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Effect of phosphate solubilizing bacteria and blue green algae on phosphorus uptake and its apparent recovery in hybrid rice

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Abstract
A pot experiment was conducted during *kharif* season of 2020 on sandy loam soil in texture to investigate the impact of phosphate solubilizing bacteria (PSB) and blue green algae (BGA) on P uptake and its apparent recovery in hybrid rice cultivation. Thirteen treatments laid in completely randomized design with two different doses of chemical fertilizers (100% and 75%), two isolates of PSB (*Enterobacter cloacae* strain BAU3 and *Bacillus aryabhattai* strain BAUMS8) and blue green algae had been used in the study. The P uptake and its apparent recovery increased significantly when supplied with 100% recommended dose of inorganic fertilizers (RDF) + PSB @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1} as compared to 100% RDF. The P uptake augmented from control treatment (T_1) 23.09 kg ha^{-1} to 30.33 kg ha^{-1} in the treatment (T_8) (100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) due to increase in the grain and straw yield and P concentration. The apparent recovery percentage was maximum in treatment T_8 (100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) i.e. 13.63 per cent. Finally, it can be concluded that application of 100% RDF along with PSB @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1} significantly increased the P uptake and its apparent recovery in hybrid rice.

Keywords: *Bacillus,* biofertilizers, *Enterobacter,* grain and straw yield, p concentration

Introduction
Phosphorus (P) is the macronutrient required for plant growth and development and is involved in several important metabolic processes, including photosynthesis, nutrient uptake, biological oxidation and cell division (Kalayu 2019) [13]. With increasing demand of agricultural production, phosphorus (P) is receiving more attention as a non-renewable resource. But available P in the soil is limited, so obtaining sufficient P is considered to be a global environmental challenge for the 21st century (Fink *et al*. 2016) [9]. Also, the lack of available P in the soil leads to an imbalance use of synthetic fertilizers, which have undesirable effects on soil health. P uptake and utilization by plants plays a vital role in growth and development and finally in the determination of final crop yield. Therefore, a holistic understanding of P dynamics from soil to plant is important to know for optimizing P management and improving phosphorus use efficiency.

Rice is one of the staple food in south-east asia and is central to the agricultural production system. With the alarming increase in population, pressure on the agricultural sector is also increasing which leads reckless use of inorganic fertilizers. Therefore, the decision to increase agricultural productivity and soil fertility through the use of better ecological management tools will ensure successful food security (Aarti Yadav 2019) [1]. Hybrid rice is capable of achieving 15-20% higher grain yield (Virmani 1996) [35] but the excessive removal of available nutrients by these varieties degrades soil health and fertility. Important strategies to increase rice productivity include the use of local hybrids and better nutrient management by integrating both inorganic fertilizers and biofertilizers to provide nutrients to crops according to their needs (Maiti *et al*. 2006; Kamble *et al*. 2008; Mondal *et al*. 2016) [19, 14, 20].

Phosphate-solubilizing bacteria (PSB) are the most important among the various phosphate-containing biofertilizers, accounting for 1% to 50% of the total soil microbial population (Alam *et al*. 2002) [2]. These microbes have ability to convert insoluble forms of P into available forms by secreting organic and inorganic acids, phosphatase enzymes, and exopolysaccharides for plant uptake (Vazquez *et al*. 2000; Richardson 2001; Emami *et al*. 2020) [34, 27, 8]. *Bacillus* and *Enterobacter* are well-known plant growth promoting rhizobacteria (PGPR) that solubilize P minerals and produce metabolites such as siderophores and phytohormones and finally accelerate plant growth (Prakash and Arora 2019) [23]. Blue-green algae (BGA) are prokaryotes that perform oxygenic photosynthesis and also release various plant growth-promoting substances which help in the solubilization of insoluble phosphates and make them available to the plant (Satapathy 1999) [29]. Although they are known N biofertilizer in rice fields, there are few reports showing that they are able to solubilize insoluble P such as rock phosphate, calcium phosphate, aluminium phosphate, ferric phosphate and hydroxyl apatite (Yandigeri *et al*. 2011) [37] in soils by releasing low molecular weight organic acids. The use of biofertilizers is an economical and environmentally friendly that play an important role in maintaining long-term sustainability and soil fertility (Aarti Yadav 2019) [1]. As the information on the conjuctive use of inorganic and biofertilizers for hybrid rice in Bihar, India, is limited a study was conducted to evaluate the effects of phosphate solubilizing bacteria and blue-green algae on P uptake and its apparent recovery.

Materials and methods

Experimental site

A pot experiment was conducted during 2020 Kharif season in the net house of the Department of Soil Science and Agricultural Chemistry, Bihar Agricultural University, Sabour, Bhagalpur ($25^0$23’ N latitude; $78^0$07’ E longitude and an elevation of 25 m from sea level), Bihar, India. The experiment was conducted with the objective to study the effect of application of recommended fertilizer dose along with phosphate solubilizing bacteria (PSB) and blue green algae (BGA) in soil on P uptake and its apparent recovery in hybrid rice of Arize-6129 variety. The experimental soil had sandy loam texture (52.40% sand, 28.00% silt and 19.60% clay) and classified as mixed hyperthermic typical haplustepts. The initial fertility status of the experimental soil collected from the research farm of Bihar Agricultural College, Sabour, was determined from a soil composite sample (at 0-20 cm depth) before the start of the pot experiment. The experimental soil had low oxidizable carbon content (4.3 g kg^{-1} soil) and had neutral soil reaction (pH 7.39) and EC (0.11 dSm-1). The experimental soil was classified as medium in terms of soil fertility, with available N, P, and K values of 213.25 kg ha^{-1}, 13.45 kg ha^{-1} and 180.40 kg ha^{-1}, respectively. The average rainfall in 2019 and 2020 during Kharif (July - October) was 366.90-22.10 mm while the maximum temperatures in 2019 and 2020 ranged from 33.65 ^{0}C to 32.30 ^{0}C and the minimum temperatures ranged from 26.45 ^{0}C to 22.15 ^{0}C. The average relative humidity varied between 74.65% and 81.92% during the growing season in 2019 and 2020. Overall, the average temperature and relative humidity during the growing season did not differ significantly from the two-year averages.

Experimental details

The experiment was laid out in a completely randomized design (CRD) comprising with thirteen nutrient management practices (T_1: Control, T_2: 100% RDF, T_3: 75% RDF, T_4: 100% RDF + PSB_1 @ 750 ml ha^{-1},T_5: 75% RDF + PSB_1 @ 750 ml ha^{-1}, T_6: 100% RDF + BGA @ 10 kg ha^{-1}, T_7: 75% RDF + BGA @ 10 kg ha^{-1}, T_8: 100% RDF + PSB_1 @ 750 ml/ha + BGA @ 10 kg ha^{-1}, T_9: 75% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}, T_{10}: 100% RDF + PSB_2 @ 750 ml ha^{-1}, T_{11}: 75% RDF + PSB_2 @ 750 ml ha^{-1}, T_{12}: 100% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg $ha^{-1}$and T_{13}: 75% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}**)** replicated thrice in pots filled with 15 kg soil pot^{-1}. The recommended dose of inorganic fertilizers (RDF) for hybrid rice was 120 kg N, 60 kg P_2O_5, 40 kg K_2O and 5 kg $ZnSO_4.7H_2O$ ha^{-1} while among biofertilizers; BGA and two isolates of phosphate solubilizing bacteria *i.e.* PSB_1 (*Enterobacter cloacae* strain BAU3) and PSB_2 (*Bacillus aryabhattai* strain BAUMS8) were procured from Biolab, Bihar Agricultural University, Sabour, Bhagalpur.

Cultivation practices

Rice seedlings were raised in a dry planting bed prepared by cross ploughing followed by flooding. Fertilizers were applied in the form of digestate, urea, DAP and MOP. A full dose of digestate, P, K, and one-third of the N dose were applied as basal fertilizer prior to planting. The remaining N fertilizer was applied in two equal parts at mid-seeding and heading stages. One seedling per hill was planted with 21-day-old seedlings. Chemical and biological fertilizers, namely BGA @ 10 kg ha^{-1} and PSB_1/PSB_2 @ 750 ml ha^{-1} (1×10^9 cfu ml^{-1}) were applied according to the treatments by dipping the seedling roots before transplanting. The crop received four irrigations at the stages of early tillering (20 DAT), panicle formation (40 DAT), grain formation (60 DAT), and grain flowering (80 DAT) with 70 mm of water per irrigation to maintain the soil under saturated conditions from planting to 25 days after flowering. Fenvalerate 0.4% DP @ 25 kg ha^{-1} was applied at the ear emergence stage to protect the crop against *Leptocorisa varicornis* infestation during the growing period.

Observations recorded

Grain and straw yield were recorded from each pot after harvest. The pH and EC of the experimental soil were determined using a pH meter and a EC meter in a soil-water suspension at a ratio of 1:2.5 (Jackson 1973) [12]. Organic carbon content (%) was determined by the wet digestion method (Walkey and Black 1934) [36], available N content by the alkaline potassium permanganate method (Subbiah and Asija 1956) [33], available P content by the Olsen method (Olsen *et al*. 1954) [21] and available K content by the neutral ammonium acetate method (Black 1965) [4]. P content were determined by the two-acid digestion method (Koening and Johnson 1942) [17]. The formulas used in this experiment for calculation of P uptake and P apparent recovery (%) are shown below:

$$\text{P uptake (kg ha}^{-1}) = \frac{Nutrient\ concentration\ (\%)\ \times\ Biomass(kg/ha)}{100}$$

$$\text{P Apparent Recovery (\%)} = \frac{Nutrient\ uptake\ (treated\ pot)\ \text{-}\ Nutrient\ uptake\ (control)}{Amount\ of\ fertilizer\ applied\ \ (kg/\ ha)}$$

Statistical analysis

The experimental data were subjected to analysis of variance. The least significant difference (LSD) test was used to separate significantly differing treatment means at $p < 0.05$.

Results and Discussion

Crop productivity

The yield of grain and rice straw, as shown in Table (1), increased positively with the application of 100% NPKZn (T_2) showing the value of 60.18 and 76.60 qt ha^{-1}, respectively, in comparison to 51.50 and 73.80 qt ha^{-1}, respectively, in the control (T_1). The highest grain yields (66.90 qt ha^{-1}) and straw (81.60 qt ha^{-1}) were recorded in T_8 (100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) which were almost at par in treatment T_4 (100% RDF + PSB_1 @ 750 ml ha^{-1}), T_9 (75% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}), T_{12} (100% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) and T_{13} (75% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) (Table 1). A significant increase in the treatment T_8 upto 11.17 and 6.53 per cent in grain and straw, respectively in comparison to 100% RDF had been observed, while treatment T_4, T_9, T_{12} and T_{13} in the case of grain yield and treatment T_4, T_6, T_7, T_9, T_{10}, T_{12} and T_{13} in the case of straw yield were statistically comparable to T_8 treatment. These parameters attained the highest value with increasing RDF levels (100% RDF) in the presence of the combined use of PSB and BGA which confirmed the findings of Rana *et al*. (2015) [25] and Singh and Shrivastava (2015) [31]. Distinct positive effects of PSB and BGA in the presence of different doses of RDF levels were noticed and this might be due the increased solubility of phosphorus by the application of PSB and BGA, also the fixation of atmospheric nitrogen by BGA which helped in increasing nutrient uptake by crop leading to increase in biological yield of rice (Chittora *et al*. 2020) [6]. Increase in yield components might also be due to higher photosynthetic activity, release of growth promoting substances like IAA, vitamins, etc, proliferation of beneficial organisms in the rhizosphere and control of plant pathogens in the presence of biofertilizers which ultimately promoted dry matter production resulting higher grain and straw yield has also been reported by (Prasanna *et al*. 2012; Garai *et al*. 2014; Singh *et al*. 2016; Kang *et al*. 2021) [24, 10, 32, 15].

Table 1: Effect of Phosphate Solubilizing Bacteria and Blue Green Algae on grain and straw yield of rice

Treatments	Grain yield		Straw yield	
	qt ha^{-1}	g hill^{-1}	qt ha^{-1}	g hill^{-1}
T_1: Control	51.50	15.45	73.80	22.14
T_2: 100% RDF	60.18	18.05	76.60	22.98
T_3: 75% RDF	54.90	16.47	75.80	22.74
T_4: 100% RDF + PSB_1 @ 750 ml ha^{-1}	62.80	18.84	78.40	23.52
T_5: 75% RDF + PSB_1 @ 750 ml ha^{-1}	59.10	17.73	77.20	23.16
T_6: 100% RDF + BGA @ 10 kg ha^{-1}	61.39	18.42	79.10	23.73
T_7: 75% RDF + BGA @ 10 kg ha^{-1}	57.78	17.33	77.70	23.31
T_8: 100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	66.90	20.07	81.60	24.48
T_9: 75% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	64.83	19.45	80.80	24.24
T_{10}: 100% RDF + PSB_2 @ 750 ml ha^{-1}	61.43	18.43	78.80	23.64
T_{11}: 75% RDF + PSB_2 @ 750 ml ha^{-1}	57.81	17.34	76.20	22.86
T_{12}: 100% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	66.74	20.02	81.30	24.39
T_{13}: 75% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	64.20	19.26	80.30	24.09
SEm (±)	1.44	0.43	1.37	0.30
LSD (0.05)	4.20	1.26	3.97	0.86

P content in grain and straw

Effect on concentration of P in grain and straw of rice due to combined application of phosphate solubilizing bacteria and blue green algae in the presence of different doses of RDF compiled in Table 2. The results indicate that there were non-significant differences between inorganic treatments and the combined use of organic and inorganic treatments on P content in grain and straw. However, the lowest content in grain and straw had been recorded in control treatment (T_1) 0.259% and 0.132% respectively, where no fertilizer was applied and highest in the treatment (T_8) (100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) 0.274% and 0.147% where combined application of 100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1} had been done. The increase of 5.80% and 2.63% had been observed in grain in treatment T_8 when compared with treatment T_1 (control) and T_2 (100% RDF), respectively while the magnitude of increase in rice straw was 11.34 and 7.28 per cent in treatment T_8 when compared with treatment T_1 (control) and T_2 (100% RDF), respectively. The increase in nutrient content in both grain and straw as compare to sole supply of nutrients through chemical fertilizers had been observed and this could be due to a balanced supply of plant nutrients from bio-fertilizers and chemical sources (Panigrahi *et al*. 2014) [22]. The P content in grain were much higher than that of straw due to increased efficiency and cumulative synergistic effect of combined application of PSB and BGA (Chinnusamy *et al*. 2006) [5]. The results are also in conformity with the findings of Raut and Mahapatra (2006) [26], Khadayate *et al*. (2005) [16], Kumar and Yadav (2009) [18], Sharma *et al*. (2009) [30] and Sahu *et al*. (2009) [28].

Table 2: Effect of Phosphate Solubilizing Bacteria and Blue Green Algae on phosphorus content in grain and straw

Treatments	P	
	Grain (%)	Straw (%)
T_1: Control	0.26	0.13
T_2: 100% RDF	0.27	0.14
T_3: 75% RDF	0.26	0.14
T_4: 100% RDF + PSB_1 @ 750 ml ha^{-1}	0.27	0.14
T_5: 75% RDF + PSB_1 @ 750 ml ha^{-1}	0.27	0.14
T_6: 100% RDF + BGA @ 10 kg ha^{-1}	0.27	0.14
T_7: 75% RDF + BGA @ 10 kg ha^{-1}	0.27	0.14
T_8: 100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	0.27	0.15
T_9: 75% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	0.27	0.14
T_{10}: 100% RDF + PSB_2 @ 750 ml ha^{-1}	0.27	0.14
T_{11}: 75% RDF + PSB_2 @ 750 ml ha^{-1}	0.27	0.14
T_{12}: 100% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	0.27	0.15
T_{13}: 75% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	0.27	0.14
SEm (±)	0.00	0.00
LSD (0.05)	0.01	0.01

Total P uptake

The P uptake was calculated by multiplying per cent concentration of nutrient with grain and straw yields. As nutrient uptake calculated from nutrient content and yield, the increase in these factors increased the uptake of P. The higher uptake of these nutrients was also due to the higher productivity of the hybrid rice (Kumar and Yadav 2009; Mondal *et al*. 2016) [18, 20]. The result (Table 3) shows that there were significant differences between inorganic treatments and combined (inorganic and bio-fertilizers) treatments. The presence of PSB and/or BGA increased the nutrient uptake rather in control treatment where no such application had been done. The P uptake augmented f0rom control treatment (T_1) 23.09 kg ha^{-1} to limit in the treatment (T_8) (100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) 30.33 kg ha^{-1} (Figure 1). The resulting increase in P uptake through a balanced supply of plant nutrients might be due to presence of both inorganic and bio-fertilizers. Beside, source of N, blue green algae also known to add considerable quantity of organic matter which improved soil structure, gave better environment for root growth, creating a more absorptive surface for nutrients uptake (Garai *et al*. 2014; Chittora *et al*. 2020) [10, 6] inoculation of PSB enhanced the P-uptake due to the solubilization of insoluble phosphate and also play a important role in root development which ultimately increases the uptake of nutrient (Elhaissoufi *et al*. 2020) [7]. The P contents in the plants positively influenced the biomass yield of rice, which could be due to higher microbial activity through the integrated use of chemical fertilizers and bio-fertilizers, which contributed to improved nutrient supply and better nourishment of hybrid rice and confirmed the findings of Khadayate *et al*. (2005) [16], Sahu *et al*. (2009) [28] Rana *et al*. (2015) [25] and Kang *et al*. (2021) [15]. The lowest value of total NPK uptake in hybrid rice was recorded in crops without fertilizer control and/or without biofertilizers and was closely followed by only chemical fertilizers (75% and 100% RDF). The results corroborate the findings of Huang *et al*. (2008) [11].

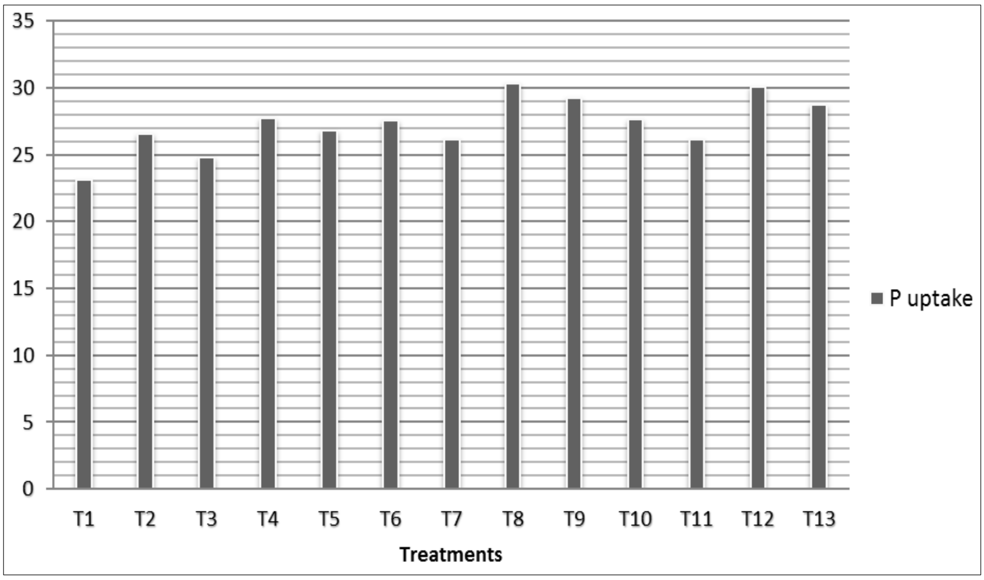

Fig 1: Total uptake of P of hybrid rice as influenced by PSB and BGA application in different treatments

P Apparent Recovery (%)

Significant result observed for P apparent recovery as shown in Table 3. After calculation it was observed that apparent recovery percentages were maximum in treatment T_8 (100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) 13.63 per cent, closely followed by treatment T_9 (75% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}) i.e. 12.07 per cent.

Table 3: Result observed for P apparent recovery

Treatments	P	
	Uptake (kg ha^{-1})	APR (%)
T_1: Control	23.09	NA
T_2: 100% RDF	26.56	5.78
T_3: 75% RDF	24.77	3.73
T_4: 100% RDF + PSB_1 @ 750 ml ha^{-1}	27.69	7.66
T_5: 75% RDF + PSB_1 @ 750 ml ha^{-1}	26.77	8.17
T_6: 100% RDF + BGA @ 10 kg ha^{-1}	27.58	7.49
T_7: 75% RDF + BGA @ 10 kg ha^{-1}	26.11	6.71
T_8: 100% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	30.33	12.07
T_9: 75% RDF + PSB_1 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	29.22	13.63
T_{10}: 100% RDF + PSB_2 @ 750 ml ha^{-1}	27.68	7.64
T_{11}: 75% RDF + PSB_2 @ 750 ml ha^{-1}	26.14	6.78
T_{12}: 100% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	30.04	11.58
T_{13}: 75% RDF + PSB_2 @ 750 ml ha^{-1} + BGA @ 10 kg ha^{-1}	28.70	12.46
SEm (±)	0.47	1.10
LSD (0.05)	1.38	3.20

This index of nutrient recovery showed increase in all the treatments where algal &/or bacterial inoculation had been done. This might be due to increased efficiency and cumulative synergistic effect of combined application of PSB and BGA resulting increased uptake of nutrients due to balanced supply of plant nutrients. This result was similar to the findings of Bahadur *et al*. (2013) [3] and they further reported that increase in apparent nutrient recovery in rice increased with increasing nutrients levels either through inorganic or including organics and/ or bio-fertilizers.

Conclusions

Combined application of Phosphate Solubilizing Bacteria (PSB) and Blue Green Algae (BGA) significantly influenced P nutrition in hybrid rice and increased the yield and apparent P recovery up to 11 and 8% respectively, as compared to 100 per cent recommended dose of fertilizers (RDF). Thus, applications of PSB and BGA have appreciable impact on the P nutrition to hybrid rice in modern intensive agriculture. Biofertilizers are cost effective, eco-friendly and renewable supply of soil nutrient which play a vital role in maintaining a long-term sustainability and fertility of soil.

Disclosure statement

No potential conflict of interest was reported by the authors.

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