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Organic amendments as ecosystem engineers: Microbial, biochemical and genomic evidence of soil health improvement in a tropical arid zone field site

K. Aparna [a], M.A. Pasha [b], D.L.N. Rao [a,*], P.U. Krishnaraj [b]

[a] AINP on Soil Biodiversity-Biofertilizers, Indian Institute of Soil Science, Bhopal, Madhya Pradesh, India
[b] Department of Agricultural Biotechnology, University of Agricultural Sciences, Dharwad, Karnataka, India

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ABSTRACT

The ability of organic biodynamic fertilizers to improve soil quality was evaluated in arid loamy sand soils in farmers' fields in Rajasthan, India in cowpea cropping and citrus orchards. Water holding capacity, organic carbon and ammoniacal nitrogen improved significantly in organic farming. Microbial community was evaluated using both a culture dependent and independent approach. Actinomycetes increased significantly in organic cropping and orchard by 92 and 100%, respectively, compared to conventional management. Bacterial populations increased significantly on nutritionally diverse media in organic farm soils over conventional, both copiotrophs (+52–119%) as well as oligotrophs (+25–79%). The arbuscular mycorrhizal protein, glomalin increased by 56–82% in organic farms. Nitrogen fixers, ammonifiers, nitrifiers and sulfur oxidizers did not show significant differences. There was a consistent increase in soil enzymatic activities in organic farms—acid phosphatase (1.5× in cropping; 3× in orchard), flourescein diacetate hydrolysis (1.8×; 3.3×), dehydrogenase (2.4×; 3.5×) and β-glucosidase (2.2×; 6.3×). Quantification of 16S rDNA abundances in soil using qPCR showed a clear 1.8 fold increase in both organic cropping and organic orchard soils. The abundance of *amoA* gene decreased by 22 and 11 folds in organic cropping and orchards. The culture independent analysis of eubacterial 16S rRNA gene showed that organically cropped farms and orchards had more diverse bacterial community compared to the conventional. The distribution of bacterial species observed in organic cropping is more even. Representation of *Proteobacteria* among the eubacterial species was 20% lesser in organic as compared to conventional cropping whereas *Actinobacteria* were higher by 10% in organic cropping. Overall, the results demonstrated unequivocally that organic amendments improved the biological quality through an alteration of the microbial community structure and function. We conclude that organic manures may thus be appropriately included in the group of 'Ecosystem Engineers' that selectively modify the environment and make soil ecosystems more sustainable.

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1. Introduction

Efficient plant growth is known to be affected by the inherent fertility properties of the soil which in turn depends on the type of the soil and its microbial community structure. Land use practices and intensification of agriculture exert varying influences on soil microbial diversity and may irreversibly affect certain microbial populations that are crucial for nutrient cycling in soil. The loamy sand soils of the tropical hot deserts (Torripsamments, a suborder of Entisols) have poor ability to support plant growth due to adverse physical, chemical and biological characteristics. The extreme climates where they occur, the poor water holding capacity of the sandy soils and excessive water losses due to evapo-transpiration makes farming a difficult proposition. But the increasing demand for food is creating a compelling need to bring them under intensive farming. Most of these regions are dependent upon rainfall and these soils of the rain-fed regions are beset with one or several forms of degradation due to low cropping intensity, low organic matter status, poor soil physical health, biological degradation, low fertility, etc.

* Corresponding author. Tel.: +91 755 2736846; fax: +91 755 2733310.
E-mail addresses: aparna.micro@gmail.com (K. Aparna), malikiabt@gmail.com (M.A. Pasha), dlnrao@iiss.res.in, desiraju.rao@gmail.com (D.L.N. Rao), krishnarajpu@gmail.com (P.U. Krishnaraj).

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The effects of physical and chemical degradation of soils are obvious. However, the effects of biological degradation which is caused due to loss of specific soil organic matter fractions and consequent loss of microbial species/communities dependent on them for nutrition are insidious. The conventional chemical practices do not answer the problems of such soils as the chemical practices are shown to affect the microbial community structure of soils negatively in the long run (Fließbach et al., 2007; Lazcano et al., 2013). Many technologies have been proposed to improve the texture of such soils like use of clay amendments (Ismail and Ozawa, 2007) or soil conditioners which are economically prohibitive in developing countries. Use of farm produce like cow dung, cow urine, etc is an economically feasible and effective practice.

Ecological engineering has been defined as “The design of sustainable ecosystems that integrate human society with its natural environment for the benefit of both” (Mitsch and Jorgensen, 2003). The problematic soil ecosystems may be engineered to improve their productivity in a sustainable manner through organic amendments which improve the soil health as they increase soil organic matter; provide plant nutrients like nitrogen, phosphates, calcium, magnesium, etc., as well as improve the soil structure (Calbrix et al., 2007; Rao, 2006). Organic farming engineers the microbial community structure by affecting the abundances of particular microbial groups (Chaudhry et al., 2012) and exerts beneficial effect on soil health (Fließbach et al., 2007).

Among the recent works reported (Wu et al., 2013), the focus was either on the qualitative assessment of the influence of organics or only a few parameters were assessed quantitatively. In the current study, 23 parameters of soil health were assessed quantitatively to judge the impact of organic farming on a low fertility tropical loamy sand soil at farmer field site in North-West India. Two different farming systems in adjacent farms, conventional and organic, in arable cropping as well as orchards were assessed to quantify the effect on physical and chemical properties, and microbial community structure and function by estimating the populations of the major microbial groups, gene pools (16S rDNA, *nifH* and *amoA*) and their products like proteins and enzymes.

2. Materials and methods

2.1. Site and soil samples

Soil samples (0–15 cm) at a loamy sand site (Torripsamments, 66.5, 16.5 and 17% of sand, silt and clay, respectively) were collected from farmers' fields under cowpea cropping or citrus orchard in Village Dhaban, Taluka Sangaria, Dt. Hanumangarh, Rajasthan. One site was organically farmed and the other was conventionally farmed with agro-chemicals. The nutrient requirements for the organic farm are met from biodynamic preparations made by fermenting cow dung, cow urine, chickpea flour, and 'Jaggery' and small amount of soil taken from beneath a 'peepal' (*Ficus religiosa*) or 'banyan' (*Ficus bengalensis*) tree. This ferment was then mixed with 200 l of water and applied through drip/trench/foliar application in one acre area. For protecting crops from insect and disease damage, a concoction is prepared from the leaves of about 11 medicinal trees in cow urine by fermentation and sprayed on fruit plants and field crops. Mulching of weed biomass was also done. The requirements of conventional farms were met only through chemical fertilizers and pesticides. The fresh soil samples collected were immediately transported to laboratory and transferred to separate sterile screw-capped plastic tubes and stored at 4 °C for microbial and enzyme analysis and soil DNA extraction.

2.2. Physical and chemical properties

The soil samples were air-dried and analyzed for pH and electrical conductivity in 1:2 soil–water suspensions. Water holding capacity was determined by funnel method using gravimetric method. Organic carbon by Walkley and Black wet oxidation method, available nitrogen by permanganate oxidation and distillation, available phosphorus by Olsen's method by spectrophotometry and exchangeable potassium in ammonium acetate extracts by flame photometry were done as per methods described by Hesse (1971).

2.3. Microbial community—Ecological structure and functional analysis

2.3.1. Quantification of major groups

Total bacteria were counted by plating serial soil dilutions on nutrient agar medium and fungal counts on potato dextrose agar (Atlas, 1995). Actinomycetes were counted on humic acid–vitamin agar (Hayakawa and Nonomura, 1987).

2.3.2. Glomalin

Glomalin, a heat stable protein produced by arbuscular mycorrhizal fungi and used as a quantitative index, was extracted as described by Wright and Upadhyaya (1996) and estimated by Lowry's method.

2.3.3. Quantification of bacterial nutritional groups

Based on the capability to grow on complex, simple or low nutrient media, soil organisms can be classified under different nutritional groups. Counts of bacteria on different media with varying nutritional complexity viz., yeast extract agar medium (YE), basal glucose salts agar medium (BGS), and soil extract agar (SE) medium (Basal glucose salts agar medium to which soil extract is added) (Atlas, 1995) were taken to assess the population of various nutritional groups. Dilute basal glucose salts medium N/10 (BGS/10) was used to count the oligotrophic bacterial populations.

2.3.4. Functional microbial groups

Quantification of different functional groups of microbes was done by most probable number (MPN) method. Appropriate soil dilutions were inoculated in 5 ml sterile medium in 5-replicate tubes and after appropriate incubation the numbers were calculated using the probability tables. Total associative N_2 Fixers were determined in N-free malic acid semi solid medium (Atlas, 1995). After incubation for 72 h at 30 °C, tubes in which a typical pellicle developed a few mm below the surface of the medium were scored as positive for associative nitrogen fixers. For autotrophic nitrifying populations, medium for autotrophic ammonium oxidizers was used for the first stage viz., ammonium oxidation. Medium for autotrophic nitrite oxidizers was used for detecting the microbial populations involved in second stage viz., nitrite oxidation. Development of pink color after addition of Griess-Ilosvay reagent in the first set of tubes indicated positive for nitrite production from ammonium salts and disappearance of the same indicated positive in the second set due to further oxidation of nitrite to nitrate (Schmidt and Belser, 1994). Autotrophic sulfur oxidizers were enumerated by MPN using modified Starkey's medium with pH adjusted to 5.4 (Lawrence and Germida, 1991). The elemental sulfur was sterilized separately by tyndallization and added to the autoclaved medium. 0.15% agar was added to the liquid broth

before autoclaving so as to maintain the insoluble elemental sulfur in a uniformly suspended state. Bromocresol green was used in the medium as a pH indicator. Color change to yellow was taken as positive to indicate sulfuric acid production from elemental sulfur.

2.3.5. Bacterial antagonists

Crowded plate technique as used to assess the population of bacterial antagonists. Soil suspensions of 10^{-2} dilution were plated on nutrient agar medium by pour plating and the plaques or inhibition zones produced by colonies were counted to enumerate the antibiotic producers in soils (Cappuccino and Sherman, 2007) expressed as plaque forming units (pfu) g^{-1} soil.

2.3.6. Soil enzymatic activity

Dehydrogenase activity of the soil was measured using triphenyl tetrazolium chloride as substrate by estimation of the red colored triphenyl formazan at 485 nm (Casida et al., 1964). Fluorescein diacetate hydrolysis was measured spectrophotometrically at 490 nm (Adam and Duncan, 2001). *p*-Nitrophenyl phosphate dissolved in modified universal buffer (MUB) of pH 6.5 was used as substrate to estimate acid phosphatase activity. The same dissolved in MUB of pH 11 was used to estimate alkaline phosphatase activity spectrophotometrically at 440 nm (Tabatabai, 1994). *p*-Nitrophenyl β-D-glucoside dissolved in MUB of pH 6.0 was used as substrate to determine the activity of β-glucosidase at 440 nm (Tabatabai, 1994).

2.3.7. Gene quantification from soil metagenomic DNA

To assess the total eubacterial community in soil, bacterial nitrogen fixation and ammonium oxidation, the 16S rRNA gene, *nif H* and *amoA* gene copies were quantified. DNA was extracted from 500 mg of homogenized soil (stored at −20 °C) in triplicates using the FAST DNA Spin Kit for soil (MP Biomedicals, Qbiogene). The metagenomic DNA was resolved on 0.8% agarose gel and documented in Alpha Imager Gel Documentation System (Alpha Innotech Corporation, San Leandro, California). Partial 16S rRNA (V1 + V2 domains) gene copy number in the soil DNA was assessed using the SYBR green® fluorescent dye based qPCR assay with a Realtime thermocycler (Stepone™ Applied Biosystems, USA). The reaction mixture contained 5 μl of 2× SYBR green qPCR mix (Fermentas, USA) (mix contains SYBR green, 1.5 mM $MgCl_2$ and dNTPs, 3 units Taq polymerase), 0.14 μl of 1% Bovine serum albumin, 0.07 μl each of the forward primer 27F (5′-AGA GTT TGA TCM T GG CTC AG-3′) and reverse primer 355R (5′-GCT GCC TCC CGT AGG AGT-3′) (Suzuki et al., 1998), 2 μl of template DNA from soil DNA and 2.72 μl of nuclease free water, totaling 10 μl final volume. For standard curve, the reaction mixture was same as above except template DNA used was 16S rDNA of *Escherichia coli* (with pTZ 57 R/T vector) serially diluted. The PCR conditions were 95 °C for 15 min (initial activation) followed by 40 cycles of 94 °C for 20 s, 55 °C for 20 s, 72 °C for 15 s and 80 °C for 15 s and data acquisition at each stage, and final melting of amplicon from 72 °C to 99 °C with gradual increment of 1 °C. The standard curve was obtained by plotting the copy numbers against the threshold cycle (CT) values and the validity of standard curve was evaluated using R^2 value (>0.99). The abundance of nitrogen-fixing gene *nifH* was assessed in the soil sample using the forward and reverse primer amplifying the partial *nifH* gene—nifH-b1 (5′-GGC TGC GAT CCC AAG GCT AG-3′) (Burgmann et al., 2004) and CDHP nif723R (5′-GAT GTT CGC GCG GCA CGA ADT RNA TSA-3′) (Steward et al., 2004). Standards for *nifH* were obtained from *Azospirillum brasilense* sp7. The PCR conditions were 95 °C for 15 min (initial activation) followed by 40 cycles of 94 °C for 20 s, 62 °C for 30 s, 72 °C for 15 s and 80 °C for 15 s and data acquisition at each stage was also done. Quantification of *amoA* was performed using amoA-1F (5′-GGG GTT TCT ACT GGT GGT) and amoA-2R (5′-CCC CTC KGS AAA GCC TTC TTC) (Rotthauwe et al., 1995). Standards were obtained from *Nitrosomonas europaea*. The qPCR conditions were same as above for *nifH* quantification.

2.3.8. Metagenomic analysis

Nearly full length 16S rDNA from all the four soil DNA samples was amplified using 8FPL and 1492RPL primers (Reysenbach et al., 1994). Each PCR reaction contained 1× PCR buffer, 1.2 mM $MgCl_2$, 250 μM of each dNTP, 5 μM of each primer, 1 unit Taq DNA Polymerase (Merck Bioscience) and 100 ng template DNA. The template DNA was denatured at 95 °C for 5 min followed by 32 cycles of denaturation at 94 °C for 50 s, primer annealing at 55 °C for 30 s and extension at 72 °C for 90 s. For addition of A at 3′ end of amplicon, an additional step of incubation at 72 °C for 30 min was included. The amplified DNA was purified by Qiagen PCR purification kit and ligated into T/A cloning vector (pTZ57R/T) as per manufacturer's instruction (InsTA cloning kit, Fermentas). The ligated product was mobilized into *E. coli* DH5α competent cells by heat shock and $CaCl_2$ method. The plasmid DNA was isolated from transformants using Qiagen quick plasmid isolation kit. The cloned DNA was sequenced by using universal M13 primer from both the ends. The vector backbone sequences were removed and contigs were assembled to get complete sequence. Nearly full length sequences were phylogenetically classified using seqmatch option of RDP version 11.2 (Cole et al., 2014). The determined sequences were aligned using Clustal W (Thompson et al., 1994). Distance matrices and the phylogenetic trees were calculated by the Jukes and Cantor (1969) and neighbor-joining (Saitou and Nei, 1987) algorithms, respectively, using MEGA5 (Tamura et al., 2011).

16S rDNA of two samples (organic and conventional cropping) were further analyzed by semiconductor based next generation sequencing (Ion torrent). Hyper-variable region (V3) of 16S rDNA was amplified using PRBA338 and PRUN518 primers (Nakatsu et al., 2000). Each PCR reaction contained 1 × PCR buffer, 1.2 mM $MgCl_2$, 250 μM of each dNTP, 5 μM of each primer, 1 unit Taq DNA Polymerase, and 100 ng template DNA. The template DNA was denatured at 95 °C for 5 min followed by 32 cycles of denaturation at 94 °C for 50 s, primer annealing at 55.0 °C for 30 s and extension at 72 °C for 50 s. The amplicons were sequenced using Ion torrent personal genome machine by semiconductor sequencing technology. Q20 reads generated by Ion torrent personal genome machine were phylogenetically classified by M5RNA annotation source of MG-RAST. Species richness, diversity, prediction and similarity between two samples were calculated by Species Prediction and Diversity Estimation (SPADE) (Chao and Shen, 2010). Distribution of bacterial species was calculated by Pielou's index (Pielou, 1966).

2.3.9. Statistical analysis

Standard error of mean (SEM) was calculated for plate counts and enzyme analysis and qPCR and presented as mean ± SEM. Confidence limits for MPN were given in parentheses in the tables. Statistical significance in Shannon diversity index between organic and conventional cropping was analyzed by Hutcheson *t* test (Hutcheson, 1970). The difference in bacterial composition (phylum and genus) between organic and conventional cropping was tested by Fischer's exact test by employing statistical analysis of metagenomic profile (STAMP) version 2.0.0. (Parks and Beiko, 2010).

3. Results

3.1. Physical and chemical properties

The results given in Table 1 show that the water holding capacity of sandy soils increased by 2.8 and 1.9% in the organic samples of

Table 1
Physico-chemical and fertility properties of organic and conventional farm soils.

Treatment	Conventional cropping	Organic cropping	Conventional orchard	Organic orchard
pH	8.4	8.4	8.35	8.25
EC ($\mu S\,cm^{-1}$)	0.39	0.27	0.28	0.38
Water holding capacity (%)	11.0	13.8	9.6	11.5
Organic carbon (%)	0.35	0.55	0.35	0.75
Organic nitrogen (ppm)	27.0	26.4	24.1	24.2
Ammoniacal nitrogen (ppm)	12.2	24.0	20.7	26.2
Olsen's phosphorus (ppm)	8.21	5.87	19.37	16.43
Exchangeable potassium (ppm)	55.5	95.0	72.0	142.5

cropping and orchards, respectively, compared to the conventional ones. No significant differences were observed in pH values or EC values. Organic carbon increased significantly in organic farming by 0.2% and 0.4% in cropping and orchards, respectively. Ammoniacal nitrogen was 2 fold higher in organic cropping compared to conventional and 1.3 fold higher in organic orchards compared to conventional (Table 1).

3.2. Microbial community—Ecological structure and functional analysis

Total bacterial counts did not differ considerably between organic and conventional in the cowpea cropping system. In orchards, organic samples showed an increase of bacterial population by 2.8 fold (Table 2). The fungal populations decreased in case of cropping by 64% but increased in case of orchards by 98% due to organic farming. Actinomycetes consistently increased in both cropping and orchards by 92 and 100%, respectively (Table 2) in organic farming. Glomalin showed an increase in organic farming in both cropping and orchards by 82% and 56%, respectively (Table 2).

In cropping system, the counts in three nutritionally different media, YE (copiotrophic), SE (low nutrition), BGS/10 (oligotrophic) showed increase in organic treatments by 52, 94 and 25%, respectively. Not much difference was observed in case of BGS medium. In orchards, increase was seen in three media, viz., YE, SE, BGS/10 by 119, 78 and 79%, respectively. The counts on BGS medium decreased in organic sites by 30% (Table 3).

No appreciable differences were observed in the MPN of functional groups like nitrogen fixers, ammonifiers and nitrifiers (ammonium oxidizers and nitrite oxidizers) (Table 4). Slight decrease in the numbers of nitrogen fixers was observed in organic farming. Ammonium oxidizers increased in organic cropping by 34 fold but decreased in organic orchards by 7.2 fold. Nitrite oxidizers were not detected in any of the samples. Sulphur oxidizers showed a slight, but statistically insignificant increase in organic cropping (2 fold) as well as orchards (1.5 fold). The number of bacterial antagonists assessed by the plaque assay using crowded plate technique showed higher numbers in organic cropping over conventional cropping but not in orchards (Table 4).

There was a consistently significant increase in the activity of all the enzymes except alkaline phosphatase in organic farming samples of both the systems. Increase in enzymatic activity in organic cropping and orchards, respectively, were: acid phosphatase (1.5; 3 fold), flourescein diacetate hydrolysis (1.8; 3.3 fold), dehydrogenase assay (2.4; 3.5 fold), β-D-glucosidase (2.2; 6.3 fold) (Table 5). Alkaline phosphatase activity increased in conventional cropping as well as orchards due chemical fertilization by 2.1 and 2.2 fold, respectively.

The 16S rRNA gene abundance increased in organic farming of both cropping and orchards by 80.0 and 80.8%, respectively, indicating a consistent increase in the total eubacterial abundance. *nifH* gene copy number was below detectible limits in all the samples. *amoA* gene was found in exponentially greater abundance in conventional farming samples (Table 6).

16S rDNA analysis of random clones by Sanger sequencing indicated that organic soil is rich in species with nearly even distribution compared to conventional soil. Details of species are given in Supplementary material 1. There was greater diversity in the organic cropping and orchards; the Shannon diversity index of bacteria in organic cropping, conventional copping, organic orchard and conventional orchard is 2.17, 1.08, 2.08 and 1.38, respectively (Table 7). Evenness i.e., distribution of the bacterial phyla was more in organic cropping and orchards (0.94 and 0.90), compared to conventional cropping and orchards (0.67 and 0.77) samples. There was greater representation of the members of Methylophilaceae among the bacterial clones in the conventional cropping as well as conventional orchards *vis-a-vis* organic cropping and orchards (Supplementary material 1). Among classified group, *Arthrobacter* was found to be most abundant in organic orchard soil (Supplementary material 1). The phylogenetic relationships among the clones from each soil are illustrated in Fig. 1.

In-depth analysis of 16S rDNA of organic and conventional cropping soil was done using next generation sequencing on Ion torrent personal genome platform. The data generated for organic and conventional cropping was 115 and 116 Mbp, respectively, with 735,775 and 738,929 quality reads (>Q20) and average read length of 156 ± 46 bp. Sampling and sequencing efforts covered 93.7% and 91.7% of the species present in organic and conventional cropping and was thus sufficient to capture the eubacterial community diversity picture as indicated by the rarefaction curves which are flattened at the right end indicates reasonable number of individuals is sampled. Comparatively, more number of species observed in conventional cropping soil (Fig. 2). Around 40–44% of the sequence reads did not match with any sequence in M5RNA database, but showed similarity to bacterial 16S rDNA (uncultured unclassified). For simplicity in further analysis, we considered only the phylogenetically classified data. Though the species richness observed by next generation sequencing is more in conventional cropping, their distribution pattern is more even and diverse in organic cropping (H = 4.19 and 4.83, respectively) (Table 8). Among the 24 phyla obtained in both organic and conventional cropping soils, 8 phyla, *Actinobacteria, Proteobacteria, Bacteroidetes, Firmicutes, Chloroflex, Acidobacteria, Gemmatimonadetes* and *Cyanobacteria* are dominant and contribute up to 97.5% (Fig. 3). The distribution of phyla in conventional cropping soil is uneven with only few phyla in large proportion whereas the phylum distribution in organic soil is more even. This is evident from the higher values of Pielou's evenness index in organic cropping soils compared to conventional farming soils (0.67, 0.57, respectively). Representation of *Proteobacteria* among the eubacterial species was 20% lesser in organic as compared to conventional cropping whereas *Actinobacteria* were higher by 10% in organic cropping.

Table 2
Microbial counts (cfu g^{-1}) groups and glomalin content of organic and conventional farm soils.

Treatment	Conventional cropping	Organic cropping	Conventional orchard	Organic orchard
Bacteria ($\times 10^7$)	36.0 ± 0.4	36.8 ± 1.3	35.5 ± 1.6	98.3 ± 2.9
Fungi ($\times 10^3$)	90.0 ± 2.1	32.3 ± 0.5	30.3 ± 0.3	60.0 ± 2.3
Actinomycetes ($\times 10^4$)	67.3 ± 5.3	129.0 ± 19.1	70.0 ± 4.6	140.3 ± 9.2
Glomalin (mg g^{-1})	9.1 ± 0.06	16.6 ± 0.17	5.4 ± 0.08	8.4 ± 0.22

Table 3
Bacterial counts (cfu g^{-1}) of organic and conventional farm soils in nutritionally rich and poor media.

Treatment	Conventional cropping	Organic cropping	Conventional orchard	Organic orchard
Yeast extract medium ($\times 10^5$)	45.5 ± 3.1	68.0 ± 1.2	37.3 ± 3.6	81.3 ± 4.8
Soil extract medium ($\times 10^5$)	63.5 ± 1.5	123.5 ± 7.8	83.5 ± 2.8	149.0 ± 5.0
Basal medium* ($\times 10^5$)	67.0 ± 1.5	72.3 ± 8.3	168.0 ± 8.8	117.3 ± 3.9
Basal medium*/10 ($\times 10^5$)	81.5 ± 1.5	101.5 ± 1.8	75.25 ± 1.9	135 ± 2.1

* Basal glucose—salts medium.

Table 4
Microbial functional groups (MPN g^{-1}) and bacterial antagonists (pfu g^{-1}) of organic and conventional farm soils.

Treatment	Conventional cropping	Organic cropping	Conventional orchard	Organic orchard
Associative N_2 fixes ($\times 10^3$)	9.4 (3.4–22)	2.6 (1.0–6.6)	2.6 (1.0–6.6)	0.7 (0.2–1.7)
Ammonifiers ($\times 10^4$)	9.4 (3.4–22)	7.9 (2.3–22)	11 (3–24)	14 (5–35)
Ammonium oxidizers	4.7 (1.4–11.3) × 10^4	1.6 (0.4–4.6) × 10^6	1.6 (0.4–4.6) × 10^6	2.2 (0.7–4.4) × 10^5
Nitrite oxidizers	ND	ND	ND	ND
Sulfur oxidizers ($\times 10^4$)	7.9 (2.3–22)	17 (6–39)	2.2 (0.7–4.4)	3.5 (1.0–7.0)
Bacterial antagonists ($\times 10^2$)	30.0 ± 2.6	38.0 ± 1.7	15.0 ± 1.0	13.0 ± 0.6

MPN = most probable number; confidence limits $p < 0.05$ given in parenthesis; pfu = plaque forming units; ND = not detected.

Table 5
Soil enzymatic activities in organic and conventional farm soils.

Treatment	Conventional cropping	Organic cropping	Conventional orchard	Organic orchard
Alkaline phosphatase*	26.0 ± 5.30	12.5 ± 0.30	40.8 ± 0.53	18.6 ± 1.02
Acid phosphatase*	6.4 ± 0.00	9.3 ± 2.17	8.7 ± 1.6	25.7 ± 2.9
FDA hydrolysis (μg fluorescein g^{-1} soil)	0.46 ± 0.12	0.80 ± 0.05	0.76 ± 0.04	2.46 ± 0.15
DHA (μg TPF g^{-1} soil d^{-1})	32.2 ± 1.7	77.3 ± 4.6	34.3 ± 5.9	118.9 ± 1.3
β-Glucosidase*	4.03 ± 0.03	8.90 ± 0.29	5.4 ± 0.58	34.06 ± 3.83

* μg *p*-nitrophenol g^{-1} soil h^{-1}; FDA = fluorescein diacetate; DHA = dehydrogenase activity.

Table 6
Gene abundances (copy number g^{-1} soil) of organic and conventional farm soils.

Treatment	Conventional cropping	Organic cropping	Conventional orchard	Organic orchard
16S rDNA ($\times 10^8$)	26.4 ± 8.1	47.5 ± 5.5	33.5 ± 2.3	60.6 ± 8.8
amoA ($\times 10^5$)	66.2 ± 34.4	3.00 ± 0.24	53.7 ± 6.7	5.07 ± 0.25

4. Discussion

Maintaining and improving soil quality is crucial for sustaining food production. In addition to the physical and chemical characteristics, the biological condition of the soil, which includes the composition of the microbial community and its activity, plays a vital role in determining the structure and function of an ecosystem. The soil microorganisms also play a crucial role in recovery of soils from stresses and disturbances and thus impart resilience. Hence evaluation of the soil microbial community structure and function is important in assessment of soil health (Fließbach et al., 2007). Also a quantitative study of the microbial ecology of a system helps to identify the magnitude of the improvements brought about by management interventions.

Table 7
Bacterial richness and diversity indices of cropping and orchard soil (calculated at genus level) under organic and conventional management based on Sanger sequencing.

Index	Organic cropping	Conventional cropping	Organic orchard	Conventional orchard
Sanger sequencing				
Richness	10	5	10	6
Shannon diversity index	2.17[a]	1.08[b]	2.08[a]	1.38[c]
Exponential of Shannon index	8.80	2.94	8.00	3.97
Simpson index	0.066	0.438	0.100	0.290
Inverse Simpson index	15.00	2.28	9.54	3.38
Pielou evenness index	0.94	0.67	0.90	0.77

Values followed by the same letter indicate non-significant difference (Hutcheson *t* test, $p = 0.05$).

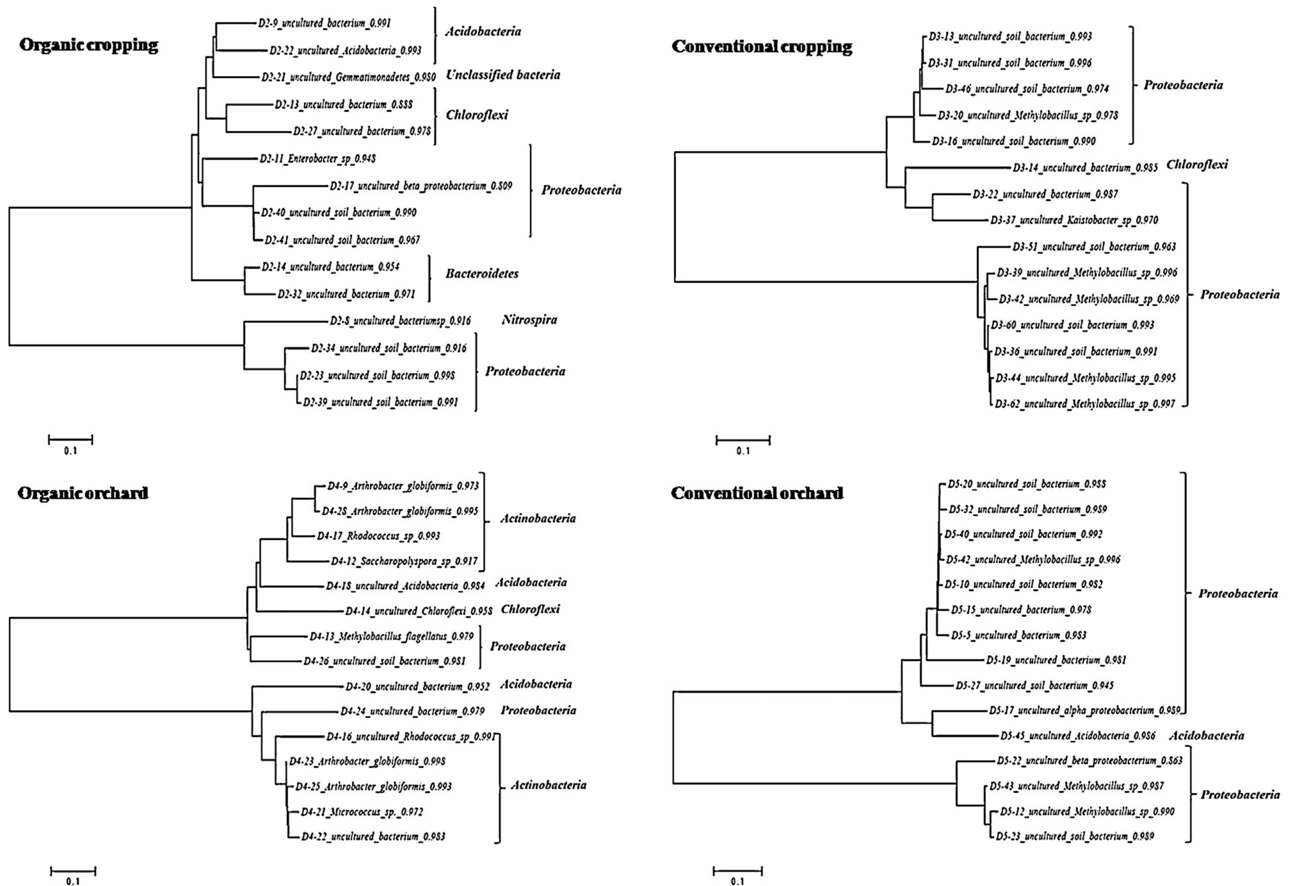

Fig. 1. Phylogenetic relationship of 16S rDNA clones. The sequences were aligned by Clustal W and the tree calculated by the Jukes and Cantor and neighbor-joining algorithms, respectively. Clones are represented by their name followed by the most closely related sequence obtained from the RDP 11.2 database and by the corresponding homology value, as determined by similarity score.

The effects of organic amendments on a low fertile tropical loamy sand soil were significant. The pH of the soil samples did not alter between conventional and organic practices and agreed with other reports (Bhaskaran and Krishna, 2009). Organic fertilizers contributed to increase in soil organic carbon content (0.2 and 0.4%) in organic samples of cropping and orchards (Table 1). Organic treatments improve the carbon sequestration in agricultural soils. Use of mineral fertilizers reduced the organic carbon

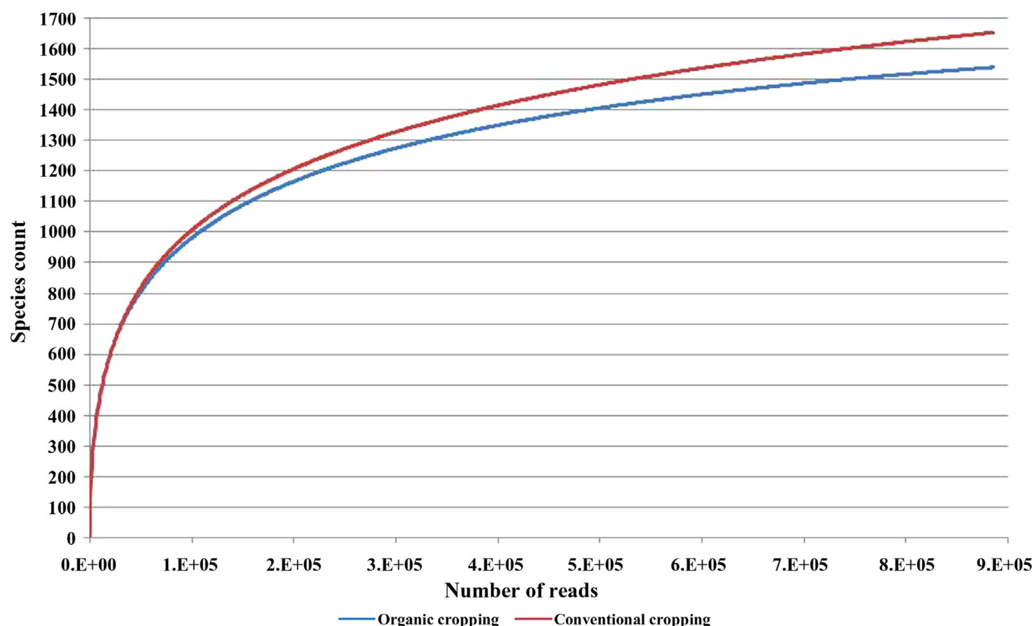

Fig. 2. Rarefaction curves for organic and conventional cropping soil metagenome.

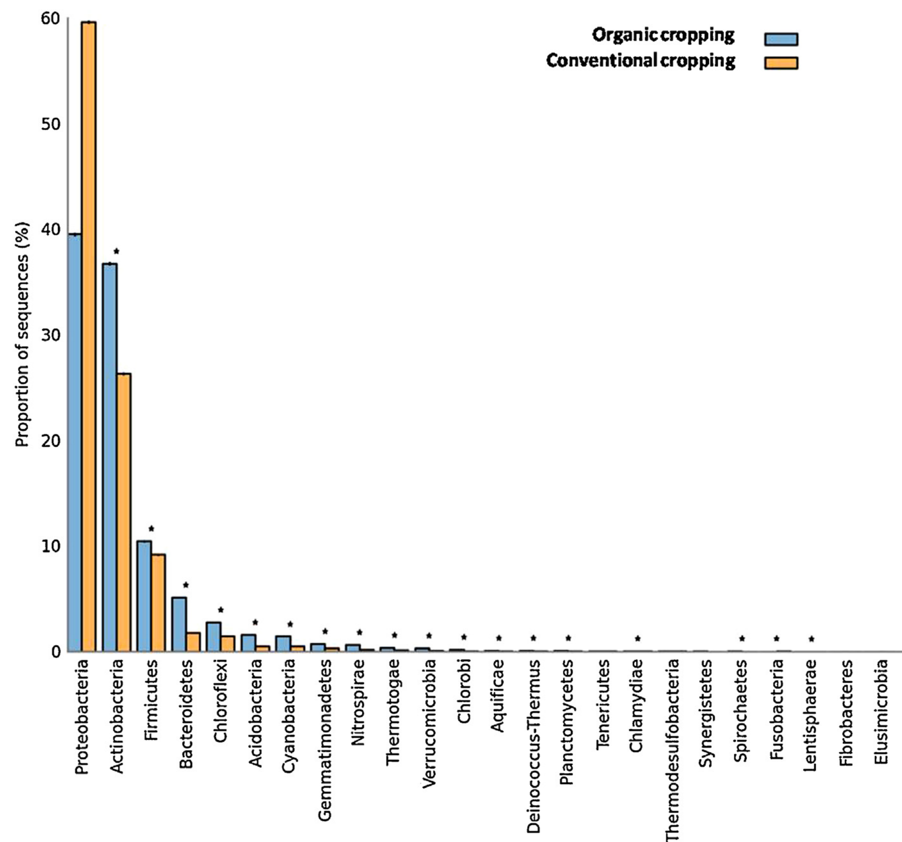

Fig. 3. Phylum level classification of 16S rDNA sequences (generated by next generation sequencing). Bars with asterisk mark indicate significance at $p < 0.05$ calculated by Fischer's exact test.

content (Fließbach et al., 2007; Reganold et al., 2010). Ammoniacal nitrogen was higher in organic cropping and orchards compared to conventional systems evidently due to higher organic carbon content.

Increase in organic matter content increases the water holding capacity of soils (Bhaskaran and Krishna, 2009) and organic amendments improve the soil physical properties and structure. This was clearly observed in the organic samples of cropping and orchards (increased by 2.8 and 1.9%, respectively). Organic farming is hence very useful in sandy soils as it improves the water retention and availability to plants.

Total bacterial counts did not alter among the samples, except that the organic orchards showed increased bacterial counts. Total fungal counts decreased in organic cropping compared to the conventional one. Parham et al. (2003) reported that cattle manure improved the counts of bacteria but not fungi. The total fungal counts increased in organic orchards. Actinomycetes counts showed significant improvement in organic farming (by 92 and 100%, respectively, in cropping and orchards) compared to the conventional ones due to increased organic carbon input and are thus a good indicator of the difference in cropping system. This supports the results of Moeskops et al., (2010) who showed greater amount of actinomycetes markers in the PLFA profiles of organic as compared to conventional farming soil samples.

Glomalin is a heat stable protein produced by arbuscular mycorrhizal (AM) fungi. AM fungi use glomalin as a carbon storage protein and thus play an important role in soil carbon sequestration. Chemical fertilization, most importantly phosphorus inputs are detrimental to AM fungi and decrease the glomalin content (Ortega-Larrocea, 2001). In our study organic soils of cropping and orchards showed increased glomalin content compared to their conventional counterparts by 82% and 56%, respectively. Glomalin content was higher in the cropping sites than the orchards since arbuscular mycorrhizal associations are found to a greater extent in annual plants than in trees.

The counts on yeast extract medium represent the copiotrophic bacterial populations which are heterotrophs and grow on energy rich substances and take part in organic matter breakdown in soil. The organic carbon compounds of the organic fertilizers acts as an energy supply for these heterotrophic populations (Elfstrand et al., 2007). The counts of copiotrophs increased in organic samples of cropping and orchards by 52 and 119%, respectively. The counts on soil extract represent those organisms which depend on some

Table 8
Bacterial richness and diversity indices of organic and conventional cropping soil based on next generation sequencing.

	Organic cropping	Conventional cropping
Nucleotide (Mbp)	115	116
Number of reads (filtered)	735,775	738,929
Avg read length (bp)	156 ± 46	156 ± 45
% unclassified sequences	44%	40%
Coverage % (species)	93.7	91.7
Richness (ACE)	1367	1502
Shannon diversity index	4.83[a]	4.19[b]
Exponential of Shannon index	124.85	66.04
Simpson index	0.023	0.080
Inverse Simpson index	43.72	12.37
Pielou evenness index	0.67	0.57

Values followed by the same letter indicate non-significant difference (Hutcheson *t* test, $p = 0.05$).

complex, unidentified growth factors present in soil, for their nutrition. Most of these are native soil bacteria and the counts of this nutritional class also were improved by the organic inputs in both cropping and orchards by 94 and 78%, respectively. The counts on BGS/10 medium represent oligotrophs, which grow on traces of organic substances and nutrients. These are representative of the bacterial flora of the extremely dilute soil solution that bathes the microorganisms in the pore spaces in soil (Killham et al., 1993). The counts of oligotrophs also increased in organic farms. Increase in oligotrophic counts indicates that the ecosystem is tending towards a more stable state (van Brüggen and Semenov, 2000). Parham et al. (2003) reported improvement of both copiotrophs and oligotrophs by organic treatment which is also confirmed by our results.

The nutrient cycling in the soils is carried out by diverse genera of microorganisms which belong to different functional groups like nitrogen fixers, ammonifiers, nitrifiers, etc. It is important that the management practices do not adversely affect these organisms. In the present study, organic inputs did not improve the numbers of associative nitrogen fixers in the bulk soils; rather a small but statistically insignificant decrease was noted in organic farming compared to conventional farming. This agrees with the results Orr et al. (2012) who found that conventional farming increases the overall nitrogen fixation activity in bulk soils (although this was not the case in rhizosphere soils). The numbers of ammonifiers also did not alter among the samples. The MPN counts did not reveal any differences in the populations of nitrogen fixers and ammonifiers among the cropping systems or management. On the other hand the counts of ammonium oxidizers increased in case of organic cropping but decreased in organic orchards. This may be due to the differences in the availability of the substrate in these two systems. In cropping system, the organic site had higher ammoniacal nitrogen compared to the conventional thus providing more substrate. In orchard system also ammoniacal nitrogen was higher in the organic site compared to conventional but the population of ammonium oxidizers was significantly lesser than that in conventional orchards. This may be due to the drastic improvement in the population of heterotrophic bacteria (+178%, Table 2) in the organic orchards which would compete with ammonium oxidizers for the ammoniacal nitrogen. This may be considered as a mechanism by which organic fertilizers promote immobilization of ammonium in microbial biomass thus ultimately leading to lower nitrification rates. This decrease in ammonium oxidizers was not evident in organic cropping as an effect of number of biodynamic sprays used, which were lesser as compared to orchards (which needed more sprays). The nitrite oxidizers were below detectible limits in all the samples. A reduction in numbers of ammonifiers and nitrifiers in some of the organic and integrated management treatments was also recorded by Filippini et al. (2012). The numbers of sulfur oxidizers also did not vary between conventional and organic samples statistically but an increasing trend was observed in organic samples. The overall picture shows that there is no significant effect of organic amendments on ammonifiers, nitrogen fixers and sulphur oxidizers in soil but ammonium oxidizers increased in cropping and decreased in orchards.

Disease suppressiveness of a soil is considered to be an important index of soil health since it depicts the frequency of plant disease incidence (van Brüggen and Semenov, 2000). It can be measured in terms the total antibiotic producers in the soil. The number of antibiotic zones was higher in organic cropping compared to conventional cropping indicating the improved disease suppressive capability by organic practices.

The most prominent and consistent evidence of the organic amendments engineering the microbial function in soils came from the enzyme analysis. Soil microorganisms produce exoenzymes which help in the breakdown and transformation of complex materials to simple and absorbable forms. They are responsible for the release of plant nutrients which are present in bound form and thus directly take part in nutrient cycling. All the enzymes studied except the alkaline phosphatase showed increase by 1.5–6.3 folds in organic farming (Table 5). Alkaline phosphatase acts on only soluble organic phosphate monoesters and is repressed by the orhtophosphates in soil (Jana, 2007). So, alkaline phosphatase activity decreased in the organic farming compared to conventional farming. This is in agreement with the work of Jiao et al. (2011), who reported increased alkaline phosphatase activity in integrated management systems compared to organic farming. Acid phosphatase is mostly involved in hydrolysis of organic phosphate monoesters which are in particulate form and is nonrepressible by the released phosphorus end product (Rodriguez et al., 2007). Addition of organic manures thus stimulated the release of acid phosphatase by soil organisms. Other workers (Marinari et al., 2006; Reganold et al., 2010) also reported increased acid phosphatase activity in organically treated fields. FDA and dehydrogenase activity are generalized indices of total microbial activity. Increased FDA, dehydrogenase and β-glucosidase activities were recorded in organic samples of both cropping and orchards as well as by other workers (Marinari et al., 2006; Moeskops et al., 2010).

The positive effects of organic amendments on microbial structure and biochemical activity were clearly evident. The effect of the organic amendments on the microbial structure was further confirmed through quantification of 16Sr RNA gene in soil which was significantly higher in organic cropping and orchard compared to the conventional samples by 80 and 80.8%, respectively. The abundance of *nif H* was below detectible limits in all the samples, which may be attributed to the low organic carbon content of the soils (Hayden et al., 2010). The abundance of *amoA* gene decreased in organic samples of both cropping and orchard by 22 fold and 11 fold, respectively, compared to the conventional samples. The gene *amoA* codes for the enzyme ammonia monooxygenase which is responsible for oxidation of ammonium salts to nitrites which either spontaneously or by further microbial oxidation convert to nitrates, a leachable form of nitrogen. A decrease in the number of ammonium oxidizers is thus considered to be a positive indication of soil health. Xue et al. (2013) reported no correlation of *amoA* gene abundance with organic amendments whereas Hayden et al. (2010) reported that *amoA* abundance was lower in managed sites compared to the fallow ones.

To capture the bacteria which are reluctant to grow under laboratory conditions, a culture independent approach was followed. There is difference in soil bacterial community composition analysed by clonal sequence and next generation sequencing. This difference could be due to the difference in primer set used for respective study. For Sanger sequencing, we used 27F and 1492R primer which covers all the nine hyper-variable regions of 16S rDNA whereas for next generation sequencing PRBA338 and PRUN518 primer pair was used which targets only one hypervariable (V3) region. There are several studies that have reported difference in community composition in same sample due to primer bias (Mou et al., 2005; Mesbah et al., 2007).

The diversity analysis through cloning gave preliminary information on diversity indices which mirrored those obtained by next generation sequencing. The diversity and evenness indices obtained for organically cropped soils were similar to those of organic orchards; similarly the indices of conventional cropping were at par with those obtained for conventional orchards. So, we analyzed the organic and conventionally cropped soils only using next generation sequencing methods. Both the sequencing methods showed greater degree of diversity and even distribution of bacterial species in organically cropped soil.

Organic amendments improve the nutrient availability, microbial biomass and microbial activity in soil (Liu et al., 2009). Our results agree with Li et al. (2012) who also observed diverse and more uniformly distributed bacterial community in organically managed soil. Organics helps in improving the physico-chemical properties and nutrient status of soil (Carter et al., 1999) and hence greater diversity of bacteria was observed in organically managed farms. This is evident from the dendrogram (Fig. 1) which represents the phylogenetic affiliation of random clones of soil bacteria. In the organic soil (both cropping and orchard) the distances between the members of clusters as well as the number of representative phyla are greater compared to conventional soils indicating greater diversity.

The structure and composition of microbes indicates the function of the ecosystem. More diverse and evenly distributed species in organic cropping and orchard soil indicate that the soil functions under organic management are more balanced. Thus, loss or disturbance to a particular species will not affect the overall functionality of the soil since it will be buffered by the presence of other species involved in similar functions. This is also supported by the enzyme activities which increased significantly in organic soils.

The rarefaction curves indicate that 93.7% and 91.7% of the species present in organic and conventional cropping were captured by the sequencing method (Fig. 2). Rarefaction curves give information about the species richness only. However, it is not only species richness, but also how they are distributed that is equally important for the functioning of an ecosystem. Although conventional soil is rich in species which is reflected by both rarefaction curve and richness index (ACE), the Shannon diversity index is more in organic cropping soil indicating a healthier ecosystem.

Organic cropping soil contained *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Chloroflexi* and *Cyanobacteria* significantly in large proportion (Fig. 3) compared to conventional cropping. Actinobacteria were in higher proportion by 10%. This is in agreement with the culturable microbial analyses which showed increased actinomycetes counts (2 fold higher) in organic soils compared to conventional soils (Table 2). Application of organic amendments to soil has significant impact on the diversity and community structure of *Actinobacteria* (Piao et al., 2008). Organic amendments also improve or maintain the soil nutrient reserve which is indicated by abundance of copiotropic bacteria like *Arthrobacter* in organic orchard soil. Unclassified bacterial members of the family *Methylophilaceae* were present in much higher proportion in conventionally managed soils. Members of *Methylophilaceae* are known for metabolism of C1 compounds (Chistoserdova, 2011). The commonly used pesticides like aldicarb, carbaryl and carbofuran contain methyl carbamate which is C1 compound (http://www.epa.gov/oppsrrd1/reregistration/status_carbamates.htm). The presence of *Methylophilaceae* in higher proportion in conventional farming (both cropping and orchard) system can be attributed to their ability to utilize C1 compounds released from applied pesticides.

An ecosystem engineer is defined as "an organism that directly or indirectly modulates the availability of resources (other than itself) to other species by causing state changes in biotic or abiotic materials. In so doing they modify, maintain and/or create habitats" (Wright and Jones, 2006). According to the definition an ecosystem engineer is a particular organism. But addition of organic manures like fermented cattle manure and cattle urine preparations as in the present study also caused changes in the physical structure, biochemical transformations and also brought out differences in the abundances of particular microbial groups. The organic manure though not a single living organism is a community of several microorganisms and fulfills all the characters described for an ecosystem engineer and we propose that it may be considered an "ecosystem engineer" on its own.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecoleng.2014.07.016.

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