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Lessons from Long-term Nutrient Management Adoptions in Semi-arid Tropical Alfisol

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Authors' contributions

This work was carried out in collaboration between all authors. Author DB designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors CC and SMT performed all analyses and managed the literatures. Authors KI and KA managed the experimental process and critically improved the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Understanding the consequences of adoption of long-term nutrient managements on the fluctuation of soil biological variables is of greater importance in relation to nutrient supplying capacity of the ecosystem and crop requirement. The knowledge of linkage between nutrient management strategies and the soil microbiome and biochemical processes would be useful for soil health sustainability. The results from comprehensive study made on a field experiment comparing the long-term (more than 100 years) effects of organic manures and inorganic chemical fertilizers on soil biological and biochemical properties are reviewed. The soils adopted with three nutrient managements viz., no fertilization (control), inorganic chemical fertilizers (IC) and organic amendments (OM) obtained from long-term fertilizer experiment were assessed for physical, chemical, microbiological and biochemical properties in three successive years. The response of most of the assessed soil variables including soil organic carbon, microbial biomass carbon, humic and fulvic acid fractions, population of different functional bacterial communities, eubacterial

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community diversity and enzymes' activity to long-term organic nutrient management was significantly higher than those from inorganic fertilization and control (OM > IC = control). The biological properties and enzymes activity of the soil was unaffected due to long-term use of inorganic chemical fertilizers, as those were on par with control. The culture-independent molecular approaches also revealed that the organic manures encourage the overall biodiversity of eubacteria and to be specific, favour to some of the eubacterial phyla viz., Acidobacteria, Actinobacteria. The functionality of total soil microbiome assessed through respiration indices implies that the metabolic quotient was unaffected due to nutrient management, while OM had highest substrate induced respiration rate compared to IC and control. The principal component analysis of assessed soil variables clearly discriminated the OM from IC and control. The evidence from these observations shows that addition of organic manures is vital to enhance the abundance, diversity and functionality of microbiome of soil and thereby the fertility sustainability. However, it is also observed in our investigations that adoption of balanced inorganic chemical fertilization, which provides instant nutrients to the crop plants, had no harm to the microbial diversity and functionality as well as the soil processes including respiration and enzymes functioning.

Keywords: Eubacterial community diversity; long-term fertilization experiment; microbial biomass; organic manures; soil enzyme activity.

1. INTRODUCTION

Agricultural soils of India are under enormous pressure to provide food and fiber for ever increasing population. Most of the Indian soils are low in soil organic carbon (SOC) and macro- and micronutrients. The agricultural system is typically a monsoon-driven, low-input farming with limited nutrient management options. Additionally, these soils are fragile and suffer continuous degradation due to over-exploitation for crop production and imbalanced source and sink of carbon and plant nutrients. The major attributes that limit the productivity include low nutrient capital, soil moisture stress and loss of soil biodiversity [1]. Even though the hybrids and modern cultivation practices improved the major food crop production, the yields have stagnated in several regions for the last 10 years. The challenge for the next 50 years is to double the food production without compromising the environmental integrity and public health [2]. The Indian farmers are using organic manures with relatively low quality and quantity to meet the plant nutrients. With reference to the inorganic chemical fertilizer use, over-dose of nitrogen (N) and low or nil quantities of phosphorus (P) and potassium (K) application is common for most of the crops. This practice further decreases the soil quality and in turn may also cause a threat for the productivity in future [3]. Therefore, continuous monitoring of these soils is essential for developing strategies to sustain the yields as well as the soil health.

Soil microbiome, phylogenetically and physiologically diversified communities, plays a vital role in nutrients cycling, organic residues decomposition for plant nutrition and soil structure and fertility. Altering the microbial communities and activities may directly or indirectly affect the soil ecosystem functioning, nutrient cycling and the crop productivity [4]. Hence, upholding the microbial diversity and abundance is much essential for sustainable agricultural production and for long-term soil fertility. Nutrient management is one among the agricultural practices greatly alters the soil microbial communities. Organic fertility management increases the microbial abundance, diversity and activity in addition to soil enzymes activity [5]. The use of inorganic synthetic fertilizers (NPK) also brings change in soil properties including enzymes and functional diversity of microorganisms [6]. However, Zhong and Cai [7] reported that long-term practice of balanced mineral fertilization may cause negligible deleterious effects to the soil biological properties than those from unbalanced fertilizations (NP, NK, PK). Under Indian perspective, only few studies have been conducted on the influence of long-term addition of mineral fertilizers and manures on soil biological properties [8-10]. No comprehensive study has been conducted elsewhere in agro-ecological soils to account the impact on biology and biochemical processes. Hence, the key objective of the present investigation is to assess the long-term impacts of application of organic manures and inorganic chemical fertilizers on biological and biochemical properties of semi-arid tropical soil. This report is the summarized results of our works being done on long-term (>100 years) organic and inorganic nutrient management enforced soils. We have adopted both culturable and culture-independent approaches to assess the soil microbiome. Our investigation suggests that adoption of organic nutrient management improves the biological properties of semi-arid tropical soil, while the balanced inorganic chemical fertilizers did not harm them.

2. PHYSICO-CHEMICAL PROPERTIES

The analyses of soils collected on 100th (2009), 101st (2010) and 102nd (2011) year of continuous adoption of different nutrient managements from 104-years old long-term fertilizer experiment (detailed as Fig. 1) revealed that organically managed soil (OM) recorded increased macro- and micronutrients content compared to balanced inorganic chemical fertilized soil (IC) and unfertilized control soil (control) (Table 1). There was no significant impact on the soil pH and electrical conductivity (EC) due to long term nutrient managements [11]. The two-way analysis of variance (ANOVA) was applied to all the data to record the impact of sampling year and its interaction with nutrient management. The results revealed that though significant impact was observed on macro- and micronutrients (except K), there was an influence of sampling year too (Table 2). This implies that flex in macro- and micronutrients concentration had many influential factors apart from nutrient managements. Additionally, these results also infer the favouring of long-term organic manuring for the nutrient buildup of soil greater than inorganic chemical fertilizers, as the former provide the available nutrients to the crops instantaneously. Our findings are in agreement with investigations conducted at different agro-ecological soils such as temperate sandy loam [12] and sub-tropical wetlands [13].

The agronomic practices have a great impact on SOC that often lead to decreased levels, as a consequence, to deterioration of soil quality [14]. Nutritional managements usually affect the organic input to the soil and thereby can affect the quantity and quality of SOC. Long-term application of mineral fertilizers leads to a net loss of SOC and mineral N fertilization alone is, therefore, insufficient to maintain SOC levels unless it is combined with a high return of crop residues [15]. During the course of our investigation, comparing the mineral fertilizers alone and unfertilized control, use of organic manures (on nutrient equivalent basis to inorganic fertilizers) more effectively increased the SOC along with major plant nutrients (Table 1) [11,16]. However, application of mineral fertilizers enhances SOC too (Table 1), which is due to enhanced crop growth and thereby increased input of crop residues (i.e., roots and stubbles) into the soil [17]. The humic (HA) and fulvic acid (FA) fractions of SOC represent the most microbially recalcitrant and stable reservoir of organic carbon in soil. These humic substances are important attributes of soil quality as they influence the physical, chemical and biological properties and processes of soil. In our investigation, as expected the HA and FA were significantly higher in OM, while the IC and control had on par levels (Table 1). This implies that the SOC build up in the inorganic chemical fertilized plot did not account for carbon sequestration.

The microbial biomass carbon (MBC) of soil, an agent of labile nutrients, is critically important for the soil quality establishment and thus one of the most sensitive indicators of sustainability of management systems [18]. An increase in MBC in tropical soil following application of compost has been reported earlier [19]. However, during our investigation, along with OM, IC also had higher MBC (Table 1) than that of unfertilized control indicating that nutrient management practice with sufficient nutrient inputs and supplements is a vital key for improvement of MBC.

Fig. 1. View of the long-term fertilizer experiment (A) and cropping in progress (B)

The long-term fertilizer experiment, being conducted in Alfisol (Typic Haplustalf) since 1909 at Tamil Nadu Agricultural University, Coimbatore, India (11°N latitude, 77°E longitude, and 426 m altitude), was selected for this study. The permanent manurial experiment area is characterized as semi-arid sub-tropical with a mean annual precipitation of about 670 mm and mean annual maximum and minimum air temperature of 34.2 and 20°C, respectively. The soil is characterized by red sandy loam, arable, enriched with aluminium- and iron- minerals and low in SOC. It has high content of available Ca, Mg, K and Na and low in available N and P. Maize followed by sunflower is the crop rotation being adopted in the experimental field. Three long-term non-replicated nutrient management adopted plots (100 m^2) were chosen for this study. IC refers to inorganic chemical fertilizer applied soil in which 250:75:75 kg ha^{-1} of inorganic N, P_2O_5 and K_2O were applied in the form of urea, super phosphate and muriate of potash respectively. OM refers to organically managed soil, which received composed cattle manure (CM) on the nutrient equivalent basis corresponding to IC plots. The CM was incorporated into the soil during last ploughing and before sowing of every crop. Control refers to the soil in which crop raised without any nutrient input. Undisturbed soil samples were collected during fallow periods (September 2009, 2010 and 2011) from 0 to 15 cm depth from each of the treatment plots respectively and six independent samples were collected per soil. The soil samples removed from stones and stubbles were powdered, packed in plastic bags and stored at 4°C for all the analyses.

3. MICROBIAL COMMUNITIES

We have enumerated eight different soil microbial communities to reveal the impact of long-term use of organic manures and chemical fertilizers. Even though the most-probable number (MPN) or plate count based enumeration of microbial communities represent only the organisms which grow in the specific medium (less than 1% of the total population), it is widely adopted technique to compare the soil influenced by management systems [10,20]. Despite the well-known bias as detailed by Kirk et al. [21], several assumptions are to be considered for the culturable enumeration methods, MPN or colony forming unit (cfu) counts on more or less specific media are still used and informative [10,22]. In the sense of soil health, total cultivable bacteria, fungi, actinobacteria, total diazotrophs, free-living diazotroph - *Azotobacter*, ammonia oxidizers, nitrite oxidizers, denitrifying bacteria and plant growth promoting *Pseudomonas* are considered as beneficial microbial communities which decide the nutrient recycling, productivity and sustainability of soil. These cultivable microbial communities could discriminate the long-term consequences of tillage and nutrient management on soils of different agro-ecological regions [10,20,23]. The eight different microbial communities quantified in three successive years clearly demonstrated that organic manures foster the soil microbial communities (except ammonifiers), while continuous addition of inorganic chemical fertilizers did not show any build-up of microbial communities and are on par with unfertilized control soil [11,16] (Tables 1 and 2). All the assessed microbial communities had positive correlation with SOC build up and MBC (Data not shown). Therefore, long-term addition of organic manures affords the diversified substrates for proliferation of diversified microbial communities. In contrast, IC plot that received only specific inorganic fertilizer inputs did not support the buildup of microbial communities in soil. Interestingly, the chemical fertilizers are being applied at recommended dose of the plant nutrients did not cause any deleterious effects on the count of microbial communities as comparable to unfertilized control soil.

Azotobacter is an obligate aerobic soil bacteria, phylogenetically belonging to gamma-Proteobacteria. It is the predominant free-living diazotroph in agricultural soils. The ecological distribution of *Azotobacter* is dictated by soil characters including SOC, C/N ratio, pH and moisture [24]. The occurrence and functionality of *Azotobacter* in soil have been used as an indicator of physical and chemical disturbances [25]. During the course of our investigation, we have assessed the molecular diversity of *Azotobacter* isolated from these three soils (OM, IC and control) by a ribosome based fingerprinting assay, amplified ribosomal DNA restriction analysis. The results imply that though *Azotobacter* population was significantly higher in OM compared to IC and control, the genetic diversity of *Azotobacter* was unaffected due to long-term addition of either organic manures or inorganic chemical fertilizers [26]. These results conclude that adoption of proper dosage of balanced fertilization (NPK) accounts the soil nutrients (especially the nitrogen) well-below the inhibitory concentrations for *Azotobacter* occurrence and functionality. Additionally, it gave an assumption that the organic and inorganic nutrient managements may cause short-term impact to the soil *Azotobacter*, not for long-term.

4. EUBACTERIAL COMMUNITY DIVERSITY

We have assessed the soil eubacterial diversity by culture independent approaches such as amplicon length heterogeneity-Polymerase chain reaction (LH-PCR) [16] and by 16S rRNA gene sequence polymorphism [11]. LH–PCR uses the inherent length variations of the hyper-variable regions of 16S rRNA gene to produce a fingerprint from a metagenomic DNA sample [27]. During our investigation, we have used three hyper-variable domains (V1, V2, V1+V2) of 16S rRNA gene to compare the abundance and diversity of eubacteria between organic and inorganic nutrient managements. Of these domains, V1+V2 could discriminate the bacterial communities between the soil types. The relative ratios of amplicons (V1+V2) differed between OM and IC and eubacterial diversity was decreased substantially due to inorganic chemical fertilizers compared to organic amendments [16].

Further, 16S rRNA gene sequence driven approach also revealed that the composition of soil bacterial communities was altered by nutrient managements (Fig. 2 adopted from Chinnadurai et al. [11]). The major bacterial community discrepancies among the three soils (OM, IC and control) become visible in the distribution of Proteobacteria, Acidobacteria, Actinobacteria and Firmicutes. Soil Proteobacteria is characterized by a high metabolic diversity and is of great importance to global carbon, nitrogen and sulphur cycling [28]. Members of this phylum are also known for the interactions with plant species, which decide the beneficial or harmful role to the plants [29].

Table 1. Impacts of nutrient managements on assessed soil variables

Soil variables	OM	IC	Control
Soil organic carbon (mg g^{-1})	3.11^{a}	2.36^{b}	2.01^{b}
Microbial biomass-C (µg g^{-1})	1629.71^{a}	1249.45^{b}	923.51^{c}
Humic acid (% SOC)	0.68^{a}	0.39^{b}	0.27^{c}
Fulvic acid (% SOC)	0.06^{a}	0.04^{b}	0.03^{b}
pH	8.66^{a}	8.63^{a}	8.57^{a}
Ec (dS m^{-1})	0.12^{a}	0.12^{a}	0.19^{a}
Available N (kg ha^{-1})	199.00^{a}	147.07^{b}	130.78^{bc}
Available P (kg ha^{-1})	32.03^{a}	28.54^{a}	21.47^{b}
Available K (kg ha^{-1})	621.67^{a}	376.78^{b}	298.11^{c}
Available Copper (µg g^{-1})	2.07^{a}	2.07^{a}	2.17^{a}
Available Manganese (µg g^{-1})	6.11^{a}	6.07^{a}	5.21^{b}
Available Iron (µg g^{-1})	5.33^{b}	7.00^{a}	7.64^{a}
Available Zinc (µg g^{-1})	0.69^{a}	0.63^{ab}	0.53^{b}
Bacteria ($\log_{10}$ cfu g^{1})	8.72^{a}	8.66^{b}	8.55^{b}
Fungi ($\log_{10}$ cfu g^{1})	6.81^{a}	5.59^{b}	5.59^{b}
Actinobacteria ($\log_{10}$ g^{-1})	6.02^{a}	5.94^{ab}	4.66^{c}
Azotobacter ($\log_{10}$ cfu g^{-1})	6.52^{a}	5.44^{b}	4.51^{b}
Diazotrophs ($\log_{10}$ cfu g^{-1})	5.79^{a}	5.22^{b}	4.82^{b}
Ammonifers ($\log_{10}$ MPN count g^{-1})	5.72^{b}	6.50^{a}	5.35^{b}
Nitrifiers ($\log_{10}$ MPN count g^{-1})	5.05^{a}	5.42^{a}	4.55^{b}
Denitrifiers ($\log_{10}$ MPN count g^{-1})	3.87^{a}	3.92^{a}	3.62^{b}
Acid phosphatase (µg p-nitrophenol g^{-1} h^{-1})	544.14^{a}	535.18^{a}	431.76^{b}
Alkaline phosphatase (µg p-nitrophenol g^{-1} h^{-1})	1207.99^{a}	1103.38^{b}	990.01^{c}
Aryl sulphatase (µg p-nitrophenol g^{-1} h^{-1})	68.55^{a}	59.43^{b}	46.87^{c}
Urease (µgNH_4-N g^{-1} h^{-1})	26.83^{a}	37.86^{b}	21.99^{c}
Dehydrogenase (µg triphenylformazan g^{-1} day^{-1})	11.13^{a}	8.87^{b}	9.13^{b}
Microbial quotient (µg C-CO_2 h^{-1})	1.78^{a}	1.94^{a}	1.88^{a}
Substrate induced respiration (µg C-CO_2 h^{-1})	8.77^{a}	5.21^{b}	2.32^{c}

Values are mean of three years data (2009, 2010 and 2011) and values followed by the same letter in each row are not significantly different from each other as determined by DMRT ($p \leq 0.05$). OM – Organic manure amended soil; IC – Inorganic chemical fertilizer amended soil; Control – Unfertilized control soil

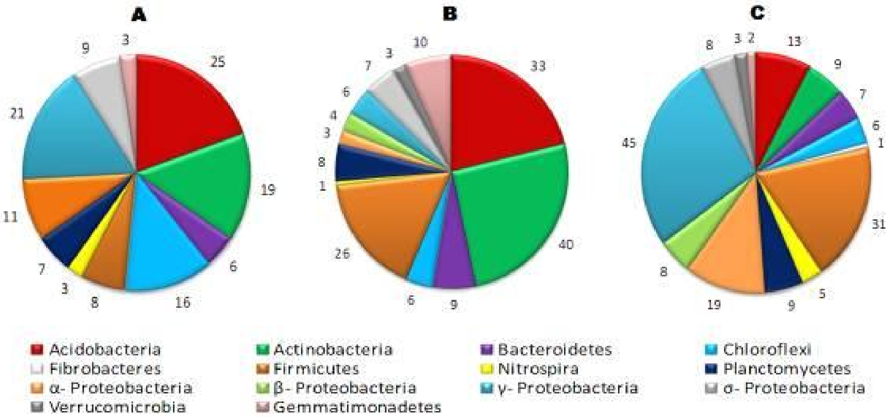

Fig. 2. 16S rRNA gene sequence based eubacterial community composition of soils (A- Control; B – OM; C – IC) influenced by long-term application of organic manures and chemical fertilizers application

Values of each panel denote the number of similar sequences. [Chinnadurai et al. Impact of long-term organic and inorganic nutrient managements on the biological properties and eubacterial community diversity of the Indian semiarid Alfisol, Archives of Agronomy and Soil Science, Volume 60, Issue 4, pp. 531-548, 2014, http://www.tandfonline.com/doi/full/10.1080/03650340.2013.803072, © Taylor & Francis, 2014]

Table 2. Two-way ANOVA of assessed soil variables indicating the interaction effects of nutrient management and time of sampling

Soil variables	***F* values**		
	Nutrient management	**Year of sampling**	**Nutr x year**
Soil organic carbon (mg g^{-1})	77.91**	NS	NS
Microbial biomass-C (µg g^{-1})	27.04**	NS	NS
Humic acid (% SOC)	37.56**	NS	NS
Fulvic acid (% SOC)	46.93**	NS	NS
pH	NS	84.07**	2.52
Ec (dS m^{-1})	NS	5.70*	0.87
Available N (kg ha^{-1})	37.98**	137.00*	26.77*
Available P (kg ha^{-1})	3.59*	32.53*	NS
Available K (kg ha^{-1})	247.54**	NS	NS
Available Copper (µg g^{-1})	NS	1713.92**	3.71*
Available Manganese (µg g^{-1})	10.75*	82.28*	7.80**
Available Iron (µg g^{-1})	9.99*	736.21**	7.24*
Available Zinc (µg g^{-1})	5.85*	48.48**	NS
Bacteria (cfu g^{-1})	87.32**	31.33*	NS
Fungi (cfu g^{-1})	6.48*	NS	NS
Actinobacteria (cfu g^{-1})	9.61*	NS	NS
Azotobacter (cfu g^{-1})	138.61**	NS	NS
Diazotrophs (cfu g^{-1})	164.23**	NS	NS
Ammonifers (MPN count g $^{-1}$)	30.55**	NS	NS
Nitrifiers (MPN count g^{-1})	19.59**	NS	NS
Denitrifiers (MPN count g^{-1})	8.21*	NS	NS
Acid phosphatase (µg p-nitrophenol g^{-1} h^{-1})	131.91**	NS	NS
Alkaline phosphatase (µg p-nitrophenol g^{-1} h^{-1})	46.78**	NS	NS
Aryl sulphatase (µg p-nitrophenol g^{-1} h^{-1})	585.71**	NS	NS
Urease (µgNH$_4$-N g^{-1} h^{-1})	27.23**	5.32*	NS
Dehydrogenase (µg triphenylformazan g^{-1} day^{-1})	253.79**	NS	NS
Microbial quotient (µg C-CO$_2$ h^{-1})	NS	NS	NS
Substrate induced respiration (µg C-CO$_2$ h^{-1})	228.34**	NS	NS

** p<0.05; ** p<0.001; NS – Not significant; Year of sampling: 2009; 2010 and 2011*

During our investigation, Proteobacteria was dominant in control (33%) and IC (48%), while the organically managed soil showed a decline in the proteobacterial proportion (20%). The relative ratios of classes of Proteobacteria also affected due to long-term nutrient managements. In most of the long-term nutrient studies elsewhere, the dominant class of proteobacteria was α-Proteobacteria followed by β- and γ-Proteobacteria and their ratio in organically manured soil significantly increased than control [30]. In contrast to this, in our investigation, the dominant class is γ-Proteobacteria (close to Pseudomonads) followed by α-Proteobacteria and less proportion of β-Proteobacteria and ε-Proteobacteria was noticed. When compare our results with other related findings, it is obvious that there is no specificity in the relative abundance of specific class of Proteobacteria in the soil. For example, Castro et al. [31] reported that the class δ-Proteobacteria was predominant in constructed old-field ecosystem maintained in Tennessee. Likewise, γ-Proteobacteria were abundant in the Cecil sandy loam soil and influenced due to inorganic chemical fertilizers [32]. Among the classes of Proteobacteria, β-Proteobacteria are considered as copiotrophic (fast growing and flourishing in soil with high amount of available nutrients) [30] and its proportion is expected to be low in organic farming [33]. In our investigation, its relative abundance to total Proteobacteria is very low; their flux due to nutrient management could not be attributed.

During our investigation, the Acidobacteria phylum was also predominant in the control and OM (20 and 21.15%, respectively), while drastically low in the IC (7.88%). Because of their recalcitrant culturability, the functions of Acidobacteria in the ecosystem are not yet completely understood. However, recent study revealed that they are metabolically active in soils and might contribute greatly to biogeochemical processes [34]. In most of the studies, the change in the acidobacterial proportion was well-correlated with change in the soil pH, which will be a strong indicator of abundance and community structure of Acidobacteria [30]. Though there is no significant differences in pH in the soils studied, application of organic manures might have triggered mineralization yielding lot of organic acids transiently lowering the pH and thus up- surging Acidobacteria. Apart from soil pH, the organic carbon availability also seems to be best predictor of Acidobacteria abundance and Acidobacteria are mostly regarded as K-strategists and oligotrophic bacteria with low growths and seem to be favoured under resource-limited conditions due to high substrate affinities [30,35]. In our study, its abundance should be low in OM due to high SOC and available nutrient contents, but did not. This disparity can be supported by findings of Naether et al. [36] that members of subgroup, Gp1 Acidobacteria had oligotrophic lifestyle, while other subgroups like Gp5, Gp6 and Gp17 showed highest relative abundance in soils of high nutrient concentrations. The decline of acidobacterial abundance in the inorganic fertilizer amended soil was already reported by Campbell et al. [37] who revealed that the change in nutrients and carbon shift due to inorganic fertilizers may harm several subgroups of Acidobacteria.

Actinobacteria play a major role in organic matter turnover, carbon cycling and humus formation. The members of this phylum can decompose the most complex carbon substances such as cellulose and chitin [38]. Organically managed soils have been reported to be rich in recalcitrant carbon sources and the diversity of Actinobacteria would be expected to be higher in those soils than inorganically managed soils. Khodadad et al. [39] also confirmed that addition of pyrogenic carbon triggered the actinobacterial abundance in soils. During the course of our investigation also, the increase in Actinobacteria relative abundance in organically managed soil was not surprising. The relative ratios of Firmicutes were increased notably both in organic (16.7%) and inorganic nutrient management (18.8%) regimes than control (6%). The members of the phylum Firmicutes have remarkable resistance to desiccation and extremes of environmental variations. They are known to thrive in environments where carbon and other nutrients are highly are available [40] which may explain the relative increase in the proportion of Firmicutes sequences invariably in the IC and OM than control.

5. SOIL ENZYMES

Soil enzymes catalyze all biochemical reactions and are an integral part of nutrient cycling in the soil ecosystem. They are believed to be primarily of microbial origin but also originate from plants and animals. Soil enzymes are usually associated with viable proliferating cells, but can be excreted from a living cell or be released into soil solution from dead cells [41]. The soil enzymes are potential indicators of soil quality because of their relationship to soil biology, ease of measurement and quick response to any disturbance [42]. Joining with soil physico-chemical and biological properties, soil enzymes as index would be used to monitor the impacts of long-term nutrient management regimes [9,43-45].

We have assessed the consequences of long-term adoption of organic and inorganic nutrient managements on soil enzymes in Alfisol [11]. Our study demonstrated that organically managed soils exhibited greater soil enzymes activity than chemically fertilized soil, agreeing with García-Ruiz et al. [46] (Table 1). Both acid- and alkaline phosphatase activities were higher in manure amended soils and were positively correlated with MBC suggesting that MBC build up due to continuous application of organic amendments favours these enzymes. Addition of organic manures in OM acts as nutrients supplement and affords as source for enzyme as well as substrates for hydrolysis. Soil dehydrogenase, the functions of total range of oxidative activity and viable microbial populations, serves as a good indicator of soil microbial activity [47]. In our study, enhanced dehydrogenase activity in OM (Table 1) [11,16] might be due to diversified, more complex nutritional amendments than IC. Meanwhile, the application of mineral fertilizers did not affect dehydrogenase, suggesting that application of optimal dose of mineral fertilizers has no deleterious effect on soil respiratory activity [7]. This is also supported by the microbial counts and in agreement with earlier findings [9,45].

Both OM and IC had significantly higher urease activity than control, with significant impact of sampling year too (Tables 1 and 2). The enhanced levels of urease in both organic and inorganic soils suggest continuous availability of substrates for the enzyme either in the form of organic sources or urea like inorganic sources [48]. Aryl sulfatase (ALS), the enzyme responsible for mineralization of ester sulfate in soils, has varied widely in the literature in relation to soil properties and management. It may be an indirect indicator of fungi as they contain rich of ester sulfate, the substrate of aryl sulfatase [49]. As this enzyme activity is associated with organic amendments [50], in our investigation, the organic amendments received soils had significantly high ALS than inorganic fertilized and control soils. Higher fungal counts recorded in OM than IC is correlated with aryl sulfatase activity ($r=0.59$, $p<0.05$). ALS also significantly correlated with MBC of soil ($r=0.73$; $p<0.001$) is in accordance with the results of Klose et al. [50].

6. SOIL RESPIRATION

Respiration indices are the potential soil variables associated with the microbial eco-physiology both in response to exogenous disturbances, as well as establishing the baseline endogenous environment, could provide the necessary information for management of biological systems [51]. The eco-physiological indices such as metabolic quotient, mineralization quotient, substrate induced respiration rate etc. are generated by basing the physiological performances (respiration, growth/death, carbon uptake) on the total microbial biomass per unit time. Any environmental or soil management impact which will affect the microbial community can be easily detected through these indices [52]. In our investigation, soil respiration rates increased with organic nutrient managements, while IC and control had significantly lower respiration are in accordance with previous works reported in different agro-ecosystems [43,53,54].

The metabolic quotient (MQ or qCO_2), the ratio between respiration rate and MBC, reflects metabolically active fraction of soil microbiome. This quotient provides a measure of specific metabolic activity that varies according to the composition and physiological state of the microbial community, carbon and energy sources and various abiotic factors [52]. The qCO_2 has been altered due to additional organic manures [55] and inorganic fertilizers [56]. The low qCO_2 reflects a more efficient use of substrates by the soil microbial biomass [52], in other words energy and carbon required for biomass maintenance in such soil is less. A high qCO_2 reveals a high maintenance carbon demand and if the soil system cannot replenish the carbon which is lost through respiration, microbial biomass will decline [57]. In our study, MBC of OM was significantly higher than IC and control, yet the qCO_2 of soils did not differ significantly due to long-term nutrient managements (Table 1) [58], indicating that introduced carbon into the system is efficiently cycled into biomass. Greater deposition of SOC and concomitant increase of MBC in OM could be the reasons for maintaining same qCO_2 as that of IC and control. In other words, metabolic state of the microorganisms of Alfisol remains same, invariable of nutrient managements, though significant difference found in the microbial communities and abundance [11].

Substrate induced respiration (SIR) measures the microbial respiration of soil after amending it with an excess of readily available nutrient source, usually glucose, to trigger the microbial activity and the respiratory response, can be related with current size and functionality of microbial biomass [52,57]. Higher SIR indicates the presence of metabolically active microorganisms including *r* and *K* strategists with the former growing faster when substrate is abundant, whereas the *K* strategic microbes can grow when resources are limited [30]. Organic manure amended soil had higher SIR rates than the soil under inorganic fertilization [53]. This shows that the type of fertilization leading to higher fluctuation of the substrate, have an influence on the distribution of *r* and *K* strategists in soil which can influence the nutrient flow through microflora [58]. During our investigation, the soil receiving organic manures as sole fertility management recorded highest SIR followed inorganic chemical fertilized soil and least in unfertilized control (Table 1) [59] and our SIR results agree with those of Enwall et al. [53] on Eutric Cambisol soil of Sweden. The organic amendments, rich source of C (SOC) encourages high abundance of microorganisms lead the physiologically active microbiome which recorded higher rates of SIR, while the unfertilized control soil having less active microorganisms (dormant stage) found low SIR. Unchanged qCO_2 and significantly differed SIR rates among the treatments (OM, IC and control) reveal that the physiological activity of soil microorganisms is under the influence of fertility managements.

Table 3. Loading values and per cent contribution of variables (mean of three years) on the axis identified by the principal component analysis

Soil variables	**PC1**		**PC2**	
	Loading values	**Contribution of variables (%)**	**Loading values**	**Contribution of variables (%)**
pH	0.07	1.15	-0.39	6.16
Ec	-0.06	0.41	**-0.72**	10.28
N	0.28	1.57	-0.36	8.17
P	0.46	3.19	0.01	0.62
K	**0.96**	7.20	-0.09	1.05
Cu	-0.26	2.29	0.06	3.73
Mn	0.39	1.21	-0.17	5.25
Fe	-0.75	4.43	0.21	2.04
Zn	-0.15	4.66	0.17	1.46
SOC	**0.82**	6.01	0.06	0.04
MBC	**0.87**	6.15	0.08	0.47
HA	**0.78**	5.46	-0.09	1.00
FA	**0.85**	6.07	-0.03	0.20
TCB	0.30	4.53	-0.02	3.38
TCF	**0.62**	3.30	-0.06	4.23
ACT	**0.62**	3.30	0.15	0.93
AZO	0.20	1.88	0.19	3.72
NIF	**0.91**	6.80	-0.29	2.49
AMO	**0.84**	5.90	0.18	0.78
NIT	**0.68**	4.85	-0.21	2.71
DNF	0.34	1.24	**-0.67**	9.93
AP	**0.50**	2.04	0.36	3.27
ALP	0.71	5.66	-0.01	1.14
ASP	**0.50**	1.52	**0.74**	10.32
URA	0.16	1.80	**-0.85**	12.48
DHA	**0.61**	3.28	0.09	1.45
MQ	0.15	0.46	-0.02	1.25
SIR	**0.68**	3.56	0.08	1.25
Eigen value	12.61		4.20	
Variability (%)	48.51		16.16	
Cumulative (%)	48.51		64.67	

Values in bold explained >50% contribution to the significant component. SOC – Soil organic carbon; MBC – Microbial biomass C; HA – Humic acid; FA – Fulvic acid; TCB – total culturable bacterial count; TCF – total culturable fungal count; ACT – actinobacterial count; AZO – Azotobacter count; NIF – Nitrogen-fixing bacterial count; AMO – Ammonifier count; NIT – Nitrifier count; DNF – Denitrifier count; AP – acid phosphatase; ALP – alkaline phosphatase; ALS – Aryl sulphatase; URA – urease; DHA – dehydrogenase; MQ – Metabolic quotient; SIR – Substrate induced respiration

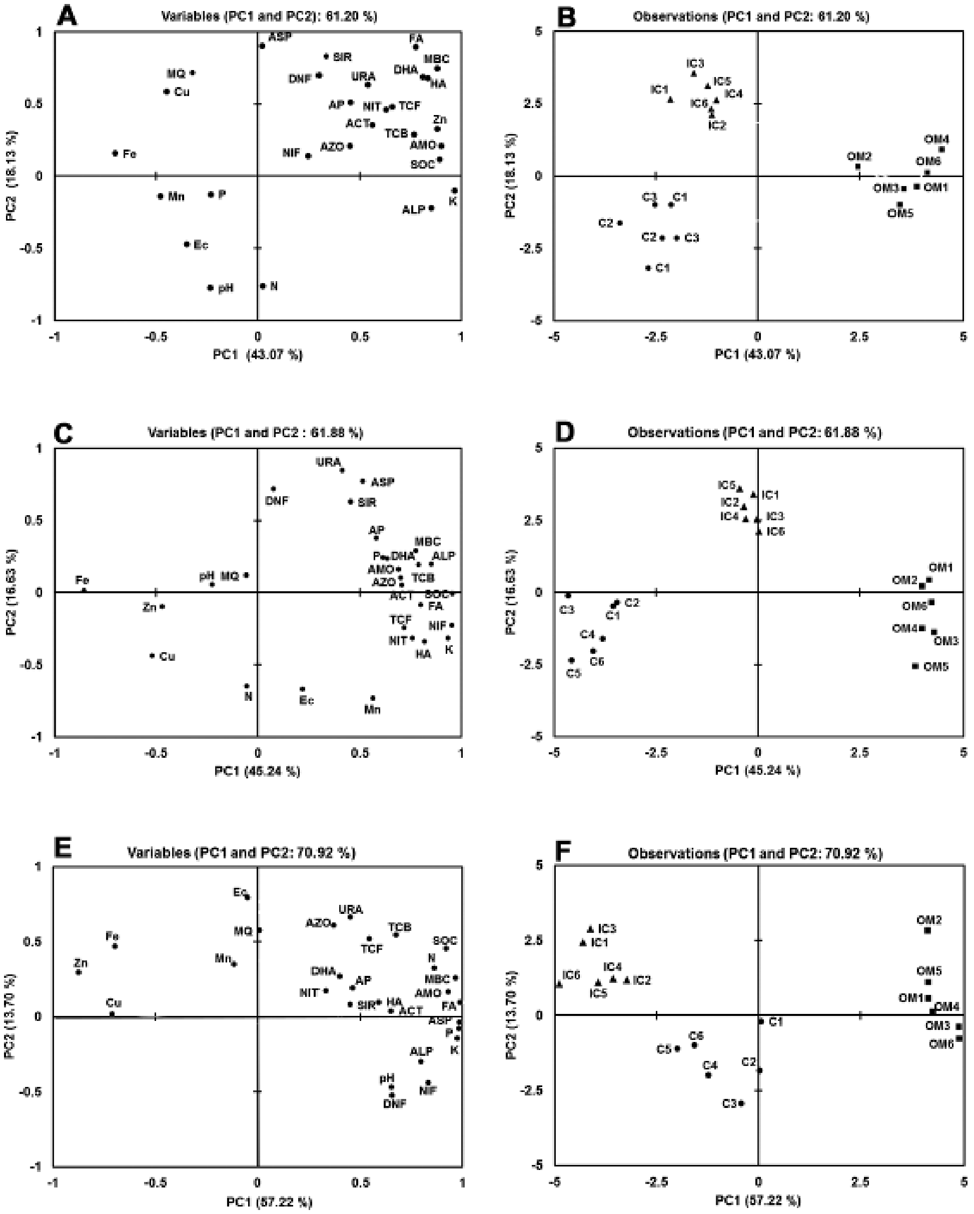

Fig. 3. Scoring plot of samples (A-2009; C-2010; E-2011) and loading plot of variables (B-2009; D-2010; F-2011) ordinated in PCA. Abbreviations of variables are explained in Table 3. The % variance explained by each component is given in parenthesis. C – Unfertilized control soil (●); IC – Inorganic chemical fertilizer amended soil (▲); OM – Organic manure amended soil (■)

7. INDICATORS FOR SOIL QUALITY

According to Doran and Zeiss [60], soil quality is defined as the continued capacity of soil to function as a vital living system within ecosystem and land use boundaries and sustain biological productivity without harm to environmental, plant, animal and human health. Since soil microorganisms and biochemical processes subjected to respond quickly to any disturbance including nutrient managements, agronomical practices, cropping system and environmental stresses and are therefore considered as monitors of soil status. The datasets collected in all the three years (2009, 2010 and 2011) were subjected for multivariate statistical analysis tool, principal component analysis (PCA) to assess the similarities and differences between soil samples and to assess the relationship between variables. The yearwise PCA plots are presented as Fig. 3 and variability added for 2009, 2010 and 2011 by first two PCs was 61.2, 61.88 and 70.92 % respectively. This indicates that the total response of assessed variables to long-term nutrient management is consistent. Most of the biological variables assessed in the present investigation showed positive correlation with PC1 (Fig. 3A, 3C and 3E). In all the assessed years, the OM samples positioned in right end of the scoring plot indicating positive score for both PCs, while the IC samples were negative to PC1 and positive to PC2 and control samples were located in plot where both PCs were negative (Fig. 3B, 3D and 3F). By comparing scoring and loading plots of PCA, it is obvious that most of the biological variables orthogonally positioned in the plot where OM samples are present, while the control soils had negative relation to the variables and IC samples positioned between OM and control. Three-year mean loading values for PC1 and PC2 were used to identify the potential indicators (Table 3). Among the assessed variables, SOC, MBC, SIR, counts of diazotrophs and nitrifiers, dehydrogenase and aryl sulphatase are the most significant (>80%) and reliable variables which can possibly be used as indicator for soil fertility.

8. CONCLUSION

The investigations revealed that the overall biological properties of Alfisol were controlled by the long-term nutrient management regimes. The biological variables were highest in organically managed soil. The inorganic nutrient amendments and no fertilizer application had same magnitude on the biological and biochemical properties of soil which indicates the less impact of chemical fertilizers. The culture independent approaches reveal that application of organic amendments expanded the eubacterial communities and favoured some of the phyla like Acidobacteria and Actinobacteria. The SOC, MBC, SIR, diazotrophic and nitrifying bacterial counts and activities of dehydrogenase and aryl sulphatase are the most sensitive soil biological indicators responded to nutrient managements, especially the organic manures. These findings validate our postulate that the long-term organic and inorganic nutrient managements had a strong influence on biological properties of semi-arid tropical Alfisols. The results reveal the inevitability of organic nutrient management and balanced inorganic fertilization for microbial sustainability of such soils.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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