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Review Article

Agricultural Microbiology Research Progress in India in the New Millennium

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The research efforts in microbiology over last two decades have focussed on assessing the microbial diversity in various soil types and cropping systems in all agro-eco-regions, including extreme environments resulting in creation of a vast repository of microorganisms, free living and endophytes. A marked departure from the past is their identification by both conventional and molecular methods, resulting in discovery of many novel species. The effects of various soil and agronomic management practices were assessed on soil microbial diversity and soil health by both classical and metagenomic approach. The beneficial effects of organic and integrated farming, and adverse effects of chemical intensification have been assessed, leading to the identification of actinobacteria as important players in maintaining soil health. A major impact has been the firm attention received from policy makers on the urgent need to conserve soil biodiversity and improve soil health. Identification of rapid methods of soil biological health assessment, improved rhizobial inoculants, microbial technology for rapid *in situ* and *ex situ* residue and waste breakdown, and mitigating climate change effects are the current priorities. Future priorities include assessment of the structure and function of the vast reservoir of unculturable microbes; deleterious microorganisms, assessment of soil-plant-microbe continuum and plant immunity modulation by microbes; rhizosphere engineering, role of archaea in maintaining soil health, microbial methods to improve soil organic matter formation; assessment and quantification of ecosystem services rendered by agriculturally important microorganisms; and improved microbial technology for biofertilizers and delivery systems. The tremendous advances in agricultural microbiology in last two decades in India are a cause for optimism that solutions will be forged for the above challenges using both classical and modern approaches, thereby ushering in the process more sustainable agricultural systems.

Keywords: Biological Nitrogen Fixation; Biofertilizers; Climate Change; Endophytes; Microbial Diversity; Plant Growth Promoting Bacteria; Soil Bioremediation; Soil Health

Introduction

The major research concerns in Agricultural Microbiology in India during the last decade have all revolved around ecological principles and practices that ensure sustainable production systems that are economically viable and environmentally safe. The adverse effects of agricultural intensification on soil biodiversity in a background of the already low soil organic matter levels, diminished organic recycling (green manuring, composts), imbalanced chemical fertilization, indiscriminate usage of agro-chemicals, land disposal of industrial effluents etc., have firmly brought the attention of the researchers and policy makers to the urgent need to conserve soil biodiversity and improve soil biology (Rao *et al*., 2015). Assessment of soil microbial diversity in different agroecosystems; identification of soil biological health indicators; improvement of legume biological nitrogen fixation through efficient and competitive rhizobial inoculants; improving plant health by inoculation with plant growth promoting rhizobacteria biofertilizers; identification of microbial cultures for rapid degradation of agricultural wastes; role of microorganisms in mitigation of green house gases are some of the major thrust areas that received (and

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continue to receive) the attention of agricultural microbiologists. A major shift in research approach-linking the classical approach to molecular approach using genomic tools was decisively made in all the laboratory investigations as well in some field studies. Another major development was the close linkages developed by microbiologists with soil chemists and physicists, agronomists, molecular biologists, bioinformaticians, climate change researchers and social scientists. A system approach replaced the piece-meal approach in field research. The outcome of these research efforts led to answers to some pressing questions like: How are some of our agricultural management practices impacting soil biodiversity, ecosystem processes and in turn soil health? How can application of microbial biotechnology help in improving soil biology, saving chemical fertilizers, improve biological nitrogen fixation, improve nutrient use efficiency and sustainable agricultural intensification? Some of those major developments are highlighted in this review.

Soil Microbial Diversity

Bacteria

India is one among 12 mega-biodiversity countries and 25 hotspots of the richest and highly endangered eco-regions of the world. Efforts were made in the last ten years to survey and enlist the biodiversity of certain groups of bacteria, fungi and cyanobacteria. The Indian Council of Agricultural Research (ICAR) initiated a program in year 2002 under the aegis of the All India Coordinated Research Project on Biological Nitrogen Fixation to survey the various districts of Madhya Pradesh for nodulation of soybean and characterize the native population of its rhizobia. Results showed that these soils largely harbored the slow growing rhizobia (Saxena 2006; Rawat *et al.*, 2008). Indigenous soybean rhizobia in Vertisols of Central India and other soils were assessed for cross nodulation, bacteriocin production and competitiveness (Ansari and Rao, 2013; 2014; Ansari *et al.*, 2014). Attempts were also made to study the diversity of rhizobia specific to chickpea (Rai *et al.*, 2012; Yadav *et al.*, 2013; Suneja *et al.*, 2016); pea (Wadhwa *et al.*, 2011) and *Vicia faba* (Gera *et al.*, 2013). Besides *Rhizobium*, diversity of *Azotobacter* and other free living diazotrophs in the rhizosphere of foot yam (Anjanadevi *et al.*, 2013) and red kidney beans (Suyal *et al.*, 2015); in salt affected soils of Haryana (Kayasth *et al.*, 2014); in the semi-arid Alfisol receiving long-term organic and inorganic nutrient amendments (Cinnadurai *et al.*, 2013) and in Indian agricultural lands (Anima and Singh, 2011) have also been reported.

Western Ghats are the hot spots of diversity and have been explored by different workers for various groups of microorganisms. Diversity of endorhizospheric diazotrophs and *Beijerinckia* (Alagawadi *et al.*, 2014 a,b); *Bacillus thuringiensis* (Ramalakshmi and Udayasuriyan, 2010); methylotrophic bacteria (Rahul Sri Balaji, 2013) and fluorescent pseudomonads (Indi and Alagawadi, 2012) were some of the reports from Western Ghats and were reviewed in detail by Naampoothiri *et al.* (2013). Few significant developments, particularly in India, to understand the relationship of soils and plants with the diversity of associated bacteria were reviewed by Tilak *et al.* (2005). It also traces contributions of Indian scientists in isolating and defining the roles of plant growth promoting bacteria to evolve strategies for their better exploitation. The diversity of plant growth promoting bacteria was assessed in the rhizosphere soil of tropical rice plant in Odisha (Bal *et al.*, 2012), wheat in northern hills and Central zone of India (Verma *et al.*, 2014, 2015); Terai-Dooars regions of North Bengal (Dey *et al.*, 2013); North Western Indian Himalayas (Yadav *et al.*, 2016a) and diverse agroecosystems of India for *Sorghum bicolor* (Praveen Kumar *et al.*, 2012). Asokan *et al.* (2013) reported the first isolation and characterization of native *Bacillus thuringiensis* isolates in soils of Great Nicobar Islands. Some of these isolates displayed toxic potential and could be adopted for future applications to control some agriculturally important insect pests in the area.

In another study epiphytic pink pigmented methylotrophic bacteria isolated from the phyllosphere of different crop plants were reported to enhance germination and seedling growth of wheat (*Triticum aestivum*) by producing phytohormones (Meena *et al.*, 2011). Indigenous formulations based on cow dung fermentation (biodynamic preparations: Panchagavya, BD500 and CPP) were analyzed by Radha and Rao (2014) who reported high counts of lactobacilli (10^9) and yeasts (10^4 ml^{-1}). *Bacillus safensis, Bacillus cereus, Bacillus subtilis, Lysinibacillus* *xylanilyticus* and *Bacillus licheniformis* were isolated and found to have PGPR attributes and improved the growth of maize. This is the first report of *L. xylanilyticus* and *B. licheniformis* in biodynamic preparations.

The microbial diversity all over the world in several specialized habitats of deserts, *viz.,* patina (desert varnish), cryptobiotic crusts, saline playas and rhizosheath was reviewed (Bhatnagar and Bhatnagar 2005). There are several reports by different research groups on the diversity of microorganisms in different extreme environments of the country which may harbor novel microflora. The extreme environments surveyed includes Sunderbans and Bhitarkanika Mangroves, thermal springs at Manikaran (Singh *et al*., 2010), Soldhar, Rajgiri, Vashist, Balarampur and Bakreshwar thermal springs, Chilka lake, Odisha, Pulicat brackish water lake, Sambhar Salt Lake, Lonar Lake; Rann of Kutch, Gujarat, Leh and Drass cold desert, Pindari, Kafni and Roopkund glacier, Himalayan mountain ranges; Kovalam district of Kerala, Jaisalmer, Rajasthan; Andaman & Nicobar island; estuarine sediments on Central west coast (Singh *et al*., 2010); deep-sea sediments of the Central Indian Basin (Singh *et al*., 2010) ; sediments of oxygen minimum zone in the Arabian Sea and Calcutta wet lands (Singh *et al*., 2012; Yadav *et al*., 2011; 2015a,b,c; 2016; Sahay *et al*., 2011; 2012a,b; Srinivasan *et al*., 2011; Thomas *et al*., 2012; Singh *et al*., 2013; Kumar *et al*., 2013b; 2014; Sharma *et al*., 2015; Sharma *et al*., 2015; Shivaji *et al*., 2011; Srinivas *et al*., 2011; Gupta *et al*., 2015; Ghosh *et al*., 2010; Pradhan *et al*., 2010). Bacterial diversity analyses of soil in the vicinity of Pindari glacier (India) showed the presence of diverse groups of organisms belonging to the genera *Arthrobacter*, *Bacillus, Sporosarcina*, *Rhodococcus*, *Lysinibacillus*, *Pseudomonas* and *Viridibacillus* (Shivaji *et al*., 2011). Culture dependent phenotypic characterization and 16S rRNA gene based phylogenetic analyses were applied to study the halophilic bacterial population of the Pulikat brackish water lake, India (Sahay *et al*., 2011). Phylogenetic analysis of bacteria isolated from Leh cold desert led to the identification of 175 bacteria, grouped in four phyla, Firmicutes (54 %), Proteobacteria (28 %), Actinobacteria (16 %) and Bacteroidetes (3 %), and included 29 different genera with 57 distinct species (Yadav *et al*., 2015a). Many different extreme environments were explored to study the diversity of *Bacillus* and *Bacillus* derived genera (BBDG) and led to the identification of 392 bacilli, grouped in two families, Bacillaceae (89.03 %) and Paenibacillaceae (10.97 %), and included 13 different genera with 75 distinct species. The major outcome of the investigation was the identification of niche-specific species and also the ubiquitous presence of selected species of BBDG, which illustrate the diversity and pervasive nature of BBDG in extreme environments (Yadav *et al*., 2015c). Lot of attention was paid to study the diversity of *Bacillus thuringiensis* in different ecological niches with a primary aim to identify efficient strains against Coleopteran, Dipteran and Lepidopteran insects and also to identify strains with variability in genes coding for crystal protein (*cry*) (Dangar *et al*., 2010; Asokan *et al*., 2011, 2013; Das *et al*., 2012; Ramalakshmi and Udayasuriyan, 2010).

In the last decade efforts have been made to study the diversity of Archaea through culture dependent and independent approaches. Phylogenetic analysis of Archaea isolated from Rann of Kutch, Gujarat, India revealed a novel lineage of three isolates. The 16S rRNA sequences of the strains 3A1-DGR, H9-DGR and 2ANA-DGR showed less than 93% similarity with the available known type strains and these three strains belong to a distinct novel lineage within the family Halobacteriaceae (Thomas *et al*., 2012). Using a metagenomic approach, 67 archaeal genera, representing more than 60 % of all known archaeal genera, were reported from Rann of Kutch (Pandit *et al*., 2015). Archaea isolated from Rann of Kutch were first time reported to possess ability to solubilize phosphorus through the production of organic acids (Yadav *et al*., 2015d).

Many novel species of bacteria have been reported from the country. *Devosia albogilva* sp. nov., *Devosia crocina* sp. nov., *Microbacterium amylolyticum* sp. nov., N5T a bacterium isolated from industrial waste soil (Anand *et al*., 2012), *Flavobacterium lin-danitolerans* sp. nov., *Devosia chinhatensis* sp. nov., *Sphingobium lactosutens* sp. nov., *Microbacterium lindanitolerans* sp. nov., *Sphingomonas histidinilytica* sp. nov., *Sphingobium quisquiliarum* sp. nov., P25T, *Sphingopyxis ummariensis* sp. nov., *Pseudoxanthomonas indica* sp. nov., *Sphingobium chinhatense* sp. nov., IP26T, *Sphingobium lucknowense* sp. nov., *Pontibacter* *lucknowensis* DM9T sp. nov., *Sphingopyxis indica* sp. nov., *Rhizobium rosettiformans* sp. nov., *Sphingomonas laterariae* LNB2T sp. nov, *Sphingobium baderi* sp. nov., *Edaphobacillus lindanitolerans* gen. nov., sp. nov., *Flavobacterium ummariense* sp. nov., *Acinetobacter indicus* sp. nov., *Novosphingobium lindaniclasticum* sp. nov., *Devosia lucknowensis* sp. nov., *Pontibacter ramchanderi* sp. nov., *Sphingomonas indica* sp. nov., *Novosphingobium barchaimii* sp. nov., *Sphingobium czechense* LL01T, á-hexachloro cyclohexane (HCH) degrading bacterium isolated from HCH contaminated soil (Verma *et al*., 2009; Kumar *et al*., 2008, Kumari *et al*., 2009, Kumari *et al*., 2011; Lal *et al*., 2010, Nigam *et al*., 2010; Bala *et al*., 2010, Dadhwal *et al*., 2009, Sharma *et al*., 2010; Garg *et al*., 2012; Dwivedi *et al*., 2013, Jindal *et al*., 2013, Kaur *et al*., 2012, 2013; Lal *et al*., 2012, Lata *et al*., 2012; Malhotra *et al*., 2012; Saxena *et al*., 2012; Devosia *et al*., 2013, Singh *et al*., 2013; Niharika *et al*., 2012a,b); *Pseudomonas panipatensis* sp. nov., *Novosphingobium panipatense* sp. nov., *Novosphingobium mathurense* sp. nov., *Sphingobium abikonense* sp. nov., isolated from an oil-contaminated site (Gupta *et al*., 2008, 2009; Kumari *et al*., 2009) were reported. *Thermus parvatiensis* RL^T sp. nov., *Lampropedia cohaerens* sp. nov. and *Fictibacillus halophilus* sp. nov. isolated from Manikaran thermal springs were also described (Dwivedi *et al*., 2015, Tripathi *et al*., 2016, Sharma *et al*., 2016).

Fungi

The diversity of fungi including AM fungi and mushrooms have also been an area of interest among different workers (Swer *et al*., 2011; Bansal *et al*., 2012; Upadhyay *et al*., 2008; Sharma *et al*., 2015; Chakraborty *et al*., 2010a,b,c; Mesapogu *et al*., 2012). A total number of 1531 wild mushroom specimens were collected from forests of Himachal, Uttarakhand, Jharkhand, Chhattisgarh, Rajasthan, Arunachal Pradesh, Tripura and Andaman & Nicobar islands. Four mushrooms, *Phellodon tomentosus, Laetiporus sulphureus, Thelephora* and *Dictyophora* were recorded for the first time in country (Upadhyay *et al*., 2008; Sharma *et al*., 2015). Two lepiotaceous mushrooms [*Lepiota indica* sp. nov. and *Lepiota attenuispora* sp. nov. (Agaricaceae)], one *Limacella magna* sp. nov. in the Amanitaceae and *Chlorolepiota brunneotincta* a new species belonging to Agaricaceae were also identified (Kumari *et al*., 2013; Atri *et al*., 2013). Diversity of culturable fungi in deep-sea sediments of the Central Indian Basin was carried out and a total of 16 filamentous fungi and 12 yeasts were isolated from 20 sediment cores indicating the presence of terrestrial fungi as a component of culturable fungi in deep-sea sediments (Singh *et al*., 2010). Genetic diversity and pathogenic variability among Indian isolates of *Fusarium udum* infecting pigeonpea (Mesapogu *et al*., 2012) and *Macrophomina phaseolina* isolates from certain agroclimatic regions of India (Babu *et al*., 2010) was also reported. Ambili *et al*., (2012) showed that arbuscular mycorhizal association and species richness was more in coconut cropping systems compared to arecanut cropping systems. Glomeraceae and Acaulosporaceae families were preponderent in both, however, Gigasporaceae was solely present in coconut cropping system. *Glomus aggregatum*, *Funneliformis mosseae*, and *Rhizophagus fasiculatum* were the uniformly abundant genera in both the cropping systems.

Cyanobacteria

Cyanobacteria are another group which has been extensively investigated for its diversity in different ecological niches. Distribution of toxic cyanobacteria and cyanotoxins in Lake Ambazari and Lake Phutala at Nagpur revealed the predominance of *Anabaena, Oscillatoria, Lyngbya, Phormidium,* and *Microcystis* (Maske *et al*., 2010). Exploration of Oscillatorian cyanobacteria of Manipur falling under Indo-Burma biodiversity hotspots was also attempted (Deepa *et al*., 2010). Choudhary and Bimal (2010) reported variations in the distribution of nitrogen fixing cyanobacteria in fertilized and unfertilized paddy fields. Kumar *et al*. (2015) carried out biochemical characterization and diversity analysis of cyanobacteria isolated from different locations. Community structure and diversity of cyanobacteria and green algae in freshwater bodies of Jodhpur, Rajasthan (Makandar and Bhatnagar, 2010a), soils of Thar Desert (India) (Makandar and Bhatnagar, 2010b), Santragachi lake, West Bengal (Ghosh *et al*., 2012), Bakreshwar thermal springs, Rajgiri thermal springs (Rastogi *et al*., 2012), high altitude lakes of Lahaul-Spiti in Himachal Pradesh (Singh *et al*., 2014), building facades and monuments in India (Keshari and Adhikary, 2013), saline soils and biological crusts on arid soils of Eastern India (Kumar *et al*., 2016) have been reported by different workers.

Biological Nitrogen Fixation

The major thrust in research on improving biological nitrogen fixation in legumes focussed on genetic diversity of rhizobia of major legumes grown in India including in arid and hyper-arid regions. Also efforts were made to quantify the inputs of biologically fixed nitrogen in Indian soils. The current status of biological nitrogen fixation research in India was subjected to a major review recently (Rao, 2014a)

Rhizobial Diversity

In a major initiative of the ICAR's All India Network Project on Soil Biodiversity-Biofertilizers launched in 2009, >2000 isolations of rhizobia of 20 major legumes (Chickpea, Pigeon pea, Groundnut, Cowpea, Soybean, Black gram, Mungbean, Pea, Lentil, Faba bean, Moth bean, Cluster bean, Lucerne, Berseem, Horse gram, *Sesbania*, Methi etc.) were made all over India including from hyper-arid and arid regions of Rajasthan and Haryana; acid soils of Jharkhand, 'Taal' lands of Bihar, soils of Uttarakhand (Ansari *et al*., 2014; Gera *et al*., 2013; Rao, 2016) and analyzed by conventional and molecular tools. In groundnut, nodulating bacteria other than *Rhizobium* were identified (*Enterobacter cloacae*, *Pantoea dispersa*, *Ochrobactrum*). Rhizobia of arid soils showed high genetic diversity and exhibited stress tolerance and PGPR attributes. Inoculation with high temperature and drought tolerant isolates significantly improved the yields of cluster bean, mungbean and pigeonpea under rain-fed conditions. Several *Rhizobium* isolates of pigeon pea, chickpea and soybean in acid soils analyzed by proteomic tools, which showed unique protein differences amongst acid-soil tolerant isolates (Dubey *et al*., 2016). Garg *et al*. (2016) found seven biotypes of `berseem' rhizobia in Haryana soils of which two types were common in three districts showing some degree of endemism even in the high temperature arid soils.

The population of soybean-rhizobia and the proportion of slow and fast growers were enumerated in Vertisols of central India in different crop rotations. Soybean based rotations stimulated the rhizobial population by 22 fold as compared to cereal rotations (Kumar *et al*., 2017). Lowest rhizobial populations were in the summer and re-bounded after rainy season at maximum vegetative growth by 13.3 fold in soybean rotations but only 1.7 fold in cereal rotations. Long term integrated nutrient management by annual application of farm yard manure in soybean along with chemical fertilizers improved the rhizobial numbers by 1.5 fold over chemical fertilization alone and by 2.9 fold over unfertilized treatments. Increased crop growth by chemical fertilizers also stimulated rhizobial populations by 1.9 fold over unfertilized soybean. The slow growing bradyrhizobia were symbiotically superior to the fast growing rhizobia but their proportion was lower in soybean based rotations as compared to cereal based rotations. Results highlight the importance of crop rotation with cereals, application of organic amendments along with chemical fertilizers and selecting slow growing rhizobia as inoculants.

BNF Quantification

Measurements of legume BNF in long term field experiments are very few in India. Rawat *et al*. (2013) in a 8 year soybean-wheat rotation experiment in Vertisol showed that fertilized and uninoculated soybeans (nodulated by native rhizobia) removed 158 kg N ha^{-1} (1.8 Mg ha^{-1} seed yield) whereas fertilized and inoculated soybeans removed 173 kg N ha^{-1} for a 2.0 Mg ha^{-1} seed yield and BNF amounted to ~92-100 kg N ha^{-1} yr^{-1}. The soil N increment in 0-15 cm soil was 27.8 kg ha^{-1} yr^{-1} after soybean harvest and 33.2 kg N ha^{-1} yr^{-1} after wheat harvest in the fertilized uninoculated plots averaged over eight years. In the fertilized and inoculated plots (*Bradyrhizobium* to soybean and *Azotobacter* to wheat) the soil N increments were 40.8 and 34.0 kg N ha^{-1} yr^{-1} after soybean and wheat harvest respectively.

Gross Estimate of BNF in India

Worldwide production of nitrogenous fertilizer through Haber-Bosch process is about 120 Tg yr^{-1}. It may reach 165 Tg yr^{-1} (1 Tg = 1 million tonnes or 10^9 kg) by 2050, bringing with it all the attendant environmental consequences, particularly the increased production of reactive nitrogen species (Nr) and nitrate leaching to ground-water. Cultivation of crops adds N inputs though biological nitrogen fixation (BNF) amounting to 55-60 Tg yr^{-1}. By adopting the agricultural cropping area data of 2014, total N harvest and Ndfa (nitrogen derived from air), the BNF input added by various crops of India was estimated at 5.2 Tg yr^{-1} by Rao and Balachandar (2017). Of this, legumes added 2.24 Tg (pulses 0.94; soybean 0.80 and groundnut 0.50 Tg); major cereals added 1.51 Tg (rice 0.76; wheat 0.65; maize 0.10 Tg); coarse cereals 0.13 Tg; sugarcane 0.20 Tg; fodder legumes 0.55 Tg; N fixing and other trees 0.41 Tg). A conservative estimate of BNF in Indian agriculture was computed as 5.20 to 5.76 Tg (9.5 to 10.6 % of global agricultural BNF).

Soil Biological Health

Soil Health Indicators

Soil microbiome is a sensitive indicator of soil health as it provides an indication of the direction and magnitude of the changes in ecosystem structure and function, earlier and better than others. Soil microbial biomass, respiration and soil enzyme activities have been extensively used to assess soil health in the past. Rao (2013) reviewed the worldwide and Indian literature on soil biological health and concluded that measurements of organic carbon, labile carbon, soil respiration, population of diazotrophs, soil dehydrogenase activity, soil enzymes like â-glucosidase and acid phosphatase, and glomalin content are easy to measure routinely and give a good idea of soil health. All are related to each other and are of the 'more is better' type.

Genomic indicators of soil health have now been developed in last five years. In a 100-year old permanent manurial trial at Coimbatore continuous application of organic manures enhanced the abundance of members of phyla *Acidobacteria* and *Actinobacteria*, while the balanced inorganic fertilization induced minimal shift in the bacterial community composition of semi-arid tropical Alfisol (Chinnadurai *et al*., 2014). The consequences of agricultural intensification with fertilizers and pesticides in Vertisols of Guntur, Andhra Pradesh showed differential effects in chilli and black gram. Very high inputs in chilies had an adverse effect on soil biological properties but not in black gram. In chilies, the counts of copiotrophs increased, of fungi and Actinobacteria reduced significantly, â- glucosidase activity increased and acid phosphatase activity reduced with very high inputs (Aparna *et al*., 2016). Metagenomic analysis of 16S rRNA gene showed a drastic reduction in the diversity of bacteria that was represented by only three phyla in very high input soils of chilli, whereas in normal there were 12 phyla (Malhotra *et al*., 2015). The proportion of Actinobacteria reduced from 30% in normal to 14% in very high input soils. The phylogenetic diversity of bacteria did not reduce in high input soils of black gram but was altered. No significant reduction in proportion of Actinobacteria was observed. The proportion of rhizobial *nifH* in black gram soil was reduced by 46% due to high inputs compared to normal inputs. Methods for measurement of soil biological health should therefore emphasize the size of Actinobacteria. In organically farmed Aridisols of Rajasthan, there was increase in counts of copiotrophs, oligotrophs, actinobacteria, higher content of glomalin (a measure of vesicular arbuscular mycorrhizal fungi), dehydrogenase activity, acid phosphatase, fluorescein diacetate hydrolysis and â-glucosidase. The eubacterial diversity analysis by sequencing of metagenomic DNA using next generation sequencing technologies showed Actinobacteria were 10% higher in organic and Proteobacteria were 20% higher in proportion in conventional management. The relative proportions of Actinobacteria and Proteobacteria thus serves as a good indicator of soil biological heath (Rao *et al*., 2014).

Studies on the impact of long term nutrient management in a 100 year old permanent manurial trial in a semi-arid Alfisol showed that soils under organic management had significantly higher microbial biomass carbon, total culturable bacterial counts and *Azotobacter* populations, whereas the chemical fertilized and unfertilized soils were similar. Though *Azotobacter* populations were higher in organic soils, however the genetic diversity was similar in all three soils (Cinnadurai *et al*., 2013). Apart from nutrient management, cropping pattern and crop growth stages also influence the soil microbial dynamics by altering the temporal and spatial distribution of available substrates from rhizodeposition and root biomass (Tamilselvi *et al*., 2015). All the assessments revealed that the active vegetative stage irrespective of crops tested recorded more abundance of diversified rhizo-deposits which significantly favoured the microbial colonization and diversity.

The role of soil microfauna as indicators of soil health has received little attention. Highest mean abundance of Collembola at different vegetational sites in Varanasi, (belonging to 18 genera that were identified) were found in floral garden (24%) and least in grassy field (16%), with mango orchard, bamboo grove and teak plantation being intermediate (20%) (Raghuraman *et al*., 2010). In a study in north-east India at the AAU, Jorhat Centre of the ICAR-All India Network Project on Soil Biodiversity-Biofertilizers, Collemobolans sampled throughout the year were highest in forest followed by fallow land and were the least in agro-ecosystem (Assam Agricultural University 2016). Collembola and mites were the highest in soils from pristine forest followed by organically cultivated vegetables, rice grown with integrated nutrient management whereas least were in paddy cultivated with conventional chemical fertilization. (Assam Agricultural University 2014).

In a study in hill ecosystem of Nagaland, metagenomic analysis of soils showed that soils with shorter *jhum* cycle (slash and burn cultivation) of 5 years had greater bacterial and archaeal abundance coupled with lesser eukaryotic abundance in comparison with a longer fallow cycle of 20 years (Thakuria and Sharma, 2014). Longer fallow cycle sites had greater woody plants, perennials and bryophytes; peroxidases and pectin esterases as the most exclusive enzymes; and led to a more stabilized N-sufficient ecosystem. This study demonstrated clearly the role of the regenerated above-ground vegetation during fallow phase in shaping the soil biodiversity and thereby regulating the ecosystem processes for stabilizing the ecosystem.

Practices to Improve Soil Health

Sustainable agriculture should manage the natural resources so as to maximize yield output with use of minimal synthetic inputs. Sustainable intensification of production should be achieved without any long-term detrimental effect on microbial communities. Some important research findings on management practices that impact soil microbial diversity and functioning are discussed here.

In a field experiment on a sandy loam soil in north India on wheat, conservation tillage significantly increased soil respiration, soil microbial biomass carbon and soil dehydrogenase activity compared to conventional tillage (Sharma *et al*., 2011). Similarly, in a field study on four tillage practices in NE India on rice-wheat/mustard/linseed, zero tillage (residue retention and double no-till) recorded higher SMBC, dehydrogenase activity and earthworm population, which in turn resulted in good growth and higher yield of all the crops (Ghosh *et al*., 2010).

A number of studies have reported beneficial effects of applying balanced dose of chemical fertilizers on crop productivity, soil organic matter and biological properties [for review see Rao (2013)]. The impact of application of animal manures, sewage sludges, composts and other organic amendments on changes in microbial properties of soil is well-documented. These amendments increase the size, activity and diversity (Chinnadurai *et al*., 2014) of soil microbial communities. Such enhancements have the benefits for crop productivity through increased nutrient cycling rate and impact climate change (Kollah *et al*., 2014).

Recent awareness of organic farming has made the recycling of agro-wastes an integral part of resource management. Managing the crop residues using microbial technology is beneficial; microorganisms producing hydrolytic enzymes e.g., laccase, peroxidase, polyphenol oxidase, cellulase, xylanase etc. are now being increasingly deployed. Thermophilic lignolytic and cellulolytic fungi, bacteria and actinobacteria such as *Trichoderma viride, T. reesei, Phanerochaete chrysosporium, Aspergillus niger, A. nidulans, A. awamori, Streptomyces* spp., are being successfully demonstrated as inoculants for rapid decomposition of paddy straw, wheat straw, jowar, pearl millet stover as also urban solid wastes (Manna *et al*., 2014). For microbial enrichment of compost, after the thermophilic phase of composting is over, N fixers and P solubilizers are added e.g., *Azotobacter*, P solubilizing fungi *Aspergillus awamori* to enrich available N and P. Addition of rock phosphate (12%), pyrite (10%), urea (1%), $ZnSO_4$ (0.1%) were also found useful for hastening the decomposition of crop residues as well as nutrient balancing of organic amendments. A consortium of four efficient lignocellulolytic psychrotrophic microbes (*Eupenicillium crustaceum, Paceliomyces* sp., *Bacillus atropheus* and *Bacillus* sp.) and commercial fungal consortia (*Aspergillus awamori, Aspergillus nidulans, Trichoderma viride* and *Phanerochaete chrysosporium*) were used to enhance the composting process of paddy straw at low temperature

(Shukla *et al*., 2016). The potential of two epigeic earthworms (*Perionyx excavatus* and *Eisenia foetida*) was compared for composting of crop residues (wheat straw and paddy straw) amended with farm yard manure. In general, *P. excavatus* exhibited better growth and mineralization efficiency, which further support the suitability of the species for large scale vermiculture operations (Singh *et al*., 2014). Another variation was to develop cyanobacteria fortified compost that was demonstrated as viable inoculation option for integrated nutrient and pest management strategies of cotton (Dukare *et al*., 2011, Prasanna *et al*., 2014).

Microbial Inoculants

The use of microbial inoculants in sustainable agriculture has been shown to reduce the inorganic fertilizer inputs (nearly 25%), improve the nutrient use efficiency of various inorganic fertilizers under integrated nutrient management and also the quality of the produce (Rao, 2014b) and soil health (Aparna *et al*., 2014). The microbial inoculant based integrated nutrient management (INM) practice (*Azospirillum*, *Rhizobium* and phosphate-solubilizing bacteria and fertilization with rock phosphate rock, compost, and muriate of potash) on rice-legume-rice rainfed production system in Assam reported significantly better cumulative grain yield, N, P and Zn uptake with positive N balance in soil than farmer's practice (Thakuria *et al*., 2009). The mechanisms by which plant growth promoting rhizobacteria improve plant health has been reviewed by Dutta and Podile (2010). Increased soil aggregates, better fungal/bacterial biomass C, high number of earthworm casts and high degree of bacterial community diversity are the other significant impacts reported in those soils used with microbial inoculants. In North-east India, in rice-rape seed rotation for two years soil health as reflected in soil enzyme activities were highest when fertilizers, composts and biofertilizers were added together (Nath *et al*., 2012).

Mitigation of Stresses

Saravanakumar *et al*. (2011) have reported the ability of *P. fluorescens* Pf1 to increase the activity of catalase and peroxidase in water stressed green gram plants when compared to untreated plants. The bacterized plants were found to tolerate stress better than the uninoculated controls. Ali *et al*. (2011) reported that inoculation of plant growth promoting thermotolerant *Pseudomonas putida* improved the survival and growth of wheat plants under heat stress. Inoculation reduced membrane injury and the activity of several antioxidant enzymes such as SOD, APX and CAT under heat stress. Inoculation improved the levels of cellular metabolites like proline, chlorophyll, sugars, starch, amino acids, and proteins compared to uninoculated plants. Scanning electron microscopy studies confirmed the colonization of the organism on the root surface. Psychrotolerant *Pseudomonas* spp. exhibiting plant growth-promoting activities at 4°C were inoculated and found effective in cold alleviation and promoting growth nutrients uptake of wheat seedling at 8°C (Mishra *et al*., 2011). Bacterization significantly improved the level of cellular metabolites and other physiological attributes involved in cold alleviation. These results suggest the possible role of microorganisms in mitigating adverse effects of climate changes on crop growth and may lead to development of microbe based climate-ready technology.

The ability of the rhizobacterial strain *Pseudomonas putida* GAP-P45 to improve the plant biomass, relative water content, leaf water potential, proline sugars, and free amino acids of maize plants exposed to drought stress was recently reported by Sandhya *et al*. (2010). *Methylobacterium* commonly referred as pink-pigmented facultative methylotrophs (PPFM) are phyllosphere dwelling proteobacteria and have the ability to induce the systemic tolerance to drought through ACC deaminase enzyme (Chinnadurai *et al*., 2009). By spraying this bacteria under large scale (24,000 litres covering 1.25 lakh ha of rice crop), the short-term drought tolerance (for about 15 days) was induced in rice, which avoided the complete yield loss due to monsoon failure in 2011-12 in Tamil Nadu Cauvery delta area (K. Ilamurugu, Personal communication). In another study co-inoculation of *B. subtilis* and *Arthrobacter* sp. could alleviate the adverse effects of soil salinity on wheat growth (Upadhyay *et al*., 2012). Inoculation with salinity tolerant *Azotobacter* strains has also been reported to significantly increase total nitrogen, biomass and grain yield of wheat in saline soils (Chaudhary *et al*., 2013). These findings provide a new dimension in inoculant technology for mitigating drought stress which is so crucial in this era of climate vagaries. Halotolerant bacteria *Bacillus* and

Halobacillus spp. mitigated salt stress on wheat growth and enhanced plant growth even at very high salinity (Ramadoss *et al*., 2013) and would thus be most appropriate as bioinoculants under such conditions. Psychrotolerant *Pseudomonas* was found effective against *Cajanus cajan* and *Eleusine coracana* (Shukla *et al*., 2015).

Subhashini (2012) reported the biocontrol potential of *Streptomyces* spp. isolated from tobacco rhizosphere and also its bioherbicidal activity (Subhashini 2014). Six strains of *Streptomyces* sp. with proven potential for plant growth-promotion (PGP) in rice were also found effective on chickpea and enhanced nodule number and biomass; root and shoot biomass, stover and grain yield and soil dehydrogenase activity and mineral nutrients (Gopalakrishnan *et al*., 2015). Scanning electron microscopy revealed the success in colonization of the chickpea roots by all six strains. Bacterial wilt caused by *Ralstonia solanacerum* in tomato was significantly reduced by >60% by application of lactic acid bacteria as biocontrol agent through seed treatment as well as soil drench (Murthy *et al*., 2013). Bacillus *amyloliquefaciens* was found to be the best PGPR for growth promotion and management of soft rot disease in ginger (Dinesh *et al*., 2015). A single strain multi-functional PGPR- *Bacillus licheniformis* was found to control white root rot disease of apple caused by *Dematophora necatrix* and improve plant health, vigour and fruit yield in both diseased and healthy orchards in Himachal Pradesh (YSPUHF, 2014). *Trichoderma* has achieved significant success as a powerful biocontrol agent at global level; Keswani *et al*. (2014) reviewed the potential applications of target specific secondary metabolites produced by different species of *Trichoderma* for controlling phytopathogens and their applications in diverse areas with the major emphasis on agriculture.

Plant Microbe Interactions

Plant-microbe interaction studies employing nitrogen fixers, PSBs, PGPRs and AM fungi were carried out in several laboratories. Two strains of *Gluconacetobacter diazotrophicus* isolated from endorhizosphere of sweet sorghum (Acd1 and Acd2) were tested for their plant growth potential and nitrogen-fixing ability along with a reference strain obtained from Brazil (Pal3) under green house and field conditions with sweet sorghum cultivar PC 121 in the presence of fertilizer nitrogen (20 and 40 kg N ha^{-1}) applied in the form of isotopically labelled ammonium sulphate [$(^{15}(NH_4)_2SO_4$] (Sahai *et al*., 2015). Priya *et al*. (2015) studied the influence of cyanobacterial inoculation on the culturable microbiome and growth of rice. Many reports on the inoculation of PGPR along with *Rhizobium* were published (Tsigie *et al*., 2011). Co-inoculation of the endophytic fungus *Piriformospora indica* with the phosphate-solubilising bacterium *Pseudomonas striata* was shown to influence the population dynamics of *P. striata* and also growth of chickpea (Meena *et al*., 2010). Dudeja *et al*. (2012) reviewed the work on legume endophytes; the quantum benefit derived by plants from an endophyte and *vice versa* is still unclear and it is still not clear which population of microorganisms (endophytes or rhizospheric) promotes plant growth and the way the interactions among endophytes influence plant productivity. Suman *et al*. (2016) reviewed the mechanism by which endophytes contribute to better plant health. Bhattacharjee *et al*. (2012) reported the beneficial effect of an IAA and ACC deaminase-producing *Rhizobium leguminosarum* bv. trifolii in promoting rice growth. Gosal *et al*. (2012b) isolated several nitrogen fixing bacteria in wheat rhizosphere in Punjab soils and using molecular methods characterised them as *Azotobacter* sp., *Azospirillum* sp., *Stenotrophomonas maltophilia, Stenotrophomonas* sp., *Sphingomonas paucimobilis, Rhizobium larrymoorei, Pseudomonas aeruginosa,* and *Xanthomonas oryzae*. Inoculation of sugarcane with *Azotobacter* in Punjab soils gave increase in cane yield of 3-4 Mg ha^{-1} (Gosal *et al*., 2012a). An endophytic *Bacillus* sp. was found very effective in promoting sugarcane yield under field conditions at Pantnagar (Chauhan *et al*., 2013). Saini *et al*. (2015) isolated endophytes from chickpea, *Bacillus subtilis* and *Bacillus licheniformis*; there was more diversity in nodule endophytes than root endophytes, they could solubilize phosphate and could promote growth of chickpea in field better than recommended biofertilizers. PGPR, *Bacillus megaterium* from coconut and *Pseudomonas putida* from cocoa were found cross-compatible with vegetables crops like chili and tomato (Rajeela *et al*., 2017).

Phosphate-solubilizing microorganisms from salt-affected soils of India were reported to significantly influence the growth of sorghum plants (Srinivasan *et al.*, 2011). Besides P solubilising bacteria, phytase producing bacteria have been implicated in influencing the growth and phosphorus uptake of Indian mustard (*Brassica juncea*) (Kumar *et al*., 2013). Potassium solubilising bacteria *Bacillus subtilis* and *Bacillus megaterium* were shown to reduce the pH and also produce exopolysaccharides and promote the growth of elephant foot yam in field (Anjanadevi *et al*., 2016). Bacteria with plant growth activities were identified in wheat rhizosphere in different agro-climatic zones of India belonging to *Bacillus*, *Exiguobacterium*, *Lysinibacillus*, *Paenibacillus* etc., that could be promoted niche-specific inoculants for crops growing in respective climatic conditions. (Verma *et al*., 2016a) Several thermo-tolerant bacterial isolates exhibiting plant growth promoting attributes at high temperature were isolated (Verma *et al*., 2016b) and may have application as inoculants for plant growth promotion and biocontrol agents for crops growing at high temperature conditions. The potential of eight thermotolerant bacteria (seven *Bacillus* spp. and one actinobacterium *Kocuria* sp.) and two cyanobacteria (*Anabaena laxa* and *Calothrix elenkinii*) as plant growth promoting (PGP) agents was evaluated with seed spices – coriander, cumin and fennel, under controlled conditions in potting mix fortified with microbial cultures (Kumar *et al*., 2013a). Application of mycorrhiza associated bacteria like *Pseudomonas putida* with *Glomus mosseae* significantly increased the mycorrhizal colonization and growth of sapota and helped in establishment of healthy seedlings which has a large impact on establishment of seedlings and germplasm maintenance (Panneerselvam 2013).

Microbes have also been used for biofortification of micronutrients in crops like soybean and wheat. Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria has also been reported (Rana *et al*., 2012). Inoculation of zinc solubilizing *Bacillus aryabhattai* strains has been reported for mobilization and biofortification of zinc in soybean and wheat (Ramesh *et al*., 2014). Two endophytes *Arthrobacter sulfonivorans* DS-68 and *Enterococcus hirae* DS-163 efficient for fortification of Fe and two endophytes *Bacillus subtilis* DS-178 and *Arthrobacter* sp. DS-179, efficient for fortification of Zn in low accumulator wheat genotype were identified. In general, the amount of Zn in grains due to inoculation of endophytes was 2 folds higher as compared to un-inoculated control (Singh *et al*., 2017). Many plants engage in interactions with rhizosphere- and root-associated microbes to survive in toxic and nutrient-limited environments. Abhilash *et al*. (2012) have given examples of how plants and their associated microbes can be screened, custom-built and utilized in field applications.

Biofertilizer Technology

Solid carrier based (peat or lignite) inoculants are used in most tropical countries, including India but they have several drawbacks like a short shelf life, high contaminants and inconsistent field performance. In India, several liquid formulations containing not only the desired microorganisms and their nutrients but also added cell protectants or chemicals that promote formation of resting spores or cysts have been developed (Trimurtulu and Rao, 2014). Several pulse crops responded well to the application of liquid inoculants than carrier inoculants and the response was better in Alfisols than in Vertisols. In cereal crops like rice, maize and wheat, liquid inoculants enhanced the grain yields over local carrier inoculants and resulted in 25-30% saving of chemical fertilizers. Liquid inoculants of good quality thus hold a great promise to farmers of tropical countries.

A microbial consortium package consisting of *Pseudomonas*-enriched mycostraw, *Azospirillum*, and cyanobacteria used in rice for four years resulted in an increase in organic carbon, preponderance of *Azospirillum* in soil, nitrogen fixation, total N and P and fertilizer use efficiency and rice yields in gangetic alluvial soils (Jha *et al*., 2013).

A novel strategy of developing multifunctional inoculants in the form of biofilms using a combination of two organisms with useful plant growth-promoting rhizobacteria (PGPR) was optimized. *Trichoderma* and *Anabaena* was employed as matrix and agriculturally important bacteria (*Azotobacter chroococcum*, *Pseudomonas fluorescens* and *Bacillus subtilis*) as partners (Prasanna *et al*., 2010; Triveni *et al*., 2012b). The synergism in terms of the PGP traits in the biofilms revealed their promise as superior PGP inoculants as compared to the individual organisms and dual cultures (Triveni *et al*., 2012c; Prasanna *et al*., 2013). Different carriers were used to develop the formulations of biofilmed inoculants (Triveni *et al*., 2012a). The influence of biofilmed formulations composed of *Trichoderma viride* and *Anabaena torulosa* as matrices was investigated in *Macrophomina phaseolina* infected cotton crop, in terms of plant growth and biocontrol parameters (Triveni *et al*., 2015). These biofilms were tested with several crops like cotton, wheat, chickpea and soybean and recorded increase in growth and yield (Swarnalakshmi *et al*., 2013; Prasanna *et al*., 2014). *Anabaena* based biofilms were found effective in improving micronutrient uptake in rice (Adak *et al*., 2016).

Mesorhizobium ciceri-based biofilms were developed and used for the antifungal and plant growth promoting activity in chickpea challenged *Fusarium* wilt (Das *et al*., 2017). These studies illustrates that such biofilms may closely mimic natural microbial communities and may represent a more viable inoculation option for integrated pest and nutrient management strategies in agriculture.

Soil Pollution and Bioremediation

Less than 1% of total applied pesticides generally get to the target pests while most of it remains unused, which causes serious ecological problems. The use of chemical pesticides played an important part in the green revolution in India but environmental concerns about the harmful consequences have led to design of newer molecules, transgenic plants etc., which have addressed some of the concerns. High inputs of pesticides are damaging and leads to loss of diversity and/or function within the soil microbial community. Over the past 15 years, use of microorganisms has shown promise in remediation of soil pollutants which include organic compounds, heavy metals, hydrocarbons, poly-chlorinated aromatic compounds and so on. Pesticide degradation may be carried out by several ways described as biotransformation, biomineralization, bioaccumulation, biodegradation, bioremediation and co-metabolism. Microbial degradation involves the use of effective microbes for pesticides belonging to organochlorines, organophosphorus or carbamate groups. Because of the tropical nature of the Indian environment and the varied ecology under which various crops are grown, Indian agro-ecosystem remains a rich source of diverse microorganisms expressing such potential of detoxification. Over the years, concept of microbial degradation of xenobiotics has taken strong roots and with the extension of knowledge, biochemical and genetic basis of biodegradation are being worked out. During the last two decades, substantial information on the microbial degradation of several pesticides was generated from various laboratories in India. Recent reports involve the isolation and screening of many microbes for degradation of xenobiotics. Of these many agriculturally important species have been reported to be involved in the degradation of Atrazine (Sagarkar *et al*., 2013; Kolekar *et al*., 2014; Bhardwaj *et al*., 2015; Dutta *et al*., 2016); Chlorpyrifos (Lakshmi *et al*., 2009; Sasikala *et al*., 2012; Maya *et al*., 2011; Dubey and Fulekar, 2012; Abraham and Silambrarasan, 2013; Maya *et al*., 2012; Silambarasan and Abraham, 2013; Silambarasan and Abraham, 2014 and Pailan *et al*., 2015); 2,4-D and phenoxy herbicides (Gauri *et al*., 2012; Singh, 2013); DDT (Bajaj *et al*., 2014a); Endosulfan (Jesitha *et al*., 2015; Singh and Singh, 2011; Bajaj *et al*., 2014b; Giri and Rai, 2012.) and monocrotophos (Jain *et al*., 2014; Acharya *et al*., 2015); Hexachlorocyclohexane (Negi *et al*., 2016); Nitroaniline, Nitrophenols, Phorate and Phenylurea herbicides. Formulation of bacterial and fungal consortia effective in biomineralisation of pesticides have been reported along with optimisation of conditions for degradation (Abraham and Silambarasan 2014, Bhattacharjee *et al*., 2014). Pesticide degradation genes of several bacteria are usually located in extrachromosomal genomes and encode the degrading enzymes. This has allowed the development of bioremediation technology based on engineered microbes. Also, scientists are planning to transfer the genetic potential of pesticide degradation in other microbes to effectively utilize their potential in the safe disposal of xenobiotic residues.

Genetically engineered or modified crops (GM crops) give superior yields with less use of pesticides but their effects on non-target organisms including soil microbial communities are the major risks. Balachandar *et al*. (2008) recorded no significant difference in the community composition of pink-pigmented facultative methylotrophs (PPFMs) between Bt- and non-Bt cotton. Soil respiration, dehydrogenase and mineral nitrogen were reduced in Bt-cotton soils while microbial biomass carbon and nitrogen and phosphatase showed an increased trend compared to non-Bt cotton (Sarkar *et al*., 2008; Sarkar *et al*., 2009).

Agricultural soils are polluted due to a variety of point and non-point sources. Tripathi *et al*. (2013) assessed the effects of application of long term application (2 decades) of pulp and paper mill effluent on bacterial diversity and found no adverse effects, rather there was an increase in diversity. Of various sources of pollution, the one issue that needs firm attention is heavy metal pollution because they do not degrade or disappear and can only be transformed from one oxidation state to another of lower solubility. Indiscriminate release of heavy metals such as cadmium, copper, lead, nickel, zinc etc. from various sources including fertilizers, urban solid wastes etc., into soil and aquatic environments has altered their geochemical cycles and biochemical balance. Heavy metals adversely affect soil respiration, microbial activity and soil enzyme activity, inhibit nutrient transformation processes such as ammonification and nitrification, reduce earthworm population, suppress algal population, crops yields and quality, soil microflora and cause health problems through accumulation in food chain (Adhya and Pathak, 2013). The bacteria showing resistance to heavy metal toxicity posses specific genes for that and there is evidence that organic farming can enhance proportions of those genes in rhizosphere (Rao *et al*., 2014). Bacterial inoculation can alleviate metal toxicity (Aluminium) of crop plants in polluted soils (Thakuria *et al*., unpublished). Researches on heavy metal bioremediation, have been fewer. While some studies were on heavy metal removal through microbial films including fungal biomass (Joshi *et al*., 2011), majority research has focussed on surfactant producing microbes. Elevated atmospheric CO_2 enhance the biomass production and metal accumulation in plants and influence the plant-microbe-metal interaction in polluted soils (Rajkumar *et al*., 2013).

Microbiology of Climate Change

Increased emissions of GHGs *viz*., carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are anticipated to raise the mean ambient global temperature by 2°C by 2050 causing a change in the global climatic patterns, and thereby, affecting the life systems on the planet (IPCC, 2013). Alternately, increased temperature and elevated CO_2 would result in an increased emission of GHGs, especially CH_4. Incidentally soil microorganisms including heterotrophs-autotrophs, methanogens-methane oxidizers and nitrifier-denitrifiers present in the environment are responsible for the production and consumption of these greenhouse gases in the environment. Most of the microbiological processes such as respiration, methanogenesis, nitrification and denitrification are significantly enhanced due to climatic change.

Studies including both culture-based and non-culture-based methods have helped in the characterization of GHG-cycling microbial population and their dynamics. Molecular methods have emerged successful in the identification and characterization of methanogens in many rice field ecosystems (Singh and Dubey, 2012; Singh *et al*., 2012; Dubey *et al*., 2013). The RFLP-based 16S rRNA gene-targeted phylogenetic analysis showed that clones obtained from rhizospheric rice soils were closely related to diverse group of methanogenic community represented by Methanomicrobiaceae, Methanosarcinaceae, Methanosaetaceae and RC I (Singh and Dubey, 2012; Singh *et al*., 2012). Following flooding of the rice fields, the fermentation of polysaccharides leads to the formation of H_2, acetate and other fermentation products. At this stage, hydrogenotrophic methanogens belonging to Methanocellales, Methanomicrobiaceae and Methanobacteriaceae are found to be more active (Dubey *et al*., 2013). Methanocellales are found to be the more preferred methanogenic archaea in Indian paddy fields in both oxic as well as anoxic soils during plantation of rice crop, thereby contributing a more active role in methane production (Singh and Dubey, 2012; Dubey *et al*., 2014).

As the earth's climate and GHG cycling are considered closely linked climatic changes including increased temperature and elevated CO_2, may also contribute to increased CH_4 emission, and changes in the methanogenic microflora). Elevated CO_2 concentration favoured methanogenesis under field conditions involving rice paddy, attributable to the decrease in the abundance of methanotrophs (Das and Adhya, 2012) as also selective stimulation of specific methanogenic population. Based on whole genome metagenomic approach, four methanogenic genera (*Methanobacterium, Methanosphaera, Methanothermus and Methanothermococcus*) were found to be absent under ambient CO_2 concentration, contrary to their presence at elevated CO_2 level (Bhattacharyya *et al.*, 2016). However, not many studies involving nitrifier-denitrifiers under field situations including their diversity and dynamics have not been worked out under Indian conditions, excepting a few related to sewage treatment plants.

In simulated experiments an increase in methane concentration in atmosphere resulted in a doubling of apparent CH_4 consumption rate (Mohanty *et al.*, 2016). Methanotrophs and heterotrophs increased and there was increase in potential denitrification rate, potential iron and sulfate reduction rate. Methane feedback cycle influenced composition of the methanotrophs community. Methane uptake in soils of soybean-wheat production system under different fertilization regimes in Vertisol showed that it was higher in organic, followed by integrated nutrient management and least in inorganic management irrespective of the crop (Mohanty *et al.*, 2015). Result highlighted that organic farming can significantly decrease global atmospheric CH_4 budget in addition to improving soil physical and biological properties. Kollah *et al.*, (2015) showed that biochar enhanced the methane consumption potential in tropical Vertisols, particularly smaller sized fractions (<0.25 mm), and could be an effective strategy to mitigate atmospheric CH_4. Organic amendments like vermicompost and FYM were more effective than poultry manure in improving methane consumption in soils. But when combined with biochar poultry manure was most effective in improving the population of methanotrophs.

Conclusions and Future Needs

There is now a large repository of microorganisms available for deployment for improving plant growth, nutrition and mitigating abiotic and biotic stresses. Improvement of soil biology is the key to improving soil health. The effects of crop and soil management on the soil microbial community and functionality are well studied. Microbial inoculants are now playing an important role in organic and sustainable agricultural systems. The application of microbial technologies for recycling of municipal, industrial and agricultural wastes is getting popular. It is also being extended for remediation of polluted soils. Some areas requiring more attention in future are:

- Improve the methods of isolation so as to bring a greater proportion of the unculturables into culture. Explore the role of *Acidobacteria* in soil functionality.
- Unravel the role of archaea in maintaining soil health and sustainability and how their activity can be modulated.
- Research the role of soil fauna in nutrient turnover, improving soil and crop productivity in various agricultural situations and practices, an area which continues to be neglected by soil microbiologists.
- Research the methods to improve soil organic matter formation by heterotrophic microorganisms and improve soil carbon sequestration.
- Understand the exact mechanisms by which PGPR alter the physiology and immune system of plant and improve the fitness to withstand biotic and abiotic stresses.
- A deeper understanding of the deleterious rhizobacteria and fungi is needed to understand the mechanisms that lead to pathogen build-up in various unsustainable agricultural practices.
- Identification of the factors that disturb the right balance between the proportion of the beneficial and deleterious microorganisms will be a major area of research that will allow microbiologists and plant breeders to 'tailor' crop varieties in a way to ultimately benefit soil health through rhizosphere engineering.
- Widen the search for more efficient consortia of mineral solubilizing microbes from organic and inorganic native forms, for meeting the deficiency of P, S, Zn etc. and preparation of organo-mineral-biofertiliser preparations.
- More emphasis on improving the inputs of symbiotic nitrogen fixation by legumes through improved rhizobial selection to maximise BNF contribution in the era of climate change.
- Develop one or two sensitive, compound indicators of soil biological health, easily measurable, cost-effective and amenable to rapid automation (based on soil respiration or

genes/gene transcripts). It is a potentially exciting goal and challenge so that scientists can use it in a practical way for advisory purpose on soil health akin to soil testing for soil fertility.

- Quantification of ecosystem services rendered by microbes.

The tremendous advances in agricultural microbiology in last two decades in India are clearly attested to by the discovery of dozens of novel microbial species, hundreds of publications in highly rated journals and microbial technologies adopted in the field by farmers. All these are a cause for optimism. However, some caution is required in microbial taxonomy overly relying on molecular approaches alone. A combination of the classical and modern methods will be required to forge solutions for the above challenges which would ultimately contribute to more sustainable agricultural systems.

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