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FULL-LENGTH RESEARCH ARTICLE

Effects of Plant Growth-Promoting Rhizobacteria and NPK Fertilizers on Biochemical and Microbial Properties of Soils Under Ginger (*Zingiber officinale*) Cultivation

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Abstract For this study, plant growth-promoting rhizobacteria (PGPR) were isolated from soils under ginger and short-listed based on their nutrient mobilization traits. The promising PGPR (*Burkholderia cepacia*, *Klebsiella* sp., *Serratia marcescens*, and *Enterobacter* sp.) were either applied alone or in combination with varying rates of NPK fertilizers to determine their effect on sensitive biochemical and microbial properties of soils under ginger (*Zingiber officinale* Rosc.). The properties studied were soil organic carbon, dissolved organic-C (DOC), and -N, microbial biomass-C (C_{MIC}), -N (N_{MIC}) and -P (P_{MIC}), net N mineralized (N_{MIN}), soil respiration (SR), metabolic quotient (q_{CO_2}) and activities of dehydrogenase (DHA), acid phosphatase (AcP), β-glucosidase (βG), and urease (UR). Results revealed a 24 % increase in mean DOC level in treatments with PGPR + NPK compared to control. Similarly, mean C_{MIC} and N_{MIC} levels were greater by 27 and 71 %, respectively, in treatments involving PGPR + NPK compared to treatments with only fertilizers. Also, combined application of PGPR and fertilizers positively influenced P_{MIC} and N_{MIN} rates compared to sole application of PGPR or NPK. While SR did not vary considerably among the treatments, q_{CO_2} levels across PGPR + NPK treatments were lower by 15–20 % relative to treatments with only NPK or PGPR. Results also revealed that DHA activity was on an average greater by 49.0 %, UR by 15 %, AcP by 40 %, and βG by 35 % in PGPR + NPK treatments compared to only NPK.

Keywords Soil microbial biomass · Soil enzyme activity · Soil respiration · Metabolic quotient

Introduction

In the present day agriculture, intensive farming practices that achieve high yield require fertilizers. However, inappropriate agricultural intensification coupled with reckless use of fertilizers has deteriorated soil quality. Therefore, there is growing awareness on the use of environment friendly sustainable nutrient management practices that lay emphasis on restoration and maintenance of soil quality both in the short- and long-term. Thus, effective biological technologies like the use of plant growth-promoting rhizobacteria (PGPR) are being exploited for enhancing crop yields. PGPR represent a wide variety of rhizosphere-inhabiting bacteria which colonize the root systems of plants and can stimulate plant growth by direct or indirect mechanisms. Direct mechanisms of plant growth-promotion include biofertilization, stimulation of root growth, rhizoremediation, and plant stress control, while mechanisms of biological control include reducing the level of disease, antibiosis, induction of systemic resistance, and competition for nutrients and niches [19]. Common PGPR include genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium*, and *Serratia* [4]. At present, PGPR are being increasingly used in combination with manures and

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fertilizers for improving crop yields and have contributed to the development of sustainable agricultural systems.

Studies have shown that PGPR had positive effects on cereals [27], fruits [18], vegetables [15], and spices [5]. PGPR have also been found to improve plant uptake of nutrients, and thereby increase the use efficiency of applied fertilizers and manures thus allowing reduced application rates of fertilizers [1]. While studies on PGPR effects on growth and yield of crops is widely reported, very little information exists on the effects of PGPR applied alone or in combination with graded doses of fertilizers on sensitive biochemical and microbial indices reflecting soil quality.

Studies employing a host of physical and chemical parameters have been made to determine the effects of nutrient inputs on soil quality. However, the present focus is on biochemical and microbial parameters that reflect the size and activity of microbial processes. This is because the biochemical properties are more sensitive to environmental stress, play a major role in degradation, and provide rapid and accurate estimates on soil quality [14]. The biochemical parameters include variables directly related to microbial activity (microbial biomass C and N, respiration, etc.), and the activities of extracellular hydrolytic enzymes involved in the C, N, S, and P cycles in soil. These soil biochemical and microbiological parameters have been considered as potential indicators of soil quality and management impacts in numerous studies [10–12, 32, 33].

Exclusive use of only fertilizers have been found to cause a significant reduction in microbial biomass C [34, 35] as well as reductions in soil respiration (SR) and dehydrogenase (DH), acid phosphatase (AcP), and β-glucosidase (βG) activities [12]. We hypothesized that use of PGPR along with fertilizers would attenuate such negative or insignificant effects of sole NPK application on important soil microbial and biochemical processes. Hence, the primary objective of the study was to determine the effects of promising native strains of PGPR (*Burkholderia cepacia*, *Klebsiella* sp., *Serratia marcescens*, and *Enterobacter* sp.) isolated from ginger rhizosphere, applied alone and in combination with NPK fertilizers (urea, rock phosphate [RP], muriate of potash [MOP]) on sensitive biochemical and microbial parameters that reflect short-term changes in soil quality. A secondary objective was to determine the inter-relationships between these parameters in soils applied with PGPR and fertilizers.

Materials and Methods

Experiment Details

The experiment was conducted at Indian Institute of Spices Research, Kozhikode, Kerala and was repeated for 3 years from 2007 to 2009. For the study, earthen pots of 20 kg capacity were filled with 15 kg sieved soil (<2 mm). The soil was a clay loam Ustic Humitropept. The initial properties of the soil are pH 5.12, organic C 14.2 g kg^{-1}, mineral N 42 mg kg^{-1}, Bray P 5.4 mg kg^{-1}, exchangeable K 84 mg kg^{-1}. For planting of rhizomes, small shallow pits were made and the seed-rhizome (20–30 g) of ginger (var. Varada) with at least two sprouted buds after pre-treatment with the respective PGPR was placed 3.5–5.0 cm deep in the pits and the soil-pressed over it.

Treatments

Inorganic Fertilization

The recommended dose (RD) of NPK for ginger in Kerala State, India is 75–50–50 kg ha^{-1}, respectively. The inorganic sources of NPK used were urea, RP and MOP, respectively. RP was applied as basal, urea in two splits (45th and 90th day after planting [DAP]) and MOP in two splits (45th and 90th DAP). This fertilization regime was considered as 100 % RD and each nutrient was reduced to either 75 or 50 % and was applied alone or in combination with PGPR (Tables 2, 3).

Isolation of PGPR

Rhizosphere soils were collected from healthy ginger growing sites in Calicut and Wayanad districts (Kerala State, India) and Kodagu district (Karnataka State, India). PGPR were isolated using dilution plate technique using Tryptic Soy Agar. The most suitable dilution was selected for estimating the population of the rhizobacteria and expressed as colony forming units per gram soil. Colonies on each plate were distinguished based on phenotypic characteristics such as shape, motility, color, growth rate, culture morphology, and Gram's staining. The representative isolates (100 nos.) were selected and cryopreserved at −80 °C in glycerol (40 %) for further use.

Selection of Isolates

The isolates were then tested for nutrient mobilization in vitro using standard parameters viz., production of indole acetic acid, solubilization of phosphorus, potassium, silica, and zinc. Four best isolates were then shortlisted for the study and preliminary identification of the isolates was done using the Bergey's Manual of Determinative Bacteriology. Identity of the isolates was confirmed using 16S rDNA sequence analysis and Biolog [7]. The four PGPR used in the experiment were identified as *B. cepacia* (GRB25), *Klebsiella* sp. (GRB36), *S. marcescens* (GRB38), and *Enterobacter* sp. (GRB70). The basic traits of the shortlisted PGPR for nutrient mobilization are given in Table 1.

Table 1 Basic information on shortlisted PGPR for nutrient mobilization traits in vitro

Isolate numbers	Identities	IAA production	NH_3 production	Nutrient mobilization			
				K	P	Zn	Si
GRB25	*Burkholderia cepacia*	−	+	+	+	+	−
GRB36	*Klebsiella* sp.	+	+	−	+	−	+
GRB38	*Serratia marcescens*	+	+	−	+	+	+
GRB70	*Enterobacter* sp.	+	+	+	+	−	−

GRB ginger rhizobacteria

Application of PGPR

The shortlisted PGPR were applied both as rhizome dip and soil application. Just before planting, the healthy ginger rhizome seeds were dipped in 1 % starch solution containing bacterial cells at the rate of 1×10^8 cells mL^{-1} for 1 h. Immediately after planting, 100 mL of 1×10^8 cells mL^{-1} suspension was applied to each pot. The soil application was repeated on 30th, 60th, and 90th day of planting. The PGPR were either applied alone or in combination with varying levels of NPK (Tables 2, 3). The study also consisted of an absolute control where no nutrients, whatsoever, were applied. The experiment consisted of six replications. The temperature in the greenhouse during the experiment hovered between 28 and 33 °C and relative humidity between 65 and 82 %.

Soil Sampling

The crop duration is 270 days. However, we collected soil samples 1 month after the final application of PGPR and fertilizers treatments, i.e., at 120 DAP. Apparently, it was presumed that the effects of the treatments would be more distinct at 120 DAP than at harvest. Besides, 120–135 DAP also happens to be the active growth stage of ginger. The soils samples were taken from bottom two-thirds of each pot, cleared of any organic debris and transferred for storage in sealed plastic bags. The soils were then sieved (<2 mm), analyzed for their moisture content, and stored at 4 °C. Subsamples for the determination of soil organic carbon (SOC) and mineral N were sieved to pass a 0.5 mm mesh.

Soil Physico-chemical Properties

SOC, $NH_4 + NO_3$-N, Bray P, and exchangeable K were estimated using standard methods [29]. Soil pH was measured in 1:2.5 soil:water suspension.

Soil Biochemical and Microbiological Analyses

Nitrogen mineralization (N_{MIN}) capacity was determined by extracting 10 g soil with 50 mL of 2 M KCl for 30 min before and after incubation for 10 days at 30 °C. The NH_4-N and total inorganic N were determined by steam distillation [20]. The difference between the values obtained before and after incubation indicates N mineralization capacity. Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were determined by the method described by Smolander and Kitunen [28]. The fumigation–extraction method [33] was used to determine soil microbial biomass-C (C_{MIC}), -N (N_{MIC}), and -P (P_{MIC}) as described by Heinze et al. [16]. SR 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 production was then measured for the next 7 days using NaOH traps and titration with HCl. The metabolic quotient (q_{CO_2}) was calculated as follows: (μg CO_2-C evolved in 7 days g^{-1} soil)/((μg biomass C g^{-1} soil)/7 days) × 1,000 = mg CO_2-C g^{-1} biomass C day^{-1} [24]. Soil enzyme activities viz., dehydrogenase (DHA), urease (UR), AcP, and βG were estimated using standard methods [31].

Statistics

All values reported are average of results across the 3 years and are expressed on an oven-dried soil basis (105 °C). The significance of treatment effects was determined by ANOVA. Where the F values were significant, post-hoc comparisons of means were made using the Least Significance Test (lsd) at the 0.05 probability level. Pearson's correlation coefficients ($n = 25$; $P < 0.05$) were calculated to determine the strength of the relationship between various parameters. Principal component analysis (PCA) was performed for reflection of any intrinsic pattern in the multidimensional data swarm. PCA often reveals previously unexpected associations among variables and thereby allows interpretation that would not be possible otherwise. Only PCs with Eigen values >1 and that explain >10 % of the total variance were retained.

Results

Soil pH, Mineral N, Bray P, and Exchangeable K

Soil pH ranged from 4.10 to 5.49 (Table 2) and among the treatments, mineral N, and Bray P levels were greatest in the treatment GRB38 + 100 % NPK (98.6 and 8.8 mg kg^{-1}, respectively) and exchangeable K level was greatest in the treatment with GRB25 + 75 % N + 100 % PK (224 mg kg^{-1}).

Table 2 Effects of PGPR, inorganic NPK fertilizers and their combinations on pH, mineral N, Bray P, exchangeable K, soil organic C (SOC), dissolved organic C (DOC), and dissolved organic N (DON) levels in soil at 120 days after planting of ginger

Treatments[a]	pH	$NH_4 + NO_3$-N (mg kg^{-1})	Bray P	Exchangeable K	SOC (g kg^{-1})	DOC (μg g^{-1})	DON (μg g^{-1})
Control	4.30 ± 0.33	37.2 ± 5.6	5.9 ± 1.1	86 ± 8	1.45 ± 0.11	133 ± 18	14.3 ± 3.2
75 % N + 100 % P + 100 % K	4.82 ± 0.55	67.2 ± 4.9	8.4 ± 1.4	212 ± 14	1.54 ± 0.23	146 ± 13	16.8 ± 2.8
100 % N + 75 % P + 100 % K	4.87 ± 0.32	92.8 ± 9.9	6.3 ± 1.4	204 ± 9	1.52 ± 0.21	150 ± 14	22.6 ± 4.3
100 % N + 100 % P + 75 % K	5.12 ± 0.51	96.8 ± 10.4	8.8 ± 1.2	146 ± 8	1.54 ± 0.18	158 ± 12	24.5 ± 3.6
100 % N + 100 % P + 100 % K	5.16 ± 0.34	94.0 ± 8.9	8.6 ± 1.5	206 ± 12	1.51 ± 0.11	157 ± 12	21.2 ± 3.9
GRB25	4.28 ± 0.48	41.6 ± 5.4	5.1 ± 1.0	87 ± 8	1.44 ± 0.14	157 ± 11	14.7 ± 2.8
GRB25 + 75 % N + 100 % P + 100 % K	5.49 ± 0.31	75.6 ± 6.7	8.5 ± 1.8	224 ± 11	1.51 ± 0.14	163 ± 13	17.4 ± 4.1
GRB25 + 100 % N + 75 % P + 100 % K	4.31 ± 0.33	93.2 ± 7.8	6.2 ± 1.2	216 ± 11	1.54 ± 0.23	161 ± 15	20.6 ± 3.5
GRB25 + 100 % N + 100 % P + 75 % K	4.32 ± 0.33	98.0 ± 6.7	8.3 ± 1.1	151 ± 9	1.54 ± 0.21	172 ± 12	21.7 ± 4.6
GRB25 + 100 % N + 100 % P + 100 % K	4.34 ± 0.32	97.2 ± 6.7	8.4 ± 1.1	209 ± 9	1.49 ± 0.13	166 ± 16	24.9 ± 3.2
GRB36	4.12 ± 0.34	39.0 ± 4.5	5.7 ± 1.3	86 ± 6	1.45 ± 0.14	146 ± 13	14.5 ± 2.8
GRB36 + 75 % N + 100 % P + 100 % K	5.44 ± 0.33	75.6 ± 5.9	8.9 ± 1.8	214 ± 11	1.53 ± 0.24	153 ± 14	17.2 ± 2.8
GRB36 + 100 % N + 75 % P + 100 % K	4.36 ± 0.35	95.4 ± 6.8	6.7 ± 1.1	221 ± 14	1.52 ± 0.21	160 ± 12	21.5 ± 4.2
GRB36 + 100 % N + 100 % P + 75 % K	4.27 ± 0.40	97.4 ± 7.5	8.5 ± 1.4	219 ± 12	1.54 ± 0.13	157 ± 15	22.8 ± 4.4
GRB36 + 100 % N + 100 % P + 100 % K	4.10 ± 0.33	94.4 ± 8.6	8.0 ± 1.3	154 ± 9	1.54 ± 0.24	165 ± 12	25.0 ± 4.0
GRB38	4.16 ± 0.35	35.4 ± 4.5	5.2 ± 1.0	88 ± 8	1.44 ± 0.21	149 ± 13	14.3 ± 2.1
GRB38 + 75 % N + 100 % P + 100 % K	5.14 ± 0.37	72.8 ± 6.8	7.9 ± 1.4	218 ± 12	1.55 ± 0.30	168 ± 16	16.3 ± 3.2
GRB38 + 100 % N + 75 % P + 100 % K	4.33 ± 0.31	96.8 ± 8.7	6.2 ± 0.9	223 ± 12	1.54 ± 0.45	168 ± 16	19.4 ± 3.8
GRB38 + 100 % N + 100 % P + 75 % K	4.51 ± 0.33	97.2 ± 6.9	8.5 ± 1.6	221 ± 13	1.55 ± 0.23	165 ± 13	20.5 ± 2.8
GRB38 + 100 % N + 100 % P + 100 % K	4.22 ± 0.40	98.6 ± 7.4	8.8 ± 1.6	146 ± 12	1.52 ± 0.25	174 ± 14	23.3 ± 4.3
GRB70	4.27 ± 0.37	38.4 ± 4.8	5.6 ± 1.4	89 ± 9	1.49 ± 0.18	156 ± 12	15.2 ± 2.3
GRB70 + 75 % N + 100 % P + 100 % K	4.53 ± 0.38	72.8 ± 6.5	8.5 ± 1.4	218 ± 11	1.49 ± 0.19	167 ± 12	17.5 ± 3.6
GRB70 + 100 % N + 75 % P + 100 % K	4.45 ± 0.44	93.6 ± 7.8	6.9 ± 0.9	221 ± 13	1.52 ± 0.16	167 ± 16	21.6 ± 2.6
GRB70 + 100 % N + 100 % P + 75 % K	4.27 ± 0.34	97.8 ± 7.5	8.6 ± 1.3	224 ± 12	1.53 ± 0.12	169 ± 13	20.4 ± 3.4
GRB70 + 100 % N + 100 % P + 100 % K	4.33 ± 0.34	98.2 ± 7.4	8.2 ± 1.3	145 ± 11	1.52 ± 0.15	170 ± 13	25.3 ± 3.6
Lsd ($P < 0.05$)	1.3	6.6	0.8	14	0.80	3.2	1.6

The recommended dose of NPK (i.e., 100 % N + 100 % P + 100 % K) for ginger in the study area is 75–50–50 kg NPK ha^{-1}, GRB (ginger rhizobacteria)25 *Burkholderia cepacia*, GRB36 *Klebsiella* sp., GRB38 *Serratia marcescens*, GRB70 *Enterobacter* sp. (GRB70), all values are mean ± SD values of six replications

SOC, DOC, and DON

The levels of SOC did not exhibit marked variations across treatments, albeit showing a slight increase in treatments with NPK and PGPR applied alone or in combination (Table 2). However, the DOC level was lowest in control and increased marginally by 9.0–19.0 % in treatments with only NPK and 9.0–17 %, respectively in treatments with only PGPR. Greatest levels of DOC were, however, registered in the treatments involving PGPR + NPK. Mean level DOC in these treatments (165.4 μg g^{-1}) indicated a 24 % increase compared to control. In contrast, DON levels increased significantly (Table 2) with increasing N levels indicating a 17.5 % increase in treatments with 75 % N (16.8 μg g^{-1}) and a significant 48–71 % increase in treatments with 100 % N relative to control. No significant variation with respect to DON existed among NPK and PGPR + NPK treatments.

Microbial Biomass-C (C_{MIC}), -N (N_{MIC}), and -P (P_{MIC})

C_{MIC} level under sole NPK application (mean 115 μg g^{-1}) was almost identical to control (114 μg g^{-1}) but lower than the treatments with sole application of PGPR (mean 128 μg g^{-1}) and PGPR + NPK (mean 146.0 μg g^{-1}; Table 3). PGPR application also positively influenced P_{MIC}. Mean level in PGPR alone treatments was 12.2 μg g^{-1} indicating an increase of 45 % relative to control (Table 3). In treatments involving only NPK, the P_{MIC} levels were, in general, greater in treatments with 100 % P suggesting positive effects of P fertilization. Relatively greater levels of P_{MIC} were, however, registered in the treatments involving 100 % P + PGPR. Contrary to C_{MIC} and P_{MIC}, N_{MIC} accumulated at markedly greater level in the treatments with NPK fertilization. The N_{MIC} level was lowest in the control (6.3 μg g^{-1}), while in treatments with only PGPR it averaged 11.9 μg g^{-1} and in treatments with sole

Table 3 Effects of PGPR, inorganic NPK fertilizers, and their combinations on microbial biomass-C, -N, -P, net N mineralized (N_{MIN}), soil respiration, metabolic quotient, soil dehydrogenase (DH), urease (UR), acid phosphatase (AcP), and β-glucosidase (βG) activities in soil at 120 days after planting of ginger

Treatments	Microbial biomass			N_{MIN}	SR	q_{CO_2}	DH	UR	AcP	βG
	C (μg g^{-1})	N (μg g^{-1})	P (μg g^{-1})							
Control	104 ± 8	6.3 ± 1.4	8.5 ± 1.7	48 ± 5	20 ± 3	192 ± 16	53.0 ± 6.5	2.0 ± 0.6	10.5 ± 1.6	2.5 ± 0.7
75 % N + 100 % P + 100 % K	114 ± 6	11.9 ± 2.6	13.8 ± 2.8	67 ± 5	21 ± 3	184 ± 18	66.2 ± 6.7	4.7 ± 0.9	9.2 ± 1.6	3.2 ± 0.7
100 % N + 75 % P + 100 % K	122 ± 6	15.8 ± 2.9	13.5 ± 2.9	76 ± 7	21 ± 4	172 ± 18	65.2 ± 8.2	5.6 ± 1.1	10.5 ± 1.4	3.4 ± 0.5
100 % N + 100 % P + 75 % K	116 ± 8	16.7 ± 3.2	16.7 ± 3.2	87 ± 8	21 ± 3	181 ± 19	57.6 ± 5.9	5.5 ± 1.1	8.7 ± 1.4	3.9 ± 0.7
100 % N + 100 % P + 100 % K	118 ± 8	17.8 ± 2.6	14.7 ± 2.4	77 ± 8	22 ± 3	186 ± 21	59.7 ± 6.1	5.0 ± 1.2	8.7 ± 1.6	3.7 ± 0.7
GRB25	128 ± 9	14.6 ± 2.1	12.2 ± 1.4	52 ± 5	25 ± 5	195 ± 23	72.4 ± 7.3	3.3 ± 0.8	11.2 ± 1.2	2.7 ± 0.4
GRB25 + 75 % N + 100 % P + 100 % K	136 ± 7	20.6 ± 2.4	14.7 ± 1.4	77 ± 5	23 ± 4	169 ± 16	87.8 ± 8.8	5.2 ± 0.9	12.4 ± 1.6	4.6 ± 0.8
GRB25 + 100 % N + 75 % P + 100 % K	133 ± 7	26.7 ± 2.8	12.2 ± 1.6	92 ± 9	22 ± 4	165 ± 15	81.5 ± 7.8	7.8 ± 1.3	13.7 ± 1.1	4.8 ± 0.8
GRB25 + 100 % N + 100 % P + 75 % K	142 ± 6	23.7 ± 2.2	17.2 ± 2.8	90 ± 8	22 ± 4	154 ± 12	82.3 ± 8.3	6.6 ± 1.3	12.2 ± 1.2	4.7 ± 0.9
GRB25 + 100 % N + 100 % P + 100 % K	144 ± 8	27.3 ± 2.4	17.5 ± 2.4	95 ± 8	23 ± 5	160 ± 15	81.0 ± 7.7	7.9 ± 1.1	13.0 ± 1.2	5.7 ± 0.9
GRB36	121 ± 9	9.3 ± 1.1	11.4 ± 1.4	54 ± 5	24 ± 5	198 ± 21	71.4 ± 6.4	3.4 ± 0.8	11.4 ± 1.4	2.7 ± 0.4
GRB36 + 75 % N + 100 % P + 100 % K	143 ± 8	23.5 ± 1.8	18.0 ± 2.7	76 ± 6	23 ± 5	161 ± 18	88.3 ± 8.8	4.8 ± 0.7	12.0 ± 1.4	4.3 ± 0.5
GRB36 + 100 % N + 75 % P + 100 % K	151 ± 8	27.0 ± 2.3	12.5 ± 1.8	89 ± 8	23 ± 4	152 ± 16	74.3 ± 8.5	5.7 ± 0.9	13.5 ± 1.3	4.9 ± 0.7
GRB36 + 100 % N + 100 % P + 75 % K	142 ± 9	27.0 ± 2.4	17.3 ± 2.0	87 ± 8	23 ± 3	162 ± 16	77.4 ± 6.8	6.1 ± 1.0	12.2 ± 1.3	5.5 ± 1.0
GRB36 + 100 % N + 100 % P + 100 % K	126 ± 8	33.5 ± 2.9	18.6 ± 2.1	96 ± 7	22 ± 3	175 ± 19	73.5 ± 6.4	6.3 ± 0.9	12.9 ± 1.4	5.2 ± 0.9
GRB38	134 ± 7	9.6 ± 1.3	12.2 ± 1.3	51 ± 4	25 ± 4	187 ± 19	76.7 ± 7.9	2.9 ± 0.6	11.1 ± 1.4	2.8 ± 0.4
GRB38 + 75 % N + 100 % P + 100 % K	148 ± 6	25.0 ± 2.1	17.4 ± 2.1	69 ± 6	23 ± 4	155 ± 14	84.6 ± 7.8	4.7 ± 0.8	13.4 ± 1.3	3.8 ± 0.5
GRB38 + 100 % N + 75 % P + 100 % K	153 ± 6	25.5 ± 1.8	13.6 ± 1.4	86 ± 8	22 ± 3	144 ± 14	73.0 ± 6.7	6.6 ± 1.1	14.4 ± 1.6	4.1 ± 0.7
GRB38 + 100 % N + 100 % P + 75 % K	152 ± 8	28.5 ± 2.4	18.2 ± 2.1	91 ± 8	22 ± 3	145 ± 16	76.8 ± 6.9	6.0 ± 1.1	13.1 ± 1.1	5.8 ± 1.1
GRB38 + 100 % N + 100 % P + 100 % K	151 ± 8	29.0 ± 3.0	18.4 ± 2.0	94 ± 8	23 ± 4	152 ± 16	71.0 ± 5.5	6.4 ± 0.9	13.4 ± 1.2	5.2 ± 0.9
GRB70	127 ± 5	14.0 ± 1.5	13.2 ± 1.6	49 ± 5	24 ± 4	189 ± 21	72.0 ± 5.7	3.1 ± 0.4	11.7 ± 1.4	3.1 ± 0.6
GRB70 + 75 % N + 100 % P + 100 % K	156 ± 7	22.5 ± 1.6	18.3 ± 1.8	68 ± 5	24 ± 5	154 ± 14	85.0 ± 7.8	4.5 ± 0.6	13.3 ± 1.5	4.2 ± 0.8
GRB70 + 100 % N + 75 % P + 100 % K	155 ± 6	27.5 ± 1.8	16.2 ± 1.3	87 ± 7	21 ± 4	135 ± 15	75.8 ± 6.9	5.8 ± 1.0	14.5 ± 1.2	3.9 ± 0.8
GRB70 + 100 % N + 100 % P + 75 % K	153 ± 6	26.5 ± 2.1	17.9 ± 2.1	87 ± 7	21 ± 4	137 ± 15	76.0 ± 6.8	6.3 ± 1.0	12.9 ± 1.1	4.7 ± 0.8
GRB70 + 100 % N + 100 % P + 100 % K	157 ± 9	33.0 ± 2.6	17.9 ± 1.7	91 ± 8	21 ± 3	134 ± 14	77.0 ± 8.3	6.2 ± 1.3	12.6 ± 1.3	5.6 ± 0.9
Lsd ($P < 0.05$)	9	4.4	2.3	13	3	12	8.0	0.5	0.8	0.4

All values are mean ± SD values of six replications

N_{MIN} mg N kg^{-1} 10 day^{-1}, *SR* soil respiration (μg CO_2-C g^{-1} day^{-1}), q_{CO_2} metabolic quotient (mg CO_2-C (g biomass C)$^{-1}$ day^{-1}), *DH* dehydrogenase (nmol TPF g^{-1} soil h^{-1}), *UR* urease (μmol NH_3-N g^{-1} h^{-1}), *AcP* acid phosphatase (μmol *p*-nitrophenol g^{-1} h^{-1}), *βG* β-glucosidase (μmol *p*-nitrophenol g^{-1} h^{-1})

application of NPK it averaged 15.6 μg g^{-1} (Table 3). This suggested large average increases of 89 and 148 %, respectively in these treatments relative to control. The degree of increase in N_{MIC} levels was, however, greatest in treatments involving PGPR + NPK (mean 26.7 μg g^{-1}) indicating a significant increase of 124 % compared to PGPR alone treatments and 71.0 % compared to treatments with only NPK.

Total N Mineralized, SR, and Metabolic Quotient

Sole application of PGPR had very little effects on N_{MIN} rates (Table 3). Contrarily, NPK fertilization markedly enhanced N_{MIN} rates; mean value across these treatments (76.8 mg N kg^{-1} 10 day^{-1}) being greater by 60 % compared to control. The results also showed a clear effect of combined application of PGPR and NPK on N_{MIN}. Mean level across these treatments (86.0 mg N kg^{-1} 10 day^{-1}) suggested an increase of 79 % relative to control. Among the treatments, the greatest levels of N_{MIN} were registered by the treatments with combined application of either of the PGPR with 100 % N. SR (Table 3) showed little variation among the treatments including control. The average q_{CO_2} levels in NPK alone and PGPR alone treatments were 181 and 192 mg CO_2-C (g biomass C)$^{-1}$ day^{-1}, respectively (Table 3). Across PGPR + NPK treatments, q_{CO_2} levels (mean 153.0 mg CO_2-C (g biomass C)$^{-1}$) decreased by 15 % relative to NPK alone treatments and by 20 % relative to PGPR alone treatments.

Soil Enzyme Activities

Control recorded lowest activity of all enzymes (Table 3) and across treatments greatest activities were always recorded by those involving NPK + PGPR. DHA activity was on an average greater by 49.0 and 23.2 % in PGPR + NPK treatments compared to NPK and PGPR alone treatments, respectively. In NPK alone treatments, UR activity was on an average greater by 43 % compared to PGPR alone treatments, while AcP activity was on an average greater by 46 % in PGPR + NPK treatments relative to NPK alone treatments. The activity of βG was almost identical in NPK alone and PGPR alone treatments (3.5 and 3.3 μmol *p*-nitrophenol g^{-1} h^{-1}, respectively) but lower by 29–33 % compared to PGPR + NPK treatments.

Discussion

Unlike soil physico-chemical properties, the treatments effects were more evident on soil microbial properties (Table 3). Our results suggested little or no change in C_{MIC} levels in treatments with only NPK. This is most likely due to application of N fertilizer as urea at rates equivalent to 100 or 75 % N. No changes or reductions in soil C_{MIC} that are attributable to N fertilization have been reported earlier [12, 22, 27]. The supply of readily metabolisable C (DOC) in the treatments with PGPR + NPK is likely to have been the most influential factor contributing to the C_{MIC} and P_{MIC} increases. This confirmed that the levels of C_{MIC} is strongly related to the steady-state substrate availability in soils as reflected by the existence of strong correlations between C_{MIC} and DOC ($r = 0.80$). Also, P_{MIC} showed strong correlations with DOC ($r = 0.74$). Interestingly, C_{MIC} levels in treatments with PGPR + NPK increased significantly even though it involved inorganic fertilization. This suggested that the application of PGPR attenuated the insignificant effects of inorganic fertilization on C_{MIC}. In this study, other nutrients (P and K) besides N were utilized, although the influence of applied nutrients other than N on soil microbial biomass has largely been regarded as inconsequential [2].

The results also suggested a positive effect of N application on N_{MIC}. After N application, N availability increased and consequently microbes immobilized N, which subsequently increased N_{MIC} [35]. Positive correlation between N_{MIC} and DON ($r = 0.72$) suggested that inorganic fertilization especially N enhanced DON levels. Earlier reports also indicate significantly positive effects of N fertilization on DON [12, 23]. A clear positive effect of combined application of PGPR and NPK on N_{MIN} was observed. This suggested that increased soil microbial pool is often associated with high net N_{MIN} rates [11] as indicated by the positive correlation between N_{MIC} and N_{MIN} ($r = 0.87$). This is further supported by positive correlations reported between N_{MIC} and N_{MIN} with UR activity ($r = 0.81$ and 0.84, respectively), which might be explained by the role played by microbial extracellular enzymes in the depolymerisation of N-containing polymers [25].

SR did not vary considerably among the treatments including control (Table 3), while the metabolic quotient (q_{CO_2}) in PGPR + NPK treatments, were considerably lower compared to NPK alone and PGPR alone treatments. The metabolic quotient (SR 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 [36]. The results in this study did show changes in the DOC pool that would suggest changes in labile C. It is, therefore, possible that this explained the difference in q_{CO_2} rates across treatments. Apparently, relatively greater q_{CO_2} levels in NPK and PGPR alone treatments and control suggested lower substrate quality, resulting in a lower C use efficiency or high maintenance C demand [6]. In contrast, lower q_{CO_2} under PGPR + NPK treatments indicated relatively more efficient microbial community and better use of the available organic substrates. Our results suggested that inorganic fertilization did not enhance SR. Similar effects on SR due to inorganic fertilization has been found when N fertilizer was added [21], possibly from the suppression of the decomposition of native SOC due to decrease in microbial biomass [13].

Similar to soil microbial biomass, the soil enzymes were activated to varying degrees by PGPR and NPK applied alone or in combination (Table 3). The stronger effects of

PGPR + NPK application on DHA might be due to the greater metabolism by soil microorganisms and the poor influence of NPK fertilization, especially N on DHA is consistent with the results of Kautz et al. [17]. The study also indicated positive effects of N fertilization on UR and is in conformity with the results of Allison et al. [3]. Conversely, we observed an inhibition in AcP activity due to inorganic fertilization. This may be partly explained by the high P-fixing capacity of our soils [30] and partly by the negative effects of inorganic P application [26]. Similarly, the activity of βG was lowered in NPK alone and PGPR alone treatments compared to PGPR + NPK treatments. Our finding is supported by the results of Debosz et al. [8] who reported that the activities of βG was markedly lowered due to exclusive mineral N fertilization. The lower values of βG in NPK and PGPR alone treatments indicated that the potential to mineralize organic matter, and so the activity of the C-cycle, is reduced.

The inter-relationships between various soil parameters were studied using PCA (Table 4). Factor 1 (PC1) explaining 53 % of the total variance was defined mainly by mineral N, Bray P, exchangeable K, SOC, DOC, DON, N_{MIC}, P_{MIC}, N_{MIN}, UR, and βG. This possibly reflected the strong relationship between the labile and easily mineralisable organic matter and microbial activity and the logical dependence of microbial biomass on soil nutrients. The negative loading of q_{CO_2} in this factor suggested a stressed microbial community with reduced substrate use efficiency in treatments with only PGPR or NPK or the control. Conversely, the strong loading of βG in this factor indicated a decrease of the microbial community maintenance energy requirement and higher C efficiency [9] in soil with PGPR + NPK. Also, positive loadings of UR, N_{MIN}, and DON in PC1 suggested positive correlations between UR activity, N dynamics, and organic matter mineralization. Factor 2 (PC2) explaining 22 % of the total variance was defined mainly by DOC, C_{MIC}, SR, DH, and AcP activities which suggested that enhanced levels of labile organic substrates positively influenced soil microbial and enzyme activities.

Table 4 Principal component loadings after Varimax rotation

	Principal components[a]	
	PC1	PC2
Eigen values	10.0	2.7
Explained variance (%)	0.53	0.22
Rotated loading on two retained components		
Soil pH	ns	ns
NH_4 + NO_3-N	0.97	ns
Bray P	0.81	ns
Exchangeable K	0.83	ns
Soil organic C	0.85	ns
Dissolved organic C	0.67	0.60
Dissolved organic N	0.87	ns
Microbial biomass C	ns	0.81
Microbial biomass N	0.83	ns
Microbial biomass P	0.73	ns
Net N mineralized	0.95	ns
Soil respiration	ns	0.58
Metabolic quotient	−0.73	ns
Dehydrogenase	ns	0.81
Urease	0.87	ns
Acid phosphatase	ns	0.83
β-glucosidase	0.83	ns

The soil parameters are grouped according to the maximum fittings to principal components (correlation coefficients >0.50; $n = 150$)

ns Loadings lower than 0.50

[a] Only principal components with Eigen values >1 and those explaining >10 % of the total variance were retained

Conclusions

Results showed that PGPR or NPK applied alone consistently registered markedly lower levels of microbial biomass and activity. Contrarily, application of PGPR in combination with inorganic NPK promoted soil biological quality as evidenced by enhanced soil microbial biomass and enzyme activities. The non-significant effects of chemical fertilization on soil microbial properties were overcome to some extent by combined application of NPK and PGPR. Though the study did not indicate any reduction in the dose of NPK fertilizers due to application of PGPR, we found that the use of PGPR along with fertilizers attenuated the negative or insignificant effects of sole NPK application on important soil microbial and biochemical processes. However, considering that *S. marcescens*, *Enterobacter* sp., and *Klebsiella* sp. are opportunistic human pathogens, we recommend the use of *B. cepaceae* for enhanced soil quality under ginger. Overall, the study suggested that in cropping systems that overly depend on inorganic fertilization for enhanced yields, it would be ideal to combine crop specific native strain of PGPR to maintain or enhance soil quality even in the short-term.

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References

1. Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929

2. Allen AS, Schlesinger WH (2004) Nutrient limitations to soil microbial biomass and activity in loblolly pine forests. Soil Biol Biochem 36:581–589
3. Allison SD, Nielsen C, Hughes RF (2006) Elevated enzyme activities in soils under the invasive nitrogen-fixing tree *Falcataria moluccana*. Soil Biol Biochem 3:1537–1544
4. Anandaraj M, Dinesh R (2008) Use of microbes for spices production. In: Parthasarathy VA, Kandiannan K, Srinivasan V (eds) Organic spices. New India Publishing Agency, New Delhi, pp 101–132
5. Anandaraj M, Sarma YR (2003) The potential of PGPR in disease management in spice crops. In: Reddy MS, Anandaraj M, Eapen SJ, Sarma YR, Kloepper JW (eds) Abstracts and short papers, 6th international PGPR workshop, vol 2. Indian Institute of Spices Research, Calicut, pp 8–12
6. Anderson T-H, Domsch KH (2010) Soil microbial biomass: the eco-physiological approach. Soil Biol Biochem 42:2039–2043
7. Bini YK, Anandaraj M, Dinesh R, Silna N, Kumar A (2011) Evaluation of PGPR strains for growth and disease suppression in ginger (*Zingiber officinale* Rosc.). In: Proceedings of 2nd Asian PGPR conference, Beijing, China, p 519
8. Debosz K, Rasmussen PH, Pedersen AR (1999) Temporal variations in microbial biomass C and cellulolytic enzyme activity in arable soils: effects of organic matter input. Appl Soil Ecol 13:209–218
9. Dilly O (2005) Microbial energetics in soils. In: Buscot F, Varma A (eds) Microorganisms in soils: roles in genesis and functions. Springer, Berlin, pp 123–138
10. Dinesh R, Ghoshal Chaudhuri S (2013) Soil biochemical/microbial indices as ecological indicators of land use change in mangrove forests. Ecol Indic 32:253–258
11. Dinesh R, Srinivasan V, Hamza S, Manjusha A (2010) Short-term incorporation of organic manures and biofertilizers influences biochemical and microbial characteristics of soils under an annual crop [Turmeric (*Curcuma longa* L.)]. Bioresour Technol 101:4697–4702
12. Dinesh R, Srinivasan V, Hamza S, Manjusha A, Sanjay Kumar P (2012) Short-term effects of nutrient management regimes on biochemical and microbial properties in soils under rainfed ginger (*Zingiber officinale* Rosc.). Geoderma 173–174:192–198
13. Ding W, Yu H, Cai Z, Han F, Xu Z (2010) Responses of soil respiration to N fertilization in a loamy soil under maize cultivation. Geoderma 155:381–389
14. García C, Hernandez T, Pascual JA, Moreno JL, Ros M (1999) Microbial activity in soils of SE Spain exposed to degradation processes. Strategies for their rehabilitation. In: García C, Hernandez T (eds) Research and perspectives of soil enzymology in Spain. Consejo Superìor de Investìgacìones Cìentificas, Madrid, pp 93–143
15. Gravel V, Antoun H, Tweddell RJ (2007) Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). Soil Biol Biochem 39:1968–1977
16. Heinze S, Rauber R, Joergensen RG (2010) Influence of mouldboard plough and rotary harrow tillage on microbial biomass and nutrient stocks in two long-term experiments on loess derived Luvisols. Appl Soil Ecol 46:405–412
17. Kautz T, Wirth S, Ellmer F (2004) Microbial activity in a sandy arable soil is governed by the fertilization regime. Eur J Soil Biol 40:87–94
18. Kavino M, Harish S, Kumar N et al (2010) Effect of chitinolytic PGPR on growth, yield and physiological attributes of banana (*Musa* spp.) under field conditions. Appl Soil Ecol 45:71–77
19. Lugtenberg B, Kamilova F (2009) Plant-growth-promoting-rhizobacteria. Annu Rev Microbiol 63:541–556
20. Mulvaney RL (1996) Nitrogen-inorganic forms. In: Sparks DL, Page AL, Helmke PA et al (eds) Methods of soil analysis, Part 3, chemical methods, SSSA book series, Number 5. Madison, pp 1123–1184
21. Ramirez KS, Craine JM, Fierer N (2010) Nitrogen fertilization inhibits soil microbial respiration regardless of the form of nitrogen applied. Soil Biol Biochem 42:2336–2338
22. Rifai SW, Markewitz D, Borders B (2010) Twenty years of intensive fertilization and competing vegetation suppression in loblolly pine plantations: impacts on soil C, N, and microbial biomass. Soil Biol Biochem 42:713–723
23. Ros GH, Hoffland E, van Kessel C, Temminghoff EJM (2009) Extractable and dissolved soil organic nitrogen—a quantitative assessment. Soil Biol Biochem 41:1029–1039
24. Salamanca E, Raubuch M, Joergensen RG (2002) Relationships between soil microbial indices in secondary tropical forest soils. Appl Soil Ecol 21:211–219
25. Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602
26. Schneider K, Turrion M-B, Grierson PF, Gallardo JF (2001) Phosphatase activity, microbial phosphorus, and fine root growth in forest soils in the Sierra de Gata, western central Spain. Biol Fertil Soils 34:151–155
27. Shaharoona B, Arshad M, Zahir ZA, Khalid A (2006) Performance of *Pseudomonas* spp. containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. Soil Biol Biochem 38:2971–2975
28. Smolander A, Kitunen V (2002) Soil microbial activities and characteristics of dissolved organic C and N in relation to tree species. Soil Biol Biochem 34:651–660
29. Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME (1996) Methods of soil analysis, Part 3, chemical methods. SSSA, Madison
30. Srinivasan V, Sadanandan AK, Hamza S (2000) Efficiency of rock phosphate sources on ginger and turmeric in an Ustic Humitoprept. J Indian Soc Soil Sci 48:532–536
31. Tabatabai MA (1994) Soil enzymes. In: Weaver RW, Angle S, Bottomley P, Bezdicek D, Smith S, Tabatabai A, Wollum A (eds) Methods of soil analysis, Part 2, microbiological and biochemical properties. SSSA, Madison, pp 775–833
32. Truu M, Truu J, Ivask M (2008) Soil microbiological and biochemical properties for assessing the effect of agricultural management practices in Estonian cultivated soils. Eur J Soil Biol 44:231–237
33. Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707
34. Wallenstein MD, McNulty S, Fernandez IJ, Boggs J, Schlesinger WH (2006) Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. For Ecol Manag 222:459–468
35. Wang QK, Wang SL, Liu YX (2008) Responses to N and P fertilization in a young *Eucalyptus dunnii* plantation: microbial properties, enzyme activities and dissolved organic matter. Appl Soil Ecol 40:484–490
36. Wardle DA, Ghani A (1995) A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of disturbance and ecosystem development. Soil Biol Biochem 27:1601–1610