Long-Term Bacterial Fertilizer Application Improves Eucalyptus Growth and Yields through Altering Soil Quality

Han Ren (✉ renhan@st.gxu.edu.cn)

Wenzhou University <https://orcid.org/0000-0002-0156-0726>

Xiaohong Qin

Guangxi University

Chengqun Lv

Guangxi University

Víctor Fernández-García

University of Leon

Chuan Xie

Huanggang Research Institute of Forestry

Research

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Abstract

Sludge and plant growth-promoting rhizobacteria (PGPR) are widely used to improve soil quality and plant growth. In this study we aim to determine the responses of soil enzyme activities, soil nutrient contents and plant growth to fertilization with sludge and PGPR (*Bacillus megaterium* strain DU07). To achieve this goal, we planted bare-root eucalyptus seedlings with soil amended with (1) filtered sludge from sugar factory (FS), (2) filtered sludge + PGPR (BF) and (3) non-amended soil (control). Soil properties (soil nutrients and soil extracellular enzymatic activities) and eucalyptus growth (diameter, height and stem volume) were studied 3, 6, 9 and 12 months after treatments. Results indicated that BF significantly increased plant height, diameter, and stem volume after the sixth month. Principal component analysis (PCA) showed that soil quality was significantly increased by BF during our trial period, especially after the ninth month. The pooled regression model indicated that soil quality significantly and positively affected eucalyptus yield, whereas soil TN was negatively and significantly related to plant stem volume. Hence, our study indicates that the application of a bacterial fertilizer increases plant growth and yield via increasing soil quality. Thus, our findings suggest that PGPR amendments could be useful as bio-fertilizer to improve soil-plant interactions in eucalyptus plantations.

1. Introduction

There is a world-wide drive to develop environmentally friendly bio-fertilizers from N-fixing bacteria and organic matter to replace or reduce the artificial pesticide and inorganic fertilizer use in agricultural and forestry land. The main mechanism for achieving benefits from bio-fertilizer applications to soils is 1) increasing soil nutrient contents (including N, available K, available P, etc.) through fast microbial vital activity (Tamreihao et al. 2018); 2) transferring the non-directly absorbed matter (litterfall drops, lignin, a nitrogen-containing compound, etc.) by plants into the absorptive format (amino acid, inorganic N) (Hassan et al. 2017); 3) elevating the content of stimulating production (phytohormone, Indole-3-acetic acid, cytokinins, etc.) in vivo (Sharma et al. 2017).

Plant Growth Promoting Rhizobacteria (PGPR) has been widely reported to improve crop yield and soil quality via improving soil fertility (Han Ren, Chengqun Lv, Víctor Fernández-García, Baoling Huang, Jiangming Yao 2019), soil microbial composition diversity (Ren et al. 2020a), and plant nutrient uptake capacity (Ren et al. 2020b). *Bacillus megaterium*, such as *B. megaterium* var. *phosphatic*, *B. subtilis*, *B. Lichens*, have the potential to protect the plant from diseases and pests (Srinivasan et al. 2016) and conduct biological N-fixing (Selbmann et al. 2010). Guangxi is in the south of China and is well known for its sugarcane as the most economically important crop in the past 10 years (Chen et al. 2013). Likewise, filtered sludge from cane sugar factories contains abundant active materials, including soluble humic acid (> 14%) and crude protein (> 6.5%), and thus potentially increase the soil dissolved oxygen content and soil fertility when applied as soil amendment (Sohi et al. 2010). However, the accumulation of bagasse either leads to soil acidification, loosen, contamination of groundwater, or other negative impacts on the soil environment (Adekayode and Olojugba 2010). One approach to solving this problem is the use of bio-fertilizer consisting of probiotics and organic matter.

Soil quality, including soil extracellular enzymes and soil nutrient content, has been investigated to evaluate soil ecological and microbial status in previous researches (Han Ren, Chengqun Lv, Víctor Fernández-García, Baoling Huang, Jiangming Yao 2019)(Chan et al. 2008). Soil quality is closely related to the sustainability of forestry ecosystems in the tropics (Agegnehu et al. 2016) and is usually assessed by studying soil biological, chemical, and physical properties (Jordan et al. 1995). In this sense, Han et al. (Han Ren, Chengqun Lv, Víctor Fernández-García, Baoling Huang, Jiangming Yao 2019) reported that soil quality was positively affected by PGPR application in acidic latosolic red soils in south China over the short-term, but there is no information on how sludge and PGPR and affect soil quality along an entire year.

The utility of bacterial fertilizers has been studied in crops such as maize (Beirinckx et al. 2020), wheat (Khatri et al. 2020), sweet potato (Habibi et al. 2014), paddy rice (AW et al. 2020), lettuce (Venancio et al. 2019), tobacco (Hamid et al. 2020) and sugarcane (Santos et al. 2018). However, to our knowledge, few studies have focused on the effects of sludge and PGPR on forest plantations, as well as on understanding the underlying mechanisms via soil quality alteration. In the current study, we aim to determine the effects of sludge and PGPR on soil quality (soil nutrients and soil extracellular enzymatic activities) and tree growth (diameter, height and stem volume) in a eucalyptus plantation during one-year study period. Moreover, we attempt to investigate the key factors of soil quality affecting eucalyptus yield using the pooled regression model one year after treatments. We hypothesized that (1) soil quality and plant growth would increase with the application of bio-fertilizers and (2) soil nutrient contents would significantly influence plant growth and yield.

2. Materials And Methods

2.1. Experimental site

The study was conducted on the Liangfengjiang National park in Nanning, China (107°45' 108°51' E, 22°13' 23°32' N). The average annual precipitation in our research site is 1304 mm and the mean annual humidity is 79% from 2005–2015. The average temperature at our research site ranges from 21.1 °C to 22.5 °C, with the highest temperature of 40.4 °C in summer and the lowest temperature of -2.4 °C in winter (Chen et al. 2013). The soil is classified as acidic latosolic red soil.

In our study site, we carried out a plantation of bare root eucalyptus seedlings (3600 eucalyptus ha⁻¹). We selected eucalyptus as the focal crops for our research because eucalyptus is the most prominent pulp and wood material in Guangxi [4]. Bare-root eucalyptus (DH32-29, a clone of *Eucalyptus urophylla* x *E. grandis*) seedlings were supplied by Dongmen tree farm, Guangxi, China (seedling quality examination certificate number 2011-029) and planted using three-dimensional holes (20 cm * 20 cm * 20 cm) for each individual. The initial average height of the bare-root seedlings was 25 cm.

2.2 Filtered sludge and PGPR characterization

Filtered sludge (FS) is the extracted residue from sugarcane juice clarification by pressure filter and rotary vacuum filter. We have selected FS as the focal raw material for bacterial fertilizer for this study because Guangxi is a major producer of sugarcane and FS (mainly sugarcane residue) is largely stocked after crop roughly processed. FS was supplied by the Nanning sugar factory (Guangxi, China) and transferred to our research site with 55 cm × 85 cm plastic bags (Ren et al. 2020b). The FS applied in our study had the following characteristics: 1.50 mg TN g⁻¹, 0.27 mg TK g⁻¹, 0.33 mg TP g⁻¹, and pH 6.10 (Ren et al. 2020b).

The PGPR *Bacillus megaterium* strain DU07 (Ren et al. 2020b) was firstly isolated from the eucalyptus rhizosphere in solid lysogeny broth (LB) in 2011 in the microbiological laboratory of the Forestry College at the Guangxi University (China) and stored in an Ultra-low Temperature Freezer at -80 °C (See [27] for genotyping of *B. megaterium* strain DU07). Stored *B. megaterium* strain DU07 cells were cultured in fluid LB at 28 °C under shaking at 120 r/min for 6 days for activation and were diluted to 9 × 10⁹ CFU/g with sterile water under strict sterile conditions in a clean bench.

2.3. Experimental set-up and treatments

In order to determine the effects of filtered sludge and bacterial fertilizer on soil quality and plant growth we applied three different treatments at the moment of eucalyptus seedling plantation: (1) 6.25 kg m⁻² filtered sludge from sugar factory (FS), (2) 6.25 kg m⁻² FS + inoculation of 2 mL per seedling of 3 × 10⁹ CFU/g PGPR (BF) (3) no amendment (control). To apply the three treatments, the study site was divided in 150 2m*2 m field plots, each treatment being applied to 50 plots (50 replicates). In order to ensure no contamination among treatments, we established a minimum distance of 2 m between plots subjected to different treatments.

2.3 Field sampling and lab measurements

We randomly collected five soil samples (5 replicates) for each treatment and the control 3 months (M3), 6 months (M6), 9 months (M9), and 12 months (M12) after the eucalyptus seedlings were planted and amended. Soil samples were collected from the top 20 cm soil layer to determine soil nutrient contents and enzyme activities (Han Ren, Chengqun Lv, Víctor Fernández-García, Baoling Huang, Jiangming Yao 2019).

Soil total N (TN) and Available N (Avail N) were analyzed in a C/N analyzer (Vario, Max, CN) following the method of Bronk et al. (Bronk et al. 1998), soil available K (Avail K), Available P (Avail P) were determined with a flow injection auto-analyzer (Tech- nicon, AA3, Germany) following the method of (Qi et al. 2011).

Soil catalase (CAT), urease, sucrase, and protease activities were colorimetrically measured as described by Akhtar et al. (2018), Piotrowska, and Wilczewski (2012), Han et al. (2017), and Li et al. (2018), respectively.

Likewise, 3, 6, 9 and 9 months after treatments we measured stem diameters with a Vernier caliper, a band tape, and a length rod plant diameter and plant height. Then, we calculated the plant stem volume

according to the following formula described by Lie and Xue (Lie and Xue 2019).

$$\text{Log (Stem volume)} = -4.182 + 1.65\log (\text{diameter}) + 1.102 \log(\text{height})$$

2.4 Statistical analysis

The effects of treatments on soil quality (soil nutrients and soil extracellular enzyme activities) and plant growth (stem diameter, height and stem volume) were evaluated by analysis of variance (ANOVA) in R (<https://www.R-project.org/>). Where the main effects were significant ($p < 0.05$), we used the LSD test to determine significant differences between pairs. We used the ggbiplot package in R to conduct the Principle Component Analysis (PCA) on soil quality to evaluate the influence of FS and BF on soil quality at four-time points. We chose the pooled regression model when the result of the Hausman test between fixed effects regression and pooled regression for plant and soil parameters in our panel data was $p > 0.05$.

3. Results

3.1 Plant growth

No clear effect of FS and BF on plant height and timber of volume were observed in M3 while plant diameter was significantly ($p < 0.01$) decreased by FS (0.73 cm) relative to the control (0.79 cm) in M3.

In M6, eucalyptus diameter and stem volume were significantly (both $p < 0.01$) increased upon FS (2.57 cm, 0.041 m³ tree⁻¹ respectively) and BF (2.78 cm, 0.045 m³ tree⁻¹ respectively) compared to the control (2.22 cm, 0.036 m³ tree⁻¹ respectively). Plant height was significantly ($p < 0.01$) increased in BF (2.10 m) compared to the control (1.85 m); whereas, no significant difference between FS and the control was observed.

Plant diameter, height, and stem volume were extreme significantly (all $p < 0.001$ vs. control) increased in all treatments in M9 and M12. Remarkably, BF enhanced plant parameters more significantly ($p < 0.001$) than FS in M9 and M12, indicating that co-application of PGPR and FS has stronger effects on plant growth and yield than FS in the long term (Fig. 1).

3.2 Soil nutrient contents

There were no clear effects of BF and FS amendments on soil nutrient contents in M3 and M9 (Table 2). In M6, none of the treatments had a significant impact on soil Avail K and Avail P. In contrast, soil TN was significantly ($p < 0.01$) increased in FS (3.43 mg. g⁻¹) and BF (3.57 mg. g⁻¹) when compared with the control (2.50 mg. g⁻¹), whereas soil Avail N significantly ($p < 0.01$) decreased after BF (9.33 µg. g⁻¹) and FS (7 µg. g⁻¹) treatments. We found no significant difference in Avail P and Avail N when comparing the treatments with the control in M12. Additionally, we observed the significant differences in the effects of BF (1.89 mg. g⁻¹ and 34 µg. g⁻¹ respectively) on soil TN and Avail K in relative to FS (1.39 mg. g⁻¹ and 26 µg. g⁻¹) and the control (1.52 mg. g⁻¹ and 21 µg. g⁻¹) in M12. Further, BF increased soil TN and Avail K

evaluated more significantly than FS in M12, indicating that co-application of PGPR and organic matter contributes more to specific soil nutrient conversion is relative to the separate application of FS in the long term (Fig. 2).

3.3 Soil enzyme activities

No clear effects of FS and BF on soil CAT, sucrase, protease, and urease activities in M9 and M12. In M3, soil sucrase activity was significantly ($p < 0.01$) increased in relative to FS ($7.54 \text{ mg. g}^{-1} \cdot (24 \text{ h})^{-1}$) and the control ($8.29 \text{ mg. g}^{-1} \cdot (24 \text{ h})^{-1}$) in bacterial fertilizer treatment ($10.96 \text{ mg. g}^{-1} \cdot (24 \text{ h})^{-1}$), whereas none of the treatments had a significant effect on soil CAT and urease activities. In M6, soil urease activity was significantly ($p < 0.01$) increased in BF ($11.71 \text{ mg. g}^{-1} \cdot (24 \text{ h})^{-1}$) when compared with FS ($8.11 \text{ mg. g}^{-1} \cdot (24 \text{ h})^{-1}$) and the control ($7.16 \text{ mg. g}^{-1} \cdot (24 \text{ h})^{-1}$). However, in M6, there were no significant differences among each of the treatments and the control on soil CAT, sucrase, and protease activities (Fig. 3).

3.4 Soil quality

PCA was used to determine the soil quality under FS and BF amendment regimens (Fig. 4 & Table 1). We drew a 68% confidence ellipse in the plot to observe whether or not treatments and the control were separated. All of the variance in soil quality in our biplots could be attributed to two factors. In M3, the first principal component (PC1) explained 43.8% of the variance and the second principal component (PC2) explained 24.5% (Fig. 2- A). Soil sucrase and Avail K were mainly correlated with PC1, whereas soil urease was mainly correlated with PC2. The principal component scores of FS (-0.52) was lower than that of the control (-0.42); whereas, that of BF (0.94) was higher (Table 1).

Biplot for soil quality in M6 showed that PC1 explained 46.4% of the variation and PC2 explained 23.9% (Fig. 2-B). Soil urease and CAT were mainly correlated with PC1; whereas, soil protease was mainly related to PC2. Sample separation between control and the treatments were marked by PC1. FS (-0.20) and BF (0.89) had higher PC scores than the control (-0.69) (Table 1).

In M9, PC1 explained 36.6% of the variance and PC2 explained 23.9% (Fig. 2- C). Soil urease and Avail K were mainly correlated with PC1, whereas soil Avail N was mainly correlated with PC2. The principal component scores of BF (-0.87) was lower than that of the control (0.41); whereas, that of FS (0.46) was higher (Table 1).

Biplot for soil quality in M12 showed that PC1 explained 49.8% of the variation and PC2 explained 19.2% (Fig. 2-D). Soil TN and CAT were mainly correlated with PC1; whereas, soil protease and Avail N were mainly related to PC2. Sample separation between control and BF was marked by PC1. FS (0.26) and BF (1.17) had higher PC scores than the control (-0.91) (Table 1).

In general, the PC scores in treatments and the control indicated that soil quality was significantly (both $p < 0.001$) positively affected by BF amendment in the short term (M3 and M6); whereas, the significant (p

< 0.01 and $p < 0.001$ respectively) influence on soil quality in BF treatments showed the contrasting trend at M9 and M12 in the long term.

3.4 Correlation between plant yield and soil quality

The specific impact factors on eucalyptus yield were determined using the pooled regression model on plant yield and soil quality during our trial period (Table 2). The pooled regression model for panel data ($F = 6.15$, $p < 0.05$, Adj. $R^2 = 0.77$) showed that eucalyptus yield was positively affected by soil urease (Estimate = 0.012, $p = 0.31$) and sucrase (Estimate = 0.0044, $p = 0.17$), whereas negatively influenced by soil CAT (Estimate = -0.26, $p = 0.17$), TN (Estimate = -0.054, $p = \mathbf{0.04}$), AK (Estimate = -0.00081, $p = 0.44$), AN (Estimate = -0.0012, $p = 0.79$) and AP (Estimate = -0.034, $p = 0.87$) (*Equation S2*).

Besides, all soil quality parameters except soil TN had no significantly ($p > 0.05$) effect on plant yield, and the effect of soil TN on the eucalyptus output was significant ($p < 0.05$), indicating that soil TN negatively and significantly affected plant yield rather than other soil quality parameters during our study period.

Plant yield was positively correlated with soil quality ($F = 6.15$, $p = 0.049$, Adjust. $R^2 = 0.77$), indicating that soil quality was the key factor of affecting eucalyptus growth in our study.

4. Discussion

4.1 Effect of FS and BF on plant growth

In our study, plant diameter was significantly decreased by FS in M3, the possible reason may be the main ingredient of FS applied in our trial was calcium carbonate, which immediately leads to the soil water retention capacity and soil pH changes while applied.

BF significantly increased plant growth, including plant diameter, height, and timber of volume since M6, which is in line with the finding of PGPR increases eucalyptus foliar chlorophyll, total nitrogen, and growth in Guangxi, China (Ren et al. 2020b). Co-application of PGPR and FS has been certified to have stronger effects on plant growth than FS in the long term because co-application of rhizobacteria and organic matters may improve soil enzyme activity, electrical conductivity, and nutrient availability (Han et al. 2019) which could be beneficial to plant growth and yield.

4.2 Effect of FS and BF on soil nutrient content

The response of soil nutrient content to seasons has been reported to be influenced by seasonal precipitation, temperature, atmospheric sedimentation, nitrogen deposition (Zhang et al. 2015). This pattern is supported by our findings of soil nutrient contents varied among treatments in different sampling time points.

Soil O_2 cycling capacity is mostly influenced by soil permeability, soil bulk density, and other soil physical properties, which in turn affecting soil microbial activity and nutrient input. When filtered sludge is

abundantly applied in our field, soil TN was significantly increased as a result of oxygen supplementary for soil microbial metabolic and growth.

Soil nitrobacteria take part in transferring ammonia into nitrous acid or transferring nitrous acid into nitric acid, which is related closely to soil available N transformation and accumulation. The optimum pH for soil *Nitrosomonas* and *Nitrobacter* abundance is 7.5–8.2. The pH of FS used in our trial was 6.10 (Ren et al. 2020b), and PGPR accumulation may contribute to soil acidification (Vitousek et al. 1997). These findings are consistent with the decrease in soil available N after the application of FS and BF in our study.

Strain DU07 applied in our trial has certified to belong to soil Firmicutes phylum (Ren et al. 2020b). Further, the positive correlation between soil TN and soil Firmicutes abundance has been found in the Loess Plateau soil of China (Liu et al. 2020). This is consistent with our result of increased soil TN in BF when compared with FS and the control in our study. Besides, PGPR may stimulate the synthesis of secondary metabolites that may increase the accumulation of potassium compounds in eucalyptus roots (Tinna et al. 2020). Han et al. (Ren et al. 2020a) also reported that major increases in soil potassium content occurred in PGPR treatment over uninoculated control. Consistent herewith, we observed that soil available K content increased with PGPR applied in the soil.

4.3 Effect of FS and BF on soil enzyme activity

Soil sucrase is the main hydrolase which takes part in decomposing soil organic matters (including cellulose, hemicellulose, lignin, etc.) into mineralized nitrogen. Further, soil C conversion status, soil respiration intensity, soil bacterial abundance were positively affected by PGPR applied in the artificial soil in China (Fu et al. 2018), which is in line with our finding that soil sucrase activity was increased upon bacterial fertilizer treatment. Soil urease activity has been reported to potentially reflect soil organic nitrogen supply and soil nutrient transformation (Han et al. 2017). (Pérez-Montañó et al. 2014) reported that PGPR applied may increase plant species diversity through benefits to plants from the host–PGPR interactions, and thus provide the hydrolysate (mainly microorganisms and litterfall) for urease in an agricultural system. This is consistent with our finding that soil urease activity was increased upon bacterial fertilizer treatment in our study.

4.4 Effect of FS and BF on soil quality

The significantly positive response of soil quality to BF application in M3, M6, and M12, whereas inverse response was observed in M9, demonstrating that application of bacterial fertilizer is beneficial to soil physical, chemical, and biological parameters in some specific time points. Soil physicochemical property takes part in the motivation of soil ecological metabolism process, and relates closely to both soil fertility and plant growth (Agegnehu et al. 2016), indicating that soil enzyme and nutrient might serve as essential soil quality indicators. This is consistent with the result of BF treatment decreased soil quality in M9, as indicated by potentially reduced nutrient contents (Avail K and Avail P) and enzymatic activities in M9. Besides, the relatively low average precipitation and low temperature in our research site in M9 may

lead to the repressed soil microbial activity and reproduction rate, and thus negatively affected soil quality.

Remarkably, BF mainly improves soil quality during our trial period except for M9, as a result of soil nutrient accumulation improvement and soil enzyme activities increases after PGPR applied. Soil quality has been reported to closely relate to soil microbial as the recycling and transformation of soil nutrient substance is mainly determined by the activity of microorganisms (Gouda et al. 2018). Han et al. (2019) reported that PGPR enhanced soil quality in a eucalyptus seedling through improving soil nutrient retention capacity and microbial activity rate. A study on PGPR has also reported that PGPR potentially increased soil bacterial and actinomyces amount while reduced soil fungal quantity, altering the soil type from fungal to bacterial, and thus improving soil fertility and quality in the lettuce in a greenhouse. Besides, *Acidobacteria* has been reported to be a predictor of soil health status and generally negatively correlated to soil quality, especially in relatively barren soil (Liu et al. 2020). PGPR potentially decreased soil *Acidobacteria* phylum abundance in a eucalyptus seedling in south China (Ren et al. 2020a). These findings are consistent with the increase in soil quality after the application of BF in our study.

4.5 Suggestion for using PGPR to improve plant yield

The positive responses of plant yield to soil urease and sucrase activities demonstrated that selected soil enzyme activities may increase plant growth in our study. The mechanism of PGPR promotes eucalyptus growth was mainly via nitrogen accumulation in eucalyptus root, stem, and foliar, including organic N and inorganic N. The increase in urease activity suggests a potential for an increase in gross N mineralization rates (Saiya-Cork et al. 2002), and thus improve the plant timber of volume. Gai et al. (Gai et al. 2015) reported that soil sucrase activity was closely related to soil microbial amount, soil microbial carbon use capacity, and soil respiration intensity, and soil sucrase has been certified to be a predictor of soil quality and fertility (Gr et al. 2005). Soil sucrase positively affected soil pH, electrical conductivity, nitrate-nitrogen content through accelerating hydrolysis soil carbohydrate into soluble nutrition, and further, supplied sufficient available nutrients for plant growth.

The negative responses of plant yield to soil CAT and nutrient contents demonstrated that soil CAT and nutrient contents may decrease plant growth in our trial. Soil CAT positively affects soil pH through decomposing soil humus content and thus inhibiting eucalyptus growth through changing soil physicochemical property. Soil nutrient content, including soil TN, available K, available P, and available N, negatively affected plant yield and growth, the main reason may be the plant nutrient content closely relates to plant function, structure and growth rhythm, and plant foliar morphosis consumes copious nutriment in the growing stage, leading to the decreases of soil nutrient contents in our study.

Soil microorganism activity contributes most to the cycling and supplementary of soil nutrient (especially N and P) as the essential soil decomposer, and potentially affect plant growth, plant-soil nutrient allocation, and plant tissue chemical compositions (e.g. foliar N). *Proteobacteria* has been reported to be a predictor of soil health status and generally negatively correlated to soil quality, especially in relatively barren soil (Liu et al. 2020), however, soil TN was certified to be positively affected by the activity of soil

Proteobacteria in a 3-month eucalyptus seedling plantation (Ren et al. 2020a). These findings are consistent with the negative correlation between soil TN and soil quality, and further soil TN negatively and significantly influenced plant yield in our trial.

5. Conclusions

Our study illustrates that the filtered sludge and plant growth promoting rhizobacteria amendments modify soil quality and plant growth in a one-year eucalyptus plantation. BF significantly increased plant diameter, height, and timber of volume in the long term. Besides, BF significantly increased specific soil enzyme activity and soil enzyme activity, contributing to the improvement of soil quality. The study also revealed that soil quality was significantly increased by BF during our trial period except M9. Results also indicate that a negative and significant relationship between soil TN and eucalyptus stem volume has occurred, and soil quality might serve as a sensitive indicator of plant growth. We encourage the co-application of FS and PGPR as bio-fertilizer, as it has the potential to reduce the heavy demand for artificial N fertilizer in eucalyptus plantations via improving soil quality.

Abbreviations

BF, bacterial fertilizer; FS, Filtered sludge; TN: total nitrogen; Avail K, Available potassium; Avail N, Available nitrogen; Avail P, Available phosphorus; CAT, Catalase; M3, Month 3; M6, Month 6; M9, Month 9; M12, Month 12.

Declarations

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Tables

Table 1
Scores of all PCAs and significances to determine the soil quality during our trial period. Significances were bold when $p < 0.05$. $p < 0.05$ and $p < 0.01$, significant different; $p < 0.001$, extreme significant different

Sampling time	Treatments	PC score	<i>p</i>
M3	Control	-0.42	
	FS	-0.52	$p < 0.001$
	BF	0.94	
M6	Control	-0.69	
	FS	-0.20	$p < 0.001$
	BF	0.89	
M9	Control	0.41	
	FS	0.46	$p < 0.01$
	BF	-0.87	
M12	Control	-0.91	
	FS	0.26	$p < 0.001$
	BF	1.17	

Table 2

Coefficients of pooled regression model for panel data to determine the relationship between eucalyptus stem volume and soil quality in our study. Significances were bold when $p < 0.05$. $p < 0.05$, significant different.

Soil quality parameter	Estimate	Std.error	Pr (> t)
(Intercept)	0.22	0.078	0.051
Protease	\	\	\
CAT	-0.26	0.15	0.17
Urease	0.012	0.01	0.31
Sucrase	0.0044	0.0027	0.17
TN	-0.054	0.018	0.04*
AK	-0.00081	0.00096	0.44
AN	-0.0012	0.004	0.79
AP	-0.034	0.2	0.87
$F = 6.15, p = 0.049, \text{Adjust. } R^2 = 0.77.$ Equation S2: Stem volume = $-0.26 \times \text{CAT} + 0.012 \times \text{Urease} + 0.0044 \times \text{Sucrase} - 0.054 \times \text{TN} - 0.00081 \times \text{AK} - 0.0012 \times \text{AN} - 0.034 \times \text{AP} + 0.22$			

Figures

Figure 1

Means and standard errors of eucalyptus growth measured at 4 time points; Different lowercase letters show the statistically significant differences at $\alpha=0.05$ level among BF, FS and the control.

Figure 2

Means and standard errors of soil nutrient contents during our trial period. Different lowercase letters showed significantly difference at $p < 0.05$.

Figure 3

Means and standard errors of soil enzyme activities during our trial period. Different lowercase letters showed significantly difference at $p < 0.05$. Protease was not determined 3 months after the treatment.

Figure 4

Soil quality PCA factor in M3 (A), M6 (B), M9 (C) and M12 (D) loadings for treatments and the control. In plot A, factor 1 accounted for 43.8% of the variance, factor 2 accounted for 24.5%; In plot B, factor 1 accounted for 46.4% of the variance, factor 2 accounted for 23.9%; In plot C, factor 1 accounted for 36.6% of the variance, factor 2 accounted for 27.9%; In plot D, factor 1 accounted for 49.8% of the variance, factor 2 accounted for 19.2%. Protease.: soil protease activity, CAT: soil catalase activity, Sucrase: soil sucrase activity, Urease: soil urease activity, TN: soil total nitrogen, AK: soil available potassium, AP: soil available phosphorus, AN: soil available nitrogen.