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Technical Note

Testing new soil enzyme assays for predicting forest fertiliser response – protease may have limited suitability

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Summary: Almost all nitrogen (N) in soils occurs as organic polymers and transformation of these to smaller polymers or mineral forms (NH_4^+, NO_3^-) that can be absorbed by tree roots is dependent on the activity of soil enzymes such as proteases. Therefore, soil enzymes may have the potential to predict soil N availability and forest growth response to N application and offer improvements over existing tests. In this initial study, the activity of the enzyme protease was determined in soils from twelve sites where N fertiliser had been applied five years previously to 7-9 year old radiata pine stands. The stands were located from Auckland to Southland and represented six major soil orders. At each site, there was an existing experimental plot that was installed at the time of planting, and a second plot that was installed several years later to examine the effect of N application at the site. Soil analyses of samples collected at age 0 and age 4 were compared with protease activity as a potential predictor of tree growth response to N fertilisation. Although protease activity accounted for 32-34% of variation in tree growth response to N fertilisation, this was not statistically significant. Protease activity accounted for more variation in tree growth response than total soil C and N in samples collected at age 4, but less than in samples collected adjacent to the plots at planting. Total soil N in the latter samples was the best predictor of N response, accounting for 38% of variation after one year and 52% of variation in N response after 5 years. Protease activity was strongly correlated with total soil C and N concentration in the soil samples collected at age 0. In contrast to previous studies foliar N concentration was not found to be a good predictor of tree growth response to N fertilisation, possibly because of the limited number of sites and use of 'surrogate' control plots for foliar N determinations in the present study. Further research is required to properly evaluate the utility of both protease and soil total N for predicting tree growth response to N.

Introduction

Almost all nitrogen (N) in soils occurs as organic N and a large proportion of phosphorus (P) can also occur in organic form. Transformation of large organic polymers of N and P into simple organic polymers or mineral forms, such as NH_4^+, NO_3^-, and HPO_4^{2-}, is dependent on enzymes (eg phosphatases, phytases, proteases) exuded by soil microbes, fungi and plant roots[1,2]. These smaller polymers and mineral forms can be absorbed by tree roots. Soil tests using enzymes, therefore, might have potential to predict N and P availability, forest fertiliser response, and offer improvements over existing soil tests. New studies have begun to test whether enzymes may be used as indicators of soil N and P availability and predictors of fertiliser N and P response in radiata pine. This report describes initial investigations examining the potential of a protease enzyme for predicting soil N availability and N fertiliser requirement.

Methods

Field methods

Twelve sites from a Long Term Site Productivity trial series were used in the study. The trial series had been set up to examine relationships between forest productivity and site quality. Soil chemical and physical data had already been collected for the trial sites[3]. The sites covered six major soil orders, spanned an annual average rainfall range of 776 to 3718 mm and average temperature range of 8.5 to 16.9˚C (Table 1). Soil total N concentrations ranged

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from 0.03% to 0.79%, being lowest in the Raw and highest in the Allophanic soil groups.

Each site had an existing 40 x 40 m permanent sample plot with 10 m buffers. One half of each of these plots received conventional weed control prior to planting, but no further herbicide was applied after planting. The other half of the plot received additional herbicide applications to keep these sub-plots in a weed free state for several years. The sub-plots receiving conventional weed control only were used as the control treatment for the present study.

An additional 40 x 40 m plot was established at each site to match, as similarly as possible, the existing plot in terms of soil, slope and aspect. Nitrogen as urea was applied to these additional plots, and also to the 10 m buffers, in November 2009 at a rate of 200 kg N ha^{-1}. The trees were 7-9 years old at the time of fertiliser application.

Table 1. Trial site, soil order, mean annual rainfall, mean annual temperature and total N concentration in topsoil (0-10 cm). Sites are arranged in order of increasing latitude.

Site	Soil Order	Rainfall (mm)	Temp. (°C)	Soil N[1] (%)
Mahurangi	Ultic	1739	16.0	0.35
Riverhead	Ultic	1520	16.5	0.11
Woodhill	Recent	1563	16.9	0.07
Kaingaroa	Recent	1611	13.8	0.13
Mamaku	Podzol	2122	11.6	0.30
Mawhera	Podzol	3718	10.8	0.53
Hochstetter	Podzol	3454	12.2	0.22
Eyrewell	Brown	776	11.5	0.17
Bottle Lake	Raw	640	11.9	0.03
Otago Coast	Brown	886	9.4	0.17
Longwoods	Allophanic	1290	8.5	0.79
Catlins	Brown	1190	10.6	0.29

[1]Soil N from profile pits at planting

Tree height, ground line diameter and diameter at breast height (DBH) of individual trees in all plots were measured prior to, and at one, two, three and five years after fertiliser application and stem volume was estimated from these measurements.

Determining protease activity

Mineral soil samples (0-10 cm depth) were collected from the fertilised plots and the no-weed-control half of the unfertilised plots in June 2014. Four 0.1 m^2 quadrats were located at 4 m intervals along a transect bisecting the plot (or control sub-plot) and three cores within each quadrat were collected using a 25 mm diameter corer. The 12 cores per plot were bulked into a single sample, kept cool and sent to the laboratory for analysis. Samples were passed through a 5 mm sieve to remove coarse material and then thoroughly mixed. The method used for determining protease activity is described in Landi et al.[4]. The method is based on determination of the amount of the amino acid tyrosine released by casein-hydrolysing proteases when the soil sample is incubated with casein.

Comparison with other soil and foliar nutrient predictors of growth response

Two soil analyses were available for comparison with protease activity. Firstly, soil samples (0-10 cm depth) had been collected at each site from a profile pit dug at undisturbed (by forest harvesting) and unfertilised locations adjacent to the plots when originally planted. Secondly, samples (also 0-10 cm depth) were collected from within the no-weed-control half of the unfertilised (control) plots when the trees were four years old. The samples had been analysed to determine pH (in water); total C, N and P; inorganic P, organic P, exchangeable-bases, and cation exchange capacity using the methods described in Blakemore et al.[5]. Mineralisable N, a measure of the amount of N mineralised after incubation of the soil under aerobic conditions for a period of eight weeks, had also been determined in the samples collected from the unfertilised control plots.

Foliar nutrient concentrations and fascicle weights were determined in current-year mature needles sampled from trees in the control plots in March 2010. Nitrogen was determined in the samples using a C/N analyser, and phosphorus was determined by inductively coupled plasma optical emission spectrometry.

Results

Soil protease activity varied 60-fold across sites in unfertilised plots from 4 to 240 µg tyrosine/g dry soil/hour (Table 2). Across site variation was 8-fold in fertilised plots. Mean protease activity did not differ significantly between the fertilised and unfertilised plots. Protease activity in fertilised plots was not significantly correlated with activity in unfertilised plots. In unfertilised plots, values were highest in Allophanic and Podzol soils and lowest in the Raw soil. Similar rankings were not seen in the fertilised plots. Fertiliser had the greatest effect on protease activity in the Podzol and Brown soils (Figure 1). Protease activity was reduced consistently by fertiliser in the Podzol soils, whereas changes in the Brown soils were variable (Figure 1). Protease activity was positively correlated with soil total C and N concentrations ($P < 0.01$) and cation exchange capacity ($P < 0.05$), and negatively correlated with pH, and Olsen and Bray-2 available P concentrations ($P < 0.05$) determined in soil samples collected from within the unfertilised control plots. The positive correlation with soil C and N concentration and CEC is consistent with studies that have found protease activity to be positively correlated with organic amendments and soil organic matter as well as the clay content of soil[4].

Differential thinning between fertilised and unfertilised plots prior to harvest at one site (Bottle Lake) and herbicide damage at one site (Mamaku) compromised tree growth data so these sites were omitted from comparisons of N response prediction.

The tree growth response to N at the remaining 10 sites amounted to 27%, but was highly variable across sites (Figure 2), and not significant (T-test P = 0.11).

Linear correlation analysis showed protease activity explained 32-34% of the variation in tree growth response to N fertilisation (Table 3): the correlation was negative and non-significant. However, protease activity explained more variation in N response than either total soil C and N concentrations or soil N mineralisation in soil samples collected from within the unfertilised control plots at year 4.

Table 2. Protease activity (µg tyrosine/g dry soil/hour) in unfertilised and fertilised plots at twelve sites.

Site	Unfertilised	Fertilised
Mahurangi	120.9	117.4
Riverhead	23.9	40.4
Woodhill	47.3	65.8
Kaingaroa	45.2	83.2
Mamaku	200.0	77.5
Mawhera	194.2	96.9
Hochstetter	199.8	124.0
Eyrewell	58.1	118.0
Bottle Lake	4.3	25.6
Otago Coast	97.5	71.9
Longwoods	240.1	196.2
Catlins	92.5	186.4
Mean	**110.3**	**100.3**

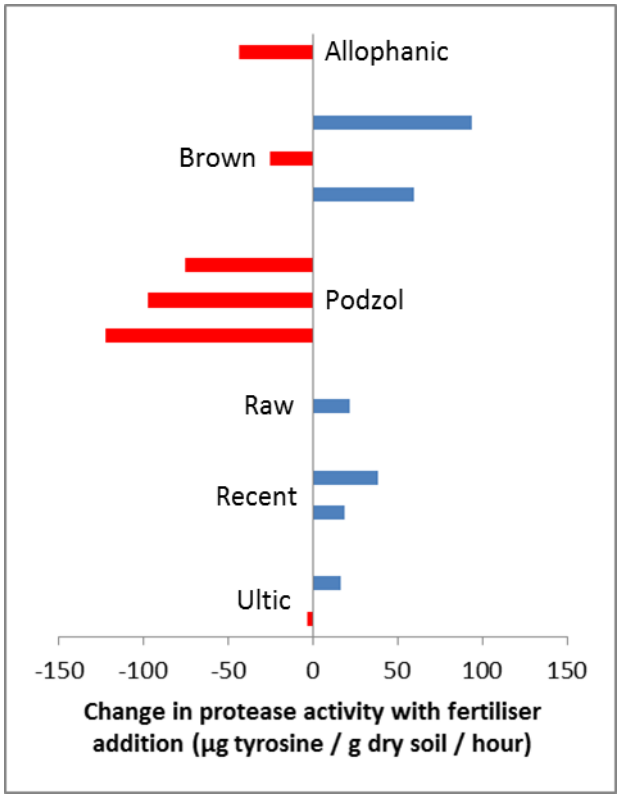

Figure 1. Change in protease activity with fertiliser addition in six soil groups.

Surprisingly, total C and N concentrations in soil samples collected at the time of planting explained more of the variance in tree growth response to N than concentrations in samples collected from within the plots at age four (Table 3). Soil C and N concentrations in these samples also explained more variance in tree growth than protease activity. The predictive ability of both these variables improved with length of time since fertiliser application (Table 3). The correlations for both C and N were negative and significant from year 2 for soil N and from year 3 for soil C.

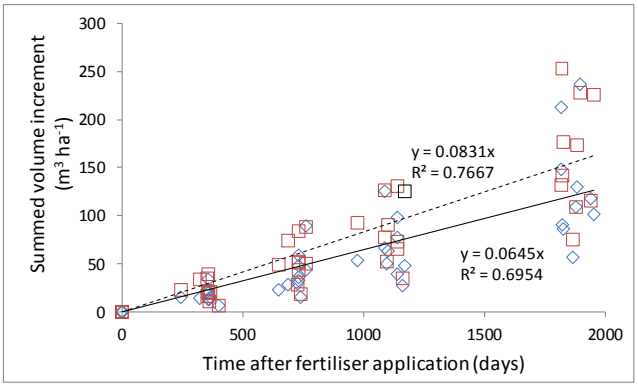

Figure 2. Tree growth response over a five year period to nitrogen applied at 200 kg N ha^{-1} as urea. Unfertilised plots, diamond markers and solid line, fertilised plots, square markers and dashed line. The response was not significant (P = 0.11).

Foliar N concentrations in unfertilised plots ranged from 1.12% (deficient) to 1.50% (adequate). Nine of the ten sites had foliar N concentrations of 1.44% or below and were considered to be potentially responsive to N fertilisation. Foliar P concentrations were marginal at two sites but adequate (>0.13 %) at the remaining sites. However, foliar N concentration and mass and N/P ratio did not explain a significant amount of variance in tree growth response to N fertilisation (Table 3). This contrasts with previous

Table 3. Amount of variance in change of volume increment after fertiliser application explained by soil and foliage variables (%).

Sampling year[1]	Variable	Years after fertiliser application			
		1	2	3	5
12-14	Protease	32	32	32	34
0[2]	Soil C (%)	38	39	43*	51*
0	Soil N (%)	38	44*	48*	52*
0	Soil C/N	17	12	15	24
4[3]	Soil C (%)	22	21	23	26
4	Soil N (%)	17	22	24	28
4	Soil C/N	8	1	2	1
4	Min[4] N ($mgkg^{-1}$)	3	6	4	3
7-9	Fol[5] N (%)	2	2	2	6
7-9	Fol P (%)	2	3	1	1
7-9	Fol N/P	<1	3	1	2
7-9	Fol N mass	30	11	11	2
7-9	Fol P mass	19	17	13	7

[1] Year after planting
[2] At year 0 samples were collected adjacent to plots
[3] At year 4 samples were collected from within non-herbicide-half of control plots
[4] Mineralisable N
[5] Foliage
* Correlation coefficient (r) significant at P < 0.05

studies[e.g. 6, 7]) which have found foliar N concentration or N mass to be good predictors of N response in radiata pine. The limited number of sites and the use of 'surrogate' control plots for determination of foliar N may have contributed to the poor ability of foliar N to account for variation in growth response to fertiliser N in the present study.

Conclusions

Soil protease activity did not explain a significant amount of variation in tree growth response to N fertilisation. While protease activity explained more variation than soil total N and mineralisable N in soil samples collected from within the same plots at age four, it explained much less variation than soil total C and N in samples collected adjacent to the plots when the trees were planted.

This pilot study was limited by the number of sites and the fact that protease activity was determined in 'surrogate' control plots five years after fertilisation rather than at or just prior to fertiliser application. Further, comparison between protease activity and other soil predictors of forest response was limited because of lack of uniformity in time and location of sample collection.

Despite these limitations, the strong correlation observed between protease activity and total soil C and N concentration ($P < 0.01$), and the further correlation between these latter variables and tree growth response to N fertilisation in samples collected at the time of planting, suggests further study of protease activity as a predictor of tree response to N fertilisation may be warranted.

Protease activity in soils is affected by many factors including soil physicochemical properties, climatic conditions, presence of substrates, and management Practises[4]. An improved understanding of how such factors influence protease activity in radiata pine soils is required to progress its use as a predictor of tree growth response to N fertilisation.

Total soil N measured in samples collected at the time of planting was the best predictor of tree growth response to N fertilisation, with total soil N explaining 52% of variation in tree growth response five years after N application. It is not known why total N measured in soil collected from within the plots at age four did not perform as well as a predictor. Both variables improved as predictors from year one to year five after fertiliser application, presumably reflecting continued tree uptake of N from the soil N pool over a period of years. This study indicates that the utility of total N as a predictor of N response requires further investigation.

Recommendation

Because of the limitations of this initial study it is recommended that, as field trial opportunities arise, further investigations be undertaken to examine the potential of protease enzymes for predicting soil N availability and N fertiliser response. Further study of soil total N as a predictor of N response should also be undertaken. A series of well replicated trials with radiata pine treated with and without N, across a number of sites, is required to properly evaluate the potential of both measures for predicting growth response to N fertilisation.

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