RHIZOBACTERIAL SWITCHING TOWARDS CLIMATE SMART AGROECOSYSTEMS

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Abstract. Climate smart agriculture is defined as a systematic and synergetic ingress for transformation and reorientation of agricultural development under constrained environment with climate risks. Enhanced plant productivity and reduced microbial respiratory C can potentially mitigate the rising of the atmospheric CO_2 however we are currently in shortfall of efficient routes to accomplish these objectives. Under future climate scenarios of exalted CO_2, rhizosphere microbes may serve important links in mediating plant productivity and soil C/N dynamics with optimization of root-soil interface mechanisms to achieve CSA goals. Study was undertaken to precisely quantify microbial biomass-carbon and microbial activity of rhizosphere region of cauliflower (*Brassica oleracea var. botrytis*) at two different agro-climatic zones. A native bacterial strain *Bacillus pumilus* isolated from cauliflower rhizosphere was employed that enhanced plant growth, nutrient uptake with improved soil nutrient status. Microbial biomass carbon (119.8 mg MB-C/ 100 g soil) was the highest with the application of *Bacillus pumilus* and 75% NP fertilizers at both locations. Moreover, the microbial activity was found to be the highest (0.18 mg CO_2/ g soil) with the same consortium up to 48 h and then followed a sudden decreasing trend. The results clearly suggest that *Bacillus pumilus* strains as bio-inoculants can be successfully employed for maintaining soil health being useful in context of climate smart agriculture goals.
Keywords: *Bacillus pumilus, microbial biomass, soil microbial activity, Pearson's correlation, principal component analysis*

Introduction

The hastily growing world population which is expected to be 8.9 billion by 2050 (UN, 2015), raised an alarming situation of increasing food demand. In a study done by Center for Study of Carbon Dioxide and Global Change revealed that about 70-100% increase in crop production is requisite to feed this consistently rising population (UN, 2015). Intensive farming, a tool of elevated yield however, promotes the use of chemical fertilizers and pesticides at peak levels with rapid existence of pests and diseases. When used frantically, these fertilizers and pesticides get accumulated in soil and in water leading to adverse impact on livestock and on human health, as well as on our ecosystem (Liu et al., 2012). Depletion of soil nutrients, diversification of croplands, genetic diversity and augmentation to global warming are the other well-known associated obstacles of intensive agriculture. Thus, considering long-term emphasis of soil quality and fertility there is an increasing interest in the use of climate smart agritechnology that can enhance crop yield as well as ensures sustainability and resilience (Hamilton et al., 2016). Climate smart agriculture (CSA) may be defined as a synergetic approach to transform and reorient agricultural development and security

under the horrified situation of climate change (Khatri-Chhetri et al., 2017). Sustainable productivity, enhanced resilience or adaptation and Green house gases (GHGs) mitigation are identified as the three interlinked pillars requisite to engender these agricultural food security goals. To achieve desired goals of food productivity, adaptation and GHG mitigation CSA approaches must involve the appropriate inclusion of beneficial bacteria. Revitalising soil quality is a major aspect of sustainable agriculture. Technologies like use of plant growth promoting microbes serves as a boon to crops with multifarious attributes. Despite of the restoration of soil fertility, rhizosphere microbes are lending with other perks such as stimulation of plant growth, boosting crop yield and resistance against diversified soil borne pathogens. Plant growth-promoting rhizobacteria (PGPR) can arouse plant growth by direct (fixation of nitrogen, solubilization of phosphorus etc.) or indirect (antibiosis, induction of systemic resistance etc.) mechanisms (Kaushal and Wani, 2016). Some of the common PGPR include genera *Acinetobacter, Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Bacillus pumilus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Rhizobium*, and *Serratia* (Anandaraj and Dinesh, 2008). Various studies have shown positive effects of PGPR on cereals (Shaharoona et al., 2006), fruits (Kavino et al., 2010) and vegetables (Kaushal et al., 2017) when either used alone or in combination with fertilizers for enhancing crop yields and have committed to the progress of sustainable agricultural systems. Impacts of PGPR on growth and yield parameters have been widely investigated, slight clue exists on the effects of PGPR on sensitive biochemical and microbial indices reflecting soil quality. Besides extra sensitive to environmental stress biochemical properties imparts rapid and authentic values on soil quality (Ding, 2010). The biochemical parameters comprise of the variables that are directly associated to microbial activity such as microbial biomass C and respiration. Microbial biomass C and respiration are considered as potential indicators of soil quality and management impacts (Dinesh and Ghoshal, 2013). Moreover, ameliorated microbial biomass C boosts plant productivity and reduced microbial respiratory C loss could potentially mitigate increasing atmospheric CO_2 concentrations. Nevertheless, our understanding of PGPR interactions with plants to regulate microbial biomass C and microbial respiration in the rhizosphere is still limited. We evaluated the effect of *Bacillus pumilus* inoculation with reduced applications of N and P on productivity of cauliflower and soil nutrient status under North Western Himalayas. *Bacillus pumilus* with 75% NP, improves cauliflower yield (by 27.65%) over recommended dose of fertilizers through several different mechanisms, such as enhancement of nutrient uptake and the suppression of plant diseases (Kaushal and Kaushal, 2015). Here, we hypothesized that use of PGPR along with reduced application of fertilizers (NP) would attenuate such negative impacts of sole NPK use on beneficial soil microbiota and their related functions.

Hence, the objective of the study was to figure out the impacts of *Bacillus pumilus* (native strains of PGPR isolated from cauliflower rhizosphere) applied with reduced application of NP fertilizers (urea and muriate of potash) on microbial biomass C and microbial activity (CO_2 evolution). Another objective was to determine the correlations between yield parameters of cauliflower, soil nutrient status and inoculated population of native strain impacting microbial biomass and activity.

Materials and methods

Site description

The study was conducted at two locations (Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan and Regional Horticultural Research Station, Kullu) of Himachal Pradesh that belongs to North-Western Himalayan region of India as presented in *Figure 1*. Experiments was replicated twice at both locations in each year 2010 and 2011. Both locations differ slightly in geographical characteristics, physico-chemical and microbiological properties of soil as presented in *Table 1*. Rhizosphere soil sampling was done before sowing (Mar 2010 and 2011) and after harvest (May 2010 and 2011) of field trails at both locations replicated twice. Three randomized soil samples were collected from each plot to make one composite sample (100 g) with the help of a soil augur and up to a depth of 15-20 cm from all the treated plots and every replicated experiment.
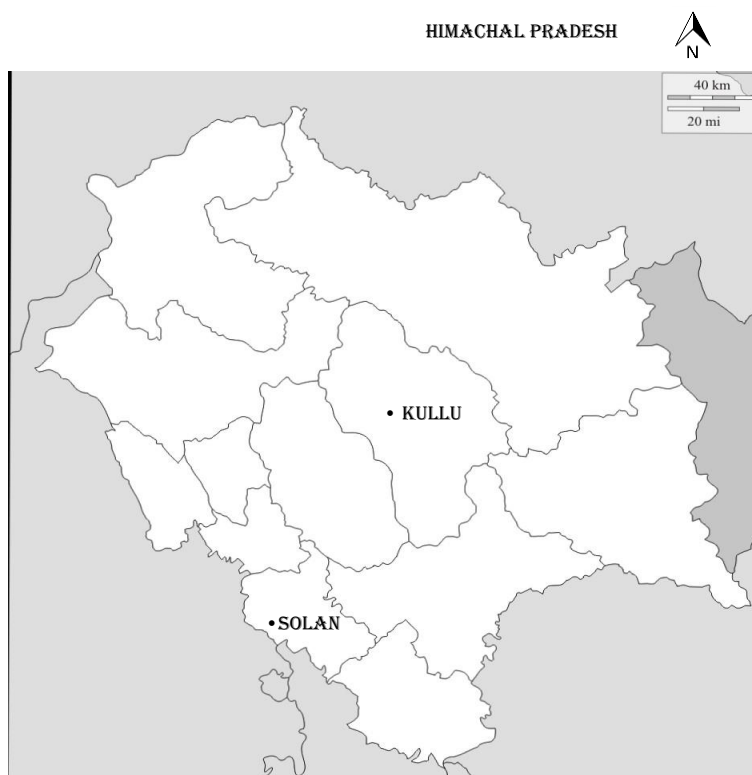

Figure 1. Study area and sampling locations of rhizospheric soil samples

Plant growth promoting rhizobacterial attributes of Bacillus pumilus

Isolated native bacterial strain from cauliflower (*Brassica oleracea var. botrytis*) was tested for its in vitro efficacy in ideal laboratory conditions before the field experimentations (Jan – Mar 2010) at Soil Microbiology Laboratory, Department of Soil Science and Water Management, Dr. Y S Parmar University of Horticulture and Forestry, Solan – India. Isolate was tested for major plant growth promoting attributes including P-solubilization, Indole acetic acid (IAA), siderophore and Hydrogen cyanide (HCN) production and antagonistic activities against phytopathogens (Kaushal et al.,

2016). Nitrogen fixation ability of native bacterial strain was also quantified indirectly by acetylene reduction assay (ARA) method and compared to standard strain (procured from the Institute of Microbial Technology, Chandigarh) of *Azotobacter chroococcum* (Hardy et al., 1968).

Table 1. *General characteristics of both locations (Solan and Kullu)*

Parameters/characteristics	Solan	Kullu
Longitude	77.0967° E	77.1095° E
Latitude	30.9045° N	31.9579° N
Altitude	1502 m	1279 m
pH	6.76	6.71
Electrical conductivity (EC) (dSm^{-1})	0.47	0.34
Bulk density (BD) (g cm^{-3})	1.04	1.50
Organic matter (OM) (%)	1.16	1.03
Nitrogen (N) (kg/ha)	301.7	313.6
Phosphorus (P) (kg/ha)	41.00	86.00
Potassium (K) (kg/ha)	195.3	191.7
Total microbial counts Nutrient agar (NA) ($10^4 \times$ cfu/g soil)	185.67	107.00

Field efficacy of Bacillus pumilus inoculation

Based on in vitro plant growth promoting traits and N_2-fixing abilities, field studies were conducted twice at both locations with *Bacillus pumilus* and reduced application of N and P fertilizers (Kaushal and Kaushal, 2015). The treatments: T1 (Uninoculated control), T2 (*Bacillus pumilus* +25% NP), T3 (*Bacillus pumilus* +50% NP), T4 (*Bacillus pumilus* +75% NP), T5 (*Bacillus pumilus* +100% NP), T6 (100% NPK), T7 (75% NPK), T8 (50% NP), T9 (*Bacillus pumilus*-inoculation) and T10 (Recommended dose of fertilizers) were arranged in randomized complete block design (RCBD) and replicated thrice. Calcium ammonium nitrate (25% N) and single super phosphate (16% P_2O_5) were the sources of nitrogen and phosphorus, respectively. Bacterial cell suspension (at OD 540 nm 1.00) of 72 h old cultures, grown in 10% nutrient broth, was used as inoculum. Data were collected on the growth and yield parameters of cauliflower, soil nutrient status and rhizospheric as well as endophytic microbial population and analysed over recommended dose of fertilizers. We used 20 plants/treated plot for analysing the data and each plant is harvested manually and recorded for growth and yield parameters which includes number of non-wrapper leaves, curd diameter and curd weight.

Efficacy of Bacillus pumilus inoculation on soil microbial biomass and microbial activity (CO2 evolution)

We analysed the incremental growth of microbial biomass-carbon and microbial activity (CO_2 evolution) over time. The fumigation–extraction method was used to analyse soil microbial biomass-C (CMIC) (Vance et al., 1987). Microbial activity was measured as the CO_2 evolved from moist soil, adjusted to 55% water holding capacity, and pre-incubated for 7 days at 20 °C in the dark. The CO_2 evolution was then measured

for the next 7 days using NaOH traps and titration with HCl and the metabolic quotient (qCO_2) was calculated (Salamanca et al., 2002).

Statistical analysis

All samples were measured in triplicate. The selected variables were compared between the tested soils using a one-way ANOVA. Significant differences were detected at the 0.05 level. Correlations between all measurable variables (plant, soil and microbial) were estimated using Pearson's r with $p < 0.05$ significance threshold. Principal components analysis (PCA) was conducted to determine and clarify the relationship between plant, soil and microbial parameters.

Results and discussion

Plant growth promoting rhizobacterial traits of Bacillus pumilus

Bacillus pumilus was the native bacterial strain isolated from rhizosphere of cauliflower. Our earlier published results confirmed that this strain displayed a very high ability towards most of the plant growth promoting attributes (Kaushal et al., 2017).

The isolate (*Bacillus pumilus*) produced a significantly higher concentration P-solubilization (664.33 µg/mL), IAA (29.67 µg/mL), maximum (51.36%) siderophore unit and HCN production (*Fig. 2*).
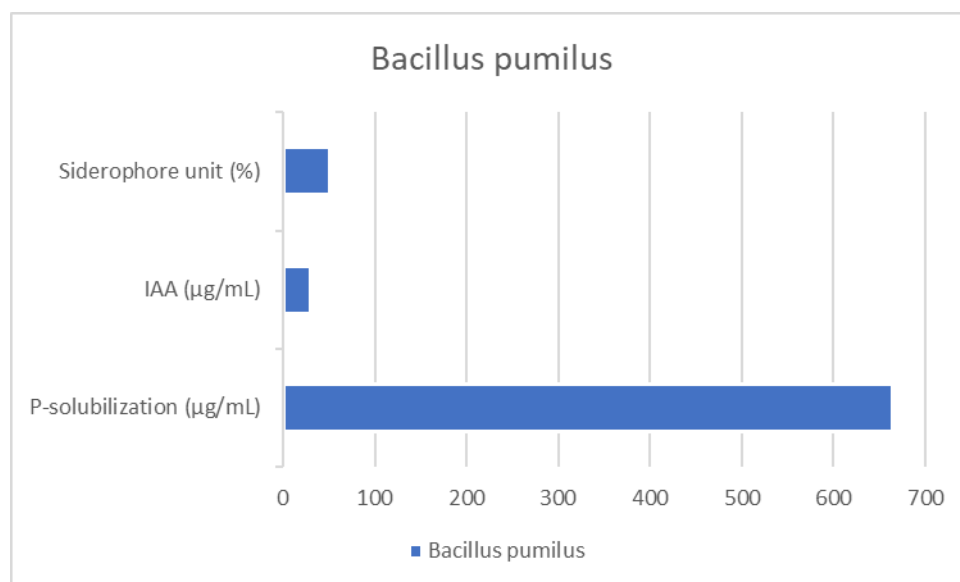

Figure 2. *Plant growth promoting activities of selected bacterial isolates (Bacillus pumilus).*
*Significance < 0.05

Inorganic phosphate is solubilized by rhizosphere microbes with the production of organic acids and chelating oxo-acids from sugar (Cao et al., 2017). Thus, most quantitative analysis to evaluate the efficacy of phosphate-solubilizing bacteria due to the production of organic acids into surrounding medium are based on lowering pH (Bini et al., 2011). IAA produced by *B. pumilus* can enhance plant growth which was

the same as synthetic PGRs produced (Dasri et al., 2016). Siderophore and HCN production was also exhibited by *Bacillus pumilus* used as potential biocontrol agent of Fusarium in tomato (Heidarzadeh and Baghaee-Ravari, 2016). With regards to antagonistic activity against *Sclerotinia sclerotiorum*, culture filtrate of *Bacillus pumilus* exhibited very high antifungal antibiotic activity with an inhibition zone ranged from 20.2 to 22.4 mm. Our results are agreement with other researchers., who demonstrated that *Bacillus pumilus* strain is a promising biological control agent for control against Sclerotinia stem rot (Gao et al., 2014). *Bacillus pumilus* also showed higher nitrogenase activity, i.e., 437.26 ηmole C_2H_4 h^{-1} mg^{-1} protein (109 mg of N_2 fixed/ha/d), as compared to standard of *Azotobacter chroococcum*, i.e., 372.85 ηmole C_2H_4 h^{-1} mg^{-1} protein (93.0 mg of N_2 fixed/ha/d). In support to our results, a representative strain of *Bacillus pumilus* was identified that is also able to fix atmospheric N_2 as determined by the acetylene-dependent production assay (Castellano-Hinojosa et al., 2016).

Field efficacy of Bacillus pumilus inoculation

Our earlier published results confirmed that bio-inoculation of *Bacillus pumilus* at 75% of N and P fertilizers significantly enhanced all the growth and yield parameters of cauliflower such as number of non-wrapper leaves, curd weight and curd diameter (Kaushal and Kaushal, 2015).

Field experiment data of two years for both locations (replicated twice) revealed that bio inoculation of *Bacillus pumilus* along with 75% NP increased curd yield by 27.65% over recommended dose of fertilizers along with the saving of 25% NP (*Fig. 3*). Application of beneficial microbes to crop plants enhanced the fixation of atmospheric N, organic decomposition and augmented the soil properties by production of bioactive compounds such as vitamins, phytohormones which accelerated plant growth and yield (Upadhyay et al., 2012). The increase in levels of chemical fertilizers (NP) along with the bio-inoculation of *Bacillus pumilus* significantly enhanced NPK content in plants. Two years data (each replicated twice) revealed that N contents increased by 4.46% and 3.29%, P contents 0.49% and 0.57%, K contents 3.84% and 3.02% over uninoculated controls at Kullu and Solan, respectively. Uptake of N (77.14 kg/ha and 90.86 kg/ha) and P (35.28 kg/ha and 26.22 kg/ha) also boosted significantly with progressive increase in the supply of N and P nutrients along with bio-inoculation of *Bacillus pumilus* to the crop in both the years and locations. As far as ameliorations in plants nutrient status is concerned, enhanced nutrients like N, P and K might be due to enhanced vegetative growth and improved soil health leading to increased root formation. Studies reported enhanced N, P and K constituent of plant which might be attributed to presence of abundant number of soil microorganisms, microbial respiration and microbial biomass C and align with the findings of other researchers (Liu et al., 2012; Santibanez and Varnero, 2014). In addition, increased level of P might be due to presence of adequate organic acids which were released after organic matter decomposition and subsequently help in the solubilization of the indigenous phosphates. It was interesting to note that larger the total rhizosphere population, more is the accumulation of nitrogen, phosphorus and potassium contents in plants. Soil physico-chemical and nutrient status, i.e. pH, electrical conductivity (EC), bulk density (BD), organic matter (OM), N, P, K, was also assessed after harvest of crop (*Fig. 3*). Concentration of nutrients increased in soil with the combined use of *Bacillus pumilus* and NP fertilizers. The available nutrient contents N and P were increased by 2.56-32.00% and 6.66-40.72%, respectively over control. Bio-inoculation of *Bacillus*

pumilus not only improved the nutritional content particularly NPK of plants but also increased their uptake significantly over uninoculated control. This increase may be attributed to atmospheric nitrogen fixation, phosphate solubilization in the rhizosphere by *Bacillus pumilus*. The higher concentration of soil available nutrients with conjoint use of *Bacillus pumilus* and NP fertilizers may be attributed to well develop root system, significant improvement in soil physical properties and metabolic activity as well as rhizospheric and endophytic microbial population (Kaushal and Wani, 2016). The microbial analysis performed at the harvest of crop in each year at both the locations. The data revealed higher population of rhizospheric and endophytic bacteria in duly inoculated plots as compare to uninoculated control. The highest 162.5 × 10^8 cfu/g soil and 158.8 × 10^8 cfu/g soil rhizospheric population was recorded at Kullu and Solan, respectively. The lowest total bacterial counts were recorded under uninoculated control at both the locations. Also, endophytic bacterial population was highest 106.4 × 10^1 cfu/g root (Kullu) and 108.8 × 10^1 cfu/g root (Solan). At both the locations, the lowest endophytic bacterial counts were recorded with uninoculated control. The rhizosphere is a niche of beneficial microflora. Higher root exudation, OC, N and moisture content in amended organic fertilizers mechanized the microbes for their proliferation (Nagar et al., 2016). Decomposition of root tissue contribute ample energy in terms of C and N for the multiplication of bacteria enabling the growth of heavy microbial population (Singh et al., 2012). Moreover, increase in beneficial microbial population may be because of balanced water supply, particulate organic matter and presence of respirable substances (Majumdar et al., 2014). The study revealed that bio-inoculation of *Bacillus pumilus* supplied plots improved the cauliflower yield, physico-chemical properties of soil leading to enhanced soil microbiome (*Fig. 3*).

Efficacy of Bacillus pumilus inoculation on soil microbial biomass and microbial activity (CO2 evolution)

Soil microbial biomass-carbon (SMBC) was significantly higher in *Bacillus pumilus* + 75% NP plots (*Figs. 4* and *5*). This might be due to enhanced mineralization of organic leading to increased microbial activity (Nagar et al., 2016) as revealed in our studies also. SMBC at Kullu was 119.8 mg MB-C/ 100 g soil and Solan was 117.6 mg MB-C/ 100 g soil both with bio-inoculation of *Bacillus pumilus* + 75% of N and P fertilizers as presented in *Figures 4* and *5*. Similarly, various studies displayed that increased population of *Azotobacter*, PSB, soil enzymes, OC exhibited positive correlation with SMBC (Majumdar et al., 2014). Moreover, significant improvement in SMBC in our experiments might be due to more stimulation in associated microbial populations. Treatments consisting of *Bacillus pumilus* bio-inoculation and chemical fertilizers (NP) increased the CO_2 evolution up to 48 h of incubation period and then followed a decreasing trend with the increase in incubation period at both the locations. However, the maximum CO_2-evolution was recorded 0.18 mg CO_2/ g soil (Kullu) and 0.17 mg CO_2/ g soil (Solan) with bio-inoculation of *Bacillus pumilus* + 75% of N and P fertilizers as presented in *Figures 6* and *7*. The basal soil respiration gives an estimate of total microbial activity, reflecting both the quantity and quality of the carbon sources (Liu et al., 2012). In present study, higher soil respiration sustained our notion that the rhizosphere soils had a dominant soil microbial activity, implying accelerated decomposition of organic residues that makes nutrients available for the consequent stimulation of heterotrophic microorganisms as observed by other researchers (Fall et

al., 2012). Moreover, the lower soil microbial metabolic quotient (qCO_2) displayed lower soil chemical stress to microbes, more worthwhile C utilization ability, low energy demand in microbial biomass supply and improved soil quality (Gonzalez-Quinones et al., 2011). Our results also suggested that inorganic fertilization did not increase soil respiration. Similar effects on soil respiration due to inorganic fertilization has been found when N fertilizer was added, possibly from the elimination of the decomposition of soil organic carbon due to reduction in microbial biomass (Yang et al., 2010; Ge et al., 2012).
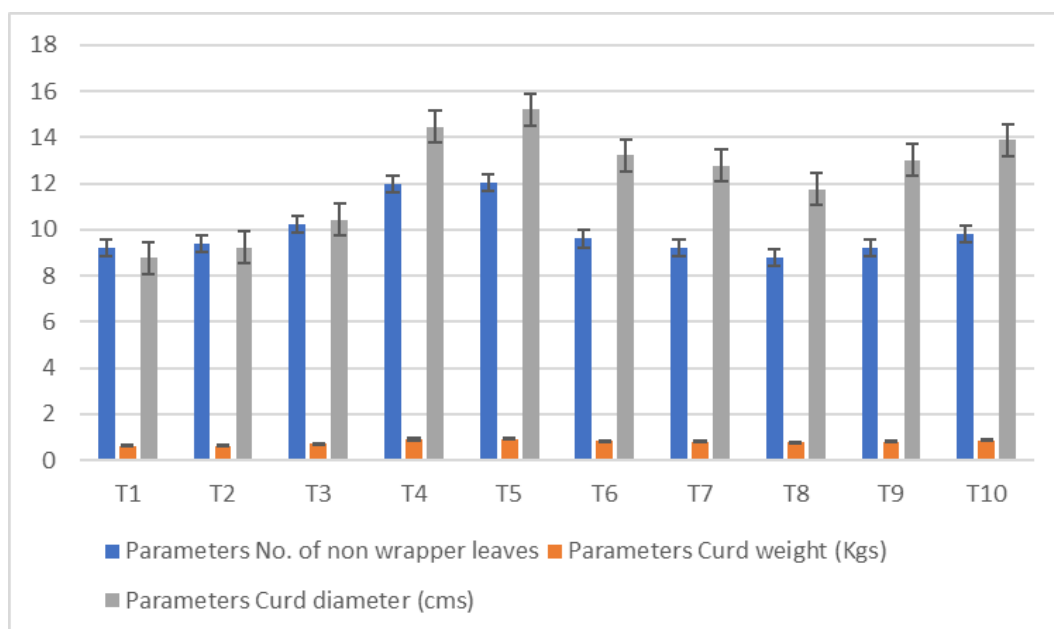
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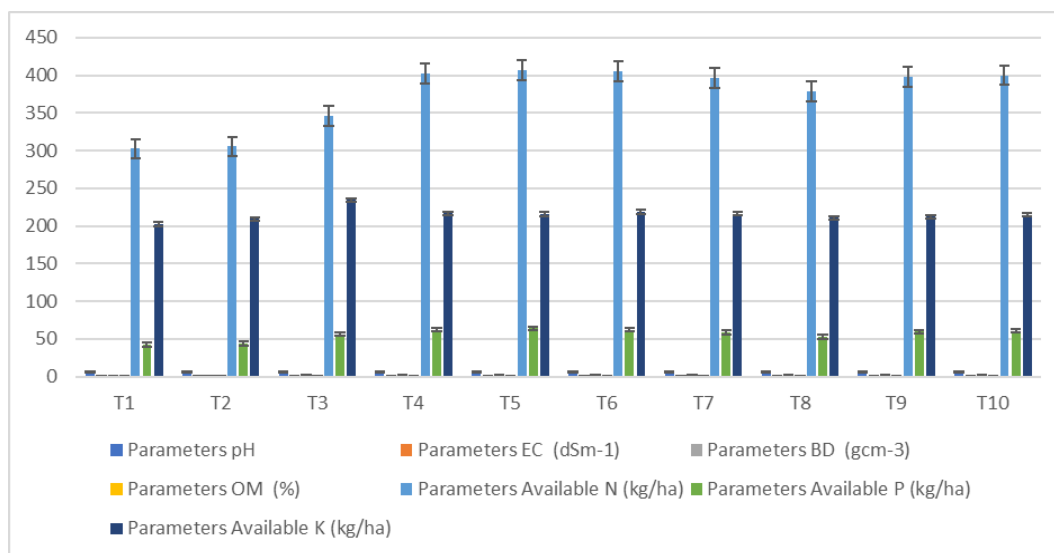
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*Figure 3. Impact of selected bacterial isolates (Bacillus pumilus) on (a) plant and (b) soil parameters. T1 (uninoculated control), T2 (Bacillus pumilus + 25% NP), T3 (Bacillus pumilus + 50% NP), T4 (Bacillus pumilus + 75% NP), T5 (Bacillus pumilus + 100% NP), T6 (100% NPK), T7 (75% NPK), T8 (50% NP), T9 (Bacillus pumilus-inoculation) and T10 (recommended dose of fertilizers) *Significance < 0.05*

*Figure 4. Impact of bio-inoculation of Bacillus pumilus on microbial biomass-carbon at Solan. T1 (uninoculated control), T2 (Bacillus pumilus + 25% NP), T3 (Bacillus pumilus + 50% NP), T4 (Bacillus pumilus + 75% NP), T5 (Bacillus pumilus + 100% NP), T6 (100% NPK), T7 (75% NPK), T8 (50% NP), T9 (Bacillus pumilus-inoculation) and T10 (recommended dose of fertilizers). *Significance < 0.05*
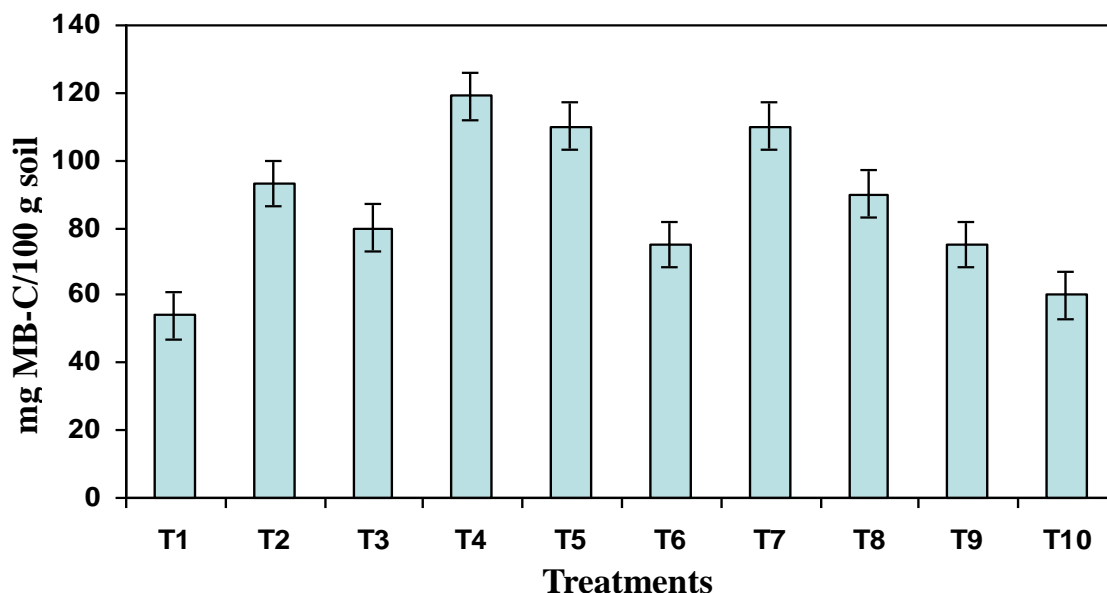

*Figure 5. Impact of bio-inoculation of Bacillus pumilus on microbial biomass-carbon at Solan. T1 (uninoculated control), T2 (Bacillus pumilus + 25% NP), T3 (Bacillus pumilus + 50% NP), T4 (Bacillus pumilus + 75% NP), T5 (Bacillus pumilus + 100% NP), T6 (100% NPK), T7 (75% NPK), T8 (50% NP), T9 (Bacillus pumilus-inoculation) and T10 (recommended dose of fertilizers). *Significance < 0.05*
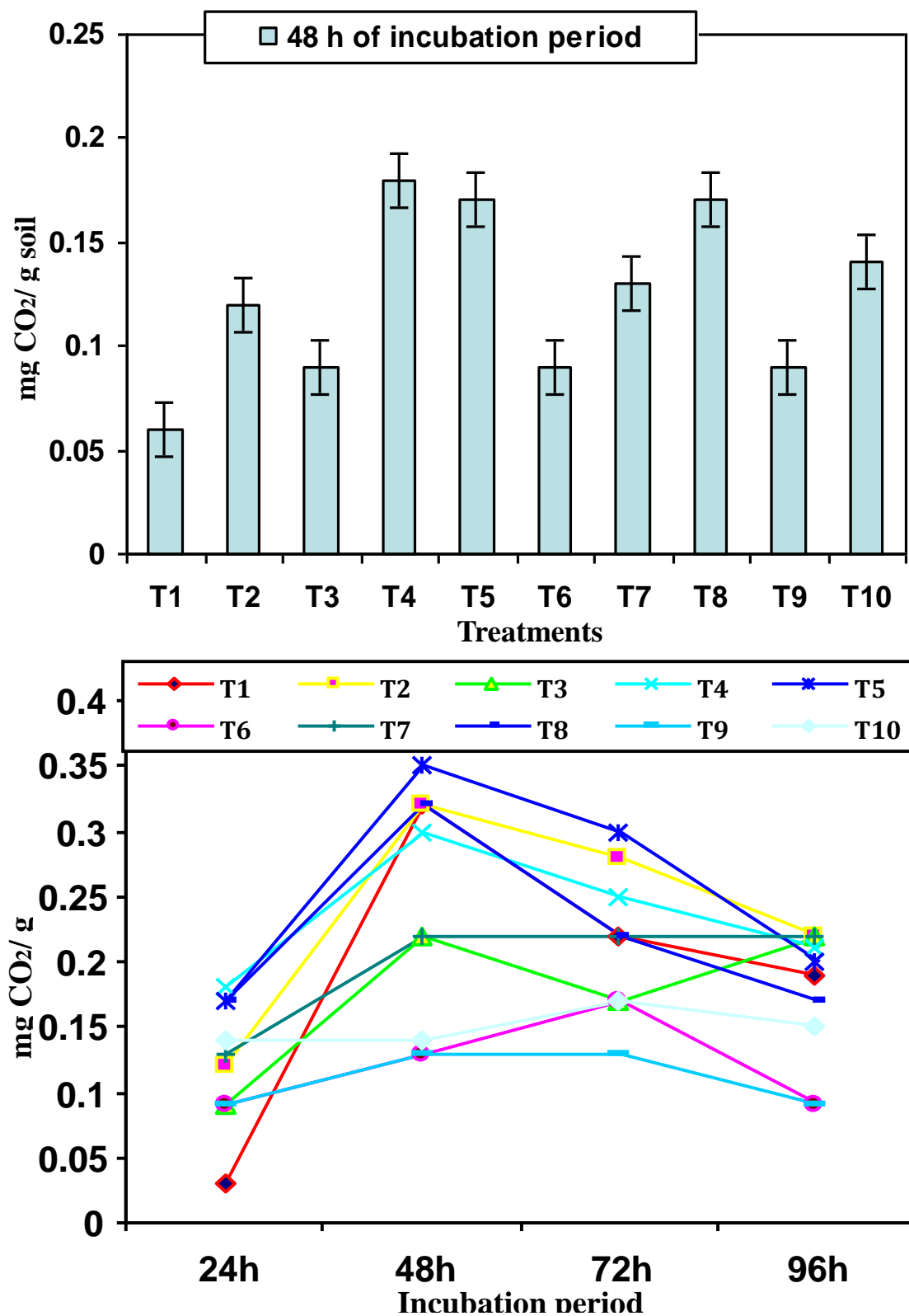
*Figure 6. Impact of bio-inoculation of Bacillus pumilus on microbial activity (CO₂ evolution) at Kullu. T1 (uninoculated control), T2 (Bacillus pumilus + 25% NP), T3 (Bacillus pumilus + 50% NP), T4 (Bacillus pumilus + 75% NP), T5 (Bacillus pumilus + 100% NP), T6 (100% NPK), T7 (75% NPK), T8 (50% NP), T9 (Bacillus pumilus-inoculation) and T10 (recommended dose of fertilizers). *Significance < 0.05*
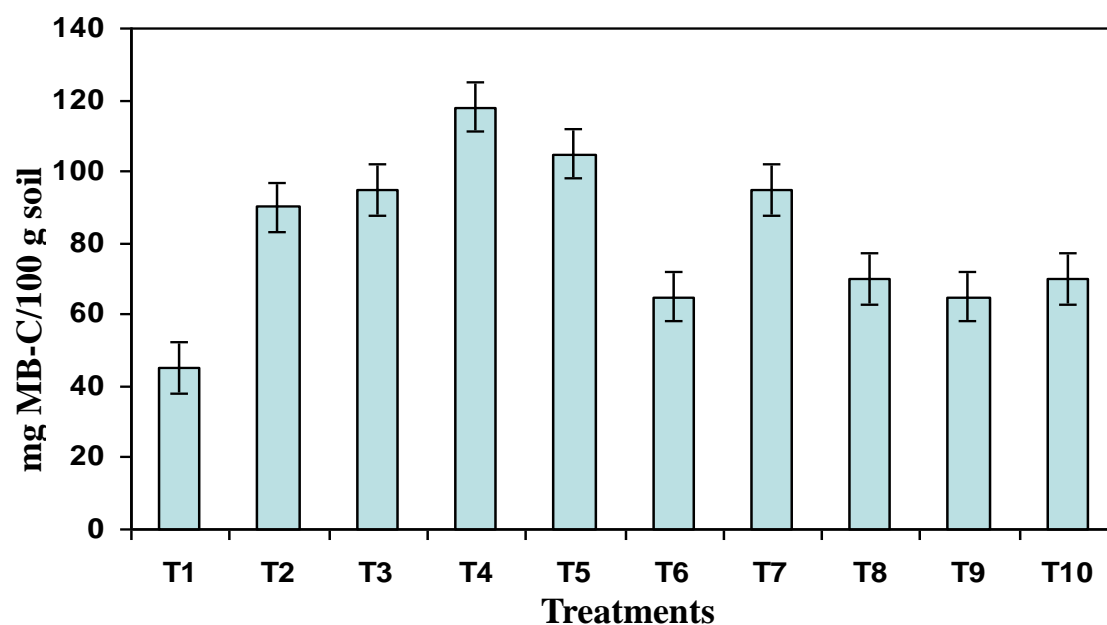
*Figure 7. Impact of bio-inoculation of Bacillus pumilus on microbial activity (CO_2 evolution) at Kullu. T1 (uninoculated control), T2 (Bacillus pumilus + 25% NP), T3 (Bacillus pumilus + 50% NP), T4 (Bacillus pumilus + 75% NP), T5 (Bacillus pumilus + 100% NP), T6 (100% NPK), T7 (75% NPK), T8 (50% NP), T9 (Bacillus pumilus-inoculation) and T10 (recommended dose of fertilizers). *Significance < 0.05*
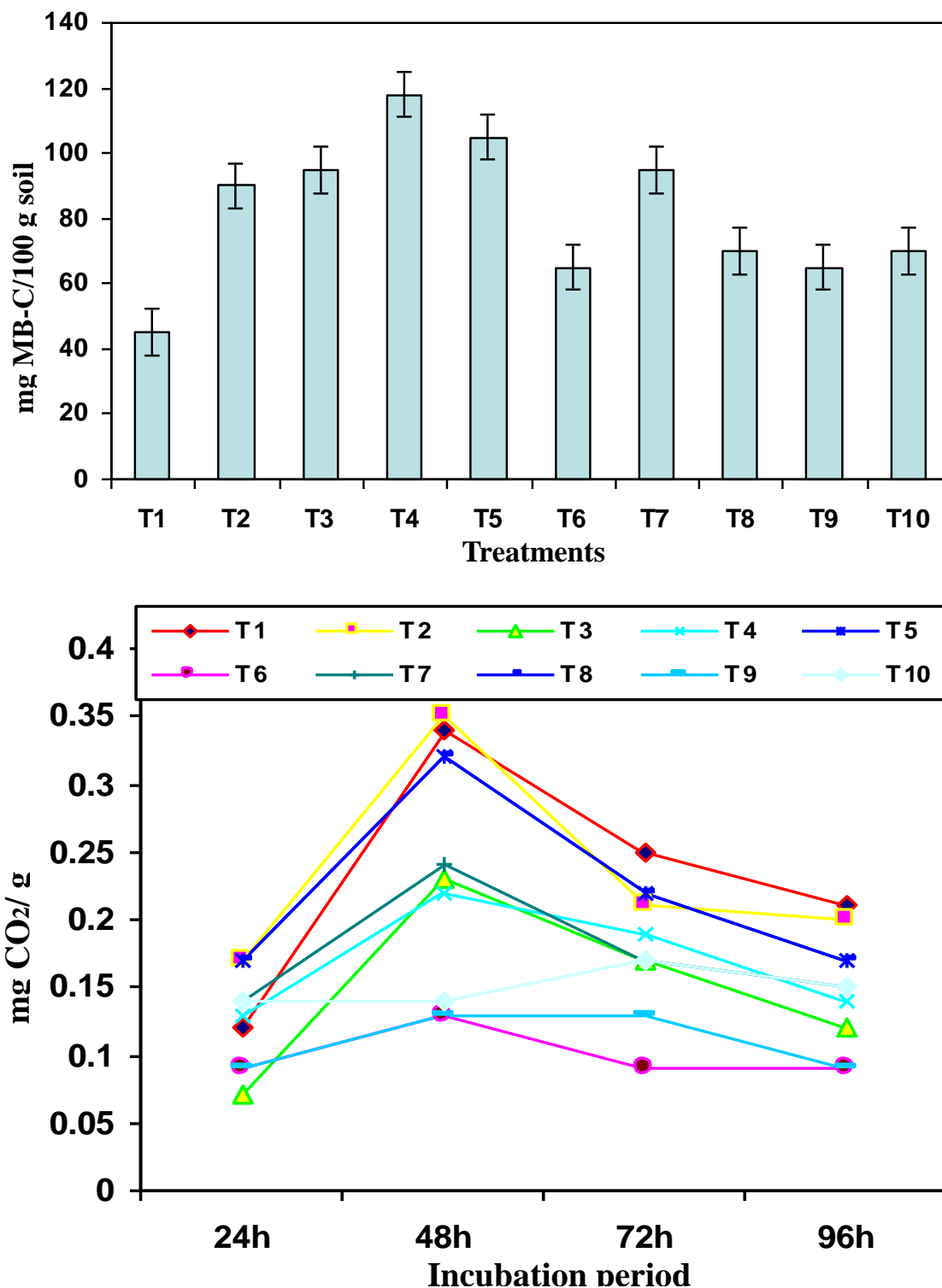
Correlations analysis

Pearson correlation matrix revealed that many plant, soil and microbial variables were significantly correlated with each other The plant parameters, i.e. number of non-wrapper leaves, curd diameter, curd weight, curd yield, curd depth, soil physico-chemical, i.e. pH, EC, BD, OM, N, P, K, rhizospheric and endophytic bacterial population was significantly correlated at 5% level of significance at Kullu as given in *Table 2*. Significant positive correlation exists between curd diameter-N, P (r = 0.49, 0.48 respectively), curd diameter-rhizospheric bacterial population (r = 0.42), curd diameter-endophytic bacterial population (r = 0.38), curd weight-N, P (r = 0.65, 0.73, respectively), curd weight-rhizospheric bacterial population (r = 0.35), curd weight-endophytic bacterial population (r = 0.50), curd yield-N, P (r = 0.65, 0.73 respectively), curd yield-rhizospheric bacterial population (r = 0.35) curd yield-endophytic bacterial population (r = 0.50), curd depth-N, P (r = 0.50, 0.51 respectively), curd depth-rhizospheric bacterial population (r = 0.32), curd depth-endophytic bacterial population (r = 0.33), N-rhizospheric bacterial population (r = 0.42), P-rhizospheric bacterial population (r = 0.43), P-endophytic bacterial population (r = 0.32) and between K-endophytic bacterial population (r = 0.53). The plant parameters, i.e. number of non-wrapper leaves, curd diameter, curd weight, curd yield, curd depth, soil physico-chemical, i.e. pH, EC, BD, OM, N, P, K, rhizospheric and endophytic bacterial population was significantly correlated at 5% level of significance at at Solan as given in *Table 3*. Significant positive correlation exists between number of non-wrapper leaves-N, P (r = 0.32, 0.52 respectively), number of non-wrapper leaves-rhizospheric bacterial population (r = 0.41), curd diameter-N, P (r = 0.47, 0.59 respectively), curd diameter-rhizospheric bacterial population (r = 0.46), curd diameter-endophytic bacterial population (r = 0.35), curd weight-N, P (r = 0.76, 0.84 respectively), curd weight-rhizospheric bacterial population (r = 0.38), curd yield-N, P (r = 0.76, 0.84, respectively), curd yield-rhizospheric bacterial population (r = 0.38), curd depth-N, P (r = 0.52, 0.68, respectively), curd depth-rhizospheric bacterial population (r = 0.37), P-rhizospheric bacterial population (r = 0.47) and between P-endophytic bacterial population (r = 0.31). In our results, positive correlation between plant, soil and bacterial variables was obtained which showed a considerably positive relationship. Enhanced SMBC might be one of the determinant factor of this positive relationship. Moreover, as predicted in our findings, MBC is the important source of C supplying higher energy to the microbes leading to enhanced metabolic activities (Liu et al., 2017). The rhizosphere microbial community is recognized to be closely related to soil health and quality (Raaijmakers and Mazzola, 2016). The positive correlation between N-rhizospheric bacterial population (r = 0.42), P-rhizospheric bacterial population (r = 0.43), P-endophytic bacterial population (r = 0.32) and between K-endophytic bacterial population (r = 0.53) revealed in the field experiments corresponds with many previous studies, suggesting that the soil quality is directly related to bacterial population occurrence and could therefore be used as an indicator for soil fertility prediction. Moreover, bio-inoculation of *Bacillus pumilus* significantly induce the community shift of culturable bacteria in the rhizosphere soil leading to improved soil microbial biomass. From a long-term perspective, appropriate application of *Bacillus pumilus* can improve the soil quality and fertility. This comprises both nutrients (e.g., aggregation of organic matter) and structure (e.g., aggregates accumulation), which is conductive to agricultural sustainable development. The results also suggested a positive effect of N application on microbial biomass-C. After N application, N

availability increased and consequently microbes immobilized N, which subsequently increased microbial biomass-C (Chaparro et al., 2014). A clear positive effect of combined application of *Bacillus pumilus* and NP on microbial biomass and microbial activity was observed. Microbial activity also varies considerably among the treatments including control. The metabolic quotient (soil respiration per unit of microbial biomass) reflects the maintenance energy requirement of soil microbes and can be a relative measure of how efficiently the soil microbial biomass is utilizing C resources, as well as the degree of substrate limitation for soil microbes (Xun et al., 2016). The results in this study did show changes in the microbial pool that would suggest changes in microbial biomass-C and activity as well (*Tables 2* and *3*). In contrast, combined application of *Bacillus pumilus* and NP treatments indicated relatively highly efficient microbial community and better use of the available organic substrates. On the contrary, the Pearson's correlation indicated strongly positive correlation between microbial biomass and plant and soil parameters (*Tables 2* and *3*). All the soil factors controlling microbial biomass-C had significant effects on microbial respiration (CO_2 evolution) as described by the correlation analysis.

Principal components analysis was used to determine the factors related to soil microbial properties. The two components (PC1 and PC2) explained 63.54% and 12.82% of the total variance. The PC1 and PC2 were chosen to draw a biplot, because of explaining 76.36% of the total variance together as presented in *Figure 8*. PCA was weighted by physico-chemical (pH, EC, BD, OM, N, P and K), which exhibited a more powerful influence on discrimination in soil samples and microbiological (rhizospheric and endophytic bacterial population) variables together with growth and yield components. Thus, PCA allowed us to detect the subtle differences in plant, soil physical-chemical and microbial factors among the studied parameters. Additionally, the high soil organic matter (SOM) and N contents as material basis largely contribute to the high microbial biomass and basal respiration. Also, changes in SOM and N application affects soil microbial biomass (SOM/N ∝ SMBC). As far as soil quality is concerned, microbial diversity should be well considered when vast of N fertilizer was added to soils in agricultural practice.
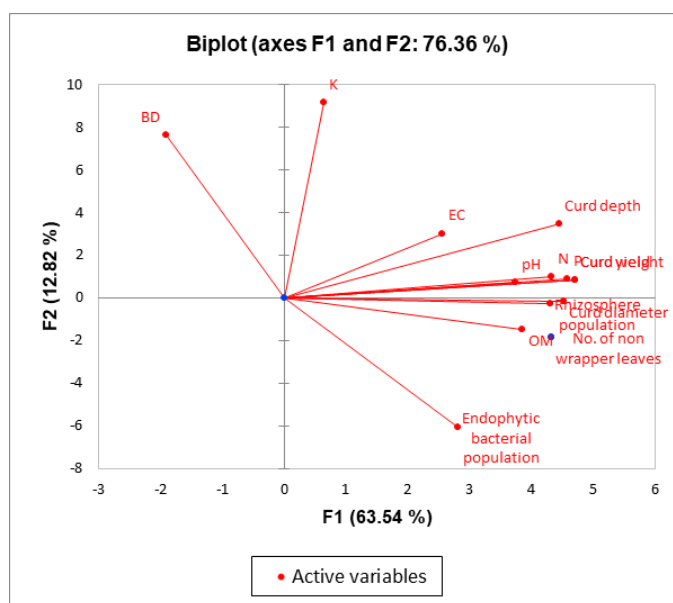

Figure 8. *PCA-ordination biplot of plant, soil and microbial parameters*

Table 2. *Pearson's correlation coefficient (r value) between plant, soil parameters, rhizospheric and endophytic population under field conditions experiment at Kullu (n = 30)*

Parameters	No. non wrapper leaves	Curd diameter	Curd weight	Curd yield	Curd depth	pH	Electrical conductivity (EC)	Bulk density (BD)	Organic matter (OM)	Nitrogen (N)	Phosphorus (P)	Potassium (K)	Rhizospheric bacterial population	Endophytic bacterial population
No. non wrapper leaves	1.00													
Curd diameter	0.49*	1.00												
Curd weight	0.28	0.64*	1.00											
Curd yield	0.28	0.64*	1.00	1.00										
Curd depth	0.21	0.68*	0.61*	0.61*	1.00									
pH	0.11	-0.04	-0.01	-0.01	0.005	1.00								
Electrical conductivity (EC)	0.15	-0.03	-0.02	-0.02	-0.18	0.14	1.00							
Bulk density (BD)	-0.12	-0.04	0.07	0.07	-0.002	-0.15	-0.01	1.00						
Organic matter (OM)	-0.04	0.06	0.16	0.16	0.01	-0.03	0.08	-0.04	1.00					
Nitrogen (N)	0.09	0.49*	0.65*	0.65*	0.50*	0.13	-0.05	0.09	0.35*	1.00				
Phosphorus (P)	0.17	0.48*	0.73*	0.73*	0.51*	0.14	-0.007	0.16	0.30*	0.88*	1.00			
Potassium (K)	-0.10	-0.10	-0.13	-0.13	-0.07	0.02	0.12	-0.03	-0.05	-0.17	0.07	1.00		
Rhizospheric bacterial population	0.17	0.42*	0.35*	0.35*	0.32*	0.13	0.16	-0.13	0.12	0.42*	0.43*	0.09	1.00	
Endophytic bacterial population	0.27	0.38*	0.50*	0.50*	0.33*	-0.001	-0.05	-0.05	-0.02	0.29	0.32*	-0.002	0.53*	1.00

*Significant correlation at α = .05 level

Table 3. *Pearson's correlation coefficient (r value) between plant, soil parameters, rhizospheric and endophytic population under field conditions experiment at Solan (n = 30)*

Parameters	No. non wrapper leaves	Curd diameter	Curd weight	Curd yield	Curd depth	pH	Electrical conductivity (EC)	Bulk density (BD)	Organic matter (OM)	Nitrogen (N)	Phosphorus (P)	Potassium (K)	Rhizospheric bacterial population	Endophytic bacterial population
No. non wrapper leaves	1.00													
Curd diameter	0.35*	1.00												
Curd weight	0.45*	0.54*	1.00											
Curd yield	0.45*	0.54*	1.00	1.00										
Curd depth	0.36*	0.40*	0.57*	0.57*	1.00									
pH	-0.17	0.26	0.21	0.21	-0.06	1.00								
Electrical conductivity (EC)	0.08	0.07	0.18	0.18	0.19	0.11	1.00							
Bulk density (BD)	0.09	-0.23	-0.02	-0.02	0.09	-0.22	0.09	1.00						
Organic matter (OM)	0.04	0.12	0.27	0.27	0.21	0.18	0.39*	0.04	1.00					
Nitrogen (N)	0.32*	0.47*	0.76*	0.76*	0.52*	0.22	0.13	-0.19	0.17	1.00				
Phosphorus (P)	0.52*	0.59*	0.84*	0.84*	0.68*	0.20	0.14	-0.17	0.26	0.83*	1.00			
Potassium (K)	0.07	-0.09	0.06	0.06	-0.07	0.17	0.15	-0.02	0.12	0.06	0.07	1.00		
Rhizospheric bacterial population	0.41*	0.46*	0.38*	0.38*	0.37*	0.10	0.07	-0.15	0.26	0.26	0.47*	0.05	1.00	
Endophytic bacterial population	0.22	0.35*	0.22	0.22	0.26	-0.12	-0.14	-0.04	0.08	0.26	0.31*	-0.05	0.42*	1.00

*Significant correlation at α = .05 level

Conclusion

The inference could be drawn that inclusion of rhizobacteria in CSA ensures win win results of enhanced sustainable productivity, resilience and reduced GHG emissions. The conjoint application of *Bacillus pumilus* + NP (75%) showed higher impacts on plant productivity as well as soil fertility. Plant nutrients (N, P and K) and microbial population registered manifold improvement in organically sound (*Bacillus pumilus* + NP) plots as compared to control. SMBC was found to be higher in *Bacillus pumilus* + 75% NP treated plots as compared to other treatment plots. Similar trends were recorded in the bacterial (rhizospheric and endophytic) populations. A significant reduction in CO_2 evolution was recorded after 48 h of incubation in the treatments receiving *Bacillus pumilus* bio-inoculation. Therefore, based on these findings it is concluded that this fertilization regime (*Bacillus pumilus* + NP) not only could produce a good yield but also offers an opportunity for savings of fertilizers and mitigation of soil pollution. Despite the information gained from this detailed study, other relationships needed to know turnover rates of microbial biomass and microbial activity for which detail work using isotope dating is in progress.

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