Sugar Tech
https://doi.org/10.1007/s12355-021-00955-x

RESEARCH ARTICLE

Intercropping in Sugarcane Improves Functional Diversity, Soil Quality and Crop Productivity

S. R. Singh[1] · Poonam Yadav[1] · Dinesh Singh[1] · S. K. Shukla[1] · M. K. Tripathi[1] · Lal Bahadur[2] · Aradhana Mishra[2] · Sanjeev Kumar[1]

Received: 15 September 2020 / Accepted: 11 January 2021

Abstract Intercropping of mustard or potato in sugarcane in relation to traditional non-intercropping rotation on microbial diversity, soil quality and crop productivity was assessed in a 3-year cropping system trial. The systems consisted of sugarcane + mustard–ratoon–cowpea (SmRC), sugarcane + potato–ratoon–wheat (SpRW) and a standard sugarcane–ratoon–wheat (SRW) rotation. The SpRW system recorded a significantly higher cane equivalent yield (120.4 t ha^{-1}) than SmRC (109.4 t ha^{-1}) and SRW (92.6 t ha^{-1}), which was 10.1% and 30.0% greater, respectively. However, the highest microbial activities (microbial counts, microbial biomass carbon and nitrogen and basal soil respiration), soil enzymes, total carbon (TC) and nitrogen (TN), available N, Zn, Cu, Fe and cation exchange capacity (CEC) were recorded for SmRC system. The available K and S content were greater in SRW, while the highest average substrate oxidation rate was recorded in SmRC (0.00291 OD h^{-1}), which was 14.1% and 7.58% more than that of SpRW and SRW systems, respectively. Moreover, SmRC significantly increased functional diversity indices and soil quality index. Total N, soil organic carbon, available P and S were identified as the key soil quality indicators, contributing 31.8, 30.9, 12.9 and 10.8% toward quality development, respectively. The highest functional diversity indices of microbial community, soil quality and crop productivity under intercropping are the result of greater SOC, TC, TN, microbial and enzymatic activities. In conclusion, intercropping of mustard or potato in sugarcane could be the way to increase crop productivity in limited land resources in subtropical areas of India.

Keywords Alluvial soil · Intercropping · Microbial diversity · Enzymatic activity · Soil quality

Abbreviations

ACP	Acid phosphatase activity
AFB	Ammonifying bacteria
ALP	Alkaline phosphatase activity
AWCD	Average well color development
AZO	*Azotobacter*
BD	Bulk density
BSR	Basal soil respiration
CEC	Cation exchange capacity
DHA	Dehydrogenase activity
FDA	Fluorescein diacetate hydrolytic activity
INFR	Infiltration rate
MBC	Microbial biomass carbon
MDS	Minimum data set
MBN	Microbial biomass nitrogen
NFB	Nitrifying bacteria
OD	Optical density
PC	Principal component
PSM	Phosphate solubilizing microorganisms
SmRC	Sugarcane + mustard–ratoon–cowpea
SpRW	Sugarcane + potato–ratoon–wheat
SOC	Soil organic carbon

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12355-021-00955-x.

✉ S. R. Singh
shivramsingh22@gmail.com

✉ Sanjeev Kumar
sanjeeviivr@gmail.com

[1] ICAR-Indian Institute of Sugarcane Research, Raibareli Road, P.O. Dilkusha, Lucknow 226002, India

[2] CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow 226001, India

Published online: 29 January 2021

SQI	Soil quality index
SRW	Sugarcane–ratoon–wheat
TC	Total carbon
TCA	Total counts of culturable actinomycetes
TCB	Total counts of culturable bacteria
TCF	Total counts of culturable fungi
TN	Total nitrogen
WHC	Water holding capacity

Introduction

In terms of area, India is the seventh largest country with 1.30 billion population (FAO 2017; Un-Pop 2017), which is expected to reach > 1.60 billion by the year 2050. This will pose a challenging task for providing food security including sugar, molasses and jaggery and bio-ethanol to 18% of the world population residing in 2.4% of the world total land area (Bhattacharyya et al. 2015). Sugarcane is a key commercial crop in India that provides sugar, molasses and jaggery. India ranks after Brazil in area (4.95 m ha) and production (395 m t) during 2017–2018 (Sugar Annual India 2018). The ever-increasing demand of sugar, molasses, jaggery and bio-ethanol is a challenging task. Apart from sugar crops, the demand of potato and oilseed is also increasing gradually due to increasing population. In India, potato is well known as the poor man's food due to its ability to provide major proportion of carbohydrates to a larger population and consumed both in fresh form and as processed products. Mustard is the second most important oilseed crop after groundnut sharing 27.8% in the Indian edible oil economy. However, due to lower productivity, shrinking coverage area and the greater demand of mustard as edible oil and monocropping, its production requirements are not fulfilled. Hence, there is an urgent need to exploit intensive/diversified crop production system so as to meet food security challenges.

Intercropping can be a viable option to increase productivity of short duration crops like vegetables, oilseeds, legumes, maize and spices. As wide spacing (90 cm) between two rows, long duration for sprouting (35–45 days), initial slow growth rate and compensating ability of losses provide ample opportunities for intercropping in sugarcane. Intercropping also minimizes weeds that draw huge amounts of nutrients and moisture. Intercropping plays a key role in increasing efficiency of water and land use, nutrient, energy, helping reducing soil erosion, environmental pollution, and decreasing the risk of crop failure or disease (Wang et al. 2014). A wide range of intercropping systems has been developed with strong synergistic effects on crop productivity compared to monoculture (Hossain et al. 2003). As roots of different plant species interact directly with each other under intercropping system; thereby, subsequent root exudation is liable to alter microbial diversity, enzymatic activity and crop productivity (Li et al. 2013; Singh et al. 2017; Lian et al. 2019). Wang et al. (2014) reported that soil organic matter does not differ significantly under mono-cropping, but did increase maize/chickpea and maize/turnip yield in intercrop systems. However, a number of physical, chemical and biological properties have been reported to alter due to intercropping. This could be due to differences in growth behavior, rhizodeposition, nutrient intake and plant residues, etc. (Thierfelder and Wall 2012; Cong et al. 2014).

Pulses fit very well as intercrop with autumn/spring planted sugarcane and exert a synergistic effect on cane yield, microbial activities and economic efficiency (Li et al. 2013). Sugarcane/soybean intercropping has been recognized as potential system for improving productivity over space and time in subsistence forming owing to greater utilization efficiency of light, stability of yields, and resilience to perturbations and reducing N-leaching (Yang et al. 2013). Intercropping gives more yield than monoculture or rotation cropping and maintains various soil chemical and enzymatic activities (Wang et al. 2015). Significantly more yield and nutrient acquisition advantages have also been reported in maize/soybean, maize/-faba bean, sorghum/soybean, maize/cowpea, wheat/chickpea, soybean/pigeonpea and wheat/mung bean as compared to sole rotation without applying extra inputs (Mei et al. 2012). Sugarcane yield was not adversely affected with potato as intercrop in the center of ridge, but potato yield responds well to higher plant density liable to increased potato yield up to 22.5 t $ha^{-1}$with good crop management (Nankar 1990). Intercropping of potato in sugarcane enhanced cane yield and net returns than sole cane due to better use of residual P and K, and to a lesser extent of N (Imam et al. 1990). Organic carbon and microbial respiration were greater as maize, wheat or mustard intercrop with sugarcane (Suman et al. 2006). Intercrop of potato and mustard with sugarcane in sugarcane–ratoon–wheat rotation affected nutrient acquisition, and fertility status that has apparent effects on microbial biodiversity and soil quality (Paungfoo-Lonhienne et al. 2015; Mariano et al. 2016; Lian et al. 2018). The bacterial community richness as well as diversity was significantly higher in mustard–eggplant and oilseed rape–eggplants than sole eggplant crop (Li et al. 2017). Also, intercropping shifted fungal community structures and accomplished important ecological functions including carbon and nutrient cycling, plant growth promotion, pathogenesis, and parasitism in agriculture ecosystems (Rachid et al. 2015). However, sugarcane monoculture had a negative

impact on soil compaction, structural degradation, soil organic carbon, abundance and diversity of macro- and micro- fauna (Cherubin et al. 2016). Intercropping of pulses with sugarcane on crop yield and net returns has been studied previously (Imam et al. 1990; Nankar 1990; Suman et al. 2006), but meager information is available on intercropping of mustard/potato in sugarcane on microbial diversity and soil quality. Therefore, the present investigation was undertaken to assess the impact of mustard or potato as intercrop with sugarcane on microbial diversity, soil quality and crop yield.

Materials and Methods

Site Description

The cropping system experiments were conducted over three consecutive years (2014–2015, 2015–2016 and 2016–2017) at ICAR-Indian Institute of Sugarcane Research, Lucknow, Uttar Pradesh, India (26°56′N, 80°52′E and 111 m above sea level). The experiment site lies in sub-tropical climate with mean annual precipitation of 763.0 mm. The mean minimum and maximum air temperatures were 21.0 and 31.3 °C, whereas the mean relative humidity at morning and noon was 92 and 62% during the crop season, respectively. The texture of experimental field is a silty loam (6.50% clay, 70.0% silt and 23.5% sand) developed by deposition of alluvium carried out through the River Ganges and its tributaries. The initial soil characteristics of the experiment site were as follows: soil organic carbon 4.70 g kg^{-1}, total N 2.89 g kg^{-1}, available N 109.5 mg kg^{-1}, P 5.44 mg kg^{-1}, K 74.4 mg kg^{-1}, Zn 0.66 mg kg^{-1}, Cu 0.76 mg kg^{-1}, Fe 8.59 mg kg^{-1}, Mn 6.62 mg kg^{-1}, CEC 14.5 $cmol^{+}$ kg^{-1}, pH 8.07, EC 0.10 dSm^{-1}.

Experimental Design

The field experiment was designed with three intercrop systems and four replications in a randomized block design. The three intercrop systems consisted of sugarcane + mustard–ratoon–cowpea (SmRC), sugarcane + potato–ratoon–wheat (SpRW) and a standard sugarcane–ratoon–wheat (SRW) rotation. After land preparation, deep furrows (> 20 cm) were made at 90 cm row spacing using tractor-drawn furrow opener. Before sugarcane planting, 1/3rd dose of N, full doses of P_2O_5 and K_2O were applied in furrow. Sugarcane was planted in furrows with three-node setts at the end-to-end sequence (∼ 35,000 setts ha^{-1}). The field was leveled immediately after cane planting using light leveler. Intercrops, viz. potato and mustard, were planted/sown between the vacant spaces of two rows of sugarcane and harvested before closing the cane canopy. Potato tubers were planted into two ridges at 30 cm and plant to plant spacing of 15 cm under SpRW system. Similarly, mustard was line sown in two rows at 30 cm and plant to plant at 10 cm under SmRC system. The rest of the test crop was grown as sole crop, according to the standard system. The planting/sowing and harvesting time of each test crops were taken within a system (Table S1). After planting/sowing of intercrop, the emphasis was on to intercrop for maintaining optimum plant population and weed-free plot to achieve the highest yield. A light hoeing was done at 15–20 days after germination to break the crust and remove the weeds. Two earthing up in potato, in which first was performed when plant attained 15–20 cm height (stolon formation) and the second was done to cover up the tubers. A light irrigation was applied in furrow as and when required in potato. The weeding is very important in mustard for maintaining plant population and weed-free plot. The first manual weeding was done at 20 days and second at 40 days after germination. Similarly, two light irrigations were applied for obtaining the highest yield of mustard. The first irrigation was done at pre-flowering and second at pod filling (siliqua formation) stage. After harvesting of intercrops, a deep manual intercultural operation was done within interspaces of cane rows for breaking hard pans and removing weeds. Before irrigation, fields were left for one week to achieve proper drying of weeds. At 4–5 days after irrigation, the 1/3rd dose of N was applied as a side dressing in rows. Intercultural operations and irrigations were continued up to last week of June as per the requirements. The remaining 1/3rd dose of N fertilizer was applied after irrigation (4–5 days) as a side dressing in the last week of June. The recommended doses of N, P_2O_5 and K_2O were applied in each test crop at critical growth stages through urea, diammonium phosphate and muriate of potash (Table S2). After June, the field was left for attaining the maximum growth as the active growth phase of sugarcane occurs from July to September (rainy season). After harvesting of plant crop at maturity (12 month), cane trash was removed immediately from experimental plots. Ratooning is the process in which cutting above ground portion (stubble cutting) but leaving the roots and growing shoots apices intact so as to allow the plants to recover and produce a fresh crop in the next season. A deep interculture operation followed manually after stubbles were removed and applied half the dose of N and full doses of P_2O_5 and K_2O in furrows. After ratoon initiation, potato tubers and mustard were planted/sown immediately, and after germination, light irrigation was applied for promoting root growth of ratoon as well as increased growth of intercrops. After harvesting of potato and mustard, a deep interculture followed manually and irrigated after one week weeding and

covered the inter-row spaces with sugarcane trash. The 1/3rd dose of N fertilizers applied in furrow by side-dressing; thus the first and second ratoons were maintained till the harvest as plant crop. Grain and straw yield of wheat, mustard and cowpea were recorded at maturity by difference methods. The dry weights of biological yield (grain + straw) and grain yields were measured separately. Straw yield was quantified by subtracting grain yield from biological yield (straw yield = biological yield - grain yield). The tuber and haulm yield were measured separately from each plot and converted into yield t ha^{-1}. Cane yield (t ha^{-1}) was recorded immediately after removing leaves from the harvested cane stalk. To compare the system performance, the cane equivalent yield (CEY) was calculated [CEY = yield of crop in sequence (t ha^{-1})- × price of the crop (Rs t^{-1})/price of sugarcane (Rs t^{-1})] by converting yield of non-cane test crop into equivalent cane yield by using minimum support price (MSP) declared by Government of India, and value of straw/stover yield was assessed separately based on local market price.

Experimental Analysis

Random soil samples were collected before initiation and after completion of the cropping cycle at four places in each plot (0–15 cm depth) and combined together into a composite sample for determining chemical and biological properties of soil. About 250 g fresh moist soil samples was packed in air-tight plastic bags and stored at − 20 °C for analysis of microbial and enzymatic activities. Another 250 g soil samples were air-dried, ground, passed through a 2-mm sieve and stored for the determination of chemical properties. Texture, soil pH, EC, available N, P, K, S and CEC were determined using the methods as suggested by Jackson (1973). The SOC, TC and TN were determined by TOC analyzers (Multi N/C-2100S-Analytic-Jena), while Zn, Cu, Fe and Mn were analyzed (USDA 1996) using atomic absorption spectrophotometer (Z2300, Hitachi Science and Technology). Separate soil samples were collected from three places in each plot (0–15 cm depth) using a core sampler (height 8 cm × 6 cm diameter). The mean value of all these three samples from each plot was used for the determination of final bulk density (BD) as suggested by Karim et al. (1988). The water holding capacity (WHC) was measured following the method of Cassel and Nielsen (1986).

The serial dilution and plate counting method was applied for total counts of culturable bacteria, fungi, actinomycetes, *Azotobacter* and phosphate solubilizing microorganisms using selective media. The ammonifying bacteria and nitrifying bacteria were determined by MPN technique. The replicated culture plates were used in each dilution (3 dilutions per microbe) for each treatment (3 × 3 = 9 plate per treatment). The microbial biomass carbon and nitrogen, basal soil respiration, dehydrogenase, fluorescein diacetate hydrolytic, urease, and alkaline and acid phosphatase activities were determined by methods as suggested by Alef and Nannipieri (1995). Community level physiological profiling (CLPP) was assessed by BIOLOG Eco Microplate system (Biolog Inc., Hayward, Ca, USA) in which three technical replications per treatment were carried out (Lin et al. 2007). The plates were incubated for 168 h at 25 °C and the optical density (OD) was recorded at 590 nm with an automatic plate reader (Thermo scientific Multiskan MK3, Shanghai, China) after color development at every 24-h intervals. The microbial activities in each micro-plate were expressed as average well color development (AWCD) was measured as follows: AWCD = $\sum$(C-R)/31, where C is the optical density within each well; R is the absorbance value of the plate control well. According to Choi and Dobbs (1999) method, 31 carbon substrates in Eco micro-plates were subdivided into six categories (polymers, carbohydrates, carboxylic acid, amino acids, amines, and phenol compounds). The optical density (at 96 h incubation time) was used to calculate diversity and evenness indices as well as principal component analysis (PCA) because it was shortest incubation time that provided the best resolution for all treatments (Gomez et al. 2006). The average substrate oxidation rate was calculated by summation of average OD value derived from AWCD per hour.

Soil Quality

Soil quality index (SQI) was computed after defining the goal, selection of a minimum data set (MDS) of indicators which exhibited best representation in relation to soil functions, scoring the MDS indicators based on their response to soil function, and drawing meaningful conclusion from indicator scores in to relative SQI. In this study, yield of test crops was mull as a defined goal as farmers would like to achieve more productivity from each of the test crop. The physical, chemical and biological variables of soil had significant variations among the cropping systems were chosen to develop MDS using PCA as suggested by Andrews et al. (2002a, b). Attributes having high eigenvalues and factor loading screened by PCA were considered to be the ideal variables, and hence, PC that had eigenvalues > 4 and explain at least 5% of the trait variation were investigated (Wander and Bollero 1999). The multivariate Pearson's correlation coefficient was applied to retain more than one factor in a single PC. The high weighted variables could be considered redundant and therefore removed from the MDS (Andrews et al. 2002a). If the highly weighted variables were not correlated to each other within the PCs, then it was treated mull

as crucial and retained in the MDS. After choosing the MDS indicators, every observation of each indicator was transmuted using a nonlinear scoring method (Andrew et al. 2002b). Indicators were arranged in order depending on whether a higher value was considered 'good' or 'bad' in terms of soil function, and were scored as 'more is better' or 'less is better'. A nonlinear scoring function (NLSF) for transformation and normalization for each indicator was used having a value between 0 and 1 which was computed as NLSF (Y) = $1/[1 + e^{-b(x-A)}]$, where 'x' is soil property value, 'A' the baseline or value of soil property where score equal to 0.5 and 'b' is slope.

Once the values of soil functions indicator transformed, the MDS attributes for each observation were assigned weightage using PCA results. Since each PC explains a certain proportion (%) of trait variation; it was divided by total variation explained by all PCs with eigenvectors > 4, given the weighted factor for variables selected under a given PC. Then the weighted MDS variables scores were summed up for each observation using the following equation:

$$\text{SQI} = \sum_{i=1}^{n} W_i \times S_i$$

where SQI is the soil quality index, 'W_i' is the weight value of each indicator, 'S_i' in the indicator score for the subscripted variable and 'n' is the number of indicators in the minimum data set. The SQI values obtained by the sum of final key indicators under different cropping system were tested for their level of significance at $P < 0.05$.

Statistical Analysis

The differences in the variables related to the different cropping system were analyzed with ANOVA employing SPSS 16.0 software for Windows (SPSS 2001). Duncan's multiple range test was applied to measure significant differences among the variables ($P < 0.05$). The mean data of three cropping years were presented with standard errors of means SEm $\pm$ = SD/$\sqrt{n}$. Correlations between the variables were assessed by determining Pearson product–moment correlation coefficients and probability at $P < 0.05$ and $P < 0.01$ levels of significance. The PCA was performed on all the data for computation of eigenvalues, variability and drawing bi-plot (Wold et al. 1987).

Results

Crop Productivity

Compared with SRW system, potato or mustard intercrop with sugarcane under SpRW or SmRC had no apparent effect on cane yield during the first and second year, but the ratoon yield decreased significantly during the third year under SmRC system (Table 1). The highest cane yield was observed in the SRW system, where sugarcane was taken as sole crop, which was comparable to the cane yield obtained under SpRW and SmRC systems during the first- and second-year crop (Table 1). The cane yield decreased by 2.80, 5.29 and 4.33% during the first, second and third year, respectively, as mustard intercrop with sugarcane under SmRC system than SRW. The potato yield decreased by 7.93% when intercropped with first sugarcane ratoon compared to plant crop. However, there was no clear trend on the mustard yield intercropped with sugarcane under SmRC during all the three years. The wheat yield increased by 21.6% when it was grown as a third crop under SpRW system. A significantly higher cane equivalent yield (CEY) was observed in SpRW (133.7 and 126.4 t ha^{-1}) over SmRC and SRW during the first and second year, but it was greater under SmRC (110.9 t ha^{-1}) during the third year. Overall, SpRW system increased CEY by 30.0 and 10.0% over SmRC and SRW, respectively (Table 1).

Microbial and Enzymatic Activity

The total counts of culturable bacteria, actinomycetes, *Azotobacter*, phosphate solubilizing microorganisms, ammonifying and nitrifying bacteria increased significantly under SmRC over SpRW and SRW, but fungal counts were the highest under the SpRW system (Table 2). The SmRC system also recorded significantly increased MBC, MBN, and BSR, which showed enhancement of 13.2 and 6.57, 4.07 and 1.89 and 23.1 and 13.1% over SRW and SpRW systems, respectively. Similarly, DHA (2.36 µg g^{-1} h^{-1}), FDA (11.9 µg fluorescein g^{-1} h^{-1}), urease (73.1 µg g^{-1} h^{-1}), ACP (175.5 µg g^{-1} h^{-1}) and ALP (260.4 µg g^{-1} h^{-1}) activities were greater in the SmRC system compared to that of SpRw and SRW systems (Table 2).

BIOLOG Analysis

The BIOLOG results showed that the lag phase under SRW and SpRW was quite longer, whereas SmRC had shorter phase prior to the color development, and it altered with an increase in incubation time (upto 168 h). The SmRC system showed a greater rate of carbon substrate utilization (CSU) up to 144-h incubation than SpWR and SRW, but it decreased and recorded the lowest at 168 h. However, SRW displayed significantly lower CSU rate across the incubation period (Fig. 1). The average substrate oxidation rate was 0.00291 OD h^{-1} in SmRC system, which was 14.1% and 7.84% greater than those of SRW and SpRW systems, respectively. The consumption of amines/amides, amino

Table 1 Effect of different cropping systems on crop productivity during 2014–2017 (mean ± standard errors of mean)

Cropping system	Crop yield (t ha^{-1})						System-wise cane equivalent yield (t ha^{-1})			
	2014–2015		2015–2016		2016–2017		2014–2015	2015–2016	2016–2017	Mean
	Intercrops	Main crop	Intercrops	Main crop	Main + Intercrop	Main crop	Main crop			
SRW	–	92.2^{a} ± 2.51	–	89.6^{a} ± 3.72	3.99^{bc} ± 0.32	74.7^{a} ± 1.92	–	92.2^{c} ± 2.51	89.5^{c} ± 3.72	96.0^{c} ± 0.76	92.6^{c} ± 2.33
SpRW	27.5^{a} ± 2.0	91.3^{a} ± 1.77	25.2^{a} ± 1.65	87.7^{a} ± 2.77	4.85^{a} ± 1.79	75.4^{a} ± 2.06	–	133.7^{a} ± 4.11	126.4^{a} ± 4.69	101.1^{b} ± 3.57	120.4^{a} ± 3.42
SmRC	0.79^{c} ± 0.19	89.7^{a} ± 2.23	1.66^{c} ± 0.06	85.1^{a} ± 1.90	1.83^{c} ± 0.92	71.6^{ab} ± 1.45	0.97 ± 0.09	111.8^{b} ± 1.80	105.6^{b} ± 1.20	110.9^{a} ± 2.84	109.4^{b} ± 1.94

Different letters within the same row indicate significant differences (Duncan's multiple range test, $p < 0.05$). For abbreviation of cropping systems, please refer to abbreviations section

acids, carbohydrates, carboxylic acids and polymers was significantly greater under SmRC (Fig. 2). Among the sources of carbon substrate, amino acids were the most preferred by soil microbial communities across the system, except amide/amines which was utilized the highest in SRW system. The phenyl ethylamine was the most utilized carbon source than putrescence by the microbial communities found across the systems (Fig. 2b). The L-asparagine, L-serine and glycyl-L-glutamic acid were maximally utilized by the microbial communities under SmRC system, whereas L-arginine, L-phenylalanine and L-threonine were highly utilized in SpRW system (Fig. 2c). Among the carbohydrates utilized by the microbial communities, D-mannitol, D-cellobiose, α-D-lactose and i-erythritol under SRW, D-xylose and N-cetyl-D-glucosamine in SmRC, and D-cellobiose and β-methyl-D-glucoside in SpRW were the most preferred sources of carbon. The α-D-lactose was the least preferred source of carbon in SmRC (Fig. 2c). Tween-80 polymer was the highest consumed by the microbial communities of SRW system followed by SmRC and SpRW and α-cyclodextrin polymer was the least utilized. The pyruvic acid methyl ester, Tween-40, α-cyclodextrin and glycogen were maximally consumed by the microbial communities of SmRC system followed by SpRW and SRW (Fig. 2d). SmRC system utilized the pollutants at maximum as carbon substrate followed by SRW and SpRW. Among the pollutants, D-galacturonic acid was consumed maximally under SmRC and D,L-α-glycerol phosphate was least utilized in SpRW. Overall, glucose-1-phosphate and γ-hydroxybutyric acid were the least utilized across the systems (Fig. 2e).

The computation of 96 h AWCD data using PCA clearly differentiated response of cropping systems on soil microbial communities through carbon substrates. The BIOLOG ECO microplate data suggested that the PC1 and PC2 contributed 53.5% and 46.5% cumulative variations, respectively (Fig. 3). In PC1, out of 31, a total of 14 (five carbohydrates, four carboxylic acid, three polymers and one each of amino acid and amine/amide) had a coefficient of > 0.2. In which, Tween-40, D-glucosaminic acid and N-acetyl-D-glucosamine discriminated most positively out of seven carbon substrates, whereas phenyl ethylamine, α-D-lactose and 2-hydroxy benzoic acid contributed most negatively out of seven substrates in PC1 (Table 3). The SmRC system had the highest positive eigenvalues in both the PCs clearly indicating that microbial communities in that system utilized most of the compounds as carbon substrate (Table 3). However, out of 10 carbon substrate, L-phenylalanine, L-threonine and β-methyl-D-glucoside were discriminated most negatively in PC2. The community level physiological profile (CLPP) diversity indices assess with AWCD data at 96 h was significantly higher under

Table 2 Effect of different cropping systems on activity and functional diversity of soil microbial communities (mean ± standard errors of mean)

Cropping system	SRW	SpRW	SmRC
Microbial counts			
TBC ($\times 10^6$ cfu g^{-1}soil)	$6.44^{c} \pm 0.34$	$7.96^{b} \pm 0.10$	$8.46^{a} \pm 0.20$
TCA ($\times 10^5$ cfu g^{-1}soil)	$4.76^{b} \pm 0.26$	$5.26^{a} \pm 0.08$	$5.31^{a} \pm 0.15$
TCF ($\times 10^4$ cfu g^{-1}soil)	$3.59^{c} \pm 0.22$	$4.84^{a} \pm 0.13$	$4.29^{b} \pm 0.11$
AZO ($\times 10^4$ cfu g^{-1}soil)	$53.4^{bc} \pm 1.08$	$56.0^{b} \pm 1.12$	$60.1^{a} \pm 1.09$
PSM ($\times 10^4$ cfu g^{-1}soil)	$4.65^{c} \pm 0.20$	$5.12^{b} \pm 0.08$	$5.45^{a} \pm 0.09$
AFB ($\times 10^4$ cfu g^{-1}soil)	$6.01^{c} \pm 0.26$	$6.63^{b} \pm 0.17$	$7.21^{a} \pm 0.20$
NFB ($\times 10^4$ cfu g^{-1}soil)	$2.32^{c} \pm 0.14$	$2.54^{b} \pm 0.10$	$3.04^{a} \pm 0.09$
MBC, MBN, BSR and soil enzymes			
MBC (μg C g^{-1} soil)	$235.9^{c} \pm 3.04$	$251.4^{b} \pm 2.49$	$267.1^{a} \pm 3.14$
MBN(mg NH_3-N $kg^{-1}d^{-1}$)	$2.70^{b} \pm 1.92$	$2.75^{ab} \pm 1.61$	$2.81^{a} \pm 1.19$
BSR (mg CO_2-C$g^{-1}d^{-1}$)	$4.89^{c} \pm 0.39$	$5.53^{b} \pm 0.27$	$6.03^{a} \pm 0.34$
DHA (μg TPF g^{-1} h^{-1})	$1.17^{c} \pm 0.06$	$1.91^{b} \pm 0.08$	$2.36^{a} \pm 0.02$
FDA (μg fluorescein g^{-1} h^{-1})	$9.63^{c} \pm 0.25$	$10.6^{b} \pm 0.16$	$11.9^{a} \pm 0.24$
Urease (μg NH_4^+ g^{-1} h^{-1})	$59.0^{bc} \pm 1.94$	$62.8^{b} \pm 1.61$	$73.1^{a} \pm 1.19$
ACP (μg PNP g^{-1} h^{-1})	$162.9^{b} \pm 0.92$	$155.4^{c} \pm 1.56$	$175.5^{a} \pm 2.42$
ALP (μg PNP g^{-1} h^{-1})	$240.3^{b} \pm 1.31$	$258.0^{a} \pm 3.23$	$260.4^{a} \pm 2.65$
Functional diversity indices			
Shannon diversity index	$3.33^{b} \pm 0.010$	$3.362^{ab} \pm 0.019$	$3.377^{a} \pm 0.007$
Shannon evenness index	$0.969^{bc} \pm 0.003$	$0.979^{b} \pm 0.006$	$0.983^{a} \pm 0.002$
Mclntosh diversity index	$0.968^{b} \pm 0.001$	$0.988^{ab} \pm 0.003$	$0.990^{a} \pm 0.001$
McIntosh evenness index	$0.901^{b} \pm 0.011$	$0.922^{a} \pm 0.002$	$0.920^{a} \pm 0.004$
Simpson diversity index	$0.958^{b} \pm 0.003$	$0.978^{a} \pm 0.006$	$0.978^{a} \pm 0.001$

Different letters within the same row indicate significant differences (Duncan's multiple range test, $p < 0.05$). For abbreviation of biological variables and cropping systems, please refer to abbreviations section

SmRC followed by SmRW. Shannon diversity index, Shannon equitability (Shan E_H), Mclntosh diversity (MacD), Mclntosh evenness (Mac E_V) and Simpson diversity (Simpson) were significantly greater for SmRC system than SRW, but it was at par with SpRW in most of the cases (Table 2).

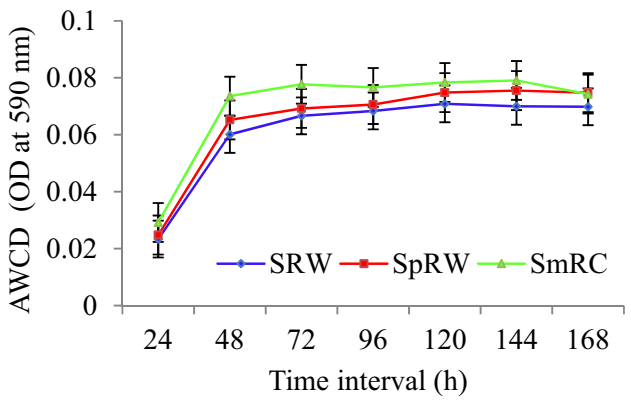

Fig. 1 Change in average well color development (AWCD) of soil microbial community with incubation time in soil samples collected from different cropping systems

Soil Physical and Chemical Properties

Intercropping had no effect on soil pH, BD and Mn but HWC, SOC, TC and TN and C:N ratio altered significantly ($P < 0.05$), though the highest were under SmRC over SpRW and SRW systems. The SmRC and SpRW systems increased SOC, TC and TN by 12.6 and 6.90%, 27.9 and 8.20%, 8.19 and 5.25% over SRW, respectively (Table 4). The SmRC system led to a significant increase in available N, P, Zn, Cu, Fe and CEC over SpRW and SRW, while levels of K and S showed highest in SRW ($P < 0.05$).

Soil Quality

Principal component analysis was employed to screen out the weighted variables by considering 28 physical, chemical and biological attributes of soil measured from various systems. The three PCs that had eigenvalue > 3.0 and

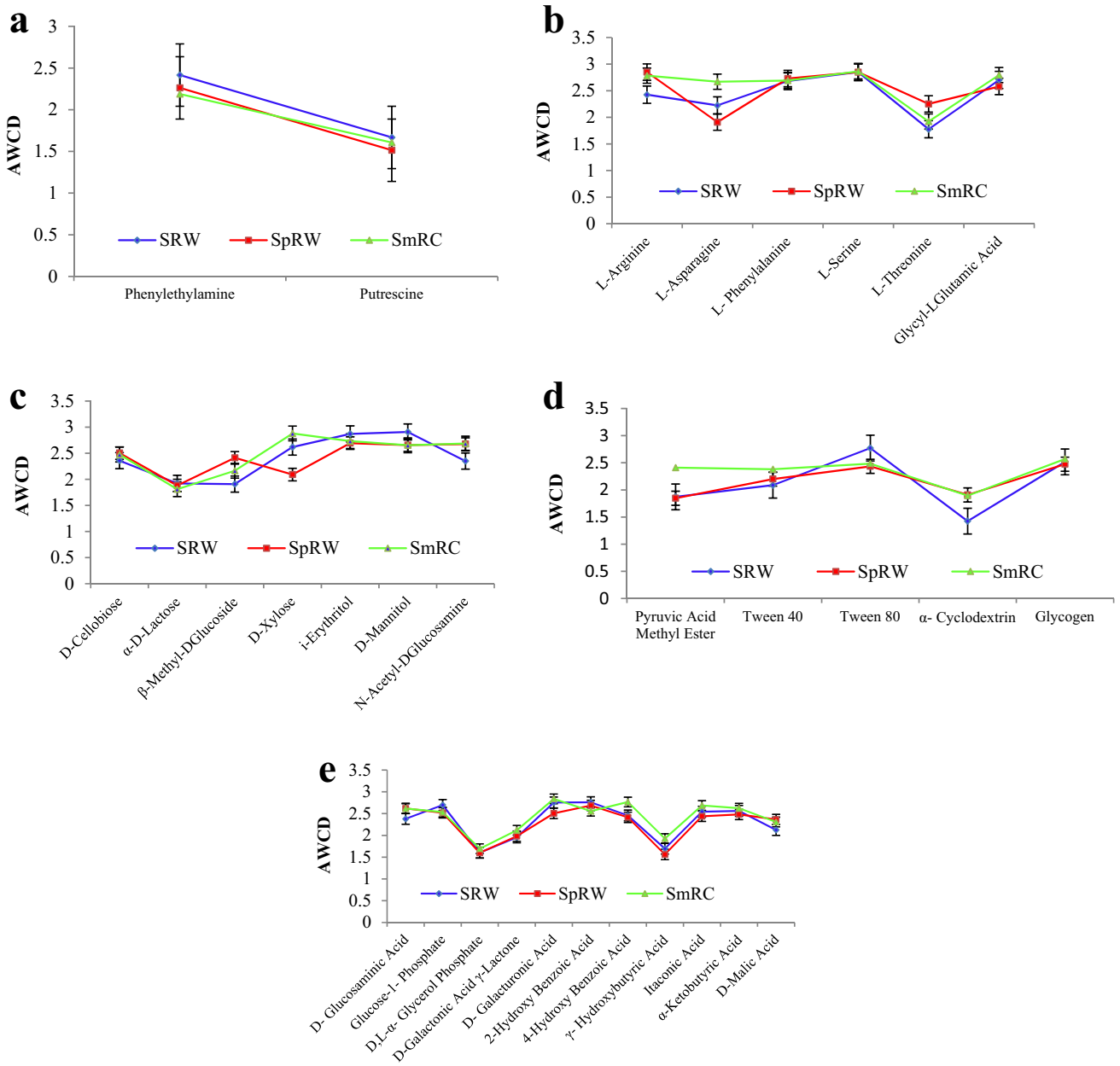

Fig. 2 Carbon source utilization pattern (derived from AWCD OD at 590 nm) of different components by soil microbial communities under different cropping systems in IGP region. **a** amines/amides utilization pattern, **b** amino acid, **c** carbohydrates, **d** polymers, **e** pollutant. For abbreviation of cropping systems, please refer to abbreviations section

89.7% cumulative variance were selected. Individually, PC1, PC2 and PC3 explained 62.2%, 14.9% and 12.6% trait variability, respectively (Table 5). The TN, SOC, BSR, AZO and TC had a higher factor loading in PC1, in which TN and SOC were retained as minimum data set (MDS). In PC2, available P and PSM dominated weighted variables, but P was chosen as MDS, while in PC3, only sulfur was selected for MDS. In this way, TN, SOC, P and sulfur comprised the MDS. After deciding the anticipated response, 'more is better' was used for the all the MDS and assigned the thresholds values considering the site-specific characteristics and management goals (Table 6). Weightage of the indicator for MDS was assigned that was equal to the variance explained by three PCs. As TN and SOC were high factor loading attributes in PC1, thus the weight (0.622) was equally divided between the two variables as non-significant positive correlation between them. Since, P and S contents were retained as indicators due to high factor loading variables in PC2 and PC3, full weight equal to 0.149 and 0.126 was assigned, respectively (Table 5).

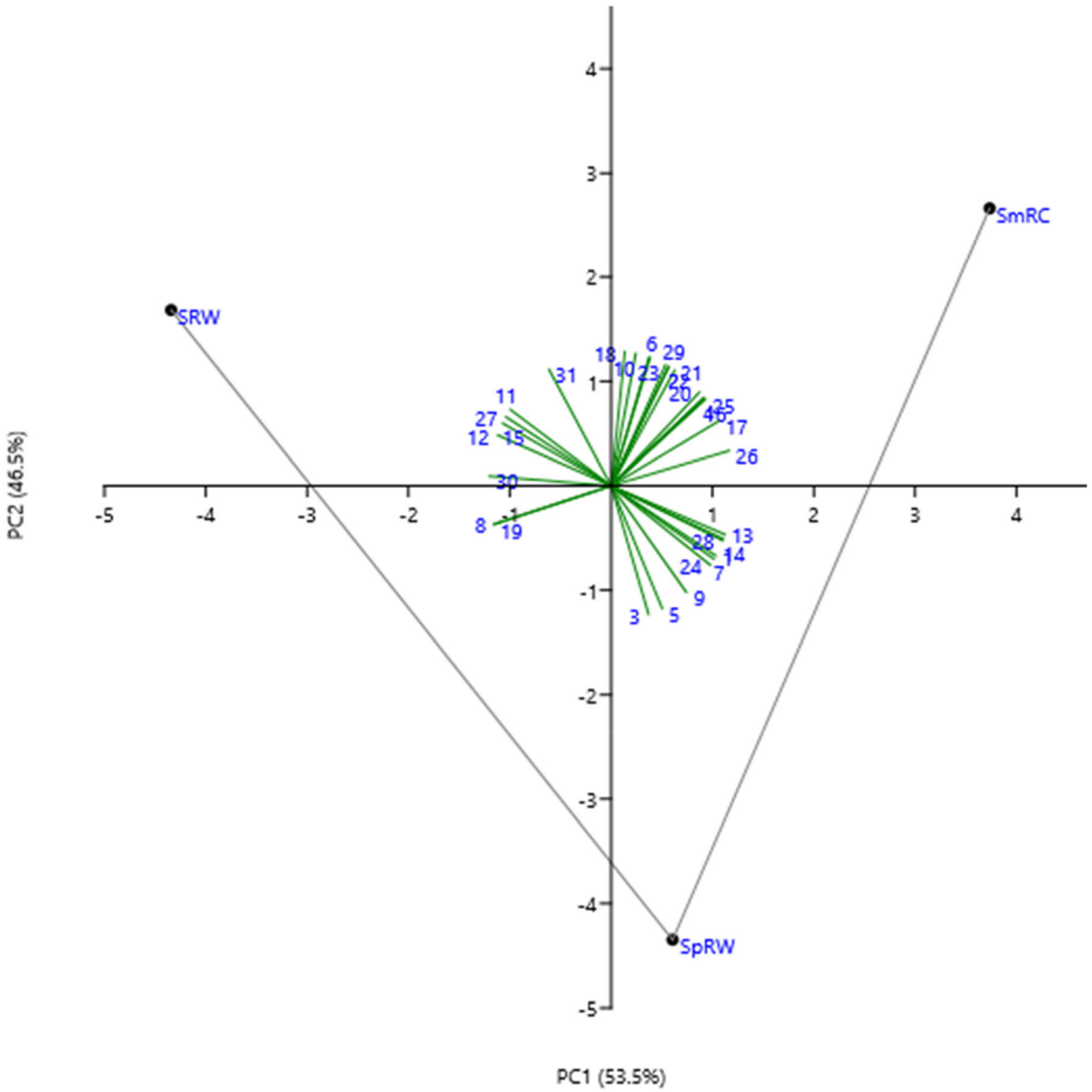

Fig. 3 Principal Component analysis (PC1, PC2) of substrate utilization patterns from different cropping systems. Variable of carbon substrate, viz., 1: L-Arginine, 2: L-Asparagine, 3: L-Phenylalanine, 4: L-Serine, 5: L-Threonine, 6: Glycyl-L-Glutamic Acid, 7: D-Cellobiose, 8: α-D-Lactose, 9: β-Methyl-D-Glucoside, 10: D-Xylose, 11: i-Erythritol, 12: D-Mannitol, 13: N-Acetyl-D-Glucosamine, 14: D-Glucosaminic Acid, 15: Glucose-1-Phosphate, 16: D,L-α-Glycerol Phosphate, 17: D-Galactonic Acid γ-Lactone, 18: D-Galacturonic Acid, 19: 2-Hydroxy Benzoic Acid, 20: 4-Hydroxy Benzoic Acid, 21: γ- Hydroxybutyric Acid, 22: Itaconic Acid, 23: α-Ketobutyric Acid, 24: D-Malic Acid, 25: Pyruvic Acid Methyl Ester, 26: Tween 40, 27: Tween 80, 28: α-Cyclodextrin, 29: Glycogen, 30: Phenyl ethylamine, 31:Putrescine. For abbreviation of cropping systems, please refer to abbreviations section

The SQI was computed by stating the estimated factors to the soil quality indicators as follows:

$$\mathrm{SQI} = [0.311S_{\mathrm{TN}} + 0.0311S_{\mathrm{SOC}} + 0.149S_{\mathrm{P}} + 0.126S_{\mathrm{S}}]/0.897$$

$$\mathrm{SQI} = 0.35S_{\mathrm{TN}} + 0.35S_{\mathrm{SOC}} + 0.16S_{\mathrm{P}} + 0.14S_{\mathrm{S}}$$

where '*S*' is the score for the subscripted variable and coefficients are the weighting factors.

These results clearly show that SmRC cropping sequence recorded significantly greater SQI than SpRW and SRW. Bi-plot study clearly indicated that maximum number of active variables was observed under SmRC system followed by SpRW and SRW contributed 82.7% under PC1 and 16.3% under PC2 (Fig. 4). Among the cropping systems, the SQI ranged from 0.837 (SRW) to 0.903 (SmRC). The specific contribution of each indicator toward the SQI is presented in Fig. 5. The TN contributed the highest (31.9%) toward the SQI, followed by SOC (30.9%), P (12.9%) and sulfur content (10.8%). The polynomial correlation between SQI and CEY yield was

Table 3 Details of different parameters of principal components (PC1, PC2) analysis of substrate utilization from different cropland grown systems derived from measurements on BIOLOG Eco Plates™

Component	Eigenvalue	Variance (%)	Substrate	Substrate type	Coefficient†
PC1	16.6	53.5	L-Arginine	A	0.210
SRW	− 4.3417		D-Cellobiose	C	0.206
SpRW	0.6064		α-D-Lactose	C	− 0.236
SmRC	3.7353		i-Erythritol	C	− 0.203
			D-Mannitol	C	− 0.228
			N-Acetyl-D-Glucosamine	C	0.229
			D-Glucosaminic Acid	K	0.226
			Glucose-1-Phosphate	K	− 0.218
			D-Galactonic Acid γ-Lactone	K	0.216
			2-Hydroxy Benzoic Acid	K	− 0.235
			Tween 40	P	0.237
			Tween 80	P	− 0.211
			α-Cyclodextrin	P	0.224
			Phenylethylamine	Am	− 0.245
PC2	14.4	46.5			
SRW	1.6841		L-Asparagine	A	0.232
SpRW	− 4.3474		L-Phenylalanine	A	− 0.251
SmRC	2.6633		L-Threonine	A	− 0.239
			Glycyl-L-glutamic acid	A	0.251
			β-Methyl-D-glucoside	C	− 0.208
			D-Xylose	C	0.258
			D-Galacturonic acid	K	0.262
			γ-Hydroxybutyric acid	K	0.225
			Itaconic acid	K	0.233
			α-Ketobutyric acid	K	0.250

†A: amino acids, C: carbohydrates, K: carboxylic acids, P: pollutant, Am: amide/amines. Only substrates sources with a coefficient > 0.20 are listed. For abbreviation of cropping systems, please refer to abbreviations section

non-significant ($y = -0.000x^2 + 0.046x - 1.616$; $R^2 = 0.713$).

Discussion

Yield

No significant differences in cane yield during first and second year were recorded when sugarcane was taken either as sole crop or intercrop with potato and mustard. This indicates that intercrop of potato or mustard with sugarcane had no antagonistic effect on cane yield (Miah et al. 1994). However, the lowest cane yield was recorded during third year when mustard intercrop with sugarcane (Table 1). This is in line with reports of Chaudhary et al. (1999), wherein 14.9% decreased cane yield was recorded when mustard was intercropped with autumn planted sugarcane. A minor reduction in sugarcane yield has also been reported with cauliflower, cabbage, knol-khol and turnip intercrop (Singh et al. 2017). Mustard or potato intercrop with sugarcane under SmRC and SpRW system increased CEY, which could be attributed to yield potential of potato (Nankar 1990). Compared to the ratoon, the potato yield as intercrop was the highest with plant crop due to the maximum plant population (Imam et al. 1990). The greater wheat yield recorded under SpRW system could be due to residual effects of excess fertilizers applied in sugarcane and potato.

Microbial Counts and Enzymatic Activity

The variations in microbial counts across the systems could be attributed to a combined effect of greater root biomass, exudates, mucilage and microclimate of community (Li et al. 2013). The greater quantity of crop residues added

Table 4 Effect of different cropping systems on physical and chemical variables of soil (mean ± standard errors of mean)

Variable	Unit	Cropping system		
		SRW	SpRW	SmRC
BD	Mg m^{-3}	$1.32^{a} \pm 0.02$	$1.31^{a} \pm 0.01$	$1.29^{a} \pm 0.01$
WHC	%	$45.9^{bc} \pm 1.59$	$49.4^{ab} \pm 1.71$	$51.8^{a} \pm 1.79$
Soil pH	1:2.5	$8.13^{a} \pm 0.12$	$8.13^{a} \pm 0.07$	$8.10^{a} \pm 0.10$
SOC	g kg^{-1}	$5.80^{b} \pm 0.06$	$6.20^{a} \pm 0.06$	$6.53^{a} \pm 0.03$
TC	g kg^{-1}	$30.5^{c} \pm 0.69$	$33.0^{b} \pm 0.82$	$39.0^{a} \pm 0.97$
TN	g kg^{-1}	$3.05^{b} \pm 0.03$	$3.21^{ab} \pm 0.05$	$3.30^{a} \pm 0.08$
C:N ratio		$10.2^{b} \pm 0.22$	$10.3^{b} \pm 0.26$	$11.8^{a} \pm 0.08$
N	mg kg^{-1}	$120.2^{b} \pm 2.31$	$119.1^{b} \pm 1.16$	$125.7^{a} \pm 1.22$
P		$6.95^{b} \pm 0.32$	$6.80^{b} \pm 0.11$	$8.00^{a} \pm 0.23$
K		$83.5^{a} \pm 0.73$	$74.1^{c} \pm 0.90$	$77.2^{b} \pm 0.68$
S		$5.9^{a} \pm 0.16$	$5.1^{b} \pm 0.13$	$5.2^{b} \pm 0.10$
Zn		$0.67^{b} \pm 0.02$	$0.76^{a} \pm 0.03$	$0.79^{a} \pm 0.02$
Cu		$1.71^{c} \pm 0.02$	$1.76^{b} \pm 0.01$	$1.91^{a} \pm 0.05$
Fe		$12.3^{c} \pm 0.56$	$16.0^{b} \pm 0.43$	$17.5^{a} \pm 0.27$
Mn		$12.5^{a} \pm 0.56$	$12.6^{a} \pm 0.43$	$12.3^{a} \pm 0.27$
CEC	$Cmol_cP^{+}kg^{-1}$	$16.4^{c} \pm 0.44$	$18.1^{b} \pm 0.46$	$20.3^{a} \pm 0.27$

Different letters within the same row indicate significant differences (Duncan's multiple range test, $p < 0.05$). For the abbreviation of physical and chemical variables and cropping systems, please refer to the abbreviations section

under SpRW and SmRC led to increased SOC, utilized as a carbon substrate resulting to increase microbial abundance. Berg and Verhoef (1998) and Li et al. (2017) reported that SOC was the primary factor affecting both soil microbial abundance and structure as greater SOC had positive relation with microbial counts. Our results also confirm the findings of Berg and Verhoef (1998) and Li et al. (2017) as the highest SOC was found in SmRC followed by SpRC and SRW (Table 4) that had a positive correlation with PSM and AFB. Lian et al. (2018) also reported that soil fungus abundance and community are linked to the alteration of TN and SOC. Contrary to that, fungal counts were highest in SpRW than bacterial counts which might be related to application of higher doses of nitrogenous fertilizers liable to inhibit the bacterial activities (Singh et al. 2010) but nutrient-rich soil environment favors bacterial activities (De-Vries et al. 2006).

The MBC, MBN, BSR and enzyme (DHA, FDA, urease, ACP and ALP) activities enhanced maximally in SmRC and SpRW system. Greater microbial activity under SmRC affected root system architecture and its biomass, exudates and secretion converted into MBC and MBN. Moreover, higher microbial counts and its functional diversity under SmRC led to increased biomass through its multiplication, synthesis of new bio-solids, and dead cells mixed in the soil. Additional roots, shoots and leaves biomass incorporated under intercropping system liable to increase organic matter, total C and C: N ratio resulting in higher microbial biomass C and N (Vuyyurus et al. 2019). Our result showed that MBC, MBN and BSR were significantly correlated with most of the microbial counts (PSM, AFB and FDA), SOC, TC and TN (Table S3). These results are in line with the findings of Lian et al. (2019), wherein it was suggested that microbial community has a significant correlation with soil respiration rate. The shift of microbial community structure had significant effects on MBC and MBN (Sun et al. 2015). The highest microbial activity (counts and its efficiency), SOC and macro- and micro-nutrients liable to enhanced enzymatic activities as they create favorable atmosphere to secrete greater amount of extra or intracellular enzymes (Gómez-Luna et al. 2012). Our results are in line with those of Li et al. (2013) as PSM was positively correlated with DHA, *Azotobacter* with FDA, nitrifying bacteria with urea and actinomycetes with ALP.

BIOLOG Assay

BIOLOG assay is a culture-based technique that provides a sensitive and reliable index to assess community-level physiological profiling (CLPP) of soil microbes altered due to cropping system. The slower microbial growth at the initial phase of incubation might mediate their adaptation to an artificial nutritional environment. Once adapted, the microbial growth increases as organisms could utilize the

Table 5 Principal components of among the variables observed under different cropping systems

	PC1	PC2	PC3
Eigenvalue	**18.7**[a]	**4.45**	**3.78**
Variance (%)	62.2	14.9	12.6
Cumulative variance (%)	62.2	77.1	89.7
Eigenvectors[b]			
AWCD	0.576	− 0.241	0.758
TCB	**0.968**[c]	− 0.098	− 0.027
TCA	0.915	− 0.393	0.023
TCF	0.790	− 0.017	− 0.163
AZO	**0.972**	− 0.067	− 0.087
PSM	0.070	**0.901**	− 0.336
AFB	0.915	− 0.096	0.160
NFB	**0.960**	− 0.097	− 0.087
WHC	0.875	0.339	0.115
SOC	**0.975**[d]	0.093	0.248
TC	**0.970**	0.188	0.017
TN	**0.984**	− 0.057	− 0.165
N	0.590	0.648	0.024
P	− 0.151	**0.955**	0.250
K	− 0.471	0.353	0.782
S	− 0.397	0.077	**0.909**
Zn	0.861	0.319	− 0.293
Cu	0.804	0.451	0.004
Fe	0.946	0.160	− 0.021
CEC	0.820	− 0.339	0.278
MBC	0.947	0.177	0.058
MBN	0.829	− 0.431	− 0.154
BSR	**0.972**	− 0.077	0.207
DHA	0.740	− 0.013	− 0.370
FDA	0.461	0.616	0.579
Urease	0.637	0.246	− 0.621
ACP	0.710	− 0.144	0.504
ALP	0.893	− 0.412	0.178

[a]Boldface eigenvalues correspond to the PCs examined for the index

[b]Variables: For abbreviations of soil variables, please refer to abbreviations section

[c]Boldface component: loadings corresponding to the indicator included in the MDS

[d]Bold underlined component: loadings are considered highly weighted

substrates in different ways (Andruschkewitsch et al. 2014). Irrespective of cropping system, amino acids were the most preferred sources utilized by the soil microbes and amide/amines were least utilized which might be related to majority of the microbes preferred amino acids as a source of energy, carbon substrate or cell protoplasm (Alexander 1972). However, carbohydrates, amino acids, carboxylic acid, polymers and amines/amides were maximally utilized as carbon sources under SmRC system (Fig. 2) could have exerted remarkable changes in the community composition and structure of the soil biota. This possibly fomented them to harness the greater amount of an amino acid as carbon and energy sources. The amino acids utilized as primary carbon sources owing to bacterial communities are better adapted to amino acids under SmRC system. Moreover, carboxylic acids used in BIOLOG plates are mainly the products of carbohydrates metabolism (modified forms of monosaccharide); thus microbes in SmRC soil jump upon those readily available substrates. As regard to amino acids, L-serine was maximally utilized as carbon source by microbial communities across the cropping systems and L-threonine was least exploited due to its ability to introduce it into the central metabolisms via pyruvate (Novak and Loubiere 2000). In addition, another distinguishing feature of L-serine is its high provision for protein synthesis as L-serine is additionally required for glycine, cysteine, tryptophan and phospholipids synthesis as well as for 1-carbon-unit generation (Stauffer 1996)). The highest utilization of Tween-80 in SRW (Fig. 2) suggested that the bacterial communities of such soils might receive polymeric substances from rhizodeposition and decomposition products of above ground biomass added through test crop.

The functional diversity indices such as Shannon diversity and evenness, Mclntosh diversity and evenness, and Simpson index were greater in SmRC (Table 2). This could be related to alterations in WHC, SOC, and TC and TN, microbial counts and soil enzymes due to changes in quantity and quality of biomass added through various test crops grown under SmRC and SpRW systems that have strong effects on functional diversity (Andruschkewitsch et al. 2014; Qin et al. 2017). Long-term monotony of plant–ratoon–wheat (SRW) significantly decreased microbial and enzymatic activities than SmRC and SpRW due to poor quality harvests, and using similar management practices (Hunsigi 2001; Kirk et al. 2004). Diversification in biomass production under SmRC and SpRW might influence carbon-limited microbes by increasing resource exudation (Liu et al. 2008), and changes in edaphic variables, corresponding to increases microbial diversity and shifts in soil microbial communities (Lian et al. 2019).

Soil Properties

The pH, BD and Mn could not affect under different cropping systems, but WHC affected significantly, being the highest in SmRC. The greater biomass under SmRC might be playing a critical role in increasing moisture retention. The SOC, TC and TN are not only the key determinant of soil organisms, but also enhancing WHC by improving soil aggregation (Manns et al. 2016). Since,

Table 6 Scoring functions (SF), threshold values and weight for the minimum data set indicators

Indicator	Soil function	L^t	U^t	B^a	Weight	Slop at base line
TN (g kg^{-1})	More is better	1.75	5.00	3.50	0.35	0.296
SOC (g kg^{-1})	More is better	3.00	9.00	6.00	0.35	0.356
P (g kg^{-1})	More is better	4.50	13.5	9.00	0.16	0.054
Sulfur (g kg^{-1})	More is better	3.50	10.5	7.00	0.14	0.913

Lt, Lower threshold value at which or below score is 0; Ut, Upper threshold value at which or above score is 1; Ba, baseline at which score is 0. For abbreviation of soil variables, please refer to abbreviations section

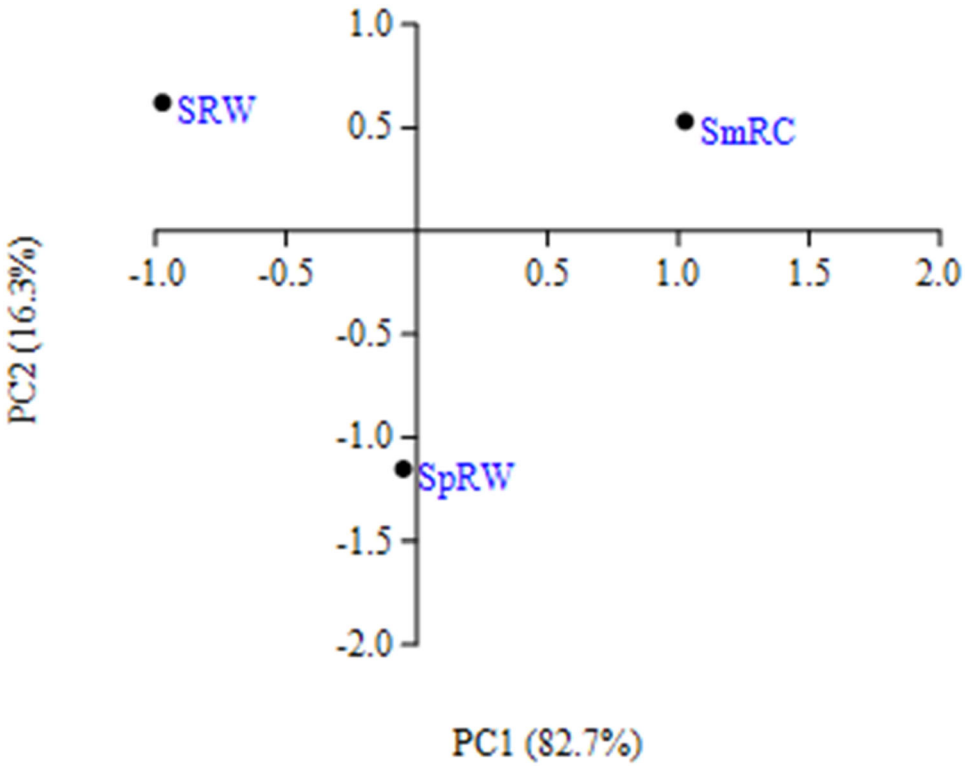

Fig. 4 PCA bi-plot (PC1 vs PC2) of active soil variables after completing third cycle of different cropping systems in IGP region. For abbreviations of soil variables and cropping systems, please refer to abbreviations section

fungal and bacterial activities act as a binding agent owing to producing fungal hyphae and polysaccharides, respectively (Tisdall and Oades 1982; Trivedi et al. 2017). A significant positive relation of SOC, TN, BSR and PSM with WHC as reflected in our results indicated crucial role of these factors in improving WHC. These results are in line with those of Franzluebbers (2002), wherein a significant positive correlation among the SOC, soil aggregates and soil water content in different soils was reported.

Intercropping of mustard in sugarcane under SmRC system improved SOC, TC and TN and C:N ratio mainly due to greater microbial counts and annual crop residue return to the soil (Thierfelder and Wall 2012; Cong et al. 2014; Sainju et al. 2017). The test crop under SmRC system had greater ability to produce more biomass through roots, shoots, leaves and trash, where it accumulated at relatively large-scale, and liable to increase SOC, TC and TN. Similarly, greater TN and C:N ratio in SmRC might be the result of N rich residues addition through plant crop, ratoon, mustard and cowpea. Inclusion of cowpea had a direct impact on biomass addition, through roots and leaves. However, absence of nodules on cowpea roots might be more active in rhizodeposition and stimulation. Photosynthetic carbon has been reported to be the primary source of rhizodeposited carbon contributes to increase SOC through cowpea (Nguyen 2003). Cowpea also provides N-rich root exudates led to enhance soil N content and stimulated microbial populations (Fustec et al. 2011). Contrary to that, the highest amount of inorganic fertilizers applied in SpRW (Table 2) as both potato and sugarcane are nutrient exhaustive crop and needed higher amounts of

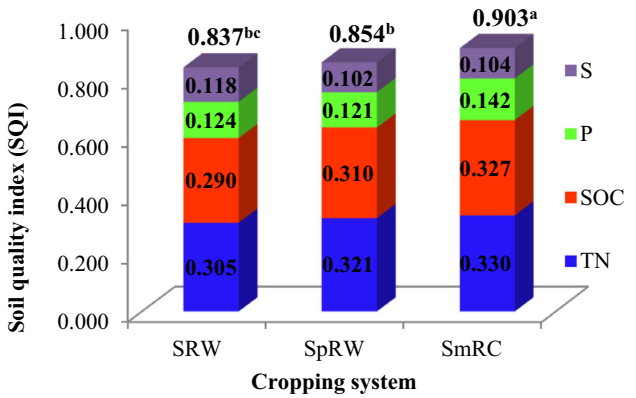

Fig. 5 Average contribution of minimum data set (MDS) indicators toward development of soil quality index (SQI) under different cropping systems. Different letters within the same column indicate significant differences (Duncan's multiple range test, $P < 0.05$). For abbreviations of cropping systems, please refer to abbreviations section

mineral fertilizers resulting in decreased microbial activity (Yu et al. 2016).

Our results indicate that SmRC and SpRW significantly improved most of the macro- and micro-nutrients in soil, except available K and S, being the highest under SRW system. These results seem to be associated with increased quantity of plant and microbial biomass mineralized into inorganic macro- and micro-nutrients. In addition, increased SOC, TC and TN resulted to accelerate microbial activity leading to enhanced mineralization, solubilization/mobilization and recycling of nutrient, thus enhanced macro- and micro-nutrients (Li et al. 2013; Lian et al. 2019). However, lower macro- and micro-nutrient status under SpRW than SmRC due to excess nutrient removal caused by sugarcane (plant and ratoon) and potato. The highest N content in SmRC may be due to greater N availability, thereby increased counts of bacteria, *Azotobacter*, ammonifying and nitrifying bacteria as microbes, plant and soil interactions played a crucial role in the N-cycle (Li et al. 2013). Greater microbial counts liable to produce siderophore and phenolic compounds which reduce carboxylic compound and root hairs resulting to increased Zn, Cu and Fe under the SmRC system. Our results also show that the bacterial counts had a significant positive correlation with Zn and Fe. Solanki et al. (2017) also ascribed that intercrop of legume with sugarcane enhanced diazotrophic population, N-fixers, chemical and biological properties of soil. The higher CEC under SmRC and SpRW might be the result of greater quantity of basic cations, WHC, SOC and microbial activities (Burle et al. 1997). The greater SOC help to release basic cation from the inorganic and organic pool of soil accumulated around the exchange complex which led to increased CEC (Iwasakia et al. 2017). The exact causes of lower CEC under the SRW system are unknown but lower biomass, SOC, macro- and micro- nutrient might be the reason for this. As MBC, MBN, *Azotobacter* counts and the FDA had a significant positive correlation with CEC (Table S3).

Soil Quality

In this study, data reduction technique (PCA) was employed to sort out the most weighted soil quality variables. Out of 28 prominent soil variables, TN, SOC, available P and sulfur were identified as high weighted variable in minimum data set (MDS). In which, TN and SOC were screen out from the PC1, available P and S from PC2 and PC3 due to higher factor loading value in respective PCs. The total N is mull as the most weighted indicators in MDS, because N is the major essential nutrient required for the synthesis of structural components of plant such as chlorophyll, amino acids and carbohydrates. Moreover, $\sim$ 96% soil N exist in organic-N converted into available N (NH_4^+–N and NO_3^-–N) after mineralization by the microbes liable to provide available N to plants. Thus, TN had direct impact on microbial activity, soil quality and crop productivity (Thierfelder and Wall 2012; Cong et al. 2014). The SOC retained in MDS as it plays a key role in determining physical, chemical and biological indicators of soil (Manns et al. 2016). The SOC is a crucial factor because of its multiple roles including improving soil biodiversity, soil structure, infiltration of air and water, promoting water retention and reducing erosion as well as acts as a source and a sink of plant nutrients (Gregorich et al. 1994; Pan et al. 2009). Available P and S also considered as a MDS due to prominent role in synthesis of structural component of plants. The P played a crucial role for the synthesis of phospholipids, DNA and RNA, ATP generation. Apart from this, P stimulates root development, stalk and stem strength, flower and seed formation and quality of grains. He et al. (2016) also found that phosphorus is the most crucial edaphic factor explaining dissimilarities in fungal communities and reported important role of P in structuring soil fungal communities. Similarly, sulfur content is also a vital nutrient for the synthesis of S-containing amino acids. Therefore, both the available P and S are crucial nutrients as they atler the microbial and enzymatic activity, microbial diversity and soil quality resulting in higher crop productivity. Both P and S are just border line of lower categories (< 10 kg P ha^{-1} and 20 kg SO_4^{2-}—S ha^{-1}) of this region; hence, their availability and dose of application become very crucial for enhanced crop productivity. The highest SQI was recorded in SmRC due to greater edaphic factor, microbial and enzymatic activities. The diversified crop residues added through root biomass, root exudates/rhizodeposition, leaf fall and trash addition under

intercropping led to increase weighted soil variables significantly under SmRC system stimulates microbial and enzymatic activities liable for improving soil quality.

Conclusions

Intercropping of potato in sugarcane under sugarcane + potato–ratoon–wheat (SpRW) system proved to be superior over sugarcane + mustard–ratoon–cowpea (SmRC) and traditional sugarcane–ratoon–wheat (SRW) in terms of crop productivity. However, SmRC system significantly improved microbial activity, function diversity and soil quality than SpRW and SRW. Thus, it is inferred that intercropping of mustard with sugarcane (plant and ratoon) under SmRC is the best system in subtropical India. Overall, diversification of mustard or potato as an intercrop with sugarcane (plant and ratoon) is the way to improve soil quality and crop productivity.

References

Alef, K., and P. Nannipieri. 1995. Enzyme activities. *Methods in Applied Soil Microbiology and Biochemistry*. https://doi.org/10.1016/B978-012513840-6/50022-7.

Alexander, M. 1972. *Introduction to soil microbiology*, 2nd ed. Calcutta: Wiley Eastern Limited.

Andrews, S.S., D.I. Karlen, and J.P. Mitchell. 2002a. A comparison of soil quality indexing methods for vegetable production systems in Northern California. *Agriculture, Ecosystem and Environment* 90: 25–45.

Andrews, S.S., J.P. Mitchel, R. Mancinelli, D.I. Larlen, T.K. Hartz, W.R. Horwarth, G.S. Pettygrove, K.M. Scow, and D.S. Munk. 2002b. On farm assessment of soil quality in California's central valley. *Agronomy Journal* 94: 12–23.

Andruschkewitsch, M., C. Wachendrof, A. Sradnick, F. Hensgen, R.G. Joergensen, and M. Wachendorf. 2014. Soil substrate utilization pattern and relation of fungal evenness of plants group and soil microbial community in five low mountains NATURA 2000. *Plant and Soil* 383: 275–289.

Berg, M.P.J.P. Kniese., and H.A. Verhoef. 1998. Dynamics and stratification of bacteria and fungi in the organic layers of a Scots pine forest soil. *Biology and Fertility of Soils* 26: 313–322.

Bhattacharyya, R., B. Ghosh, P. Mishra, B. Mandal, C. Rao, and D. Sarkar. 2015. Soil degradation in India: challenges and potential solutions. *Sustainability* 7 (4): 3528–3570.

Burle, M.L., J. Mielniczuk, and S. Focchi. 1997. Effect of cropping systems on soil chemical characteristics with emphasis on soil acidification. *Plant and Soil* 190: 309–316.

Cassel, D.K., and D.R. Nielsen. 1986. Field capacity and available water capacity. In *Methods of soil analysis. Part 1. Physical and mineralogical methods. Agronomy monograph 9*, 2nd ed., ed. A. Klute, 901–924. Madison, WI: ASA.

Chaudhary, D.C., S.N. Singh, K.S. Shekhar, A. Saxena, and T. Pratap. 1999. Companion cropping of oilseeds with autumn sugarcane in bhat soils of eastern Uttar Pradesh. *Cooperative Sugar Journal* 31 (2): 117–119.

Cherubin, M.R., D.L. Karlen, and A.L.C. Franco. 2016. Soil physical quality response to sugarcane expansion in Brazil. *Geoderma* 267: 156–168.

Choi, K.H., and F.C. Dobbs. 1999. Comparison of two kinds of Biologmicroplate (GN and ECO) in their ability to distinguish among aquatic microbial communities. *Journal of Microbiological Methods* 36: 203–213.

Cong, W.F., E. Hoffland, L. Li, J. Six, J.H. Sun, X.G. Bao, F.S. Zhang, and W. Van Der Werf. 2014. Intercropping enhances soil carbon and nitrogen. *Global Change Biology* 21 (4): 1715–1726.

De Varies, F.T., E. Hoffland, N. van Eekeren, L. Brussaard, and J. Bloem. 2006. Fungal/bacterial ratio in grasslands with contrasting nitrogen management. *Soil Biology and Biochemistry* 38: 2092–2102.

FAO. 2017. FAOSTAT: Food and Agricultural data. http://www.fao.stat/en/#home. Accessed 15 Mar 2020.

Franzluebbers, A.J. 2002. Water infiltration and soil structure related to organic matter and its stratification with depth. *Soil and Tillage Research* 66: 197–205.

Fustec, J., F. Lesuffleur, S. Mahieu, and J.B. Cliquet. 2011. Nitrogen rhizodeposition of legumes. *Journal of Sustainable Agriculture and Environment* 2: 869–881.

Gomez, E., L. Ferreras, and S. Toresani. 2006. Soil bacterial functional diversity as influenced by organic amendment application. *Bioresource Technology* 97: 1484–1489.

Gómez-Luna, B.E., G.M. De la Luz Ruiz-Aguilar, G. Vázquez-Marrufo, L. Dendooven, and V. Olalde-Portugal. 2012. Enzyme activities and metabolic profiles of soil microorganisms at KILN sites in *Quercus* spp. temperate forests of central Mexico. *Applied Soil Ecology* 52: 48–55.

Gregorich, E.G., M.R. Carter, D.A. Angers, C.M. Monreal, and B.H. Eliert. 1994. Towards a minimum data set to assess soil organic matter in agricultural soils. *Canadian Journal of Soil Science* 74: 367–385.

He, D., X. Xiang, J.S. He, C. Wang, G. Cao, J. Adams, and H. Chu. 2016. Composition of the soil fungal community is more sensitive to phosphorus than nitrogen addition in the alpine meadow on the Qinghai-Tibetan Plateau. *Biology and Fertility of Soils* 52: 1059–1072.

Hossain, G.M.A., S.M. Bokhtiar, S.K. Paul, and M.R. Anam. 2003. Intercropping of sugarcane with onion and potato followed by sesame in paired row system. *Journal of Agronomy* 2: 85–91.

Hunsigi, G. 2001. *Sugarcane in agriculture and industry*. Bangalore: Prism Books Pvt Ltd.

Imam, S.A., A.H.M. Delwar-Hossain, L.C. Sikka, and D.J. Midmore. 1990. Agronomic management of potato/sugarcane intercropping and its economic implications. *Field Crops Research* 25: 111–122.

Iwasakia, S., Y. Endob, and R. Hatanoa. 2017. The effect of organic matter application on carbon sequestration and soil fertility in upland fields of different types of Andosols. *Soil Science and Plant Nutrition* 63: 200–220.

Jackson, M.L. 1973. *Soil chemical analysis*. New Delhi, India: Prentice Hall of India Pvt. Ltd.

Karim, Z., S.M. Rahman, M. Idris, and A.J.M.S. Karim. 1988. *Soil bulk density. A manual for determination of soil physical parameters*. India: Soils and Irrigation Division, BARC.

Kirk, J.L., L.A. Beaudette, M. Hart, P. Moutoglis, J.N. Klironomos, H. Lee, and J.T. Trevors. 2004. Methods of studying soil microbial diversity. *Journal of Microbiology Methods* 58: 169–188.

Li, D., J. Liu, H. Chen, L. Zheng, and K. Wang. 2017. Soil microbial responses to forage grass cultivation in degraded karst soils, South China. *Land Degradation and Development* 29: 4262–4270.

Li, X., X. Mu, Y. Cheng, Y. Liu, and H.X. Nian. 2013. Effects of intercropping sugarcane and soybean on growth, rhizosphere soil microbes, nitrogen and phosphorus availability. *Acta Physiologiae Plantarum* 35: 1113–1119.

Lian, T., Y. Mu, Q. Ma, Y. Cheng, R. Gao, Z. Cai, B. Jiang, and H. Nian. 2018. Use of sugarcane-soybean intercropping in acid soil impacts the structure of the soil fungal community. *Scientific Reports* 8: 14488.

Lian, T., Y. Mu, J. Jin, Q. Ma, Y. Cheng, Z. Cai, and H. Nian. 2019. Impact of intercropping on coupling between soil microbial community structure, activity and nutrient use efficiencies. *PeerJ* 7: e6412.

Lin, R.Y., H. Rong, J.J. Zhou, C.PYu.C.Y. Ye, L.S. Chen, and W.X. Lin. 2007. Impact of allelopathic rice seedling on rhizospheric microbial populations and their functional diversity. *Acta Ecologia Sinica* 27: 3644–3654.

Liu, Z., G. Lui, B. Fu, and X. Zheng. 2008. Relationship between plant species diversity and soil microbial functional diversity along a longitudinal gradient in temperate grasslands of Hulunbeir, Inner Mongolia, China. *Ecological Research* 23: 511–518.

Manns, H.R., G.W. Parkin, and R.C. Martin. 2016. Evidence of a union between organic carbon and water content in soil. *Canadian Journal of Soil Science* 96: 305–316.

Mariano, E., D.L. Jones, P.W. Hill, and P.C.O. Trivelin. 2016. Mineral nitrogen forms alter 14C-glucose mineralization and nitrogen transformations in litter and soil from two sugarcane fields. *Applied Soil Ecology* 107: 154–161.

Mei, P.P., L.G. Gui, P. Wang, J.C. Huang, H.Y. Long, P. Christie, and L. Li. 2012. Maize/faba bean intercropping with rhizobia inoculation enhances productivity and recovery of fertilizer P in a reclaimed desert soil. *Field Crops Research* 130: 19–27.

Miah, M.A.M., S.A. Sabur, and M.S. Islam. 1994. Comparative economics of sugarcane with intercrops in Jaipurhat sugar mill area. *Bangladesh Journal of Sugarcane* 16: 5–9.

Nankar, J.T. 1990. Scope and prospects for intercropping of potato with sugarcane in Maharashtra State, India. *Field Crops Research* 25: 123–132.

Nguyen, C. 2003. Rhizodeposition of organic C by plants: Mechanisms and controls. *Agronomie* 23: 375–393.

Novak, I., and P. Loubiere. 2000. The metabolic network of *Lactococcus lactis*: distribution of 14C-labeled substrate between catabolic and anabolic pathways. *Journal of Bacteriology* 182: 1136–1143.

Pan, G.X., P. Smith, and W.N. Pan. 2009. The role of soil organic matter in maintaining the productivity and yield stability of cereals in China. *Agriculture, Ecosystems and Environment* 129: 344–348.

Paungfoo-Lonhienne, C., Y.K. Yeoh, N.R.P. Kasinadhuni, T.G.A. Lonhienne, N. Robinson, P. Hugenholtz, M.A. Ragan, and S. Schmidt. 2015. Nitrogen fertilizer dose alters fungal communities in sugarcane soil and rhizosphere. *Science Reporter* 5: 8678.

Qin, S., S. Yeboah, L. Cao, J. Zhang, S. Shi, and Y. Liu. 2017. Breaking continuous potato cropping with legumes improves soil microbial communities, enzyme activities and tuber yield. *PLoS ONE* 12 (5): e0175934.

Rachid, T.C.C., F.C. Caio, E. Balieros, R.S. Fonseca, G.M. Peixofo, J.M. Tiedje. Cheer, and A.S. Rosado. 2015. Intercropped silviculture system, a key to achieve soil fungal community management in Eucalyptus plantation. *PLoS ONE* 10: e0118515.

Sainju, U.M., A.W. Lenssen, B.I. Allen, W.B. Stevens, and J.D. Jabro. 2017. Soil total carbon and crop yield affected by crop rotation and cultural practices. *Agronomy Journal* 109: 1–9.

Singh, S.N., A.D. Pathak, and A.K. Sharma. 2017. Introducing technology of intercropping winter vegetables with autumn planted sugarcane (*Saccharum* sp. hybrid) for enhancing productivity and profitability in real framings of north-central India. *Proc DSTA* 66: 37–40.

Singh, S.R., M.Y. Zargar, U. Singh, and M. Ishaq. 2010. Influence of bio-inoculants and inorganic fertilizers on yield, nutrient balance, microbial dynamics and quality of strawberry (*Fragariaananassa*) under rainfed conditions of Kashmir valley. *Indian Journal of Agricultural Sciences* 80: 275–281.

Solanki, M.K., Z. Wang, F.Y. Wang, Chang-Ning. Li, Tao-Ju. Lan, R.K. Singh, P. Singh, L.T. Young, and Y.R. Li. 2017. Intercropping in sugarcane cultivation influenced the soil properties and enhanced the diversity of Vital Diazotrophic Bacteria. *Sugar Tech* 19: 136–147.

SPSS. 2001. Statistical Package. Version 11.0 for Windows. SPSS Inc., Chicago, IL.

Stauffer, G.V. 1996. Biosynthesis of serine, glycine and one-carbon units. In *Escherichia coli and Salmonella: Cellular and molecular biology*, 2nd ed., ed. F.C. Neidhart, R. Curtiss III., J.L. Ingraham, E.C.C. Lin, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, and H.E. Umbarger. 506–513. Washington, DC: ASM Press.

Sugar Annual India. 2018. USDA Gain Report Number IN8047 dated 19/04/2018. https://gain.fas.usda.gov.

Suman, A., M. Lal, A.K. Singh, and A. Gaur. 2006. Microbial biomass turnover in Indian subtropical soils under different sugarcane intercropping system. *Agronomy Journal* 98: 698–704.

Sun, R., X.X. Zhang, X. Guo, D. Wang, and H. Chu. 2015. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. *Soil Biology and Biochemistry* 88: 9–18.

Thierfelder, C., and P.C. Wall. 2012. Effects of conservation agriculture on soil quality and productivity in contrasting agro-ecological environments of Zimbabwe. *Soil Use and Management* 28: 209–220.

Tisdall, J.M., and J.M. Oades. 1982. Organic matter and water-stable aggregates in soils. *Journal of the Indian Society of Soil Science* 33: 141–163.

Trivedi, P., M. Delgado-Baquerizo, T.C. Jeffries, C. Trivedi, I.C. Anderson, K. Lal, M. McNee, K. Flower, B. Pal Singh, D. Minke, and B.K. Singh. 2017. Soil aggregation and associated microbial communities modify the impact of agricultural management on carbon content. *Environmental Microbiology* 19 (8): 3070–3086.

United States Department of Agriculture (USDA). 1996. Soil survey laboratory methods manual: Washington D.C. U.S. Department of Agriculture, National Resources Conservation Services, National Soil Survey Centre, Soil Survey Investigations, Report No. 42.

Un-Pop. 2017. World population prospects: The 2017 Revision, Online Demographic Profiles. http://www.population.Un.org/wpp/Graphs/DemographicProfiles/. Accessed 12 Mar 2020.

Vuyyurus, M., H.S. Sandhu, J.E. Erickson, and A.V. Ogram. 2019. Soil chemical and biological fertility, microbial community structure and dynamics in successive and fallow sugarcane planting systems. *Agroecology and Sustainable Food Systems* 44: 68–794.

Wander, M.M., and G.A. Bollero. 1999. Soil quality assessment of tillage impacts to Illinois. *Soil Science Society of America Journal* 63: 961–971.

Wang, Z., X. Bao, X. Li, Z. Jin, J. Zhao, J. Sun, P. Christie, and L. Li. 2015. Intercropping maintains soil fertility in terms of chemical properties and enzyme activities on a timescale of one decade. *Plant and Soil* 391: 265–282.

Wang, Z.G., X. Jin, X.G. Bao, X.F. Li, and J.H. Zhao. 2014. Intercropping enhances productivity and maintains the most soil

fertility properties relative to sole cropping. *PLoS ONE* 9: e113984.

Wold, S., K. Esbensen, and P. Geladi. 1987. Principal component analysis. *Chemometrics and Intelligent Laboratory Systems* 2 (1–3): 37–52.

Yang, W.T., Z. Li, J.W. Wang, P. Wu, and Y. Zhang. 2013. Crop yield, nitrogen acquisition and sugarcane quality as affected by inter specific competition and nitrogen application. *Field Crops Research* 146: 44–50.

Yu, H., Q. Gao, Z. Shao, A. Ying, Y. Sun, J. Liu, W. Mao, and B. Zhang. 2016. Decreasing nitrogen fertilizer input had little effect on microbial communities in three types of soils. *PLoS ONE* 11 (3): e0151622.