Bioprotection - enhancing growth and health Research Programme

LINCOLN UNIVERSITY
TE WHARE WĀNAKA O AORAKI

Can *Trichoderma* increase nutrient uptake in planted radiata?

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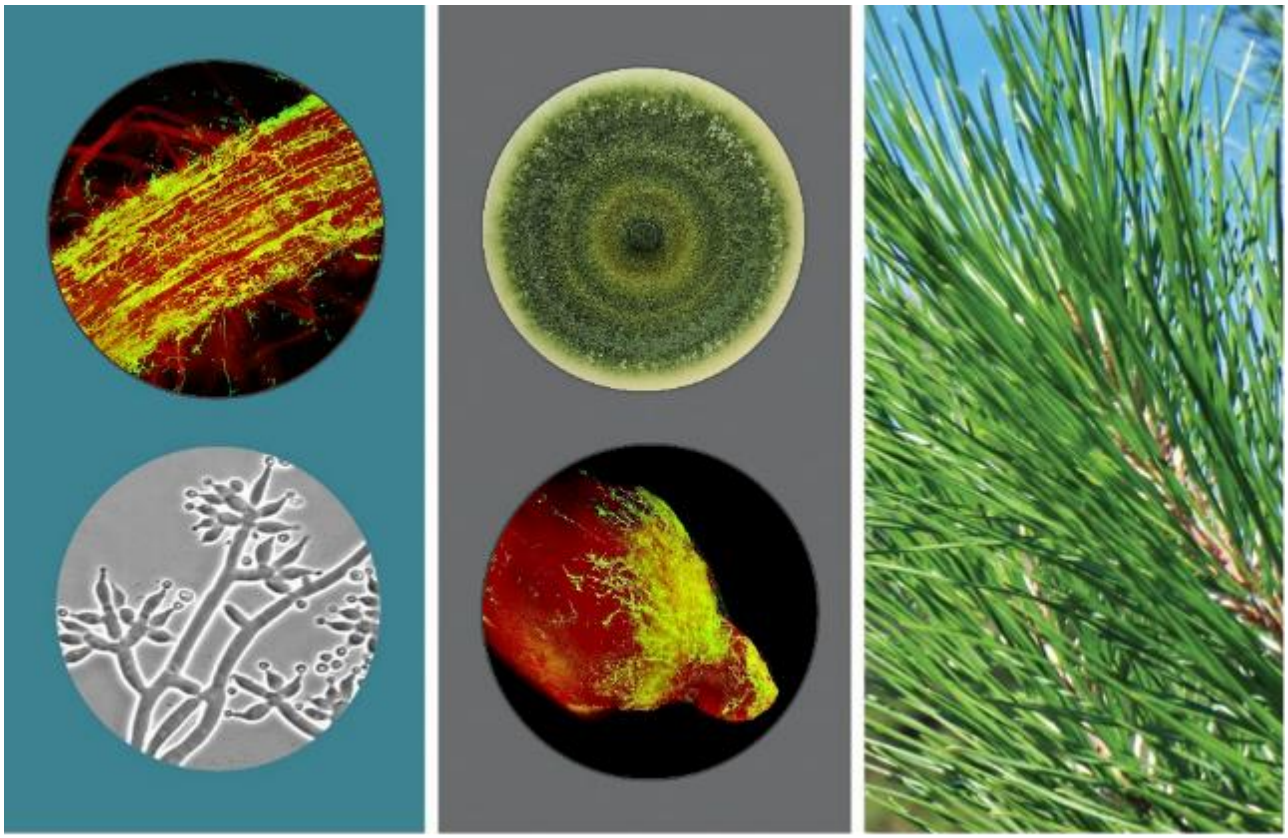

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EXECUTIVE SUMMARY

Forestry in New Zealand is generally situated in areas with poor quality and low fertility soils. Silviculture technologies that can increase growth rates by improving the uptake of important nutrients, are beneficial for timber production irrespective of the intensity of management operation. The objective of this study was to determine if previously observed growth benefits in young trees inoculated with *Trichoderma* influenced the uptake of soil nutrients into the foliage.

This research programme has shown that nursery inoculation with *Trichoderma* PR6 and PR3a mixtures is an important management tool for radiata pine tree cultivation. *Trichoderma* treatments significantly ($P<0.05$) increased year four productivity (stem volume per hectare) by up to 40.2% across eight sites. Stand productivity followed an optimum response relationship, where *Trichoderma* treatments may have contributed to gains within different abiotic stress environments and tree growth potentials.

Inoculation with *Trichoderma* resulted in volume gains of:

- -5.0 to 19.9% in the low growth potential stands (four each in Nelson and Bay of Plenty, ranging from 0.65 to 8.2$m^{3}.ha^{-1}$)
- 25.1 to 40.2% in the medium growth potential stands (four in Northland, ranging from 11.2 to 18.7 $m^{3}.ha^{-1}$) and
- 0.9 to 14.6% in the high growth potential stands (four in Gisborne, ranging from 29.9 to 34.6 $m^{3}.ha^{-1}$).

The response in the low growth stands may have been restricted due to environmental limitations, whereas in the medium growth sites, some nutrient limitations may have been overcome. The high growth sites typically reflect adequate nutrition with improved environmental conditions. The response of *Trichoderma* inoculated trees to existing soil nutrition could be further investigated by measuring needle nutrients in the five additional trials of this research programme. These unique trials were established in colder or drier parts of the South Island and represent different soil types to those measured for this report.

Status of macro and micronutrients was determined by analysis of foliar and soil samples in seven trials. *Trichoderma* treatments did not significantly ($P<0.05$) improve nutrient uptake; however, the samples were collected in winter and so potentially reflect optimal soil moisture, nutrient availability, and low foliage nutrient demand conditions. However, the efficiency of phosphorus uptake was increased by 5.6 and 10.4% for PR3a and PR6 on average across sites respectively. Future sampling at the more critical and typical time of February or March when trees are under high nutrient stress, may provide greater treatment contrasts.

Vector analysis techniques were used to interpret the important influences of tree volume (surrogate of biomass), foliar nutrient concentration, and the predicted nutrient content of individual nutrients across sites and treatments. Responses showed that sites with volume gains associated with *Trichoderma* inoculation absorbed larger amounts of nutrients than uninoculated trees, and trends suggest a current accumulation phase or that individual nutrients were previously limited. Part of this response may also have been that growth increased needle biomass to the extent that nutrients were diluted relative to that in the control trees. The technique was also useful in predicting sufficient or deficient levels and luxury and excessive uptake of nutrients into needles.

These data show complex relationships between inoculated *Trichoderma* species and radiata pine trees grown in New Zealand's soil and climatic conditions. The best responses were found across mid to low growth sites and this may help to target *Trichoderma* applications better in the future.

INTRODUCTION

Plantation forests in New Zealand are typically established on low fertility or steep terrain land that is less suitable for agriculture. Soils are generally young and naturally acidic with low levels of nitrogen and phosphorus (Parfitt et al. 2008). Silviculture management practices that conserve accumulated nutrients will enable the uptake of faster and greater nutrient supplies in the following rotation and this will become more important as increased carrying capacity and productivity is a key goal of the forest industry. New Zealand's planted forest soils are now supporting rotations at higher stocking with genotypes that grow faster and have greater nutrient demands. In extensive or low-cost, and intensive management systems, priority should be given to silviculture techniques that facilitate more efficient nutrient uptake and are cost-effective and practical to use.

Trichoderma spp. are endophytic plant symbionts that have been used successfully in this research programme to enhance young tree growth and manage foliar diseases in radiata pine. Practical techniques for the efficient application of these fungal spores to seeds or cuttings in the nursery have been developed. In eight trials, PR6 and PR3a *Trichoderma* mixtures were found to increase year four height and DBH by a mean of 6.8 to 7.7% compared to untreated trees, with the estimated stem volume per hectare increased by 13.0 and 14.6% (Whelan 2023). These mixtures were also useful at reducing Dothistroma needle blight severity by a mean of 38% (PR6) and 46% (PR3a) in six trials where the disease was present (Whelan 2023) and increasing uniformity in tree plot volume by approximately 10% (Whelan 2022).

The mechanisms for these responses are likely to be numerous and may include mycoparasitism, antibiosis by production of secondary metabolites, competition for space and nutrients, plant defensive activation and stimulation of plant growth. Root colonisation by *Trichoderma* spp. may also enhance root growth and development and aid in the ability of the tree to exploit available nutrients over a greater soil volume than occupied by the tree root system alone (Li et al. 2017, Khan et al. 2020). Improved uptake of soil nutrients by *Trichoderma* inoculants has been demonstrated in many crops (Howell et al. 2000, Mbarki et al. 2016, Li et al. 2015) but little work has been done in forestry species. The objective of this study was to determine if the growth benefits of radiata pine trees inoculated with nursery-applied *Trichoderma* influenced the uptake of soil nutrients into tree foliage.

METHODS

The effect of *Trichoderma* treatments on tree nutrient status was determined by analysis of foliar and soil samples taken across seven radiata pine plantation trials (establishment details in Whelan 2019 and 2021). In brief, six of the trials were established in 2018 in four important forestry regions (Patunamu in Gisborne, Whatoro and Topuni in Northland, Kaingaroa 209_4 and 660_2 in Bay of Plenty and Berrymans in Nelson), and one was established in Nelson in 2020 (Kings Ridge). Experimental design consisted of randomised complete blocks with 7 to 10 replications within each site. Two *Trichoderma* treatments (PR6 and PR3a mixtures) were applied as part of a seed coat recipe to a single radiata pine seed-lot and the seedlings were raised under standard containerised tray management practices in a commercial nursery. Treated and untreated (control) seedlings were then out-planted in commercial plantation forests that coincidentally had different site preparation methods and tree stocking rates (ranging from 800 to 1190 stems per hectare). Herbicide management was also variable in the first two years of growth, but no insecticides, fungicides or fertilisers were applied. Plots contained 81 trees in a 9 x 9 grid pattern. Tree height, diameter at breast height (DBH at 1.4m) and foliar disease (percentage incidence and severity; method according to Whelan 2019) were measured in the central 25 plants (5 x 5) four years after trial establishment.

Soil and foliar samples were taken on 29 and 30 May (Berrymans and Kings Ridge), 4 and 7 July (Topuni and Whatoro), 16 August (Kaingaroa 660_2 and 209_4) and 8 September 2022 (Patunamu). Because of Covid-19 travel restrictions, the sampling dates were delayed from the typical sampling time of February or March (autumn) when trees have high growth demands for nutrients, are exposed to environmental stress and foliage nutrient concentrations are relatively stable (Knight, 1978, Mead and Will, 1976) to winter 2022. Two additional trials (Sherry in Nelson and Tauwhareparae in Gisborne) in the 2018 series were not sampled due to resource constraints.

In all trials, treatments were nursery-applied PR6 and PR3a *Trichoderma* mixtures or an untreated control. Plots for sampling were further prioritised due to resource funding restrictions. Plots (replicates within treatments) with the highest and lowest mean tree height were removed from nutrient selection and four plots were randomly selected from the remaining set of plots.

Foliage samples consisted of current season fully-grown needles (approximately 10 fascicles per tree) exposed to full sunlight on the youngest second-order branches in the top third of the crown (method according to Will, 1985). Samples were collected from 20 to 25 unstressed trees in the centre of each plot. Hands were covered with nitrile laboratory gloves and gloves were replaced when sampling each plot. Needles were bulked for each plot, placed in a clean paper bag, and sent to the Veritec Laboratory in Rotorua (https://www.scionresearch.com/services/laboratory-services) within two days of collection. At all stages after harvest, the needles were kept chilled. Needles were dried at 70°C for at least three days and ground to pass through a 1.0mm screen before analysis of C and N using a modified high temperature Dumas combustion method with a LECO Trumac CNS instrument (Rayment and Lyons, 2011). In addition, samples were digested with a mixture of nitric acid and hydrogen peroxide and analysed by inductively coupled plasma mass spectrometry for aluminium (Al), arsenic (As), barium (Ba), boron (B), cadmium (Cd), calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), potassium (K), lead (Pb), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), phosphorus (P), selenium (Se), silver (Ag), strontium (Sr), vanadium (V) and zinc (Zn) using the Environmental Protection Agency Method 3052, SW-846, 1996. Due to the low levels of some micro-nutrients (Ag, As, Cd, Cr, Mo, Pb, Se and V), concentrations were below detection limits across some sites and therefore removed from the analysis.

Soil samples were collected from the same plots that were sampled for foliage concentrations. The overlying soil organic layer was removed at each sample point and mineral soil was collected using a 17mm diameter Hoffer tube from 0 to 10 cm depth. Sampling points were within 500mm of the tree trunk, and approximately 20 samples were collected in each plot. Soil from the same treatment was bulked together, mixed, and approximately 500g was subsampled, placed in a clean plastic bag and sent to Veritec Laboratory for analysis. Soil samples were air-dried and ground to pass through a 2.0-mm screen prior to

analysis. Samples were then analysed for chemical properties of pH (using the method of Rayment and Lyons, 2011) and total carbon (C) and nitrogen (N) using a modified high temperature Dumas combustion method with a LECO Trumac CNS instrument (Rayment and Lyons, 2011). In addition, aluminium (Al), boron (B), calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), potassium (K), sodium (Na) and zinc (Zn), were measured using the Mehlich 3 extraction method and inductively coupled plasma mass spectrometry (Mehlich, 1984). All soil and foliage results are expressed on an oven dry (104°C) basis and reported as percentage or parts per million (ppm).

Soil types were estimated using the New Zealand Manaaki Whenua – Landcare Research soil digital mapping facility (https://soils-maps.landcareresearch.co.nz) but no soil profile pits were dug to confirm soil types.

The nutritional interpretation was determined by two techniques, primarily with known deficiency and adequate nutrient concentration levels (Table 1) and secondly, Vector analysis, to show an alternative interpretation in trees of different sizes.

Table 1: Foliar nutrient concentrations (%) for radiata pine needles in New Zealand or Australian forests and their interpretation relative to adequate and deficiency levels according to Davis et al. (2015).

Interpretation	Nutrient concentration									
	%					ppm				
	N	K	Ca	P	Mg	Mn	Zn	Fe [b]	B	Cu
Deficient (<)	1.2	0.3	0.1	0.1	0.06	10	10	35	8	2
Adequate (>)	1.45	0.5	0.1	0.13	0.1	20	20	70	12	4

[b] sampled in late May to late June in Australian forests (Boardman et al. 1997); the rest sampled in mid-February to end-March in New Zealand forests.

Vector analysis is a bivariate model to simultaneously depict changes in plant yield and nutritional response in a single diagram, facilitating the identification of nutritional status such as growth dilution, deficiency, sufficiency, luxury uptake, and toxicity (Isaac and Kimaro 2011). In vector analysis, nutrient concentration is plotted as a function of plant biomass and nutrient content, where nutrient content (x), nutrient concentration (y), and biomass (z) satisfy the function: x = *f* (y,z). Responses are expressed relative to the control (that is normalised to 100) to facilitate comparisons between various treatments and nutrients. This approach eliminates differences associated with plant size and initial nutrient status (Imo and Timmer 1997, Isaac and Kimaro 2011). Relative values, instead of absolute values, in association with the normalised controls, allow for comparisons between multiple nutrients and sites (Haase and Rose 1995). In this study, the controls were the untreated trees at each site and provided the baseline nutrient concentration and growth values. All sites and treatment effects were plotted on a single diagram for comparison.

Needle dry mass is often used for vector analysis because it is often highly correlated with long-term stem wood responses and provides an easily measured and rapid estimate of future tree growth (Haase and Rose 1995). In this study, year four under-bark stem volume per hectare (formula 1) in six trials, and year two tree height in the Kings Ridge trial were selected as suitable proxies for needle mass because these variables also represent long-term stand stem-wood and the plot data was available from previous measurements of height and trunk diameter at breast height.

The formula for under-bark stem volume was:

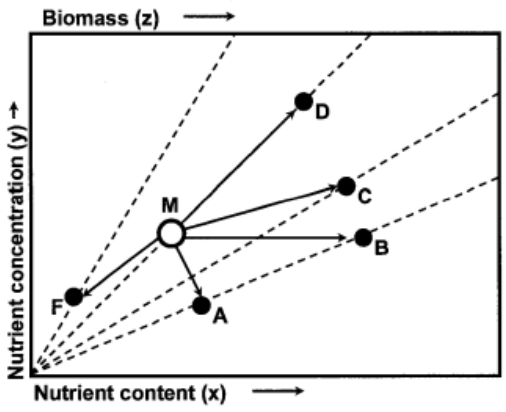
$$v=h*ba*(B1*(h-1.4)**(-B2)+ B3 \quad (1)$$

where h = height of an individual tree (m) in measurement plot, ba = basal area of an individual tree in measurement plot ($ba = 7.85\times10^{-5}\times DBH^2$, m^2) and f = individual-tree breast height volume form factors (derived from $f = v/(ba\times h, m^3$; B1=0.86, B2=0.972 and B3=0.304; Kimberley and Beets 2007). Under-bark stem volume per hectare = v*tree stocking rate (trees per hectare) as found in winter 2022.

Also, the ratio of foliage concentration (ppm) divided by the soil concentration (ppm) for individual nutrients (B, Ca, Co, Cu, K, Mg, Mn, N, Na, P and Zn) was calculated. A mixed-model analysis of variance (ANOVA) was used in R version 4.2.0 (2022-04-22 ucrt) with the lme package to explore differences in the uptake ratio of the treatments (fixed effect), nutrients (fixed) and their interactions (fixed) across sites (random effect). Quadratic regression analysis of the relationship between stem volume ($m^3.ha^{-1}$) and gain in volume to *Trichoderma* treatments relative to the control at each site was performed.

At each site, two summation climatic variables, mean annual air temperature (°C) and rainfall (mm), were taken from nearby climate stations (sourced from the National Institute of Water and Atmospheric Research website (https://cliflo.niwa.co.nz/)). The variables were normalised to the same scale using the statistic Z Score method (where the number of standard deviations by which a data point is above or below the mean value of the dataset is calculated) and an additive interaction was performed. Linear regression analysis of the relationship between this climate variable interaction and gain in volume to *Trichoderma* treatments relative to the control at each site was performed. A similar analysis was also done with site altitude. The significance of relationships was determined with an F-test at the 0.01, 0.1, 1 and 5% level.

The nutritional diagnosis in Vector analysis was determined from the direction and magnitude of each vector based on the interpretation diagram of Imo and Timmer 1997 (Figure 1).



Vector	z	y	x	Interpretation	Possible diagnostic
A	+	-	+	Dilution	•Growth dilution
B	+	0	+	Sufficiency	•Steady-state
C	+	+	+	Deficiency	•Limiting
D	0	+	+	Luxury consumption	•Accumulation
F	-	-	-	Excess	•Antagonistic

Figure 1: Diagram of relative response in biomass (z), nutrient content (x) and nutrient concentration (y). The reference condition (M) is normalised to 100. Diagnosis (A to F) is based on shifts (increase [+], decrease [-] or no change [0]) in biomass, nutrient concentration and nutrient content response to treatment effect (modified from Imo and Timmer 1997). Vector interpretation and possible diagnosis are given in the associated table.

RESULTS AND DISCUSSION

Gain in productivity from *Trichoderma*

The *Trichoderma* treatments significantly (P<0.05) increased productivity (estimated using stem volume x tree stocking in winter 2022) by up to 40.2% in the eight trials established in 2018 (Whelan 2023). Gains in productivity followed an optimum response relationship (Figure 2a) where the *Trichoderma* treatments contributed to gains at different levels of growth potential. The gains in volume to *Trichoderma* were between -5 to 19.9% in the lowest productivity trials (ranging from 0.65 to 8.2$m^3.ha^{-1}$ at Berrymans, Sherry and Kaingaroa 209_4 and 660_2), and between 0.9 to 14.6% in the highest productivity trials (ranging from 29.9 to 34.6$m^3.ha^{-1}$ at Tauwhareparae and Patunamu). In comparison, the trials with medium productivity (ranging from 11.2 to 18.7$m^3.ha^{-1}$ at Topuni and Whatoro) had the greatest gains of between 25.1 to 40.2%. The tree productivity response to *Trichoderma* at each site may have been dependent on factors including the interaction between environmental conditions as well as the nutritional status of the soil.

Recently in New Zealand, a 300 Index model for radiata productivity of sites showed that productivity is strongly regulated by available soil water, air temperature and site fertility (Watt et al. 2021). The effect of climate variables on the productivity of *Trichoderma* inoculated trees was determined using two summary climate variables, annual air temperature and annual rainfall (being a surrogate for available soil water because few sites had these data measured). Similarly to Watt et al. (2021), the gain in productivity from *Trichoderma* was found to be significantly (P<0.05) higher in sites with warmer air temperatures and more rainfall (Figure 2b) and was also inversely correlated to the altitude of the site (Figure 2c).

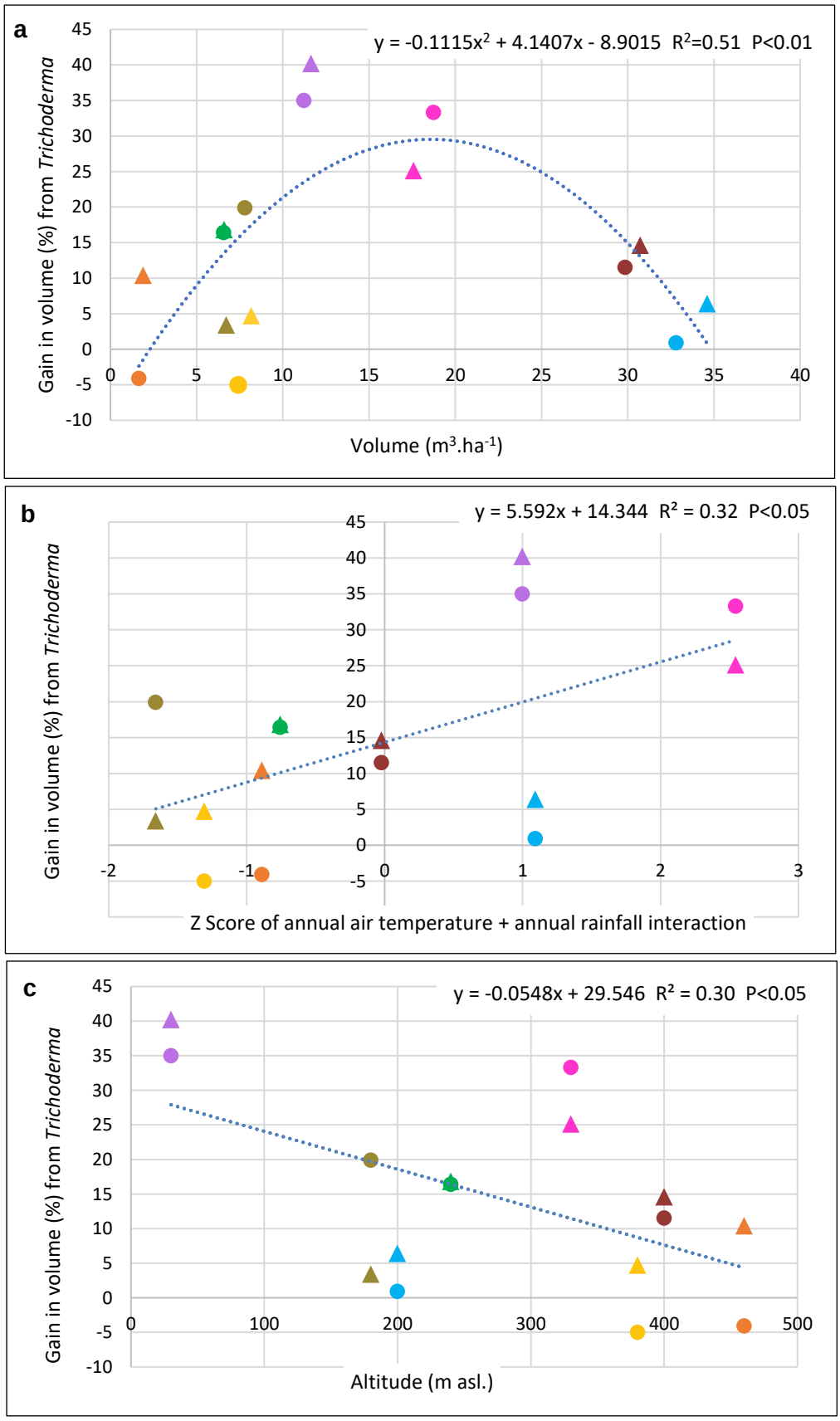


Figure 2: The relationship between a) 2022 stem volume ($m^3.ha^{-1}$), b) Z Score of annual air temperature and annual rainfall (data obtained from Table 3) or c) altitude (m asl.), and percentage gain in volume in the *Trichoderma* treatments (PR6 circle, PR3a triangle) compared to the untreated controls in the eight trials.

Available soil nutrients

The wide spread of the soil nutrient concentrations across sites shows the broad range in site types explored (Table 2). Some sites had especially high concentrations of Mn, N, P and Zn and were therefore 'a-typical' compared with the other sites (Figure 3). No significant (P<0.05) difference in soil nutrient concentrations was found in any treatment plots.

Table 2: Mean total C and N, pH and available soil nutrients across sites.

Region	Site	Soil Parameters														
		Total C (%)	Total N (%)	pH	Mehlich 3 Extraction (ppm)											
					K	Ca	P	Mg	Al	Mn	Na	Zn	Fe	B	Cu	Co
Gisborne	Patunamu	4.8	0.29	5.7	198	1024	15.3	186	1924	26	24	12.5	96	0.30	0.37	0.07
Northland	Topuni	3.5	0.15	5.0	149	1076	4.6	431	1354	41	79	2.2	307	0.19	0.88	0.11
	Whatoro	8.4	0.53	4.9	132	452	3.9	113	1676	6	41	0.9	175	0.31	2.00	0.02
Bay of Plenty	Kaingaroa 660_2	4.0	0.19	5.3	83	49	4.3	10	2186	4	19	0.8	137	0.14	2.59	0.04
	Kaingaroa 209_4	1.9	0.12	5.8	270	115	7.2	16	2032	5	43	1.8	70	0.18	0.85	0.01
Nelson	Berrymans	2.9	0.13	5.3	98	365	3.9	72	1493	9	10	1.5	243	0.23	0.38	0.07
	Kings Ridge	3.5	0.17	5.7	198	1905	46.2	318	981	16	7	2.7	216	0.18	0.63	0.14

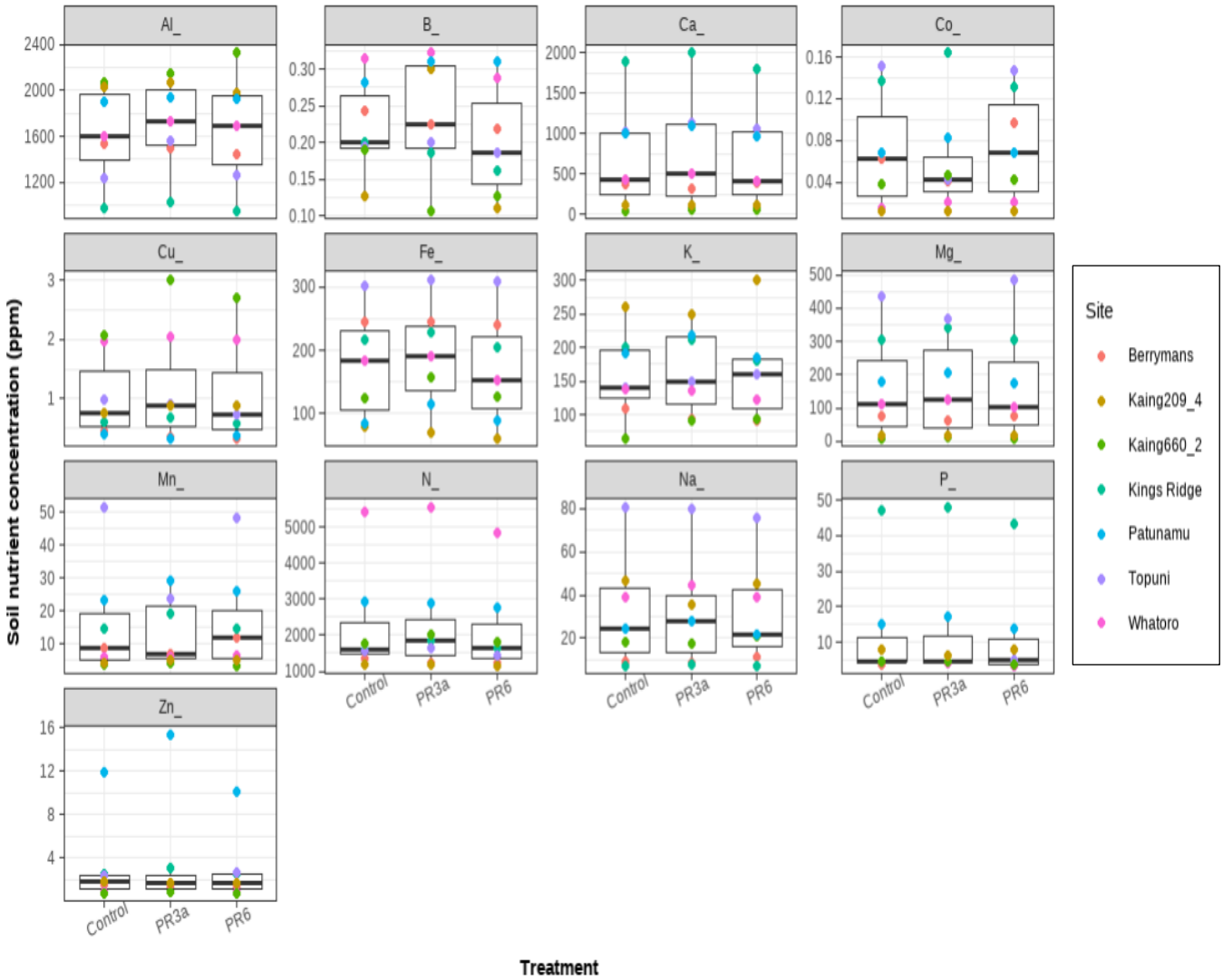


Figure 3: Boxplots of individual available soil nutrients across sites (indicated by coloured dots), split by treatment of Control, PR3a and PR6**.** Horizontal lines represent the lower 25%, upper 75% quartile and median (dark line). Whisker lines represent scores outside the middle 50% with outliers above or below the whisker lines**.**

Site characteristics and impact on available soil and foliar nutrients

a) High productivity site:

The most productive site was the Gisborne Patunamu trial with a mean 4-year volume of 33.3$m^3.ha^{-1}$ across all treatments. The gains to *Trichoderma* treatments were small (PR6 0.9% and PR3a 6.4%) possibly because the trees were near the expected maximum growth potential for the region (35-40$m^3.ha^{-1}.yr^{-1}$; Palmer et al. 2010). At Patunamu, the soil reserves (Table 2) and foliar nutrient levels (Table 4) were at sufficient levels for strong tree growth (interpretations according to Davis et al. 2015, Table 1) and an optimal balance of most essential foliar nutrients was present. For example, the soil carbon: nitrogen ratio (17) suggested that carbon in the organic matter did not greatly restrict the availability of nitrogen, and Total N (0.29%) and P (Mehlich-3 extraction method; 15.3ppm) were balanced with an N/P ratio of 10 (Davis et al. 2015). The site also had few climatic limitations to growth, compared to some other sites, with warm temperatures, sufficient spring, summer and autumn rainfall (Table 3), and low weed pressure. The foliage samples at Patunamu indicated that only one nutrient, Cu (2.5ppm) was below adequate levels for growth (4ppm; Table 4). This may have been due to the high natural zinc levels in these orthic pumice soils (Table 2). However, no long-term deficiency symptoms (ie. twisting of the branches and main stem, Davis et al. 2015) were observed in the September 2022 sampling.

The inability of *Trichoderma* to strongly enhance growth in sites with low, or the absence of abiotic stress, due to their services not being required by the plant, has been observed in other studies (Maherali 2014, Huey et al. 2020). The fungi can create an environment in which they do not germinate or grow but when plant roots release sugars and hormones and form an association with mycorrhizal spores, the spores can reactivate and commence ecological services (Huey et al. 2020). This ongoing relationship between the plant and endomycorrhizal fungi may be important as soil and environment conditions flux both seasonally and long-term, particularly with the potential impact of future climate change (Bauer et al. 2020).

Table 3: Seasonal site-level variation in climate.

Climate Parameter	Period	Region and Site [a]							
		Gisborne		Northland		Bay of Plenty		Nelson	
		Patunamu	Tauwhare-parae	Whatoro	Topuni	Kaing 209_4	Kaing 660_2	Berry-mans	Sherry
Mean air temperature (°C)	Dec to February	16.1 [d]	18.0 [c]	17.3 [d]	18.0 [b]	15.8 [c]	14.1 [c]	15.7 [c]	16.6 [d]
	March to May	12.3	14.0	15.1	15.5	11.0	9.9	11.3	12.0
	June to August	7.0	9.0	10.7	10.5	5.9	4.8	5.7	6.4
	September to November	10.8	13.0	12.8	13.6	10.5	8.7	10.7	11.5
	Annual	11.5	13.5	14.0	14.4	10.8	9.4	10.9	11.6
Mean monthly rainfall (mm)	Dec to February	129 [d]	58 [d]	109 [d]	99 [b]	108 [d]	128 [c]	97 [b]	81 [d]
	March to May	165	117	140	105	82	127	119	86
	June to August	166	121	221	130	101	149	116	100
	September to November	142	92	130	99	92	140	117	105
	Annual Total	1804	1163	1800	1301	1148	1631	1347	1115

[a] meteorological stations were within 8km of each trial site except for Tauwhareparae and Topuni that was 15km apart. Data for periods 1951-1980 ([b]), 1971-2000 ([c]) and 1981-2010 ([d]) according to National Institute of Water and Atmospheric Research (https://cliflo.niwa.co.nz/).

b) Medium productivity sites:

The Northland sites consisted of strongly weathered Ultic clay (Topuni) and Granular (Whatoro) soils with significant chemical and physical limitations that can severely restrict root and stem growth (Ross et al. 2009). The soils were the most acidic in the trial series (pH of 4.9 and 5, Table 2) and this may have contributed to low soil availability of P (3.9 or 4.6ppm) due to P being fixed by the soil clay components. In Topuni, the luxurious uptake of N (possibly due to large plant numbers of the legume gorse) may have further reduced the availability of P and led to a potential deficiency (Davis et al. 2015, Li et al. 2017).

The low soil pH (Table 2) and high rainfall (Table 3) also increased the solubility of soil Fe, Cu and Co in both trials, and Zn and Mn in Topuni, increasing the availability of these elements for uptake by the trees (Adams and Walker 1973). Further limiting factors in both trials may have been the excessive soil Fe that led to low foliar Cu levels. Topuni had a very high level of weeds (mainly gorse and pampas grass - *Cortaderia selloana*) that had developed since trial establishment and this may have caused plant stress. Root development may also have been restricted in control plots due to clayey subsoils with poor permeability. This was the only site that received remediation (ripping) to improve water drainage.

The Whatoro and Topuni trials were the most responsive (25.1 and 40.2% increase, Figure 2) to *Trichoderma*, suggesting that these treatments substantially contributed to the trees overcoming the poor-quality and low-fertile nature of the soils. *Trichoderma* spp are known to generate a symbiotic relationship with host plants in soil with low nutrients, including P (Huey et al. 2020) and to enhance tolerance to abiotic stresses during plant growth (Yildirim et al. 2006). This is in part due to induced root growth in the plant, increasing the root absorption surface area and enhancement of nutrient uptake by solubilization of phosphates and micronutrients in soil (Altomare et al. 1999).

c) Low productivity sites:

In the trials with low productivity (Berrymans, Sherry and Kaingaroa 209_4 and 660_2) *Trichoderma* contributed to gains in growth of up to 19.9% (Figure 2) but a full response may have been restricted due to many nutrients and other environmental limitations. The Berrymans trial was situated on Brown soils that have low natural levels of numerous nutrients (including P (3.9ppm), Ca (365ppm), K (98ppm), Mg (72ppm), Mn (9.1ppm), Na (9.7ppm) and Zn (1.4ppm, Table 1)) and being inland, little opportunity for coastal nutrient inputs. Foliar N was relatively high (1.46%, Table 4) and likely to be a limitation for uptake of P, K and other nutrients. Foliage analysis indicated that P (0.11%), Mg (0.08%), Na (42.5ppm), B (9ppm) and Cu (3.4ppm) were near or at deficiency levels and likely to limit growth (Table 4, Adams and Walker 1973).

The Kaingaroa Forest is primarily on pumice soils that have low reserves of macro nutrients, including N (0.12 and 0.19%), P (4.3 and 7.2ppm) and B (0.14 and 0.18ppm). They also have shallow topsoils with low C% and a low water holding capacity, although rainfall generally maintains adequate plant available water during the year. Production was limited in the two Kaingaroa sites mainly due to a likely deficiency in magnesium (foliar Mg at 0.06ppm, Table 4) because of very low magnesium in the soil (10 and 16 ppm, Table 2) and high K, particularly in Kaingaroa 209_4 (270ppm). Severe deficiency symptoms (needle tips golden yellow, remainder of needle normal green, Davis et al. 2015) were observed in some trees in the Kaingaroa 660_2 trial in August 2022 (Figure 4). The Kaingaroa and Nelson trials also have lower growth potential compared to the Northland and Gisborne sites due to colder temperatures, particularly between spring and autumn (Table 3). Seasonal rainfall was less critical in the Kaingaroa and Nelson trials, although Sherry may experience water stress in summer and autumn (Table 3).

In the three Nelson trials established in 2018 (Berrymans and Sherry) and 2020 (Kings Ridge), the least responsive *Trichoderma* treatment was PR6. Productivity was 4.1 and 5.0% less in the Berrymans and Sherry trials respectively although this was not significantly different ($P<0.05$) from the control (Figure 2).

The repeated inability of PR6 to stimulate growth in the Nelson forests suggests that the four isolates that make up the mixture (3 x *T. harzianum* and 1 x *T. atrobrunneum*) were not suited to the Golden Downs Forest soil environment or could not activate a growth response. Soil temperature was unlikely to be a limiting factor (Table 3) in isolate survival or development because both *Trichoderma* species have a broad range of tolerance. In laboratory studies, these isolates had medium to high mycelial growth rates at 12, 17, 22 and 27°C, whilst still being able to grow, albeit slowly, at 2 to 7°C (Whelan 2018). In addition, the PR6 mixture has performed well in five much colder sites in Southern Otago and Canterbury (at year one the mean tree height increase was 10.2% in the PR6 treatment compared to the control; Whelan 2023). The survival rate of the PR6 isolates in the tree roots is unknown because of the unavailability of specific molecular identification tests. In contrast, the PR3a mixture (containing *T. asperellum, T. atroviride* and *T. crassum*) fared better with 2.0 to 10.4% gains in volume, and 3.4 to 5.5% gains in tree survival, in the three Nelson trials, although these were not significantly different to the controls at the 5% level due to variation within the trials. Interestingly, at Kings Ridge, another *Trichoderma* mixture PBI (consisting of four *T. atroviride* isolates) resulted in a very large height increase in year one (21%) and year two (13%) compared to the control (Whelan 2023). These strains were able to thrive and induce a large growth response. This mixture is recommended for the promotion of radiata pine growth in the Nelson Golden Downs Forest region, instead of PR6 and, to a lesser extent PR3a (due to its ability to improve survival). Future studies to determine the mechanisms of this strong response would be helpful.

Figure 4: Magnesium deficiency in a radiata pine tree severely affected by *Dothistroma septosporum* at Kaingaroa 660_2 trial on 15 August 2022.

The Kings Ridge trial was not considered in the volume productivity data because the two-year-old trees were too small for DBH measurement, but they were measured for nutrients. Although the trial was mapped as having an Othic Brown soil type (similar to the Berrymans site) the soil test suggested an Allophanic Brown soil type may be more appropriate. Allophanic Brown soils can retain high levels of phosphorus due to the large amounts of iron oxides weathered from the soil parent material. This soil was considerably different from those in the other trials with adequate levels of available P (46.2ppm), Ca (1905ppm), Mg (318ppm), Mn (16ppm), Fe (216ppm) and Co (0.14ppm). Although foliar N was high (1.71%), the trees were able to absorb sufficient P from the soil (0.23%) for growth not restricted substantially by P supply. Foliar magnesium may also be at adequate levels for growth at 0.1%.

Winter foliage nutrient concentrations

Foliage nutrient concentrations sampled in winter varied substantially across nutrients and sites (Table 4 and Figure 5). The soil nutrient analysis shows potential for limitations that would impact growth of the tree during late spring conditions when growth demand increases.

Table 4: Mean foliage nutrient concentration (% and ppm) across sites.

Site	Nutrient Concentration															
	%					ppm										
	N	K	Ca	P	Mg	Al	Mn	Zn	Fe	B	Cu	Co	Li	Sr	Ba	Ni
Patunamu	1.21	0.85	0.28	0.12	0.08	325	242	56	25	16	2.5	0.08	0.089	8.9	4.2	0.08
Topuni	1.54	0.81	0.24	0.11	0.11	365	647	57	41	18	3.6	0.13	0.065	3.8	2.2	0.36
Whatoro	1.58	0.71	0.18	0.12	0.09	450	236	39	58	16	3.8	0.09	0.015	1.5	0.2	0.50
Kaingaroa 660_2	1.33	0.66	0.20	0.12	0.06	497	351	43	30	9	4.2	0.07	0.048	2.3	1.9	0.74
Kaingaroa 209_4	1.37	0.80	0.24	0.14	0.06	478	303	57	30	10	6.0	0.02	0.070	4.9	4.0	0.11
Berrymans	1.46	0.80	0.21	0.11	0.08	454	332	43	37	9	3.4	0.12	0.024	8.4	1.5	0.22
Kings Ridge	1.71	0.98	0.29	0.23	0.10	156	146	49	35	13	3.9	0.09	0.015	9.8	3.1	0.77

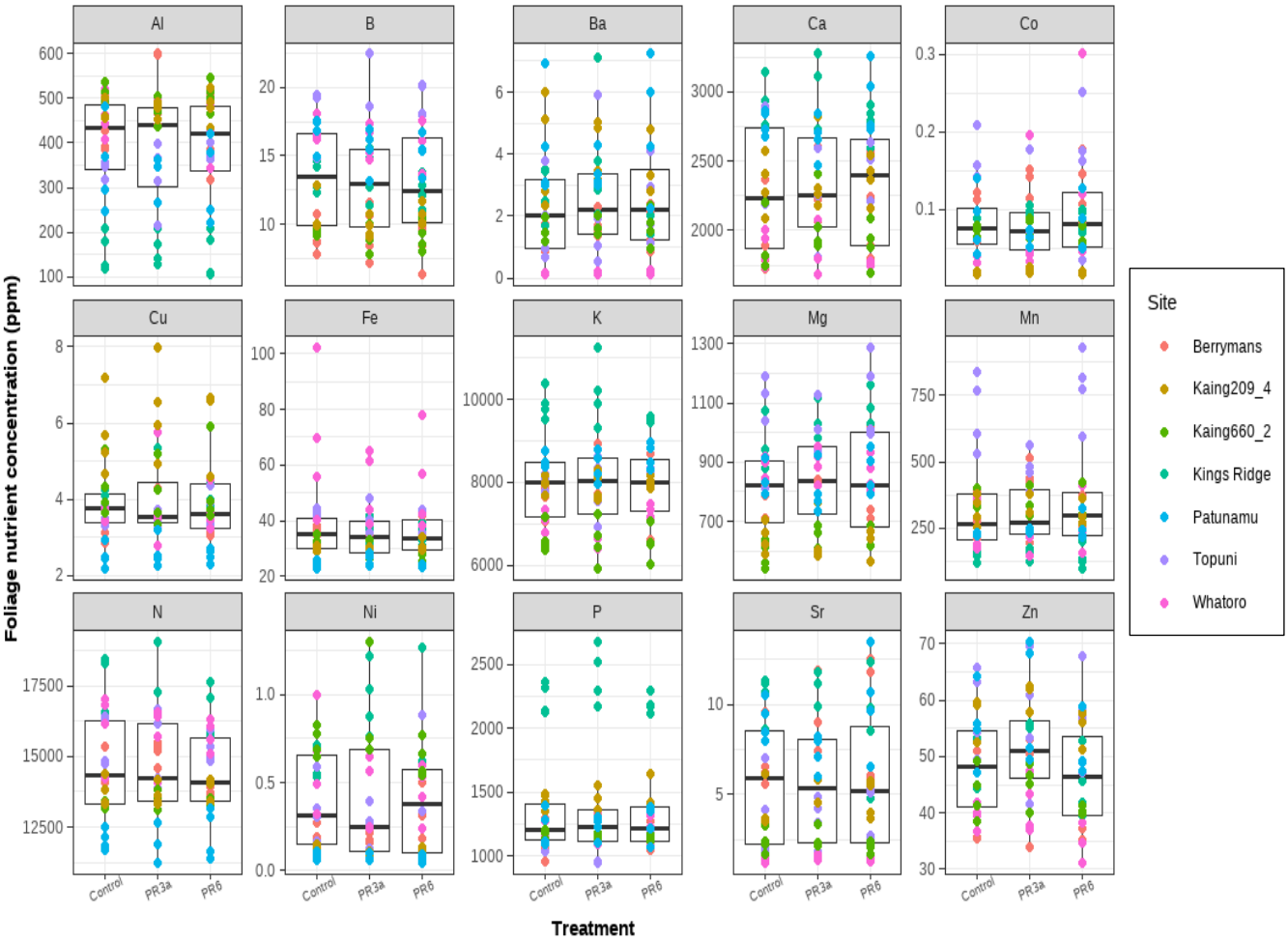


Figure 5: Boxplots of individual available foliage nutrient concentrations across sites (indicated by coloured dots), split by treatment of Control, PR3a and PR6. Horizontal lines represent the lower 25%, upper 75% quartile and median (dark line). Whisker lines represent scores outside the middle 50% with outliers above or below the whisker lines.

Nutrient Uptake efficiency

Nutrient uptake efficiency reflects a plant's absorption of nutrients from the soil and the internal transport, storage and remobilisation of nutrients and might be used to determine effects of *Trichoderma* inoculation. The ratio of foliage to soil nutrient concentrations varied across all nutrients and all sites (Figure 6). The uptake ratio of B, K, Mn, P and Zn (Table 5) was significantly greater than all other nutrients. This demonstrates the effort of the radiata pine trees to accumulate these nutrients despite the low concentrations in the soil relative to plant demand. Accumulation of phosphorus was the greatest compared with requirements of other nutrients, indicating its fundamental importance in large amounts for plant structural development. On average across the sites, approximately 200 parts per million (Table 5) of phosphorus was accumulated in the foliage for every ppm as measured by soil available phosphorus (Mehlich-3 extraction method). Boron accumulation (important for cell wall synthesis and plant metabolism) was also high at approximately 65 parts per million accumulated for every ppm of soil available boron. This may have approached excessive levels at some sites, like Topuni, due to the wet soil conditions and the narrow range of concentration from essential to toxic levels (Landi et al. 2018). Accumulation of K was similar to that of B at 58 ppm, and although is not involved in structural formation, it is essential for regulating plant photosynthesis and development. In contrast, nutrients with low uptake efficiency (eg. Al, Na and Co) may be nutrients that are not required or potentially inhibitory to plant growth. Finally, nutrients with about a 10-fold concentration ratio (eg. Ca, Mg and N) may be readily available from the soil or they are relatively easy for the plant to extract them.

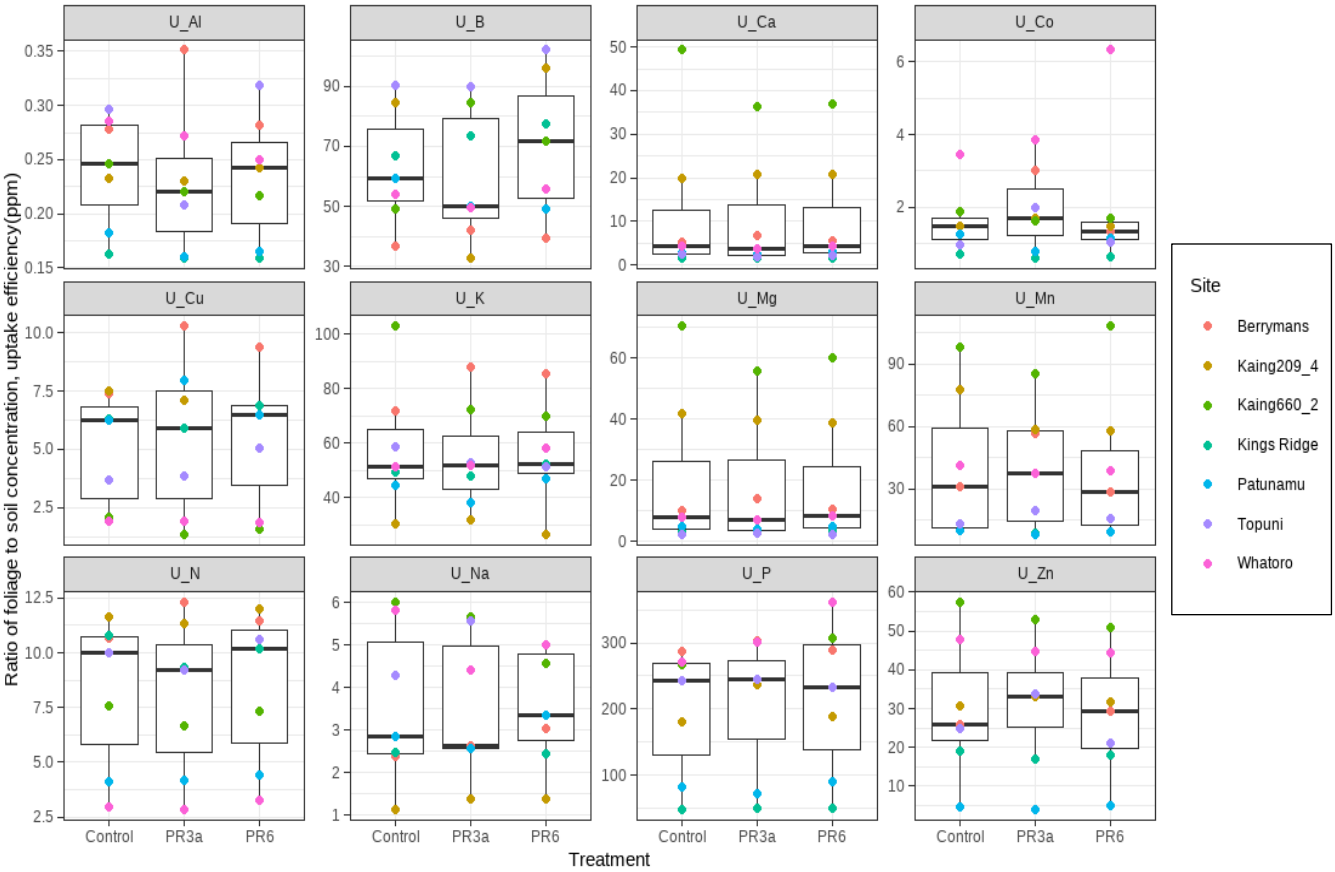


Figure 6: Boxplots of the ratio of foliage to soil nutrient concentrations across sites (indicated by coloured dots), split by treatment of Control, PR3a and PR6. Horizontal lines represent the lower 25%, upper 75% quartile and median (dark line). Whisker lines represent scores outside the middle 50% with outliers above or below the whisker lines.

There was no statistical evidence at P=0.05 level to support the view that the *Trichoderma* treatments improved nutrient uptake based on this dataset (Table 5). However, the samples were collected in winter and so reflect sub-optimal conditions. The efficiency of P uptake improved by 5.6-10.4% for PR3a and PR6 on average across sites respectively.

Table 5: Mixed-model ANOVA of mean foliage and soil concentration ratios (*n* = 4) per site (*n* = 7).

Fixed effects	Estimate	Standard Error	Degrees of Freedom	t-value	P-value	Significance
Intercept	0.24	13.01	210	0.018	0.9853	
PR3a	-0.01	16.94	210	-0.001	0.9994	
PR6	-0.01	16.94	210	0.000	0.9997	
B	62.80	16.94	210	3.707	***0.0003***	***
Ca	11.96	16.94	210	0.706	0.4810	
Co	1.37	16.94	210	0.081	0.9358	
Cu	4.77	16.94	210	0.281	0.7787	
K	58.19	16.94	210	3.435	***0.0007***	***
Mg	19.81	16.94	210	1.169	0.2436	
Mn	40.03	16.94	210	2.363	***0.0190***	*
N	8.01	16.94	210	0.473	0.6369	
Na	3.33	16.94	210	0.197	0.8443	
P	196.53	16.94	210	11.600	***0.0000***	***
Zn	29.80	16.94	210	1.759	***0.0801***	.
PR3a:B	-2.65	23.96	210	-0.111	0.9121	
PR6:B	7.18	23.96	210	0.300	0.7648	
PR3a:Ca	-1.67	23.96	210	-0.070	0.9446	
PR6:Ca	-1.56	23.96	210	-0.065	0.9481	
PR3a:Co	0.35	23.96	210	0.015	0.9883	
PR6:Co	0.35	23.96	210	0.015	0.9882	
PR3a:Cu	0.48	23.96	210	0.020	0.9841	
PR6:Cu	0.44	23.96	210	0.018	0.9854	
PR3a:K	-3.73	23.96	210	-0.156	0.8764	
PR6:K	-2.67	23.96	210	-0.111	0.9115	
PR3a:Mg	-2.10	23.96	210	-0.088	0.9303	
PR6:Mg	-1.70	23.96	210	-0.071	0.9437	
PR3a:Mn	-0.73	23.96	210	-0.030	0.9759	
PR6:Mn	-1.72	23.96	210	-0.072	0.9427	
PR3a:N	-0.24	23.96	210	-0.010	0.9920	
PR6:N	0.22	23.96	210	0.009	0.9928	
PR3a:Na	-0.02	23.96	210	-0.001	0.9994	
PR6:Na	-0.02	23.96	210	-0.001	0.9994	
PR3a:P	11.01	23.96	210	0.460	0.6462	
PR6:P	20.40	23.96	210	0.852	0.3954	
PR3a:Zn	1.26	23.96	210	0.053	0.9579	
PR6:Zn	-1.38	23.96	210	-0.058	0.9540	

Significance of relationships P< 0.001 (***),P < 0.05 (*) and P less than 0.1 ('.').

Low concentrations of the foliar nutrients N, P, B and Cu in radiata pine may occur in spring and summer (Knight, 1978) when rapid growth is taking place but before the autumn and winter rainfall commences that increase the availability of these nutrients. Future sampling of these trials during the summer months, after most reserves of nutrients have been mobilised for growth, may increase the likelihood of detecting treatment differences in foliage N, P, B and Cu. In contrast, summer maxima may occur for Na, Mg, Mn, Ca

and Zn, as a result of decreased demand when summer growth becomes more constrained, and an increased supply of these nutrients from microbial and root activity during the months that still have high moisture availability (Knight, 1978).

Vector analysis was used graphically to show the effect of the two *Trichoderma* treatments on nutrient concentration, with contrasting trends of different nutrients apparent over the sites (Figure 7). Nutrient contents were lowest in Kings Ridge, Patunamu and Berrymans, and highest, in Whatoro and Topuni, largely reflecting the impact of *Trichoderma* on tree size. Kaingaroa 209_4 had the largest difference in nutrient content between the two *Trichoderma* treatments, suggesting the PR3a mixture in this situation, had better ability to utilise soil nutrients than PR6. Some sites had a narrow range of nutrient concentrations (Kaingaroa 209_4, Patunamu and Whatoro) whilst others had a wide range (eg. the PR3 treatments of Kings Ridge PR3a, Berrymans and Topuni). Some plots had an increase in nutrient concentration as well as tree volume (eg. PR6 in Berrymans), indicating these nutrients were approaching or at deficiency levels. In contrast, some inoculated plots had increased growth without nutrient addition (ie values below the nutrient concentration horizontal line of 100) and resulted in a dilution in needle nutrient concentration. Dilution of nutrients was observed in both *Trichoderma* treatments including examples of reduced P and Ca concentration in the PR6 treatment in Topuni and the PR3a treatment in Kaingaroa 660_2 (Figure 7). These data support the hypothesis that an improved nutrient efficiency did occur in some plots but the dilution of nutrient concentration in the needles possibly masked the effects of *Trichoderma*. The rest of the datapoints reflected sufficiency trends with no change in concentration and growth and nutrition was balanced.

Similar definitions of nutrient status were found using the known deficient and adequate nutrient concentrations (Davis et al. 2015) and vector analysis methods. However, vector analysis provides more information because factoring in tree size considers non-nutrient interactions (eg. weeds, moisture, and micro-climate). In this study, the definition of luxurious uptake of Mg was made in both *Trichoderma* treatments in Kings Ridge (being an increase in nutrient concentration and no change in volume) as well as an excessive uptake of P, K, N and Ca in the Kings Ridge PR3a treatment (a reduction in the two parameters).

Interpretation of responses should be viewed with caution as other factors may impact on nutrient responses, eg. preceding climatic conditions (such as drought or unusually moist conditions) and disease infection or insect attack. In this dataset foliar disease, mainly *Dothistroma septosporum*, was present in six trials in winter 2022 although not at high levels (the highest mean disease severity of 12% was in Kaingaroa 660_2). Foliar disease may have had an impact on nutrient uptake in both the treated and untreated control trees.

Sampling techniques were chosen to minimise variation in nutrient concentrations associated with foliage age and crown positions (ie. sampling the current-season fully-grown needles exposed to full sunlight on the youngest second-order branches in the top third of the crown) and handling contamination (replacing gloves between sampling plots for foliage). In future studies, additional replicate samples for increased precision of measurements could allow smaller differences in nutrient concentration to be statistically detected. Soil and foliar nutrient measurement of the recently established South Island trials (Whelan 2019) would substantially expand the range of soils, temperatures, rainfall and soil water storage capacities that were experienced in the current trials, and better quantify the effect of *Trichoderma* on nutrient uptake. Further monitoring of the existing trials, particularly in the wetter and warmer sites would also better define the relationship between climate and production gain in the inoculated trees. Intensive greenhouse experiments with nutrient gradients, particularly of N and P may also help refine the nutrient response of trees to *Trichoderma* inoculation.

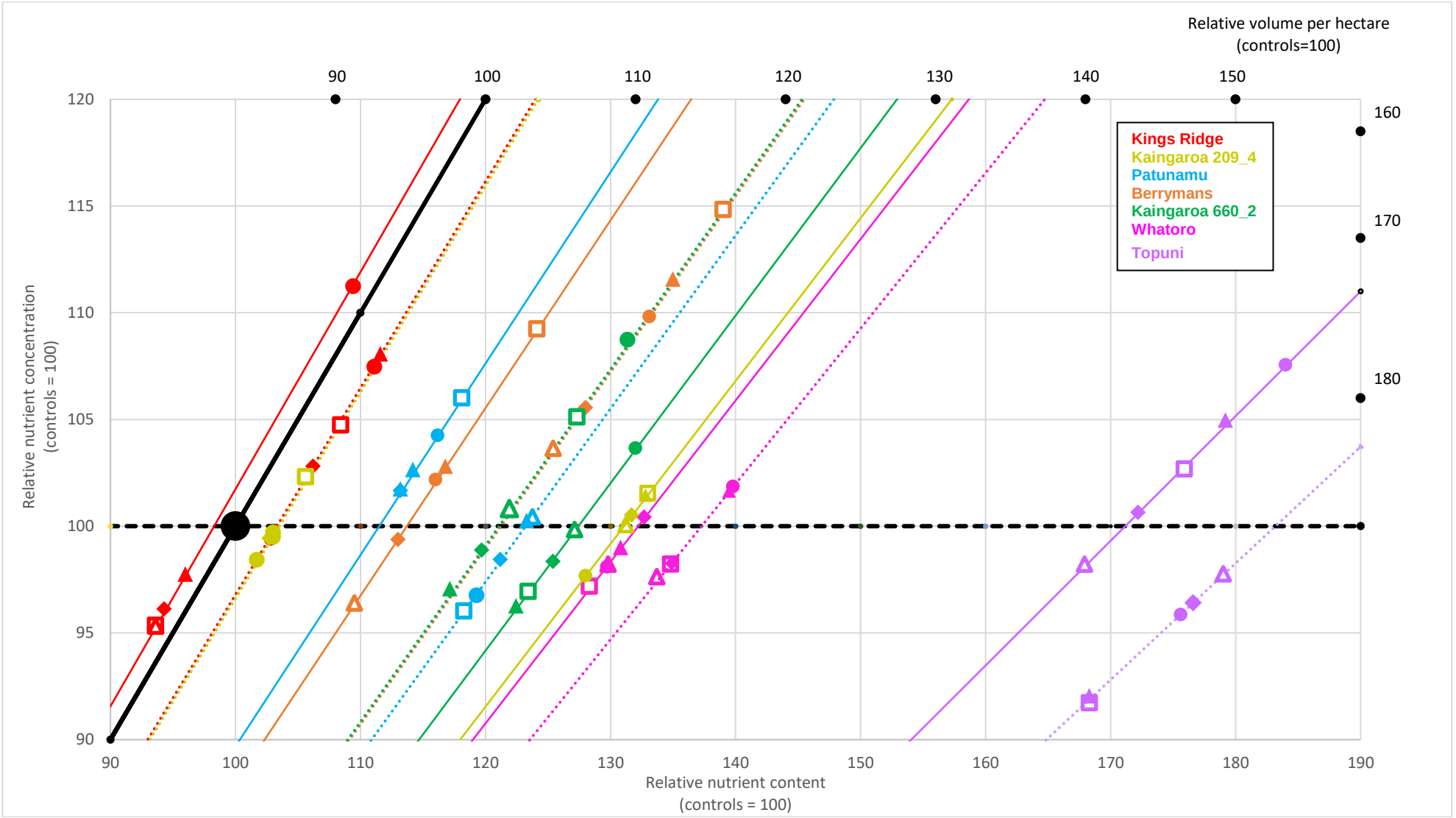


Figure 7: Diagram of relative response in volume.ha^{-1} and nutrient content (N △, P ▲, K ◆, Ca □ and Mg ●) of foliage in radiata pine for the *Trichoderma*-treated plots (PR3a ·····; PR6 ——) in Kings Ridge, Kaingaroa 209_4, Patunamu, Berrymans, Kaingaroa 660_2, Whatoro and Topuni trials. Responses are relative to the untreated control plots that were normalised to 100 (●). For simplicity vector arrows from the reference point were not drawn on this diagram. Height in the Kings Ridge trial and volume.ha^{-1} in the other trials were used to represent biomass.

CONCLUSION

This research programme showed that nursery *Trichoderma* inoculation is a potentially important management tool for improving radiata pine tree productivity. In this dataset plants were often larger when grown with the endomycorrhizal fungi *Trichoderma,* but the productivity response may have been dependent on the site's growth potential. It was not possible to determine a relationship between tree growth response to *Trichoderma* and nutrient uptake. However, *Trichoderma* may have improved the efficiency of nutrient uptake in the sites with treatment associated productivity responses (Topuni, Whatoro, Kaingaroa 660_2, Tauwhareparae, and the PR3a treatment in the Kaingaroa 209_4 and Berrymans) but this was not evident in the sub-optimal winter sampling. Part of this response may also have been that growth increased to the extent that nutrients were diluted within the needles relative to uptake. Sampling in late summer or early autumn when trees are under climatic stress and essential foliar nutrients are generally at low and stable levels may determine whether *Trichoderma* can be used as a management tool to improve nutritional requirements by increasing uptake efficiency. In addition, the effect of *Trichoderma* on nutrition in other soil types or environmental conditions needs further evaluation, and the inclusion of the newly established trials in the colder or drier parts of the South Island would be suitable testing sites. In forests with high growth potential due to low-stress environments and balanced soil nutrients (ie. similar to the Gisborne Patunamu site) *Trichoderma* treatment may be less beneficial. However, many existing or future forestry sites in New Zealand will have low to medium growth potential with limiting soil nutrient conditions, and beneficial growth responses to these *Trichoderma* strains would be expected.

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