- Meghalaya Agricultural Profile 2006. Agriculture Information Officer, Department of Agriculture, Government of Meghalaya, Shillong.
- Motavalli, PP and Miles, RJ 2002. Soil phosphorus fractions after 111 years of animal and fertilizer application. *Biology and Fertility of Soils*, 36: 35-42.
- O'Donnell AG, Goodfellow M, Hawksworth DL 1994. Theoretical practical aspects of the quantification of biodiversity among microorganism. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*. 345: 65-73.
- Pansombat, K, Kanazawa, S, Horiguchi, T, 1997. Microbial ecology in tea soils I. Soils properties and microbial populations. *Soil Science and Plant Nutrition*, 43: 317-327.
- Parham, JA, Deng, SP, Raun, WR, Johnson, GV 2002. Long-term cattle-manure application in soil. Effect on soil P levels, microbial biomass carbon and dehydrogenase activity and phosphatase activity. *Biology and Fertility of Soils*, 35: 328-337.
- Plaza, C, Hernandez, D, Garcia-Gil, JC, Polo, A 2004. Microbial activity in pig-slurry-amended soils under semiarid conditions. *Soil Biology and Biochemistry*, 36: 1577-1585.
- Perrucci, P, Bonciarelli, U, Santicocchi, R, Bianchi, AA 1997. Effect of rotation, nitrogen fertilization and management of crop residues on some chemical, microbiological and biochemical properties of soil. *Biology and Fertility of Soils*, 24: 311-316.
- Raper, KB, and Thom, C 1949. *A Manual of the Penicillia*. The Williams and Wilkins Company, Baltimore.
- Sadej, W and Przekwas, K 2008. Fluctuations of nitrogen levels in soil profile under conditions of a long-term fertilization experiment. *Plant Soil and Environment* 54: 197-203.
- Saleh, SI 1989. *Nagaland's Economy in Transition since 1964*, Omson Publications.
- Schiavon, M, Perringanier, C Portal, JM 1995. The pollution of water by pesticides-state and origin. *Agronomie*, 15: 157-170.
- Schmit, JP and Mueller, GM 2007. An estimate of the lower limit of global fungal diversity. *Biodiversity Conservation*, 16: 99-111.
- Shannon, CE and Weaver, W. 1948. *The Mathematical Theory of Communication*. University of Illinois Press, Urbana.
- Sharma, N, Srivastava, LL and Mishra, B 1983. Studies on microbial changes in soil as a result of continuous application of fertilizers, farmyard manure and lime. *Journal of Indian Society of Soil Science*, 31: 202-206.
- Simpson, E.H. 1949. Measurement of diversity. *Nature*, 163: 688.
- Song, YN, Zhang, FS, Marschner, P, Fan, FL, Gao, HM, Bao, XG, Sun, JH, Li, L 2007. Effect of intercropping on crop yield and chemical and microbiological properties in rhizosphere of wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and faba bean (*Vicia faba* L.). *Biology and Fertility of Soils*, 43: 565-574
- Subramanian, CV 1983. *Hyphomycetes – Taxonomy and Biology*. Academic Press, London.
- Tate, RL 1995. *Soil Microbiology*. Wiley, New York.
- Tokuda, S and Hayatsu, M 2002. Nitrous oxide emission potential of 21 acidic tea field soils in Japan. *Soil Science and Plant Nutrition*, 47: 637-642.
- Van Bruggen, AHC and Semenov, AM 2000. In search of biological indicators for soil health and disease suppression. *Applied Soil Ecology*, 15: 13-24.
- Van Elsas, JD and Trevors, JT 1997. *Modern Soil Microbiology*. Marcel Dekker, New York.
- Venkateswarlu. B and Srinivasaroa, CH 2000. Soil microbial diversity and the impact of agricultural practices. Central Research Institute for Dryland Agriculture, Santoshnagar, India.
- Wang, H, Hyde, KD, Soyong, K, Lin, F 2008. Fungal diversity on fallen leaves of *Ficus* in northern Thailand. *Journal of Zhejiang University Science*, 9: 835-841.
- Watanabe, T 1994. *Pictorial Atlas of Soil and Seed Fungi: Morphologies of cultured fungi and key to species*. Lewis Publishers, USA.