Soil quality parameters vis-a-vis growth and yield attributes of sugarcane as influenced by integration of microbial consortium with NPK fertilizers

S. K. Shukla*, Lalan Sharma, V. P. Jaiswal, A. D. Pathak, Raghvendra Tiwari, S. K. Awasthi & Asha Gaur

Intensive agriculture involving high crop intensity, unavailability of organics, and higher use of straight fertilizers causes imbalanced use and deficiencies of several other macro and micronutrients. Nutrients supply through the integration of microbial consortium containing *Gluconacetobacter diazotrophicus*, *Trichoderma harzianum*, and *Pseudomonas fluorescens* can reduce the requirements on the one hand and can also increase the response of chemical fertilizers. Thus we had planned the present experiment with the objectives (i) to determine the effect of integrated application of microbial consortium (MC) and NPK fertilizer on soil quality parameters and crop growth and yield attributes and (ii) to assess the effect of integration on agronomic efficiency of N, P and K and find out the possibilities for reduction in applied doses of NPK, if any. Five treatments viz., T1: N0P0K0; T2: N75P13K25; T3: N150P26K50; T4: N75P13K25 + microbial consortium and T5: N150P26K50 + microbial consortium containing new strains of *Trichoderma harzianum*, *Gluconacetobacter diazotrophicus*, and *Pseudomonas fluorescens* (CFU 10⁹–10 per ml liquid culture) were evaluated under four replications in a randomized block design (RBD). Experimental results indicated that integrating microbial consortium and NPK fertilizers’ application proved effective in improving soil organic carbon, soil microbial population, microbial biomass carbon, microbial biomass nitrogen, and soil respiration. Integrated use of microbial consortium with NPK also improved the cation exchange capacity of soil and roots. However, the growth and yield attributes, nutrients uptake, sugarcane, and sugar yields also revealed a positive effect of microbial consortium’s integrated application with NPK. The integration of MC and NPK also improved the agronomic efficiency of applied nutrients (NPK). Reduction of 50% NPK with these microbial consortia (*Trichoderma harzianum*, *Gluconacetobacter diazotrophicus*, and *Pseudomonas fluorescens*) was found better than the application of full NPK through chemical fertilizers. Thus application of N150P26K50 with microbial consortium can sustain soil fertility besides improving sugarcane and sugar yields in subtropical Indian conditions.

Microbes’ role in modern agriculture is increasing due to declining soil organic carbon content¹ and decreasing partial factor productivity. Sustainability of the crop production system is a vital component to feed the growing population¹. Intensive agriculture involving high crop intensity, unavailability of organics, and higher use of straight fertilizers causes imbalanced use and deficiencies of several other macro and micronutrients¹. The higher use of chemical fertilizers is also affecting NO₃⁻ pollution in soil water⁴ and CH₄ and NO₂ emission in the environment causing global warming besides adversely affecting soil chemical and biological properties⁵. Sugarcane crop-producing 100 tonnes cane yield per ha removes about 208 kg N, 53 kg P, and 280 kg K⁶. The requirement of nutrients varies with planting seasons, growing regions, and nutrient status availability in fields. Sugarcane is a heavy feeder crop supplied with 150–350 kg N; 60–100 kg P₂O₅, and 60–120 kg K₂O ha⁻¹ annually depending upon planting time and growing regions of the country⁶.
Soil microbes and their functions related to C transformation and turnover are influenced by N application. Microbes also play an important role in decomposition processes, including lignin and cellulose degradation and soil carbon turn over. Gluconacetobacter diazotrophicus is an endophytic bacteria and survives under anaerobic conditions in plant roots, rhizomes, stalk, and leaves of sugarcane. Flavonoids exuded by the crop sources serve as signals to enter, colonize, and fix atmospheric dinitrogen by reducing into ammonia. Several experiments in India and other countries revealed saving of 25–50% N through the application of Gluconacetobacter bacteria in sugarcane. Pseudomonas fluorescens acts as P solubilizer and improves solubility of P of plant by converting PO_{4}^{3−} to HPO_{4}^{2−} and H_{2}PO_{4}^{−}. Increasing P content in sugarcane juice is an utmost important task for better crystallization of sugar during crushing. Usually > 300 ppm phosphate concentration in sugarcane juice provides better crystallization of sugar, which improves sugar recovery in mill. The phosphorus uptake in cane and its diversion in juice could be increased through efficient use of unavailable P in soil. Trichoderma fungus controls soil-borne pathogens and also makes the rhizospheric environment congenial for the growth of sugarcane crops through the release of cell wall degrading enzymes, secondary metabolites, etc. Thus, Trichoderma also acts as bio decomposer and having the potential to increase soil organic carbon besides improving crop growth and yields.

Various experiments have been conducted to determine the effect of the above microorganisms on the growth and yield of sugarcane. However, an integrated application of these microbes with chemical fertilizers has great scope keeping in view the reduction of macronutrients supplied through chemical fertilizers and improving the nutrient use efficiencies besides increasing crop yields. Gluconacetobacter diazotrophicus can reduce the N level. However, up to 50% requirement of P can also be supplemented/substituted with P solubilizers with chemical fertilizers. Trichoderma also acts as a growth promoter and, if augmented with chemical fertilizer, increased sugarcane yield and soil quality parameters. Thus the individual effect of these microbes on the growth and yield of crops has been reported by several researchers. Work has also been done on the aspect of containing pathogen by Trichoderma spp. The reports on P solubilizer also revealed the performance of various Pseudomonas or Bacillus spp on P solubilizing capacity and its effect on reducing P level in various crops. The interactions of diverse microbes in soil play the most crucial role in optimizing the rhizospheric environment for better plant growth. The performance of these microorganisms varies in different ways. Complementarities between Trichoderma, Gluconacetobacter, and Pseudomonas spp is an essential issue for efficient utilization of these microbes and assessing their effect on soil quality parameters, crop growth, and yields as well. The scientific information on reducing NPK through the combined use of these microorganisms is lacking. It holds great promise for sustaining soil fertility and crop productivity in the sugarcane-based system. The variations in these microbes’ efficiency have also been observed, and potential strains of these microbes have been identified at the India Institute of Sugarcane Research, Lucknow. These microbes have also been deposited in GenBank, and accession numbers had also been provided. Therefore, a need was felt for a systematic study on assessing microbial consortium, its effect on soil quality parameters, sugarcane growth, yields, and possibilities to reduce NPK level to sugarcane crop.

Thus present experiment was planned, keeping the following objectives in view viz., (i) to determine the effect of integrated application of microbial consortium (MC) and NPK fertilizer on soil chemical and biological parameters. (ii) to assess the impact of integrated application of MC and NPK fertilizer on dry matter accumulation, nutrients uptake pattern, yield attributes, and sugar yield (iii) to assess the effect of integration on nutrients use efficiencies (agronomic efficiencies of N, P and K) exploring the possibilities of reduction in applied doses of NPK, if any.

Materials and methods
The Experimental site, climate, and soil. A field experiment was conducted during 2016–18 at the ICAR-Indian Institute of Sugarcane Research, Lucknow, located at 26.50° North and 80.50° East at an elevation of 123 m above sea level. The climate of the area comes under the category of subtropical (semi-arid region) having dry, hot summer (April–June), rainy season (July–September), winter (November–January). Autumn (October) and spring (February–March) are moderately cooler with moderate temperatures and humidity and are considered best for the planting sugarcane. The data on meteorological parameters viz., temperature, rainfall, and humidity have been presented through Fig. 1a,b. The soil of the experimental field was loam (45% sand, 35% silt, and 20% clay) having pH 7.60, Electrical conductivity (0.26 dS m^−1), soil organic carbon (19.75 Mg Mg ha^−1), available N (304.2 kg Na ha^−1), available P (16.32 kg ha^−1) and available K (376.3 kg ha^−1). Initial soil sampling from five spots in the experimental field was done through a Core Sampler of 8 cm diameter in 0–15 cm depth to determine physical, chemical, and biological properties. The initial bulk density of soil at 0–15 cm depth was recorded as 1.40 Mg m^−3. Soil organic carbon content (Mg ha^−1) was calculated by the formula given below.

\[
\text{SOC (Mg ha}^{-1}) = \text{Soil organic carbon (g kg}^{-1}\text{soil) \times 2.20 \times bulk density (Mg M}^{-3})
\]

Initial soil microbial population viz., bacteria (48×10^3 CFU per g soil), fungi (2.50×10^4 CFU per g soil), and actinomycetes (2.10×10^6 per g soil) in 0–15 cm depth was determined. Other soil microbial activities viz., soil microbial biomass carbon (264.4 mg CO_{2}-C/kg soil/day), soil microbial biomass nitrogen (12.4 mg NH_{4}-N/kg soil/day), and soil respiration (204 mg CO_{2}-C/kg soil/day) were also determined before experimentation. The initial cation exchange capacity of the soil was determined as 42.8 Meq/100 g soil.

Preparation and application of microbial consortium of P. fluorescens, G. diazotrophicus and T. harzianum. The pure culture of Pseudomonas fluorescens strain PSB28 (GenBank Acc. No. MH817418), Glu-
conacetobacter diazotrophicus strain NB73 (GenBank Acc. No. MT012287), and Trichoderma harzianum strain T6 (GenBank Acc. No. MH151122) were isolated at the Soil–Plant–Water Analysis Lab of ICAR-Indian Institute of Sugarcane Research, Lucknow. The bacterial culture was prepared from a single colony of P. fluorescens and G. diazotrophicus in 1000 ml of sterilized Pikovskaya broth and LGI broth. The inoculated bacterial culture flask was incubated at 30 °C for five days. After five days of incubating bacterial culture at 30 °C, the bacterial culture was counted for colony-forming units (CFUs). It was recorded that bacterial culture broth of P. fluorescens strain PSB28 had approximately 4.5 × 10⁹ cells/ml and broth of G. diazotrophicus strain NB73 had about 3.9 × 10⁹ cells/ml, respectively. The mass inoculum of T. harzianum strain T6 was prepared by preparing starter culture on potato dextrose broth medium and incubated at 30 °C for 20 days. For good sporulation of T. harzianum culture, every third day's proper inoculum shaking and mixing was done. The prepared T. harzianum (Th) based broth culture inoculum containing approximately 5 × 10⁵ Th fungal counts/ml. The broth-based inoculum of these microbial cultures (bacterial and fungal) at equal proportion were mixed and diluted with sterilized distilled water. The microbial inoculum carrying sterilized water was sprayed on sugarcane setts in furrows before soil covering.

**Assay of microbial population determination.** For microbial population determination before experimentation, the spread plate method was used. The spread plate method provides information on total counts/viable cells. The broth multiplied bacterial (Pseudomonas fluorescens and Gluconacetobacter diazotrophicus) and fungal (Trichoderma harzianum) samples (0.1 ml) of suitably diluted suspensions were surface plated on to nutrients agar and potato dextrose agar plates, respectively. All the platings were made in triplicate to minimize errors on the microbial population count. The microbial colonies were counted after incubation at 30 °C for 48 h. The presence of single type colonies on the surface plated petri dish indicates the pure culture of microbes. It was recorded that bacterial culture broth of Pseudomonas fluorescens and Gluconacetobacter diazotrophicus has approximately 4.5 × 10⁹ and 3.9 × 10⁹ cells/ml, respectively. The prepared Trichoderma harzianum based broth
culture inoculums were plated and counted about $5 \times 10^7$ Th fungal counts/ml. The broth-based inoculums of these microbial cultures (bacterial and fungal) were mixed in equal proportion at the application time. The quantified appropriate microbial inoculums carrying sterilized water was sprayed on sugarcane setts in furrows before soil covering. The formula adopted for microbial population determination was $\sim$ Colony-forming units/ml = (no. of colonies $\times$ dilution factor)/volume of culture plate.

**Crop culture.** The experimental field was prepared for planting after one plowing through a moldboard plough and two ploughings through tractor operated disc harrow/cultivator. After planking, the area was leveled, and the layout was done. Deep furrows (15–20 cm) were opened at 75 cm row spacing, and three bud setts of sugarcane (Cv. Co0238) were used for planting. A basal dose of 1/3rd N and full P and K were applied as per treatments. Basal application of 1/3rd N and full P and K (as per treatments) was used through urea, diammonium phosphate, and muriate of potash (KCl). Three bud setts were placed manually in furrows keeping end to end adjustment and pressed in soil. About 53,300 three bud setts were required for planting of one ha area. After manually placing, setts were covered through soil not more than 2–3 inches on setts. Topdressing of the remaining 2/3rd N was done during the tillering stage (April–June) in two equal splits. Planting was done on 16th February 2016 and on 18th February 2017 during the first and second cropping seasons. Another package of practices recommended for raising sugarcane crop in the region was followed. The sugarcane crop was harvested on 28th December 2016 and 29th December 2017 during the first and second cropping seasons.

**Treatments.** Five treatments, viz., T1: N0P0K0; T2: N75P13K25; T3: N150P26K50; T4: N75P13K25 + microbial consortium and T5: N150P26K50 + microbial consortium containing Trichoderma harzianum, Gluconacetobacter diazotrophicus, and Pseudomonas fluorescens (CFU $10^6–10^7$ per ml liquid culture) were applied. These treatments were evaluated under four replications in a randomized block design (RBD). Planting of eight rows (at 75 cm spacing) of 6-m length was done in each plot. Thus minimum plot size was kept at 36 m$^2$. Destructive sampling was done through the 2nd row of each plot. Net sugarcane yield at the harvest was worked out based on four rows leaving border rows and sample rows in each plot.

**Soil studies.** Various soil quality parameters were determined at different growth stages (tillering, grand growth, and harvest). Sampling for tillering, grand growth, and harvest stages was done in June, September, and December, respectively, during both the seasons. The bulk density of soil was determined by the Core Sampler method (Blake, 1965). Soil texture (sand, silt, and clay percent) was determined through the Bouyoucos hydrometer method. Soil pH and electrical conductivity (EC) were determined in 1:2.5 soil–water suspensions by pH Meter and Electrical conductivity meter, respectively. Available N in soil (alkaline permanganate method), available P (Olsen P ascorbic acid method), and available K were determined. The determination of soil pH was done through the Rose Bengal medium method. However, total bacteria through plate count agar method and actinomycetes through Kenknight method were determined. At different growth stages (tillering, grand growth, and harvest), soil microbial biomass carbon and soil microbial biomass nitrogen were determined through the chloroform fumigation-incubation method. Soil respiration was also determined through unfumigated soil incubation method. The soil’s cation exchange capacity was determined through the method prescribed by Chapman (1965) and calculated by the following formula.

\[
\text{CEC (meq/100 g soil)} = \frac{X^{**} - Y^* \times 100 \times \text{Normality of titrant}}{\text{soil wt (g)}}
\]

*Volume of acid used for titrating blank.
**Volume of acid used for titrating the sample.

**Crop studies.** At various growth stages, ten sugarcane plants were randomly harvested in each plot. Tillers were partitioned in leaf and stalk after harvesting. These samples were oven-dried at 65 °C for 72 h after proper sun drying. Tillers counting at different growth stages were done and expressed in thousand per ha. Thus total number of tillers viz., individual length, diameter, and weight were recorded after sampling. Quality parameters of cane juice (°brix, pol percent, and purity) were determined through Auto Pol. Commercial cane sugar (%) was determined through the following formula.

\[
\text{CCS (%)} = \frac{[S - (B - S) \times 0.4]}{0.73}
\]

where S is the Sucrose percent juice, and B is the °Brix. Commercial cane sugar (CCS) or sugar yield per ha was estimated by multiplying CCS (%) with sugarcane yield.
Agronomic efficiency of N, P, and K was also worked out as per the following formula:

\[ AE = \frac{\text{Crop yield (kg)}/N, P \text{ or } K \text{ applied}}{\text{N, P or K applied}} \]

Cation exchange capacity of roots was determined through the method given by Chamuah.

\[ \text{CEC meq/100 g root} = \left( X^{**} - Y^{**} \right) \times 100 \times \text{Normality of titrant} \times \text{Root dry wt (g)} \]

* Volume of acid used for titrating blank.
** Volume of acid used for titrating the sample.

Statistical analysis. The data on various soil and plant characters recorded were statistically analyzed in RBD. Pooled analysis of two years crop data was done, and mean results have been presented for analysis on scientific principles, discussions, and conclusions. Statistical analysis was done through a software program on RBD, and the critical difference (CD) at a 5% probability level was determined to assess the significant differences among various treatments.

Results

Soil organic carbon (SOC), soil microbial biomass carbon (SMBC), soil microbial biomass nitrogen (SMBN), and soil respiration (SR). The data on soil organic carbon (SOC) during various crop growth stages (Table 1) indicated inoculation of *Trichoderma harzianum*, *Pseudomonas fluorescens*, and *Gluconacetobacter diazotrophicus* (microbial consortium-MC) with recommended dose of NPK increased SOC up to grand growth stage. However, after the grand growth stage, a marginal decrease in SOC was recorded with all the treatments, including NPK + MC. Despite this, the application of MC with NPK increased SOC by 17.86% (21.25 Mg ha\(^{-1}\)) compared to NoPoKo and 10.05% compared to N150P26K50. Thus inoculation of MC with NPK fertilizers increased SOC as compared to inorganic fertilizers. Reduction of 50% NPK and integration of microbial consortium increased SMBN compared to recommended NPK (N150P26K50) at all the growth stages. However, with recommended NPK, the application of MC further improved the soil microbial biomass nitrogen and increased the response.

Soil respiration is an indication of the microbial population in the rhizosphere. Mean soil respiration (231.00 mg CO\(_2\)-C/kg soil/day) was higher during the tillering stage than the harvest stage. However, the most increased soil respiration (299.8 mg CO\(_2\)-C/kg soil/day) was also recorded during the grand growth phase. The increasing dose of NPK favoured soil respiration at all the growth stages. However, the application of MC with recommended NPK further improved soil respiration by 13.645 to 33.33% at various growth stages than

| Treatment                  | SOC (Mg ha\(^{-1}\)) | SMBC(CO\(_2\)-C mg kg\(^{-1}\) soil day\(^{-1}\)) | SMBN(NH\(_3\)-N mg kg\(^{-1}\) soil day\(^{-1}\)) | Soil respiration(CO\(_2\)-C mg kg\(^{-1}\) soil day\(^{-1}\)) |
|----------------------------|----------------------|-------------------------------------------------|-------------------------------------------------|--------------------------------------------------|
|                            | Tillering            | Grand growth                                   | Harvest                                         | Tillering                                        | Grand growth                                   | Harvest                                         | Tillering                  | Grand growth             | Harvest                  |
| T\(_1\): N0P0Ko            | 19.96                | 21.25                                           | 18.03                                           | 574.5                                           | 1533.3                                          | 831.1                                           | 11.4                      | 14.7                    | 12.4                      | 198.0                    | 242.0                    | 165.0                                           |
| T\(_2\): N75P13K25         | 20.44                | 23.18                                           | 19.64                                           | 634.5                                           | 1757.8                                          | 855.6                                           | 13.7                      | 15.2                    | 14.8                      | 209.0                    | 297.0                    | 176.0                                           |
| T\(_3\): N150P26K50        | 21.73                | 22.85                                           | 19.31                                           | 684.5                                           | 1906.7                                          | 880.0                                           | 15.6                      | 17.4                    | 16.0                      | 242.0                    | 319.0                    | 198.0                                           |
| T\(_4\): N75P13K25 + MC    | 21.62                | 22.64                                           | 20.13                                           | 712.4                                           | 1967.4                                          | 912.3                                           | 15.9                      | 17.9                    | 16.3                      | 263.4                    | 325.3                    | 214.5                                           |
| T\(_5\): N150P26K50 + MC   | 21.57                | 23.50                                           | 21.25                                           | 855.6                                           | 2102.2                                          | 953.3                                           | 17.1                      | 19.6                    | 17.3                      | 275.0                    | 341.0                    | 264.0                                           |
| CD for years               | 0.529                | 0.516                                           | 0.577                                           | 116.8                                           | 224.3                                            | 105.9                                           | 1.70                      | 2.06                    | 1.761                     | 14.39                     | 16.45                    | 13.45                                           |
| CD for years × treatments  | 1.20                 | 1.32                                            | 1.25                                            | 67.35                                           | 134.45                                           | 67.34                                           | 0.87                      | 0.92                    | 0.78                      | 14.39                     | 16.45                    | 13.45                                           |
| CD for treatments          | NS                   | NS                                              | NS                                              | NS                                              | NS                                               | NS                                              | NS                        | NS                      | NS                        | NS                        | NS                      | NS                                              |
| Initial                    | 19.75                | 624.6                                           | 12.4                                            | 204                                             |                                                  |                                                  |                          |                         |                          |                          |                         |

Table 1. Soil organic carbon and available nutrients status in soil during crop growth at various stages.
recommended NPK. At the harvest stage, soil respiration with recommended NPK + MC was increased by 60% as compared to N0P0K0 and about 33.3% over N150P26K50. Integration of MC with reduced NPK also improved soil respiration (SR) and proved superiority over recommended NPK. Thus SOC, SMBC, SMBN, and soil respiration showed improvement due to inoculation of microbial consortium along with NPK fertilizers. Reducing the NPK level to N75P13K25 also improved soil chemical and biological activities with microbial consortium integration. However, the integration of MC with recommended NPK further increased the soil quality parameters and proved superiority to integration with a reduced level of NPK.

Available nutrients (NPK) in soil. Integration of microbial consortium enhanced nutrients availability at all the growth stages (Table 2). Available N during all the growth stages ranged from 308.0 to 348.9 kg ha⁻¹. The highest available N content was recorded during the grand growth stage. Increasing doses of NPK also increased the available N content of soil. Available N increased during the grand growth phase as compared to the tillering stage, but after completion of the grand growth phase, it declined during the maturity of the crop (Table 2). About 40 kg N ha⁻¹ in soil was decreased during the grand growth to maturity phase. At the lower doses of NPK, approximately 14.28% available N could be increased at the grand growth stage with the integration of microbial consortia. However, at the recommended level of NPK, it could be improved by 9.16% during the same phase. The soil’s highest P content was recorded during the tillering phase (20.25 kg P ha⁻¹). It decreased to 17.45 kg ha⁻¹ during the grand growth phase and further improved during maturity (18.16 kg ha⁻¹). The inclusion of PSB in microbial consortium improved the available P content in soil during all the growth stages. Integration of microbial consortium improved P availability, and with 50% NPK showed higher P content than recommended NPK without MC. At the tillering stage, the application of microbial consortium also improved P content in soil by 16.38% and 7.47% with reduced NPK and recommended level, respectively. Potassium content in the soil was the highest (425.8 kg ha⁻¹) during the tillering phase. It decreased to 37.7 kg ha⁻¹ during the grand growth phase and further reached 343.8 kg ha⁻¹ at the harvest stage. Inoculation of MC with a reduced level of NPK and at the recommended level improved 12.06% and 8.18% available K, at the tillering and grand growth stages, respectively. Sugarcane crops heavily remove potassium, and the application of K through inorganic fertilizer enhanced K availability in soil. The microbial consortium also improved K accumulation and ensured higher availability of sugarcane crop.

Cation exchange capacity of root and soil. Cation exchange capacity of soil represents the capacity to hold the exchangeable cations on clay complex. The improved cation exchange capacity of soil and roots showed the active ions adsorption and governed nutrients availability to crop. Cation exchange capacity of roots was

| Treatment | CEC root Meq per 100 g dry wt of roots | CEC soil meq per100 g dry wt of soil |
|-----------|----------------------------------------|-------------------------------------|
|           | Tilling | Grand growth | Harvest | Tilling | Grand growth | Harvest |
| T1: N0P0K0 | 39.34  | 37.78     | 34.43   | 4.46   | 4.73        | 3.38    |
| T2:N75P13K25 | 45.30  | 39.02    | 36.03   | 5.36   | 5.47        | 3.60    |
| T3:N150P26K50 | 48.57  | 43.44    | 38.85   | 5.38   | 5.60        | 3.70    |
| T4:N75P13K25 + MC | 47.27  | 44.27    | 38.12   | 5.42   | 5.73        | 3.81    |
| T5:N150P26K50 + MC | 56.09  | 48.06    | 40.69   | 5.75   | 5.94        | 3.98    |
| CD for years | 5.70   | 4.673    | 4.53    | 0.645  | 0.674       | 0.447   |
| CD for Treatments | 2.76   | 2.45     | 2.82    | 0.32   | 0.34        | 0.28    |
| CD for years × treatments | NS     | NS        | NS      | NS     | NS          | NS      |

Table 3. Cation exchange capacity of root and soil during crop growth.
worked out on 100 g dry weight of roots during different growth stages (Table 3). The mean cation exchange capacity (CEC) during the tillering stage was the highest (47.33 meq per 100 g dry weight of roots) as compared to grand growth (42.08 meq per 100 g dry weight of roots) and harvest stage (37.5 meq per 100 g dry weight of roots). Increasing the NPK level and integration of MC improved cation exchange capacity of roots at all the growth stages because of the higher number of cations available. Microbial consortium affected 13.45% and 10.64% improvement with reduced NPK and recommended NPK level, respectively. Cation exchange capacity of soil also followed a similar pattern as root CEC. However, the CEC of soil increased during the grand growth phase (5.44 meq/100 g soil) and declined during maturity because of a lower level of nutrients. Integration of MC with recommended NPK improved soil CEC by 17.75% at the harvest stage compared to control (N150P26K50). However, improvements during the tillering and grand growth phase were recorded as 28.92% and 25.58%, respectively, with N150P26K50 + MC over N0P0K0. Application of MC with NPK showed the highest CEC of soil (5.94 meq/100 g soil) during the grand growth phase of the sugarcane crop. Microbial consortium affected 5.83% and 7.57% improvement with reduced NPK and recommended level, respectively.

**Soil microbial population (bacteria, fungi, and actinomycetes).** The data on soil microbial population (bacteria, fungi, and actinomycetes) during various growth phases of sugarcane have been presented through Fig. 2a–c. The logarithmic transformation of data revealed that the MC’s integration with NPK improved the population of all microbes at all the growth stages. Mean bacterial numbers during all the growth stages ranged from 61.0 × 10^5 CFU per g soil to 78.2 × 10^5 CFU per g soil. However, the lower population of fungi was recorded during the tillering and grand growth phase in the range of 14.6 × 10^3 CFU per g soil to 17.5 × 10^5 CFU per g soil) as compared to bacteria. The population of actinomycetes was recorded at the lowest (4.0 × 10^5 CFU per g soil) during the tillering stage. The population of all the microbes improved due to the application of NPK and MC. Reducing NPK dose and inclusion of microbial consortium increased microbial population as compared to recommended NPK. During the grand growth stage, integration of MC improved 14.97% bacterial population at the reduced NPK level. However, the population could be increased by 52.23% at the harvest stage. Inoculation of MC also recorded a higher increase (38.49%) in population of fungi at the harvest stage with the recommended level of NPK as compared to a reduced level. The higher population of all the microbes was recorded during the grand growth phase except actinomycetes. The population of actinomycetes was recorded at the highest level during the maturity phase. Recommended NPK + MC improved bacterial population in the range of 16.66% and 64.75% during the tillering and harvest stage, respectively, as compared to control (N0P0K0). The fungi population also improved in the range of 42.84% and 75.57%, respectively, and the corresponding increase for actinomycetes during the tillering and harvest stage was found as 17.98% and 53.81%, respectively, over the control (N0P0K0). Inoculation of MC at the reduced level of NPK also improved the population of actinomycetes by 41.42% compared to the recommended NPK (9.38% increase) during the grand growth stage.

**Tiller population and partitioning of dry matter accumulation.** The periodic tiller population increased from 60,800 ha⁻¹ in March to 98,700 ha⁻¹ in September (Table 4). Tiller number increased till June during high temperature and low humidity and reached at the highest level (166,700 ha⁻¹). After that, the tiller population declined due to their mortality in high temperature and high humid conditions prevailing during the monsoon/rainy season. In September, the mean tiller population reached 98,700 ha⁻¹. Application of NPK along with MC increased tillering in sugarcane during all the growth stages. An improvement of 38.45% to 58.55% was obtained with recommended NPK + MC compared to control (N0P0K0). However, the additional effect of MC on increasing tiller population in September was recorded as 11.24% higher than N150P26K50. Integration of MC with the reduced level of NPK increased tiller population by 13.48% in September. An increase in tiller population showed improved growth in the integrated application of inorganic fertilizer with MC. Dry matter accumulation (DMA) at the different growth stages is partitioned into leaf and stalk and depicted through Fig. 3a–c. About 38.8% DMA of total dry matter (TDM) at the harvest (37.1 Mg ha⁻¹) was recorded during tillering stage. The DMA increased at a faster rate and reached 34.1 Mg ha⁻¹ (91.9% of TDM) at the grand growth phase. Partitioning in leaf and stalk showed that leaf contributed about 46.5% during the tillering phase-out of total biomass produced at tillering. Thus contribution of stalk was higher (53.5% of TDM at tillering). However, during the grand growth phase, the contribution of leaf reduced to 25.2%, and stalk increased to 74.8%. During the maturity stage also, the contribution of leaf and stalk to the TDM was recorded to 26.9% and 73.1%, respectively. Thus increasing biomass during the grand growth phase increased dry matter accumulation in stalk and showed diversion of photosynthates from source to sink. Tiller mortality was recorded during the grand growth phase, and elongation of cane diverted a higher share of biomass in stalk as compared to leaf. At all the growth stages, and improved dose of NPK accelerated biomass production in leaf and stalk. During the tillering phase, the application of NPK + MC recorded about 112.5% higher biomass accumulation as compared to control (N0P0K0) in leaf.

However, the increase in stalk was recorded at a level of 95.6% with the integration of NPK and MC over N0P0K0. Reduced NPK and inoculation of MC increased 34.51% TDM at the harvest stage as compared to N150P26K50. However, at the recommended NPK level, MC could increase the TDM by 26.65%. In both groups, improvement in dry stalk matter was greater than the total dry matter improvement. At the reduced NPK level, MC could improve dry stalk matter by 38.25% vis-a-vis 34.27% at the recommended NPK. It indicated the effect of microbial consortium in increasing biomass production. Reduction in NPK and integration with MC proved superior over recommended NPK (N150P26K50), and it increased 11.16% higher biomass accumulation in stalk at the harvest stage as compared to recommended NPK.
Root weight and volume. An increase in dry matter production resulted from vigorous root growth due to the higher absorption of nutrients from the soil. Fresh weight and Volume of active roots in 0–15 cm plough depth was determined at different growth stages (Table 5). The mean fresh weight of sugarcane roots increased to the grand growth phase (1.19 Mg ha⁻¹) and decreased after that till harvest. Root volume also followed a similar trend as of root weight. During the tillering stage, it was about 1.03 M³ ha⁻¹ and increased to 1.5 M³ ha⁻¹ during the grand growth phase and declined at 0.91 M³ ha⁻¹ during maturity. Thus approximately 39.33% root volume diminished during the grand growth phase to the maturity phase. However, since the tillering phase, about 45.63% root volume increased up to the grand growth phase. It was because of the elongation phase of the crop,
Table 4. Periodic tiller population (000/ha) in sugarcane.

| Treatment               | March | April | May  | June | July | August | Sep  |
|-------------------------|-------|-------|------|------|------|--------|------|
| T1: N0P0Ko             | 48.6  | 68.5  | 90.4 | 134.5| 112.4| 94.3   | 74.4 |
| T2:N75P13K25           | 52.75 | 88.00 | 104.6| 164.7| 139.7| 113.17 | 96.23|
| T3:N150P26K50          | 67.61 | 96.56 | 138.7| 181.1| 144.8| 124.29 | 106.04|
| T4:N75P13K25 + MC      | 65.32 | 99.23 | 156.2| 183.4| 149.2| 128.4  | 109.2 |
| T5:N150P26K50 + MC     | 74.39 | 104.23| 167.1| 186.4| 155.6| 133.16 | 117.96|
| CD for years            | 7.425 | 10.493| 10.493| 20.588| 17.000| 14.375 | 12.211|
| CD for treatments       | 4.65  | 6.38  | 7.62 | 8.20 | 7.34 | 6.84   | 6.72 |
| CD for years × treatments| NS    | NS    | NS   | NS   | NS   | NS     | NS   |

Figure 3. (a) Bacteria population in soil (log 10 × 10^5 transformed data) as influenced by various treatments. (b) Fungi population in soil (log 10 × 10^3 transformed data) as influenced by various treatments. (c) Actinomycetes population in soil (log 10 × 10^3 transformed data) as influenced by various treatments.
which favored an increase in root dry weight and root volume also. About 8.2% increase in mean root biomass was recorded during the grand growth phase as compared to the tillering stage. Thus it was observed that despite a lesser increase in fresh root weight, root volume increased at the higher rates during the grand growth phase. Improved fine roots (active) influenced root volume despite a lower rate of increase of root biomass. Integration of microbial consortium increased root weight and Volume at all the growth stages. Microbial consortium alone contributed higher root biomass in the range of 26.62% (at the harvest stage) to 62.87% (at tillering stage). However, root volume could be increased in the range of 26.62% and 79.34% at harvest and tillering stages, respectively. The effect of NPK on root biomass and Volume was upheld by the integration of microbial consortium with chemical fertilizer. Integration of MC with reduced NPK dose also showed superiority over recommended NPK. Increased root volume (40.19%) and root weight (18.78%) were recorded with NPK + MC as compared to the application of inorganic source (NPK) only.

| Treatment | Tillering | Grand growth | Harvest |
|-----------|-----------|--------------|---------|
| Fresh root weight (Mg ha⁻¹) | Root volume (M³ ha⁻¹) | Fresh root weight (Mg ha⁻¹) | Root volume (M³ ha⁻¹) | Fresh root weight (Mg ha⁻¹) | Root volume (M³ ha⁻¹) |
| T₁: N₀P₀K₀ | 0.612 | 0.519 | 0.663 | 0.924 | 0.625 | 0.432 |
| T₂: N₇₅P₁₃K₂₅ | 0.763 | 0.627 | 0.887 | 1.263 | 0.850 | 0.65 |
| T₃: N₁₅₀P₂₆K₅₀ | 1.230 | 1.163 | 1.440 | 1.657 | 1.033 | 1.07 |
| T₄: N₇₅P₁₃K₂₅ + MC | 1.367 | 1.264 | 1.526 | 1.724 | 1.143 | 1.173 |
| T₅: N₁₅₀P₂₆K₅₀ + MC | 1.803 | 1.827 | 1.750 | 2.027 | 1.227 | 1.50 |
| CD for years | 0.122 | 0.130 | 0.157 | 0.175 | 0.100 | 0.122 |
| CD for treatments | 0.153 | 0.172 | 0.163 | 0.146 | 0.163 | 0.153 |
| CD for years × treatments | NS | NS | NS | NS | NS | NS |

Table 5. Fresh weight and volume of active roots during various stages of crop growth.

| Treatment | Tillering | Grand growth | Harvest |
|-----------|-----------|--------------|---------|
| N uptake kg ha⁻¹ | P uptake kg ha⁻¹ | K uptake kg ha⁻¹ |
| Leaf | Stalk | Total | Leaf | Stalk | Total | Leaf | Stalk | Total |
| T₁: N₀P₀K₀ | 25.11 | 20.49 | 45.6 | 38.2 | 77.6 | 115.8 | 39 | 85 | 124 |
| T₂: N₇₅P₁₃K₂₅ | 32.82 | 36.75 | 69.57 | 46.23 | 112.5 | 158.73 | 47.5 | 116.7 | 164.2 |
| T₃: N₁₅₀P₂₆K₅₀ | 43.88 | 47.13 | 91.01 | 63.25 | 124.34 | 187.59 | 64.2 | 128 | 192.2 |
| T₄: N₇₅P₁₃K₂₅ + MC | 43.29 | 46.27 | 89.56 | 64.12 | 134.2 | 198.32 | 65.12 | 134.5 | 199.62 |
| T₅: N₁₅₀P₂₆K₅₀ + MC | 47.12 | 52.26 | 99.38 | 65.34 | 142.3 | 207.64 | 66 | 148 | 214 |
| CD for years | 4.638 | 4.894 | 9.558 | 6.720 | 14.311 | 21.014 | 6.821 | 14.834 | 21.65 |
| CD for treatments | 2.65 | 3.20 | 5.43 | 3.25 | 7.45 | 12.43 | 3.45 | 7.43 | 12.32 |
| CD for years × treatments | NS | NS | NS | NS | NS | NS | NS | NS | NS |

Table 6. Nutrients (NPK) uptake and partitioning in leaf and stalk at various growth stages of sugarcane.
Nutrients (NPK) uptake. Nutrients uptake (NPK) presented in Table 6 indicated the positive influence of applying NPK and microbial consortium. About 31.2% N was accumulated in leaf at the harvest stage, (54.18 kg ha⁻¹) and the remaining 69.8% (119.43 kg ha⁻¹) in stalk. During the tillering phase, about 44.0% N accumulation was recorded in sugarcane compared to total N uptake at harvest. Partitioning of N in leaf and stalk showed a narrower margin (48.7% in leaf and 51.3% in stalk) during the tillering stage. However, during the grand growth phase, total N accumulation reached 167.44 kg ha⁻¹, and leaf and stalk contributed 31.8% and 69.2%, respectively. The only marginal increase in N accumulation was recorded after the grand growth phase. Microbial inoculation with a reduced level of NPK brought forth 21.57% to 28.73% higher N accumulation during various growth stages. However, inoculation with the recommended NPK level also significantly influenced total N accumulation at all the stages (9.20% to 11.34% higher than recommended NPK). Thus, it was proved that by reducing NPK level and MCs inclusion, N accumulation could be improved. However, the response could be further enhanced with the recommended level of NPK + MC. About 15.44% higher N accumulation in stalk at the harvest stage could be analyzed due to the inclusion of microbial consortium with recommended NPK.

P uptake in leaf, stalk, and total accumulation at the harvest stage indicated the positive influence of NPK and the inclusion of microbial consortium. About 29.55% P of total uptake (33.40 kg ha⁻¹) was accumulated during the tillering stage only. P accumulation increased to 31.57 kg ha⁻¹ (94.5% of total) during the grand growth phase. Reduction in N at 75 kg ha⁻¹ produced out in kg cane produced per kg of N, P, and K applied (Table 7). N’s agronomic efficiency could be increased with inoculation of MC improved about 9.90% higher P uptake in stalk than N150P26K50 (24.35 kg ha⁻¹). It showed a similar trend, as shown by sugarcane yield. Thus about 24.08% improvement in CCS was achieved by the application of NPK + MC as compared to control (N0P0K0). Thus NPK application and inoculation of MC showed the importance of K nutrition in sugarcane crops.

Growth, yield attributes, sugarcane and sugar yields. Application of NPK with microbial consortium improved individual cane length, diameter, and weight. The higher number of millable canes was recorded with NPK + MC compared to reduced NPK/N0P0K0. About 180.95% higher mean length of cane was recorded with NPK + MC as compared to N0P0K0. However, MC’s integration increased mean cane length by 8.99% at the 50% NPK level. NPK + MC recorded a 16.12% improvement in cane diameter and 87.67% in individual cane weight over N0P0K0. NPK + MC also increased the number of millable canes (91,440 ha⁻¹) by 23.07% over N0P0K0. These canes also recorded higher length and improved weight. The higher individual cane weight and number of millable canes improved cane yield (110.1 t ha⁻¹) with NPK + MC. Inoculation of microbial consortium with NPK increased sugarcane yield by 21% (110.1 t ha⁻¹) over recommended NPK (91.4 t ha⁻¹). Reduction in NPK to half significantly affected sugarcane yield (76.04 t ha⁻¹). However, the integration of MC with reduced NPK improved cane yield (95.3 t ha⁻¹) by 25.33% over N0P1K150. Thus, concerning sugarcane yield, integration of MC with reduced NPK level (95.3 t ha⁻¹) showed superiority over recommended NPK (91.4 t ha⁻¹). Commercial cane sugar was the product of sucrose % juice and sugarcane yield. Sucrose % juice (19.16) could also be significantly influenced by NPK + MC over N0P0K0 (18.34). Commercial cane sugar also followed a similar trend, as shown by sugarcane yield. Thus about 24.08% improvement in CCS was achieved by the inoculation of MC with recommended NPK. However, with reduced NPK levels, also, the increase was recorded to 28.1% over N0P1K150. Inoculation of MC with N0P1K150 could improve CCS by 10.17% (12.67 t ha⁻¹) over N150P26K50. Thus application of microbial consortium saved 50% NPK (N75P13K25) and improved sugarcane and sugar yields with N75P1K25. However, the application of N150P3K50 further improved sugarcane and CCS yields.

Agronomic efficiency of NP & K. Agronomic efficiency (Apparent recovery of N, P, and K) was worked out in kg cane produced per kg of N, P, and K applied (Table 7). N’s agronomic efficiency could be increased with the inclusion of microbial consortium and inorganic sources of fertilizers. Reduction in N at 75 kg ha⁻¹ produced 220.5 kg cane per kg N applied. However, increasing N dose to 150 kg ha⁻¹ marginally decreased agronomic efficiency to 212.7 kg cane per kg N applied. The inclusion of MC with 50% reduced N increased it to 477.3 kg cane per kg N used. However, with the application of 150 kg N (RD), it could be reduced (337.4 kg cane per kg N applied). Recommended N with microbial consortium showed 116.5% improvement over no N and 58.63% improvement over recommended N (150 kg N ha⁻¹).

Agronomic efficiency of P (AEp) was higher than N. The highest AEp (2753.8 kg cane/kg P applied) was recorded with P13 + MC. The agronomic efficiency of P with full P (26 kg ha⁻¹) + MC was higher (1946.5 kg cane
per kg P applied) as compared to a similar P level without MC (1226.9 kg cane per kg P applied). This showed that the agronomic efficiency of P could be increased with the integration of MC and inorganic fertilizer. Agronomic efficiency of K ranged from 661.16 kg cane per kg K applied to 1432.0 kg cane/kg K applied in various treatments. Recommended K level (K_{25}) reduced AEK to 638.0 kg cane/kg K applied as compared to K_{50} (661.6 kg cane/kg K applied). However, the response at both levels could be increased with the inclusion of microbial consortium. Reduction at a higher level of K was due to a decreasing rate of increase beyond K_{25}. Although sugarcane yield significantly increased up to N_{150}P_{26}K_{50} and at this level, the inclusion of microbial consortium further improved the yield sugarcane and sugar yields.

### Discussion

At a higher level of NPK, the inclusion of microbial consortium improved soil organic carbon by improving biological activities and decomposing crop residues containing lignin, hemicellulose, and cellulotic materials. The mean increase in soil organic carbon during the grand growth phase was the primary source of higher degree of microbial activity at higher temperature and moisture during monsoon/rainy season. NPK application increased tiller population, and during the grand growth phase, higher mortality of tillers also increased SOC at higher levels of NPK + MC. Soil microbial biomass carbon (SMBC), soil microbial biomass nitrogen (SMBN), and soil respiration (SR) are mainly governed by microbial population and activities. Higher rates of NPK also imparted higher soil enzymatic activity. Soil enzyme activity is controlled by microbial population.

Higher microbial populations improved soil enzyme activity resulting in the degradation of complex compounds such as protein, phospholipids, lignin & cellulose significantly at a higher level of NPK and inclusion of microbial consortium. The higher population improved soil enzyme activity resulting in the degradation of complex compounds such as protein, phospholipids, lignin & cellulose significantly at a higher level of NPK and inclusion of microbial consortium.

Higher SOC leads to improved soil microbial biomass carbon. Higher application of NPK and MC also made available higher N to crop and affected soil microbial biomass nitrogen. Soil respiration is governed by bacterial/microbial population and represents the life of soil. Higher soil organic carbon and SMBC also improved soil respiration. Residue decomposition due to increased microbial community during the tillering and grand growth phase also improved soil respiration under N_{150}P_{26}K_{50} and MC. Significant improvement of SMBC during the grand growth phase was recorded due to the higher microbial population in the congenial environment, i.e., rainy season-high temperature and high humidity. Thus the availability of nutrients was improved in the rhizosphere that could be made available to sugarcane crops without an external supply of nutrients during the grand growth phase.

The microbial population increased due to the congenial rhizospheric environment during the grand growth phase (elongation phase) improved at the recommended level of NPK. Inoculation of *Trichoderma*, *Gluconacetobacter*, and *Pseudomonas* in microbial consortium further improved the soil microbial activities. *Trichoderma harzianum* induced several enzymes and inhibited soil pathogens. *Pseudomonas fluorescens* solubilized unavailable P and produced organic acids. *Gluconacetobacter* also makes Indole-3-Acetic Acid. Thus congenial conditions for microbial activities were created. Improved activities of microbes also increased SMBC, SMBN, and SR at all the growth stages.

The available nutrients (NPK) status of soil was improved due to the application of NPK. Further, the addition of microbial consortium enhanced nutrients availability during all the growth stages. The mean N level increased during the grand growth phase as compared to the tillering and maturity phase. The decomposition of organic residues, higher microbial activities, and improved SMBC and SMBN also positively influenced N availability during the grand growth phase. The highest P availability was recorded during the tillering stage. The marginal decrease in P was recorded during the grand growth phase, and further availability of P increased till maturity. P fixation and availability are greatly influenced by atmospheric temperature and moisture contents of the soil. The application of P fertilizer along with PSB in MC increased P availability during the tillering phase. However, initial uptake of P was found higher, so a marginal decrease in P availability in soil during the grand growth phase occurred. During the grand growth phase, loss of P due to leaching and erosion because of monsoon season might decrease P content in soil, and after passing monsoon season, the P accumulation increased during winter season.
The availability of K in soil was reduced as crop growth advanced towards maturity. The amount of removal of K was higher as compared to nitrogen and phosphorus in the sugarcane crop. During the maturity phase, higher nutrients uptake from crop depleted the availability of K in soil. Higher application of K through inorganic fertilizers increased K availability in soil. However, experimental soil (alluvial soil) was dominant in illite clay mineral, and the release of non-exchangeable K and availability of exchangeable K was largely dependent on K concentration in soil solution and the presence of NH4+. Microbial inoculation regulates soil C and N turnover, cellulose degradation, fixes atmospheric dinitrogen, releases organic acids, influences soil enzyme activity and release of nutrients uptake and availability in soil. In vitro activity of P solubilizers could be observed by protein production, organic acid binders, and organic P mobilization, probably due to the production of phytase.

Cation exchange capacity (CEC) characterizes the number of fixed negative charges of plant cell walls and is an important parameter in studies dealing with the uptake of ions into roots. However, the cation exchange capacity of soil represents the capacity to hold the exchangeable cations on the clay complex. It influences the soil’s ability to hold onto essential nutrients and provides a buffer against soil acidification. The higher rates of application of fertilizers released a higher number of exchangeable cations on clay micelle. Microbial population improves the soil structure and binds the particles through fungal hyphae. Thus improved chemical and biological conditions affect the cation exchange capacity of soil and roots. In our experiment, the cation exchange capacity of roots was higher during the tillering stage as compared to grand growth and harvest stages. Efficiency of roots and improved cation levels due to fertilization and inoculation of MC improved CEC of roots during the tillering period. Cation exchange capacity of roots was greatly reduced in control plots (N0P0K0) because of the poor availability of cations, particularly K+, Ca++, and Mg++. Poor availability of exchangeable cations also affected the population of anions HPO4–, H2PO4– and NO3–. When clay particles are negatively charged, they attract and hold on to cations stopping them from being leached down the soil profile. This created a deficit of N, P, and K in the rhizosphere and decreased root CEC. The CEC of soil increased during the grand growth phase and decreased after that till maturity. The higher availability of nutrients during the grand growth phase, higher microbial communities and their activities, and congenial environment for grand growth improved the CEC of soil also. After completion of the grand growth phase, the demand for nutrients was diminished due to sugar accumulation during the maturity of the crop. Lack of fertilization and drying of active roots in canopy reduced CEC of soil in the later phase of the crop. Improved CEC of soil due to fertilization and the microbial consortium was observed due to a higher amount of available cations and root activities.

The population of bacteria, fungi, and actinomycetes was influenced by nutrients application and microbial inoculation. Application of Trichoderma influenced the activity and releases organic acids binding agents through hyphae and decomposed cellulose compounds at faster rates. Gluconacetobacter is IAA producing bacteria and as endophytic bacteria, fixes N in sugarcane crop. Rhizodepositions also favoured mineralization processes. This increased mineralization was governed by nitrobacteria. P solubilization was also improved by the bacterial population. The addition of NPK fertilizers also released inorganic acids controlled by microbes in soil. An increased population of bacteria during the grand growth phase improved the availability of nutrients and their removal through faster residue decomposition. Population of fungi also increased during the grand growth phase. It was due to higher moisture (high humidity) and congenial temperature for fungi. However, a marginal increase in the fungi population during maturity was recorded due to closed canopy, less interception of light on soil, and low temperature during maturity phase of the crop. Actinomycetes inhabit the rhizosphere of agricultural crops, where they increase soil fertility through the recycling of organic matter and solubilizing phosphates. The population of actinomycetes was the lowest as compared to bacteria and fungi; however, an increasing trend was observed till harvest. Slightly saline pH of soil favoured population builds up of actinomycetes.

The tillering pattern in sugarcane showed an increasing trend till the month of June. It was due to high temperatures along with low humidity. After that, due to the onset of monsoon, crop entered the elongation phase, and the tiller population decreased due to the mortality of tillers. Tiller mortality continued till September, and in the month of October, millable canes were counted. Sugarcane tillering was greatly influenced by NPK application because of the role of these nutrients in chlorophyll formation, protein metabolism, root growth, and K in translocation of carbohydrate. Improved dry matter accumulation during the grand growth phase was higher in stalk as compared to leaf. Diversion of photosynthates from leaf to stalk and deposition of sucrose in parenchyma tissues during maturity of sugarcane crop increased dry matter of stalk. Besides, tiller mortality during the grand growth phase and stabilizing maximum leaf area during the grand growth period also decreased the contribution of dry matter in leaf. The application of MC with chemical fertilizer increased dry matter accumulation. Thus, being a high biomass producer, the sugarcane crop had great potential in increasing crop yield. Maximization in cane yield could be achieved with the integrated use of various sources for efficient nutrient supply in a balanced proportion. Higher biomass could be achieved due to improvement in soil physical, chemical, and biological conditions, higher nutrients absorption, and improved physiological and biochemical processes through the application of microbial consortium with chemical fertilizers.
Root weight and Volume were greatly influenced by nutrients application with microbial consortium. Application of NPK increased fresh root weight and Volume. Root growth and Volume increased up to the grand growth phase. However, during maturity, the root weight and volume diminished. Initially, the more adventitious roots developed, and when the crop attained the grand growth phase, roots started decaying in the soil. It was also due to interculturing, earthing-up, and crop management practices. However, the weight and Volume of active roots increased up to the grand growth phase. Percent increase in root weight due to NPK application, and MC was greater during the tillering stage compared to the harvest stage. This signified contribution of plant nutrition during the early phase of crop in sugarcane. N is applied for increasing tiller emergence in sugarcane crop. During the grand growth phase, a favourable rhizospheric environment supports the growth of earlier formed tillers. These tillers receive maximum light interception, nutrients supply for their growth. Thus these tillers are converted into millable canes of higher length and weight. Increased tiller population was also recorded due to the application of MC. Higher weight and Volume of roots supported early and vigorous tillers. Microbial consortium improved root weight and Volume at both the levels of NPK. However, higher percent increase in the Volume of roots was observed as compared to fresh weight. This was due to more number of fine roots having lesser comparative weight per unit area. This affected nutrient absorption in a positive manner. Also, MC supported the growth of active roots having comparatively higher dry weight and higher volume.

Nutrients uptake (NPK) was also affected by dry matter production and nutrients composition. The higher biomass was accumulated in NPK + MC plots, and these plots also removed higher nutrients per unit area. However, the differences in nutrients uptake patterns among N, P, and K were also obtained. The higher nutrient contents during tillering as compared to grand growth and maturity brought forth variations in nutrient accumulation patterns as compared to dry matter accumulation. During the tillering stage, about 38.8% dry matter production of total at harvest was recorded. However, N uptake during the tillering was about 44%. Similarly, P & K uptake also contributed 29.55% and 54% during the tillering stage as compared to the total accumulation of these nutrients at the harvest stage. This signified the application of full P and K as basal and splitting of N up to the tillering phase. Higher demand for P & K by the crop during the tillering phase improved higher rates of accumulation. Application of NPK significantly affected Chlorophyll formation, carbohydrate metabolism, protein metabolism, and influenced dry matter accumulation and nutrients uptake in sugarcane crop. Besides, the role of Gluconacetobacter diazotrophicus, Pseudomonas, and Trichoderma in producing organic acids, nutrients availability, soil enzyme activities, containing soil pathogens, decomposing cellulosic material, and improving mineralization processes improved nutrients uptake in crop.

Effect on increasing tiller population affected the number of millable canes. The role of NPK in plant physiological and biochemical processes improved cane length having higher individual cane diameter. Cane weight was the product of individual cane length and its diameter. Thus the improvement in cane weight and number of millable canes per unit area affected sugarcane yield. Inclusion of microbial consortium at a higher rate of NPK application favoured growth, yield attributes, and sugarcane yield. The carbohydrate, protein, and fat metabolisms in plants are governed by NPK application and resulted in improvement of growth, yield attributes, cane, and sugar yields. Commercial cane sugar increased due to higher sugarcane yield and improved sucrose content through the inoculation of microbial consortium with NPK. About 24.08% higher CCS (14.27 t ha−1) was recorded with NPK + MC as compared to sole NPK. Although, NPK + MC produced 21% higher sugarcane yield with a similar level and clearly showed the contribution of sucrose content in sugar yield (CCS) besides cane yield. Effect of microbial consortium on tillering, millable canes, growth, and yield attributes and sucrose content also brought forth improvement in sugarcane and sugar yields.

The highest agronomic efficiency of P was recorded as compared to N and K. It was due to reduced application of P (13 and 26 kg ha−1) as compared to N (75 and 150 kg ha−1) or K (25 and 50 kg ha−1). However, AEK decreased to 212.7 kg cane/kg N applied with increasing N level from 75 to 150 kg ha−1. This indicated that although increasing N levels improved sugarcane yield after 75 kg N ha−1, however, the rate of increase was decreased. At the reduced level of N also, AEK increased and proved the superiority of integration of MC with chemical fertilizers (N) as compared to sole N. At the recommended level of NPK, however, further N, P, K use efficiencies (AE) reduced as compared to 50% (NPK) + MC despite higher yields obtained at recommended NPK. Agronomic efficiency of N, P, and K were found in order of AE > AEK > AEY. Lower doses of P and K were required to produce one kg of sugarcane as compared to N. Improved utilization of P & K by sugarcane crop as compared to N reflected higher AE of P & K than N. Higher mobility of nitrogen in soil and losses through leaching, runoff and volatilization decreased agronomic efficiency of N (AE3) Thus additional N application was required to compensate for the productivity.

Conclusions

Inoculation of microbial consortium containing new strains of Pseudomonas fluorescens, Gluconacetobacter diazotrophicus and Trichoderma harzianum with NPK fertilizers increased soil organic carbon as compared to inorganic fertilizers. However, soil microbial biomass carbon at the grand growth phase was increased by 137% over the control. Soil respiration with recommended NPK + microbial consortium also increased by 60% as compared to N, P, K. Increasing NPK level and integration of microbial consortium improved cation exchange capacity of roots and soil at all the growth stages. The population of microbes was increased due to the application of microbial consortium. Reduction in NPK and integration with microbial consortium increased 11.16% higher biomass accumulation in stalk at the harvest stage as compared to recommended NPK. Microbial consortium alone contributed higher root biomass in the range of 26.62% (at the harvest stage) to 62.87% (at tillering stage). About 15.44% higher N accumulation in stalk at the harvest stage could be analyzed due to the inclusion of microbial consortium with recommended NPK. Reduced NPK with inoculation of microbial consortium improved about 9.90% higher P uptake in stalk than N150P26K50 (24.35 kg ha−1). About 61.78% higher

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The highest agronomic efficiency of P was recorded as compared to N and K. It was due to reduced application of P (13 and 26 kg ha−1) as compared to N (75 and 150 kg ha−1) or K (25 and 50 kg ha−1). However, AEK decreased to 212.7 kg cane/kg N applied with increasing N level from 75 to 150 kg ha−1. This indicated that although increasing N levels improved sugarcane yield after 75 kg N ha−1, however, the rate of increase was decreased. At the reduced level of N also, AEK increased and proved the superiority of integration of MC with chemical fertilizers (N) as compared to sole N. At the recommended level of NPK, however, further N, P, K use efficiencies (AE) reduced as compared to 50% (NPK) + MC despite higher yields obtained at recommended NPK. Agronomic efficiency of N, P, and K were found in order of AE > AEK > AEY. Lower doses of P and K were required to produce one kg of sugarcane as compared to N. Improved utilization of P & K by sugarcane crop as compared to N reflected higher AE of P & K than N. Higher mobility of nitrogen in soil and losses through leaching, runoff and volatilization decreased agronomic efficiency of N (AE3) Thus additional N application was required to compensate for the productivity.

Conclusions

Inoculation of microbial consortium containing new strains of Pseudomonas fluorescens, Gluconacetobacter diazotrophicus and Trichoderma harzianum with NPK fertilizers increased soil organic carbon as compared to inorganic fertilizers. However, soil microbial biomass carbon at the grand growth phase was increased by 137% over the control. Soil respiration with recommended NPK + microbial consortium also increased by 60% as compared to N, P, K. Increasing NPK level and integration of microbial consortium improved cation exchange capacity of roots and soil at all the growth stages. The population of microbes was increased due to the application of microbial consortium. Reduction in NPK and integration with microbial consortium increased 11.16% higher biomass accumulation in stalk at the harvest stage as compared to recommended NPK. Microbial consortium alone contributed higher root biomass in the range of 26.62% (at the harvest stage) to 62.87% (at tillering stage). About 15.44% higher N accumulation in stalk at the harvest stage could be analyzed due to the inclusion of microbial consortium with recommended NPK. Reduced NPK with inoculation of microbial consortium improved about 9.90% higher P uptake in stalk than N150P26K50 (24.35 kg ha−1). About 61.78% higher
K accumulation at the harvest stage was recorded with NPK + microbial consortium as compared to control (N₀P₀K₀). The higher individual cane weight and number of millable canes improved cane yield (110.1 t ha⁻¹) with NPK + microbial consortium. Inoculation of microbial consortium with NPK increased sugarcane yield by 21% (110.1 t ha⁻¹) over the recommended NPK (91.4 t ha⁻¹). Allot 24.08% of improvements in commercial cane yield was achieved by microbial inoculation with recommended NPK.

Thus it could be concluded that N₁₅₀P₂₆₀K₅₀ with microbial consortium containing *Trichoderma harzianum*, *Glucanacetobacter diazotrophicus*, and *Pseudomonas fluorescens* can sustain soil quality parameters besides improving sugarcane and sugar yields in subtropical India.

**Data availability**

Authors are ready to share the whole data of the study to Scientific Reports after publication.

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**References**

1. Dawe, D. et al. Do organic amendments improve yield trends and profitability in intensive rice systems?. *Field Crops Res.* **83**, 191–213. https://doi.org/10.1016/S0378-4290(03)00074-1 (2003).
2. Benbi, D. K. & Brar, J. S. A 25 year record of carbon sequestration and soil properties in intensive agriculture. *Agron. Sustain. Dev.* **29**, 257–265 (2009).
3. Raihana, K., Ahmad, D. L. & Sabah, P. Problems and prospects of secondary and micronutrients management in India. *SKUAST J. Res.* **19**, 29–52 (2017).
4. Ongley, E. D. Control of water pollution from agriculture. FAO Irrigation and drainage paper **55** (1996).
5. IPCC Climate Change Impacts, adaptation and vulnerability. In *Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Parry, M. L. et al.) (Cambridge University Press, Cambridge, 2007).
6. Shukla, S. K., Sharma, L., Awasthi, S. K. & Pathak, A. D. Sugarcane in India: Package of practices for different agro-climatic zones, pp 1–64 (2017).
7. Compton, J. E., Watrud, L. S., Porteous, L. A. & Degrood, S. Response of soil microbial biomass and community composition to chronic nitrogen additions at Harvard forest. *For. Ecol. Manage.* **196**, 143–158. https://doi.org/10.1016/j.foreco.2004.06.023 (2004).
8. Lei, L. et al. Thinning but not understory removal increased heterotrophic respiration and total soil respiration in *Pinus massoniana* stands. *Sci. Total Environ.* **621**, 1360–1369. https://doi.org/10.1016/j.scitotenv.2017.10.092 (2018).
9. Mele, P. M. & Crowley, D. E. Application of self-organizing maps for assessing soil biological quality. *Agric. Ecosyst. Environ.* **126**, 139–152. https://doi.org/10.1016/j.agee.2007.12.008 (2008).
10. Sun, S. et al. Response of soil microbial community structure to increased precipitation and nitrogen addition in a semiarid meadow steppe. *Eur. J. Soil Sci.* **68**, 524–536. https://doi.org/10.1111/1365-2389.12441 (2017).
11. Hallmann, J., Quadt-Hallmann, A., Mahaffee, W. F. & Klopper, J. W. Bacterial endophytes in agricultural crops. *Can. J. Microbiol.* **43**, 895–914 (1997).
12. Döbereiner, J. Isolation and characterization of root associated diazotrophs. *Plant Soil* **110**, 207–212 (1988).
13. Reis, V. M., Olivares, F. L. & Döbereiner, J. Improved methodology for isolation of *Acetobacter diazotrophicus* and confirmation of its endophytic habitat. *World J. Microbiol. Biotechnol.* **10**, 101–104 (1994).
14. Urquiaga, S., Cruz, K. H. S. & Boddy, R. M. Contribution of nitrogen fixation to sugarcane: N-15 nitrogen balance estimates. *Soil Sci. Soc. Am. J.* **56**, 105–114 (1992).
15. Alori, E. T., Glick, B. R. & Babalola, O. O. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Front. Microbiol.* **8**, 971. https://doi.org/10.3389/fmicb.2017.00971 (2017).
16. Satyaprakash, M., Nikitha, T., Reddi, E. B., Sadhana, B. & Satyavani, S. A Review on phosphorous and phosphate solubilising bacteria and their role in plant nutrition. *Int. J. Curr. Microbiol. Appl. Sci.* **6**, 2133–2144. https://doi.org/10.20546/ijcmas.2017.604.251 (2017).
17. Sundara, B. Sugarcane Cultivation (Vikas Publishing House Pvt. Ltd., New Delhi, India, 2000).
32. Subbaiah, B. V. & Asija, G. L. A rapid procedure for the estimation of available nitrogen in soil. *Carr. Sci.* 25, 259–260 (1956).

33. Watanabe, F. S. & Olsen, S. R. Test of ascorbic method for determining phosphorus in water and sodium bicarbonate extracts of soil. *Proc. Soil Sci. Soc. Am.* 9, 677–678 (1965).

34. Toth, S. S. & Prince, A. C. Estimation of cation exchange capacity and exchangeable Ca, K and Na content of soil by flame photometer techniques. *Soil Sci.* 67, 439–445 (1949).

35. Martin, J. F. Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. *Soil Sci.* 69, 215–232 (1950).

36. Buchbinder, L. et al. Studies to formulate new media for the standard plate count of dairy products. *Public Health Rep.* 66, 327–340 (1951).

37. Kuster, E. & Williams, S. T. Selective media for the isolation of Streptomyces. *Nature* 202, 928–929 (1964).

38. Jenkinson, D. S. & Powlson, D. S. The effects of biocidal treatments on metabolism in soil I. Fumigation with chloroform. *Soil Biol. Biochem.* 8, 167–177 (1976).

39. Jackson, M. L. *Soil Chemical Analysis* (Pub. Prentice Hall of Indian Pvt. Ltd, New Delhi, 1973).

40. Meade, G. P. & Chen, J. C. P. *Cane sugar hand book (10th)* Wiley inter science (1964).

41. Martin, J. P. Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. *Soil Sci.* 69, 215–232 (1950).

42. Buchbinder, L. et al. Studies to formulate new media for the standard plate count of dairy products. *Public Health Rep.* 66, 327–340 (1951).

43. Kuster, E. & Williams, S. T. Selective media for the isolation of Streptomyces. *Nature* 202, 928–929 (1964).

44. Toth, S. S. & Prince, A. C. Estimation of cation exchange capacity and exchangeable Ca, K and Na content of soil by flame photometer techniques. *Soil Sci.* 67, 439–445 (1949).

45. Martin, J. F. Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. *Soil Sci.* 69, 215–232 (1950).

46. Buchbinder, L. et al. Studies to formulate new media for the standard plate count of dairy products. *Public Health Rep.* 66, 327–340 (1951).

47. Kuster, E. & Williams, S. T. Selective media for the isolation of Streptomyces. *Nature* 202, 928–929 (1964).

48. Jenkinson, D. S. & Powlson, D. S. The effects of biocidal treatments on metabolism in soil I. Fumigation with chloroform. *Soil Biol. Biochem.* 8, 167–177 (1976).

49. Jackson, M. L. *Soil Chemical Analysis* (Pub. Prentice Hall of Indian Pvt. Ltd, New Delhi, 1973).

50. Meade, G. P. & Chen, J. C. P. *Cane sugar hand book (10th)* Wiley inter science (1964).

51. Martin, J. P. Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. *Soil Sci.* 69, 215–232 (1950).

52. Buchbinder, L. et al. Studies to formulate new media for the standard plate count of dairy products. *Public Health Rep.* 66, 327–340 (1951).

53. Kuster, E. & Williams, S. T. Selective media for the isolation of Streptomyces. *Nature* 202, 928–929 (1964).

54. Toth, S. S. & Prince, A. C. Estimation of cation exchange capacity and exchangeable Ca, K and Na content of soil by flame photometer techniques. *Soil Sci.* 67, 439–445 (1949).

55. Martin, J. F. Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. *Soil Sci.* 69, 215–232 (1950).

56. Buchbinder, L. et al. Studies to formulate new media for the standard plate count of dairy products. *Public Health Rep.* 66, 327–340 (1951).
75. Sugiyama, T. & Goto, Y. Physiological role of potassium in the carbohydrate metabolism of plants (part II). Soil Sci. Plant Nutr. 12, 19–23. https://doi.org/10.1080/00380768.1966.10431962 (1966).
76. Arévalo-Gardini, E., Canto, M., Alegre, J., Loli, O. & Julca, A. Changes in soil physical and chemical properties in long term improved natural and traditional agroforestry management systems of Cacao genotypes in Peruvian Amazon. PLoS ONE 10, e0132147. https://doi.org/10.1371/journal.pone.0132147 (2015).
77. Fageria, N. & Moreira, A. The role of mineral nutrition on root growth of crop plants. Adv. Agron. 110, 251–331. https://doi.org/10.1016/B978-0-12-385531-2.00004-9 (2011).
78. Badiger, N. M., Jagadeesh, K., Krishnaraj, P. U. & Suma, M. Effect of inoculation of microbial consortia on growth parameters of green gram (Vigna radiata L.). Int. J. Carr. Microbiol. Appl. Sci. 8, 562–567 (2019).
79. Gusmaini, S., Arifin, A., Abdul, M., Didy, S. & Nurliani, B. Isolation and selection of endophytic bacteria consortia from medicinal plant (Andrographis Paniculata) as plant growth promoting agents. J. Agron. 12, 113–121 (2013).
80. Camagnu, C. J. R. et al. Cellulase decomposing ability of Trichoderma in relation to their saprophytic survival. Arch. Phytopathol. Plant Protect. 42, 698–704. https://doi.org/10.1080/03235400701492731 (2009).
81. Zhao, D., Kane, M., Borders, B. & Harrison, M. Pine growth response to different site-preparation methods with or without post-plant herbaceous weed control on North Florida's lower coastal plain. For. Ecol. Manage. 255, 2512–2523 (2008).
82. Jones JR, Downing JA (2009) In: Encyclopedia of Inland Waters. (Ed) Likens JE, published by Academic Press.
83. Leghari, S. J. et al. Role of nitrogen for plant growth and development: a review. Adv. Environ. Biol. 10, 209–218 (2016).

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Author contributions
S.K.S. wrote the manuscript, planned experiment and executed in field. L.S. identified the bioagents viz., Gluconacetobacter diazotrophicus, and Pseudomonas ﬂuorescens characterised and submitted to Gen Bank. V.P.J. was associated in analysis of soil/plant quality parameters. A.D.P. provided guidance and extended all the facilities of institute. R.T. was associated in isolation of Trichoderma harzianum. S.K. was associated in execution of field experiment and compilation and tabulation of data. A.G. was associated in lab work related to soil, plant, microbes etc.

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Competing interests
The authors declare no competing interests.

Additional information
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