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Assessing Microbial Community in Andisol Differing in Management Practices by Biochemical and Molecular Fingerprinting Methods

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Abstract: Several biochemical and molecular methods are used to investigate the microbial community structure and microbial diversity in soils. Biochemical (Phospholipid fatty acid: PLFA profiles) and molecular (Denaturing gradient gel electrophoresis of polymerase chain reaction amplified DNA: PCR-DGGE) fingerprinting methods were employed to quantify soil microbial community in Andisol differing in management practices. No-tillage (NT) soil had significantly higher microbial biomass carbon and microbial biomass nitrogen than conventional tillage (CT) soil. PLFA for gram-positive bacteria, gram-negative bacteria, aerobes, cyanobacteria and fungi were significantly higher in NT than CT. On the other hand, PLFA for sulfate-reducing bacteria, methane-oxidizing bacteria and mycorrhizae were significantly higher in CT than PD and NT. The total DNA extracted from Andisol with differing management practices ranged from 21.0 to 33.0 $\mu g\ g^{-1}$ soil. Soil DNA yielded from puddling (PD) showed highest amount and NTg showed lowest amount. There were no significant variations in DNA yield obtained from Andisol of CT and NTg. Highest bacterial diversity evaluated by DNA band number in DGGE analysis based on PCR amplification of 16S rDNA fragments was observed in PD and can be arranged as: PD>CT>NT. On the other hand, highest fungal diversity evaluated by DNA band number in DGGE analysis based on PCR amplification of 18S rDNA fragments was observed in NT and can be arranged as: NT>CT>PD. Results indicated that microbial community was responsive to management practices demonstrating their usefulness as indicators of soil quality in temperate Andisol.

Key words: Andisol, microbial community, biochemical (PLFA) and molecular (PCR-DGGE) techniques, tillage

INTRODUCTION

Management practices have a strong influence on the soil physical, chemical and microbiological properties. Microbial-based indicators of soil quality are believed to be more dynamic than those of physical and chemical properties. Therefore, only physical and chemical properties do not draw detailed conclusions on soil fertility. Microbial community is an important determinant of soil organic matter decomposition rates and nutrient turnover and availability in agricultural soils. Microbial characteristics of soils are being evaluated increasingly as sensitive indicators of soil health because of the clear relationships between microbial diversity, soil and plant quality and ecosystem sustainability (Doran *et al*., 1994). While the understanding of microbial properties such as biomass, activity and diversity are important to scientists in furthering knowledge of the factors contributing to soil health, results of such analyses may also be useful to extension personnel and farmers in devising practical measures of soil quality (Hill *et al*., 2000).

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Soil ecosystems are highly complex, containing a tremendous amount of microbial species. Indigenous microbial populations in soil are of fundamental importance for ecosystem functioning, through determining nutrient cycling, organic matter decomposition and energy flow (Doran and Zeiss, 2000). Despite all attempts to measure fluxes and gross microbial pools, the soil and its microbiota still remain a black box. Most soil micro-organisms are still unknown, while very few have been isolated, cultured and identified and directly related to their function in agroecosystems. The development of effective methods for studying the diversity, distribution and behavior of micro-organisms in soil habitats is essential for a broader understanding of soil health. Traditionally, the analysis of soil microbial communities has relied on culturing techniques using a variety of culture media designed to maximize the recovery of diverse microbial populations. Although new culture media have been recently developed to maximize the recovery of diverse microbial groups from soils (Balestra and Misaghi, 1997; Mitsui *et al*., 1997), only a small fraction (<0.1%) of the soil microbial community has been accessible with this technique (Atlas and Bartha, 1998; Hill *et al*., 2000). Widmer *et al*. (2001) reported that standard microbiological culture techniques leave over 90% of the micro-organisms unaccounted for in the environment. Prosser (2002) reported that less than 5% of soil micro-organisms are cultivable. To overcome these problems, several methods have been utilized in an attempt to assess a greater proportion of the soil microbial community. On the other hand, recent techniques like Phospholipid Fatty Acid (PLFA) and Denaturing Gradient Gel Electrophoresis (DGGE) analyses have provided a new understanding of microbial communities in soil. The analysis of PLFA is a useful assay for soil microbial community because (i) the concentration of total PLFA can be used as an index of viable microbial biomass since phospholipids are rapidly degraded after cell death, (ii) certain fatty acids may be used as molecular markers for specific taxa and as indicators of microbial stress and (iii) multivariate analysis of the PLFA profiles can be used to detect changes in community composition (White *et al*., 1996). More recently, molecular techniques for identification that do not require cultivation have been developed to study of different groups of micro-organisms. One of those techniques is DGGE of PCR-amplified genes, which can be used to evaluate the diversity of complex microbial systems (Muyzer *et al*., 1993). We used (i) PLFA analysis with gas chromatography and mass spectrometry and (ii) DGGE analysis of polymerase chain reaction-amplified genes coding for 16S rDNA and 18S rDNA for bacteria and fungi, respectively to investigate the microbial communities in Andisol. This is the first attempt done to check whether the two methods give identical results in detecting the soil microbial communities especially in Andisol. Such information will improve our understanding in investigating microbial communities in Andisols.

MATERIALS AND METHODS

Collection of Soil Samples

Soil samples were collected from the experimental field of Iwate University, Japan established in 1980 and located in about the latitude of 39°42′ N and longitude 141°10′ E with an altitude of about 155.2 m above sea level. The mean monthly maximum and minimum temperature, precipitation and humidity of the study area for last 20 years are shown in Table 1.

Three sites under NT, CT and PD systems were selected in this study. The samples were collected from A horizons at a depth of 0-10 cm of sandy clay pumice Andisol (Melanudands) which was partially modified by siltation of Kitakami river from apple orchard in which apple was grown with NT, grassland in which fodder crops (Orchardgrass: *Dactylis glomerata* L., *Kentucky bluegrass*: *Poa prantensis* L., White clover: *Trifolium repens* L., Tall fescue: *Lolium arundinaceum* [Schreb.] SJ Darbyshire, Meadow fescue: *Lolium pratense* [Huds.] SJ Darbyshire, *Rumex: Rumex obtusifolius* L. Sere etc.) were grown with no-tillage (NT_g), dryland soil cultivated with sweet potato, corn, wheat,

Table 1: Meteorological data of field area during 1980-2006

Months	High temp. (°C)	Lowest temp. (°C)	Total ppt (mm)	Mean humidity (%)
January	6.9	-11.7	53	73
February	8.6	-11.4	48	70
March	14.8	-8.1	79	67
April	23.9	-2.8	84	65
May	27.5	2.0	106	69
June	29.2	7.9	112	75
July	32.2	12.8	185	81
August	33.3	13.8	180	80
September	29.3	7.4	159	80
October	23.4	0.4	97	77
November	17.5	-4.0	89	75
December	11.5	-8.1	64	74

Morioka meteorological station, Iwate, Japan

barley, rye and colza in rotation with conventional tillage (CT) and puddling with continuous rice (PD) systems for more than 20 years. It can be noted that two or three grass species (Orchardgrass: *Dactylis glomerata* L., White clover: *Trifolium repens* L., Tall fescue: *Lolium arundinaceum* [Schreb.] SJ Darbyshire, Rumex: *Rumex obtusifolius* L. Sere etc.) existed or were present in NT-apple orchard as interplants. Tillage methods, cropping patterns and changes in soil properties were measured as per the methods followed by Rahman *et al.* (2003).

The soil was sampled in September 2006 (after ploughing) from surface horizon because this is where the maximum difference in soil properties occurs among treatments (Rahman *et al*., 2003). Ten samples were collected from each plot and made into three composite samples. Each soil sample was placed in a plastic bag in the field and kept cool until it was processed in the laboratory. Soil samples were sieved (<4 mm) in the laboratory. After removing visible plant litters, rocks and roots, a portion of soil was immediately frozen for microbial analysis.

Chemical Analysis

The total C and N contents of soils were determined using a Sumigraph-90A automatic analyzer (Sumitomo Chemical Co. Ltd., Osaka, Japan) and expressed as g kg^{-1} soil. Since there was no indication of the presence of carbonates, total C was equivalent to organic C. The flush of C was converted to total microbial biomass carbon (MBC) by multiplying by 2.64 (Vance *et al*., 1987) and the flush of N was converted to total microbial biomass nitrogen by multiplying by 1.86 (Brookes *et al*., 1985).

Microbial Community Structure

PLFA analysis was done using a composite mixture of three replicates in 20 samples for each site. The method described by Frostegård *et al.* (1993) was followed to obtain PLFA profiles for soil samples. Briefly, lipids were extracted from fresh soils (equivalent to 10.0 g of dry soil) following 4 h shaking using a mixture of chloroform: methanol: citrate buffer (1:2:0.8 v/v/v). The chloroform phase of the extraction was collected and then separated into functional classes using silicic acid column chromatography (Sep-Pak Silica, Waters, Milford, USA). The polar lipid was eluted with 5 mL methanol and then dried under N_2 gas. Then, fatty acid methyl esters (FAMEs) were extracted. The dried samples were saponified with 1 mL of saponification reagent (NaOH 15 g, methanol 50 mL and distilled water 50 mL) and heated in a 100°C water bath for 30 min. The samples were then cooled with water stream and then methaylated by adding methylation reagent (6.0N HCl 130, 110 mL methanol) and heated in a 80°C water bath for 10 min. FAMEs were extracted by adding a 1.25 mL mixture of 1:1 methyl-t-butyl ether and n-hexane (v:v) and shaked for 10 min. The organic phase was then transferred to another test tube, dried under a stream of N_2 gas, resuspended in 10 µL of n-hexane and 1 µL was analyzed on a gas chromatography (GC-14B, Shimadzu, Kyoto, Japan) equipped with

a capillary column of 30 m with 0.25 m inner diameter (SPB-1, Supelco, Bellefonte, USA) in a condition of oven temperature of 150°C (4 min) to 250 at 4°C increment min^{-1}. Helium was used as carrier gas, 20 cm sec^{-1}, at 150°C and FID at 280°C. In addition, PLFA mixtures extracted from representative soils were analyzed by GC-MS (PQ2010, Shimadzu, Kyoto, Japan) and chemical structure of each PLFA peak was identified. We also used a commercial bacterial FAME mixture for peak identification (FAME, Supelco, Bellefonte, USA). PLFAs of each soil sample were identified from the chromatographic retention time by comparison with nonadecanoic acid (19:0, Supelco, Bellefonte, USA) as an internal standard. In this study PLFA peaks with more than 0.071 μg g^{-1} soil equivalent proportion to the amount almost higher than 0.5 % of total PLFA were used (Kourtev *et al*., 2003). A total of 58 PLFA peaks were found and 45 of them were identified and 34 were considered for evaluation or further studies. For each sample, the abundance of individual fatty acid methyl-ester was calculated by comparison with internal standard and was expressed as nmol g^{-1} soil.

According to Hill *et al*. (2000), common fatty acid signatures were used for groups of organisms viz., common bacteria, aerobes, anaerobes, sulfate-reducing bacteria, methane-oxidizing bacteria, cyanobacteria and fungi. The fatty acid signatures i15:0, a15:0, 15:0, 16:0, 16:1ω5, 16:1ω9, i17:0, a17:0, 17:0, 18:1ω7t, 18:1ω5, i19:0 and a19:0 were chosen to represent bacterial PLFA. The fatty acid signatures 16:1ω7, 16:1ω7t and 18:1ω7t were chosen to represent aerobes. The fatty acid signatures cy17:0 and cy190 were used for anaerobes. For sulfate-reducing bacteria, fatty acid signatures 10Me16:0, i17:1ω7 and 17:1ω6 were used. Fatty acid signatures 16:1ω8c, 16:1ω8t, 16:1ω5c, 18:1ω8c, 18:1ω8t and 18:1ω6c were considered for methane-oxidizing bacteria. The polyenoic, unsaturated PLFA 18:2ω6 was used as an indicator for cyanobacteria. The PLFAs 18:1ω9, 18:2ω6, 18:3ω6 and 18:3ω3 were used for fungi. On the other hand, the branched, saturated PLFAs 10Me16:0, i15:0, a15:0, i16:0, i17:0 and a17:0 were chosen to represent Gram-positive bacteria (O'Leary and Wilkinson, 1988; Zogg *et al*., 1997). The monoenoic and cyclopropane unsaturated PLFAs 16:1ω7t, 16:1ω9c/a16:0, 18:1ω5c, 16:1ω7c, cy17:0, 18:1ω7c and cy19:0 were chosen to represent Gram negative bacteria (Zogg *et al*., 1997; Porazinska *et al*., 2003). The PLFAs 16:1ω5c and 10Me18:0 were used to represent mycorrhizae and actinomycetes, respectively (Bossio and Scow, 1998; Zak *et al*., 2003).

Bacterial and Fungal Diversity

DNA extraction was performed with the Ultra Clean Soil DNA kit (MoBio Laboratories, Solana Beach, USA). One gram soil was processed according to the protocol. Concentration of DNA was measured by BIO-RAD SmartSpec™ Plus Spectrophotometer, USA and expressed as μg g^{-1} soil.

Bacterial 16S rDNA were amplified using the primers 341f-GC and 534r (Muyzer *et al*., 1993). Amplification of fungal 18S rDNA required nested PCR. First round PCR used the primers NS5 and NS8 (White *et al*., 1990) and the nested-PCR used FR1-GC and FF390 (Vainio and Hantula, 2000). The sequence, band-size obtained and PCR thermal cycles of each primer are shown in Table 2. The reaction mixture (25 μL) consisted of 5 ng template DNA, 2.5 μL of 10×PCR buffer (100 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl_2$), 2 μL of dNTP Mixture (2.5 mM of eath dNTP), 0.5 μM of each primer and 0.3U of Smart Taq DNA Polymerase (Abgene®, UK).

Bacterial DGGE was performed with 8% polyacrylamide gels (Acrylamide/bis-acrylamide: 37.5/1) containing 45% to 65% vertical gradient of denaturant (100% was defined as containing 7 M Urea and 40% Formamide). On the other hand, 7% polyacrylamide gels containing 25 to 55% gradient were used for fungal DGGE. Approximately 24 μL of each PCR product was loaded onto the gels. Electrophoresis was performed in 1xTAE buffer (40 mM Tris/Acetate, pH 8;1 mM EDTA) at 80 V and 60°C for 16 h for bacterial DNA, while 65 V and 60°C for 13.5 h for fungal DNA. After electrophoresis, gels were stained in 1xTAE containing 0.01% SYBR Green I Nucleic Acid Gel Stains (Cambrex Bio Science Rockland, Inc. Rockland, ME USA) for 30 min. The gels were visualized by UV

Table 2: Properties of each primer and PCR conditions

Micro-organisms	Primer	Sequence (5'→3')	Product size (bp)*[1]	Thermocycle programs
Bacteria	341f-GC	CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG	200	5 min at 95°C followed by 30 cycle of 30 sec at 95°C, 1 min at 65°C, 1.5 min at 72°C and final extension for 5 min at 72°C
	534r	ATT ACC GCG GCT GCT GG		
Fungi	NS5	AAC TTA AAG GAA TTG ACG GAA G	690	5 min at 95°C followed by 30 cycle of 30 sec at 95°C, 45s at 62°C, 1.5 min at 72°C and final extension for 7 min at 72°C
	NS8	TCC GCA GGT TCA CCT ACG GA		
	FR1-GC	CCC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GCC GAI CCA TTC AAT CGG TAI T	390	5 min at 95°C followed by 30 cycle of 30 sec at 95°C, 45s at 54°C, 1 min at 72°C and final extension for 7 min at 72°C
	FF390	CGA TAA CGA ACG AGA CCT		

*: [1]The size described is estimated from this experiment and/or references

illumination and the gel images were digitally captured by a CAMEDIA digital camera C-750 Ultra Zoom (OLYMPUS CO., LTD., Japan) with a Sybr Green filter. DGGE fingerprints were processed to waveform with Image J 3.14n (National Institutes of Health, USA) to normalize background. After background subtraction, the clustering of patterns was calculated with NTSYSpc Ver.2.11 (Biostatistics Inc.) using the unweighted pair group method using average linkages (UPGMA).

Experimental Design and Statistical Analysis

Soil samples were collected according to a systematic sampling design across the S-shaped transects. The least significant differences (LSD) test was also used to determine whether differences between means were statistically significant ($p<0.05$). Statistical analysis was conducted by JMP 4.0 (SAS Institute, Cary, NC, USA). Unless otherwise noted, all the results were calculated using oven-dried soil (105°C, 24 h).

RESULTS AND DISCUSSION

Microbial biomass-C was influenced by management practice (Fig. 1a) being highest in the NTg and lowest in the CT. Soil under CT had lowest microbial biomass-N (Fig. 1b). The lowest microbial biomass-C in CT associated with the lowest microbial biomass-N.

NT systems generally show higher amount of soil organic carbon (Rahman *et al*., 2003) as well as microbial biomass-C (Paustian *et al*., 1997). Franchini *et al*. (2007) found 80 and 104% higher microbial biomass-C and microbial biomass-N in NT than CT, respectively.

Many of the individual fatty acids were affected by management practice (Table 3). In CT soils individual fatty acids of i14:0, a14:0, 16:1ω7t, i17:0, a17:0, 18:2ω6, 18:1ω9 and 18:0 were least abundant while 3-OH 14:0, 16:1ω5c, cy17:0, 2-OH 16:0 and i18:0 had more abundance than PD and NT. On the other hand, individual fatty acids of a15:0, 15:0, 16:1ω9c, i17:1ω5, 17:0, 20:2 and 20:1ω9c did not show any observable difference. Total PLFA, aerobes, gram-positive bacteria, gram-negative bacteria, cyanobacteria and fungi were significantly higher in NT than CT (Table 4). Bardgett *et al.* (1996) observed that, the ratio of fungal to bacterial PLFA was higher in unfertilized upland grassland soil than in adjacent fertilized treatments. Correlation between microbial biomass-C and N and microbial community indicated that MBC and MBN were highly and positively correlated with total PLFA ($r = 0.777$ and 0.878, respectively), fungal PLFA ($r = 0.917$ and 0.868, respectively), gram-positive bacteria ($r = 0.665$ and 0.911, respectively), gram-negative bacteria ($r = 0.911$ and 0.871, respectively) and cyanobacteria ($r = 0.617$ and 0.873, respectively) while highly and negatively

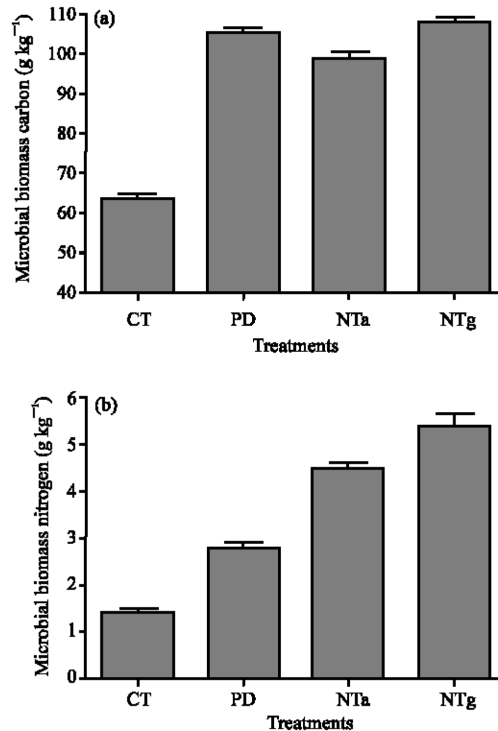

Fig. 1: Microbial biomass carbon (a) and microbial biomass nitrogen (b) of temperate Andisol Bars showed the standard errors

correlated with methane-oxidizing bacteria (r = -0.969 and-0.827, respectively) and mycorrhizae (r = -0.968 and -0.825, respectively). Sulfate-reducing bacteria showed nonsignificant correlation with either MBC or MBN. PLFA for methane-oxidizing bacteria and mycorrhizae were higher in CT than PD and NT while for sulfate-reducing bacteria it was higher in NTa than CT (Table 4). Methane-oxidizing microbial community is very important because of their importance for global methane cycling. Methane is considered as the most potential greenhouse gas after carbon dioxide (IPCC, 2001). Due to the fact that methane concentration in the atmosphere has more than doubled in the post-industrial era, much research effort has been expended to identify sources and sinks of methane and the organisms involved. The balance between the production of methane by methanogenic bacteria under anoxic conditions and the consumption of methane by methanotrophic bacteria (MOB) under oxic conditions determines whether a particular environment acts as a source or a sink for atmospheric methane. Submerged wetland soils like. rice are regarded as the most important source of atmospheric methane while non-flooded upland soilslike grasslands are regarded to be the only biological sink of atmospheric methane (LeMer and Roger, 2001). In both wetland and upland soils, obligate aerobic methane-oxidizing bacteria use molecular oxygen to oxidize methane to carbon dioxide and cell carbon (Hanson and Hanson, 1996). In wetland soils these bacteria are active in the surface soil layers and in the rhizosphere of oxygen releasing plants (Frenzel, 2000). Rahman *et al*. (2007) conducted a research on Andisol, observed that under dry condition, soils has higher methane-oxidizing bacteria although those soils have been under puddling for long time. In this study, it was observed that Andisol with puddling contained substantially less methane-oxidizing bacteria than conventional tillage under submerged condition. It is necessary to carry out intensive research on methane-oxidizing microbial communities with the special reference to Andisols.

Table 3: Phospholipid fatty acids (PLFAs: nmol g^{-1} soil) profiles of temperate Andisol

	Treatments				
Phospholipid fatty acids	CT[1]	PD[2]	NTa[3]	NTg[4]	LSD[5]
i14:0	0.73	1.78	1.77	1.78	0.33
a14:0	1.91	2.71	2.53	2.62	0.23
l4:0	0.62	0.67	1.81	0.93	0.16
i15:0	0.74	0.32	0.67	0.49	0.12
a15:0	1.05	1.26	1.21	1.24	0.06
l5:0	0.70	0.65	0.68	0.66	0.04
2-OH 14:0	0.60	0.29	0.74	0.52	0.12
3-OH 14:0	1.85	0.70	0.65	0.67	0.37
i16:0	1.44	1.95	5.21	3.58	1.07
16:lω9c	8.31	8.52	6.45	7.49	0.59
16:lω7c	0.83	1.67	7.90	4.78	2.03
16:lω7c	1.20	9.90	5.40	7.65	2.34
16:lω5c	2.03	0.70	0.53	0.61	0.45
16:0	0.33	0.35	1.69	1.02	0.41
i17:lω5	1.20	2.09	1.33	1.71	0.25
10Me16:0	1.18	0.59	1.40	1.04	0.22
i17:0	0.36	1.33	1.52	1.43	0.34
a1:17	0.57	1.97	2.29	2.13	0.50
cy:17:0	0.60	0.45	0.46	0.45	0.07
17:0	0.54	0.52	0.69	0.61	0.05
2-OH- 16:0	10.71	0.79	0.90	0.80	3.11
i18:0	1.40	0.47	0.46	0.46	0.29
18:2ω6	0.51	2.16	7.53	4.84	1.94
18:1ω9	2.40	8.01	5.65	6.83	1.52
18:1ω7c	0.00	0.00	2.97	1.48	0.89
18:1ω9c	0.00	0.00	0.52	0.26	0.16
18:1ω5c	0.61	0.00	0.77	0.39	0.21
18:0	0.66	2.59	1.61	2.10	0.52
10Me 18:0	0.71	0.58	1.10	0.84	0.14
cy19:0	0.53	0.68	1.38	1.03	0.24
20:4	0.00	0.69	0.98	0.83	0.27
20:2	0.29	0.37	0.51	0.44	0.06
20:1ω9c	0.52	0.39	0.75	0.57	0.09
20:0	0.72	1.96	1.31	1.63	0.33

CT[1]: Conventional Tillage, PD[2]: Puddling, NTa[3]: No-tillage (apple), NTg[4]: No-tillage (grass), LSD[5]: (Least Significant Different) at $p<0.05$ between treatments (rows)

Table 4: Microbial community (nmol g^{-1} soil) of temperate Andilsol

	Treatments				
Microbial community	CT[1]	PD[2]	NTa[3]	NTg[4]	LSD[5]
Total PLFAs	45.91	57.11	70.74	63.93	6.66
Gram-positive bacteria	5.34	7.42	12.29	9.90	1.90
Gram-negative bacteria	12.13	21.23	25.33	23.28	3.66
Aerobes	0.83	1.67	7.90	4.78	2.03
Anaerobes	1.19	1.13	1.83	1.48	0.20
Sulfate-reducing bacteria	1.18	0.59	1.40	1.04	0.22
Methane-oxidizing bacteria	2.03	0.70	0.53	0.61	0.45
Cyanobacteria	0.51	2.16	7.53	4.84	1.94
Fungi	2.91	10.17	13.18	11.68	2.86
Fungi/bacteria	0.68	1.59	1.51	1.54	0.27
Mycorrhizae	2.03	0.70	0.53	0.61	0.45
Actinomycetes	0.71	0.60	1.10	0.84	0.14

CT[1]: Conventional Tillage, PD[2]: Puddling, NTa[3]: No-tillage (apple), NTg[4]: No-tillage (grass), LSD[5]: (Least Significant Different) at $p<0.05$ between treatments (rows)

DNA extracted from Andisols were colorless indicating no humic acid contamination. Soil DNA of sufficient purity for reproducible PCR amplification yielded 21.0 to 33.0 μg g^{-1} soil (Fig. 2). Highest bacterial diversity evaluated by DNA band number in DGGE analysis was observed in PD and can be arranged as: PD>CT>NTg>NTa (Fig. 3a and 4a) while, highest fungal diversity evaluated

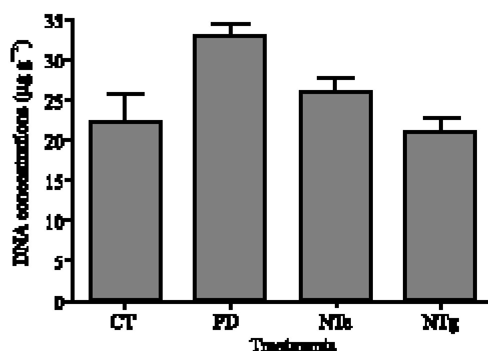

Fig. 2: Concentration of DNA reproducible PCR amplification of temperate Andisol (Bars showed the standard errors)

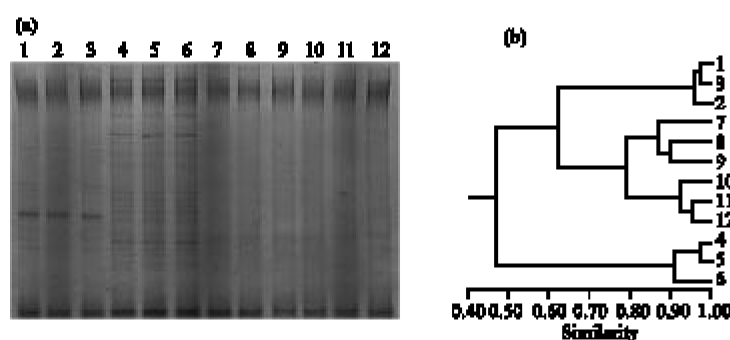

Fig. 3: DGGE fingerprint (a) and dendrogram analysis (b) of bacterial community (1, 2, 3: Conventional tillage; 4, 5, 6: Puddling; 7, 8, 9: No-tillage-apple; 10, 11, 12: No-tillage-grass)

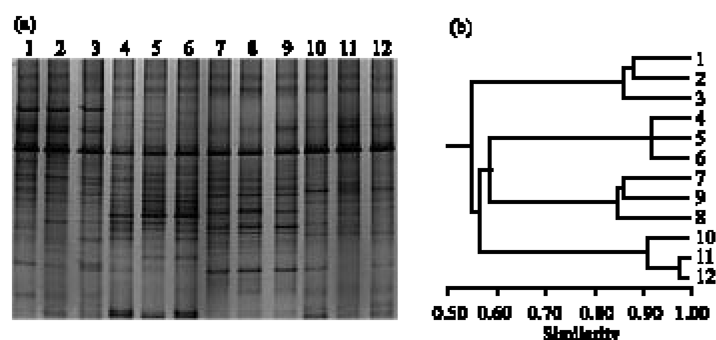

Fig. 4: DGGE fingerprint (a) and dendrogram analysis (b) of fungal community (1, 2, 3: Conventional tillage; 4, 5, 6: Puddling; 7, 8, 9: No-tillage-apple; 10, 11, 12: No-tillage-grass)

by DNA band number in DGGE analysis was observed in NTa. In this study inconsistent relationship was observed between bacterial as well as fungal abundance and diversity measured by number of PLFA peaks and DNA bands, respectively. Rahman *et al*. (2007) observed inconsistent relationship between bacterial abundance and diversity measured by number of PLFA peaks and DNA bands, respectively. They also observed consistent relationship between fungal abundance and diversity

evaluated by the number of PLFA peaks and DNA bands. We inferred that microbial abundance could be high although the diversity might be low. In addition methods like PLFA and DGGE used for measuring microbial community in mineral soils may not give parallel results for microbial community composition in organic soils (Andisols) hence, more studies are necessary to understand the reason behind.

The dendrogram was generated by UPGMA and percentage of similarity among the lanes was calculated taking into account the band migration distance and the relative intensity of all bands (Fig. 3b and 4b). Cluster analysis of the data from PCR-DGGE methods grouped the replicates of the different soils. For bacterial community, cluster analysis revealed closer association of the characteristics of NTa and NTg with a relative distance value of 80%. On the other hand, for fungal community, cluster analysis revealed closer association of the characteristics of NTa and PD with a relative distance value of 57%. The result suggested a linkage between fungal diversity and tillage practices in temperate Andisol. This study justified the need of measuring soil quality based on soil microbial communities. These findings indicated that management inputs, such as fertilizer, herbicide and irrigation were associated with the distinctive microbial community composition of the different management practices.

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