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Short communication

Plant growth promoting rhizobacteria increase the efficiency of fertilisers while reducing nitrogen loss

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ABSTRACT

More than half of the applied conventional fertiliser nitrogen (N) in cropping systems can be lost to the environment, resulting in water and air pollution. Farming systems that ensure efficient fertiliser use are crucial to sustain crop productivity without harming the environment. One avenue to achieve this is the use of bio-fertilisers with recognised benefits for plant nutrition and soil heath. Within this area, plant growth promoting rhizobacteria (PGPR) are increasingly applied to enhance plant nutrient acquisition and assimilation. Here, we investigated if PGPR can improve fertiliser performance. We show that the addition of PGPR to soils amended with 50% organic and 50% conventional N fertilisers increased the growth of kikuyu grass (*Pennisetum clandestinum*), producing yields similar to those obtained using 100% conventional N fertiliser. Encouragingly, this combination also reduced mineral N leaching by 95% relative to the all conventional fertiliser treatment. These findings suggest that using organic and synthetic fertilisers together in the presence of PGPR is a promising approach for sustaining plant growth while reducing potential pollution from inefficient use of conventional N fertilisers.

1. Introduction

In modern agricultural systems, less than 50% of nitrogen (N) applied is utilised by crops and much of the remaining nitrogen pollutes waterways and generates large amounts of the potent greenhouse gas, nitrous oxide (Lassaletta et al., 2014). Therefore, N pollution is now considered as a pressing issue in the world (Rockström et al., 2009). The development of systems that ensure efficient fertiliser use without negatively impacting the environment is crucial to sustain crop productivity into the future (Tilman et al., 2002).

Application of organic residues such as organic wastes and manures was a traditional agricultural practice before shifting to synthetic fertilisers in high-production cropping (Paungfoo-Lonhienne et al., 2012). In recent decades, the importance of organic fertilisers in plant growth has received renewed attention (Bolan et al., 2010; Kumar and Maiti, 2015). This is because organic fertilisers not only supply nutrients to plants but also provide beneficial effects on soil health due to its organic matter content, a key factor for soil fertility owing to its role for soil structure, biological processes and nutrient cycling (Haynes et al., 1998; Johnston et al., 2009; Manlay et al., 2007).

There is also much interest in improving plant nutrient supply with plant growth promoting rhizobacteria (PGPR). These microorganisms promote plant growth by numerous mechanisms such as increasing the supply of nutrients, increasing root biomass or root area, and increasing nutrient uptake capacity of the plants (Madhaiyan et al., 2009; Malusá et al., 2012; Vessey, 2003). Bio-fertilisers containing PGPR increasingly show economic promise and potential for environmental benefits. The global plant-bio-stimulant market, including bio-fertilisers, is estimated to be increasing by 12% per year (Calvo et al., 2014) and will reach over $3.29 billion by 2022 (https://www.marketsandmarkets.com/PressReleases/biostimulant.asp).

Reliable techniques for PGPR delivery will provide optimal conditions to maintain the abundance and robustness of PGPR in the soil, and to maximise their activity in the rhizosphere. Currently, there are several types of formulation with or without use of carrier materials (e.g., liquid, freeze-dried powder, peat and granules) and each type has its own advantages and limits (Herrmann and Lesueur, 2013). Carrier-based formulations have shown successful results for maintaining high bacterial viability (Berninger et al., 2017; Tripti et al., 2017) and this method has been chosen in this study.

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Recognizing the importance of organic fertilisers and beneficial effects of PGPR, we investigated if PGPR can improve organic fertiliser performance, with the aim to replace leaky conventional N fertilisers with organic fertilisers in proper circumstances. We assessed the effects of poultry manure-based organic fertiliser with or without PGPR on pasture grass growth and N leaching compared to conventional urea fertiliser.

2. Materials and methods

2.1. Preparation of plant growth promoting rhizobacteria (PGPR)

The SOS3 PGPR bacterium (Sustainable Organic Solution Pty Ltd, patent application number PCT/AU2016/050453) used in this study belongs to the *Paraburkholderia* genus. It was grown to the mid-log phase in nutrient broth medium (Sigma-Aldrich), centrifuged at $4500 \times g$ for 10 min, washed twice and resuspended in 0.85% NaCl. Bacterial solution at an optical density of 1 (approximately 10^9 cell ml^{-1}) was applied onto zeolite at a ratio of 1 mL: 3 g zeolite, resulting in 10^8 cells g^{-1}. Zeolite was chosen as a carrier material for introducing and holding the PGPR (Berninger et al., 2017). One gram of this PGPR product was used per pot in inoculation treatments whereas zeolite only was added in non-inoculated treatments.

2.2. Plant growth conditions

Thirty kikuyu grass (*Pennisetum clandestinum*) seeds were grown on 0.5-L plastic pots containing 250 g of sandy soil collected from a sugarcane farm in Ayr, Queensland, Australia (Table 1). Nitrogen was added as poultry manure-based organic fertiliser (Org, CropUp™ containing 4.1% N, 1.7% P, 2.7% K with a C/N ratio of 6.8; Sustainable Organic Solution Pty Ltd, Australia) or conventional urea fertiliser (Conv), at rates of 0, 50 kg N ha^{-1} (28 mg pot^{-1}), 100 kg N ha^{-1} (56 mg pot^{-1}), 150 kg N ha^{-1} (84 mg pot^{-1}), and 200 kg N ha^{-1} (112 mg pot^{-1}) with duplicates of each N form at each N rate. In addition, a treatment with combined organic fertiliser and PGPR/biofertiliser (Conv + B) was included. To eliminate the effects of other nutrients on plant growth, each pot received basal nutrients containing 0.25 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 g $CaSO_4.2H_2O$, 0.3 mg $CuSO_4.5H_2O$, 0.66 mg $ZnSO_4.7H_2O$, 15 mg $MnCl_2.4H_2O$, 1 mg H_3BO_3, 0.15 mg $Na_2MoO_4.2H_2O$, and 45 mg Fe-EDTA. Plants were watered daily with rainwater and grown for 4 weeks to simulate the establishment phase of pasture/crop production. The plants were grown in an accelerated growth cabinet fitted with an automated irrigation system and high-intensity growth lights (25 °C, 22 h/2 h day/night). At harvest, shoots were cut, dried at 60 °C for 5 days and weighed.

A follow-up targeted experiment was established to assess treatment effects at a typical N application rate of 100 kg N ha^{-1}. In this experiment, the Org treatment was supplemented with urea N inputs to ensure sufficient N supply during the plant establishment phase. Also, the Org + Conv treatment was compared with an Org + Conv + B treatment to ascertain any effects caused by the PGPR. Plant growth conditions were as described above, except the total amount of N applied was the same in all treatments at 100 kg N ha^{-1} (56 mg per pot). N-free pots served as control. Each pot contained 30 kikuyu grass seeds, and each treatment consisted of six replicates.

In both experiments, the pots were leached with 1.5 pore volumes of rainwater (135 mL per pot) after plants were grown for 3 weeks. The leachates were analysed for NH_4^+-N and NO_3^--N contents. After leaching, the plants were allowed to grow for another week until harvest (4 weeks after growth). The harvested grass seedlings were separated into roots and shoots and then dried at 60 °C for 5 days before weighing. The dried shoots were homogenised and analysed for N and C contents by combustion using a CHN analyser (LECO TruSpec, USA). The soils were homogenised and analysed for NH_4^+-N and NO_3^--N contents (see details below).

2.3. Physicochemical analyses

NH_4^+-N and NO_3^--N contents were determined using the 2 M KCl extraction and colorimetric spectrometry method (Rayment and Lyons, 2010). Soil pH and electrical conductivity (EC) were measured in 1:5 soil:water extracts with calibrated electrodes at about 25 °C. Primary particle size distribution was determined using the pipette method (Standards Australia, 2003). Total organic C and N contents were determined with the Dumas combustion method using a TruMac® CN analyser (LECO, St Joseph, MI, USA).

2.4. Statistical analysis

Normality and homogeneity of group variances were tested and the differences among treatments were statistically analysed using ANOVA. Fisher's Protected Least Significant Difference (LSD) post hoc test at $P < 0.05$ was conducted to identify significant differences in the effects of different nitrogen forms and PGPR. A Kruskal Wallace test was used to test for differences between treatments of non-normally distributed data. All statistical analyses were performed using the GraphPad Prism4 software package (GraphPad Software, Inc., San Diego CA, USA).

3. Results and discussion

3.1. Effects of PGPR on kikuyu grass growth

The kikuyu grass biomass increased with increasing nitrogen application rates in all treatments. The growth of kikuyu was similar in all treatments up to 50 kg N/ha. However, at ≥100 kg N/ha, the grass grown with the organic fertiliser (without or with PGPR) produced less biomass in comparison to those with the inorganic fertiliser (Fig. 1). This result suggested that N supply (including existing $NH_4^+ + NO_3^-$ and mineralized N) from the organic fertiliser alone was insufficient to meet demand for maximum plant growth, especially for fast growing plants such as grass. In addition, the PGPR tested had little beneficial effects when applied with organic fertiliser alone. Supplementary addition of conventional N fertiliser may overcome this initial N limitation.

In order to address this problem, the plants were grown in soils at 100 kg N ha^{-1} with conventional urea N fertiliser (Conv), or a combination of 50% urea and 50% organic fertilisers without (Conv + Org) or with PGPR (Conv + Org + B). The kikuyu grass grown in soil amended with mixed N source in the presence of PGPR (Conv + Org + B) significantly ($P < 0.05$) increased shoot and root biomass by 48% and 45%, respectively, compared with the Conv + Org treatment (Fig. 2). This treatment produced plant biomass N similar to the conventional urea N fertiliser treatment (Conv), suggesting that PGPR played a key role in either mineralisation of the organic fertiliser N or plant N acquisition and assimilation, leading to enhancement of plant growth. This result suggests a great potential for PGPR to improve the efficiency

Table 1
Characteristics of the soil used in this study, reported on an oven dry basis. Electrical conductivity = EC. Standard error of mean = SEM.

Parameters	Units	Mean ± SEM (n = 3)
pH		6.4 ± 0.0
EC	dS m^{-1}	0.087 ± 0.003
Sand (> 20 μm).	%	78.6 ± 0.6
Silt (2–20 μm)	%	11.3 ± 0.5
Clay (< 2 μm)	%	15.63 ± 0.56
Total N	%	0.08 ± 0.01
Total C	%	0.99 ± 0.08
Organic C	%	0.86 ± 0.07

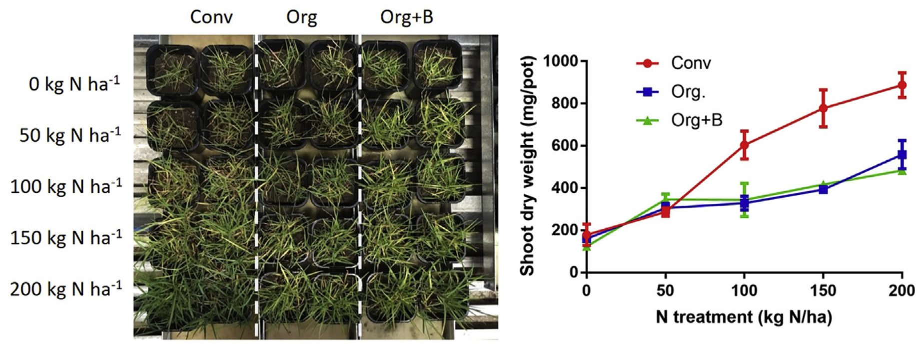

Fig. 1. Effect of different nitrogen forms and the plant growth promoting rhizobacteria (PGPR) on the biomass of kikuyu grass. Conv = conventional N fertiliser, Org = organic fertiliser, Org + B = organic fertiliser + PGPR. Data are averages and standard errors of two replicates with thirty grass seeds sown in each pot.

of N fertilisers while reducing the use of urea N fertilisers.

3.2. Mineral N in leachate and soil

To assess whether the Org + PGPR combination could reduce N loss, we analysed the mineral N composition of leachates collected in week three. The results show that the combination of Org + Conv significantly decreased mineral N in leachates compared to urea alone, resulting in a similar level to that in the non-fertilised soil control (Fig. 3 a-c). Compared with the conventional urea treatment, the mineral N in leachate decreased by 67% ($P < 0.01$) in the Org treatment and by 95% ($P < 0.001$) in the presence of PGPR (Fig. 3c). In contrast to the leachate N, soil mineral N contents were similar in the Org + Conv and the conventional treatment, indicating that N mineralisation from the Org was small. However, adding PGPR in Org + Conv decreased soil mineral N (mainly in NO_3 form) concentration by 45% (relative to Conv) and 65% (relative to Conv + Org) (Fig. 3f). These results in combination with the greater plant growth (Fig. 2) suggested that PGPR enhanced plant N acquisition and assimilation.

Our results confirmed the finding in a previous study that beneficial effects of PGPR will not occur in conditions where there is not enough available N (Paungfoo-Lonhienne et al., 2016). A mixture of urea and organic fertilisers overcame the N limitation, with PGPR generating clear benefits for plant growth. Furthermore, the Org + Conv + B combination also reduced N loss from leaching, likely due to the plant growth promotion effect of PGPR by increasing nutrient uptake of the plant (Lugtenberg and Kamilova, 2009; Vessey, 2003). Remarkably, the treatments containing the PGPR resulted in similar shoot and root growth to the Conv treatment, despite having clearly much lower soil mineral N concentrations. This result suggests that the PGPR conferred a very efficient mechanism of plant nutrient uptake, whereby nutrient leaching risks were minimised.

Our observations may have important implications in the quest to deliver improved nutrient use efficiency. Currently, more than half of the applied conventional N fertiliser in cropping systems can be lost to the environments, resulting in a loss of biota, and jeopardizing the integrity of ecosystem and global biogeochemical cycles (Gruber and Galloway, 2008; Rockström et al., 2009). Thus, reducing the pollution derived from inefficient use of conventional N fertilisers has enormous importance for agriculture. The results from our study suggests that one avenue to remediate the problem associated with conventional N fertilisers is the use of organic fertilisers along with conventional N fertilisers in combination with PGPR.

Several studies have shown the beneficial effects of combined organic and inorganic fertilisers on soil chemical and biological properties (Goyal et al., 1999; Kaur et al., 2005). Yield-enhancing effects of this application method were observed in intensified rice cropping systems (Thakur et al., 2010; Zhao et al., 2010). In accordance, our results demonstrated that PGPR had a great potential to enhance plant growth in non-paddy agricultural practices by maximising the N use efficiency of

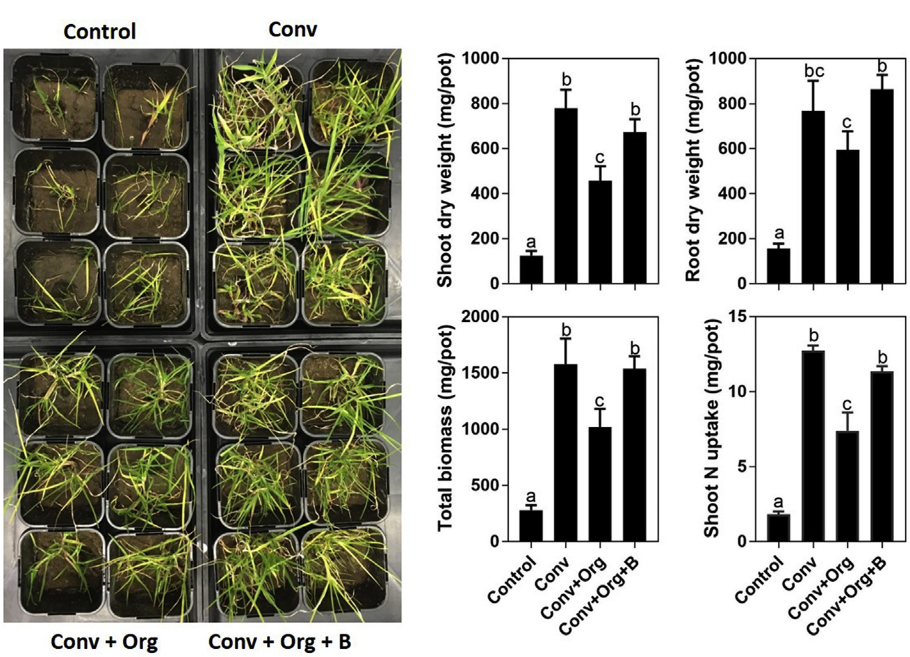

Fig. 2. Performance of kikuyu grass grown for 4 weeks with different fertiliser sources and PGPR. All treatments received nitrogen at the rate of 100 kg N ha^{-1} from the applied fertilisers. N-free pots served as control. Conv = conventional N fertiliser, Org = organic fertiliser, Org + B = organic fertiliser + PGPR. The Bars represent averages and standard errors of six replicates. Differing lowercase letters indicate statistically significant differences between treatments ($P < 0.05$; ANOVA, LSD post hoc test).

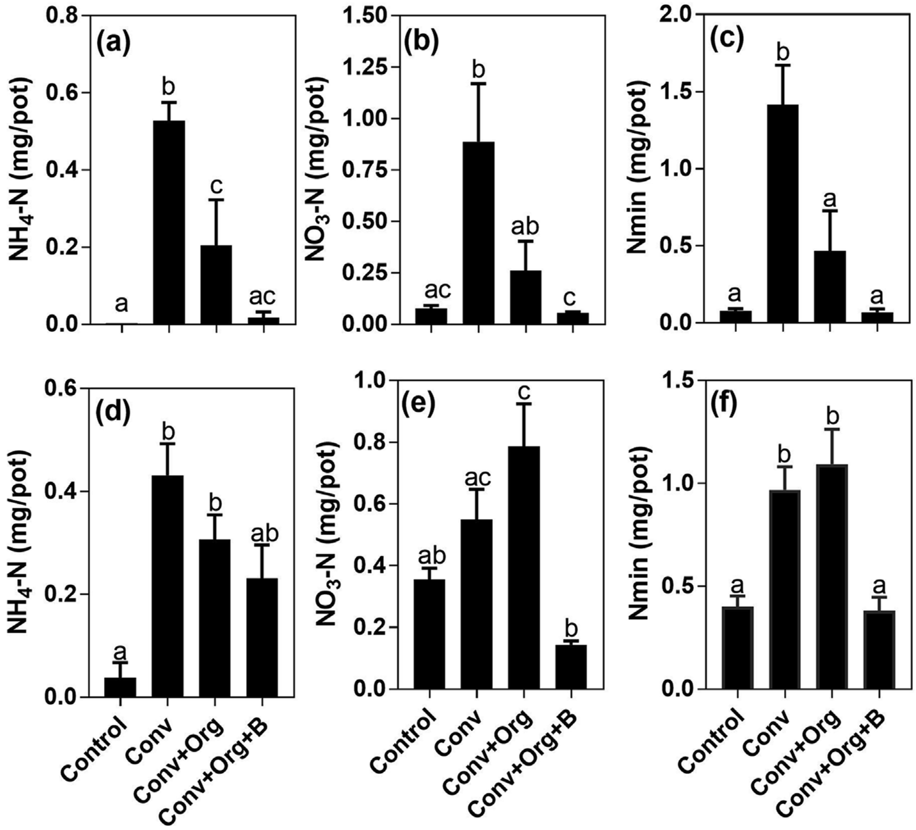

Fig. 3. NH_4-N and NO_3-N concentrations in the leachates (a–c) three weeks after planting and in the leached-soils at harvest (d–f). Conv = conventional N fertiliser, Org = organic fertiliser, Org +B = organic fertiliser + PGPR. Bars represent averages and standard errors of four replicates. Same lowercase letters indicate statistically non-significant ($P > 0.05$) differences between treatments (ANOVA, LSD post hoc test).

combined inorganic and organic fertilisers.

4. Conclusion

In summary, a combined use of PGPR with inorganic and organic fertilisers has the potential to improve the efficiency of fertiliser N, and decrease environmental risks associated with N leaching. Future research will evaluate whether this combination also benefits other plants grown in different soil types with different application rates of conventional N fertilisers, and how it will benefit crops growing in field conditions.

Disclosure statement

CPL was employees of Sustainable Organic Solution Pty Ltd that owns CropUp™ and the PGPR SOS3.

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