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EFFECT OF INTERSPECIFIC ROOT INTERACTION ON SOIL NUTRITION, ENZYMATIC ACTIVITY AND RHIZOSPHERE BIOLOGY IN MAIZE/PEANUT INTERCROPPING SYSTEM

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Pot experiment was carried out at different nitrogen levels in maize and peanut intercropping system to investigate the mechanisms that govern interactions between intercropped species in soil rhizosphere. Three different nitrogen levels 100%, 75% and 50% of normal nitrogen application rate were used in maize monoculture. Two peanut plants were intercropped with single maize plant per pot. Effect of reduced nitrogen application was investigated in soil rhizosphere by measuring soil enzymatic activity, nutrition, soil microbial diversity and composition. Phospholipid fatty acid (PLFA) was carried out to understand about composition of viable soil micro flora and community level physiological profile (CLPP) was applied to work out the microbial diversity. The results revealed that soil nutrition and enzymatic activities were higher in intercropping treatments as compared to mono-cropping with similar level of nitrogen. The results of PLFA analysis showed that intercropping inhibited the fungal population and promoted the bacterial community. The results of AWCD showed that 4 kinds of carbon utilization sources (carboxylic acid, polymer, amines & carbohydrates) increased by the decrease of nitrogen levels in intercropping treatments and other 2 kinds of carbon sources (phenolic acids & amino acids) showed different trend. Cluster analysis (CA) and Principal component analysis (PCA) carried out from the results of PLFA and CLPP indicated distinct separation amongst all treatments of monocropping and intercropping as well as with different level of nitrogen application that reflected a variation in soil microbial activity and composition. However intercropping treatment with 75% nitrogen level performed similar to 100% monocropping treatment of maize. The results revealed that by intercropping of peanut with maize can be used to decrease the dependence on artificial fertilizer which provides a convenient organic farming model for growers across the world.

Keywords: Intercropping, maize, peanut, nitrogen, enzymes, PLFA, CLPP

INTRODUCTION

With the increase of population and decrease of arable land in China for non-agriculture purposes, large amount of fertilizer are being used to get the maximum production to fulfill the demand of food, the main concern about the negative impact of high use of fertilizer regarding distortion of the quality of water and food and decrease in diversity of the flora and fauna (Zhang *et al*., 2004). It is emphasized worldwide to focus on the well-organized utilization of soil nutrients and controlling the input of chemical fertilizers by establishment of better cropping systems to handling the rhizosphere environment and moving rhizosphere processes towards ecological improvement (Zhang and Shen, 1999) because in cropping systems, the rhizosphere is not only an interface between root and soil for an individual plant, but also is a vital factor of interaction for plant community (e.g. plant species in intercropping systems), soil, microorganisms and their environment (Zhang *et al*., 2002).

Intercropping (or multicropping) is mainly culturing of two or more crops together at the same period in the same situation, which is a serious dealing of crop production both in space and phase (Li *et al*.,1999) and in this cropping system interspecies root interactions play an important role in nutrient acquisition (Li *et al*., 2001). It is best utilization way of soil nutrients (Zhang and Li, 2003; Rowe *et al*., 2005), land (Dhima *et al*., 2007), water (Walker and Ogindo, 2003; Xu *et al*., 2008), and radiation resources (Awal *et al*., 2006) as compared to monocropping system (Rodrigo *et al*., 2001).

Most of researchers worked on the interspecific relation mainly focused on interspecific competition with respect to light, space, heat and time (Jolliffe and Wanjau, 1999), although positive interaction in which intercropped species

The first three author's contributed equally in this paper.

enhances the plant growth, survival and fitness of both crops has been found in many plant communities (Callaway and Pugnaire, 1999), however, very few studies have been found on the facilitation mechanism of both crops.

About 50% of nitrogen fertilizer applied to the soil is not taken by the agriculture crops (FAO, 2009) which cause the accumulation of nitrates in soil responsible for N losses and pollution of water and soil. Due to small holding of land in China large amounts of fertilizer are being used to get higher yield during intercropping system (Zhang *et al*., 2004), therefore, it is dire need of new era to optimize the level of chemical fertilizer by introducing better cropping system for efficient use of soil nutrients (Zhang and Shen., 1999). Intercropping showed great influence on major soil nutrient elements (Phosphorus P, Nitrogen N and Potassium K) mobilization in soil rhizosphere and contribute efficiently to nutrient acquisition (Wasaki *et al*., 2003). Pearl millet intercropping with legume showed the improvement in soil nitrogen and phosphorus (Sharma and Gupta, 2002) and accumulation of potassium (K) enhanced in pea and barley intercropping system (Andersen *et al*., 2007; Eriksen, 1997). The improvement in major soil nutrients may lead to the better enzymatic activity in rhizosphere soil.

Metabolic properties of soil such as enzymatic activities and soil nutrition are the main indicators of soil degradation and physico-chemical parameters (Dick, 1994). Soil enzyme activities are the important element for soil microbial activity and soil fertility (Guo and Niu, 2010), Sucrase is involved in the disintegration of organic matter which effect the soil fertility and also used as an indicator regarding power of soil oxidation (; Zhang *et al.*, 2004), urease catalyzes the urea into water, carbon dioxide, and ammonia that are important for soil nitrogen and nitrogen cycle and is produced by soil microbes (Zhang *et al*., 2004; Robertson *et al.*, 2011). Phosphatase is important in soil by transforming organic P into inorganic phosphate that is directly available to plant and soil microorganisms (Margesin and Schinner, 1994), in addition phenol oxidase and per oxidase are important in the breakdown of lignin and in the cycling of soil organic substances (Hammel, 1997).

According to Nannipieri *et al*. (1990) various activities carried out by soil microorganism are called microbial activities; however, biological activity means not only the microbial activity but also the activities of other soil organism such as plant roots. Concentration and activity of soil microorganisms can be changed due to many ecological activities (Horner *et al*. 2003) just like association with plants, plant productivity gradients that all can alter the soil microbial communities (Kuske *et al*., 2003). The composition of microbial communities is strongly affected by the plants in the soil through roots, the decay of litter and rhizodeposition. Being the result of co-evolution, the relation is strict between microbial communities and plant species in the soil rhizosphere (Brimecombe *et al*., 2001).

Soil quality is mainly dependent on its physical, chemical and biological properties but microbial and biochemical characteristics are taken as potential indicators (Kennedy and Papendick, 1995). In the past, the counting methods such as plate count technique or the Most Probable Number (MPN) technique were used to measure the microbial diversity (Johnsen *et al*., 2001). The method used to determine the microbial diversity through the number of isolates is less popular due to laborious procedure and also less number of microorganisms can be cultured. It is well recognized that by using plate count methods just only 1-10% of total soil micro-flora can be estimated, however, fluorescence microscopy can get 100-1000% times more than results obtained by plate counting (Johnsen *et al*., 2001) which are approximately 90 % of total soil micro flora (Porteous *et al*., 1997). At present, many molecular methods are being practiced in these days because no isolation and cultivation of microorganism are involved in these advanced techniques (Johnsen *et al*., 2001). Investigators often measure microbiological activity in soil by determining soil respiration in the absence (basal respiration) or in the presence of specific organic substrates or organic residues (Nannipieri *et al*., 1990). The use of available carbon is an important aspect regarding microbial growth in the soil; therefore, BIOLOG® is most preferred method in these days to investigate the microbial functional diversity (Insam and Rangger, 1997). The methodology, named community-level physiological profiling (CLPP) (Lehman *et al*., 1995), is established on the multivariate profile of colour development on this basis of utilization of sole carbon sources and concomitant decline of tetrazolium tint in the 95 isolated wells. The average well colour development (AWCD) is an effective methodology dealing with estimated standardization of inoculum density and investigation of samples at similar overall amount of colour development. The approximate estimation of the density of active cells can be calculated by overall amount of carbon source utilization; being reasonably inexpensive method, CLPP is a quick measure to identify the relative change in microbial communities (Garland *et al.*, 2001). Various kinds of microorganisms synthesize diverse varieties of PLFA by different biochemical pathways and Phospholipid fatty acids (PLFA) are the main essentials of membrane of all living cells (Dai *et al*., 2013). Two different methods PLFA (Söderberg *et al*., 2002) and CLPP (Baudoin *et al*., 2001 & 2002) have been used for rhizosphere biological studies.

In this study our main objective is to work out the influence on soil micro-ecology includes soil nutrients, enzymatic activity, soil microbial density and dynamics of soil microbial communities in maize and peanut intercropping system under different nitrogen treatments.

MATERIAL AND METHODS

***Experiment site*:** The pot experiment was conducted at experimental station of College of Crop Sciences in Fujian Agriculture and Forestry University, Fuzhou, China. Three different levels of nitrogen (100%, 75%, 50% of normal nitrogen application rate, i.e., 200 kg /hm^2) were used both in intercropping and monocropping systems of Maize and Peanut with 3 replications of each. Intercropping maize and peanut was abbreviated as IMP (100%, 75% & 50%), sole maize crop as M (100%,75% & 50%) and sole peanut crop as P(100%,75% & 50%). The soil physical and chemical properties before experiment were as follow: sandy loam, pH value 6.2, total nitrogen (TN) 1. 53 mg/kg, available nitrogen (AN) 59.5 mg/kg, total phosphorus (TP) 1.25mg/kg, available phosphorus(AP) 20.6mg/kg, total potassium (TK) 1.05 mg/kg, available potassium(AK) 201.6 mg / kg.

***Soil sampling*:** The soil samples were collected from each pot at tasseling time of maize from root zone of crop; fresh soil was used at once for BIOLOG® and PLFA analysis regarding macro-organisms. However, rest of soil was air dried and sieved through 2 mm for enzymatic and nutrients analysis.

***Soil nutrients*:** All sieved soil samples were used to determine the nitrogen (N), potassium (K), and phosphorus (P). Total nitrogen was worked out by using Gunning & Hubbard's procedure of sulphuric acid digestion and distillation with Marco Kjeldahl apparatus (Jackson, 1962), however, available nitrogen was calculated as the sum of nitrate nitrogen (NO_3-N) and ammonium nitrogen (NH_4- N). Available phosphorus (AP) was calculated as described by Watanabe and Olsen (1965) and available potassium (AK) was determined by Gallenkamp Flame Analyzer (Method 18). The total Phosphorus and potassium (TP and TK) were determined by first digesting the soil using the H_2SO_4–$HClO_4$ method and then measured the level as followed for AP and AK.

***Soil enzyme*:** Soil enzymes (Urease, Sucrase, Peroxidase, Phosphomonoesterase, and Polyphenol Oxidase) were measured by the procedures as described by the different scientists (Guan and Shen, 1984; Guan, 1986, 1989; Wan and Ping, 2004). (1) H_2O_2 as the medium and a 0.1 mol $KMNO_4$ titration method for peroxidase (2) Measuring NH_3-N content (NH_3-N mg·g^{-1}, 37°C, 24 h) by the colour comparison method, with urea as medium, for urease (3) Measuring glucose content (glucose mg·g^{-1}, 37 °C, 24 h) by the colour comparison, glucose as the medium for sucrose by using Photo-spectrometer (4) Phosphomonoesterase was determined according to Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977) with some modifications as described by Schinner *et al*. (1993), (5) Polyphenol oxidase was measured by following the method as described by Tabatabai (1994).

***Community level physiological profiles (CLPP); Biolog method*:** Similar procedures were carried out as described by Lin *et al.,* (2007) and Wu *et al.,* (2013). Community level physiological profiles (CLPP) were evaluated by the Biolog Eco Microplate™ system (Biolog Inc., CA, and USA). Each microplate consists of 96 wells which were divided into three replicates of 31 sole carbon substrates and water blank. 150 µl volumes from each treatment were poured into each well. The plates were incubated at 25 °C for 168 h, and the colour development in each well was recorded at regular 24 h intervals as optical density (OD) at 590 nm with a plate reader (Thermo Scientific Multiskan MK3, Shanghai, China). Microbial activity in each microplate, expressed as average well-color development (AWCD) was determined as follows:

$$\mathrm{AWCD} = \sum \frac{C - R}{31}$$

Where C is the optical density within each well and R is the absorbance value of the control well.

According to Choi and Dobbs (1999) ECO micro plates having 31 carbon substrates were segmented into six categories: carbohydrates, polymers, phenolic compounds, carboxylic acids, amino acids, and amines. Cluster analysis (CA) and principal component analysis (PCA) was carried out by using the optical density at 96 h incubation time (Han *et al*., 2007).

***PLFA analysis*:** The structure and biomass of the soil microbial community was assessed by analyzing the ester linked by analyzing the phospholipids fatty acids (PLFA) composition of soil by the method described by Bardgett *et al*. (1996) and Denef *et al*. (2007), since certain groups of microorganisms have different "signature" fatty acids (Tunlid and White, 1992). Briefly, lipid fractions were extracted from 5 gram fresh soil according to procedure of Bligh and Dyer (1959). Then, FAMEs were analyzed using a 450GC/240MS system (Varian, Inc., USA) equipped with a capillary column CP8944 (30 m, 0.25 mm i.e., 0.25 m film thickness; Varian, Inc., USA). The column temperature was programmed to start at 70°C for 1 min, then ramp up at a rate of 20°C min^{-1} to 170°C which was held for 2 min, and followed by a ramp of 5°C min^{-1} to 280°C which was held for 5 min. Finally, the oven temperature was increased to 300°C at 40°C min^{-1} and held for 1.5 min. The peaks were identified based on relative retention times vs. several external standards: a mixture of 37-Component FAME Mix (47885-U, Supelco Inc., USA), a mixture of 26 Bacterial Acid Methyl Esters (47080-U, Supelco Inc., USA) and several individual FAMEs (Larodan Inc., Sweden). Individual fatty acids were quantified by comparing peak areas from the sample with peak areas of the internal standard 19:0 (non-adeconoic methyl ester) of known concentration.

In total 21 PLFA were isolated and identified. The rules of fatty acid nomenclature used, were described by Wilkinson

et al. (2002). Branched, saturated PLFAs a12:0w, i13:0w, a14:0w, i15:0w, a16:0w, a17:0w, and i18:0w indicator the Gram-positive bacteria (Gram (+)), while monoenoic, unsaturated and cyclopropyl PLFAs 16:1w7c, 16:1w9 were used as biomarker of Gram-negative bacteria (Gram (−)).The methyl-substituted PLFAs, 10Me17:0 and 10mel19:0w were regarded as representative of actinomycetes. 15:0w represents the bacteria and PLFA 20:4w6 ascribed the protozoan. PLFA of 18:2w6 is used as biomarker of fungi (Wu *et al.,* 2013; Brockett *et al*., 2012; Huygens *et al*., 2011; Joergensena and Potthoff, 2005). However straight chain PLFAs (12:0w, 16:0w, 19:1w10c, 20:4w6, 20:5w3, 24:0w, 26:0w) are used as biomarkers of non-specific PLFA's. The quantitative fingerprint data of identified PLFAs was displayed and after transformation was used for statistical analyses.

***Statistical analysis*:** This study was designed with three replicates of each treatment in a completely randomized block design (CRBD). Statistical analysis was carried out by SPSS software and analysis of variance (ANOVA) was used to define the significance of difference @ $P \geq 0.05$. However, PLFA and BIOLOG principal component analysis (PCA) and cluster analysis (CA) was carried out by using UPGMA (un-weighted pair group method with average linkage) and clustering algorithm through DPS software version 7.05 and SPSS software version 11.5, respectively.

RESULTS

***Effect of intercropping on soil nutrition*: *(i) Nitrogen*:** The results of available nitrogen can be observed from the Table 1. It showed that IMP100%, IMP75% and P100% showed higher level of available nitrogen (83.53, 82.1 and 85.87 mg/kg respectively) and found significantly different than other treatments. However, sole maize crop showed lower level of nitrogen contents (73.97mg/kg) in soil as compared to intercropping treatments. Total nitrogen levels are also found higher and significantly different in intercropped treatments (100% & 75%) as compared to sole maize and peanut crop.

***(ii) Phosphorus*:** The concentration of available phosphorus in the sole peanut treatments was higher (15.96mg/kg) as compared to intercropped and sole maize crop (Table 1). However, it can be observed from the results that with the increase of nitrogen level in intercropping treatments, the level of phosphorus contact also significantly different from each other. The total phosphorus contents were found lowest in the sole peanut treatments; however, sole maize 100% treatment (544.13mg/kg) was at higher level followed by intercropped treatment with 75% nitrogen (410.75mg/kg).

***(iii) Potassium*:** The contents of available potassium showed different trends in this experiment and found that sole peanut and sole maize crop having lesser level of nitrogen application showed higher level of available potassium contents. The intercropping treatments with 75% of nitrogen fertilizer showed higher level of total potassium (7344.62 mg/kg) as compared to other treatments (Table 1).

***Effect of intercropping on soil enzymatic activity*:** The activity of urase enzyme was recorded much higher in the treatments intercropped maize with peanut and significantly different as compared to sole maize and peanut. It was observed that intercropped treatments having higher level of nitrogen showed high concentration of urease. (Table 2). It is clear from the results that with increase of nitrogen level both in monocropping and intercropping the level of sucrase affected badly. However, the activity of sucrase was recorded higher in the treatment of maize grown without nitrogen followed by maize intercropped with 100% nitrogen levels. The concentration of peroxidase observed higher in maize sole treatments with 100% nitrogen levels followed by intercropped treatments with peanut. It can be observed that there is not much effect on the concentration of peroxidase with intercropping of maize by peanut crop. Higher level of phosphomono-estrase was recorded in the intercropping treatments as compared to sole maize crop; however, results showed that level of nitrogen influenced the

Table 1. Effect of reduced nitrogen on soil nutrients (NPK) in maize/peanut intercropping system

Treatments	Available N(mg/kg)	Available P(mg/kg)	Available K(mg/kg)	Total N(mg/kg)	Total P(mg/kg)	Total K(mg/kg)
IMP 100%	83.53a	13.87bc	59.99d	1925.00a	394.86cd	6010.91c
IMP 75%	82.10a	12.41cde	55.91e	1822.33a	440.75b	7344.62a
IMP 50%	70.23cd	10.61ef	52.78fg	872.67e	411.50bcd	7018.00ab
M 100%	73.97bc	13.16bc	50.88g	1099.00bc	544.13a	6827.47b
M 75%	78.40ab	16.29a	51.69g	1136.33b	402.42bcd	5956.47c
M 50%	79.57ab	12.95bcd	53.73efg	1163.66b	432.17bc	6827.46b
Ck(M)	67.90cd	10.03f	55.64ef	974.87cde	402.92bcd	4922.16d
P 100%	85.87a	15.96a	101.37c	1077.67bc	405.45bcd	6092.57c
P 75%	67.90cd	11.11def	103.27c	1057.01bcd	395.36cd	5902.04c
P 50%	65.80d	10.49f	110.49b	896.00e	302.07e	5194.35d
Ck (P)	55.53e	14.66ab	116.47a	914.67de	379.22d	5711.50c

Means sharing similar letters in a column are statistically non-significant (P>0.05)

Table 2. Effect of reduced nitrogen on soil enzymatic activities in maize peanut intercropping system

Treatments	Urase ($\mu g \cdot g^{-1}.h^{-1}$)	Sucrase ($\mu g.g^{-1}.h^{-1}$)	Per-oxidase ($\cdot g^{-1} \cdot h^{-1}$)	Phosphomono-estrese ($\mu g.g^{-1}.h^{-1}$)	Polyphenole oxidase ($\mu g \cdot g^{-1} \cdot h^{-1}$)
IMP 100%	7.82a	45.70cd	290.79bc	535.92a	462.52b
IMP 75%	8.36a	42.86de	261.67c	517.89ab	449.60bc
IMP 50%	7.03b	42.29de	278.83bc	480.89bc	502.45b
M 100%	5.87d	55.34b	364.16a	404.23ef	600.68a
M 75%	6.22cd	50.17bc	286.08bc	403.04ef	481.66b
M 50%	6.91bc	44.70cde	286.87bc	465.12cd	378.03c
Ck(M)	5.06e	67.65a	280.29bc	384.19f	427.11bc
P 100%	5.85d	41.47de	285.22bc	433.84de	631.90a
P 75%	5.61de	34.56fg	257.83c	426.69e	473.72b
P 50%	5.97d	32.72g	292.43bc	515.60ab	474.67b
Ck (P)	5.50de	39.85ef	298.03b	473.89c	422.09bc

Means sharing similar letters in a column are statistically non-significant (P>0.05)

Table 3. Table showing different specific PLFAs, sum of total, non-specific and group-specific of different microbes by reducing nitrogen in maize peanut intercropping system

Treatments	Bacteria	G+	G-	Actinomycetes	fungi	Protozoa	non-specific	Total PLFA
IMP100%	$3.29x10^7$a	$2.27x10^7$a	$7.65x10^6$a	$2.24\ x10^6$a	$2.09\ x10^6$d	738130a	$3.49\ x10^6$a	$5.09\ x10^7$a
IMP 75%	$2.51x10^7$b	$1.78x10^7$b	$5.48x10^6$b	$1.73\ x10^6$ab	$2.21x10^6$cd	541342b	$2.15\ x10^6$ab	$3.79\ x10^7$b
IMP 50%	$2.21x10^7$b	$1.72x10^7$b	$3.73x10^6$d	$1.41x10^6$b	$2.44\ x10^6$c	710319a	$2.34\ x10^6$ab	$3.56x10^7$b
M 100%-	$2.44x10^7$b	$1.73x10^7$b	$5.34x10^6$bc	$1.67\ x10^6$ab	$3.30\ x10^6$a	544705b	$1.81\ x10^6$b	$3.68\ x10^7$b
P 100%	$2.23x10^7$b	$1.60x10^7$b	$4.97x10^6$c	$1.47x10^6$b	$2.77\ x10^6$b	540889b	$2.46\ x10^6$ab	$3.43x10^7$b

Means sharing similar letters in a column are statistically non-significant (P>0.05)

activity of this enzyme, higher activity was recorded in IMP100% followed by IMP75% and IMP50%. The results of Table 2 revealed that activity of polyphenol oxidase was higher in the both maize and peanut monocropping treatments with 100% nitrogen. It can also be observed that level of nitrogen influenced the activity of polyphenol oxidase. Same results were found in the treatments of similar nitrogen levels both in mono and intercropping treatments of both crops.

Effect of intercropping on microbial community structure: Difference between monocropping and intercropping treatments with different level of nitrogen (3 replication from each treatment soil) for total PLFA, bacteria, fungi and actinomycetes are shown in Table 3. Total PLFA was affected by intercropping and with the application of nitrogen fertilizer, being greatest in the intercropping treatments with 100% nitrogen followed by 75% nitrogen with value of $5.09\ x10^7$ and $3.79\ x10^7$ respectively. Bacterial PLFA (15:0w) was significantly higher (P=0.05) in the intercropping treatments with 100% nitrogen (IMP100) as compared to other treatments. The results revealed that Gram-positive bacteria (a12:0w, i13:0w, a14:0w, i15:0w, a16:0w, a17:0w, and i18:0w), Gram-negative bacteria (16:1w7c, 16:1w9), actinomycetes (10Me17:0,10mel19:0w) tended to be higher in the intercropping treatments as compared to both monocropping of peanut and maize crop with similar level of nitrogen fertilizer. Similar to some specific PLFA, the non-specific PLFAs (12:0w, 16:0w, 19:1w10c, 20:4w6, 20:5w3, 24:0w, 26:0w) also showed same trends in all the treatments and found higher in intercropping treatments as compared to monocropping and the trend was IMP100% > IMP 75% > IMP50%. The sum of fungal PLFA (18:2w6) showed different trend as compared to other PLFAs. It observed from the results that fungal PLFA was higher in monocropping as compared to intercropping treatments and they were found significantly different from each other and fungal PLFA was mainly responsible for discrimination between intercropping and monocropping treatments. The result table revealed that most of PLFAs was statistically similar in intercropping treatments with 75% nitrogen (IMP75%) and maize sole crop (M100%) with 100% nitrogen application.

The PLFA data of all soil treatments (each has 3 replications) was subjected to principal component analysis (PCA). The first two principal components (PC) grouped the replicate data sets of same soil treatment and separated by different treatments. From the results of PCA, the combine data revealed a very tight clustering of replications and very clear separation of different treatments of monocropping and intercropping. From the Figure 1 (B), it is clear that intercropping treatments with 75% nitrogen is much closed to the monocropping treatments of maize crop with 100% nitrogen, amplifying that they might have similar soil microbial structures and these results are confirmed by

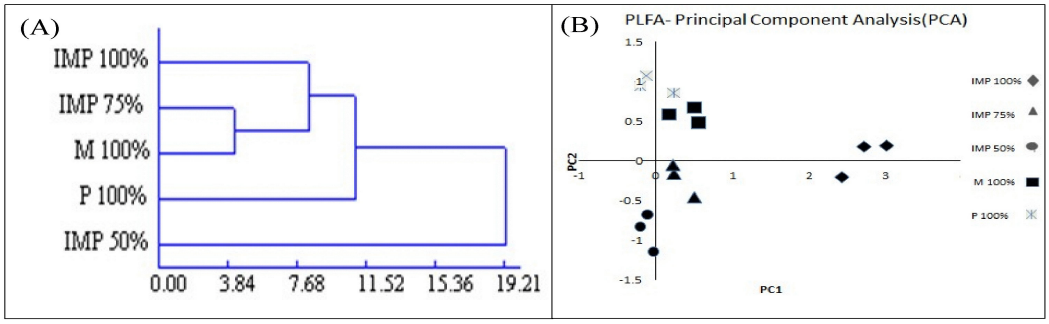

Figure 1. (A) is for PLFA cluster analysis, (B) is for PLFA-Principal component analysis

cluster analysis. The dendrogram of PLFA showed that Intercropping treatment with 25% lesser amount of nitrogen fertilizer showed similar trend as in 100% monocropping treatment of maize (Fig. 1A).

Effect of intercropping on community level physiological profiles (CLPP):

***1) Average well-colour development of carbon source (AWCD) changes over time*:** 31 single carbon sources of Biolog ecological plate was divided into 6 categories, namely: amino acids, polymers, phenolic compounds, amines, carboxylic acids, and carbohydrates. However, these were further divided into 4 kinds of polymers, 10 kinds of carbohydrates, 7 different kinds of carboxylic acids, 6 kinds of amino acids, 2 kinds of amines and 2 kinds of phenolic acids as described by Insam (1997)**.**

Percentage of carbon source utilization in different intercropping and monocropping treatments can be observed in Fig. 2. From the Fig. 3 it can be observed that with different level of nitrogen application during intercropping of maize and peanut showed significant change in soil micro flora in all 6 different kinds of carbon source utilization. The results of AWCD showed that 4 kind of carbon sources (carboxylic acid, polymer, carbohydrates, amino acids) increased with the increase of nitrogen levels in intercropping treatments and 2 kinds of carbon sources (phenolic acid, amines) showed different trend, however all 6 carbon sources increased with the increase of incubation time.

***2) Effect of different levels of nitrogen on Principal Component Analysis (PCA) on Soil microbial communities and carbon source utilization*:** The results of principal component analysis clearly showed that every treatment has different microbial biomass as in Fig. 4. The correlation between PCA1 and PCA 2 showed that treatments of intercropping with various level of nitrogen exhibited different level of carbon source utilization. Different level and various kind of biochemical of carbohydrates, polymers, carboxylic acid and amines were found in intercropping and monocropping treatments.

***3) Cluster Analysis characteristics of soil microbial communities and different carbon source utilization under intercropping of maize and peanut with different level of nitrogen*:** Based on the results of average well-colour development (AWCD) of carbon sources 6th day (120h) was selected for further study regarding cluster analysis. By cluster analysis, we can understand the relationship between individual affinity levels along with affinities between the variable degrees. In this study, we used the R-language software with 5 different kinds of soils along with 31 carbon source-map as shown in Fig. 5. From the hot spot graph clustering we can observe that all the 5 different kind of soils showed distinct difference with different carbon sources and represents changing trends in different treatments. However, the results of cluster analysis revealed that intercropping treatment with 75% level of nitrogen is closely related to 100% monocropping of maize crop regarding soil communities.

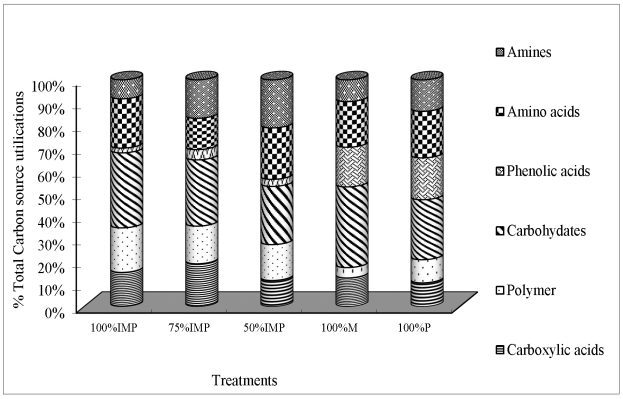

Figure 2. Percentage of total carbon source utilization in Maize peanut intercropping

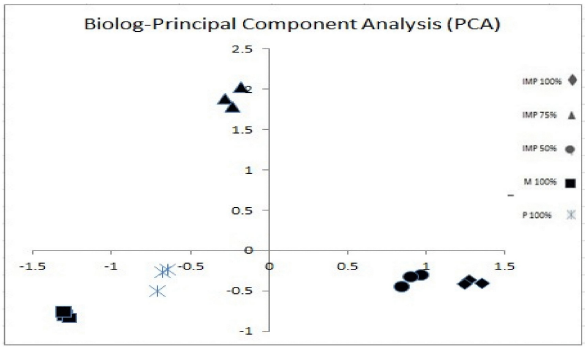

Figure 3. Average well color development (AWCD) of carbon source in Biolog micro ecological plates

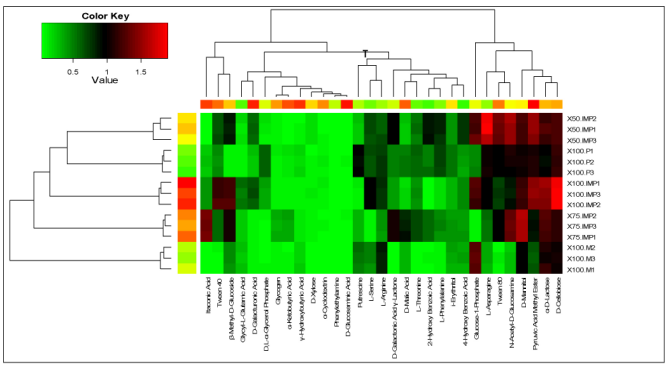

Figure 4. Biolog-Principal component analysis in maize peanut intercropping system

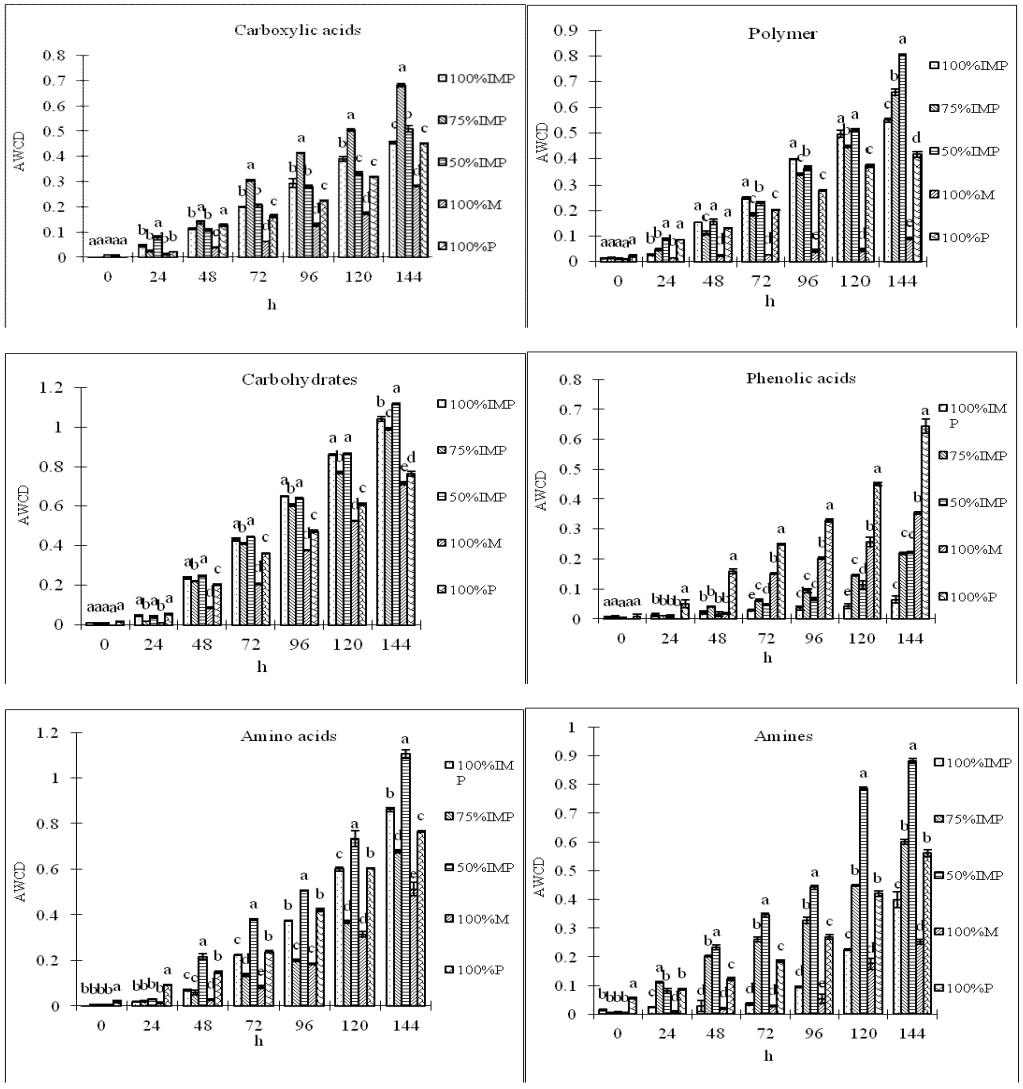

Figure 5. Biolog-Cluster analysis (Heat Map) in maize peanut intercropping system with different level of nitrogen

DISCUSSION

***Influence of maize and peanut intercropping systems on Soil Nutrition*:** Since the crop management system has strongly influence on the soil structural properties and soil environment. Mineral fertilization can provide readily available nutrients to the plant growth but it does not contribute to the soil physical condition. In cropping system, rhizosphere is not only an interface between root and soil for an individual plant but also a center of interaction of plant communities (i.e. plant species in intercropping systems), soil, microorganisms and their environment (Zhang *et al.*, 2002). In the plant, soil surface rhizosphere processes are the linkage between plant processes, soil processes and exchange of matter and energy between plant and soil that can affect the productivity (Zhang *et al.*, 2002). It is very important to clarify the mechanism of nutrient utilization and metabolization related to the rhizosphere physical, chemical and biological processes in cropping systems. Nitrogen is a very important plant nutrient and constituents of many organic compounds proteins, amino and, chlorophyll (Peries and Fernando, 1983). Phosphorus is a major component of nucleoproteins, enzymes and lecithin and major role is physiological process i.e. transfer of energy to plant body (Jalil, 1988), however, potassium (K) seems to be necessary for carbohydrate, protein and oil synthesis in plants. For this purpose intercropping is the best utilization of soil nutrients and energy uptake. In our experiment, the intercropping of maize with peanut under different nitrogen treatments, we found that soil nutrients levels were enhanced as compared to monocropping with similar level of nitrogen fertilizer. The results revealed that higher level of both available & total nitrogen under intercropping soil might be due to intercropping of maize with peanut, a leguminous crop. However, total P and available P were higher in monocropped treatments as compared to intercropping; it means higher level of phosphorus was used in intercropping treatments. Our results were consistent with that as described by Dahmardeh and others (2010). This finding suggests that intercropping can increase available nutrients of soil and improve conservation of soil fertility compared to sole cropping.

***Influence of maize and peanut intercropping systems on soil enzyme activities*:** Since the measurement of soil enzymes activity is very important for soil assessment related to sustainability (Bergstrom *et al*., 1998). Soil enzymes activity plays a vital role in the biochemical functioning of soil including decomposition of xenobiotic, nutrient cycling and soil organic matter formation & degradation (Acosta-Martinez *et al.*, 2007). Micro-organisms are the main source of soil enzymes and activities of soil enzymes can provide the information about degradation potential of soil (Tabatabai, 1994; Trasar-Cepeda *et al*., 2000). Many changes in soil management and land use is reflected in soil enzymes and can anticipate changes in soil quality before they are detected by other soil analysis (Ndiaye *et al*., 2000).

Phosphatase is one of very important enzymes and plays a vital role to transfer organic P into inorganic phosphate which is directly available to plants and soil organisms. Phosphomonoesterase is different from other phosphatase due to their substrate specificity. High level of phos-phomonoestrase contents indicates low contents of available P and it is negatively correlated with available phosphorus contents and it can be influenced by organic P contents (Gevorkyan *et al*., 1987; Badalucco *et al*., 1992). Similar findings have been recorded in our experiment that monocropping treatments showed higher activity of phosphomonoestrase as compared to intercropping that might be due to higher level of organic phosphorus and low level of available phosphorus.

The abundance and functional distribution of phenol oxide and peroxidase can vary with plant and microbial composition (Sinsabaugh, 2010). In our experiment, we recorded that peroxidase activity was higher with increase of nitrogen levels both in monocropping and intercropping treatments; however, phenol oxidase activity showed negative correlation with nitrogen levels and found that less level of nitrogen, 50% of normal nitrogen application rate showed higher enzymatic activity. It is clear from the result that higher level nitrogen performs better with respect to phenolic oxidation enzymes as the phenolic molecules are inherently toxic, therefore, it may affect activity and composition of soil microbial communities (Sinsabaugh, 2010).

Soil urease is produced by soil microbes and plays a vital role in utilization of soil nitrogen and nitrogen cycle by decomposition of urea into ammonia, carbon dioxide and water which are beneficial for plants absorption, however, soil sucrase used to indicate the strength of soil oxidation and closely related to soil decomposition of soil organic matter can affect the soil quality and fertility (Zhang *et al*., 2004). In our experiment the activity of soil urease is better in 100% and 75% of normal nitrogen treatments in intercropping system as compared to monocropping system; however soil sucrase activity was not affected significantly by intercropping or level of nitrogen fertilizer. Our results are supported by the work of different scientists (Klose *et al*., 1999) as stated that enzymatic activity is sensitive to soil changes due to cropping systems.

***Influence on microbial community structure*:** PLFA analysis is a measurement of viable community structure of microorganisms which interacts immediately before sampling. Phospholipid fatty acid (PLFA) are the major component of membrane of all living cells and different groups of microorganisms produce different varieties of PLFA through different biochemical pathways (Dai *et al*, 2013). PLFA analysis showed that soil microbial

communities in intercropping soil differed from monocropping soil. The results of PLFA analysis showed that intercropping inhibited the fungal population and promoted the bacterial population. Similar results have been recorded in aerobic rice and watermelon intercropping soil (Ren *et al.*, 2008). Analysis of PLFA composition showed the concentration of G^+ was higher in intercropping treatment with 100% of normal nitrogen supply as compared to monocropping and these results are similar to the findings of Zhong *et al*. (2010). Results showed that increase of fertilizer levels in intercropping treatments also increase the G^- biomass to some extent that might be because of more aromatic and allelochemicals in the soil (Kong *et al*., 2008). The results suggest that by intercropping of peanut with maize is beneficial for soil microbial communities which lead to soil fertility. PLFA analysis helps to observe the dynamics of soil microbial communities to overcome the problems of monocropping for assessing the composition of soil micro flora.

***Influence on Soil Microbial diversity*:** Biolog experiment provide metabolic profiles of fungal and bacterial community's ability to utilize different carbon sources and are relatively easy to use, reproducible and produce a large amount of data reflecting metabolic characteristics of the communities (Zak *et al*., 1994). By Biolog method functional diversity of microbial communities can be achieved. Mainly through AWCD overall situation reflected regarding metabolism of micro-organism and microbial diversity on different carbon sources. In this study of pot experiment, 5 different kinds of soils were used on 6 different level of carbon source utilization. Further, cluster analysis (heat map) and principal component analysis (PCA) showed that 5 different kinds of soil on carbon source utilization can fall into 6 categories, namely amino acids, polymers, phenolic compounds, amines, carboxylic acids, and carbohydrates. Both PLFA and CLPP based principal component analysis (PCA) and cluster analysis showed distinct separation between all treatments of monocropping and intercropping as well as with different level of nitrogen application that reflected an alteration of soil microbial community activity and composition. These results are confirmed by different scientists by stating that plant species can affect the composition of rhizosphere microbial communities (Rooney and Clipson, 2008; Junier *et al*., 2009). According to Hao and others (2003), organic acid in root exudates was increased in wheat/maize intercropping as compared to monocropping which might affect some acid sensitive microbes.

***Conclusion*:** By this experiment we concluded that intercropping of maize with peanut influenced the soil rhizosphere by increasing bacterial community and decreasing soil fungi. Intercropping peanut with maize enhanced the nutrition and enzymatic activity of soil to prevent soil from deterioration. These results also suggest that intercropping can be used to decrease the dependence on artificial fertilizer which provides a convenient organic farming model for growers across the world that is dire need of new era. It further needs to work out isolation and identification of allelochemicals that influence the soil rhizosphere.

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