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Soil Bacterial Properties Linked to Pinus radiata Genotype

Summary

This pilot study was conducted to determine the optimum methodology for examining microbial contributions to site productivity across 26 radiata pine plantations. This will be followed by a much larger (and more expensive) study. As such, the results from this pilot study are mainly to direct research strategy and refine scientific techniques rather than to provide conclusive results or recommendations to industry.

Soil samples were collected from underneath trees randomly selected from within three radiata pine genotypes of interest. The effect of weed control was also included in the sampling regime. Analysis of the soil samples determined that the activity of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase differed significantly with radiata pine genotype, but only in the absence of weed control. ACC deaminase increases plant stress tolerance, and is therefore important to helping plants overcome nutrient and water deficiencies. In this study we determined that in the absence of weed control, the most successful radiata pine genotype was associated with soil microbes that had the greatest ACC deaminase activity, while the worst genotype was associated with soil microbes with the lowest ACC deaminase activity.

The ability of the soil bacterial community to break down different molecules was also examined, as this has previously been linked to substantial differences in site productivity. The ability of the bacterial community to consume different substances varied significantly with genotype, providing further evidence of the importance of the soil microbiota to above-ground productivity.

This pilot study successfully identified the techniques that will be used to examine the microbial contributions to site productivity across New Zealand. However, the identification of a strong link between radiata pine genotype, growth performance and soil microbes is also a significant new finding. This study has demonstrated that some genotypes can develop a microbial community that provide them with a greater tolerance to stress, allowing them to grow in conditions that cause other genotypes to perform poorly.

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Background

Soil microbes influence the productivity of terrestrial plants through various pathways^[7]. Of these pathways, the best understood is the capability of soil microbes to increase the supply of nitrogen, phosphorus and cations that would otherwise be unavailable to plants^[1,4,12]. Some soil microbes can also directly alter plant productivity through the expression and manipulation of phytohormones^[11], and these processes may be of greater interest.

For example, various soil bacteria produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. This enzyme regulates the production of ethylene, increasing stress tolerance and therefore productivity^[2,5]. Soil bacteria can also produce the phytohormone indole-3-acetic acid (IAA) which influences cell enlargement and division. Both of these processes have been demonstrated to enhance plant growth rates^[8,10,15].

A selection of processes by which soil microbes can influence plant productivity will be examined under

various climatic conditions and management practices across 26 locations around New Zealand^[13].

Prior to conducting a full scale research study, a pilot study was developed to test and optimise the techniques that will be used to analyse specific aspects of the soil microbial community. The location chosen for this pilot study also allowed the interactions between radiata pine genotypes and aspects of the soil microbial community to be examined. This technical note presents the results of this pilot study and comments on the results of interest.

Pilot Study Details

The site chosen for the pilot study was the Rolleston Long Term Site Productivity (LTSP) 3 trial. This trial contains 35 different radiata pine genotypes established in the presence and absence of chemical weed control treatments. The trial was installed in 2005.





From the 35 different genotypes, three were chosen based on their representative growth behaviour: P31C7, 1637 and P26C2. These genotypes were identified as a poor, average and good performers at the Rolleston site based on mean diameter at breast height (DBH) and height measurements, as shown in Tables 1 and 2.

Table 1: Average DBH for three radiata pine genotypeswith or without weed control at the Rolleston trial site.

Genotype	Plus weed control (mm)	No weed control (mm)
P31C7	49.5	21.5
1637	57.3	45.5
P26C2	80.2	54.1

Table 2: Average height for three radiata pinegenotypes with or without weed control at theRolleston trial site.

Genotype	Plus weed control (cm)	No weed control (cm)	
P31C7	317	201	
1637	355	303	
P26C2	421	355	

Soil was collected from beneath 12 randomly selected trees from each genotype; six from within the weed control plots and six from within the plots that did not receive weed control. The activity of ACC deaminase and the concentration of IAA in the soil samples were measured^[14]. Community level physiological profiles (CLPP) of the bacterial species were constructed by assessing the range of substances consumed by the bacteria^[3,9].

Pilot Study Results

The activity of ACC deaminase was measured by the production of α -ketobutyrate; more production indicates greater enzyme activity. Activity in the soil samples was found to fall within the range of expected values^[6]. The values differed significantly with tree genotype in the plots that did not receive weed control, but no statistical differences were observed in the soil samples collected from the plots that did receive weed control (Table 3).

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Table 3: Differences in ACC deaminase activity withradiata genotype and weed control.

Genotype	Plus weed control (μg ketobutyrate)	No weed control (μg ketobutyrate)
P31C7	70 ± 5 a	49 ± 3 a
1637	66 ± 6 a	61 ± 6 ab
P26C2	57 ± 5 a	77 ± 6 b

Letters indicate statistically significant differences at α < 0.01.

The accuracy of the methods used to extract and quantify IAA concentrations in the soil samples will be confirmed by work to be carried out at the University of Calgary. To date, the preliminary results suggest that IAA concentrations do vary with radiata pine genotype, but additional analysis using a new technique is being conducted to confirm this result.

Analysis of the CLPP data identified several significant differences in the consumption of different substances by the bacterial community. Threequarters of the differences in carbohydrate utilisation between the bacterial communities was explained by the presence or absence of weed control and genotype (P = 0.016).

Discussion

With regards to developing a protocol for the large scale trial, the results generated from this pilot study confirm the suitability of the selected ACC deaminase and CLPP methodology. Although the IAA data are yet to be verified by additional testing, the preliminary results suggest that the techniques used to identify and quantify this phytohormone will be appropriate. These microbial methods will be added to the suite of physical (e.g. tree DBH, height and form) and chemical (e.g. soil and foliar nitrogen concentrations) assessments that will be carried out at the 26 sites.

The results of this pilot study have also raised an important issue regarding the relationship between radiata pine genotypes and soil bacterial activity. It appears that the different genotypes alter the soil conditions sufficiently to encourage the development of different bacterial species. This difference in bacterial community structure and function may then cause the tree to behave differently in response to stimuli such as nutrient deficiencies and water stress, based on the feedback between the tree and soil bacterial community.





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Hypothetically, in the plots without weed control, it is possible that the increased levels of ACC deaminase activity (and therefore stress tolerance) associated with the P26C2 genotype contributed to the greater growth rates of this genotype. The presence of weeds increased competition for moisture and nutrients, creating a more stressful environment where ACC deaminase can provide growth benefits (Fig. 1). The performance of the genotypes was also related to ACC deaminase activity in the soil (Fig. 2), although this analysis is of limited power as only three genotypes were compared.

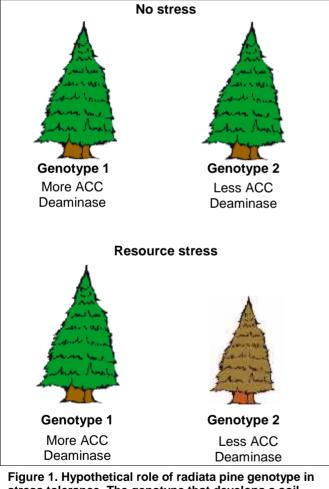


Figure 1. Hypothetical role of radiata pine genotype in stress tolerance. The genotype that develops a soil bacterial community capable of making more ACC deaminase does no better in good conditions, but performs better in stressful conditions.

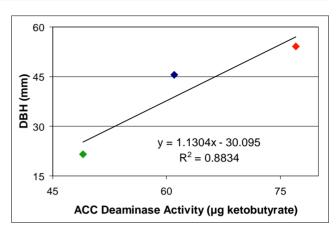


Figure 2. Relationship between DBH and ACC deaminase activity in the plots that did not receive weed control. Red = P26C2; blue = 1637; green = P31C7.

However, as P26C2 outperformed P31C7 in all plots regardless of weed control status, we must assume that the differences between the tree genotypes influence other facets of growth behaviour. Consequently, the degree of increased productivity that is directly caused by increased ACC deaminase activity cannot be determined at this time, but it is highly likely that ACC deaminase does contribute to productivity. The potential for radiata pine genotypes to influence the functions of the soil bacterial community significantly is supported by the CLPP results, which clearly showed differences in activity with genotype, potentially affecting nutrient supply to the tree.

There is substantial value in further researching the effect of radiata pine genotypes on soil bacterial activity. Given the sensitivity of fungal populations to environmental stimuli, this is also probably true for mycorrhizal species as well.

The research programme that will follow this pilot study does not include further assessments of variation in soil microbial community function with tree genotype. If a project can be initiated, the LTSP 3 trials provide an excellent pre-existing framework to integrate radiata pine genotypes into our understanding of the feedback between soil microbes and plantation management.





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References

- Balogh-Brunstad, Z., Keller, C.K., Gill, R.A., Bormann, B.T., and Li, C.Y., *The effect of bacteria and fungi on chemical weathering and chemical denudation fluxes in pine growth experiments.* Biogeochemistry, **88**, pp. 153-167. (2008).
- 2. Davies, P.J., *The plant hormones: their nature, occurrence and functions.* In P.J. Davies (Ed.), Plant hormones and their role in plant growth and development. Martinus Nijhoff Publishers, Dordrecht, The Netherlands, pp. 1-11. (1987).
- 3. Epelde, L., Becerril, J.M., Hernández-Allica, J., Barrutia, O. and Garbisu, C., *Functional diversity as indicator of the recovery of soil health derived from* Thlaspi caerulescens *growth and metal phytoextraction.* Applied Soil Ecology, **39**, pp. 299-310. (2008).
- 4. Gaskins, M.H., Albrecht, S.L., and Hubbell, D.H., *Rhizosphere bacteria and their use to increase plant productivity: A review.* Agriculture, Ecosystems and Environment, **12**, pp. 99-116. (1985).
- 5. Glick, B.R., Penrose, D.M. and Jiping, L., *A* model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. Journal of Theoretical Biology, **190**, pp. 63-68. (1998).
- Honma, M. and Shimomura, T., *Metabolism of* 1-aminocyclopropane-1-carboxylic acid. Agricultural Biology and Chemistry, 42, pp. 1825-1831. (1978).
- Kennedy, A.C., and Gewin, V.L., Soil microbial diversity: present and future considerations. Soil Science, 167, pp. 607-617. (1997).
- 8. Mayak, S., Tirsoh, T. and Glick, B.R., *Plant growth-promoting bacteria that confers resistance to water stress in tomatoes and peppers.* Plant Science, **166**, pp. 525-530. (2004).
- 9. Mijangos, I., Becerril, J.M., Albizu, I, Epelde, L., and Garbisu, C., *Effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions by cultivationdependent and –independent methodologies*

Soil Biology and Biochemistry, **41**, pp. 505-513. (2009).

- Patten, C.L. and Glick, B.R., Role of Pseudomonas putida indoleacetic acid in development of the host plant root system. Applied and Environmental Microbiology 68, pp. 3795-3801. (2002).
- 11. Ping, L., and Boland, W., Signals from the underground: bacterial volatiles promote growth in Arabidopsis. Trends in Plant Science, **9**, pp. 263-266. (2004).
- Sashidhar, B., and Podile, A.R., Mineral phosphate solubilization by rhizosphere bacteria and scope for manipulation of the direct oxidation pathway involving glucose dehydrogenase. Journal of Applied Microbiology, **109**, pp. 1-12. (2010).
- Smaill, S., *Examination of microbial contribution* to site productivity. Radiata Management Technical Note RTN-018. (2010a).
- 14. Smaill S.J., Leckie, A.C., Clinton, P.W. and Hickson, A.C., *Plantation management induces long-term alterations to bacterial phytohormone production and activity in bulk soil*. Applied Soil Ecology, **45**, pp. 310-314. (2010b)
- 15. van Loon, L.C., *Plant responses to plant growthpromoting rhizobacteria*. European Journal of Plant Pathology, **119**, pp. 243-254. (2007).