

# Fertilisation with P, N and S requires additional Zn for healthy plantation tree growth on low fertility savanna soils

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**Handling Editor:**

Leônidas Melo

**Received:** 30 June 2023  
**Accepted:** 12 December 2023  
**Published:** 8 January 2024

**Cite this:**

Rance SJ *et al.* (2024)  
*Soil Research* **62**, SR23128.  
doi:[10.1071/SR23128](https://doi.org/10.1071/SR23128)

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## ABSTRACT

**Context.** Soil nutrient limitations characterise savanna soils globally and are one of several constraints to establishing productive tree plantations and enhancing economic opportunities in tropical regions. Fertilisation offers an approach to overcome soil nutrient limitations to maximise tree growth and health, but requires research on nutrient contents, composition, rates and methods of delivery in the context of soil characteristics. **Aims.** To determine the optimal contents, rates and methods of application of fertiliser to maximise the growth and health of the plantation timber species *Pinus caribaea* on low fertility savanna soils. **Methods.** Factorial field experiments tested growth responses to applications of phosphorus (P), nitrogen (N) and sulfur (S) on three soils near Darwin, Australia. Further experiments tested effects of zinc (Zn), copper (Cu) and potassium (K) application and small-scale variation in soil characteristics on tree performance. **Key results.** Positive growth responses to P, N and S were recorded, yet unhealthy trees developed, particularly in better-performing treatments. Second phase experiments addressing potential causes of ill health confirmed Zn limitations. Intense spatial soil sampling demonstrated substantial variation in cation exchange capacity and composition over short distances. **Conclusions.** Nutrient additions to enhance plantation tree growth will need to encompass minor and trace elements in addition to N, P and S, specifically Zn, and consider the mechanism of application. **Implications.** Small-scale variability in cation exchange capacity and composition indicates that optimal fertilisation rates will vary spatially, and that soil sampling for site characterisation would be more accurate with replicated dispersed samples.

**Keywords:** cation exchange capacity, nutrient limitation, *Pinus caribaea*, plant nutrition, soil limitation, soil variation, tree health, tropical soil.

## Introduction

There has been longstanding interest in establishing tree plantations in the northern parts of the Northern Territory of Australia (the area north of 15°S) to satisfy existing and potentially increasing timber demand, as is the case in other savanna regions globally (Kanowski 1997). Initial research concentrated on testing tree species and provenances from other parts of the tropics with potential to tolerate the conditions in the region (Stocker 1972; Cameron 1985), but broad similarities in tree performance across species has led to an increasing focus on climatic, soil and pest limitations on tree growth. Savanna regions are characterised by an extended dry season, low fertility soils with typically limited phosphorus (P) and nitrogen (N), and antagonistic activities of termites and other pests (Pellegrini 2016; Rance *et al.* 2020).

Generally, the soils of the Northern Territory savanna zone most structurally suitable for plantations or agriculture have low fertility with very low cation exchange capacities. Responses to N, P and potassium (K) have long been known (Stocker 1969). Yet, fertiliser application rates commonly used in agriculture elsewhere could induce imbalances in the low fertility soils, tree growth responses were sensitive to rates, combinations and proportions of fertilisers, and there seemed to be step-wise responses (Rance *et al.* 2020). Growth responses to P appear ubiquitous, and positive responses to P plus N common

(Aldrick and Robinson 1972; Winter and Jones 1977). Significant  $P \times T$  ( $T$  being a combined minor and trace element treatment) and  $K \times T$  interactions have been recorded (Cameron et al. 1982), as have positive responses to  $N$  or the  $T$  treatment in the presence of  $P$  and negative responses in the absence of  $P$  (Cameron et al. 1981). Sulfur ( $S$ ), either with or separate from  $T$ , increased growth in the presence of  $P + N$  in glasshouse trials (Rance et al. 1983, 2020). However, trees in the field frequently develop unhealthy new growth near the end of their second or third dry season, more commonly or sooner on fertilised trees than unfertilised trees (Rance et al. 2022). The availability of cations also appears to play an important role in plant growth, mediated through soil pH which in turn can be altered by fertiliser addition (Rance et al. 2020). Zinc ( $Zn$ ) deficiency was identified as a contributing factor (Rance et al. 1982). This combination of soil limitations is typical of savanna soils globally (Pellegrini 2016), and consistent with a long history of nutrient loss through fire and weathering (Orians and Milewski 2007; Devi Kanniah et al. 2010; Rance et al. 2020).

Despite this growing body of work on tree responses to fertilisation on savanna soils, uncertainty remains over field responses (c.f. glasshouse), the timing of expression of nutrient deficiencies, the optimal rates, formulations, timing and methods of application of nutrients, and the effects of local variation in soils. The general pattern was a response to  $P$ , followed by one or more of  $N$ ,  $S$  and  $K$ , not necessarily in a consistent order, but some other factor related to soil pH seemed to be influencing nutrient uptake and susceptibility to termite attack. The aim of this study was to examine field responses of  $N$ ,  $P$  and  $S$  on three of the soils examined in preliminary glasshouse factorial experiments (Rance et al. 2020). This was a study of threes; testing three levels of  $N$ ,  $P$  and  $S$  on three sites (soils). If symptoms of ill health

appeared through the factorial experiments, the capacity to initiate second phase experiments testing minor and trace element treatments (e.g.  $Zn$ , copper ( $Cu$ ) and  $K$ ) and methods of application was built into the experimental program. Renewed interest in establishing agriculture and tree plantations in the north of Australia (Office of Northern Australia 2015) underpins the importance of this research on savanna soil limitations.

## Materials and methods

The work described here was centred on Darwin, Northern Territory, Australia. Darwin's climate is monsoonal with marked wet and dry seasons, where ~90% of the annual rainfall occurs from November to April, and no rain recorded from May to September in most years. The three sites were Three Ways on Melville Island (TW; 11°40'S, 130°42'E on a Red Kandosol (gravelly red earth)), Humpty Doo (HD; 12°33'S, 131°20'E, on a Rudosol (sandy loam)) and Gunn Point (GP; 12°21'S, 131°9'E, a Red Kandosol). The wet season arrived earliest and total rainfall was highest at TW, with HD being the furthest inland and driest. Two sites seemed to be free of *Mastotermes darwiniensis* Froggatt termites (TW and HD; Fox and Clarke 1972), and were the sites examined by Cameron et al. (1982). Some soil data for the sites are presented in Table 1, showing the general low nutrient content and low cation exchange capacity.

The sites were cleared and root raked to approximately 20–25 cm depth during the dry season to remove stumps and residual roots to minimise resprouting from lignotubers, concentrating woody plant material in windrows off site which were burned. Initial soil cores to 1.5 m across the three sites indicated uniform soil profiles. Weeds that germinated

**Table 1.** Some chemical analyses on the soils at Three Ways (TW), Humpty Doo (HD) and Gunn Point (GP) (pH, electrical conductivity, organic carbon, total N, P, S and Zn and cation exchange capacity (CEC)).

Site	Depth (cm)	pH <sup>A</sup>	EC <sub>2.5</sub> <sup>A</sup> (μS cm <sup>-1</sup> )	Org.C <sup>B</sup> (mg g <sup>-1</sup> )	Total				CEC <sup>G</sup> (cmol kg <sup>-1</sup> )
					N <sup>C</sup> (mg g <sup>-1</sup> )	P <sup>D</sup> (μg g <sup>-1</sup> )	S <sup>E</sup> (μg g <sup>-1</sup> )	Zn <sup>F</sup> (μg g <sup>-1</sup> )	
TW	0–10	5.1	23.4	15.9	0.64	95	125	3.7	3.7
	10–20	5.2	16.4	14.4	0.34	82	120	4.9	2.2
HD	0–10	5.0	26.3	21.7	1.66	280	255	5.5	7.0
	10–20	5.3	19.2	14.4	1.03	260	240	5.4	4.8
GP	0–10	5.7	32.0	15.2	0.92	120	175	nd	8.4
	10–20	5.3	27.0	6.5	0.48	120	170	nd	8.4

nd, not determined.

<sup>A</sup>pH, EC<sub>2.5</sub> – 1:5 water extract of soil.

<sup>B</sup>Organic carbon – Walkley and Black (1934).

<sup>C</sup>Total N – microkjeldahl, Black (1965).

<sup>D</sup>Total P – determined on a perchloric acid digest (Chapman and Pratt 1961) by a molybdenum blue colorimetric method (Murphy and Riley 1962).

<sup>E</sup>Total S – determined on a perchloric acid digest (Chapman and Pratt 1961) by a sorbitol turbidimetric method (Verma et al. 1977).

<sup>F</sup>Total Zn – determined by atomic absorption spectroscopy on a perchloric acid digest.

<sup>G</sup>Cation Exchange Capacity – Tucker (1974).

after early wet season storms were mowed and the sites cultivated, and plots pegged. Factorial field experiments tested three levels each of N, P and S, with the 27 treatments replicated twice, at the three sites.

There were few weeds on site at the time of planting and woody weed seedlings were removed by hand. At TW, trees were planted at 1.5 m × 1.5 m spacing (4444 stems ha<sup>-1</sup>), 49 trees per plot but only the inner 5 × 5 net plot trees were measured and sampled. At GP and HD, trees were planted at 2.1 m × 1.05 m spacing (4535 stems ha<sup>-1</sup>), 56 trees per plot with 5 × 6 net plot trees. An additional isolation row was planted around the perimeter at each site. This was a much higher planting density than the plantation standard (at that time) of 1111 stems ha<sup>-1</sup> but restricted each experiment to 0.6 ha to minimise site heterogeneity and constraining the trees to extract necessary nutrients from limited soil volumes (Richards 1961). The close spacing was expected to allow the trees to rapidly occupy the site and compete with weeds. It was anticipated that responses to P, N or S were likely and would emerge rapidly, but competition between trees for water and light could mask treatment effects after 3 or 4 years.

*Pinus caribaea* was one of the more successful plantation species tested in the Northern Territory (Craciun 1978), and seedlings of *P. caribaea* var. *hondurensis* Barr. & Golf. (Belize provenance) raised in tubes in a nursery were planted into holes made with a motorised soil auger after planting tubes were removed. Planting the 9600 seedlings started at TW with the early onset of the wet season and finished at HD.

Fertiliser was broadcast by hand evenly across each plot at the rates and forms shown in Table 2. To minimise uneven distribution, each plot was divided into 14 or 16 sub-plots and the appropriate amount broadcast to each. The scheduled N<sub>1</sub>, P<sub>1</sub> and S<sub>1</sub> fertiliser application rates were near the estimated recommended optimum levels, the N<sub>2</sub>, P<sub>2</sub> and S<sub>2</sub> rates higher. However, applications of N as NH<sub>4</sub>NO<sub>3</sub> to TW and HD soils in the glasshouse induced symptoms of ill health (Cameron *et al.* 1982) so the 0.12- and 0.15-year applications were made at half the rates shown in Table 2. Basal dressings of KCl and a 'Complete' minor and trace element fertiliser mixture were not applied to allow symptoms of ill health to

**Table 2.** Scheduled fertiliser applications – rates of compound (kg ha<sup>-1</sup>) and times of application.

Age (year)		0.12	0.15	0.92	1.87	2.88
NH <sub>4</sub> NO <sub>3</sub>	N <sub>1</sub>	188 <sup>A</sup>		188	188	188
	N <sub>2</sub>	188 <sup>A</sup>	188 <sup>A</sup>	377	377	377
NaH <sub>2</sub> PO <sub>4</sub> ·2H <sub>2</sub> O	P <sub>1</sub>	377		377	377	377
	P <sub>2</sub>	377	377	753	753	753
CaSO <sub>4</sub>	S <sub>1</sub>	251		251	251	251
	S <sub>2</sub>	251	251	502	502	502

N<sub>1</sub> and N<sub>2</sub> refer to lower and higher rates of nitrogen fertilisation respectively, with the N<sub>0</sub> treatment receiving no added N (and similar notation for P and S). <sup>A</sup>Applications at Humpty Doo and Three Ways were made at 94 kg ha<sup>-1</sup>.

appear, and if so, to attempt to relate them to treatments in second phase experiments (described below).

Tall grasses (mainly annual sorghum *Sorghum* spp., together with *Mesosphaerum suaveolens* at HD), soon overtopped the trees at GP and HD, and were slashed with a sickle mower during the wet season in the first year to reduce competition, and near the end of the wet season in years 1 and 2. Mowings were retained on site to maintain soil cover and nutrient capital (Cunningham 1963), and to settle to reduce the risk of dry season fires. Feral horses grazed the TW site, maintaining a low grass cover without disturbing the pines.

Heights of all net plot trees were measured 0.15, 0.55, 1.03, 1.54, 2.73 and 3.81 years after planting. Diameters at breast height ( $D_{BH}$ , 1.3 m) were also measured at HD at 3.81 years. Foliar samples (10 fascicles of the most recent fully expanded needles from near the top of each net plot tree) were collected around 1.6 years, bulked for each plot and dried at 70°C, and ground to pass a 40 mesh (0.42 mm) sieve for chemical analysis. Samples were analysed for N (microKjeldahl method; Black 1965), P (perchloric acid digest (Chapman and Pratt 1961) by a molybdenum blue colorimetric method (Murphy and Riley 1962)), S (perchloric acid digest (Chapman and Pratt 1961) by a sorbitol turbidimetric method (Verma *et al.* 1977)) and K (atomic absorption spectroscopy (AAS) on perchloric acid digest). Needles and tips (newly expanded growth flushes on branches before needles had extended) were collected from all three sites around 2.7 and 3.7 years and analysed for N, P, K, S, Ca, Mg, Na, Fe, Mn, Zn, Cu, Al and B by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Two isolation rows were removed from the HD experiment at 3.7 years for access for a truck-mounted elevating platform (cherry picker) for foliar sampling. Fresh stem biomass (kg) was measured on harvested stems.

We used  $D_{BH}$  to estimate diameter at ground level ( $D_{GL}$ ) (Eqn 1), then used  $D_{GL}$  and height ( $H$ ) to estimate volume as cones (Eqn 2) which was then correlated with stem fresh weight.

$$D_{GL} = \frac{D_{BH} \times H}{H - 1.3} \quad (1)$$

$$V_{c, GL} = \pi D_{GL}^2 (H) / 12 \quad (2)$$

where  $D_{GL}$  is diameter (m) at ground level,  $D_{BH}$  is diameter (m) at 1.3 m,  $H$  = height (m) and  $V_{c, GL}$  (m<sup>3</sup>) is stem volume above ground level.

Based on results of correlations of  $V_{c, GL}$  with stem fresh weight (see Results), we used  $D_{BH}$  to estimate stem volumes above 1.3 m as cones (Eqn 3).

$$V_{c, BH} = \pi D_{BH}^2 (H - 1.3) / 12 \quad (3)$$

where  $V_{c, BH}$  (m<sup>3</sup>) is stem volume above 1.3 m,  $D_{BH}$  is diameter (m) at 1.3 m and  $H$  = height (m).

Volumes were summed for the plot and scaled to a per hectare basis, recognising that this ignored the stem below breast height.

### Statistical analysis

Factorial analyses of variance were carried out using the statistical package GenStat (VSN International Inc.; <https://vsni.co.uk/software/genstat>), testing three levels each of N, P and S, the 27 treatments replicated twice and plots allocated randomly in each replication on the three sites in randomised complete block designs, resulting in 22 degrees of freedom for the residual term (Cochran and Cox 1957). Linear regression was used to test whether stem fresh biomass, a commercially important indicator of plantation yield, could be estimated from standard stem dimensions (height and  $D_{BH}$ ) as combined into  $V_{c,BH}$ .

### Second phase experiments

The N, P and S experiments described above were terminated after the 3.81-year measurements. At that time, late in the dry season, termites were killing trees at GP, and trees had become unhealthy at all three sites with symptoms ranging from short needles to fasciation and stem tip die-back, similar to symptoms on other plantation species nearby (Rance et al. 2022). The incidence of affected trees was highest at TW and lowest at HD, although occasionally a tree or two appeared to be healthier than those surrounding. The experiments became test beds for other work.

#### Gunn Point

*M. darwiniensis* termite activity was noted at GP at 0.94 years, many trees had died by a year later and few were expected to survive, effectively terminating the experiment. Areas with active termites were tagged, and the experiment became a site for termite studies (Paton and Miller 1980; Spragg and Paton 1980).

#### Humpty Doo

A bush fire jumped both fire breaks and the site was burnt, the experiment aged 5.52 years. At that stage, trees in the experiment had a near continuous canopy and only a light litter layer, so the fire burned through slowly, removing the litter layer, and only a few trees experienced crown scorch. Needle and tip samples were collected from each net plot tree and bulked for each plot at age 5.91 years, and were analysed for N, P, K, S, Mg, Ca, Na, Zn, Cu, B and Al by ICP-AES. Heights and  $D_{BH}$  were measured, and the main treatments reapplied at the 2.88-year rate (Table 2). Each plot was then divided into four subplots of four trees in each plot corner.  $ZnSO_4$  (40 g  $ZnSO_4$  tree<sup>-1</sup>, 73.4 kg Zn ha<sup>-1</sup>) and  $CuSO_4 \cdot 5H_2O$  (10 g  $CuSO_4 \cdot 5H_2O$  tree<sup>-1</sup>, 15.5 kg Cu ha<sup>-1</sup>) was applied to two four-tree sub-plots in each plot in factorial

format. Heights and  $D_{BH}$  were measured at age 6.54 and 7.57 years, and  $D_{BH}$  only at 8.79 years.

### Three Ways

A patch of unhealthy trees, approximately a third of the experiment and extending across N, P and S treatments, was defined from healthier trees by a clear boundary, indicating consistent soil differences without obvious differences of soil colour or texture. Severely unhealthy trees had very short needles, commonly approximately 5 cm long, compared with 15–20 cm on healthy trees, some with no extended needles, premature needle fall, some with fasciation and dead tops (Fig. 1). Stunted tip growth hinted at Zn, Mn or B deficiency.

KCl (28 g tree<sup>-1</sup>) was applied in a ring around two rows of trees in each net plot (2.91 years). Twelve matched trios, ranging from mildly unhealthy to very unhealthy, were selected and measured. One of each trio was sprayed to wet all needles with a commercial 9.8 g Zn chelate L<sup>-1</sup> solution, a second matching set had 0.5 L of  $ZnSO_4$  solution



**Fig. 1.** An unhealthy tree with a dead stem tip and multiple leaders, and uneven needle lengths at Three Ways, some with no extended needles. Photo: S. Rance.

(10 g ZnSO<sub>4</sub> L<sup>-1</sup>, 9 kg Zn ha<sup>-1</sup>) applied to the soil surface in a ring around the trees, the third untreated.

At age 5.7 years (6.2 years after clearing the site for plantation establishment), soil cores of 4.8 cm internal diameter by 0–5 cm deep were collected along two transects, the first in the undisturbed native forest adjacent to the experiment, the second between trees along the diagonals of plots, starting in the healthier part of the experiment, through three plots in the badly affected area, and back into the healthier zone. Samples were taken at intervals between 1 and 2 m along the transects, spaced to minimise damage to tree roots. Ten samples were taken in the native forest, nine in the better part of the experiment and 19 among the severely affected trees. Exchangeable cations were leached with 1N NH<sub>4</sub>Cl (pH 7) solution, shaken and filtered, and cations (Ca, Mg, K, Na, and Mn) were determined by atomic absorption spectroscopy (AAS).

## Results

Consistent changes in needle colour and length, crown size and stem diameter growth between treatments were noted, indicating interactions. Treatment effects were most apparent early in the dry season, until fast growing trees entered a dormant phase, allowing slower growing trees to catch up before they too entered a dormant phase, and treatment responses decreased by the end of the dry season. Competing weed growth declined as trees in the close-spaced plots approached canopy cover, and needle colour improved and growth appeared to become more vigorous. Diameters of newly expanded stem tip growth flushes varied from ~0.5 cm in poorer-performing plots to ~0.9 cm in the best-performing plots; subsequent increments were added to these, and trees in better-performing plots tended to have more branches.

At TW there were significant main effect growth responses to N and P applications ( $P < 0.001$ , Table 3), along with significant changes of N, P and S concentrations in needles. There was a small response to P or N singly, and a larger response to P + N, and these responses persisted (Fig. 2a). By 2.0 years, the best growth was on plots treated with N + P + S. By 2.3 years, early in the dry season, some trees, mostly in the better-performing plots, were showing signs of ill health.

At HD, there were small responses to one of N, P or S by 0.87 years ( $P < 0.05$ , Table 4), but little response to higher applications. At later measurements, the response to P was not statistically significant, but P appeared to be necessary for healthy growth, and there were significant changes of N, P and S needle concentrations. At later measurements, heights were increased by applications of one of N, P or S, a larger response to two of N, P or S, a further increase to N + P + S excluding N<sub>2</sub>P<sub>2</sub> treatments, and a further

**Table 3.** Mean height increments of the main treatments (N, P and S) after the initial measurement at 0.07–0.15 years by time, and the mean main effect N, P and S concentrations in needles at Three Ways.

Age (years)	Height (m)					Needle (mg g <sup>-1</sup> )		
	0.4	0.9	1.4	2.6	3.7	1.5	2.7	3.7
<b>N</b>								
N <sub>0</sub>	0.03	0.12	0.29	1.03	1.98	6.49	5.90	6.54
N <sub>1</sub>	0.05	0.17	0.47	1.47	2.44	7.44	7.52	8.14
N <sub>2</sub>	0.06	0.18	0.52	1.60	2.57	7.96	8.18	9.45
	***	**	***	***	***	***	***	***
<b>P</b>								
P <sub>0</sub>	0.04	0.12	0.31	1.10	2.05	0.45	0.48	0.64
P <sub>1</sub>	0.05	0.17	0.47	1.47	2.43	0.71	0.87	0.99
P <sub>2</sub>	0.05	0.18	0.49	1.53	2.51	0.76	0.90	1.02
	ns	**	***	***	***	***	***	***
<b>S</b>								
S <sub>0</sub>	0.04	0.14	0.39	1.28	2.24	0.82	0.48	0.60
S <sub>1</sub>	0.05	0.17	0.45	1.41	2.41	0.77	0.58	0.96
S <sub>2</sub>	0.04	0.16	0.44	1.41	2.34	0.84	0.60	0.94
	ns	ns	ns	ns	ns	*	***	*

ns, not significantly different.

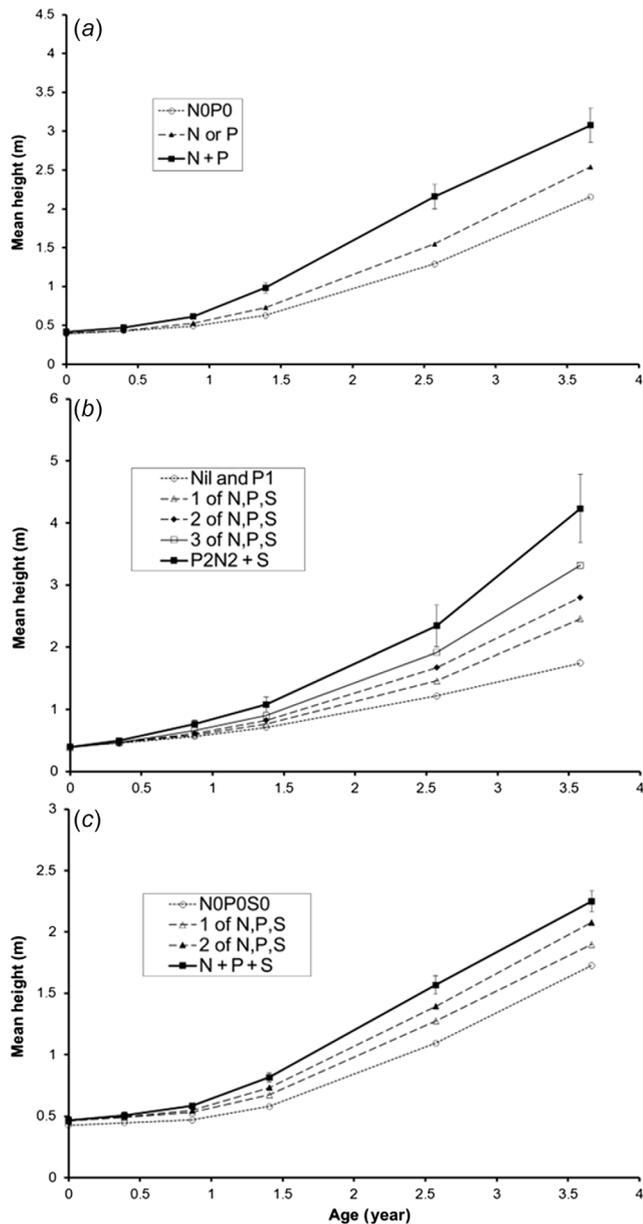
\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

increase to the mean of N<sub>2</sub>P<sub>2</sub>S<sub>1</sub> and N<sub>2</sub>P<sub>2</sub>S<sub>2</sub> which were not significantly different (Fig. 2b).

At GP, the responses were similar to HD, but heights were only approximately half those at HD (Table 5, Fig. 2c). These responses were repeated at the 1.4-, 2.6- and 3.6–3.7-year measurements. The best height growth at all sites occurred with multiple nutrient additions.

When height increments from the three sites were combined, there were significant responses to N, P and S (Table 6). Most response was to the first application level, with usually negligible additional response from higher application rates. These responses were repeated at later measurements. Rearranging the data presented in Tables 3–5 showed the additive effects were substantial. The addition of one of the main treatments, N<sub>1</sub>P<sub>0</sub>S<sub>0</sub> or N<sub>0</sub>P<sub>0</sub>S<sub>2</sub> for instance, increased growth over the N<sub>0</sub>P<sub>0</sub>S<sub>0</sub> treatment; two of the main treatments, N<sub>1</sub>P<sub>1</sub>S<sub>0</sub> or N<sub>0</sub>P<sub>2</sub>S<sub>1</sub> for instance greater again, and the mean of the 16 N + P + S plots much larger than N<sub>0</sub>P<sub>0</sub>S<sub>0</sub> (Table 7). The general pattern was a response to P applications, mainly on N<sub>1or2</sub> plots, a response to N on P<sub>1or2</sub> plots but not on P<sub>0</sub> plots, and a response to S on N + P plots. The addition of D<sub>BH</sub> measurements at HD at age 3.74 years to calculate a stem volume (V<sub>c,BH</sub>) resulted in an increased spread between treatments (Table 7).

By age 1.5 years after planting, some trees, usually on higher growth plots at TW and GP and becoming more frequent as the dry season progressed, were showing signs



**Fig. 2.** Effects of N, P and S factorial experiment on mean tree heights at (a) Three Ways to illustrate the N and P responses; (b) Humpty Doo; and (c) Gunn Point to illustrate the N × P × S interaction.

of ill health, a slight irregular shortening of needles near the growing tips of some trees. Later in the dry season, trees developed a tufted appearance, needles at the distal end of growth flushes were shorter than those at the proximal end or did not extend at all (Fig. 1). This did not seem to be related to treatments. By age 2.3 years, trees treated with N + P + S had the best needle colour and growth, but trees started to show signs of ill health on new growth at all three sites. By age 2.9 years, late in the dry season, most trees at GP and TW were affected, but at TW an irregular patch of trees, approximately a third of the experiment, had

**Table 4.** Mean height increments of the main treatments (N, P and S) after the initial measurement at 0.07–0.15 years by time, and the mean main effect N, P and S concentrations in needles at Humpty Doo.

Age (years)	Height (m)					Needle (mg g <sup>-1</sup> )		
	0.3	0.9	1.4	2.6	3.67	1.6	2.7	3.6
<b>N</b>								
N <sub>0</sub>	0.06	0.2	0.37	1.09	2.04	6.98	7.46	5.96
N <sub>1</sub>	0.08	0.25	0.49	1.40	2.68	7.98	8.36	6.81
N <sub>2</sub>	0.08	0.26	0.50	1.48	2.86	7.89	8.27	7.29
	*	*	**	***	***	**	**	**
<b>P</b>								
P <sub>0</sub>	0.07	0.21	0.41	1.21	2.35	0.59	0.67	0.57
P <sub>1</sub>	0.07	0.24	0.46	1.34	2.58	0.69	0.88	0.79
P <sub>2</sub>	0.08	0.26	0.49	1.42	2.65	0.79	0.98	0.94
	ns	*	ns	ns	ns	***	***	***
<b>S</b>								
S <sub>0</sub>	0.07	0.2	0.39	1.07	1.92	0.51	0.26	0.37
S <sub>1</sub>	0.08	0.25	0.48	1.49	2.81	0.60	0.37	0.71
S <sub>2</sub>	0.08	0.26	0.49	1.41	2.85	0.60	0.50	0.68
	ns	*	*	***	***	***	***	***

ns, not significantly different.  
\*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

**Table 5.** Mean height increments of the main treatments (N, P and S) after the initial measurement at 0.07–0.15 years by time, and the mean main effect N, P and S concentrations in needles at Gunn Point.

Age (years)	Height (m)					Needle (mg g <sup>-1</sup> )	
	0.4	0.9	1.4	2.6	3.7	1.6	2.6
<b>N</b>							
N <sub>0</sub>	0.03	0.07	0.22	0.77	1.36	5.36	5.94
N <sub>1</sub>	0.04	0.10	0.30	0.94	1.69	6.50	6.88
N <sub>2</sub>	0.04	0.10	0.32	1.12	1.79	7.21	6.87
	**	*	***	***	***	***	***
<b>P</b>							
P <sub>0</sub>	0.04	0.08	0.24	0.91	1.58	0.37	0.62
P <sub>1</sub>	0.03	0.09	0.29	0.91	1.60	0.57	1.13
P <sub>2</sub>	0.04	0.11	0.31	1.01	1.66	0.64	1.14
	ns	*	*	*	*	***	***
<b>S</b>							
S <sub>0</sub>	0.03	0.07	0.24	0.85	1.55	0.42	0.49
S <sub>1</sub>	0.04	0.10	0.30	0.99	1.59	0.55	0.52
S <sub>2</sub>	0.04	0.10	0.29	0.99	1.70	0.53	0.51
	**	*	*	**	ns	**	ns

ns, not significantly different.  
\*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

the condition more severely than those at GP, and the condition was present but less severe at HD. This was

**Table 6.** Mean height increments (m) from data combined across all sites in response to the three levels of each of N, P and S.

Application level	Element		
	N***	P***	S***
N <sub>0</sub> /P <sub>0</sub> /S <sub>0</sub>	0.289	0.316	0.316
N <sub>1</sub> /P <sub>1</sub> /S <sub>1</sub>	0.413	0.401	0.403
N <sub>2</sub> /P <sub>2</sub> /S <sub>2</sub>	0.411	0.411	0.404

\*\*\**P* < 0.001.**Table 7.** Mean height increments (m) after the initial measurement at Three Ways, Humpty Doo and Gunn Point, and *V*<sub>c,BH</sub> at Humpty Doo (HD) (m<sup>3</sup> ha<sup>-1</sup>) to illustrate the additive effects of the N × P × S interaction.

Age (year)	Three Ways			Humpty Doo			Gunn Point			HD
	1.4	2.6	3.7	1.4	2.6	3.6	1.4	2.6	3.67	<i>V</i> <sub>c,BH</sub> 3.6
N <sub>0</sub> P <sub>0</sub> S <sub>0</sub>	0.29	1.03	1.98	0.30	0.81	1.37	0.18	0.62	1.25	0.49
1 of N, P or S	0.28	1.05	1.93	0.36	1.02	1.93	0.21	0.81	1.44	1.58
2 of N, P or S	0.46	1.44	2.49	0.44	1.31	2.46	0.27	0.93	1.62	3.52
N + P + S	0.59	1.74	2.66	0.55	1.64	3.23	0.35	1.10	1.78	6.53
Best treatment	N <sub>2</sub> P <sub>2</sub> S <sub>2</sub>			N <sub>2</sub> P <sub>2</sub> S <sub>2</sub>			N <sub>2</sub> P <sub>1</sub> S <sub>1</sub>			N <sub>2</sub> P <sub>2</sub> S <sub>2</sub>

reflected in mean height increments, best growth at HD, worse growth overall at GP, and the worst growth on the affected patch at TW. Fast growing trees seemed to be more likely to have unhealthy growing tips than slower growing trees. For instance, at TW 28% of N<sub>0</sub>P<sub>0</sub> trees had short needles (<5 cm), little different on N<sub>1</sub> or 2P<sub>0</sub> trees (33%), a little lower on N<sub>0</sub>P<sub>1</sub> or 2 (21%), and higher on N + P trees (43%, *P* < 0.01).

Needle concentrations of N, P and S tended to respond in tandem with treatments (Tables 3–5), and for the most part concentrations of Ca, Mg, Na, Fe, Mn, Cu, B, and Al were not significantly affected by treatments. At 2.6–2.7 years, mean foliar Zn concentration at TW was 4.6 μg g<sup>-1</sup>, (3.4 μg g<sup>-1</sup> in the poor patch and 5.2 μg g<sup>-1</sup> in the better parts), 4.1 μg g<sup>-1</sup> at HD and 3.3 μg g<sup>-1</sup> at GP (Table 8). Mean needle Al

**Table 8.** Mean needle Zn, B, Cu, Mn and Al concentrations (μg g<sup>-1</sup>) at Three Ways, Humpty Doo and Gunn Point, and growing tips at Humpty Doo (HD).

Age (year)	Three Ways		Humpty Doo			Gunn Pt	HD Tips
	2.73	3.71	2.70	3.64	6.02	2.64	6.02
Zn	4.64	4.33	4.13	4.68	5.79	3.34	7.32
B	na	17.2	16.5	15.7	35.7	14.8	38.7
Cu	2.26	2.43	2.27	4.22	1.52	2.14	2.15
Mn	na	184	216	271	272	55	129
Al	475	464	388	410	357	314	178

na, not analysed.

concentration at GP at 3.71 years was 314 μg g<sup>-1</sup>, a little higher at HD and increased by P applications (P<sub>0</sub> 431.8 μg g<sup>-1</sup>, P<sub>1</sub> 482.8, P<sub>2</sub> 481.3, *P* < 0.01), and an N × P interaction at TW (N<sub>0</sub> and P<sub>0</sub> 434 μg g<sup>-1</sup>, N + P 505 μg g<sup>-1</sup>, *P* < 0.05).

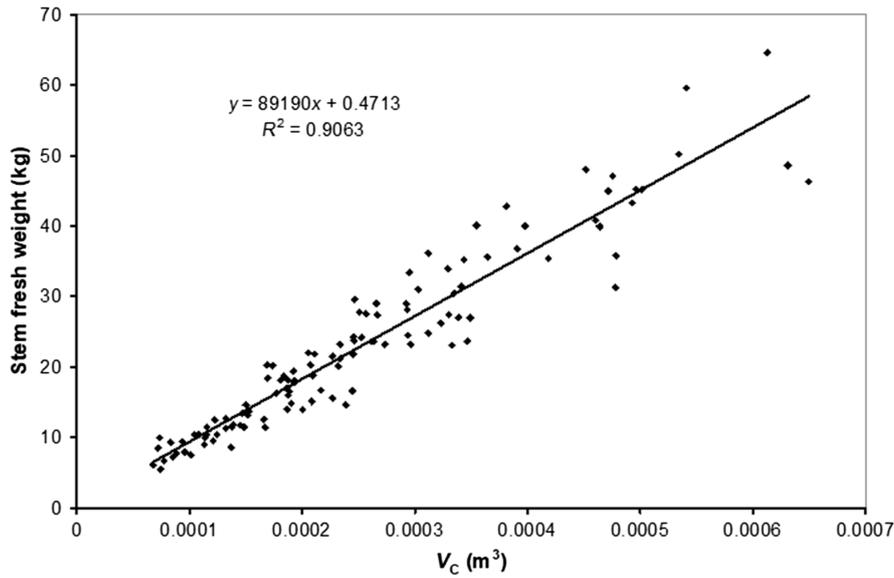
There was a significant relationship between stem volume (*V*<sub>c,GL</sub>) and stem biomass for trees more than 5 m tall (*R*<sup>2</sup> = 0.91, Fig. 3), but the relationship was not precise enough to discriminate between treatments due to variable stem taper, particularly at the base.

## Second phase experiments

After fire had removed the litter layer at the start of the second phase experiments at HD, interrupting nutrient cycling, responses to factorial N, P and S treatments remained similar to the first phase (Supplementary Tables S1 and S2). The effect of S<sub>1</sub> applications was perhaps more pronounced, but with little further growth to S<sub>2</sub>. The additive effects of the main treatments were substantial.

Responses to K applications at TW increased foliar K concentrations (K<sub>0</sub> = 2.5 mg/g, K<sub>1</sub> 3.2 mg/g, *P* < 0.001), but there was no significant height increase. Unhealthy trees with Zn chelate sprayed onto the needles improved rapidly in visual health, but there was no apparent response to 9 kg Zn ha<sup>-1</sup> applied to the soil surface around the trees within 1 year. Height increments after soil-surface Zn and Cu was applied at HD at 5.91 years were not significantly different by age 6.54 years, but diameter increments were increased by Zn applications (*P* < 0.01; Table 9), and the response persisted. By age 7.57 years, some trees were over 12 m tall. When expressed as *V*<sub>c,BH</sub> increments from 5.91 to 7.57 years, Zn applications tended to suppress growth on N<sub>0</sub>P<sub>0</sub>S<sub>0</sub> treatment, had little consistent effect on combinations with one or two of the main treatments, and increased growth in N + P + S combinations (Table 9). There was no significant growth response to Cu applications.

A striking feature of exchangeable cation concentration measurement at TW was the wide range of values over short distances. Soil samples a metre apart had very different cation profiles, both in absolute concentrations and in ratios between cations (Table 10). Concentrations of cations along the transects were examined in relation to land preparation, fertiliser treatment, and tree growth and health. Variation of Ca and Na concentration along the transects was only partly related to applied treatments in plots (S was applied as CaSO<sub>4</sub> and P as NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O). Mean exchangeable Mg concentrations were significantly lower on the cleared (now plantation) site than those on the undisturbed forest (*P* < 0.001), and mean concentrations of Ca, Na and the total of the five concentrations in the poor patch were significantly lower than the undisturbed forest soil (*P* < 0.01). Mean K and Mn concentrations were not significantly different. High Ca concentrations were associated with low K/Ca ratios, and low Ca concentrations with high K/Ca ratios (Fig. 4).



**Fig. 3.** Stem fresh biomass estimated by the conical stem volume ( $V_{c, GL}$ ) model for trees taller than 5 m.

**Table 9.** Effects at Humpy Doo plots from age 5.91 years of treatment with  $ZnSO_4$  and  $CuSO_4 \cdot 5H_2O$  on (A) mean height and  $D_{BH}$  increments, and (B)  $V_{c, BH}$  increments.

<b>(A) Zn effects on height and <math>D_{BH}</math> increment</b>					
Age (year)	Height increment (m)		$D_{BH}$ increment (cm)		
	6.54	7.57	6.54	7.57	8.79
$Zn_0$	0.52	1.70	0.50	1.55	2.70
$Zn_1$	0.55	1.80	0.58	1.77	3.03
	ns	ns	**	**	***
<b>(B) Zn and Cu effects on mean <math>V_{c, BH}</math> (<math>m^3</math>) from 5.91 to 7.57 years</b>					
	$Zn_0Cu_0$	$Zn_0Cu_1$	$Zn_1Cu_0$	$Zn_1Cu_1$	
$N_0P_0S_0$	0.0055	0.0055	0.0048	0.0048	
1 of N, P or S	0.0060	0.0062	0.0085	0.0077	
2 of N, P or S	0.0078	0.0088	0.0086	0.0073	
N + P + S	0.0104	0.0089	0.0121	0.0116	

ns, not significantly different.  
\*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

## Discussion

### N, P and S fertilisation

The combined primary growth response was to P, N and S, and probably in that order, the best growth on N + P + S treatments. The main responses were consistent with other work in the Northern Territory (Cameron et al. 1981, 1982; Rance et al. 1982, 1983), and with preliminary glasshouse experiments (Rance et al., 2020). Indeed, multiple element responses are common across a variety of savanna and grassland ecosystems (Devi Kanniah et al. 2010; Fay et al. 2015),

**Table 10.** Exchangeable Ca, Mg, Mn, K and Na concentrations and total of those five cations ( $cmol\ kg^{-1}$ ), and concentration range along transects at Three Ways.

	Poor-performing plantation soil		Better-performing plantation soil		Undisturbed native forest	
	Mean	Range	Mean	Range	Mean	Range
Ca	0.340	(0.144–1.023)	1.094	(0.148–2.187)	1.041	(0.343–4.363)
Mg	0.448	(0.114–1.003)	0.263	(0.114–0.396)	1.219	(0.227–1.794)
Mn	0.018	(0.005–0.048)	0.020	(0.006–0.059)	0.021	(0.006–0.046)
K	0.102	(0.060–0.205)	0.098	(0.048–0.159)	0.117	(0.090–1.151)
Na	0.069	(0.030–0.114)	0.093	(0.062–0.161)	0.109	(0.046–0.166)
Total	0.976	(0.461–2.226)	1.568	(0.513–2.948)	2.507	(0.697–6.100)

indicating that these responses were widespread. Growth in the field experiments was poorest at GP, yet best in the glasshouse on GP soil where it was not water stressed.

Despite positive growth responses to all main experimental treatments, the emergence of symptoms of ill health, most prominently on better-performing trees, provided strong evidence of further nutrient limitation. Symptoms of ill health started to appear near the tops of stems as the second dry season progressed; indicating uptake of a key element(s) was restricted in the drying soil profile, as was translocation from old growth to new. Accordingly, foliar sampling was standardised to the youngest fully expanded needles near the top of the tree, and newly extended growth flushes (branch tips) before needle extension/formation, collected near the end of the dry season when trees were stressed and conditions stable. Mean height increments increased in response to fertiliser applications when needle N concentrations were less than approximately  $7\ mg\ g^{-1}$



(Rance et al. 2020), so part of the growth responses could have been related to changes in pH and Al availability.

As a guide, most agricultural species with foliar Zn concentrations of 16–20  $\mu\text{g g}^{-1}$  were likely to respond to Zn fertiliser applications, those with concentrations of 15  $\mu\text{g g}^{-1}$  or less were probably Zn deficient (Loneragan et al. 1979; Mengel and Kirkby 1982). Foliar concentrations of Zn less than 10  $\mu\text{g g}^{-1}$  (Table 8) were consistent with a likely Zn deficiency at all three sites. Needle Zn concentrations in *P. caribaea* above ~6–7  $\mu\text{g g}^{-1}$  were associated with short needles and abnormal stem tip growth but trees still maintained reasonable growth; those <4  $\mu\text{g g}^{-1}$  were less healthy. Foliar applications of Zn chelate at TW improved the appearance and increased growth within weeks of application, confirming a Zn response (Rance et al. 1982). However, the foliar spray resulted in a negligible change in needle Zn concentrations, indicating the response would be transient, but there seemed to be little uptake from 9 kg  $\text{ZnSO}_4 \text{ ha}^{-1}$  applied to the soil surface at TW by the following year. This application rate was commonly used in agriculture, so the application rate on the second phase experiment at HD was increased to 73.4 kg  $\text{ZnSO}_4 \text{ ha}^{-1}$ . This returned a small mean diameter increment increase ( $P < 0.01$ ) within 0.63 years (Table 9), a larger response a year later. A more careful examination indicated  $V_{\text{c,BH}}$  increments were only increased with Zn in N + P + S treatments. In the second year, increments were reduced by 10% in  $\text{N}_0\text{P}_0\text{S}_0$  treatments, increased in N + P + S treatments, intermediate or inconsistent in treatments with one or two of N, P or S. Zn applications at HD indicated that the response was delayed and secondary to other responses. The role of P fertilisation in inducing or enhancing symptoms of Zn deficiency by depressing Zn absorption or diluting available Zn supplies in greater growth (Loneragan et al. 1979), also need to be considered.

A study of grassland sites across six continents identified nutrients other than N and P that were important for plant production, Zn and Fe well represented (Radujković et al. 2021). Unlike other countries, Australia does not appear to have a widespread response to Fe, perhaps related to the lack of large herds of herbivores migrating with the seasons, exporting Fe from the grasslands in haemoglobin and myoglobin. In the short term, foliar sprays seem the most efficient method of Zn application, until nutrient cycling through litterfall is re-established.

The transect soil sampling at TW indicated that clearing and root raking the sites before the trees were planted removed nutrient capital in the tree and shrub fraction off-site, concentrating it in windrows, interrupting internal nutrient cycling from old growth to new, nutrient cycling through litter fall, and exposed surface soil to erosion and further nutrient loss (Leuning et al. 1991; Saur et al. 2000; O'Connell et al. 2004). Site preparation and tree harvesting were recognised as the major mode of nutrient removal from the plantation system (Mendham et al. 2003;

O'Connell et al. 2004). A significant reduction of soil Mg concentration (Table 10), and probably in chlorophyll in foliage, indicated a likely response to Mg fertilisation in later years. Clearing trees and shrubs without burning and with minimum soil disturbance, leaving the litter/soil exposed to full sun, removed litter fall from the growth cycle (Cunningham 1963). Unshaded soil experienced higher day temperatures and lower moisture contents, reduced organic C, organic P and total N concentrations, lowered cation exchange capacity, exchangeable K and soil pH within 3 years compared with shaded soil (Cunningham 1963). Shaded soil also contained more N fixing and nitrifying bacteria than exposed soil. Litter removal reduced soil moisture content down to 90 cm and increased water stress in longleaf pine (Ginter et al. 1979), and interrupted nutrient cycling (Stocker 1981; West 1984). Further, repeated harvest residue removal reduced productivity of *E. globulus* plantations (O'Connell et al. 2003; Mendham et al. 2014).

The variability of cation concentrations across the site must be considered for any soil sampling strategy to assess nutrient stocks for site description (e.g. Table 1), assessing tree performance to experiment treatments and determining optimal fertilisation rates and composition. The mean of the five highest readings of Ca concentration in the poor patch was 0.606  $\text{cmol.kg}^{-1}$  soil (Table 10), and had no overlap with the mean of the five lowest readings (0.169). The sum of the five measured exchangeable cations (Ca, Mg, Mn, K and Na) represented approximately 40% of the cation exchange capacity in the native forest, 31% in the better-performing parts of the experiment and 20% in the poor-performing patch.

Large variation of soil conditions over small spatial scales has been attributed to historical processes affecting soil development (Phillips and Marion 2004). In our study, the substantial variation in cations at small scales might be echoes of past events such as ash beds from burning large structures like tree stems (Fig. 5), or foraging ants or grass-eating termites concentrating nutrients at nest sites. The K/Ca ratios (Fig. 4) are consistent with a long history of recurrent fires. K is volatilised at 779°C and Zn at 907°C (Latimer and Hildebrand 1956), temperatures experienced in burning wood and likely to be lost to the atmosphere, while Ca is volatilised at 1482°C, so likely to remain in ash beds. In these experiments, most of the wood and crowns in the windrows containing a significant proportion of the site nutrient capital burned during the first 2 days, but some smouldered for more than a week. Much of the site N, P, S, K and Zn, and possibly B nutrient capital would have been volatilised in high-temperature combustion in the first 2 days or lost in the smoke fraction when the windrow smouldered (Raison et al. 1985; Cook 1994; Alves et al. 2011; Pereira et al. 2011; Radujković et al. 2021), leaving refractory cations of Al, Fe, Ca and Mn in the ash bed on the exposed soil surface where they could be washed downhill or down the profile during high-intensity storms in the build-up to the wet season.



**Fig. 5.** A tree felled during a severe wet season storm and burned during the dry season. In the following wet season, grass seed blown into the ash bed germinated but died soon after. Photo: S. Rance.

## Conclusion

The close spacing in these experiments reduced the stands to a near monoculture and revealed the main treatment responses but exposed faster growing trees to environmental stresses. Establishing new crops in savanna regions of northern Australia, including exotic plantation species, are likely to need broad-spectrum fertilising and protection from fire, especially in the establishment phase. Nutrient additions will need to encompass minor and trace elements in addition to N, P and S, specifically Zn, and consider the mechanism of application. Fertiliser application rates will need to be low to minimise induced nutrient deficiencies and site run off, and consider small-scale variation in cation exchange capacity and composition. Changes of soil pH or increased risks of insect attack also need to be considered.

## Supplementary material

Supplementary material is available [online](#).

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**Data availability.** The authors will provide raw data upon reasonable request.

**Conflicts of interest.** The authors declare no conflicts of interest.

**Declaration of funding.** This work was supported by CSIRO and the Australian Government.

**Acknowledgements.** We acknowledge the Tiwi and Larrakia traditional owners of country where this work was conducted and their continuing connection to land, sea and community. We pay our respects to them and their cultures and to their elders both past, present and emerging. The close-spaced planting in these experiments was modelled on nutritional research on tropical pines by B.N. Richards in Queensland and by H.D. Waring working with *Pinus radiata*. We thank Doug Johnson as a link with the Research Advisory Committee for refining the treatments, George Petru who provided templates for statistical analysis using a desktop adding machine, and field staff of the Forest Research Institute, in particular G. Milne, J. Cusack, M. Farmer and P. Lukitsch for weed control, measuring and fertilising, and J. Maggs and C. Martin for laboratory analyses.

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