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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

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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.

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.

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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