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Physical, chemical and microbiological properties of an Andisol as related to land use and tillage practice

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ABSTRACT

The effects of land use and management practice on soil physical, chemical and microbiological properties may provide essential information for assessing sustainability and environmental impact. This study compared the effects of 41 years of no-tillage (NT) with continuous apple orchard, with those of conventional tillage (CT) with wheat–soybean rotation and another of puddling (PD) with continuous rice on the characteristics of a pumice Andisol in a temperate region of northern Japan. Higher values for bulk density, penetration resistance, pH, C/N ratio, exchangeable Na (X-Na), Fe, and Mn were observed for PD than NT and CT. On the other hand, organic matter, EC, N, exchangeable K (X-K), exchangeable Ca (X-Ca) and Cu were significantly higher for NT than CT and PD. Highest content of Zn was found in CT compared to other practices. The three-phase composition at pF 2.0 was significantly affected by land use and tillage practices. The solid phase and liquid phase were greater under PD than under NT and CT, while air phase was greater under CT than under NT and PD. Significantly higher values for saturated hydraulic conductivity was found in CT than NT and PD. Total phospholipid fatty acid (PLFA) and PLFA for bacteria, aerobes and cyanobacteria were remarkably higher in NT than CT and PD, regardless of depth. On the other hand, PLFA for methane-oxidizing bacteria, sulfate-reducing bacteria and mycorrhizae were significantly higher in CT than NT and PD. PLFA for fungi was significantly higher in surface (0–10 cm) soils than subsurface (10–20 cm) soils regardless of treatments. Highest bacterial and fungal diversity evaluated by DNA band number in DGGE analysis based on PCR amplification of 16S rDNA and 18S rDNA fragments, respectively, were observed in surface soil of PD. The result suggests a linkage between microbial community and tillage practices in temperate Andisol. This study also justifies the need of measuring soil characteristics based on soil microbial communities.

1. Introduction

Soil quality essentially means "the capacity of a soil to function" (Doran and Parkin, 1994). Soil characteristics influence basic soil functions, such as moderating and partitioning water and solute movement and their redistribution and supply to plants; storing and cycling nutrients; filtering, buffering, immobilizing and detoxifying organic and inorganic materials; promoting root growth; and providing resistance to erosion (Karlen et al., 1997). Management practices may alter soil quality based on soil physico-chemical and hydrological properties. Dao (1996) reported that no-till soil had lower bulk density than that under conventionally tilled soil. On the other hand, Roseberg and McCoy (1992) found that conventional tillage (CT) increased total porosity of the soil, but the macropores (effective pores) decreased in number, stability and continuity compared with no-till soil. Varsa et al. (1997) reported that penetrometer resistance (PR) and BD were greatly reduced in deep tilled plots compared with reduced till and no-till treatments. However, Hill and Cruse (1985) observed no significant effect of no-till and plow till on BD of a Mollisol. Ishaq et al. (2002) also observed no significant effect of tillage methods on soil bulk density. These contradictory results may be due to differences in crop species, soil properties, climatic characteristics and their complex interactions (Rasmussen, 1999).

Tillage affects soil nutrient concentrations and availability (Etana et al., 1999). The response of soil chemical fertility to tillage is site-specific and depends on soil type, cropping systems, climate,

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fertilizer application and management practices. Conventional tillage practices involving soil turnover usually decrease soil organic carbon (SOC) concentration (Balesdent et al., 2000). Conservation tillage increases SOC concentration leading to enhancement in soil quality and resilience (Lal, 1993). Powlson and Jenkinson (1981) reported that when changes in BD were accounted for, there were no significant differences in the organic N concentration (on a weight/weight basis) of the plow layer under no-till and plow till treatments. In Mollisol, no-tillage (NT) exhibited higher SOC and N concentrations in only 0–5 cm soil layer (Puget and Lal, 2005).

Galantini et al. (2000) reported that tillage and crop rotations have a significant impact on nutrient distribution and transformation in soils. Franzluebbers and Hons (1996) reported that soil managed by no-till had greater P and K concentrations in the 0–0.05 m layer than with conventional disk-and-bed tillage. Matowo et al. (1999) reported that after 10 years of grain sorghum (*Sorghum bicolor*) production the extractable P was significantly greater in no-till compared with chisel till in the 0.05-m layer, while extractable K was not influenced by tillage treatments. Prihar (1990) stated that because of site-specificity, tillage investigations need to be carried out under different soil and climatic conditions on a long-term basis. Soil pore geometry (pore size, shape and distribution) and soil structure are affected by tillage management and influence soil water storage and transmission (Azooz and Arshad, 1996).

Some researchers have found no or a negative effect of tillage on soil water transmission characteristics (Heard et al., 1988), while others found beneficial effects of zero-tillage (ZT) on soil water retention properties compared to CT (Datiri and Lowery, 1991). Many researchers have reported that saturated hydraulic conductivity (K_{sat}) and unsaturated hydraulic conductivity [$k(h)$] were significantly and positively affected by ZT owing to either greater continuity of pores (Benjamin, 1993) or to water flow through few very large pores (Allmaras et al., 1977) or more depth (Ehlers, 1977).

It has been conferred that tillage may expose previously protected organic matter that may serve as a substrate for microbial growth (Rovira and Greacen, 1957). Soil quality is strongly influenced by microbe-mediated processes (Powlson et al., 2001) and microbial-based indicators of soil quality such as microbial biomass are believed to be more dynamic than those based on physical and chemical properties (Karlen et al., 1994). Therefore, such indicators have the potential to serve as early signals of soil degradation or soil improvement (Salinas-Garcia et al., 2002).

Traditionally, the analysis of soil microbial communities has relied on culturing techniques using a variety of culture media maximizing recovery of diverse microbial populations. However, only a small fraction (<0.1%) of the soil microbial community has been accessible with this approach (Atlas and Bartha, 1998). Widmer et al. (2001) stated that standard microbiological culture techniques, leave over 90% of the microorganisms in the environment unaccounted for. Prosser (2002) reported that less than 5% of soil microorganisms are cultivatable. On the other hand, the analysis of PLFA is a useful assay for soil microbial community because (i) the concentration of total PLFA serves 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 detected changes in community composition (White et al., 1996).

Molecular techniques, now available, allow the identification of microbial groups without extensive plating. One of these techniques is denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR)-amplified genes, which can be used to evaluate the diversity of complex microbial systems (Muyzer et al., 1993). To evaluate possible changes in the soil microbial community due to land use and management practice, we used (i) PLFA analysis with gas chromatography and mass spectrometry and (ii) denaturing gradient gel electrophoresis analysis of PCR-amplified genes coding for 16S rDNA and 18S rDNA for bacteria and fungi, respectively.

In Japan, Andisols cover 16.4% of the total land surface and are characterized by dark color, wide range of organic carbon content and low bulk density which attributed to the development of high porosity (Rahman et al., 1998). Soil moisture conservation is a critical issue in Andisols of Japan since it has high porosity. In an Andisol, the puddling system is an effective means in reducing water loss from the soil and improving soil regime for rice culture. We hypothesized that physico-chemical properties of Andisols may fluctuate due to management practices, which alter soil microbiological properties. To test the hypothesis the objectives of this study were to evaluate the influence of land use and tillage practices on the changes in physical, chemical and microbiological properties of a temperate Andisol, which had been under three different management practices for 41 years.

2. Materials and methods

2.1. Site description, climatic condition and soils

The experimental sites were established in 1965, located latitude of 39°42′N and longitude 141°10′E and mean elevation of 135 m above sea level (Iwate University Agricultural Field, Japan). The annual average temperature, precipitation and humidity of the study area were 14.7 °C, 105.2 cm and 74.2%, respectively, for last 41 years. The pumice Andisol (Melanudands) at all sites was formed from the same loess of volcanic parent material deposited here over the last 2000.

2.2. Treatment and management practice

The three sites included in this study were (i) apple orchard with NT, (ii) a dryland soil cropped to wheat and soybean with CT and (iii) a paddy soil in which rice was grown with puddling (PD). Few 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 in NT-apple orchard as interplants.

2.3. Soil sampling

Soils were sampled in April 2006 (before plowing) from surface horizon at a depth of 0–10 cm as well as subsurface horizon at a depth of 10–20 cm of loamy sand pumice Andisol (Melanudands). The soils were evaluated from surface horizon (0–10 cm) for physico-chemical properties only because this is where the maximum difference in soil properties occurs among treatments (Rahman et al., 2003). Ten samples were collected from each plot from each sampling time using 5 cm diameter × 5 cm length cores. Disturbed soil samples were also collected from same place. Each soil sample was placed in a plastic bag in the field and kept cool until processed in the laboratory within 1 h. Disturbed soil from each place was sieved (<4 mm) in the laboratory. After removing visible plant litter, rocks and roots, a proportion of each soil was immediately frozen (<20 °C) for microbial analysis, and the rest were stored at 4 °C for later analysis of physical, chemical and hydrological properties.

2.4. Physical analyses

Bulk density (ρb) and particle density of soils were determined by core method (Blake and Hartge, 1986a) and pycnometer method (Blake and Hartge, 1986b), respectively. Total porosity (St) of the soil was also calculated. Soil penetration resistance (PR) was determined at each soil surface by using a cone penetrometer (Soil Hardness Tester, Yaman Fujiwara Seisakusyo Ltd., Japan) at field moisture condition. The three-phase distributions (solid phase: SP; liquid phase: LP; gaseous: GP) were calculated by volumetric method on field soils collected using 5 cm diameter × 5 cm length diameter core samples (Kezdi, 1974). Sand, silt and clay contents of samples were measured as per the method of Kalra and Maynard (1994a). Regardless of treatments, soils had similar particle size distribution with loamy sand textural class (USDA soil classification system). Soil moisture content was measured after drying at 105 °C overnight (Gardner, 1986). Saturated hydraulic conductivity (K_{sat}) was measured in the laboratory using Darcy's law by constant head method (Klute and Dirksen, 1986).

2.5. Chemical analyses

The pH of soils was determined in a 1:2.5 soil to water suspension (Jackson, 1973) by a digital pH meter (Horiba pH meter, model D-52, Japan) and electrical conductivity (EC) of soil was measured (Kalra and Maynard, 1994b) by digital conductivity meter (Iuchi TDS/Conductivity meter, CyberScan 100, Singapore). The total carbon (C) and nitrogen (N) contents of soils were determined using a Sumigraph-90A automatic analyzer (Sumitomo Chemical Co. Ltd., Osaka, Japan). Since there was no indication of the presence of carbonates, total C was equivalent to organic C. Exchangeable cations, viz. sodium (X-Na), potassium (X-K), calcium (X-Ca) and magnesium (X-Mg) were extracted using 1 M-ammonium acetate (Thomas, 1982) and the extracts were analysed by atomic absorption spectroscopy (Z8200 Hitachi, Tokyo, Japan). The soil pH, EC, N and exchangeable cations were measured from field-moist soil. Air-dried soil samples were digested with HNO_3–$HClO_4$ (2:1) and iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn) were determined by atomic absorption spectroscopy (Z8200 Hitachi, Tokyo, Japan) as described by Jackson (1973).

2.6. Microbial community structure

Phospholipid fatty acid (PLFA) analysis was done in three replicates of 20 samples for each site. To obtain PLFA profiles, we followed the method described by Frostegård et al. (1993). 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. Samples were cooled with water stream, methylated by adding methylation reagent (6.0 N HCl 130 ml, 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 shaking 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. One microliter was analyzed on a gas chromatograph (GC-14B, Shimadzu, Kyoto, Japan) equipped with a capillary column of 30 m with 0.25 m inner diameter (SPB-1, Supelco, Bellefonte, USA). The column temperature was held at 150 °C for 4 min and then ramped to 250 °C at 4 °C increment min^{-1}. Helium was used as carrier gas, 20 cm s^{-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 nonadecanoaic acid (19:0, Supelco, Bellefonte, USA) as an internal standard. In this study we took into account PLFA peaks with more than 0.071 μg g^{-1} soil being proportional to amounts higher than 0.5% of total PLFA (Kourtev et al., 2003). A total of 58 PLFA peaks were found and 45 of them were identified and 31–34 were considered to evaluate. For each sample, the abundance of individual fatty acid methyl-esters was calculated from the 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 of 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 (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 (Zak et al., 2003).

2.7. Bacterial and fungal diversity

DNA extraction was performed with the Ultra Clean Soil DNA kit (MoBio Laboratories, Solana Beach, USA). One-gram fresh soil was processed according to the protocol. The concentration of DNA was measured by NanoDrop ND-1000 Spectrophotometer, Asahi Technoglass, Corporation, Japan.

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 1. 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 each dNTP), 0.5 μM of each primer, and 0.3 U of 'Smart' Taq DNA Polymerase (ABgene®, UK).

Bacterial DGGE was performed with 8% polyacrylamide gels (acrylamide/bis-acrylamide, 37.5/1) containing 45–65% vertical gradient of denaturant (100% was defined as containing 7 M urea and 40% formamide). Seven percent polyacrylamide gels containing 25–55% gradient were used for fungal DGGE. Approximately 24 μl of each PCR product was loaded onto the gels. Electrophoresis was

Table 1
Properties of each primer and PCR conditions

Primer	Sequence (5′ → 3′)[a]	Product size (bp)[b]	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 cycles of 30 s 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 cycles of 30 s at 95 °C, 45 s 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 cycles of 30 s at 95 °C, 45 s at 54 °C, 1 min at 72 °C, and final extension for 7 min at 72 °C
FF390	CGA TAA CGA ACG AGA CCT		

[a] Underline means GC-clump.
[b] The size described is estimated from this experiment and/or references.

performed in 1× TAE buffer (40 mM Tris/acetate, pH 8; 1 mM EDTA) at 80 V and 60 °C for 16 h used for bacterial DNA, while 65 V and 60 °C for 13.5 h was used for the fungal DNA separations. After electrophoresis, gels were stained in 1× TAE 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 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., USA) using the unweighted pair group method using average linkages (UPGMA). We used data of three replicates of each soil layer for the cluster analysis and expressed the results as dendrograms so that differences of soil microbial communities with treatments could be more easily ascertained.

2.8. Experimental design and statistical analyses

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 ($p < 0.05$). Pearson correlation coefficients among the soil attributes and nutrients were performed. Statistical analyses were conducted by JMP 4.0 (SAS Institute, Cary, NC, USA). Unless otherwise noted, all the results were calculated using oven-dried soil basis (105 °C, 24 h).

3. Results and discussion

3.1. Physical and chemical properties

The bulk density value obtained for CT was significantly lower compared to NT and PD (Table 2). Our results are in good agreement with those of Bhattacharyya et al. (2006) who reported that bulk density of soil under CT was lower than NT in 0–75 cm depth. In contrast, our results are not in agreement with the findings of Ishaq et al. (2002) and Puget and Lal (2005). This discrepancy could be due to the differences in crop species, soil properties, climatic characteristics and their complex interactions (Rasmussen, 1999).

Total porosity was significantly greater in CT compared to PD (Table 2). Gupta and Kathavate (1972) reported that the increase in dry bulk density and the decrease in porosity were attributable to puddling, respectively. Gantzer and Blake (1978) also reported the increases in bulk density and lower porosity values in the surface soil of no-tillage compared to tilled treatments. Soil hardness under PD (7.45 kg cm^{-2}) was significantly greater than under NT (5.25 kg cm^{-2}) and CT (1.88 kg cm^{-2}) (Table 4). Rahman et al. (2003) observed that soils under PD treatment have significantly higher soil hardness than soils under NT and CT. They also observed the significant variations in soil hardness at different matric suction. Tillage treatments and associated cropping systems caused variation in pH values of soils and significant difference was found between NT, CT and PD treatments (Table 2). The lowest value for pH was obtained with NT treatment. Similar effects in changes of pH under no-tillage treatment were also reported by Thomas (1975).

Table 2
Physico-chemical properties of Andisol (0–10 cm)

Properties	NT[a]	CT[b]	PD[c]	LSD[d]
Bulk density, ρb (Mg m^{-3})	0.82	0.65	0.91	0.10
Porosity (%)	70.01	72.57	68.41	1.58
Penetration resistance (kg cm^{-2})	5.25	1.88	7.45	2.12
Solid phase (%)	29.08	25.05	31.10	2.32
Liquid phase (%)	64.51	56.54	66.80	4.06
Gaseous phase (%)	6.41	18.41	2.10	6.37
K_{sat} (×10^{-4} mm s^{-1})	21.1	118.9	6.9	46.2
pH	5.16	5.70	6.01	0.32
Organic matter (OM) (g kg^{-1})	66.61	37.43	64.12	12.20
EC (dS m^{-1})	15.76	12.88	5.03	4.19
N (g kg^{-1})	3.52	1.74	2.75	0.67
C/N	10.86	12.33	13.36	0.95
X-Na (cmol$_c$ kg^{-1})	4.40	7.05	23.45	7.78
X-K (cmol$_c$ kg^{-1})	0.67	0.57	0.45	0.09
X-Ca (cmol$_c$ kg^{-1})	14.80	6.74	6.15	3.64
X-Mg (cmol$_c$ kg^{-1})	2.53	2.38	1.03	0.62
Total X-cations (TXC) (cmol$_c$ kg^{-1})	22.39	16.74	31.07	8.19
Fe (μg g^{-1})	186.35	155.10	260.32	40.74
Cu (μg g^{-1})	18.61	8.42	15.35	3.92
Mn (μg g^{-1})	90.55	74.61	198.43	50.79
Zn (μg g^{-1})	4.13	7.91	1.75	2.34

[a] NT, no-tillage with continuous apple.
[b] CT, conventional tillage with wheat-soybean rotation.
[c] PD, puddling with continuous rice.
[d] LSD (least significant difference) at $p < 0.05$ between treatments (columns).

Significantly lower values for organic matter were obtained with CT treatment in comparison to NT and PD (Table 2). Tillage treatment enhanced the decomposition of organic matter and thereby reduced its content. The highest amount of organic matter was observed under NT. Franzluebbers et al. (1999) observed that the concentration of organic matter was higher in the surface soil under no-tillage systems. Less disruption resulted in greater accumulation of surface residue carbon. Any minor mixing of residue with soil would allow residue to remain wetter and, therefore, provide ideal conditions for microbial decomposition that leads to loss of surface residue. The roots of fodder crops and the slower rate of organic matter decomposition might have contributed to the build up of organic matter in soil with zero tillage treatment. Lal (1976) found more soil organic matter under no-tillage compared to plowed condition. Blevins et al. (1983) reported that organic carbon was approximately twice as high with no-tillage as with conventional tillage. Organic matter accumulates in the upper few centimeters under NT compared with CT soil (Rhoton, 2000) causing increases in the concentration of electrolytes and reductions of soil pH. These effects may offset the benefits of soil organic matter and P accumulation on Al toxicity in NT soils (Erani et al., 2002). Heenan et al. (1995) also found greater amount of organic matter under NT than CT. The content of organic matter was lower under PD condition compared to NT treatment, but this difference was not significant. The results with Andisol are in agreement with the findings of Lal (1986), who reported that puddling reduced the organic matter content in surface layer. As organic matter content of the studied soils is more than 2.6%, soils could be classified as suitable land for rainfed rice (Sys, 1985).

The highest value of EC was observed in NT treatment (Table 2) and the EC of the studied soils associated with pH. The present findings are in good agreement with Gupta and Gupta (1987). On the basis of EC, it has been reported that salinity effects are mostly negligible if EC is below 2 dS m^{-1} and that yields of many crops are restricted at EC of 4 through 8 dS m^{-1} while only tolerant crops may yield satisfactorily at EC between 8 and 16 dS m^{-1}. Above 16 dS m^{-1} only a few very tolerant crops yield satisfactorily (US Salinity Laboratory Staff, 1954). Because of the high salt content (EC), this soil has limited suitability for rice production.

The effects of long-term tillage practices on total N were similar to organic matter (Table 2). The reduced total N loss under NT compared to CT was also observed by Dalal (1992) in a Vertisol.

Significantly highest X-Na content was found in PD and graded as PD > CT > NT (Table 2). Significantly highest values for the exchangeable-K, -Ca and -Mg were observed in NT than in CT and PD. Higher X-K under NT than under CT was observed by Loch and Coughlan (1984). Ishaq et al. (2002) reported that tillage methods did not have any effect on concentration of N and K. These findings indicated that management inputs such as fertilizers, herbicides and irrigation affect the distinctive physicochemical properties especially in this Andisol soil.

Variations were found in concentrations of all measured micronutrient (Fe, Cu, Mn and Zn) (Table 2). Under CT, Cu and Zn contents showed significantly lowest and highest, respectively. The highest values of Fe and Mn were obtained under puddling condition. It is generally accepted that puddling caused dispersion of soil aggregates, which reduced water percolation by blocking the pore spaces and thereby created anoxic environment where the availability of iron and manganese increased due to reducing conditions in the soil. Ponnamperuma (1972) reported that puddling and submergence markedly reduced redox potentials as compared with unpuddled and unsaturated soil, thus increased the availability of nutrients, especially Mn.

3.2. Three-phase composition and hydraulic conductivity

A higher air content was observed under CT than under NT and PD while the highest values of solid and liquid at pF 2.0 were found with PD treatment (Table 2). Rahman et al. (2003) found the highest values of solid and liquid phases and the lowest value in gaseous phase with puddled treatment. Conventional tillage treatment produced the highest value in gaseous phase and the lowest value of both solid and liquid phases. The greater porosity in the conventionally tilled soil should allow it to easily drain, giving it the lowest liquid phase value of the three tillage treatments. The result is also consistent with the findings of Sharma and De Datta (1985) though they had worked with the different soils.

The highest value of saturated hydraulic conductivity was obtained with conventional tillage treatment (Table 2). Saturated hydraulic conductivity is highly dependent upon the size, continuity and arrangement of pores. Higher saturated hydraulic conductivity in CT soils was an indication of better pore continuity, as the proportion of large pores was comparatively less. Heard et al. (1988) found reduced saturated hydraulic conductivity under NT. However, Bhattacharyya et al. (2006) observed greater saturated hydraulic conductivity under ZT than those under CT. They also observed that concentration of N and K did not affect by tillage methods. Kribaa et al. (2001) found that tillage increased soil hydraulic conductivity at low potential (0.06 and 0.3 kPa). Sharratt et al. (2006) stated that no tillage resulted in greater saturated hydraulic conductivity than other tillage treatments.

3.3. Microbial community

The data in Tables 3 and 4 show the effects of tillage on soil microbial community structure and composition, respectively. Our results provided evidence that the several PLFAs, viz. i14:0, 14:0, i15:0, 2-OH 14:0, i16:0, 16:1ω7t, 16:0, i17:1ω5, a17:0, i18:0, 18:2ω6, 18:1ω7c, 18:1ω9t, 18:1ω5c, 20:4 and 20:1ω9c were highest in NT; a14:0, a15:0, 3-OH 14:0, 16:1ω9c, 16:1ω7c, 16:1ω5c, 10Me16:0, 18:1:ω9, 18:0, 10Me 18:0 and 20:0 were higher under CT and 2-OH 16:0 and cy19:0 were highest in PD, regardless of soil depths.

Total PLFA, and PLFA for bacteria, aerobes, cyanobacteria and fungi were significantly higher in NT than CT (Table 4) irrespective of soil depths. Relative abundance of fungi biomarker increased in NT in response to lower C:N ratio (Table 2). This finding is in agreement with that of Yao et al. (2006) who studied microbial community with land use and turfgrass management.

Regardless of soil depth, PLFA for methane-oxidizing bacteria, mycorrhizae and actinomycetes were highest in CT while anaerobes were highest in PD (Table 4). Methane-oxidizing bacteria are a phylogenetically diverse group defined by the ability to use methane as a sole carbon and energy source. Methane-oxidizing microbial communities are very important because of their importance for global methane cycling. Methane is considered as the most potential greenhouse gas after carbon dioxide (IPCC, 2001). Because the 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, viz. rice paddies are regarded as the most important source of atmospheric methane while non-flooded upland soils viz. grasslands are regarded to be the only biological

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

Phospholipid fatty acids	NT[a]		CT[b]		PD[c]		LSD[d]
	0–10 cm	10–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm	
i14:0	1.68	1.36	1.78	0.67	0.73	0.00	0.11
a14:0	2.36	1.60	2.71	1.56	1.91	0.38	0.28
14:0	1.69	0.47	0.67	0.54	0.62	1.22	0.32
i15:0	1.02	0.91	0.32	1.14	0.74	0.00	0.19
a15:0	1.17	0.64	1.26	0.76	1.05	0.00	0.17
15:0	0.72	0.54	0.65	0.68	0.70	0.00	0.04
2-OH 14:0	1.20	0.65	0.29	0.38	0.60	0.00	0.23
3-OH 14:0	0.61	0.65	0.70	1.99	1.85	0.00	0.02
i16:0	8.46	7.32	1.95	4.46	1.44	0.00	1.72
16:1ω9c	4.38	3.21	8.52	1.44	8.31	0.31	1.38
16:1ω7t	14.13	10.94	6.67	7.44	0.83	2.99	1.85
16:1ω7c	0.90	0.61	9.91	0.67	1.20	0.32	2.62
16:1ω5c	0.36	0.38	0.70	2.02	0.32	0.24	0.09
16:0	3.03	2.38	0.35	2.13	0.33	0.00	0.69
i17:1ω5	0.57	3.49	2.09	1.42	1.20	0.49	0.72
10Me16:0	2.20	2.15	2.59	2.93	1.18	0.50	0.45
i17:0	1.70	1.41	1.33	1.62	0.36	0.32	0.10
a17:0	2.61	2.13	1.97	0.40	0.57	0.00	0.16
cy17:0	0.46	0.45	0.45	0.84	0.66	0.25	0.00
17:0	0.87	1.07	0.52	0.92	0.54	0.70	0.14
2-OH 16:0	1.01	0.41	0.79	0.92	10.71	0.00	0.15
i18:0	0.45	5.05	0.47	4.19	1.40	3.14	1.31
18:2ω6	12.90	4.72	2.16	1.21	0.51	0.63	2.78
18:1ω9	3.29	0.00	8.01	2.16	2.40	0.99	1.99
18:1ω7c	5.94	0.74	0.00	0.31	0.61	0.00	1.60
18:1ω9t	1.04	2.62	0.00	2.21	0.00	0.00	0.65
18:1ω5c	1.55	0.00	0.00	0.25	0.00	0.00	0.44
18:0	0.62	0.00	2.59	0.72	0.66	0.25	0.67
10Me18:0	1.62	1.14	0.58	2.62	0.71	0.34	0.26
cy19:0	0.47	1.34	0.68	0.00	2.46	0.00	0.22
20:4	1.26	0.88	0.69	0.62	0.00	1.43	0.14
20:2	0.66	1.84	0.37	1.99	0.29	0.00	0.39
20:1ω9c	1.11	0.91	0.39	0.34	0.52	0.30	0.18
20:0	0.65	0.35	1.96	0.84	0.72	0.42	0.42

[a] NT, no-tillage with continuous apple.
[b] CT, conventional tillage with wheat-soybean rotation.
[c] PD, puddling with continuous rice.
[d] LSD (least significant difference) at $p < 0.05$ between treatments (columns).

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) observed that under dry condition, Andisol soils had higher numbers of methane-oxidizing bacteria although those soils have been puddled for many years. In this study, it was observed that an Andisol with puddling contained substantially fewer methane-oxidizing bacteria than conventional tillage during submerged condition.

Table 4
Microbial community (nmol g^{-1} soil) of Japanese Andisol

Microbial community	NT[a]		CT[b]		PD[c]		LSD[d]
	0–10 cm	10–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm	
Total PLFAs	82.68	62.34	64.11	52.41	46.13	15.25	5.85
Total bacteria	11.11	9.07	6.39	7.66	4.28	1.02	1.17
Gram-positive bacteria	17.16	14.55	9.42	11.32	5.34	0.82	2.50
Gram-negative bacteria	27.83	17.29	26.23	10.95	14.06	3.88	2.81
Aerobes	14.13	10.94	6.67	7.44	0.83	2.99	1.85
Anaerobes	0.94	1.79	1.13	0.84	3.12	0.25	0.22
Sulfate-reducing bacteria	2.20	2.15	2.59	2.93	1.18	0.50	0.45
Methane-oxidizing bacteria	0.36	0.38	0.70	2.02	0.32	0.24	0.09
Cyanobacteria	12.90	4.72	2.16	1.21	0.51	0.63	2.78
Fungi	16.19	4.72	10.17	3.37	2.91	1.62	2.84
Fungi/bacteria	1.46	0.52	1.59	0.44	0.68	1.59	0.29
Mycorrhizae	0.36	0.38	0.70	2.02	0.32	0.24	0.09
Actinomycetes	1.62	1.14	0.58	2.62	0.71	0.34	0.26

[a] NT, no-tillage with continuous apple.
[b] CT, conventional tillage with wheat-soybean rotation.
[c] PD, puddling with continuous rice.
[d] LSD (least significant difference) at $p < 0.05$ between treatments (columns).

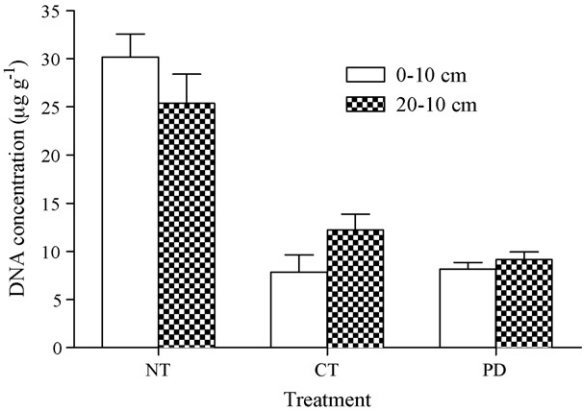

Fig. 1. Concentration of DNA reproducible PCR amplification of temperate Andisol (bars showed the standard errors). NT, no-tillage with continuous apple; CT, conventional tillage with wheat–soybean rotation; PD, puddling with continuous rice.

Sulfate-reducing bacteria were significantly higher in CT than PD (Table 4) regardless of soil depth. The relationship of sulfate-reducing bacteria to oxygen has been of particular interest since the publication of earlier reports of exceptionally high rates of sulfate reduction in the oxygenated surface regions of some microbial mats (Fründ and Cohen, 1992).

The DNA extracts from this Andisol were colorless indicating absence of humic acid contamination (Peixoton et al., 2005). The DNA isolated from this soil was of sufficient purity for reproducible PCR amplification content. The DNA content was higher in surface soil than subsurface soil, regardless of treatments (Fig. 1). Significantly higher concentrations of DNA were extracted from NT than from CT and PD soil, regardless of soil depth. In NT treatment higher DNA content was recorded in surface soil, however, the reverse was true in CT and PD treatments.

The PCR–DGGE of bacterial and fungal communities showed many bands that were unique to each soil treatment (Figs. 2A and 3A). In surface soil, highest bacterial diversity evaluated by DNA band number in PCR–DGGE analysis was observed in PD while under CT subsurface soil showed more bands than PD and NT (Fig. 2A). Surface soil under PD also showed highest fungal diversity evaluated by DNA band number in DGGE analysis (Fig. 3A). In this study a disparate relationship was observed between bacterial abundance and diversity measured by number of PLFA peaks and DNA bands, respectively (Table 6 and Figs. 2A–3A). Rahman et al. (2007) observed an inconsistent relationship between bacterial abundance and diversity measured by number of PLFA peaks and DNA bands, respectively. Nevertheless they observed a consistent relationship between fungal abundance and diversity evaluated by the number of PLFA peaks and DNA bands. In this study, we found no significant relationship between fungal abundance and diversity evaluated by the number of PLFA peaks and DNA bands.

The data for PCR–DGGE of bacterial and fungal communities were subjected to cluster analysis and the results were compared. In this context, the dendrogram was generated by UPGMA and percentage of similarity among the lanes was calculated taking

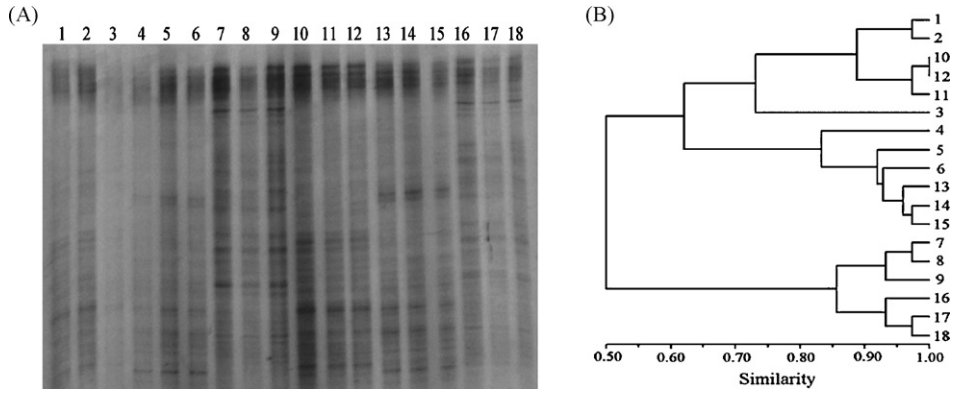

Fig. 2. DGGE fingerprint (A) and dendrogram analysis (B) of bacterial community (1–3 = NT: 0–10 cm; 4–6 = CT: 0–10 cm; 7–9 = PD: 0–10 cm; 10–12 = NT: 10–20 cm; 13–15 = CT: 10–20 cm; 16–18 = PD: 10–20 cm). NT, no-tillage with continuous apple; CT, conventional tillage with wheat–soybean rotation; PD, puddling with continuous rice.

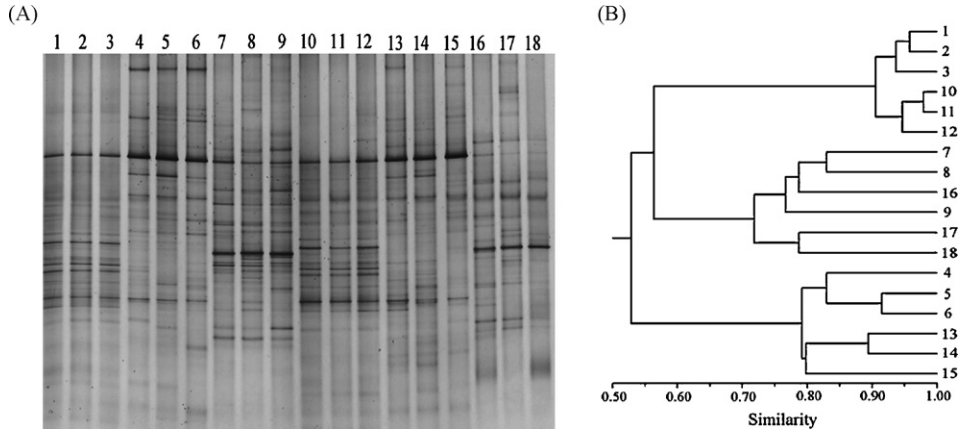

Fig. 3. DGGE fingerprint (A) and dendrogram analysis (B) of fungal community (1–3 = NT: 0–10 cm; 4–6 = CT: 0–10 cm; 7–9 = PD: 0–10 cm; 10–12 = NT: 10–20 cm; 13–15 = CT: 10–20 cm; 16–18 = PD: 10–20 cm). NT, no-tillage with continuous apple; CT, conventional tillage with wheat–soybean rotation; PD, puddling with continuous rice.

Table 5
Correlation among measured soil attributes of Andisol (0–10 cm)

	St	PR	pH	OM	EC	N	C/N	X-Na	X-K	X-Ca	X-Mg	TXC	Fe	Cu	Mn	Zn	SP	LP	GP	K_{sat}
ρb	−0.999	0.999	0.192	0.911	−0.572	0.701	0.244	0.676	−0.390	0.115	−0.704	0.965	0.919	0.786	0.836	−0.999	1.000	0.991	−0.996	−0.973
St		−1.000	−0.234	−0.892	0.607	−0.670	−0.286	−0.708	0.429	−0.071	0.734	−0.948	−0.935	−0.759	−0.859	1.000	−0.998	−0.984	0.991	0.963
PR			0.245	0.887	−0.616	0.661	0.297	0.716	−0.440	0.060	−0.742	0.976	0.939	0.751	0.865	−1.000	0.998	0.982	−0.990	−0.959
pH				−0.230	−0.915	−0.565	0.999	0.853	−0.979	−0.953	−0.832	0.487	0.564	−0.456	0.699	−0.236	0.178	0.059	−0.103	0.038
OM					−0.183	0.933	−0.177	0.313	0.025	0.514	−0.348	0.735	0.674	0.971	0.536	−0.892	0.917	0.958	−0.945	−0.981
EC						0.184	−0.935	−0.991	0.978	0.749	0.985	−0.719	−0.850	0.057	−0.928	0.609	−0.561	−0.458	0.496	0.369
N							−0.520	−0.051	0.383	0.789	0.013	0.456	0.363	0.992	0.195	−0.668	0.711	0.790	−0.762	−0.846
C/N								0.879	−0.988	−0.936	−0.861	0.517	0.607	−0.408	0.736	−0.288	0.231	0.113	−0.156	−0.015
X-Na									−0.942	−0.654	−0.999	0.876	0.912	0.076	0.970	−0.709	0.666	0.572	−0.607	−0.490
X-K										0.870	0.929	−0.653	−0.722	0.263	−0.831	0.431	−0.377	−0.264	0.305	0.168
X-Ca											0.625	−0.178	−0.287	0.704	−0.449	−0.069	0.128	0.246	−0.203	−0.339
X-Mg												−0.888	−0.927	−0.114	−0.978	0.735	−0.694	−0.603	0.637	0.522
TXC													0.995	0.574	0.955	−0.966	0.953	0.921	−0.933	1.000
Fe														0.478	0.985	−0.936	0.913	0.858	−0.880	−0.804
Cu															0.318	−0.757	0.795	0.861	−0.838	−0.907
Mn																−0.860	0.828	0.756	−0.783	−0.688
Zn																	−0.998	−0.984	0.991	0.962
SP																		0.993	−0.997	−0.977
LP																			−0.999	−0.995
GP																				0.990

into account the band migration distance and the relative intensity of all bands (Figs. 2B and 3B). Cluster analysis of the data from PCR–DGGE methods grouped the replicates of the different treatments. For bacterial community, two clusters were observed, one made up of NT and CT samples and the other from PD. The cluster analysis revealed closer association of the characteristics of NT and CT with a relative distance value of 0.62. For fungal community, two clusters were observed, one made up of NT and PD samples and the other from CT. The cluster analysis revealed closer association of the characteristics of NT and PD with a relative distance value of 0.56. Our results for fungal diversity were consistent with those of Rahman et al. (2007), who studied the changes of microbial communities in response to tillage.

3.4. Correlations among the soil attributes

Correlation analysis among the 21 soil attributes (physical and chemical) was conducted (Table 5). Out of 210 soil attribute pairs, 110 were positively correlated. Highest positive correlations were obtained for bulk density versus solid phase and porosity versus Zn. On the other hand, highest negative correlations were obtained for porosity versus penetration resistance and penetration resistance versus Zn. Turpin et al. (2007) observed negative correlations between hydraulic conductivity and soil water content. In this study pH showed negative correlations with total N, OM and EC which are in close conformity with the results of Shukla et al. (2006) and Thomas et al. (2006).

Correlation between physico-chemical properties and biological attributes was depicted in Table 6. Bulk density and penetration resistance showed significantly negative correlation while porosity and saturated hydraulic conductivity showed significantly positive correlations with methane-oxidizing bacteria and mycorrhizae. Electrical conductivity, X-K and X-Ca showed positive correlations with all the biological attributes except anaerobes. On the other hand, C/N ratio, Na, Fe and Mn showed negative correlations with all the biological attributes except anaerobes.

Table 6
Correlation between physico-chemical and biological properties of Andisol (0–10 cm)

	Total PLFAs	Total bacteria	Gram-positive bacteria	Gram-negative bacteria	Aerobes	Anaerobes	Sulfate-reducing bacteria	Methane-oxidizing bacteria	Cyanobacteria	Fungi	Fungi/bacteria	Mycorrhizae	Actinomycetes	DNA concentration
ρb	−0.272	−0.130	0.010	−0.693	−0.274	0.712	0.520	−0.969	0.053	−0.391	−0.844	−0.969	0.287	0.186
St	0.313	0.173	0.033	0.723	0.316	−0.742	−0.482	0.957	−0.010	0.431	0.866	0.957	−0.245	−0.143
PR	−0.325	−0.185	−0.045	−0.731	−0.327	0.750	0.472	−0.953	−0.002	−0.441	−0.872	−0.953	0.234	0.132
pH	−0.259	−0.117	0.024	−0.683	−0.261	0.703	0.531	−0.972	0.067	−0.378	−0.836	−0.972	0.300	0.200
OM	−0.141	0.003	0.143	−0.590	−0.144	0.612	0.629	−0.993	0.185	−0.265	−0.765	−0.993	0.412	0.315
EC	0.184	0.041	−0.100	0.625	0.187	−0.646	−0.594	0.987	−0.142	0.307	0.792	0.987	−0.372	−0.274
N	0.044	−0.100	−0.239	0.509	0.047	−0.533	−0.702	1.000	−0.280	0.170	0.698	1.000	−0.499	−0.406
C/N	−0.997	−0.998	−0.980	−0.841	−0.996	0.825	−0.739	0.059	−0.970	−0.978	−0.689	0.059	−0.885	−0.929
X-Na	0.149	0.290	0.422	−0.334	0.147	0.360	0.826	−0.985	0.460	0.023	−0.547	−0.985	0.656	0.575
X-K	0.945	0.888	0.814	0.988	0.946	−0.983	0.404	0.350	0.789	0.979	0.923	0.350	0.622	0.699
X-Ca	0.495	0.616	0.720	0.029	0.494	−0.001	0.974	−0.857	0.749	0.382	−0.209	−0.857	0.884	0.831
X-Mg	−1.000	−0.993	−0.967	−0.869	−1.000	0.855	−0.702	0.005	−0.956	−0.988	−0.727	0.005	−0.859	−0.907
TXC	−0.594	−0.441	−0.455	−0.878	−0.549	0.888	−0.993	−0.849	−0.214	−0.664	−0.965	−0.885	−0.010	−0.890
Fe	−0.893	−0.819	−0.730	−1.000	−0.894	0.999	−0.278	−0.471	−0.700	−0.942	−0.966	−0.471	−0.512	−0.598
Cu	0.992	0.964	0.917	0.934	0.993	−0.924	0.584	0.148	0.899	1.000	0.823	0.148	0.771	0.832
Mn	0.925	0.970	0.995	0.637	0.924	−0.616	0.908	−0.358	0.998	0.870	0.437	−0.358	0.985	0.997
Zn	0.875	0.796	0.703	1.000	0.876	−1.000	0.242	0.504	0.672	0.929	0.975	0.504	0.479	0.567
SP	−0.630	−0.511	−0.386	−0.921	−0.632	0.932	0.140	−0.791	−0.346	−0.723	−0.987	−0.791	−0.115	−0.217
LP	0.381	0.511	0.626	−0.098	0.379	0.126	0.937	−0.915	0.659	0.262	−0.331	−0.915	0.818	0.754
GP	−0.755	−0.653	−0.540	−0.975	−0.757	0.981	−0.034	−0.673	−0.504	−0.832	−1.000	−0.673	−0.286	−0.384
K_{sat}	0.315	0.175	0.035	0.725	0.317	−0.743	−0.480	0.956	−0.008	0.432	0.867	0.956	−0.243	−0.141

4. Conclusions

In this study the effects of more than 40 years of continuous orchard under zero tillage, rice cultivation under puddling, and grain crops under conventional tillage on the properties of an Andisol were evaluated. Soil management practices significantly changed the basic soil properties. We conclude that the effect of puddling, compared to continuous cropping or no-till orchard, was more pronounced with changes in bulk density, soil pH and on the concentrations of acid extractable iron and manganese. Significant differences were also noted among the PLFA and DNA composition characterizing the biological properties of the three differently managed Andisol soils used in this study.

Three inferences can be drawn from this study. First, the microbial abundance could be high although the diversity might be low. Secondly, the PLFA and PCR–DGGE characterization of the microbial community in mineral soils may be significantly different than that describing the microbial community in organic soils. Thirdly, when characterizing many soils, it may be extremely important to include the results of the biochemical (PLFA profiles) and molecular (DNA: PCR–DGGE) fingerprinting methods. In future studies, we intend to link the two methods in assessing microbial communities of different organic Andisols.

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