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# Predictive relationships to assist fertiliser use decision-making in eucalypt plantations

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# **Predictive relationships to assist fertiliser use decision-making in eucalypt plantations**

Prepared for

**Forest & Wood Products Australia**

by

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## **Publication: Predictive relationships to assist fertiliser use decision-making in eucalypt plantations**

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## Executive Summary

*Background:* Managers of short-rotation *Eucalyptus globulus* plantations in southern Australia have insufficient information to confidently determine when and where fertiliser should be used to maximise productivity. Many fertiliser experiments have been installed by industry, academic and government researchers over time; however, each set of experiments is limited to a small group of sites.

*Aims:* The aim of this study was to synthesise the results of such datasets to: (i) describe the magnitude and duration of growth response to fertiliser applied at establishment (age 0+1), mid-rotation (age 4-5) and both establishment and mid-rotation (age 0, 1 and 4) and (ii) to develop a robust method of identifying sites more likely to respond to establishment and/or mid-rotation fertiliser application.

*Methods:* Results from 49 experiments were compiled for this study. The magnitude and duration of growth response to 250 kg ha<sup>-1</sup> nitrogen (N) and 90 kg ha<sup>-1</sup> phosphorus (P) fertiliser, split between age 0 and age 1 (at establishment; EST) was studied at 28 sites, while response to 250 kg ha<sup>-1</sup> N-only at mid-rotation (at mid-rotation; MID) was studied at 11 different sites. Response to combined establishment and mid-rotation fertiliser application was also studied at a further 10 sites (EST+MID). Study sites covered Mediterranean and temperate climatic zones across south-western and south-eastern Australia. The number of sites and available explanatory data also facilitated use of multiple linear regression analysis to build models to predict growth response to establishment fertiliser from pre-treatment soil, foliar and climatic site variables.

*Results:* Fertiliser applied at establishment increased final standing volume by 5.6%, whereas mid-rotation applications increased volume by 20.8% at responsive sites. The subset of sites receiving both establishment and mid-rotation fertiliser showed a 10.6% increase in volume relative to control treatments. Differences in volume growth response, particularly with regard to the magnitude of response to mid-rotation fertiliser were most likely due to lower N-status or higher N-demand of MID sites compared with EST+MID. Relative volume growth responses to N lasted approximately 3-4 years, suggesting that at least two applications of fertiliser are required at sites which require fertiliser. To that end, we also made significant gains in identifying sites more likely to respond to fertiliser. Multiple linear regression analysis identified several models, with the most accurate accounting for 74% of variation in volume growth response to establishment fertiliser at age 2; using pre-treatment soil (0-10 cm) Hot KCl NH<sub>4</sub>+NO<sub>3</sub>-N, the foliar N/P ratio and the long-term climate wetness index of a site. An alternative soil-based model eliminated the need for both a diagnostic soil and foliar sample with only a 4% loss in accuracy. Volume response to fertiliser could also be predicted out to age 4; however, the best (soil-based) model only accounted for 60% of variation. Volume growth responses to mid-rotation fertiliser were more difficult to predict, with the best model using foliar N:P and long-term mean annual rainfall to predict response with only 43% accuracy.

*Conclusions:* Significant increases in yield can be achieved through targeting sites highly responsive to fertiliser. Mid-rotation N-only applications at sites with low N-status/high N-demand have significant potential to yield greater volume at end of rotation. We have successfully developed methods to identify those sites more likely to respond to fertiliser applied at establishment, based on basic soil tests and/or foliar nutrient concentrations in combination with long-term climate data. More work is required to develop models which can predict response to mid-rotation applications of N.

*Practical Applications:* We suggest that plantation managers can utilise the models presented here as tools to rank sites according to predicted fertiliser response. Depending on estate size, fertiliser budget and anticipated economic returns, plantation managers can set a threshold volume growth response, below which they can elect not to apply fertiliser. In this way, limited fertiliser resources can be deployed across the estate to maximise returns and minimise application of fertiliser where it is least likely to result in a volume growth response. This study presents the opportunity for forest managers and researchers to now validate the models we present for a wider range of sites, to continually improve accuracy of prediction and confidence in nutrition management. To that end, we briefly outline a methodology for proceeding with validation and suggest avenues of future research based on our findings.

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## Introduction

Since the 1990's there has been a rapid expansion of hardwood plantations in Australia, presently covering a total area of almost 1 million hectares (Gavran and Parsons, 2010). The national plantation estate is dominated by *Eucalyptus globulus* (55%) and *E. nitens* (24%) mainly in the southern regions of Western Australia, South Australia, Victoria and Tasmania covering a range of soil types and climatic conditions. The majority of plantations are managed for pulpwood on 10 to 15 year rotations. Many plantations were established on ex-agricultural land with elevated nutrient resources achieved through annual inputs of fertiliser (Wang *et al.*, 1998). However, many sites showed significant growth responses to nitrogen (N) and phosphorus (P) fertiliser addition (Cromer *et al.*, 1975; Birk and Turner, 1992; Cromer *et al.*, 1993a; Misra *et al.*, 1998a), albeit often constrained by availability of other macro- and micronutrients, as well as by water availability (Bennett *et al.*, 1997; White *et al.*, 2009). Fertiliser application remains the major method of ameliorating nutrient deficiencies and increasing productivity of Australian plantations (May *et al.*, 2009b).

In short-rotation eucalypt plantations, fertiliser additions are divided into two phases: prior to canopy closure and post-canopy closure, with canopy closure typically occurring between ages 3-6 in Australian plantations (Forrester *et al.*, 2010a). Fertiliser application is targeted pre-canopy closure as growth rates and therefore demand for nutrients are higher (Miller, 1981; Stape *et al.*, 2004; Laclau *et al.*, 2010). During this phase, N and P fertiliser, often blended with sulphur (S), potassium (K), copper (Cu) and zinc (Zn), fertiliser is typically applied (i) at age 0, where a low rate of fertiliser is typically applied 4-6 weeks post planting via a spot application next to each seedling or banded along planting lines, (ii) at age 1, where larger rates of fertiliser are banded along planting lines and (iii) at age 4-5, where high rates of fertiliser are broadcast across the plantation just prior to canopy closure (May *et al.*, 2009b). At age 0 and 1 (at planting and during establishment), trees may be limited by nutrient availability depending on site nutrient capital; however, they are typically not limited by water (Gonçalves *et al.*, 2004; Gonçalves *et al.*, 2008). The benefits of pre-plant cultivation in accelerating root growth, combined with weed control in limiting competition are as, if not more important than fertiliser addition, depending on site nutrient capital (Pallett and Sale, 2004; Gonçalves *et al.*, 2008; du Toit *et al.*, 2010). At age 4-5 (mid-rotation), stands are typically approaching canopy closure and plantations are thought to be more limited by both nutrients and water as inter-tree competition increases while roots are yet to access water deep in the soil profile (Gonçalves *et al.*, 2004).

While responses to P are generally long-lasting, responses to N are of short-duration (4-6 years) and several applications may be required to maximize growth over a rotation (McGrath *et al.*, 2003). Low N supply was identified as the main limitation to growth of *E. nitens* on ex-native forest sites in Tasmania (Smethurst *et al.*, 2004) and two-year responses to N (200 kg ha<sup>-1</sup>) were well correlated with total N in surface soils and significantly increased growth at levels below 4 g kg<sup>-1</sup>. Multiple applications of N fertiliser were required to prevent N deficiency and maximize productivity over 10 years on soils with low N. Treatment with N fertiliser has only a transient impact on N levels in eucalypt foliage but increases foliage biomass and leaf area index (LAI), a key driver of the rate of growth. LAI responses to N were shown to be strongly correlated with growth of fertilized *E. nitens* in Tasmania (Smethurst *et al.*, 2003). Likewise variation in LAI and growth of *E. globulus* for a range of sites in Western Australia was related to climate wetness index and soil depth (White *et al.*, 2009). Growth and LAI increased in response to N fertiliser when total N in surface soils was less than 2 g kg<sup>-1</sup>; however this also increased water stress and enhanced the risk of drought death on water limited sites. However, fertiliser in combination with thinning to 600 stems

ha<sup>-1</sup> reduced water stress and also maintained site productivity compared with unthinned stands (White *et al.*, 2009).

A recent review of the nutrient management of Australian hardwood plantations (May *et al.*, 2009b) reinforced the considerable potential for improved growth and productivity with N and P fertilisers, but found large inconsistencies with regard to: (i) the expected magnitude and duration of growth response to fertiliser and (ii) methods of identifying sites likely to respond to fertiliser and predicting the impact on productivity. Fertiliser use for hardwood plantations is a significant component of the cost of wood production and therefore identification of sites requiring treatment and modelling of fertiliser responses over time are important in terms of the financial management of plantations (May *et al.*, 2009b). Operational prescriptions are often over-generalised by climate region, soil class and prior land use, leading to inefficient and or poor targeting of fertiliser use. The inability to make validated estimates of growth response to fertiliser, coupled with a poor understanding of the frequency at which fertiliser should be applied; reduces confidence in financial analyses based on these estimates. The prediction of growth responses to fertiliser was identified by forest managers as a major knowledge gap (May *et al.*, 2009b). Process-based models (e.g. Landsberg and Waring, 1997; Battaglia *et al.*, 2004) are increasingly being used in plantation management and have the functionality to incorporate application of fertiliser in silvicultural regimes. Unfortunately, these models are not well-validated and as such are rarely used for nutrition management by the industry.

Significant gains have been made with regard to predicting response to fertiliser in eucalypt plantations. These methods mainly focussed on soil testing to identify responsive sites and to predict early growth responses, many of them related to N-status indicators including total N, total C, ammonium (NH<sub>4</sub>) and nitrate (NO<sub>3</sub>) (Cromer *et al.*, 2002; Moroni *et al.*, 2004; Smethurst *et al.*, 2004; Mendham *et al.*, 2009). Indicators of P-status including both extractable P and total P have also shown the capacity to predict response to N and P fertiliser (Mendham *et al.*, 2002; Smethurst *et al.*, 2004). For example, extractable soil P (Bray2 P and CaCl<sub>2</sub> P) was used to identify P deficient sites and was shown to correlate well with first year growth responses to phosphate fertiliser for a limited range of P-fixing soil types (Mendham *et al.*, 2002). Despite these successes, this work has not been expanded across a wide range of sites and many of these soil indicators are still not used as standard operational practice for the nutrient management of eucalypt plantations. A wide range of other variables including soil texture, available water, availability of potassium (K) and magnesium (Mg) have also been related to responses to N and P fertilisers applied early in the rotation (Bennett *et al.*, 1996; Judd *et al.*, 1996; Bennett *et al.*, 1997; Watt *et al.*, 2008); although not in a predictive capacity. Water limitations and deficiencies in macro- and micro-nutrients other than N and P often affect responses to N and P fertilisers (Turnbull *et al.*, 1994; Bennett *et al.*, 1996), making it difficult to develop generic models to predict response. Apart from N and P, few soil tests have been developed for the diagnosis of deficiencies of other nutrients essential for tree growth and therefore foliage analysis is widely used for the diagnosis of nutrient deficiencies and disorders, particularly in young (pre-canopy closure) stands (Dell *et al.*, 2001; May *et al.*, 2009b). Foliar analysis has been used to identify copper (Cu) deficiency induced by N and P fertilisers applied to *E. nitens* plantations in Tasmania (Turnbull *et al.*, 1994) and in *E. maculata* plantations in Western Australia (Dell and Bywaters, 1989). Foliar ratios of N and P have been used to track response to fertiliser (Cromer and Williams, 1982; Schönau and Herbert, 1982; Judd *et al.*, 1996); however, there are no models which can predict growth response to fertiliser from foliar nutrient concentrations.

Foresters need robust, reliable tools to predict growth response to fertiliser. The development of plantation management tools can take longer than a single plantation rotation which is an

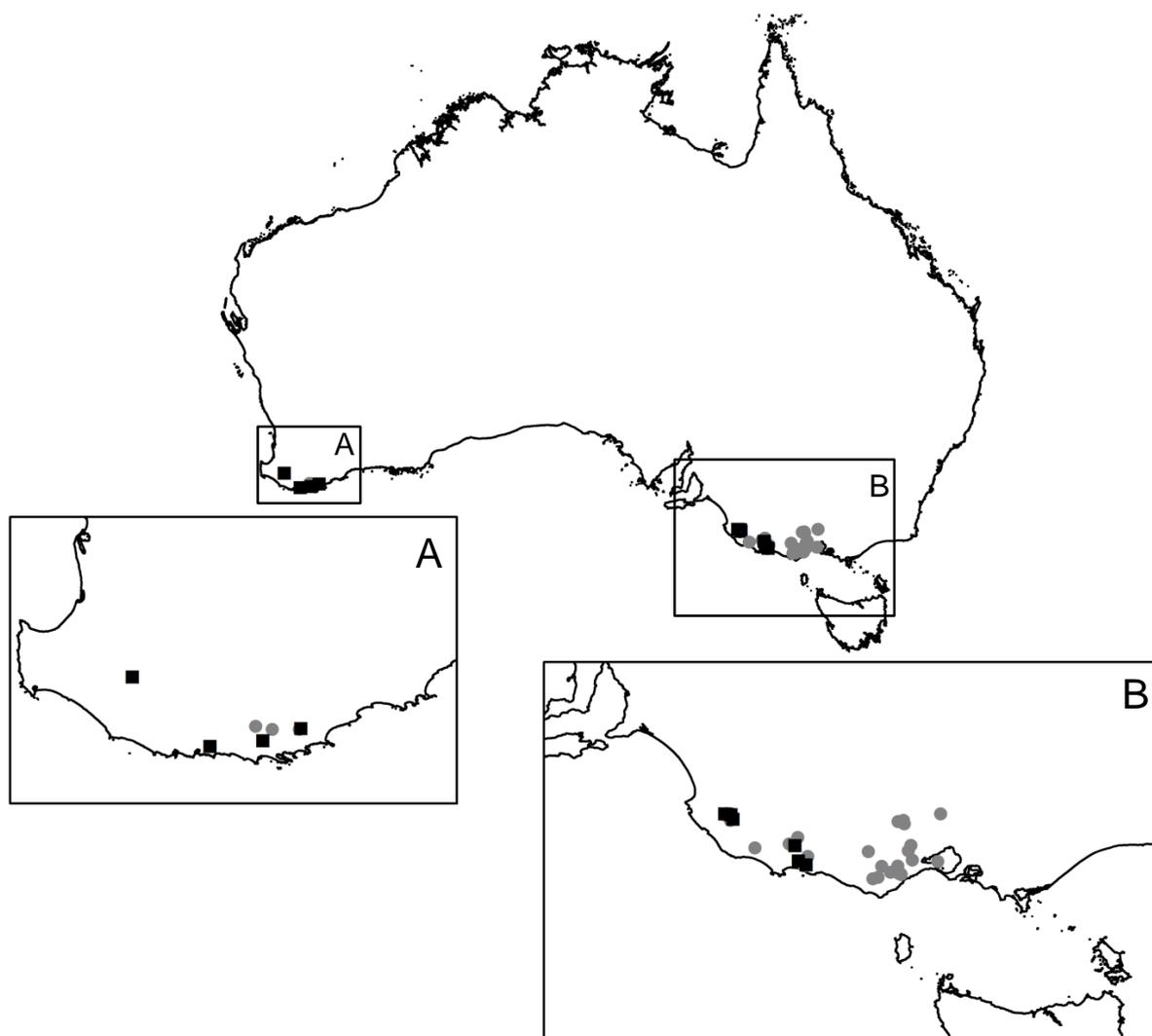
unacceptable time-frame for the industry. Through industry initiative and in collaboration with research organisations, there exists a large body of data from isolated fertiliser trials held by the industry; representing a significant untapped resource for improving management of plantation nutrition. Fertiliser trials in particular which included plant and soil based nutrition data prior to application of fertiliser, when combined with rotation-length growth data, have the capacity to be developed into robust models which can be used to predict growth response to fertiliser.

This study represents an inter-organisational synthesis of data designed specifically to meet the needs of foresters and plantation managers. International research efforts have previously developed links between plantation productivity and site factors including nutrition (Stape *et al.*, 2006; Watt *et al.*, 2008); however, relationships which predict site response of eucalypt plantations to fertiliser for a diverse range of sites have not been explored in Australia. In this study, we will collate and analyse several long-term fertiliser trial datasets for *Eucalyptus globulus* plantations across a wide range of sites with a view to: (i) describing the magnitude and duration of response to fertiliser and (ii) developing practical predictive relationships of growth response to fertiliser application.

## **Methodology**

### **Study sites and fertiliser treatments**

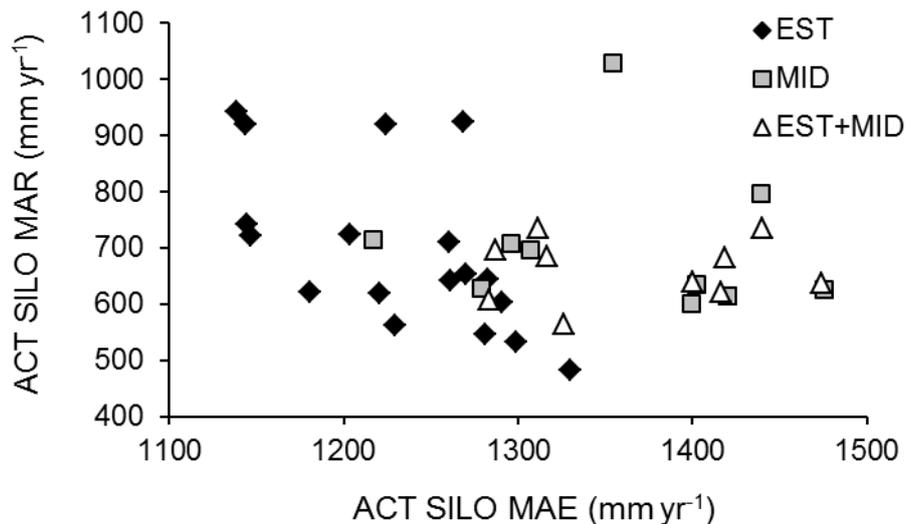
Results from 49 experiments in blue gum plantations across southern Australia were analysed to: (i) quantify the magnitude and duration of growth responses to fertiliser application and (ii) identify site variables which can be used to predict the magnitude of growth responses to fertiliser. The experiments encompassed a range of climatic conditions and site qualities across southern Australia (Figures 1-3; Tables 2-4). The data contributed to this study represent the research activities carried out by plantation managers: Australian Bluegum Plantations and Midway Limited. Fertiliser experiments ran between 1993 and 2010 and while experimental design focussed on addition of N and P, the timing and rate of fertiliser applied differed slightly (Table 1). Where the design of such experiments was similar (Table 1), data were grouped by type (~age) of application. Response to applications at age 0 and 1 (hereafter ‘establishment’ or ‘EST’) were measured at 28 sites and response to age 4-5 (hereafter ‘mid-rotation’ or ‘MID’) applications of fertiliser at 11 sites. Ten of the sites used to study response to establishment fertiliser also had additional treatments which included a combined application of both establishment and mid-rotation fertiliser, hereafter referred to as ‘EST+MID’ (i.e., fertiliser applied at age 0, 1 and 4 years). Establishment N and P fertiliser was applied as a DAP or MAP/Urea blend, applied as a spot treatment at age 0 and as a band along the planting line at age 1. Mid-rotation N fertiliser was applied as urea and broadcast across the site.



**Figure 1.** Study site locations in south-western (A) and south-eastern (B) Australia. Grey circles represent sites where establishment fertiliser was applied (n=28) and black squares represent sites where mid-rotation fertiliser was applied (n=11). Establishment fertiliser sites in western Victoria, South Australia and Western Australia were also used to study the combined effect of establishment and mid-rotation fertiliser (n=10). Base map sourced from Geoscience Australia.

**Table 1.** Details of experiments used to determine magnitude and duration of, as well as to predict, growth response to fertiliser.

<b>Application type</b>	<b>Establishment</b>	<b>Mid-rotation</b>	<b>Establishment and mid-rotation</b>
<b>Code</b>	<b>EST</b>	<b>MID</b>	<b>EST+MID</b>
~Age at application (years)	Age 0 and 1	Age 4-5	Age 0, 1 and 4
Number of experiments	28	11	10
Fertiliser applied at age 0	40-52 kg ha <sup>-1</sup> N and 27-35 P kg ha <sup>-1</sup>	0	40-52 kg ha <sup>-1</sup> N and 27-35 P kg ha <sup>-1</sup>
Fertiliser applied at age 1	200 kg ha <sup>-1</sup> N and 50-62 kg ha <sup>-1</sup> P	0	200 kg ha <sup>-1</sup> N and 50-62 kg ha <sup>-1</sup> P
Fertiliser applied at age 4-5	0	250 kg ha <sup>-1</sup> N	200 kg ha <sup>-1</sup> N
~Age at measurement (years)	2-5, 7 and 10	5-8 and 10	2 and 4-10



**Figure 2.** Mean annual rainfall (MAR) and mean annual evaporation (MAE) for all study sites over the duration of each experiment. Symbols indicate whether sites were used to study responses to establishment (EST; n=28), mid-rotation (MID; n=11) or the combined effect of both establishment and mid-rotation (EST+MID; n=10) fertiliser. Sites used to study EST+MID were also used to study response to EST fertiliser only. Data were sourced from SILO and represent average values for the actual growth period for each experiment.

### Data eligibility for inclusion in analysis

Data from 115 experiments provided by a number of plantation managers were initially compiled for this study; however, many did not meet the basic criteria required to achieve our objectives. To be eligible for use in this study, each experiment required a minimum of two fertiliser treatments: a high rate of nitrogen (with or without phosphorus) and an unfertilised control. A minimum of 3 replicates (plots) were required for each treatment and fertiliser treatments needed to be applied during spring. Regular growth measurements (every 1-3 years) from time of fertiliser application until age 10 were required to assess both the magnitude and duration of fertiliser growth response. Minimum growth measurements required for each plot were: (i) the diameter over bark of all stems at 1.3 m and (ii) the height of the 100 largest-diameter trees ha<sup>-1</sup>; to determine changes in stand level volume and sub-stand level basal area. Plots required a minimum threshold of 60% survival relative to original stocking to be included in the analysis. Further, the pre-treatment volume of fertilised and control plots needed to be similar. Finally, to predict the magnitude of growth response to fertiliser application, it was also critical that each experiment have either pre-treatment topsoil or foliar nutrient analysis data.

### Approach to analysis of magnitude and duration of response to fertiliser

A range of yield and growth responses were found within the 28 sites used for establishment fertiliser application, the 11 sites used for mid-rotation applications and the 10 sites receiving both. Rather than combining all experiments into a single average for each application type; we instead split each set of the experiments into those identified as ‘**more responsive**’ and ‘**less responsive**’ to fertiliser; i.e. those which showed a large growth response and those which showed little or no change in growth following fertiliser application. More responsive experiments were defined as those where fertilised treatments increased volume by more than 10% compared with control treatments for at least 2 years post-application of fertiliser (at any point in the rotation); and less responsive sites where fertiliser responses were less than 10%.

For establishment application experiments, 20 sites were identified as more responsive and 8 as less responsive, while for mid-rotation applications; 5 experiments were identified as more responsive and 6 as less responsive. For combined establishment and mid-rotation experiments, 7 were more responsive and 3 were less responsive.

Descriptive site data in the form of long-term climatic, as well as pre-treatment soil and foliar data (see section: *‘Variables used to predict relative volume growth response to fertiliser’*), were analysed (see section *‘Statistical analyses’*) to determine whether any inherent differences existed between sites identified as more or less responsive, as well as between application ages. This was particularly important when comparing the magnitude and duration of growth response to fertiliser between application ages, to determine whether observed differences in response were more likely due to application age, or to other site or climatic factors. For example, higher relative growth responses at mid-rotation compared to establishment may be due to higher mean annual rainfall at the mid-rotation sites compared with the establishment sites. This descriptive site data is presented in Tables 2-4 (see results section: *‘Pre-application differences in climate, soil and foliar variables within and between application age and response type’* for analysis).

### **Magnitude and duration of volume growth response to fertiliser (stand level)**

Growth response to fertiliser at the stand level was calculated by first calculating standing volume ( $\text{m}^3 \text{ha}^{-1}$ ) of each plot using a stand volume equation:  $1/3 \times \sum \text{BA} \times \text{MDH}$ ; where  $\sum \text{BA}$  is the sum of the basal area of all trees in a plot ( $\text{m}^2 \text{ha}^{-1}$ ) and MDH is the mean dominant height of the 100 largest-diameter trees  $\text{ha}^{-1}$  (m). Mean standing volume was then calculated for both fertilised and unfertilised control treatments for each experiment at each measurement age.

Site quality naturally varied between experiments (Figure 3), therefore the magnitude and duration of volume growth response to fertiliser was analysed on both a relative and absolute basis for ‘more’ and ‘less responsive’ sites. Assessing relative responses (i.e. volume of treatment relative to volume of control) allowed us to (i) present the mean volume growth response to fertiliser for both more and less responsive site types of the same application age over time and (ii) use multiple linear regression analysis to predict volume growth response from pre-treatment site variables for establishment, as well as mid-rotation applications. The relative volume growth response for each experiment was calculated as:  $(\text{mean treatment volume} - \text{mean control volume}) / \text{mean control volume} \times 100\%$ .

Interpretation of relative growth responses alone can be misleading or difficult to interpret without some understanding of absolute responses; therefore both total standing volume and current annual increment of basal area were calculated for both control and fertilised treatments for each experiment within each application age. Periodic annual increment (PAI) of basal area (BA) was calculated as, for example:  $\text{PAI (at age 2)} = (\text{BA at age 3} - \text{BA at age 2})$ . Relative and absolute volume growth response was calculated for each experiment at each measurement age, according to available measurement data (Table 1).

Growth response to fertiliser application was calculated every 1-3 years, depending on available data, to allow analysis of changes in the magnitude of volume growth response over time. Statistical comparisons of growth were not made between ‘more’ and ‘less responsive’ sites as they were sorted into these categories based on volume growth response, therefore comparison of differences between these groups is irrelevant.

## **Magnitude and duration of relative basal area growth response to fertiliser (sub-stand level)**

Sub-stand (tree-level) analysis was limited to assessing changes in tree basal area, rather than volume, as the bulk of the dataset only contained height measurements for the 100 largest-diameter trees. Basal area growth response to fertiliser for each application age was first determined by calculating individual tree basal areas for each plot at each measurement age. Each plot was then split into quarters (quartiles) based on stand basal area distribution. For example, the first quartile (0-25%) was comprised of the smallest 25% of trees in a stand. The second quartile (25-50%) was delineated at the top by identifying the value below which 50% of trees in a stand fell and at the bottom by removing trees already represented in the first quartile and so on. The sum of all individual tree basal areas within each quartile was calculated for both fertilised and control plots at each site, at each measurement age. The magnitude and duration of basal area growth response to fertiliser of each quartile was analysed on relative terms as per volume growth response. The relative basal area (BA) growth response of each quartile at each site was therefore calculated as:  $(\text{mean treatment BA [quartile]} - \text{mean control BA [quartile]}) / \text{mean control BA [quartile]} \times 100\%$ . Basal area growth response for each quartile was calculated at each measurement age according to available measurement data (Table 1). As per volume growth response calculations, basal area growth response for each quartile could be calculated every 1-3 years, allowing analysis of changes in the magnitude of basal area growth response over time.

To assist with interpretation of basal area response to fertiliser of each cohort, the relative contribution of each quartile to total basal area was determined for both fertilised and control plots. To calculate the proportion of total basal area represented by each quartile, the sum of the basal area of each was divided by the total basal area for each plot and the result multiplied by 100%. The results of this calculation were already expressed in relative terms and as such were averaged across all sites of the same fertiliser trial design at each measurement age.

## **Variables used to predict relative volume growth response to fertiliser applied at establishment or mid-rotation**

The central aim of this study was to identify pre-fertiliser application variables at a site which could be used to predict growth response to fertiliser. The establishment application dataset, including all sites; not only those identified as 'more responsive', presented the best opportunity to achieve this objective due to the relatively large number of sites (n=28). Further, the establishment application was a combination of N and P fertiliser, therefore it presented the best opportunity to assess and predict responses to both N and P, rather than N alone. Sites where establishment fertiliser was applied also covered a wide climatic range across southern Australia (Figure 1 and 2).

Models for predicting response to mid-rotation fertiliser do not exist and would be extremely valuable for forest managers. Therefore, to gain some understanding of the drivers of response to mid-rotation fertiliser, data were pooled from (i) the 11 MID sites and (ii) the 10 EST+MID sites, to give a total of 21 sites. To isolate the mid-rotation response in EST+MID experiments, volume growth response was determined relative to treatments receiving the same rate of establishment fertiliser, rather than relative to an unfertilised control. The relative volume growth response for each EST+MID experiment, only for the purpose of building predictive models, was therefore calculated as:  $(\text{mean volume of plots receiving establishment and mid-rotation fertiliser} - \text{mean volume of plots receiving only establishment fertiliser}) / \text{mean volume of plots receiving only establishment fertiliser} \times 100\%$ .

Multiple linear regression analysis was used to identify the best predictors (explanatory variables) of growth response to fertiliser (response variable). As a plantation manager, the capacity to predict additional volume at end-of-rotation from a single fertiliser application would be desirable; however, many factors other than site nutrient requirement can affect growth response to fertiliser over time. Therefore, several response variables were used in separate multiple linear regressions (see section: *'Regression analysis approach to predicting relative volume growth response'* for detail). For establishment fertiliser models, volume growth response was assessed: (i) 1-year post-application (at age 2) to predict the maximum site response, (ii) 4-years post-application (at age 5) to predict response until mid-rotation, and (iii) 9-years post-application (at age 10) to predict end-of-rotation response. For mid-rotation models, volume growth response was assessed: (i) 1-year post-application (at age 5), (ii) 3-years post-application (at age 7) to predict maximum site response and (iii) 6-years post-application to predict end-of-rotation response. Site factors including: (i) climatic variables, (ii) soil tests and (iii) foliar nutrient analysis were used as explanatory variables to predict volume growth response; with the aim of demonstrating that pre-application site factors can be used to predict volume growth response at a site.

### **(i) Climatic explanatory variables**

The method of acquiring and analysing climatic data was similar for both establishment and mid-rotation (including EST+MID) sites. Two sets of climate data were obtained for each site: ESOCLIM and SILO data drill. Both data sources rely on a synthetic coverage interpolated from meteorological monitoring stations across Australia. ESOCLIM data was provided as monthly values between 1921 and 1995. SILO data was sourced from the Science Delivery Division of the Department of Science, Information Technology, Innovation and the Arts (Queensland State Government) and was provided as daily values from 1889 to 2012 (Jeffrey *et al.*, 2001). Both datasets were summarised into long-term site averages for: mean annual maximum and minimum temperature, mean annual solar radiation, mean annual rainfall (MAR) and mean annual evaporation (MAE) and climate wetness index ( $CWI = MAR/MAE$ ) to develop relationships with volume growth response. SILO data was also used to determine 'actual' values for each site during the actual growth period for each experiment (ACT SILO). An average of the 6 climate variables mentioned above was calculated for the 10-year growth period; all of which occurred between 1993 and 2010.

Aside from rainfall, groundwater can be a significant source of water for eucalypt plantations (Morris and Collopy, 1999; Benyon *et al.*, 2006) and therefore a potentially important predictor of response to fertiliser. Only MID sites used here had information on depth to groundwater obtained from site surveys (presence/absence), therefore it was necessary to use two online applications to provide an estimate for EST and EST+MID sites, available from: Visualising Victoria's Groundwater (<http://www.vvg.org.au/>) and WaterConnect (<https://www.waterconnect.sa.gov.au>). Sites were considered to have significant access to groundwater where the water table was within 5 m of the soil surface (Benyon *et al.*, 2006). Given only partial information on depth to groundwater was available, it was treated as a categorical (i.e., present or absent), rather than a continuous variable. Groundwater was therefore not used as an explanatory variable in simple or multiple linear regression analysis. Sites likely to have access to groundwater are indicated (e.g. Figure 3).

### **(ii) Soil test explanatory variables**

For establishment fertiliser sites, composite topsoil (0-10 cm and 10-20 cm) samples were collected from each unfertilised control plot in autumn or winter in the first year. The mean

value for each site represented 40-50 cores distributed across 4-5 replicates. Samples were kept cool in transit to the laboratory, then dried (40°C), ground (<2 mm) and analysed (Rayment and Higginson, 1992) for: 1:5 EC, 1:5 pH (in 0.01M CaCl<sub>2</sub> and water), potentially available nitrogen extracted with Hot-KCl NH<sub>4</sub>+NO<sub>3</sub>-N (Wang *et al.*, 1996; hereafter referred to as 'Min-N'), extractable Bray2 P (Bray and Kurtz, 1945), total N and total C. Relationships with volume response were developed using both soil sample depths, as well as a composite of both depths to represent 0-20 cm (calculated as an average of 0-10 and 10-20 cm). At sites fertilised at mid-rotation, a 0-10 cm sample was collected in the winter preceding fertiliser application in spring. Samples were kept cool in transit to the laboratory, then dried (40°C), ground (<2 mm) and analysed for total N and total C. Relationships with volume response were developed with total N and total C, as well as the C/N ratio.

### **(iii) Foliar nutrient explanatory variables**

The method and analysis of foliar sample capture was similar for both establishment and mid-rotation (including EST+MID) sites. Pre-treatment foliar nutrient samples were collected from control plots for each experiment; at age 1 for EST, age 4 for MID and age 1 and 4 for EST+MID SITES. Each sample was typically a composite of 4-6 leaves from 5-6 trees and collected from the top third of the crown. Samples were youngest fully-expanded leaves and leaf phenology (juvenile or mature) was noted at time of sampling, as it differed between experiments depending on age of application (juvenile for establishment applications and mature for mid-rotation (including EST+MID applications)). Foliar samples were analysed for total N, P, K, S, Na, Ca, Mg, Cu, Zn, Mn, Fe and B (Reuter and Robinson, 1997). All variables, including derived foliar ratios (N:P, N:S and N:K) were used to develop relationships with volume growth response. Foliar nutrient concentrations alone may not indicate total nutrient status of the foliage; therefore we also calculated a foliar N to volume ratio (N:MAI); where foliar N concentration was effectively 'corrected' for pre-treatment tree yield. The FOL N/MAI ratio was calculated by dividing the pre-treatment foliar N concentration by the pre-treatment MAI. Pre-treatment MAI was determined by dividing the pre-treatment standing volume by the pre-treatment stand age.

### **Regression analysis approach to predicting relative volume growth response**

Up to 70 explanatory variables were available for each experiment in this study; therefore our approach focussed on identifying and testing *a priori* predictive models, rather than identifying the 'best' predictive models by performing several all subsets multiple linear regressions. As fertiliser treatments applied in this study were all based on delivery of N and P (establishment) or N-only (mid-rotation), the models identified used all climatic variables in combination with a subset of soil and/or foliar variables strongly associated with N and/or P. For establishment models, variables included: topsoil (0-10 and 10-20 cm) pH (CaCl<sub>2</sub> and H<sub>2</sub>O), total N, total C, C/N ratio, extractable P (Olsen, Colwell, Bray2 and/or CaCl<sub>2</sub>) and Min-N; as well as foliar N, P, S, K, Ca and Mg (including foliar ratios of N:P, N:S and N:K). As soil variables were not available for predicting volume growth response to mid-rotation fertiliser, only foliar variables were used, including: N, P, S, K, Ca and Mg (including foliar ratios of N:P, N:S and N:K). As groundwater was a categorical explanatory variable, it could not be included in standard models. Instead, all models were run for sites with and without groundwater separately, and then all sites combined.

Simple linear and non-linear regression analyses were performed to firstly predict volume growth response at age 2, 4 and 10 (establishment fertiliser) or age 5, 7 and 10 (maintenance

fertiliser) from each explanatory variable. For non-linear regression analysis, functions which best described (highest adjusted  $R^2$  and significance) the data were used, particularly ‘linear-by-linear’; i.e.:  $y = a + b/(1 + dx)$ . For the pooled mid-rotation dataset, despite our attempts to isolate the mid-rotation fertiliser response (see “*Variables used to predict relative volume growth response to fertiliser applied at establishment or mid-rotation*”); the magnitude of volume growth response to fertiliser at EST+MID may have differed to MID sites because of on-going response to establishment fertiliser at these sites. As an additional means of avoiding this complication, simple linear and non-linear regression analysis was initially performed separately on the two datasets: MID (n=11) and EST+MID (n=10) to determine whether the slope of the relationship differed between groups. Regression equations were similar for the MID and EST+MID groups for any combination of explanatory and response variables (all  $P \geq 0.05$ ; data not shown), therefore the datasets were pooled to develop mid-rotation models for all sites. Although 21 sites were available, some had missing data or atypical values for certain parameters; therefore the models built to predict response to mid-rotation fertiliser were based on a total of 17 sites.

Prior to multiple linear regression analysis (MLR), correlation analysis was performed between all explanatory variables. Pearson’s correlation coefficient ( $r$ ) was used to describe the strength and direction of relationships and significance of relationships was determined using correlation analysis. All explanatory variables were used in MLR; however, if two variables were strongly correlated ( $r \geq 0.60$ ), only one was used in the same model. Regression analysis including all subsets was used to identify the strongest combination of predictors of (i.e., the ‘best’ explanatory variables for) relative volume growth response. Three model types were developed through MLR based on different combinations of explanatory variables: (i) soil-based (using only soil and climate variables), (ii) foliar-based (using only foliar and climate variables) and (iii) ‘best’ overall (using any combination of soil and/or foliar and climate variables). Mallows’s Coefficient ( $C_p$ ) was used to identify the ‘best’ combination of variables for multiple linear models of each model type, with the best model showing the highest adjusted  $R^2$  and lowest  $C_p$ . Models with the minimum  $C_p$  are more likely to represent the best subset of explanatory variables (Mallows, 1973). All model variables were required to be significant ( $P < 0.05$ ); this was also a requirement for the overall model as determined by MLR analysis. Correlation analysis was used to determine the strength and significance of relationships between predictor variables identified in the ‘best’ models and other related explanatory variables. Variables strongly correlated with the strongest predictors were substituted into the ‘best’ models to analyse the effect on model accuracy.

## **Other statistical analyses**

Other than linear, non-linear and multiple linear regression analyses (see previous section), all other data were analysed using ANOVA with Genstat v15.2, VSN International, Hemel Hempstead, UK. These analyses included comparisons of: pre-treatment volumes to exclude sites with pre-existing differences between control and fertilised treatments and descriptive site data in the form of long-term climatic, as well as pre-treatment soil and foliar data to determine whether any inherent differences existed between sites identified as more or less responsive as well as between application ages. ANOVA methods were also used to compare volume and basal area growth responses between measurements within each application age and site response type (i.e. ‘more’ or ‘less’ responsive); as well as between application ages; but only for relative, not absolute responses. ANOVA was also used to determine significant differences between fertilised and control plots within each measurement age, application type and site response type. Significant differences in the contribution of each quartile to total basal area were determined for both fertilised and control treatments between measurement years within each application type using one-way ANOVA. All data were tested for

normality using the Shapiro-Wilk test and transformed where necessary to achieve normal or near-normal distributions, typically through standardisation or log transformations. All presented data are non-transformed.

## Results

### Site productivity and differences in climate, soil and foliar variables

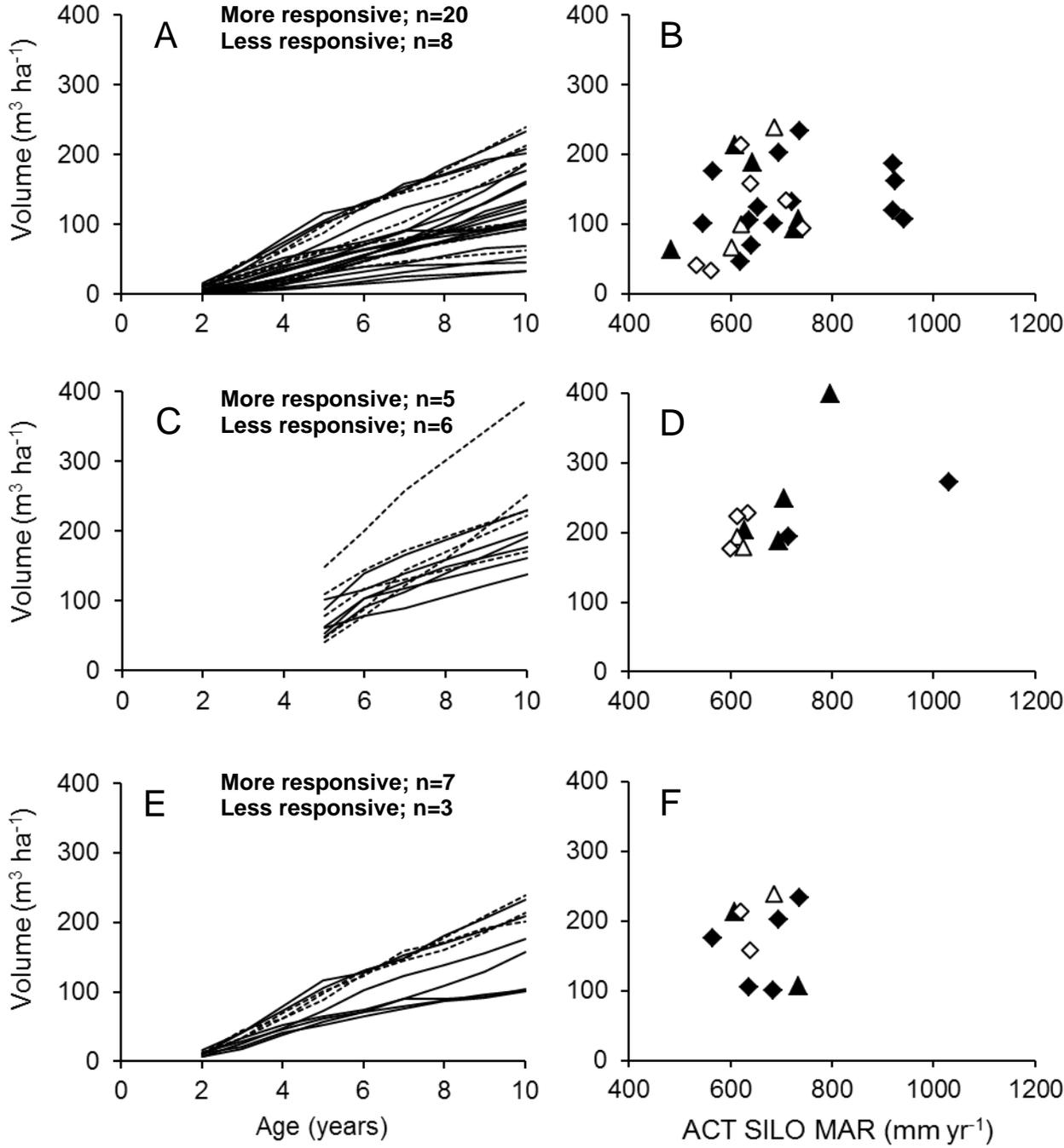
Different sets of sites were used to study growth and yield responses to fertiliser applied at establishment and at mid-rotation. Therefore it is important to first describe any fundamental differences regards site quality, between study sets/sites to aid interpretation of any differences in magnitude and duration of fertiliser responses. That is, are differences in response the result of the application timing, rather than inherent differences between the sites used for establishment (age 0+1; EST), mid-rotation (age 4-5; MID) and combined (age 0, 1 and 4; EST+MID) applications. Further, for the sake of building models predicting response to mid-rotation fertiliser, it was important to also determine whether sites used for mid-rotation only responses differed to those used for the combined establishment and mid-rotation responses.

There were differences in the baseline (i.e., unfertilised) productivity of sites used for the different fertiliser application ages. On average, the sites used to study response to establishment fertiliser had lower productivity, with an average final total volume (age 10 years) of control treatments of  $128 \text{ m}^3 \text{ ha}^{-1}$  (Figure 3A); ranging from as low as  $33 \text{ m}^3 \text{ ha}^{-1}$  up to  $239 \text{ m}^3 \text{ ha}^{-1}$ . This low average contrasted with  $214 \text{ m}^3 \text{ ha}^{-1}$  for the mid-rotation sites ( $197 \text{ m}^3 \text{ ha}^{-1}$  if one very high-productivity site was removed (Figure 3C) was caused by the inclusion of 10 'pilot' (i.e. non-routine planting) sites with MAI less than  $10 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$ . The subset of 10 establishment fertiliser sites which also received mid-rotation fertiliser were more similar in productivity to the mid-rotation only sites; yielding  $172 \text{ m}^3 \text{ ha}^{-1}$  on average ( $99\text{-}239 \text{ m}^3 \text{ ha}^{-1}$ ; Figure 3E).

Despite this study incorporating a range of site productivities, final volume was not related to relative growth response to fertiliser ( $P=0.504$ ); i.e. the baseline productivity of a site had no bearing on whether it was responsive to fertiliser application. Figure 3 distinguishes between sites 'more responsive' and 'less responsive' to fertiliser and also shows no clear relationship between final standing volume and responsiveness to fertiliser application. The underlying difference between productivity and response to fertiliser at sites fertilised at establishment versus mid-rotation was explored in relation to basic indices of water availability; specifically mean annual rainfall (MAR) and availability of groundwater (Figure 3B, D and F). There was no relationship between MAR and final volume for EST, MID or EST+MID sites, regardless of whether sites were split between more and less responsive to fertiliser, or with/without possible access to groundwater (all  $P=>0.05$ ); therefore neither high rainfall or access to groundwater adequately explained differences between sites in either baseline (i.e. unfertilised) productivity or responsiveness to fertiliser (Figure 3B, D and F).

Table 2 shows a comparison of 6 long-term average climate variables sourced from ESOCCLIM and from SILO for the two application ages; split between sites identified as more responsive and less responsive. For the ESOCCLIM data, both mean annual evaporation and mean maximum temperature were significantly higher at MID sites compared with EST sites ( $P=0.009$  and  $0.002$ ). All other ESOCCLIM climate variables showed no significant difference between application type and responsiveness to fertiliser. For the SILO climate data, more responsive establishment fertiliser sites showed lower mean maximum temperature compared

with the mid-rotation more responsive sites ( $P=0.005$ ). No other SILO climate variables showed any difference between groups.



**Figure 3.** Total volume yield with age (A, C, E) and total volume yield at age 10 years in relation to mean annual rainfall as sourced from SILO (B, D, F) for control treatments at sites used for analysis of response to fertiliser applied at establishment (A and B), mid-rotation (C and D) and establishment + mid-rotation (E and F). Within each fertiliser application age, sites were defined as ‘more responsive’ (solid lines) or ‘less responsive’ (dashed lines) according to their relative volume growth response to fertiliser; where ‘more responsive’ sites show >10% increase in yield (A, C, E). Diamonds (B, D, F) indicate ‘more responsive’ and triangles indicate ‘less responsive’ sites. Open symbols indicate sites with possible access to groundwater within 5 m of the soil surface and solid symbols indicate sites with groundwater greater than 10 m.

No significant differences were found within or between fertiliser application or response types for selected 0-10 cm pre-treatment soil variables (Table 3). Due to differences in sampling and analysis between EST and MID sites, only total C, total N and C:N at 0-10 cm depth were common to both age groups. At EST sites, all variables were significantly different between 0-10 cm and 10-20 cm sampling depths (all  $P < 0.05$ ). Total C, Total N, Hot KCl  $\text{NH}_4 + \text{NO}_3\text{-N}$  and Bray2 P concentrations decreased with depth, while the C/N ratio increased (Table 3).

Several pre-treatment differences in foliar nutrient concentrations existed between application ages, but not between response types (Table 4). Foliar N, P, K and S were all lower for mid-rotation sites compared with establishment sites ( $P < 0.001$ , 0.001, 0.007 and  $< 0.001$ ). Foliar Zn and Mn were also lower at mid-rotation sites ( $P < 0.001$  and 0.039); while Ca and B were higher compared with establishment sites ( $P = 0.001$  and 0.005). Similar differences existed for sites used for both establishment and mid-rotation applications (Table 4). There were no significant differences between pre-treatment foliar samples collected at age 1 at EST compared with EST+MID sites; nor between pre-treatment samples collected at age 4 between MID and EST+MID sites (all  $P \geq 0.05$ ). According to critical concentrations described by Dell *et al.* (2001), average values for all establishment (and EST+MID at age 1) foliar variables (juvenile leaves) were in the adequate range, but included some sites with deficiencies in N, P, K, Zn and B (Table 4). Applying the same concentration thresholds to mature leaves at mid-rotation sites (and EST+MID sites at age 4), average N, P, K, S and Zn levels were deficient, with some sites also showing low B. It may not be valid to apply thresholds provided by Dell *et al.*, (2001) for juvenile leaves of *E. globulus* to mature leaves; however, no other thresholds are available to describe foliar nutrient status of mature leaves at mid-rotation.

Table 5 shows pre-treatment foliar ratios at each site. There were no differences between foliar N:P, N:S or N:K between EST and MID sites; however, the N/MAI ratio was significantly higher at EST compared with MID sites, for both more responsive and less responsive sites (both  $P \geq 0.001$ ). This was expected due to differences in pre-treatment volume between sites. N/MAI ratios were different between EST and EST+MID sites at the same age, with EST+MID sites showing significantly lower N:MAI at age 1 for both more and less responsive sites (both  $P < 0.001$ ; Table 5). There were no other differences between application or site response types.

### **Magnitude and duration of volume growth response to fertiliser (stand level)**

Sites fertilised at establishment which were classified as more responsive to fertiliser showed 34.3% ( $1.5 \text{ m}^3 \text{ ha}^{-1}$ ) more volume 1-year after application (at age 2) compared with control stands (Figure 4A). This relative response declined over time, such that fertilised stands showed only 5.6% more volume at age 10; however, the absolute volume response increased from 1.5 to  $5.3 \text{ m}^3 \text{ ha}^{-1}$ . There was no difference in relative volume growth response between age 7 and age 10. Despite the large initial relative volume growth response, there was no significant difference in absolute volume or PAI between fertilised and control treatments at any point in time for more responsive sites fertilised at establishment (Figure 5A and B). Sites classified as less responsive showed no difference in volume growth response over time on a relative basis (Figure 4A; all  $P \geq 0.05$ ), nor did they show any difference between fertilised and control treatments in absolute standing volume or PAI at any age (data not shown; all  $P \geq 0.05$ ).

**Table 2.** Mean (standard error; SE) and range for selected climate variables at sites fertilised at establishment and /or mid-rotation, split into sites which showed > a 10% yield response to fertiliser ('more responsive') and those which did not ('less responsive'). Abbreviations (codes) used here are used in subsequent tables and figures. Uppercase letters indicate significant differences ( $P < 0.05$ ) between application ages, i.e., within row.

Climate variable	Code	Units	Establishment		Mid-rotation		Establishment and mid-rotation	
			Mean (SE)	Range	Mean (SE)	Range	Mean (SE)	Range
<b>MORE RESPONSIVE SITES</b>								
<i>ESOCLIM (1921-1995)</i>								
			<i>n=20</i>		<i>n=5</i>		<i>n=7</i>	
Mean annual rainfall	ESO MAR	mm yr <sup>-1</sup>	727 (22)	606-968	781 (90)	638-1096	702 (17)	666-756
Mean annual evaporation	ESO MAE	mm yr <sup>-1</sup>	1211 (25) <sup>A</sup>	1042-1439	1347 (15) <sup>B</sup>	1292-1376	1326 (21)	1282-1396
Climate wetness index	ESO CWI	Ratio	0.61 (0.03)	0.46-0.86	0.58 (0.07)	0.46-0.82	0.53 (0.02)	0.50-0.59
Mean maximum temperature	ESO MAX TEMP	°C	18.0 (0.2) <sup>A</sup>	16.5-19.8	19.9 (0.1) <sup>B</sup>	19.5-20.3	18.1 (0.3)	18.1-19.7
Mean minimum temperature	ESO MIN TEMP	°C	7.8 (0.2)	5.8-10.2	8.3 (0.4)	7.8-9.8	8.1 (0.5)	7.3-9.9
Mean radiation	ESO Rad	MJ m <sup>-2</sup>	15.5 (0.1)	14.9-16.5	16.0 (0.1)	15.8-16.4	15.7 (0.2)	15.2-16.4
<i>SILO (1912-2012)</i>								
Mean annual rainfall	SILO MAR	mm yr <sup>-1</sup>	742 (29)	583-991	773 (97)	613-1111	690 (28)	622-758
Mean annual evaporation	SILO MAE	mm yr <sup>-1</sup>	1264 (22)	1124-1449	1359 (29)	1248-1407	1347 (23)	1298-1423
Climate wetness index	SILO CWI	Ratio	0.60 (0.03)	0.45-0.88	0.57 (0.08)	0.44-0.81	0.51 (0.02)	0.47-0.57
Mean maximum temperature	SILO MAX TEMP	°C	18.2 (0.3) <sup>A</sup>	15.9-20.6	20.3 (0.2) <sup>B</sup>	19.9-21.0	19.4 (0.4)	18.6-20.5
Mean minimum temperature	SILO MIN TEMP	°C	8.4 (0.2)	6.2-10.4	9.0 (0.4)	8.4-10.5	8.7 (0.4)	8.0-10.3
Mean radiation	SILO Rad	MJ m <sup>-2</sup>	14.9 (0.1)	14.3-15.8	15.5 (0.2)	15.2-16.2	15.0 (0.2)	14.6-15.7
<b>LESS REPNOSIVE SITES</b>								
<i>ESOCLIM (1921-1995)</i>								
			<i>n=8</i>		<i>n=6</i>		<i>n=3</i>	
Mean annual rainfall	ESO MAR	mm yr <sup>-1</sup>	689 (25)	549-758	729 (27)	636-835	711 (23)	638-758
Mean annual evaporation	ESO MAE	mm yr <sup>-1</sup>	1222 (34) <sup>A</sup>	1091-1396	1333 (21) <sup>B</sup>	1278-1385	1345 (33)	1264-1439
Climate wetness index	ESO CWI	Ratio	0.57 (0.03)	0.45-0.65	0.55 (0.02)	0.46-0.61	0.53 (0.03)	0.46-0.60
Mean maximum temperature	ESO MAX TEMP	°C	18.1 (0.3) <sup>A</sup>	17.2-19.7	18.7 (0.3) <sup>B</sup>	17.9-19.9	18.9 (0.5)	17.6-19.8
Mean minimum temperature	ESO MIN TEMP	°C	7.8 (0.4)	6.4-9.9	8.7 (0.4)	7.4-10.2	8.8 (0.5)	7.8-10.2
Mean radiation	ESO Rad	MJ m <sup>-2</sup>	15.5 (0.2)	15.0-16.4	15.7 (0.2)	15.0-16.5	15.8 (0.3)	15.0-16.5
<i>SILO (1912-2012)</i>								
Mean annual rainfall	SILO MAR	mm yr <sup>-1</sup>	674 (31)	504-771	724 (37)	629-878	706 (25)	637-753
Mean annual evaporation	SILO MAE	mm yr <sup>-1</sup>	1275 (32)	1114-1423	1362 (27)	1297-1449	1372 (31)	1287-1449
Climate wetness index	SILO CWI	Ratio	0.53 (0.03)	0.38-0.65	0.53 (0.03)	0.45-0.62	0.52 (0.03)	0.45-0.59
Mean maximum temperature	SILO MAX TEMP	°C	18.5 (0.4)	17.4-20.5	19.3 (0.4)	18.2-20.4	19.5 (0.5)	18.1-20.6
Mean minimum temperature	SILO MIN TEMP	°C	8.5 (0.4)	7.0-10.3	9.2 (0.4)	8.0-10.5	9.4 (0.4)	8.3-10.4
Mean radiation	SILO Rad	MJ m <sup>-2</sup>	15.0 (0.4)	14.4-15.8	15.1 (0.2)	14.5-15.8	15.2 (0.3)	14.4-15.8

**Table 3.** Mean (standard error; SE) for selected soil variables (0-10 and 10-20 cm depth) at sites fertilised at establishment and mid-rotation, split into sites which showed > a 10% yield response to fertiliser ('more responsive') and those which did not ('less responsive'). Dashes indicate that data was not collected. Abbreviations (codes) used here are used in subsequent tables and figures. At establishment sites only, all variables were significantly different between 0-10 cm and 10-20 cm sampling depths (all  $P < 0.05$ ), but there were no other significant differences between means.

Soil variable	Abbreviation	Units	Establishment		Mid-rotation	
			Mean	Range	Mean	Range
<b>MORE RESPONSIVE SITES</b>						
<i>0-10 cm depth</i>						
pH (CaCl <sub>2</sub> )	0-10 pH CaCl <sub>2</sub>	-	4.40 (0.10)	3.38-5.43	-	-
EC	0-10 EC	dS m <sup>-1</sup>	0.090 (0.005)	0.054-0.123	-	-
Total C	0-10 TOT C	g kg <sup>-1</sup>	29.5 (2.7)	11.8-58.0	35.1 (11.5)	17.6-73.1
Total N	0-10 TOT N	g kg <sup>-1</sup>	2.04 (0.17)	0.73-3.53	2.11 (0.37)	1.49-3.31
C:N	0-10 C:N	Ratio	14.6 (0.7)	11.6-23.1	15.4 (2.1)	12.0-22.1
Hot-KCl NH <sub>4</sub> +NO <sub>3</sub> -N	0-10 Min-N	mg kg <sup>-1</sup>	33.2 (2.7)	12.9-48.8	-	-
Bray2 P	0-10 Bray2 P	mg kg <sup>-1</sup>	19.6 (2.5)	4.1-49.8	-	-
<i>10-20 cm depth</i>						
pH (CaCl <sub>2</sub> )	10-20 pH CaCl <sub>2</sub>	-	4.47 (0.13)	3.36-6.08	-	-
EC	10-20 EC	dS m <sup>-1</sup>	0.068 (0.008)	0.025-0.130	-	-
Total C	10-20 TOT C	g kg <sup>-1</sup>	16.1 (1.5)	6.5-31.2	-	-
Total N	10-20 TOT N	g kg <sup>-1</sup>	1.07 (0.12)	0.46-2.58	-	-
C:N	10-20 C:N	Ratio	16.2 (1.2)	10.7-33.1	-	-
Hot-KCl NH <sub>4</sub> +NO <sub>3</sub> -N	10-20 Min-N	mg kg <sup>-1</sup>	18.3 (1.9)	5.9-40.1	-	-
Bray2 P	10-20 Bray2 P	mg kg <sup>-1</sup>	10.3 (1.4)	1.7-24.4	-	-
<b>LESS RESPONSIVE SITES</b>						
<i>0-10 cm depth</i>						
pH (CaCl <sub>2</sub> )	0-10 pH CaCl <sub>2</sub>	-	4.30 (0.16)	3.60-4.70	-	-
EC	0-10 EC	dS m <sup>-1</sup>	0.097 (0.005)	0.078-0.115	-	-
Total C	0-10 TOT C	g kg <sup>-1</sup>	29.8 (3.1)	19.9-40.0	35.6 (6.4)	16.9-53.3
Total N	0-10 TOT N	g kg <sup>-1</sup>	2.21 (0.21)	1.45-2.99	2.71 (0.54)	1.48-4.56
C:N	0-10 C:N	Ratio	13.5 (0.4)	11.4-15.0	13.1 (0.9)	11.5-16.4
Hot-KCl NH <sub>4</sub> +NO <sub>3</sub> -N	0-10 Min-N	mg kg <sup>-1</sup>	40.4 (3.9)	23.7-55.3	-	-
Bray2 P	0-10 Bray2 P	mg kg <sup>-1</sup>	19.4 (4.0)	6.9-38.1	-	-
<i>10-20 cm depth</i>						
pH (CaCl <sub>2</sub> )	10-20 pH CaCl <sub>2</sub>	-	4.40 (0.10)	3.93-4.68	-	-
EC	10-20 EC	dS m <sup>-1</sup>	0.065 (0.006)	0.031-0.086	-	-
Total C	10-20 TOT C	g kg <sup>-1</sup>	14.8 (2.4)	8.6-28.2	-	-
Total N	10-20 TOT N	g kg <sup>-1</sup>	1.02 (0.19)	0.53-2.07	-	-
C:N	10-20 C:N	Ratio	15.1 (1.3)	11.1-22.8	-	-
Hot-KCl NH <sub>4</sub> +NO <sub>3</sub> -N	10-20 Min-N	mg kg <sup>-1</sup>	18.1 (2.2)	10.0-26.7	-	-
Bray2 P	10-20 Bray2 P	mg kg <sup>-1</sup>	9.2 (2.7)	2.7-23.7	-	-

**Table 4.** Mean (standard error; SE) and range for selected foliar variables at sites fertilised at establishment and /or mid-rotation, split into sites which showed > a 10% yield response to fertiliser ('more responsive') and those which did not ('less responsive'). Abbreviations (codes) used here are used in subsequent tables and figures. Uppercase letters indicate significant differences ( $P < 0.05$ ) between application ages, i.e., within row.

Foliar variable	Code	Units	Establishment		Mid-rotation		Establishment and mid-rotation			
			Mean (SE)	Range	Mean (SE)	Range	Mean (SE) [at age 1]	Range [at age 1]	Mean (SE) [at age 4]	Range [at age 4]
<b>MORE RESPONSIVE SITES</b>			<i>n=20</i>		<i>n=5</i>		<i>n=7</i>			
Nitrogen	FOL N	g kg <sup>-1</sup>	22.0 (0.9) <sup>B</sup>	15.2-32.0	14.8 (0.8) <sup>A</sup>	13.7-17.6	22.5 (1.0)	19.0-32.0	15.9 (1.4) <sup>A</sup>	11.2-18.8
Phosphorus	FOL P	g kg <sup>-1</sup>	1.81 (0.11) <sup>B</sup>	0.97-2.76	1.14 (0.06) <sup>A</sup>	1.06-1.35	2.02 (0.06)	1.72-2.56	1.14 (0.07) <sup>A</sup>	1.00-1.38
Potassium	FOL K	g kg <sup>-1</sup>	8.9 (0.4) <sup>B</sup>	5.9-12.3	6.3 (0.3) <sup>A</sup>	5.3-6.9	10.2 (0.3)	7.8-12.3	5.6 (0.2) <sup>A</sup>	5.0-6.1
Sulphur	FOL S	g kg <sup>-1</sup>	1.73 (0.06) <sup>B</sup>	1.36-2.30	1.12 (0.03) <sup>A</sup>	1.01-1.18	1.60 (0.07)	1.36-2.21	1.26 (0.08) <sup>A</sup>	1.00-1.43
Sodium	FOL Na	g kg <sup>-1</sup>	2.3 (0.2)	1.1-3.9	2.18 (0.26)	1.52-3.12	2.2 (0.2)	1.1-3.5	2.02 (0.13)	1.75-2.42
Calcium	FOL Ca	g kg <sup>-1</sup>	6.8 (0.5) <sup>A</sup>	3.7-10.7	9.1 (1.6) <sup>B</sup>	3.7-12.1	6.0 (0.4)	3.7-8.1	11.4 (0.5) <sup>B</sup>	9.4-12.6
Magnesium	FOL Mg	g kg <sup>-1</sup>	2.18 (0.12)	1.31-3.43	2.18 (0.15)	1.68-2.39	1.93 (0.09)	1.31-2.46	2.35 (0.08)	2.07-2.55
Copper	FOL Cu	mg kg <sup>-1</sup>	5.97 (0.72)	2.08-12.78	5.85 (0.82)	4.09-8.15	4.81 (0.39)	2.08-7.76	3.92 (0.27)	3.33-4.84
Zinc	FOL Zn	mg kg <sup>-1</sup>	20.0 (1.0) <sup>B</sup>	13.3-27.6	12.8 (0.4) <sup>A</sup>	11.7-13.7	23.4 (0.7)	18.9-27.6	13.1 (1.0) <sup>A</sup>	9.9-15.3
Manganese	FOL Mn	mg kg <sup>-1</sup>	787 (141) <sup>B</sup>	137-2222	195 (38) <sup>A</sup>	71-258	401 (90)	137-1159	205 (68) <sup>A</sup>	60-444
Iron	FOL Fe	mg kg <sup>-1</sup>	59.5 (6.0)	24.6-143.6	35.3 (1.5)	32.0-39.7	56.7 (3.5)	39.7-79.1	39.6 (4.3)	24.5-50.3
Boron	FOL B	mg kg <sup>-1</sup>	15.9 (1.3) <sup>A</sup>	7.3-26.0	21.9 (3.3) <sup>B</sup>	11.9-28.0	17.4 (1.5)	8.7-26.0	25.8 (2.9) <sup>B</sup>	19.3-33.7
<b>LESS RESPONSIVE SITES</b>			<i>n=8</i>		<i>n=6</i>		<i>n=3</i>			
Nitrogen	FOL N	g kg <sup>-1</sup>	25.5 (1.5) <sup>B</sup>	20.1-30.2	14.2 (0.7) <sup>A</sup>	11.3-15.8	25.2 (1.6)	21.5-30.2	15.8 (0.7) <sup>A</sup>	14.2-18.0
Phosphorus	FOL P	g kg <sup>-1</sup>	1.96 (0.16) <sup>B</sup>	1.29-2.62	1.11 (0.09) <sup>A</sup>	0.84-1.37	2.21 (0.15)	1.76-2.62	1.13 (0.05) <sup>A</sup>	1.01-1.27
Potassium	FOL K	g kg <sup>-1</sup>	10.8 (1.0) <sup>B</sup>	5.8-13.5	7.0 (1.2) <sup>A</sup>	4.8-11.9	11.6 (0.6)	9.7-13.2	5.9 (0.6) <sup>A</sup>	4.7-8.1
Sulphur	FOL S	g kg <sup>-1</sup>	1.90 (0.14) <sup>B</sup>	1.44-2.31	1.17 (0.08) <sup>A</sup>	0.94-1.40	1.80 (0.16)	1.46-2.31	1.23 (0.03) <sup>A</sup>	1.13-1.29
Sodium	FOL Na	g kg <sup>-1</sup>	2.3 (0.2)	1.1-4.9	2.07 (0.23)	1.62-2.84	2.6 (0.7)	4.8 (0.1)	2.03 (0.17)	1.67-2.59
Calcium	FOL Ca	g kg <sup>-1</sup>	5.6 (0.4) <sup>A</sup>	4.4-7.4	11.2 (1.4) <sup>B</sup>	5.9-15.2	4.8 (0.1)	4.4-5.2	11.1 (0.8) <sup>B</sup>	9.4-14.4
Magnesium	FOL Mg	g kg <sup>-1</sup>	1.90 (0.15)	1.35-2.68	2.23 (0.13)	1.76-2.61	2.04 (0.24)	1.35-2.68	2.34 (0.15)	1.80-2.67
Copper	FOL Cu	mg kg <sup>-1</sup>	7.93 (1.34)	4.15-15.75	5.78 (0.44)	4.64-7.24	5.93 (0.59)	4.15-7.44	3.67 (0.35)	2.48-4.54
Zinc	FOL Zn	mg kg <sup>-1</sup>	23.4 (2.1) <sup>B</sup>	15.0-31.5	11.5 (0.6) <sup>A</sup>	9.4-12.9	27.5 (2.2)	20.2-31.5	12.1 (0.5) <sup>A</sup>	11.3-13.3
Manganese	FOL Mn	mg kg <sup>-1</sup>	823 (130) <sup>B</sup>	265-1300	201 (57) <sup>A</sup>	46-374	631 (166)	265-1161	134 (44) <sup>A</sup>	54-303
Iron	FOL Fe	mg kg <sup>-1</sup>	56.8 (6.4)	32.1-84.0	43.4 (5.6)	28.7-64.9	60.3 (7.8)	40.7-84.0	37.6 (2.9)	26.5-43.6
Boron	FOL B	mg kg <sup>-1</sup>	15.1 (1.8) <sup>A</sup>	6.6-20.8	26.8 (3.8) <sup>B</sup>	14.4-37.1	14.2 (2.0)	7.6-17.6	30.3 (6.0) <sup>B</sup>	17.7-52.7

**Table 5.** Mean (standard error; SE) and range for selected foliar ratio variables at sites fertilised at establishment and /or mid-rotation, split into sites which showed > a 10% yield response to fertiliser ('more responsive') and those which did not ('less responsive'). Abbreviations (codes) used here are used in subsequent tables and figures. Uppercase letters indicate significant differences ( $P < 0.05$ ) between application ages, i.e., within row.

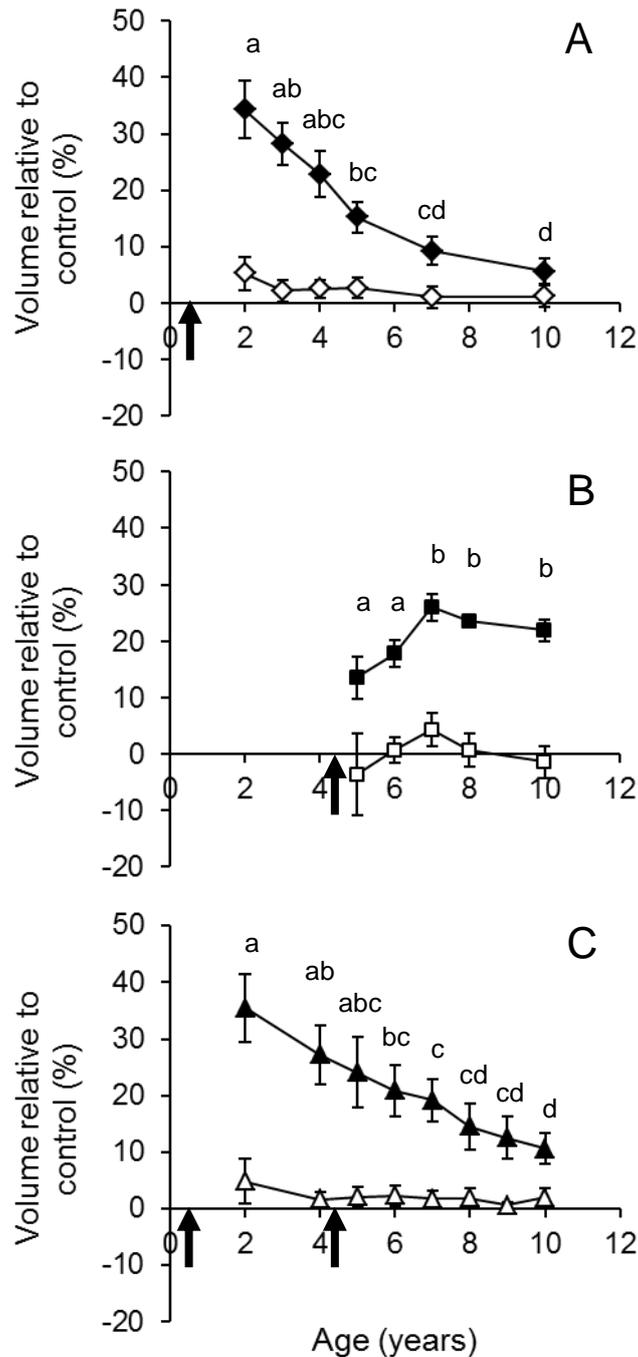
Foliar Variable	Code	Units	Establishment		Mid-rotation		Establishment and mid-rotation			
			Mean (SE)	Range	Mean (SE)	Range	Mean (SE) [at age 1]	Range [at age 1]	Mean (SE) [at age 4]	Range [at age 4]
<b><i>MORE RESPONSIVE SITES</i></b>			<b><i>n=20</i></b>		<b><i>n=5</i></b>		<b><i>n=6</i></b>			
N:P	FOL N:P	Ratio	12.6 (0.5)	8.7-16.3	12.9 (2.6)	12.7-13.3	11.2 (0.3)	8.9-12.7	13.9 (0.8)	11.1-15.6
N:S	FOL N:S	Ratio	12.7 (0.3)	9.9-14.5	12.4 (2.5)	11.9-13.7	14.0 (0.1)	12.7-14.5	12.5 (0.4)	11.2-13.3
N:K	FOL N:K	Ratio	2.56 (0.12)	1.79-3.49	2.35 (0.49)	1.99-2.68	2.21 (0.06)	1.85-2.61	2.85 (0.32)	1.84-3.72
N:MAI	FOL N:MAI	Ratio	23.5 (5.9) <sup>B</sup>	2.4-102.3	0.88 (0.20) <sup>A</sup>	0.51-1.12	4.6 (0.3) <sup>A</sup>	2.4-6.6	1.03 (0.15) <sup>A</sup>	0.62-1.30
<b><i>LESS RESPONSIVE SITES</i></b>			<b><i>n=8</i></b>		<b><i>n=6</i></b>		<b><i>n=3</i></b>			
N:P	FOL N:P	Ratio	13.3 (0.6)	10.7-15.5	13.0 (2.0)	10.1-15.2	11.5 (0.3)	10.7-12.3	14.0 (0.6)	12.7-15.9
N:S	FOL N:S	Ratio	13.5 (0.3)	12.7-14.8	12.1 (2.0)	11.0-13.3	14.1 (0.3)	13.1-14.8	12.8 (0.4)	11.9-14.1
N:K	FOL N:K	Ratio	2.46 (0.17)	2.00-3.49	2.50 (0.55)	1.90-3.01	2.17 (0.05)	2.00-2.28	2.79 (0.35)	1.78-3.69
N:MAI	FOL N:MAI	Ratio	16.0 (4.8) <sup>B</sup>	3.6-36.1	1.05 (0.32) <sup>A</sup>	0.42-2.15	4.2 (0.3) <sup>A</sup>	3.6-5.1	1.03 (0.14) <sup>A</sup>	0.54-1.41

In contrast, more responsive sites fertilised at mid-rotation showed a smaller initial relative response of 13.5% ( $9.4 \text{ m}^3 \text{ ha}^{-1}$ ) more volume 1-year post-application and a maximum response of 26.0% ( $33.2 \text{ m}^3 \text{ ha}^{-1}$ ) by age 7 (3 years post-application; Figure 4B). Relative volume growth response did not decline significantly between age 7 and age 10 (21.9%;  $42.9 \text{ m}^3 \text{ ha}^{-1}$ ); however, without measurements beyond this point it is impossible to determine whether this relative difference persisted over time (Figure 4B). On an absolute basis, fertilised stands showed significantly more standing volume at ages 7 and 10 (Figure 5C) and significantly higher PAI at ages 5 and 6 (Figure 5D) compared with control stands (more responsive sites only). The PAI at age 7 and 10 was similar for fertilised and control stands (Figure 5 D). Again, less responsive sites showed no difference in relative volume growth response over time (Figure 4B), or any difference in standing volume or PAI (data not shown; all  $P \geq 0.05$ ) between fertilised and control treatments at any age.

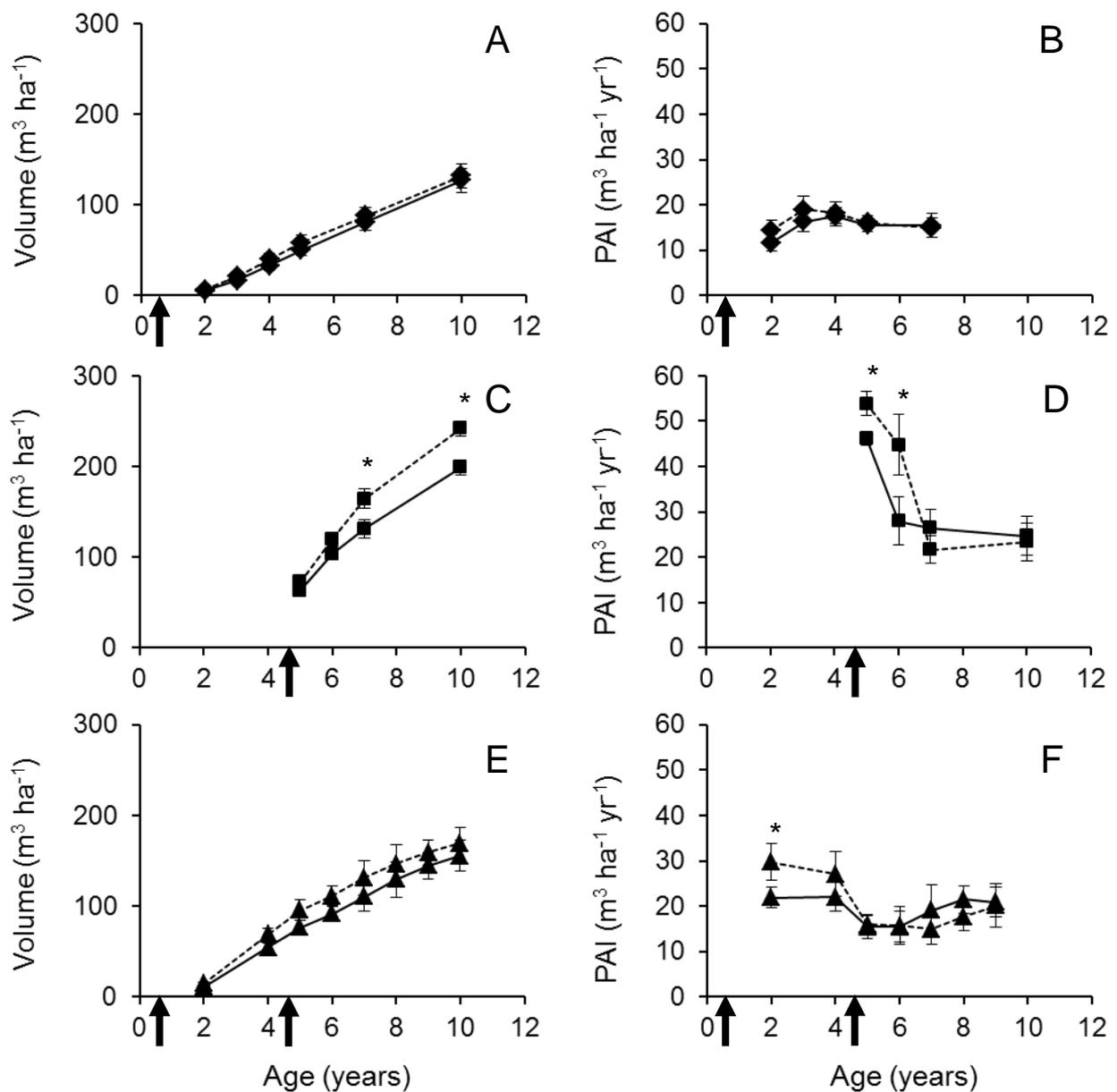
Sites fertilised at both establishment and mid-rotation showed similar initial relative responses to sites where only establishment fertiliser was applied (Figure 4C). More responsive EST+MID sites showed 36.2% more volume 1-year after application (at age 2) compared with control stands (Figure 4C); however this related to a larger difference in standing volume of  $3.8 \text{ m}^3 \text{ ha}^{-1}$  at age 2, compared with  $1.5 \text{ m}^3 \text{ ha}^{-1}$  for EST sites. As per EST sites, the relative response declined over time, such that fertilised stands showed 10.6% more volume at age 10 ( $13.7 \text{ m}^3 \text{ ha}^{-1}$ ). There was no difference between relative volume growth response at age 8 (14.5%;  $16.8 \text{ m}^3 \text{ ha}^{-1}$ ) and age 10. Also similar to EST sites, more responsive EST+MID sites showed no significant difference in absolute volume between fertilised and control treatments at any point in time (Figure 5E). Fertilised treatments did show a significantly higher PAI early in the rotation at age 2 compared with control treatments ( $P < 0.001$ ; Figure 5F). Sites classified as less responsive showed no difference in volume growth response over time on a relative basis (Figure 4C; all  $P \geq 0.05$ ), nor did they show any difference between fertilised and control treatments in absolute standing volume or PAI at any age (data not shown; all  $P \geq 0.05$ ).

### **Magnitude and duration of relative basal area growth response to fertiliser (sub-stand level)**

Sub-stand level analysis showed that when any given stand was divided into four quartiles (based on individual tree basal areas); the magnitude and duration of response to establishment fertiliser application differed between quartiles for the more responsive sites (Figure 6). At more responsive EST sites, the smallest 25% of trees (first quartile) showed 49.1% ( $0.09 \text{ m}^2 \text{ ha}^{-1}$ ) more basal area at age 2 when fertilised compared with 24.3% ( $0.22 \text{ m}^2 \text{ ha}^{-1}$ ) for the largest 25% of trees (fourth quartile;  $P < 0.001$ ; Figure 6A). As per volume growth response at the stand level, basal area response of all quartiles declined over time. By age 10, 9 years after the last application of fertiliser, basal areas of fertilised trees were 16.1, 9.9, 3.3 and 0.6% ( $0.30, 0.29, 0.15$  and  $0.01 \text{ m}^2 \text{ ha}^{-1}$ ) greater than unfertilised trees for quartiles 1-4 (Figure 6A-D). Fertilised trees in quartiles 1 and 2, which combined represented the smallest 50% of trees in a stand, showed significantly higher basal areas relative to control trees compared with quartiles 3 and 4 ( $P < 0.001$ ; Figure 6A and 6B). To interpret these trends it was also necessary to determine what contribution each quartile made to total basal area. There was no difference in the contribution made to total basal area for control trees compared with fertilised trees at any age (data not shown; all  $P \geq 0.05$ ); therefore both treatments and all ages were combined for each quartile. The four quartiles contributed: 13.8 ( $\pm 0.2$ ), 22.0 ( $\pm 0.2$ ), 27.0 ( $\pm 0.1$ ) and 40.8 ( $\pm 0.4$ ) % to the total basal area of any given stand; with each quartile significantly different from the others ( $P < 0.001$ ).



**Figure 4.** Relative volume growth response to fertiliser applied at establishment (A), mid-rotation (B) and establishment + mid-rotation (C), with arrows indicating timing of fertiliser addition. Solid symbols represent sites classified as ‘more responsive’ (n=20 at establishment, 5 at mid-rotation and 5 at establishment + mid-rotation sites) and open symbols represents ‘less responsive’ sites (n=8 at establishment, 6 at for mid-rotation and 5 at establishment + mid-rotation sites). Different letters represent significant differences between years for for more responsive sites only (derived from one-way ANOVA; both P-values = <math>\leq 0.001</math>).



**Figure 5.** Total yield response for sites with fertiliser applied at establishment (A and B), mid-rotation (C and D) and at establishment and mid-rotation (E and F). Arrows indicate timing of fertiliser addition. Growth is calculated on an absolute basis as: volume (A, C and E) and volume periodic annual increment (PAI; B, D and F) for sites identified as ‘more responsive’ to fertiliser only. Dashed lines represent fertilised stands and unbroken lines represent control stands. ‘\*’ represent significant differences ( $P < 0.05$ ) between fertilised and control stands within each year (derived from one-way ANOVA).

Sub-stand analysis of more responsive sites receiving mid-rotation fertiliser showed a different trend to those receiving establishment fertiliser (Figure 6E-H), as per the stand level trends in volume growth responsive. Unlike the establishment application, all quartiles showed smaller initial basal area growth responses 1 year post-application which increased over time to reach their maximum at age 10 (all  $P < 0.001$ ). Like the EST application, however; the first quartile showed the largest relative basal area response to fertiliser (40.2%, equivalent to  $1.13 \text{ m}^2 \text{ ha}^{-1}$  more basal area at age 10) and the fourth quartile showed the smallest relative response (20.8%, or  $2.08 \text{ m}^2 \text{ ha}^{-1}$  more basal area, at age 10). There was no difference in the contribution made to total basal area for control trees compared with fertilised trees (data not shown) and the four quartiles contributed proportions to the total basal area of any given stand similar to those observed for EST sites: 12.6 ( $\pm 0.2$ ), 21.2 ( $\pm 0.2$ ), 26.9 ( $\pm 0.2$ ) and 41.9 ( $\pm 0.3$ ) %; again with each quartile significantly different from the others ( $P < 0.001$ ).

More responsive sites receiving both establishment and mid-rotation fertiliser showed similar trends to EST sites (Figure 6I-L). The smallest 25% of trees showed 49.3% ( $0.25 \text{ m}^2 \text{ ha}^{-1}$ ) more basal area at age 2 when fertilised compared with 24.2% ( $0.27 \text{ m}^2 \text{ ha}^{-1}$ ) for the largest 25% of trees (fourth quartile;  $P < 0.001$ ; Figure 6A). As per EST sites, basal area response of all quartiles declined over time (Figure 6I-L). By age 10, 6 years after the last application of fertiliser, basal areas of fertilised trees were 24.3, 15.0, 6.5 and 7.9% ( $0.53$ ,  $0.24$ ,  $0.01$  and  $0.06 \text{ m}^2 \text{ ha}^{-1}$ ) greater than unfertilised trees for quartiles 1-4 (Figure 6I-L). Again, fertilised trees in quartiles 1 and 2 showed significantly higher basal areas relative to control trees compared with quartiles 3 and 4 ( $P < 0.001$ ; Figure 6I and 6J). There was no difference in the contribution made to total basal area for control trees compared with fertilised trees at any age (data not shown; all  $P > 0.05$ ); therefore both treatments and all ages were again combined for each quartile. The four quartiles contributed: 15.3 ( $\pm 0.4$ ), 22.1 ( $\pm 0.2$ ), 26.5 ( $\pm 0.2$ ) and 39.2 ( $\pm 0.4$ ) % to the total basal area of any given stand; with each quartile significantly different from the others ( $P < 0.001$ ).

### **Predicting volume growth response to establishment fertiliser from site climate variables**

There were no significant relationships between any single climatic variable and volume growth response to establishment fertiliser at age 2, 4 or 10 (all  $P > 0.05$ ; data not shown). Regression equations were similar for sites with and without groundwater for climate variables, as well as all other explanatory variables tested (all  $P > 0.05$ ; data not shown); therefore all simple and multiple linear regression analysis was performed without distinguishing between these groups.

### **Predicting volume growth response to establishment fertiliser from site soil variables**

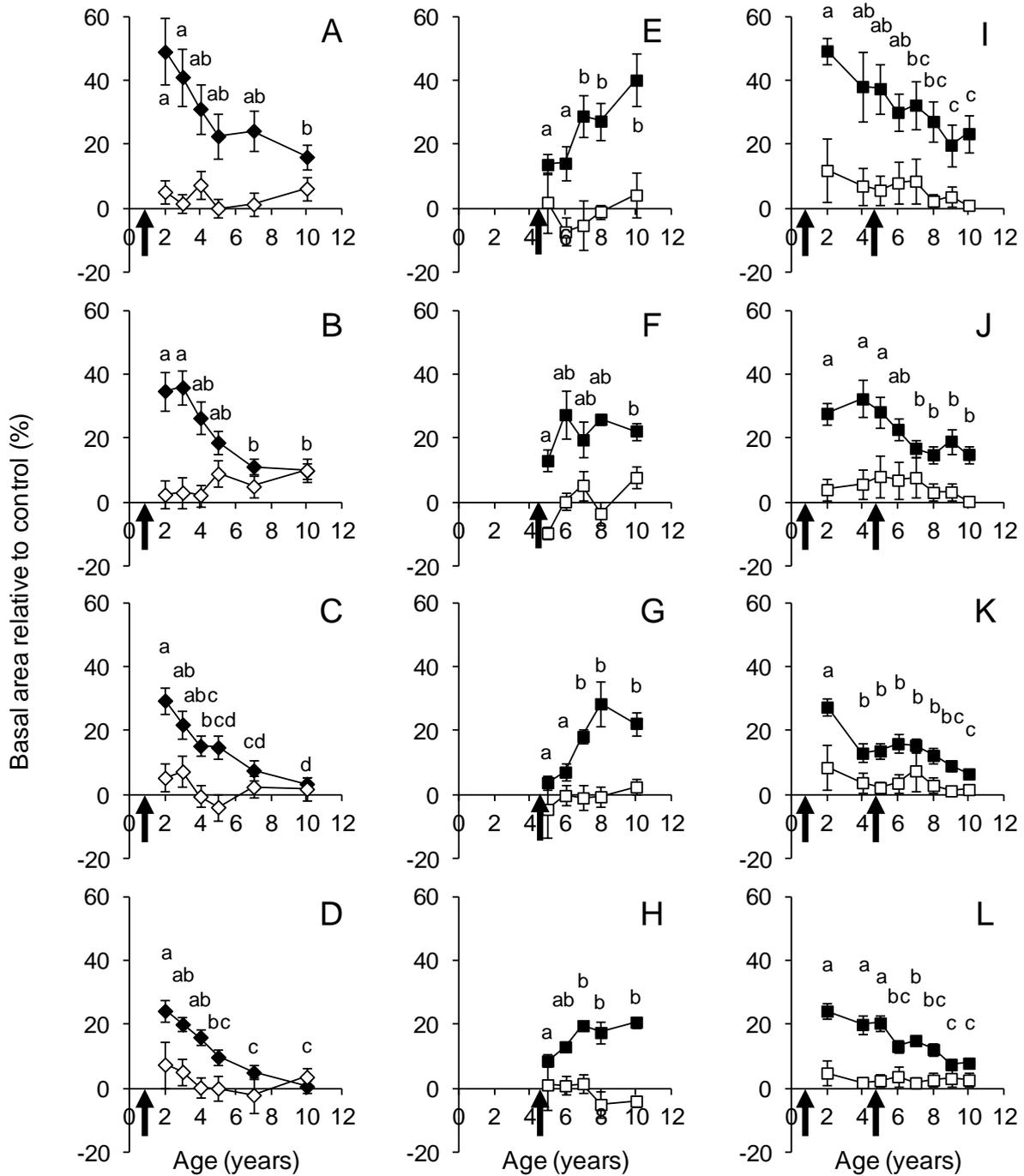
Several significant single 0-10 cm soil variables were related to volume growth response at age 2 (1 year after application) for establishment applications (Figure 7). Simple linear regression analysis suggested that sites with  $< 20 \text{ mg kg}^{-1}$  Min-N showed greater volume responses to fertiliser ( $R^2 = 0.62$ ). However, the variability accounted for by the model based on Min-N declined to 28% for volume growth response at age 4 ( $y = -0.79 * \text{NH}_4 + \text{NO}_3\text{-N} + 44.98$ ;  $P = 0.004$ ). Total C, total N, extractable (Bray2) P and 1:5 EC showed weak negative relationships with volume growth response at age 2 (Figure 7). The relationship between total C and volume growth response at age 2 was slightly improved (from  $R^2 = 0.19$ ;  $P = 0.041$  to  $R^2 = 0.31$ ;  $P = 0.003$ ) by removing an outlier, but remained weak.

The relationship between Bray2 P and volume growth improved at age 4 ( $R^2=0.43$ ;  $P<0.001$ ;  $y = 12.12 - 5.43/(1 - 0.266*\text{Bray2 P})$ ); while the relationship with 1:5 EC declined ( $R^2=0.15$ ;  $P=0.043$ ;  $y = -364.2*EC + 50.4$ ). Total N and total C were not related to volume growth response at age 4 ( $P=0.140$  and  $0.664$ ) and pH  $\text{CaCl}_2$  was not related to growth at age 4 ( $P=0.141$ ). No soil variables (0-10 cm) were related to volume growth at age 10 (all  $P=>0.05$ ; data not shown).

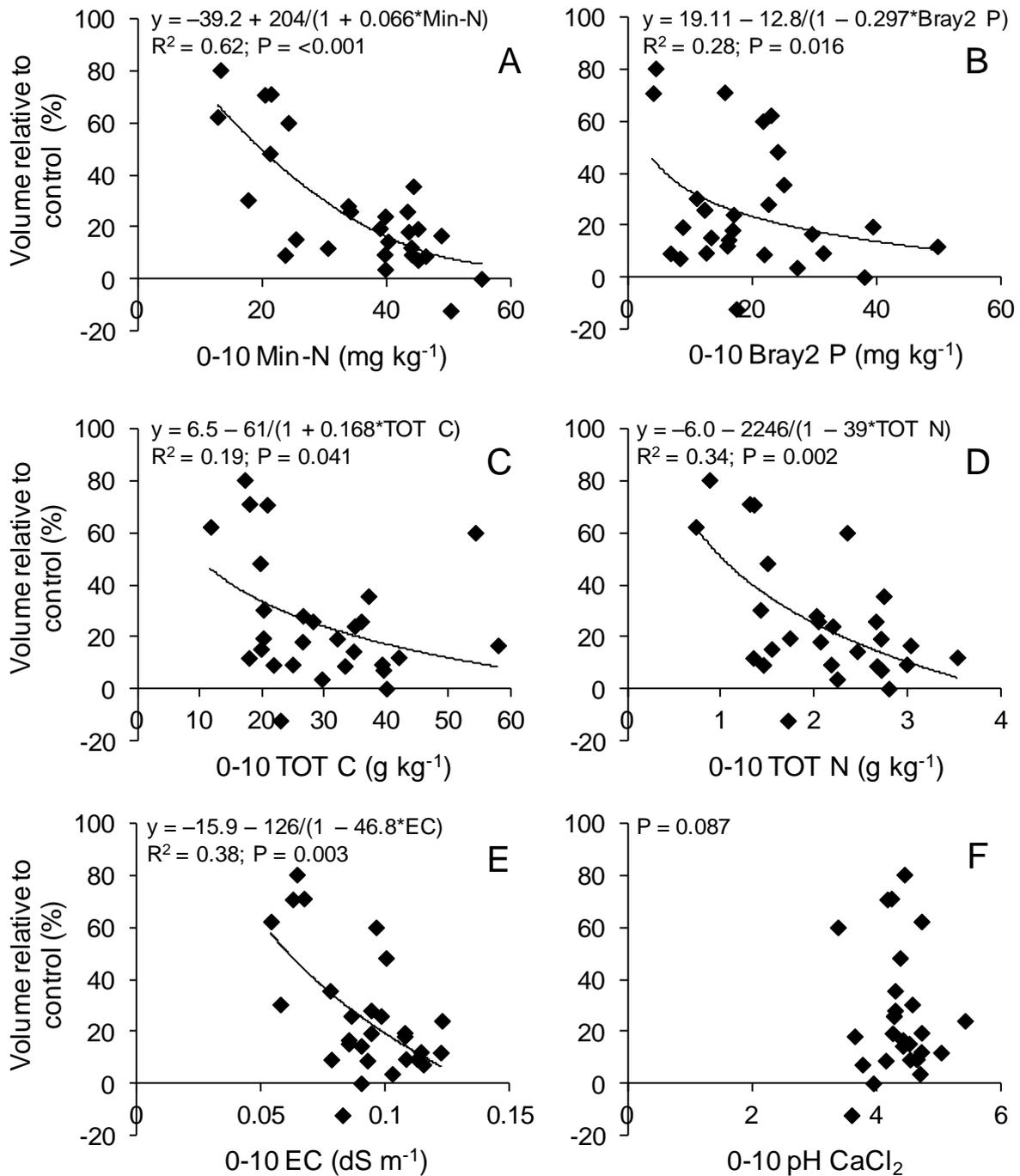
The strength and significance of the relationships between soil variables at depths 10-20 cm and 0-20 cm (the calculated average 0-20 cm) and volume growth response were lower than for soil variables for the surface layer (0-10 cm). Min-N at depth 10-20 cm was the only soil variable for this layer that significantly related to volume growth response at age 2 ( $R^2=0.40$ ;  $P<0.001$ ;  $y = -0.6 - 197/(1 - 0.564* \text{NH}_4+\text{NO}_3\text{-N})$ ) and at age 4 ( $R^2=0.15$ ;  $P=0.038$ ;  $y = -0.840*\text{NH}_4+\text{NO}_3\text{-N} + 32.87$ ), but not at age 10 ( $P=0.393$ ). No other soil variables at 10-20 cm were related to volume growth response at any age (all  $P=>0.05$ ; data not shown). Average Min-N for the combined layers (0-20 cm) was also related to volume growth response at age 2 ( $R^2=0.59$ ;  $P<0.001$ ;  $y = -26.5 + 268/(1 + 0.171* \text{NH}_4+\text{NO}_3\text{-N})$ ) and age 4 ( $R^2=0.28$ ;  $P=0.004$ ;  $y = -1.035* \text{NH}_4+\text{NO}_3\text{-N} + 45.05$ ). Soil 1:5 EC (0-20 cm) also predicted volume growth response, but only at age 2 ( $R^2=0.27$ ;  $P=0.011$ ;  $y = 0.4 - 62.4/(1 - 49.7*EC)$ ). No other single soil variables for the combined layers (0-20 cm) were related to volume growth at age 10 (all  $P=>0.05$ ; data not shown).

### **Predicting volume growth response to establishment fertiliser from site foliar variables**

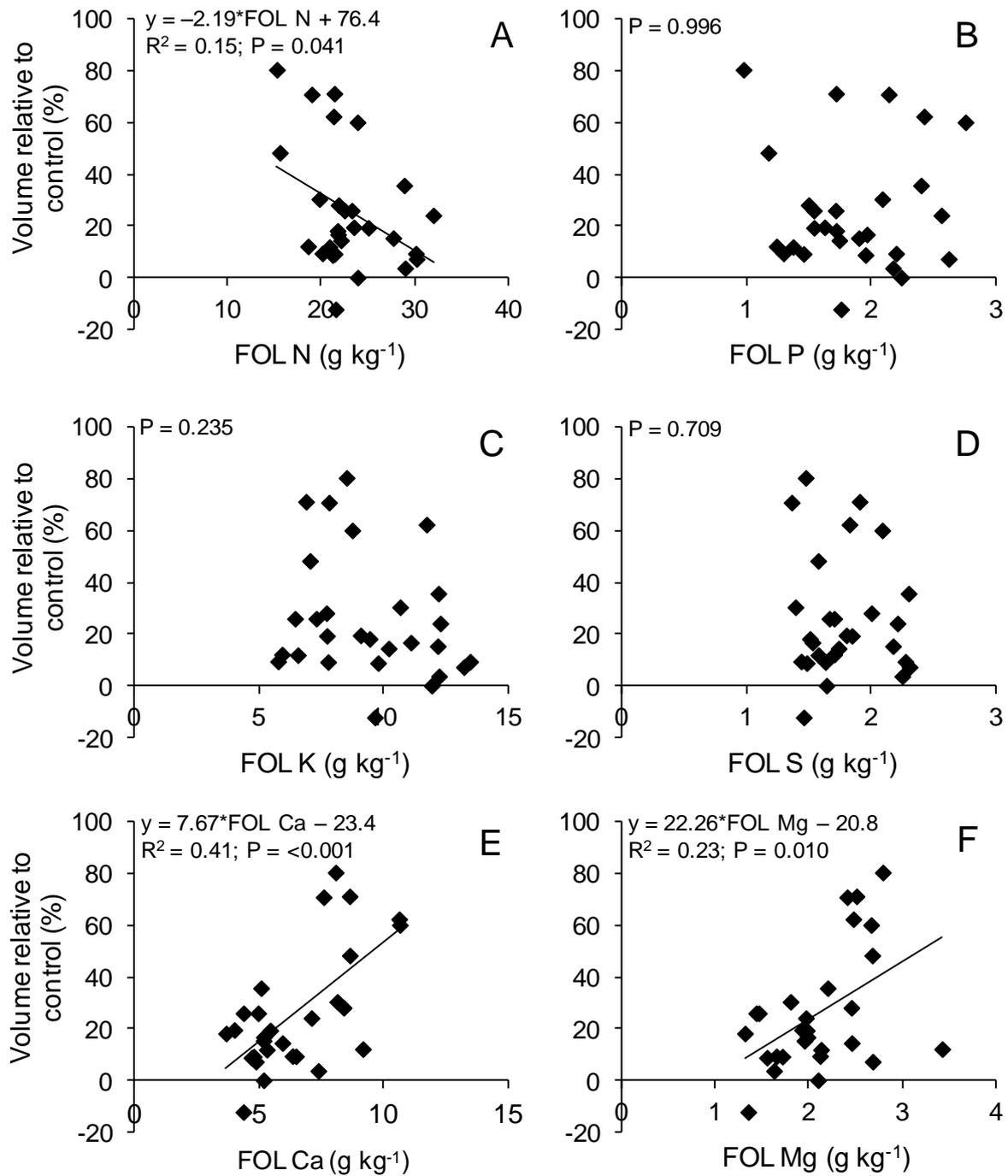
Single foliar variables showed weak relationships with volume growth response at age 2, including foliar N, Ca and Mg (Figure 8). Foliar N appeared to show two types of responses, with 7 of 18 sites indicating a higher level of responsiveness to the same fertiliser. Possible causes for the difference in response were investigated but no site factors including potential limiting factors such as low water availability or differences in foliar nutrient levels or trace element deficiencies explained this apparent difference in response (data not shown). Foliar N was still related to volume growth response at age 4, but only showed a weak negative relationship ( $R^2=0.19$ ;  $P=0.019$ ;  $y = -1.88*\text{FOL N} + 60.3$ ). Foliar P, K and S showed no relationship with volume growth response at any age (Figure 8 for age 2; all  $P=>0.05$  for age 4 and 10, data not shown). Foliar Mg was related to volume growth response at age 10; albeit weakly ( $R^2=0.16$ ;  $P=0.034$ ;  $y = 7.17*\text{FOL Mg} - 10.68$ ). The foliar ratio of N/S was the only other foliar variable related to volume growth response at age 2 (Figure 9B). Neither foliar N:P, N:K, nor the ratio of foliar N to MAI (N:MAI) were related to volume growth response at any age (Figure 9 for age 2; all other ages showed  $P=>0.05$ ; data not shown). There were no other single foliar predictors related to volume growth response at age 2, 4 or 10 (all  $P=>0.05$ ; data not shown).



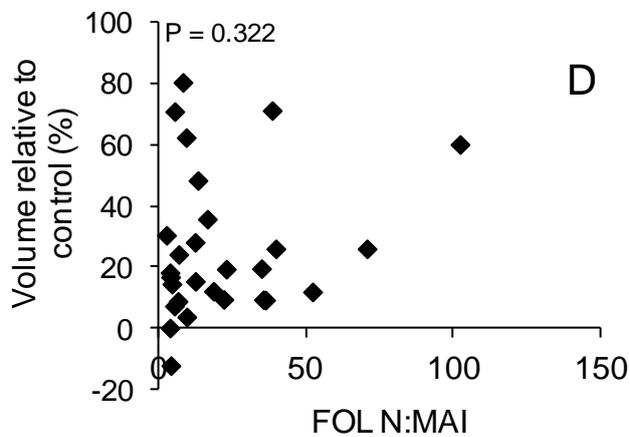
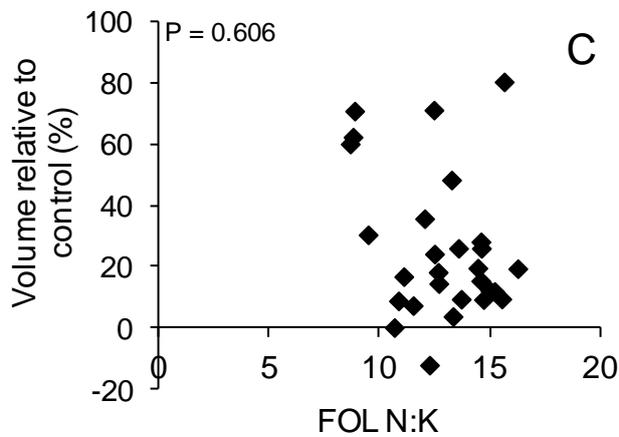
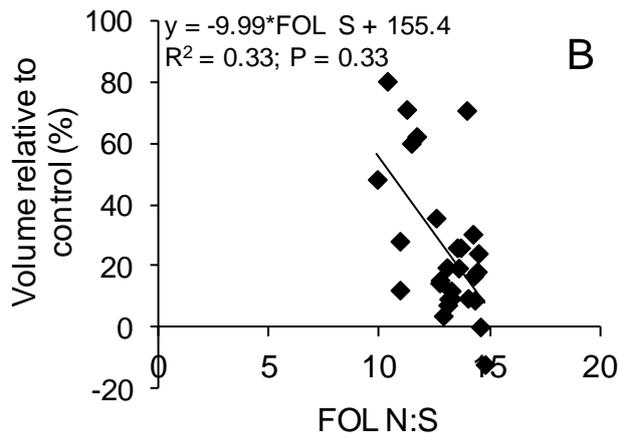
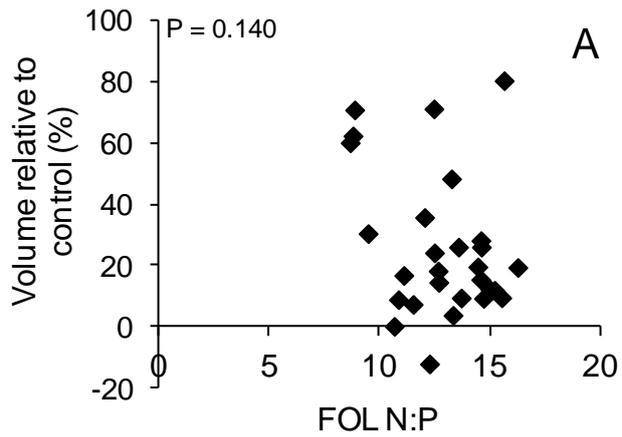
**Figure 6.** Basal area growth response to establishment fertiliser (left-hand side: A-D), mid-rotation fertiliser (centre; E-H) and establishment + mid-rotation (right-hand side; I-L) over time for four basal area quartiles: 0-25% (i.e. the smallest 25% of trees in a stand; A, E and I), 25-50% (B,F and J), 50-75% (C, G and K) and 75-100% (i.e. the largest 25% of trees in a stand; D, H and L). Solid symbols represent more responsive sites and open symbols less responsive sites. Arrows indicate fertiliser addition. Different letters indicate significant differences (all  $P$ -values  $\leq 0.05$ ) between ages within each basal area quartile (cohort) for more responsive sites only, as determined from one-way ANOVAs.



**Figure 7.** Relationships between volume growth response at age 2 to fertiliser applied at establishment and selected 0-10 cm soil variables. Equations and P-values were all derived from non-linear regression analysis (n=28).



**Figure 8.** Relationships between volume growth response at age 2 to establishment fertiliser and foliar (FOL) variables. Equations and P-values were derived from linear regression analysis (n=28).



**Figure 9.** Relationships between volume growth response at age 2 to establishment fertiliser and selected foliar (FOL) variables. All equations and P-values were derived from linear regression analysis (n=28).

## Predicting volume growth response to establishment fertiliser from a combination of variables

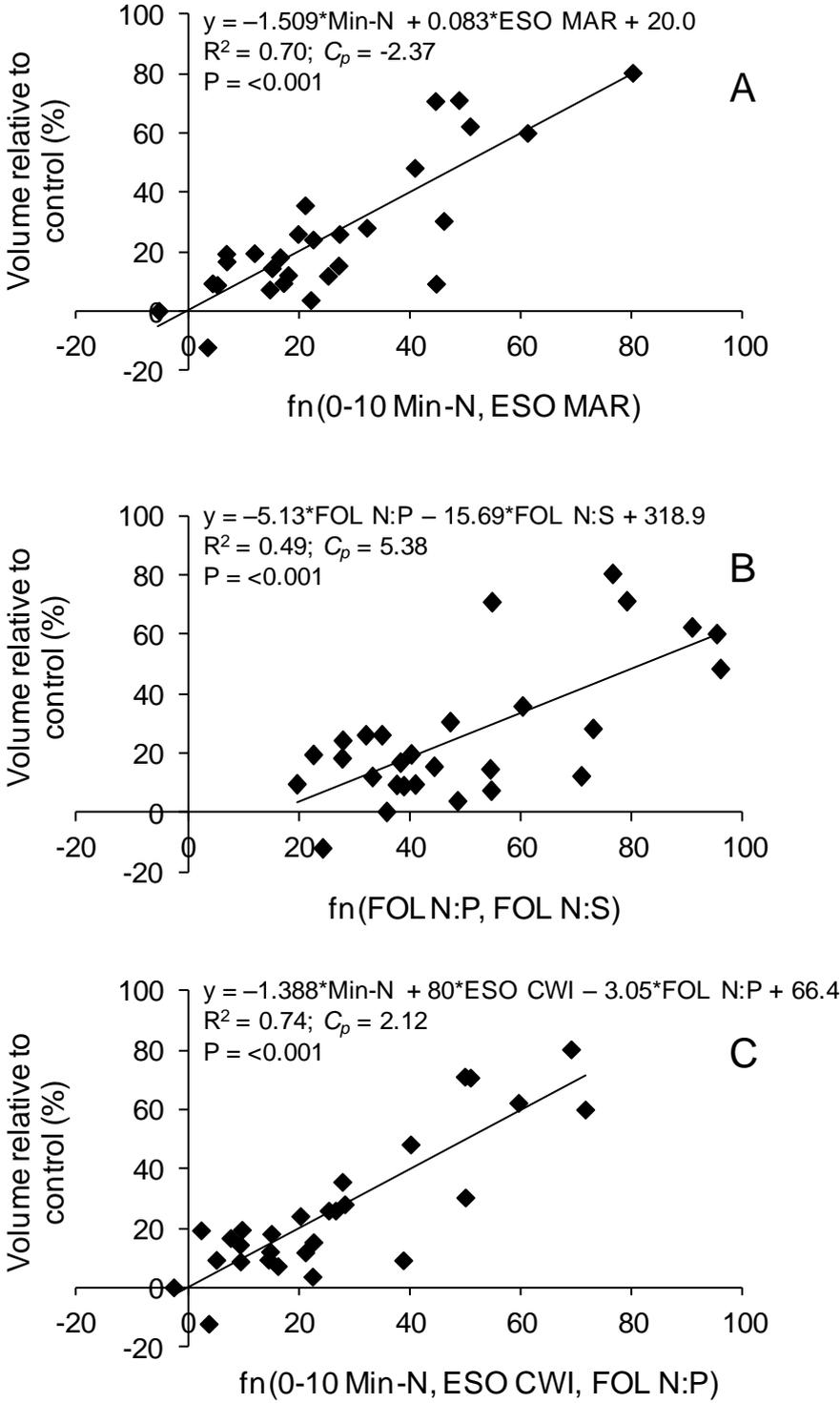
Three model ‘types’ were developed to predict volume growth response to establishment fertiliser: ‘soil-based’ (using only soil and climate data), ‘foliar-based’ (using only foliar and climate data) and ‘combined’ (using a combination of climate, soil and foliar variables). The ‘best’ soil-based model identified by multiple linear regression analysis used 0-10 cm Min-N and ESOCLIM mean annual rainfall (ESO MAR) to predict volume response to establishment fertiliser at age 2 with 70% accuracy (Figure 10A). Addition of ESO MAR only increased model accuracy by 8% compared with using 0-10 cm Min-N as a single explanatory variable (Figure 7A). A different soil-based model best described response to establishment fertiliser at age 4; however, 0-10 cm Min-N remained a significant variable (Figure 11A). In combination with 0-10 cm Min-N, mean annual rainfall (MAR), mean maximum (MAX TEMP) and mean minimum (MIN TEMP) temperature, all sourced from SILO, described 54% of the variation in response to establishment fertiliser at age 4 (Figure 11A). Multiple linear regression analysis also identified models which predicted age 4 volume growth response with up to 60% accuracy; however, several climatic terms were strongly correlated ( $r > 0.650$ ) and therefore were excluded (Table 6 and 7). No significant soil-based models could predict volume growth response at age 10.

**Table 6.** Pearson correlation coefficients ( $r$ ) for climate variables used to develop establishment fertiliser models (via multiple linear regression analysis). Climate variables were sourced from three datasets: long term averages (LTA) for SILO (1889-2012) and ESOCLIM (1921-1995) data as well as averages of SILO data during the actual duration of each experiment (Actual). Climate variables include: mean annual rainfall (MAR), mean annual evaporation (MAE), climate wetness index (CWI; equal to MAR/MAE) mean monthly maximum (MAX TEMP) and minimum (MIN TEMP) temperature and mean monthly radiation (RAD). All  $r$  values are highly significant ( $P < 0.001$ ;  $n=28$ ).

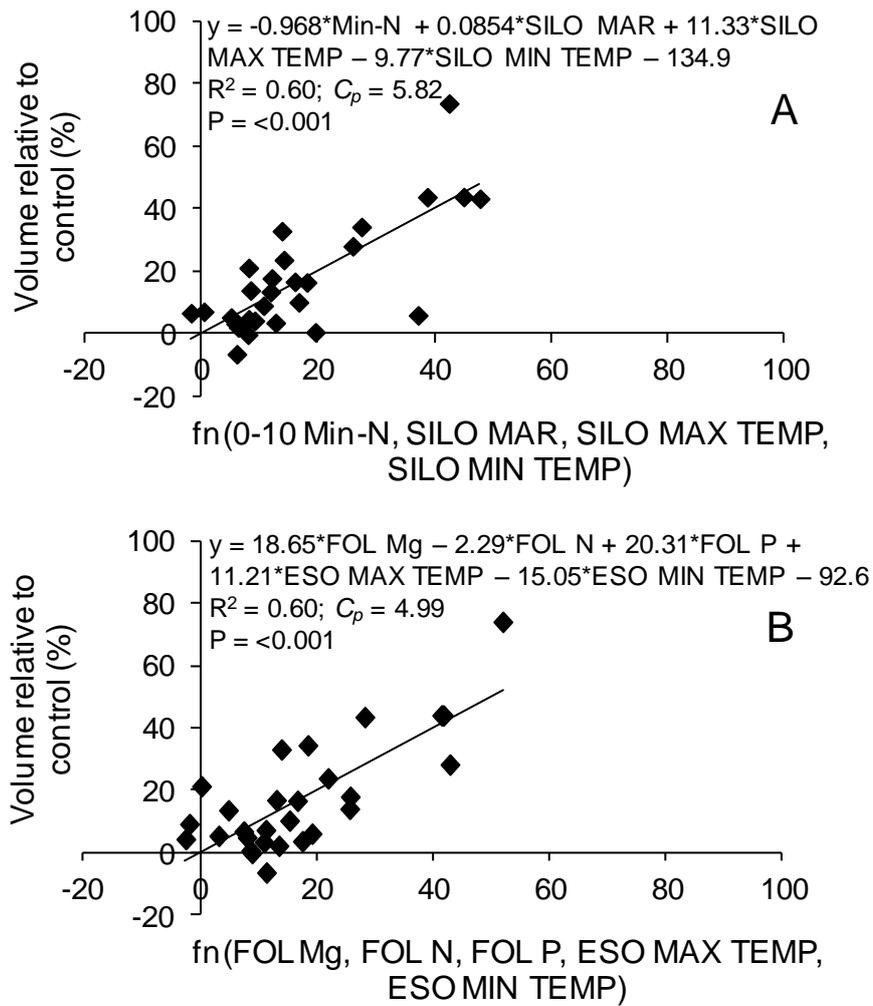
	MAR	MAE	CWI	MAX TEMP	MIN TEMP	RAD
<i>ESOCLIM (LTA)</i> <i>SILO (LTA)</i>	0.871	0.933	0.866	0.951	0.961	0.963
<i>ESOCLIM (LTA)</i> <i>SILO (Actual)</i>	0.874	0.922	0.868	0.927	0.945	0.966
<i>SILO (LTA)</i> <i>SILO (Actual)</i>	0.985	0.984	0.987	0.986	0.983	0.938

The ‘best’ foliar-based model was also significantly, albeit weakly, related to volume growth response at age 2 ( $R^2=0.49$ ; Figure 10B) and relied on pre-treatment foliar ratios of N:P and N:S rather than concentrations of individual nutrients. Foliar N:P and N:S were not correlated and therefore were both included in the model ( $r=0.33$ ; Table 9). Inclusion of climatic variables actually reduced foliar model accuracy and they were therefore removed from the model (see next section). In contrast, the ‘best’ foliar model predicting volume growth response to establishment fertiliser at age 4 used Foliar Mg, N and P, in combination with mean maximum and minimum temperatures sourced from ESOCLIM, to predict response with 60% accuracy (Figure 11B). No foliar-based models could predict volume growth response to fertiliser at age 10.

The ‘best’ overall model predicting volume growth response to establishment fertiliser at age 2 used a combination of climate, soil and foliar variables: ESOCLIM CWI, 0-10 cm Min-N and Foliar N:P (Figure 10C). Sites with lower Min-N (0-10 cm) and foliar N:P and higher ESOCLIM CWI were more likely to show higher volume response to fertiliser ( $R^2=0.74$ ). No combination of soil, foliar and climate variables produced a significant model which could predict volume growth response to establishment fertiliser at age 4 or age 10.



**Figure 10.** Relationships between volume growth response to establishment fertiliser at age 2 and the ‘best’ (A) soil-based, (B) foliar-based and (C) combined models as determined by multiple linear regression analysis (n=28).



**Figure 11.** Relationships between volume growth response to establishment fertiliser at age 4 and the ‘best’ (A) soil-based and (B) foliar-based models as determined by multiple linear regression analysis (n=28).

**Table 7.** Pearson correlation coefficients ( $r$ ) between climate variables used to develop establishment fertiliser models (via multiple linear regression analysis). Climate variables were sourced from three datasets: long term averages (LTA) for SILO (1889-2012) and ESOCCLIM (1921-1995) data as well as averages of SILO data during the actual duration of each experiment (Actual). Climate variables include: mean annual rainfall (MAR), mean annual evaporation (MAE), climate wetness index (CWI; equal to MAR/MAE) mean monthly maximum (MAX TEMP) and minimum (MIN TEMP) temperature and mean monthly radiation (RAD). Values in **bold** are highly significant ( $P < 0.001$ ), values in *italics* are significant ( $P < 0.05$ ) and normal text indicates no significant correlation ( $n=28$ ).

Variables	<i>ESOCCLIM (LTA)</i>				
	MAR	MAE	CWI	MAX TEMP	MIN TEMP
MAR					
MAE	-0.310				
CWI	<b>0.885</b>	<b>-0.713</b>			
MAX TEMP	<i>-0.571</i>	<b>0.857</b>	<b>-0.830</b>		
MIN TEMP	-0.233	<b>0.723</b>	<i>-0.522</i>	<i>0.577</i>	
RAD	-0.248	0.319	-0.305	<i>0.429</i>	-0.060

Variables	<i>SILO (LTA)</i>				
	MAR	MAE	CWI	MAX TEMP	MIN TEMP
MAR					
MAE	<i>-0.489</i>				
CWI	<b>0.955</b>	<b>-0.720</b>			
MAX TEMP	<b>-0.650</b>	<b>0.942</b>	<b>-0.828</b>		
MIN TEMP	<i>-0.277</i>	<b>0.750</b>	<i>-0.467</i>	<b>0.622</b>	
RAD	-0.268	0.213	-0.269	<i>0.396</i>	-0.119

Variables	<i>SILO (Actual)</i>				
	MAR	MAE	CWI	MAX TEMP	MIN TEMP
MAR					
MAE	<i>-0.433</i>				
CWI	<b>0.957</b>	<b>-0.678</b>			
MAX TEMP	<i>-0.570</i>	<b>0.957</b>	<b>-0.773</b>		
MIN TEMP	-0.130	<b>0.671</b>	-0.318	<i>0.496</i>	
RAD	-0.215	0.242	-0.250	0.326	-0.268

**Table 8.** Pearson correlation coefficients ( $r$ ) for a selection of 0-10 cm soil variables to develop models predicting response to establishment fertiliser (via multiple linear regression analysis). Values in **bold** are highly significant ( $P < 0.001$ ), values in *italics* are significant ( $P < 0.05$ ) and normal text indicates no significant correlation ( $n=28$ ).

0-10 cm Soil variables	0-10 cm Soil variables				
	NH <sub>4</sub> -N	NO <sub>3</sub> -N	Min-N	Total C	Total N
NH <sub>4</sub> -N					
NO <sub>3</sub> -N	<i>0.402</i>				
Min-N	<b>0.997</b>	<i>0.467</i>			
Total C	<b>0.775</b>	0.367	<b>0.778</b>		
Total N	<b>0.807</b>	0.315	<b>0.804</b>	<b>0.958</b>	
Bray2 P	0.323	-0.209	0.296	0.056	0.066

## **Substitution of variables strongly related to those in the ‘best’ establishment fertiliser models identified by multiple linear regression analysis**

Several site variables not identified in the ‘best’ models were strongly correlated with the most significant model terms (strongest explanatory variables). This indicates that several alternative models can be used, other than those described above, potentially with only a small loss in accuracy of predicting growth response to fertiliser. This section describes the relationships (correlations) between explanatory variables in the ‘best’ models and presents a number of alternative models. This is intended to increase utility of the outputs of this study, in case the explanatory variables required to run the ‘best’ models are not available to forest managers.

Climate variables were significant terms in most of the ‘best’ models (Figure 10 and 11); however, some models used variables from ESOCLIM, while others used SILO variables, including: MAR, CWI, MAX TEMP and MIN TEMP. These variables were strongly correlated between ESOCLIM and SILO long-term averages (Table 6). Long-term averages from both data sources were also strongly correlated with actual meteorological conditions during each experiment (SILO Actual; Table 6). Further to this, strong relationships existed between climate variables within each meteorological data source (Table 7). ESOCLIM MAR was (understandably) strongly related to ESOCLIM CWI ( $r=0.885$ ; Table 7). ESO MAX TEMP was strongly correlated with mean annual evaporation (MAE) as well as CWI. SILO long-term average CWI was strongly correlated with MAR and MAE as well as MAX TEMP. Long-term average SILO MAX TEMP and MIN TEMP also showed a weak, positive relationship (Table 7). Actual SILO variables were not significant in any of the ‘best’ models; yet showed very similar relationships to long-term average SILO variables (Table 6).

Table 10 presents a number of new models which best described response to fertiliser at age 2, with key climate, soil and/or foliar variables substituted with strongly correlated alternatives. For the ‘best’ soil-based model, substitution of ESO MAR with SILO MAR, ESO CWI or SILO CWI only decreased model accuracy by 2, 3 and 5%. In the best ‘combined’ model, substitution of ESO CWI with ESO MAR, SILO MAR or SILO CWI decreased model accuracy by only 2, 3 and 4%. SILO MAR, MAX TEMP and MIN TEMP were significant terms in the ‘best’ soil-based model predicting volume growth response at age 4 (Figure 11; Table 11). Table 11 shows that substitution of SILO variables with ESOCLIM variables reduced model accuracy from 54 to 47%. Models using CWI in combination with MAX TEMP and MIN TEMP (from either data source) showed higher accuracy (up to 60%) compared with those shown in Table 11; however, CWI and MAX TEMP were strongly correlated ( $r=-0.828$  for SILO and  $-0.830$  for ESOCLIM; Table 7) and therefore at least one of these terms needed to be dropped; causing large reductions in predictive accuracy and model statistical significance. ESO MAX TEMP and MIN TEMP were significant terms in the ‘best’ foliar-based model at age 4 and substitution with correlated variables (i) SILO MAX TEMP and SILO MIN TEMP or (ii) SILO MAE and SILO MIN TEMP reduced accuracy by 9 and 10% (Table 11).

With regard to soil variables (0-10 cm), total C and total N were strongly correlated with Min-N ( $r=0.778$  and  $0.804$ ; Table 8); a significant term in the ‘best’ soil-based and combination models predicting volume growth response at age 2 (Figure 10A and 10C). Substitution of  $\text{NH}_4+\text{NO}_3\text{-N}$  with total N reduced variance accounted for by the ‘best’ soil-based model from 70 to 47%; while substitution with total C reduced variance accounted for to 43% (Table 10). The same substitutions reduced variance accounted for by the ‘best’ combination model from 74% to 57 and 53% (Table 10). In predicting volume response to establishment fertiliser at

age 4, substitution of Min-N with total N or total C in the ‘best’ soil-based model produced models which were not significant (and therefore not shown in Table 11).

Foliar ratios of N:S and N:P were identified as significant in the ‘best’ foliar-based model and foliar N:P was a significant variable in the ‘best’ combination model for predicting growth response to fertiliser at age 2 (Figure 10B and 10C). Aside from those variables from which they were derived, the foliar N/S ratio was only weakly correlated with foliar Ca and Mg ( $r=-0.454$  and  $-0.456$ ); while foliar N:P was correlated with foliar K, Ca and Zn (Table 9). Substitution of foliar N:S and N:P produced only one significant (single-factor) model which used foliar Ca to predict volume growth response at age 2 with 41% accuracy (Table 10; also Figure 8E). Foliar Mg, N and P were significant explanatory variables in the ‘best’ foliar-based model for predicting growth response to fertiliser at age 4 (Figure 11B). As mentioned, foliar Mg was weakly correlated with foliar N:S, while foliar N was well correlated with foliar P, S and K (Table 9). Foliar P was strongly correlated with foliar K, Zn, Fe, N:P and N:K (Table 9). Foliar P was also correlated with Foliar Ca, B and the N/MAI ratio, albeit weakly (Table 9). Table 11 shows that, despite many variables being correlated with foliar Mg, N and P, only one significant alternative model could be identified, achieved by substituting foliar S for foliar N, reducing model accuracy from 60 to 50%.

### **Predicting volume growth response to mid-rotation fertiliser from site climate variables**

Volume growth response to mid-rotation fertiliser at age 5 (one year post-application) was weakly related to MAR sourced from all three climate sources. The strongest relationship was between volume growth response and actual MAR sourced from SILO:  $y = 0.0398 \cdot \text{ACT SILO MAR} - 22.56$  ( $R^2=0.25$ ;  $P=0.033$ ). The relationship was not as strong for long-term MAR sourced from SILO and ESOCLIM, with both showing an  $R^2=0.22$  and  $P=0.050$ . No single climate predictors were related to volume growth response at age 7 or age 10 (all  $P=>0.05$ ; data not shown).

### **Predicting volume growth response to mid-rotation fertiliser from pre-application site foliar variables**

No single foliar variables including: N, P, K, S, Ca or Mg were related to volume growth response at age 5 (Figure 12). However, consistent with our best establishment fertiliser models, the foliar N/P ratio was a significant predictor of volume growth response (at age 5) to mid-rotation fertiliser; albeit with only 20% accuracy (Figure 13A). The ratio of foliar N to pre-treatment MAI (N:MAI) was the strongest single predictor of volume growth response to mid-rotation fertiliser (37%; Figure 13D). No foliar variables were related to volume growth response at age 7 (peak response); however, foliar N:P predicted growth response at age 10 with 22% accuracy ( $y = -2.52 \cdot \text{FOL N:P} + 38.9$ ;  $P=0.045$ ).

### **Predicting volume growth response to mid-rotation fertiliser from a combination of pre-application variables**

Only foliar and climatic variables were available for building models to predict response to mid-rotation fertiliser, therefore the ‘best’ foliar-based and combined models were the same. Compared with establishment models, mid-rotation models had low predictive accuracy, with the best model using the foliar N/P ratio and long-term average SILO MAR to predict volume growth response with 43% accuracy (Figure 14). No models predicted volume growth response to mid-rotation fertiliser at age 7 or age 10 (all  $P=>0.05$ ; data not shown).

**Table 9.** Pearson correlation coefficients (*r*) for a selection of foliar variables used to develop models predicting response to establishment fertiliser (via multiple linear regression analysis). Values in **bold** are highly significant ( $P < 0.001$ ), values in *italics* are significant ( $P < 0.05$ ) and normal text indicates no significant correlation (n=28).

Foliar variables	Foliar variables													
	FOL N	FOL P	FOL S	FOL K	FOL Ca	FOL Mg	FOL Cu	FOL Zn	FOL Mn	FOL Fe	FOL B	FOL N:P	FOL N:S	FOL N:K
FOL N														
FOL P	<i>0.583</i>													
FOL S	<b>0.949</b>	<i>0.560</i>												
FOL K	<b>0.683</b>	<b>0.847</b>	<b>0.675</b>											
FOL Ca	-0.027	<i>0.438</i>	0.081	0.249										
FOL Mg	0.091	0.381	0.222	0.226	0.419									
FOL Cu	<i>0.489</i>	0.152	<i>0.560</i>	0.189	0.146	-0.086								
FOL Zn	<i>0.467</i>	<b>0.855</b>	<i>0.432</i>	<b>0.861</b>	0.284	0.412	0.113							
FOL Mn	0.028	-0.294	0.053	-0.315	-0.230	<i>-0.607</i>	0.394	<i>-0.427</i>						
FOL Fe	<i>0.628</i>	<b>0.691</b>	<i>0.646</i>	<b>0.814</b>	0.253	<i>0.539</i>	0.046	<b>0.774</b>	<i>-0.505</i>					
FOL B	0.189	<i>0.524</i>	0.217	0.409	<b>0.728</b>	<i>0.593</i>	-0.068	<i>0.459</i>	<i>-0.629</i>	<i>0.500</i>				
FOL N:P	0.188	<b>-0.680</b>	0.187	<i>-0.438</i>	<i>-0.490</i>	-0.294	0.291	<i>-0.621</i>	0.369	-0.277	-0.410			
FOL N:S	-0.232	-0.128	<i>-0.524</i>	-0.197	<i>-0.454</i>	<i>-0.456</i>	-0.393	-0.048	-0.084	-0.294	-0.190	0.033		
FOL N:K	-0.081	<b>-0.653</b>	-0.099	<b>-0.771</b>	-0.304	-0.193	0.204	<b>-0.759</b>	<i>0.478</i>	<i>-0.566</i>	-0.368	<b>0.763</b>	0.099	
FOL N:MAI	-0.045	<i>-0.511</i>	0.014	<i>-0.529</i>	-0.284	-0.285	0.214	<i>-0.624</i>	<i>0.557</i>	<i>-0.508</i>	-0.380	<i>0.595</i>	-0.218	<b>0.700</b>

**Table 10.** Alternative models to predict volume growth response at age 2 to establishment fertiliser. The ‘best’ model for each model ‘type’ (i.e. soil, foliar and combined) is presented and compared with alternative models with alternative variables strongly correlated with one or more variables in the ‘best’ model. Climate variables from long-term average (LTA) SILO (1889-2012) and ESOCLIM (1921-1995) including mean annual rainfall (MAR) and climate wetness index (CWI) are included, along with selected 0-10 cm soil variables and foliar (FOL) variables. All models are highly significant ( $P < 0.001$ ) and the ‘best’ alternative for each model is highlighted in grey. Models with  $R^2 < 0.40$  are not shown.

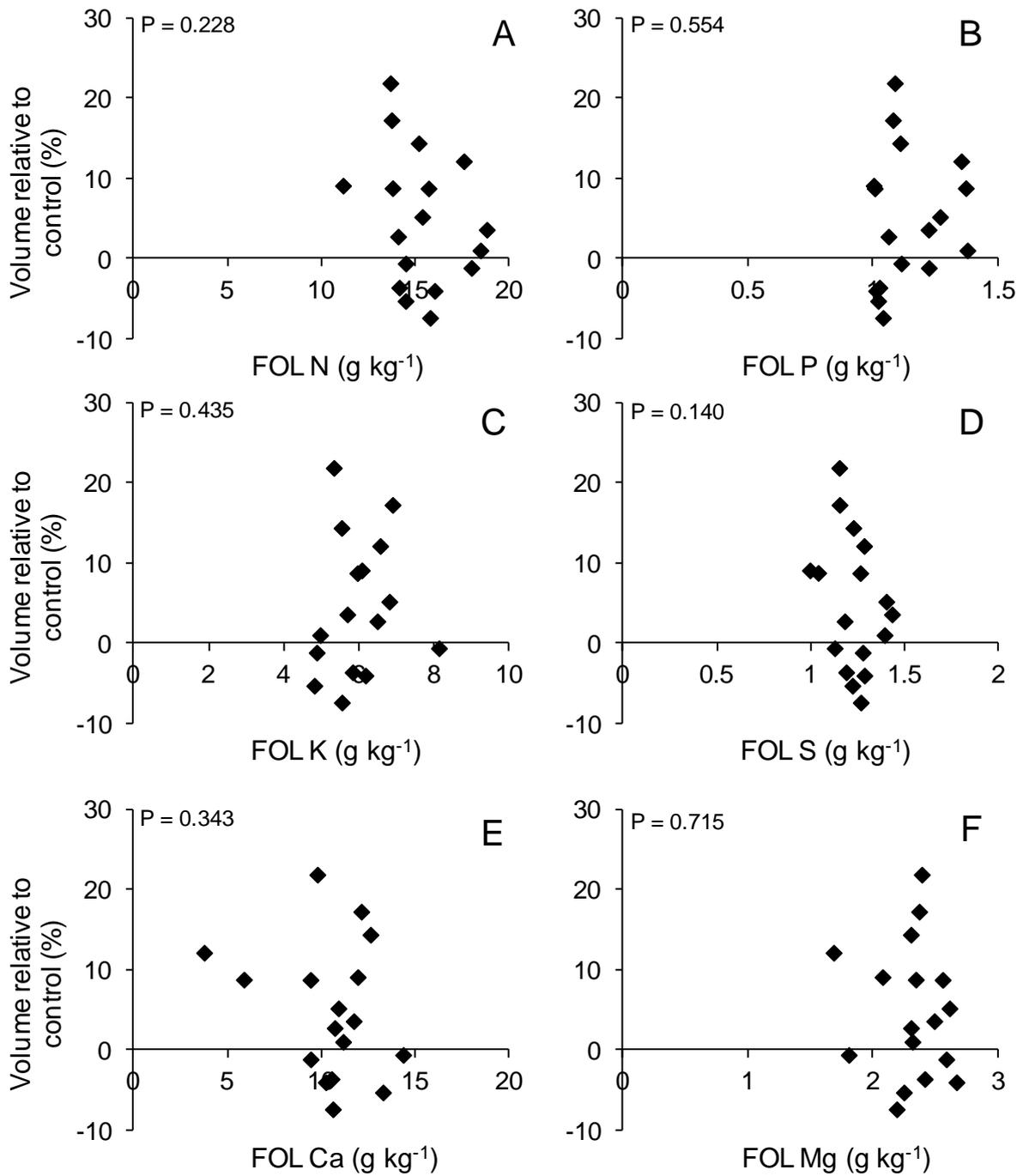
MLR models	Model statistics	
	$C_p$	$R^2$
<b><i>‘Best’ soil-based model</i></b>		
<b><math>y = 0.0828 * \text{ESO MAR} - 1.509 * \text{Min-N} + 20.0</math></b>	3.00	0.70
<i>Alternative soil-based models (using alternative climate data)</i>		
$y = 0.0573 * \text{SILO(LTA) MAR} - 1.567 * \text{Min-N} + 39.9$	3.00	0.68
$y = 62.3 * \text{ESO CWI} - 1.479 * \text{Min-N} + 41.0$	3.00	0.67
$y = 45.0 * \text{SILO(LTA) CWI} - 1.546 * \text{Min-N} + 54.6$	3.00	0.65
<i>Alternative soil-based models (using alternative soil data)</i>		
$y = 0.1149 * \text{ESO MAR} - 20.96 * \text{TOT N} - 12.6$	3.33	0.47
$y = 0.1212 * \text{ESO MAR} - 1.777 * \text{TOT C} - 11.8$	2.79	0.43
<b><i>‘Best’ foliar-based model</i></b>		
<b><math>y = 5.13 * \text{FOL N:P} - 15.69 * \text{FOL N:S} + 318.9</math></b>	5.38	0.49
<i>Alternative foliar-based models (using alternative foliar data)</i>		
$y = 7.67 * \text{FOL Ca} - 23.4$ (note: this is a single-variable model)	5.62	0.41
<b><i>‘Best’ combination model</i></b>		
<b><math>y = 80.0 * \text{ESO CWI} - 1.388 * \text{Min-N} - 3.05 * \text{FOL N:P} + 66.4</math></b>	0.94	0.74
<i>Alternative combination models (using alternative climate data)</i>		
$y = 0.0817 * \text{ESO MAR} - 1.461 * \text{Min-N} - 1.85 * \text{FOL N:P} + 42.7$	2.24	0.72
$y = 0.0580 * \text{SILO(LTA) MAR} - 1.515 * \text{Min-N} - 2.00 * \text{FOL N:P} + 63.2$	4.00	0.71
$y = 55.2 * \text{SILO(LTA) CWI} - 1.482 * \text{Min-N} - 2.66 * \text{FOL N:P} + 80.5$	3.86	0.70
<i>Alternative combination models (using alternative soil data)</i>		
$y = 117.2 * \text{ESO CWI} - 19.75 * \text{TOT N} - 3.99 * \text{FOL N:P}$	4.00	0.57
$y = 142.0 * \text{ESO CWI} - 1.685 * \text{TOT C} - 5.09 * \text{FOL N:P}$	4.00	0.53
<i>Alternative combination models (using alternative foliar data)</i>		
$y = -1.351 * \text{Min-N} + 11.38 * \text{FOL Mg} + 49.8$	7.97	0.64

**Table 11.** Alternative models to predict volume growth response at age 4 to establishment fertiliser. The ‘best’ model for each model ‘type’ (i.e. soil, foliar and combined) is presented and compared with alternative models with alternative variables strongly correlated with one or more variables in the ‘best’ model. Climate variables from long-term average SILO (1889-2012) and ESOCIM (1921-1995) including mean annual rainfall (MAR) and climate wetness index (CWI) are included, along with selected 0-10 cm soil variables and foliar (FOL) variables. All models are highly significant ( $P < 0.001$ ) and the ‘best’ alternative for each model is highlighted in grey. Models with  $R^2 < 0.40$  are not shown.

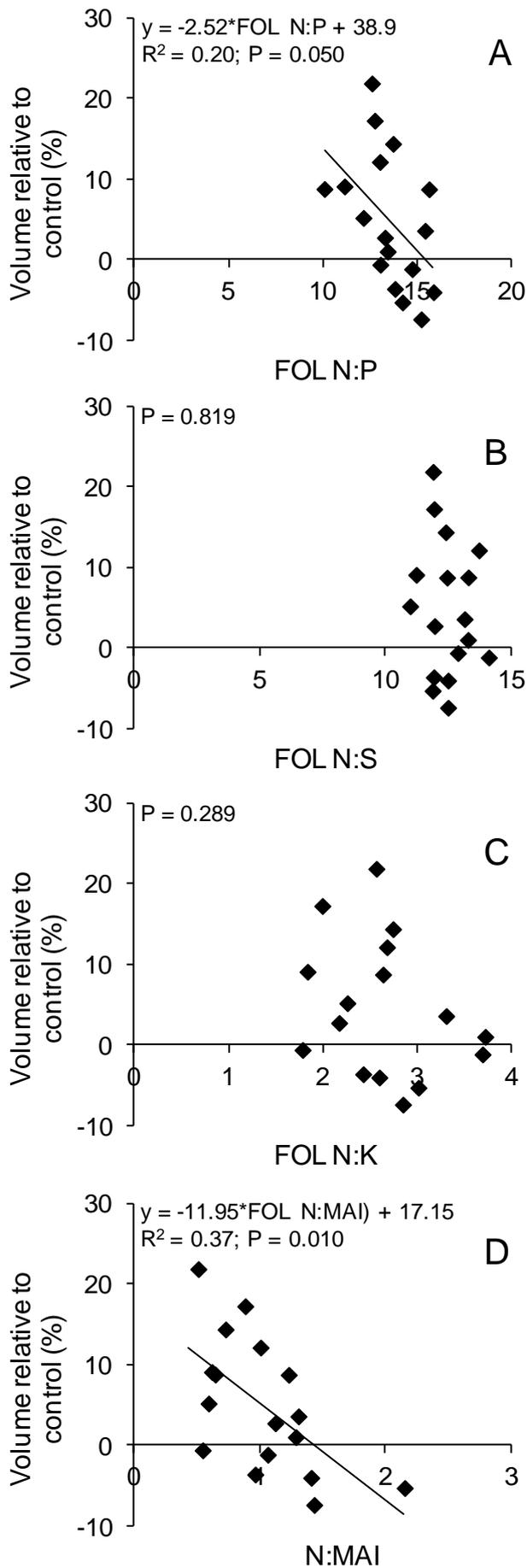
MLR models	Model statistics	
	$C_p$	$R^2$
<b><i>‘Best’ soil-based model</i></b>		
$y = -0.968 * \text{Min-N} + 0.0854 * \text{SILO MAR} + 11.33 * \text{SILO MAX TEMP} - 9.77 * \text{SILO MIN TEMP} - 134.9$	6.67	0.60
<i>Alternative soil-based models (using alternative climate data)</i>		
$y = -0.747 * \text{Min-N} + 0.0862 * \text{ESO MAR} + 13.18 * \text{SILO MAX TEMP} - 8.15 * \text{SILO MIN TEMP} - 193.1$	-0.55	0.47
<b><i>‘Best’ foliar-based model</i></b>		
$y = 18.65 * \text{FOL Mg} - 2.29 * \text{FOL N} + 20.31 * \text{FOL P} + 11.21 * \text{ESO MAX TEMP} - 15.05 * \text{ESO MIN TEMP} - 92.6$	4.99	0.60
<i>Alternative foliar-based models (using alternative climate data)</i>		
$y = 20.95 * \text{FOL Mg} - 2.65 * \text{FOL N} + 24.53 * \text{FOL P} + 8.12 * \text{SILO MAX TEMP} - 16.16 * \text{SILO MIN TEMP} - 23.2$	2.52	0.51
$y = 19.34 * \text{FOL Mg} - 2.62 * \text{FOL N} + 22.06 * \text{FOL P} + 0.120 * \text{SILO MAE} - 17.38 * \text{SILO MIN TEMP} - 9.4$	2.91	0.50
<i>Alternative foliar-based models (using alternative foliar data)</i>		
$y = 25.65 * \text{FOL Mg} - 25.3 * \text{FOL S} + 16.68 * \text{FOL P} + 10.53 * \text{ESO MAX TEMP} - 15.33 * \text{ESO MIN TEMP} - 93.6$	6.20	0.50

### Substitution of variables strongly related to those in the ‘best’ mid-rotation fertiliser models identified by multiple linear regression analysis

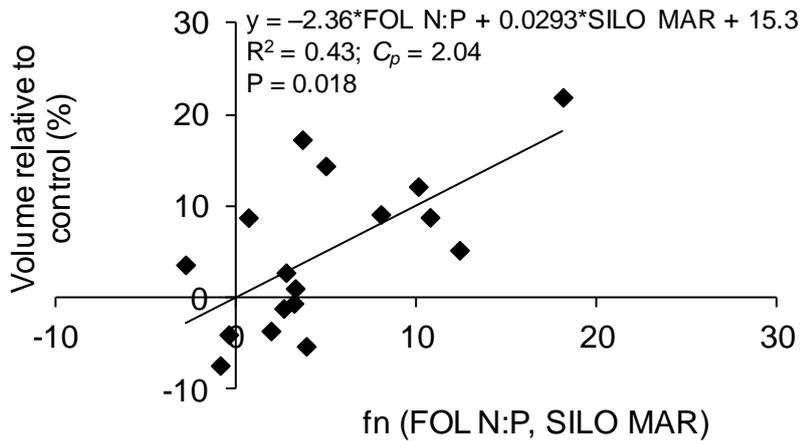
Long-term average SILO MAR was a significant model term in predicting response to mid-rotation fertiliser (Figure 14). Again climate variables from the three sources were strongly correlated for each variable (Table 12) and SILO MAR was strongly correlated with SILO CWI, MAE, MAX TEMP and RAD (Table 13). Substitution of ESO or SILO (long-term average) CWI for SILO MAR only produced significant models with low accuracy (31%;  $P=0.036$  and 34%;  $P=0.028$ ; data not shown). Further, substitution of any other ESO or SILO variables did not produce any significant models (all  $P > 0.05$ ; data not shown). Substitution of ACT SILO MAR and ACT SILO CWI did; however, produce models with only 1-3% less predictive accuracy compared with the best model (Table 15). Table 14 shows that FOL N:P was only weakly correlated with FOL N ( $r=0.507$ ;  $P=0.032$ ) and the foliar-derived N/MAI ratio ( $r=0.590$ ;  $P=0.007$ ). Substitution of these terms did not produce any statistically significant models. As no multiple-factor models predicted growth response to fertiliser at age 7 or 10, no substitutions were possible.



**Figure 12.** Relationships between volume growth response at age 5 to mid-rotation fertiliser and selected foliar (FOL) variables. Equations and P-values were derived from linear regression analysis (n=17).



**Figure 13.** Relationships between volume growth response at age 5 to mid-rotation fertiliser and selected foliar (FOL) variables. Equations and P-values were derived from linear regression analysis (n=17).



**Figure 14.** Relationships between volume growth response to mid-rotation fertiliser at age 5 and the ‘best’ predictive model as determined by multiple linear regression analysis (n=17).

**Table 12.** Pearson correlation coefficients ( $r$ ) for climate variables used to develop mid-rotation fertiliser models (via multiple linear regression analysis). Climate variables were sourced from three datasets: long term averages (LTA) for SILO (1889-2012) and ESOCLIM (1921-1995) data as well as averages of SILO data during the actual duration of each experiment (Actual). Climate variables include: mean annual rainfall (MAR), mean annual evaporation (MAE), climate wetness index (CWI; equal to MAR/MAE) mean monthly maximum (MAX TEMP) and minimum (MIN TEMP) temperature and mean monthly radiation (RAD). All  $r$  values are highly significant ( $P < 0.001$ ;  $n=28$ ).

	<b>MAR</b>	<b>MAE</b>	<b>CWI</b>	<b>MAX TEMP</b>	<b>MIN TEMP</b>	<b>RAD</b>
<i>ESOCLIM (LTA)</i>	0.979	0.942	0.980	0.945	0.985	0.971
<i>SILO (LTA)</i>						
<i>ESOCLIM (LTA)</i>	0.928	0.922	0.964	0.955	0.984	0.920
<i>SILO (Actual)</i>						
<i>SILO (LTA)</i>	0.964	0.988	0.978	0.984	0.997	0.962
<i>SILO (Actual)</i>						

**Table 13.** Pearson correlation coefficients ( $r$ ) between climate variables used to develop mid-rotation fertiliser models (via multiple linear regression analysis). Climate variables were sourced from three datasets: long term averages (LTA) for SILO (1889-2012) and ESOCLIM (1921-1995) data as well as averages of SILO data during the actual duration of each experiment (Actual). Climate variables include: mean annual rainfall (MAR), mean annual evaporation (MAE), climate wetness index (CWI; equal to MAR/MAE) mean monthly maximum (MAX TEMP) and minimum (MIN TEMP) temperature and mean monthly radiation (RAD). Values in **bold** are highly significant ( $P < 0.001$ ), values in *italics* are significant ( $P < 0.05$ ) and normal text indicates no significant correlation ( $n=28$ ).

Variables	<i>ESOCLIM (LTA)</i>				
	MAR	MAE	CWI	MAX TEMP	MIN TEMP
MAR					
MAE	<i>-0.781</i>				
CWI	<b>0.973</b>	<b>-0.904</b>			
MAX TEMP	<b>-0.914</b>	<b>0.902</b>	<b>-0.959</b>		
MIN TEMP	<i>-0.050</i>	<i>0.650</i>	<i>-0.274</i>	0.315	
RAD	<i>-0.788</i>	<b>0.954</b>	<b>-0.891</b>	<i>0.834</i>	0.561
Variables	<i>SILO (LTA)</i>				
	MAR	MAE	CWI	MAX TEMP	MIN TEMP
MAR					
MAE	<i>-0.636</i>				
CWI	<b>0.966</b>	<i>-0.814</i>			
MAX TEMP	<i>-0.798</i>	<b>0.955</b>	<b>-0.922</b>		
MIN TEMP	0.030	<i>0.673</i>	<i>-0.202</i>	0.444	
RAD	<i>-0.736</i>	<b>0.971</b>	<b>-0.878</b>	<b>0.957</b>	0.601
Variables	<i>SILO (Actual)</i>				
	MAR	MAE	CWI	MAX TEMP	MIN TEMP
MAR					
MAE	<i>-0.362</i>				
CWI	<b>0.912</b>	<i>-0.712</i>			
MAX TEMP	<i>-0.545</i>	<b>0.929</b>	<b>-0.823</b>		
MIN TEMP	0.254	0.561	<i>-0.050</i>	0.277	
RAD	<i>-0.343</i>	<b>0.899</b>	<i>-0.650</i>	<i>0.756</i>	<i>0.751</i>

**Table 14.** Pearson correlation coefficients ( $r$ ) for a selection of foliar variables used to develop models predicting response to mid-rotation fertiliser (via multiple linear regression analysis). Values in **bold** are highly significant ( $P < 0.001$ ), values in *italics* are significant ( $P < 0.05$ ) and normal text indicates no significant correlation ( $n=28$ ).

Foliar variables	Foliar variables													
	FOL N	FOL P	FOL S	FOL K	FOL Ca	FOL Mg	FOL Cu	FOL Zn	FOL Mn	FOL Fe	FOL B	FOL N:P	FOL N:S	FOL N:K
FOL N														
FOL P	<b>0.768</b>													
FOL S	<b>0.985</b>	<b>0.731</b>												
FOL K	-0.278	-0.124	-0.227											
FOL Ca	-0.493	-0.517	-0.418	0.082										
FOL Mg	0.332	0.085	0.545	-0.319	-0.070									
FOL Cu	0.133	0.182	0.121	0.065	-0.381	-0.365								
FOL Zn	0.682	0.437	<b>0.759</b>	-0.187	-0.234	0.660	0.068							
FOL Mn	0.441	0.267	0.257	-0.345	-0.023	-0.139	0.375	0.241						
FOL Fe	0.255	0.273	0.467	0.195	-0.035	0.296	0.082	0.141	0.031					
FOL B	-0.051	-0.122	0.147	-0.332	0.111	0.502	-0.246	0.099	-0.204	0.431				
FOL N:P	0.507	-0.159	0.415	-0.246	-0.086	0.404	-0.022	0.457	0.299	0.062	0.107			
FOL N:S	0.600	0.556	0.292	-0.001	-0.700	-0.389	0.420	0.089	0.362	-0.076	-0.436	0.176		
FOL N:K	<b>0.806</b>	0.594	0.690	<b>-0.776</b>	-0.302	0.380	0.009	0.529	0.506	0.239	0.164	0.432	0.372	
N- index	0.454	0.089	0.468	-0.481	-0.134	0.292	0.150	0.417	0.406	-0.069	0.162	0.590	0.128	0.571

**Table 15.** Alternative models to predict volume growth response at age 5 to mid-rotation fertiliser. Soil variables were not available for mid-rotation sites, therefore only the best foliar-based models are presented. Alternative models are also presented, where terms from the best models are substituted for strongly correlated variables. Variables include climatic factors sourced from long-term average (LTA) SILO (1889-2012) and ESOCLIM (1921-1995), as well as ‘actual over rotation’ SILO (SILO ACT), namely: mean annual rainfall (MAR) and climate wetness index (CWI). Selected foliar (FOL) variables are also included, such as the foliar N/P ratio (FOL N:P) and the N-uptake index (FOL N-uptake). All models are significant ( $P < 0.05$ ) and the ‘best’ alternative for each model is highlighted in grey.

MLR models	Model statistics	
	$C_p$	$R^2$
<i>‘Best’ foliar-based model</i>		
$y = -2.36 * \text{FOL N:P} + 0.0293 * \text{SILO MAR} + 15.3$	2.04	0.43
<i>Alternative foliar-based models (using alternative climate data)</i>		
$y = -2.27 * \text{FOL N:P} + 0.0366 * \text{ACT SILO MAR} + 10.5$	2.80	0.42
$y = -2.52 * \text{FOL N:P} + 44.7 * \text{ACT SILO CWI} + 16.4$	2.99	0.40

## Discussion

### Magnitude and duration of volume growth response to fertiliser (stand level)

Applications of fertiliser at establishment (ages 0 and 1), representing a total application of  $\sim 250 \text{ kg N ha}^{-1}$  and  $\sim 90 \text{ kg P ha}^{-1}$ , initially increased volume by 34.3% which declined to only 5.6% by age 10 (typically rotation end). The decay of this relative response over time stabilised by 5 years post-treatment, suggesting that the volume gained by mid-rotation is unlikely to increase further. Relative responses to N have been shown to last 4-6 years at ex-native forest and ex-pine plantation sites in Tasmania (Cromer *et al.*, 2002; Smethurst *et al.*, 2004), with the peak response 2-3 years post-application. Smethurst *et al.* (2004) showed soil N-status indicators increased 1-2 years post-application then declined, indicating prolonged growth response most likely due to increased leaf area (Smethurst *et al.*, 2003). Addition of high-N fertiliser in eucalypts increases basal area growth by increasing leaf area (Wiseman *et al.*, 2006; Wiseman *et al.*, 2009), therefore it is not surprising that growth response to fertiliser continues even after available N in the soil returns to pre-application levels.

Despite the large initial relative increase in volume of stands fertilised at establishment, in terms of absolute volume gain there was no significant effect of fertiliser addition, even when only considering the more responsive sites. It is likely that the relative difference between fertilised and control trees at age 2 was undetectable on an absolute basis because at this age, trees are small and even a large relative difference translates into a small difference in absolute volume. For instance, 34.3% more volume at age 2 translated to an absolute increase of only  $1.5 \text{ m}^3 \text{ ha}^{-1}$ ; whereas 5.6% more volume at age 10 was equivalent to  $5.3 \text{ m}^3 \text{ ha}^{-1}$ . It is highly likely that establishment fertiliser only marginally increased leaf area shortly after application, facilitating only a small gain in basal area and therefore standing volume. Our results suggest that although fertilised stands responded to fertiliser applied early in the rotation, the response is unlikely to substantially increase yield by more than 6% by age 10. From work on ex-forest sites in Tasmania, Smethurst *et al.* (2004) recommended that

applications of fertiliser at planting and during establishment should be followed up with 100-200 kg ha<sup>-1</sup> of N within the next 6 years. Given our analysis of the magnitude and duration of response to establishment fertiliser focussed on more responsive sites, we too would suggest that further applications prior to canopy closure are required to increase productivity. Indeed, for the subset of 10 establishment fertiliser sites which received an additional 200 kg ha<sup>-1</sup> of N fertiliser at mid-rotation, relative volume yield increased from 5.6% (EST only) to 10.6%; increasing absolute yield of fertilised treatments from 5.3 m<sup>3</sup> ha<sup>-1</sup> (EST only) to 13.7 m<sup>3</sup> ha<sup>-1</sup> (EST plus MID) relative to control treatments.

In contrast to the establishment fertiliser response, a single mid-rotation application of 250 kg N ha<sup>-1</sup> increased the volume of fertilised stands by 26.0% after three years and 21.9% by age 10, equivalent to an increased yield of 42.9 m<sup>3</sup> ha<sup>-1</sup>. There are several possible explanations as to why growth responses to mid-rotation fertiliser were sustained through to end of rotation, where establishment applications were not: (i) mid-rotation stands were better able to convert fertiliser into increased leaf area and basal area growth compared with stands fertilised at establishment, (ii) mid-rotation stands had significantly lower N-status compared with establishment stands and/or (iii) mid-rotation sites were unconstrained in response by, for example, water supply or micronutrient deficiency compared with establishment sites.

Significant response to fertiliser at mid-rotation sites follows our current understanding of fertiliser response, where the greatest gains in growth occur immediately prior to canopy closure (see Forrester *et al.*, 2010b for a recent review). Immediately prior to canopy closure, trees typically have developed sufficient root systems to fully capture the site, increasing inter-tree competition for nutrients and therefore increasing their potential responsiveness to additional nutrients, compared with at establishment (e.g. Cromer *et al.*, 1993a; Stape *et al.*, 2006; Turnbull *et al.*, 2007). At this growth stage, trees potentially have greater demand for and capacity to uptake and convert additional nutrients supplied through fertiliser into leaf area, basal area and therefore final volume yield (Cromer *et al.*, 1993a; Bennett *et al.*, 1997; Smethurst *et al.*, 2003; Stape *et al.*, 2004; Gonçalves *et al.*, 2008). Addition of mid-rotation fertiliser at responsive sites most likely accelerated stand growth (Miller, 1981) to achieve peak leaf area (Cromer *et al.*, 1993a; White *et al.*, 2010); setting fertilised stands on a higher growth trajectory which persisted to the end of rotation, i.e. a 'type 2 response' (Snowdon, 2002). Nutrient demands required to sustain growth response would most likely have been met through re-translocation over time (Saur *et al.*, 2000; Fife *et al.*, 2008). PAI in our study indicated that fertilised stands returned to the same volume growth rate as control stands between age 7 and 10, suggesting duration of response to urea-N (250 kg N ha<sup>-1</sup>) of 3 to 4 years. Larger growth responses to fertiliser applied at mid-rotation have been found previously, e.g. Stape *et al.* 2006 showed greater relative growth response to fertiliser in older stands (5-6 years) compared with younger stands (2-3 years); however they did not explore underlying mechanisms as they were unable to rule out whether changes to silvicultural regimes (i.e. higher rates applied to older plantations) were responsible.

The foliar nutrient status of mid-rotation sites was significantly lower than at establishment sites for N, P K and S which may indicate: (i) morphological differences between juvenile (establishment sites) and adult (mid-rotation sites) leaf types and/or (ii) low nutrient availability due to lack of early fertiliser application in mid-rotation stands. Foliar N and P concentrations have been shown to decrease with stand age (Bennett *et al.*, 1996; Judd *et al.*, 1996), therefore it may not be valid to apply the thresholds described by Dell *et al.* (2001) to mature leaves at mid-rotation sites. However, a decline in nutrient concentration with leaf age may indeed be indicative of decreasing soil nutrient status, rather than a physiological change in nutrient requirement. Without foliar nutrient thresholds for mature leaves, it is difficult to

conclude that foliar levels, particularly of N, P, K and S, were low and limiting growth at mid-rotation sites and therefore whether they explain the greater volume growth response.

While MID sites showed lower foliar nutrient status compared with EST sites, there were no differences in N, P, K and S between 'more' and 'less responsive' MID sites. This suggests that foliar levels of N, P, K and S were not related to fertiliser response at mid-rotation and that less responsive sites were limited by other factors. Both the more and less responsive mid-rotation sites showed similar foliar micronutrient levels and it is therefore unlikely that responsiveness was constrained by trace element deficiency at less responsive sites. The climate wetness index (CWI) and mean annual rainfall (MAR) were also similar between more and less responsive mid-rotation sites, suggesting that water was not limiting the less responsive sites either. However, water availability is also affected by the storage capacity of the soil profile and by access to groundwater, as well as rainfall and evaporative demand (Morris and Collopy, 1999; Benyon *et al.*, 2006). It therefore remains a possibility that total water availability limited the responsiveness of the less responsive mid-rotation sites, and that low nutrient availability was responsible for mid-rotation sites showing greater growth responses than establishment sites. Our attempt to attribute the influence of groundwater on response to fertiliser was limited, as detailed survey information was not available at all sites. As a categorical explanatory variable, the presence or absence of groundwater within 5 m of the soil surface is probably inadequate to discern an influence of groundwater on growth and fertiliser response. We therefore cannot conclude that water availability did not explain response to fertiliser at mid-rotation, or explain why sites fertilised at mid-rotation showed a large, sustained increase in productivity in response to fertiliser compared with sites fertilised at establishment. Irrespective of water availability, there was also no significant difference in 0-10 cm topsoil N-status between the more responsive establishment and mid-rotation sites which does not support the theory that N-deficiency caused the greater response to mid-rotation fertiliser; despite total N from topsoil samples having previously been related to fertiliser response (Smethurst *et al.*, 2004).

Mid-rotation sites showed higher mean annual evaporation and maximum temperature (ESOCLIM) compared with establishment sites which cannot be ruled out as a factor contributing to the difference in magnitude and duration of response. The majority of sites (18 of 28) used for establishment applications were located in central Victoria (temperate climate); while the remaining 10 covered approximately the same climatic zones as the mid-rotation sites. This was an unavoidable complication of the available data and is the most likely explanation for the difference in MAE and MAX TEMP between fertiliser application ages. Despite this, key climatic indicators related to rainfall (MAR and CWI) were similar for both establishment and mid-rotation sites, therefore it is unlikely that differences in the magnitude and duration of response occurred due to differences in climate.

Relative and absolute volume growth responses to mid-rotation fertiliser clearly differed between sites receiving only mid-rotation fertiliser (MID) and those which received both establishment and mid-rotation fertiliser (EST+MID). There were no significant differences in climate or pre-treatment soil and foliar nutrient status which can explain why EST+MID sites showed lower unfertilised volume growth and smaller responses to fertiliser compared with MID sites. Several possible explanations for this discrepancy remain: (i) faster growth rates (higher productivity; age 10 yield) of sites used for mid-rotation applications increased N-demand and therefore responsiveness to N-fertiliser relative to EST+MID sites, (ii) EST+MID sites were constrained by factors other than N, or by N-related factors undetected through pre-treatment soil and foliar sampling and/or (iii) EST+MID sites received only 200 kg N ha<sup>-1</sup> at age 4; whereas MID sites received 250 kg N ha<sup>-1</sup>.

The sites used to assess volume growth response to mid-rotation fertiliser on average yielded  $\sim 40 \text{ m}^3 \text{ ha}^{-1}$  more volume at age 10 (control treatments) compared with those used for EST+MID responses. Given pre-treatment soil and foliar nutrient status did not differ between these two application ages; it is likely this difference was the result of one or a number of unknown factors, such as soil water availability or total site nutrient capital. PAI at MID sites was rapid at mid-rotation, even for control plots; therefore their higher, sustained response to fertiliser may have been due to a higher N-demand; rather than the sites having an inherently higher N-supply (Miller, 1995; Nambiar, 1995). It is also possible that EST+MID sites showed a lower volume growth response to mid-rotation fertiliser as they were constrained by limiting factors, such as water availability which can significantly limit response to fertiliser (Kreuzwieser and Gessler, 2010); or that MID sites had access to additional soil water or groundwater resources (White *et al.*, 2009). Finally, the difference in N-rate applied at MID and EST+MID sites at mid-rotation may have contributed to the smaller volume growth response observed at EST+MID sites. The response to N-fertiliser supplied as urea is non-linear, with May *et al.* (2009a) showing growth responses 10 times higher for a  $200 \text{ kg ha}^{-1}$  N application in thinned stands of *Pinus radiata* compared with  $100 \text{ kg ha}^{-1}$  N. Regardless of the cause, the fact that responses to mid-rotation fertiliser differed between the relatively small number of MID and EST+MID datasets suggests that the hypothesis that MID stands are better able to convert fertiliser into increased leaf area and basal area growth compared with stands fertilised at establishment requires further evaluation across a wider range of sites.

The magnitude and duration of response to fertiliser seen here is clearly dependent on the responsiveness of sites used in this analysis and will not apply to all sites. Response to fertiliser will be dependent on site requirement for fertiliser rate and type as well as limiting factors, such as available soil depth and water (Nambiar, 1995; Gonçalves *et al.*, 2004; Kreuzwieser and Gessler, 2010). The rates of fertiliser used in this study were higher than those typically used operationally; with N-applications typically  $16\text{-}34 \text{ kg ha}^{-1}$  at establishment to  $58\text{-}90 \text{ kg ha}^{-1}$  for mid-rotation stands, adjusted for total area fertilised (May *et al.*, 2009b). N-uptake rates in the first three years of growth have been shown to be  $\sim 100 \text{ kg ha}^{-1} \text{ yr}^{-1}$  (Cromer *et al.*, 1993b; Misra *et al.*, 1998b); however, we are not suggesting that this rate of fertiliser should be directly applied as much of it can be supplied by nitrogen mineralisation depending on the site (Moroni *et al.*, 2002). Smethurst *et al.* (2004) suggested no significant advantage in any single application exceeding  $200 \text{ kg N ha}^{-1}$  based on ex-native sites in Tasmania. The supply of nutrients, whether provided by the natural fertility of a site or through fertiliser application, needs to meet the demands of the plantation to avoid losses in productivity (Nambiar, 1995).

The benefit of studying large application rates is that mechanisms of growth response can be described (Nambiar, 1995). Our analysis provides useful insight into the duration of response to fertiliser and illustrates the difference between applying approximately the same rate of N at two alternative points in the rotation. The results suggest a substantial increase in volume at age 10 if fertiliser is applied mid-rotation rather than at establishment. The magnitude of the relative growth response one year after the mid-rotation application was similar to that of the establishment application at the same age (5); which suggests no volume growth advantage in applying establishment fertiliser. This analysis does not acknowledge site-specific beneficial effects of establishment fertiliser, such as increased survival and competitive advantage over weeds through targeted spot applications (Pallett and Sale, 2004; du Toit *et al.*, 2010). Studies have shown significant benefits in rapid crown development at an early age and applications at age 2 and 3 which are also likely to show significant growth responses (Stape *et al.*, 2004; Laclau *et al.*, 2010). A large caveat to our observed responses is that these sites were all first rotation, ex-agricultural land with (most likely) higher plant-

available nutrients (Wang *et al.*, 1998) compared with, for example, a second or third rotation plantation site (Mendham *et al.*, 2003; O'Connell *et al.*, 2003; Corbeels *et al.*, 2005). Sites with depleted nutrient capital, such as 2R and 3R sites, may benefit much more from earlier intervention compared with the sites presented here.

### **Magnitude and duration of basal area growth response to fertiliser (sub-stand level)**

Plantation managers are often interested in sub-stand level growth (tree-level) rather than stand level standing volume as this can drive decisions on end-use, harvesting and logistics. To address this, each stand in this study was split into quartiles based on the basal area of individual trees. Each quartile could then be compared to determine whether different size cohorts differ in response to fertiliser application. Interestingly, this analysis showed that while all quartiles showed large initial relative responses to establishment fertiliser, only the first 2 quartiles (i.e., the smallest half of trees in a stand) showed any difference in basal area by age 10. This result must be interpreted carefully when attempting to explain the impact on total basal area and therefore volume gain at the stand level. Combined, the first two quartiles represent only approximately one third of total stand basal area; with the largest 50% of trees representing the remainder. Therefore, smaller relative increases in the basal area of larger trees can have a greater impact on the total basal area of a stand. However, given that the basal area of fertilised trees in the largest two quartiles was no different from unfertilised trees, it is unlikely that the 5.6% overall volume gain in response to establishment fertiliser was caused by an increase in the basal area of larger trees. It is far more likely that the overall volume gain was due to an increase in the size of smaller trees, with basal areas less than the median tree size. At sites receiving both establishment and mid-rotation fertiliser, the smallest 50% of trees (quartiles 1 and 2) showed similar responses to sites receiving only establishment fertiliser. However, the largest 50% (third and fourth quartiles) did show higher relative responses compared with establishment fertiliser sites which may explain the 5% increase in relative yield for EST+MID sites compared with EST sites.

In contrast, the mid-rotation application of fertiliser increased basal area at age 10 across all quartiles; however greater relative gains were still achieved in the first quartile and the smallest gains in the last quartile. Again, the absolute contribution of the 3<sup>rd</sup> and 4<sup>th</sup> quartiles to stand volume was far greater than the first two. Larger gains in small fertilised trees relative to small control trees may be the result of the calculation; i.e., a small absolute difference in basal area between a fertilised tree and a control tree can translate into a large relative difference. Competition from larger trees may have increased demand for nutrients in smaller trees; however, the majority of studies suggest that larger trees have more below-ground resources and therefore should have a greater capacity to capture additional nutrients supplied by fertiliser (Weiner and Thomas, 1986; Schwinning and Weiner, 1998). Competition for light is low pre-canopy closure which suggests that the majority of competition occurs below-ground. While competition for light is thought to favour larger, dominant trees, the ability of both large and small trees to access below-ground resources is thought to be more similar, relative to size (Weiner, 1990). West (1984) studied competition dynamics in even-aged *E. obliqua* and found that larger, more competitive trees were randomly distributed throughout plots, rather than at regular intervals. It was suggested that root grafting (Ashton, 1975) and therefore movement of water and nutrient resources between trees, combined with irregularly shaped root and crown systems common in eucalypts may reduce below-ground competition for resources (West, 1984). The underlying mechanism which explains our result is unclear; however, the implications are that volume gains from 0-1 fertiliser applications only benefit smaller trees. For pulpwood growers this may represent an

increase in merchantable yield; but only if the diameter of small trees is increased to above the minimum harvest diameter. If not, any additional growth will merely increase waste, rather than merchantable yield. For sawlog regimes, increased growth of the smallest trees in a stand indicates no benefit of early fertiliser addition for the trees most likely to be retained post-thinning (Forrester *et al.*, 2013).

### **Predicting volume growth response to establishment fertiliser**

Our analysis of magnitude and duration of response to fertiliser is restricted to those sites identified as ‘more responsive’, i.e. those most likely to demonstrate economic benefits at harvest. To utilise the knowledge gained on the magnitude and duration of fertiliser response at these sites, it is critical to identify why those sites responded and why the less responsive sites did not. We have identified several models which can predict volume growth response to fertiliser from pre-treatment climate, soil and foliar variables and therefore be used to identify sites which are more likely to respond to fertiliser.

Multiple linear regression analysis successfully identified a number of models which could predict volume growth response to N and P fertiliser at age 2 (1 year after last application) and at age 4 (3 years after last application), but not at age 10 (9 years after last application). The most accurate model (74%) relied on a combination of 0-10 cm soil Min-N, foliar N:P and ESOCLIM CWI to predict growth response 1-year after fertiliser application. Although the ‘best’ predictor, this model would require plantation managers to sample both soil and foliar samples as well as sourcing climate data in order to make fertiliser use decisions. The cost of this intensity of diagnostic sampling may be prohibitive to some plantation operations. For only a 4% loss in accuracy, plantation managers could use the ‘best’ soil-based model which used 0-10 cm soil Min-N and ESOCLIM MAR, eliminating the need for foliar sampling. The ‘best’ soil-based model to predict relative response to establishment fertiliser out to age 4 ( $R^2=0.60$ ) also relied on Min-N in combination with a different set of climate variables: SILO MAR, MAX TEMP and MIN TEMP.

Min-N, which in our case refers to a Hot-KCl extraction of  $\text{NH}_4+\text{NO}_3\text{-N}$ , has been used by many studies to represent the potentially mineralisable nitrogen supply of a soil (Ros *et al.*, 2011). Aerobic and anaerobic biological incubation, as well as N-uptake by plants from either field or glasshouse experiments, represent the best means to estimate potentially mineralisable N (Ros *et al.*, 2011). However, these methods are expensive, time-consuming, labour-intensive and the results are very dependent on environmental conditions such as temperature and moisture (Ros *et al.*, 2011). Therefore, laboratory chemical extractions have been developed and correlated with biological incubations and/or plant N uptake as a means to providing a fast and reliable method of estimating potentially mineralisable N. Hot-KCl extractions have previously been well-correlated with biological aerobic and anaerobic incubation methods, as well as actual plant N-uptake (Whitehead, 1981; Gianello and Bremner, 1986a; Wang *et al.*, 1996; Ros *et al.*, 2011); although not in all cases (Moroni *et al.*, 2004). Hot-KCl  $\text{NH}_4+\text{NO}_3\text{-N}$  is not a standard soil test; however, industry-driven demand could cause commercial laboratories to offer this analysis, given its success as a predictor of fertiliser response in our study. Hot-KCl  $\text{NH}_4+\text{NO}_3\text{-N}$  is a relatively simple extraction where 2M KCl is added to a small soil sample and heated at 95-100 °C (Øien and Selmer-Olsen, 1980; Selmer-Olsen *et al.*, 1981; Gianello and Bremner, 1986b; Wang *et al.*, 1996). By applying heat, this method most likely extracts N from soil microorganisms and relatively labile soil organic matter, in addition to that extracted by shaking soil samples with 2M KCl at room temperature. Unfortunately, KCl extraction at room temperature is the standard technique used in commercial laboratories and we had no means of assessing it as an

explanatory variable. In a recent meta-analysis on the ability of soil tests to predict N-mineralisation, Ros *et al.* (2011) showed that room-temperature KCl extractions explained an equivalent amount of variation in predicting N-mineralisation (determined through biological incubation) as total N (~45%). Hot KCl extractions explained almost 60% of this variation (n=215); while interestingly, extractions with acid  $K_2Cr_2O_7$  explained 74% of the variation (n=49). There remains scope to use a range of alternative extraction methods to represent the potentially mineralisable nitrogen supply of a soil.

Total N and extractable N-fractions including soil solution  $NH_4$  and  $NO_3$ , as well as KCl-extractable  $NO_3^-$  (room temperature extraction) have previously been shown as indicators of current soil N status (Wang *et al.*, 1996; Cromer *et al.*, 2002; Moroni *et al.*, 2004; Smethurst *et al.*, 2004). Smethurst *et al.*, (2004) suggested a critical concentration of  $1 \text{ mg kg}^{-1}$  KCl-extractable  $NO_3^-$ ; with higher concentrations indicative of a less responsive site. In our study, Hot-KCl  $NO_3^-$  represented on average only 3% of Min-N at sites used for establishment fertiliser applications. As such, when used as a single predictor of fertiliser response, our results suggest a that a higher concentration of  $<20 \text{ mg kg}^{-1}$  Min-N in the top 0-10 cm is more likely to result in a significant response to fertiliser, while  $>40 \text{ mg kg}^{-1}$  is less likely to show a response ( $R^2=0.62$ ). Sampling only the top 0-10 cm does not capture total available nutrient capital; however, the topsoil (0-10 cm) concentration of Min-N is clearly indicative of site responsiveness, despite N availability of the subsoil (10-120 cm) having been estimated as twice that of that of the top 10 cm (Moroni *et al.*, 2004). Relationships between volume growth response and soil variables at 10-20 cm and 0-20 cm were not as strong as the 0-10 cm depth which also suggests the shallow depth as being a strong indicator of site N-status.

Hot KCl-N appears to be relatively stable in the topsoil, showing little seasonal variation (Moroni *et al.*, 2004). This suggests that soil samples can be collected at a convenient time, to allow for analysis and interpretation of results such that plantation managers can prioritise allocation of fertiliser resources in any given year. Soil sampling is a desirable diagnostic for plantation nutrition management, as sample capture is simple and samples can be dispatched to a laboratory without the need for pre-treatment, depending on required analysis. It remains critical that if Min-N, or any other soil variable, is used as a diagnostic tool that the timing and methods of sample capture, handling and analysis are consistent and follow the recommendations of Rayment and Higginson (1992) and Rayment and Lyons (2010). According to the methods used by both Gianello and Bremner (1986b) and Wang *et al.* (1996), samples for Min-N do not need to be refrigerated, but can be collected and air-dried prior to sending to a laboratory for analysis. However, keeping samples cool ( $<4^\circ\text{C}$ ) after collection is generally recommended, to minimise biological and chemical transformations of nutrients; therefore precaution would suggest following this recommendation (Rayment and Lyons, 2010). Further, the samples used in this study were collected between autumn and winter and were kept cool after collection. Therefore, if the models based on Min-N are to be used, then samples should be collected using the same methods.

With regard to sampling intensity, 10 cores per plot were captured then bulked, such that each plot represented a single sample and each site contained 3-5 samples depending on experimental design. Samples can be easily collected with a 'pogo stick'-type soil corer which is pushed into the ground via a footrest to the appropriate depth. Typically 15-30 subsamples are bulked into a single sample for each sampling point, collected from the undisturbed inter-rows to avoid cultivation effects (Rayment and Lyons, 2010). To capture spatial variation at a sampling point, subsamples should be collected several metres apart and from at least 3 inter-rows. One approach is to imagine the soil (or foliar) sampling point as having approximately the same footprint as a permanent sample plot. Prasolova *et al.* (2000) showed no difference in the levels of most major soil test variables when sampled at very

small scale (2.6 m<sup>2</sup> plots) compared with 42 m<sup>2</sup> plots; however testing across a larger plot may reduce the probability of collecting an unrepresentative sample. The number of samples required to adequately represent a plantation, or management unit within a plantation, can only be determined by an intensive analysis of variability (e.g. Prasolova *et al.*, 2000). This is a costly and time-consuming process, unlikely to be exhaustively conducted; therefore the intensity of sample collection will be a combined effect of site variability (soil type, slope, previous landuse) and economic analysis (cost of higher sampling intensity weighed up against cost of fertiliser operation).

For diagnostic sampling, be it soil or foliar, samples should be stratified by soil type across plantations using soil maps from site assessments. Site history is as important as soil type (Aggangan *et al.*, 1998; Kasel and Bennett, 2007), therefore sampling by compartment or section is required, even if soil type does not change. Plantation compartments often follow the layout of the previous landuse due to restrictions on size, vehicle access and the requirement for firebreaks; therefore each compartment most likely represents a paddock in the case of ex-agricultural sites, each of which has its own management history. Sampling by section or compartment is also a practical way to approach diagnostic sampling, as they typically represent the smallest management unit within a plantation. A critical consideration for any diagnostic sampling program is to retain enough material for re-analysis or new analyses which may be identified as important in the future. One approach is to collect more material when sampling, bulk and mix all material thoroughly, then split the sample into two. One sample can be sent to a laboratory for analysis and the second sample can be stored, often referred to as 'legacy samples'. Laboratories may be willing to organise storage of legacy samples for a fee. For long-term storage within an organisation, the difficulty lies not in the physical space requirements, but in developing administrative and organisational systems which are managed consistently over time.

When soil indicators including total C and total N were introduced into our models in place of Min-N, model accuracy decreased to 43 and 47% for predicting the age 2 response and became not significant for predicting response at age 4; despite total C and total N showing strong correlations with Min-N. Total C and total N also predicted response to fertiliser at age 2 (but not at age 4), however not with an acceptable level of accuracy to have confidence in their use as prediction tools ( $R^2=0.19$  and  $0.34$ ). This is unfortunate from a practical point of view, as total C and N represent low-cost standard tests in commercial laboratories and are regularly captured by plantation managers. Smethurst *et al.* (2004) analysed the growth response to a single application of 200 kg ha<sup>-1</sup> N at 14 sites in Tasmania and found topsoil (0-10 cm) total N and total P to be accurate single variable predictors ( $R^2=0.64$  and  $0.71$ ). Significant responses could be expected at total N <3 mg g<sup>-1</sup> and/or total P <1 mg g<sup>-1</sup> and no response when total N >6 mg g<sup>-1</sup> and/or total P >3 mg g<sup>-1</sup>. Smethurst *et al.* (2004) suggested net mineralisable nitrogen could be predicted from total P ( $R^2=0.90$ ) and total N ( $R^2=0.70$ ). This result was based on 5 sites and dominated by two high-P sites; a fact which, in combination with no obvious causal link between [particularly] total P and net mineralisable nitrogen, caused Smethurst *et al.* (2004) to doubt the usefulness of the finding. Mixed results have been found for pine plantations, with some studies showing nitrogen mineralisation can be predicted from total N and organic P (Carlyle and Nambiar, 2001) and others finding no relationship (May *et al.*, 2009a). Other than Bray2 P which as a single variable predicted growth response at age 2 with 28% accuracy, none of the predictive models in our study incorporated any measure of 0-10 cm soil P status (Olsen P, Colwell P, or CaCl<sub>2</sub> P). Total P was not analysed in the soil samples used in this study, therefore we cannot provide further insight on its usefulness as a predictor of fertiliser response. Total P, Olsen P and the C/N ratio have previously been identified as strong predictors of growth (not growth response to fertiliser) in a large study of *Pinus radiata* and *Cupressus lusitanica* in New Zealand (Watt *et*

*al.*, 2008). In our study, the relationship between Bray2 P (as a single predictor) and response to fertiliser improved at age 4 ( $R^2=0.43$ ) which may indicate that P-status of a site (sampled at planting) has a larger bearing on relative growth several years after application of N and P fertiliser. Bray 2 P has been shown to be a better indicator of P-requirement compared with total P (Mendham *et al.*, 2002) in P-fixing soils in Tasmania and Western Australia.

The 'best' overall (combined) establishment model included another P-status indicator in the form of the foliar N/P ratio. The foliar ratio of N/P was a significant term in the best overall model predicting growth response at age 2 ( $R^2=0.74$ ); as well as in the best foliar-based model in combination with N/S ( $R^2=0.49$ ). Foliar N and P were also significant terms in predicting growth response to fertiliser at age 4, in combination with mean maximum and minimum temperature sourced from ESOCIM ( $R^2=0.60$ ). The predictive capacity of pre-treatment foliar N in isolation is highly variable, as eucalypts tend to respond to increased N-supply by producing more foliage and increasing growth, often only showing slight increases in foliar N concentration compared with applications of P (Cromer *et al.*, 1975; Bennett *et al.*, 1996; Judd *et al.*, 1996; Bennett *et al.*, 1997). Further, as sampling only 0-10 cm of a soil profile does not indicate total nutrient resource, so too foliar sampling of a small number of leaves gives no indication of total nutrient status of a tree (May *et al.*, 2009b) or dilution effects (Cromer and Hansen, 1972; Cromer and Williams, 1982); prompting exploration of leaf area index as nutrition management tool (Smethurst *et al.*, 2003; May *et al.*, 2009a; White *et al.*, 2010). Foliar nutrient status, including ratios of N/P, has been shown many times to be responsive to addition of fertiliser (Judd *et al.*, 1996; Bennett *et al.*, 1997); however, there is little evidence to show it can predict response to N-fertiliser in eucalypts (Smethurst *et al.*, 2004). Despite this, many forestry operations in Australia rely exclusively on foliar nutrient analysis for guidance in nutrition management presumably because it can identify growth limiting deficiencies or disorders of essential nutrients (May *et al.*, 2009b). Furthermore, several commercial plant analysis laboratories make recommendations for fertiliser application using foliar nutrient concentrations presented by Dell *et al.* (2001) as critical levels, despite these authors advising against it.

Foliar Ca and Mg were significant single predictors and foliar Mg could be substituted for foliar N:P in the best model with a 10% loss in accuracy ( $R^2=0.64$  at age 2). Higher responses to fertiliser were observed at higher foliar Ca and Mg levels. There is considerable doubt as to whether foliar levels of Mg (and K) reliably indicate their status in the tree (Mitchell and Smethurst, 2009). Mitchell and Smethurst (2009); however, recently achieved a growth response to Mg and K fertiliser with *E. globulus* in a highly-leached field soil in the glasshouse. Over-use of N-fertiliser can result in leaching of base cations to the point where they can limit growth (Mitchell and Smethurst, 2004; Ringrose, 2005; Mitchell and Smethurst, 2008). Mitchell and Smethurst (2008) found that although fertiliser application in *E. nitens* increased  $\text{NH}_4$ ,  $\text{NO}_3$ , Ca, Mg and K in soil solution for at least 1 year post-application, topsoil (0-10 cm) pH, exchangeable Mg and K declined in the longer term, most likely the result of leaching. As all sites used in this study were previously used for agriculture, it is possible that regular additions of N-fertiliser caused leaching of Mg and Ca, therefore sites with higher Mg and Ca, less affected by previous N-addition and leaching, showed greater relative responses to fertiliser.

The accuracy of foliar-based models in predicting growth response to fertiliser was higher at age 4 (60%) compared with age 2 (49%), in contrast to the soil-based model which decreased in accuracy with age. Furthermore, accuracy of the foliar-based model was the same as the soil-based model at age 4, indicating that both models can be used equally to predict responses to mid-rotation age. Inclusion of climate variables in the age 4 foliar-based model may explain why it improves in variation accounted for (Watt *et al.*, 2008). It is possible that

in predicting growth response at age 2, climate is not an important factor; however, at age 4 climatic factors related to growth limitations may become more important. In the case of the foliar-based model predicting growth response at age 4 this is unlikely, as significant climatic variables were maximum and minimum temperature, as opposed to an indicator of water availability; such as CWI or MAR.

Diagnostic foliar sampling can be extremely valuable in identifying particularly trace element deficiencies (Dell *et al.*, 2001) and should not be ignored as a silvicultural operation. It is highly likely that deficiencies in a range of macronutrients including K and Mg (Judd *et al.*, 1996; Bennett *et al.*, 1997) and micronutrients including Cu will limit growth response to N and P fertiliser applications (Dell and Bywaters, 1989; Turnbull *et al.*, 1994), despite the fact they were not significant in any of the models identified here. Targeted diagnostic foliar sampling where obvious nutrient deficiencies arise remains the best method of remedial nutrition management in eucalypt plantations. Foliar nutrient levels vary seasonally and with leaf age, therefore sampling should be consistent and follow the guidelines provided by Dell *et al.* (Dell *et al.*, 2001).

Soil- and foliar-based indicators of site requirement for fertiliser-alone give no indication of climatic factors which may limit the response to fertiliser (White *et al.*, 2009). Inclusion of climate variables related to rainfall, particularly climate wetness index (CWI) and mean annual rainfall (MAR), in our 'best' models predicting response to fertiliser at age 2 provides a level of confidence that limitations related to water availability have been taken into account (Watt *et al.*, 2008). Although coarse metrics for water availability to a plantation, especially at sites with stored soil water at depth (e.g. White *et al.*, 2009), climatic factors such as CWI and MAR can be useful at the estate level in identifying where larger responses to fertiliser are likely to occur (Stape *et al.*, 2004; Stape *et al.*, 2006). Of all the factors likely to limit response to fertiliser, available water is typically the most important. Greatest responses to fertiliser have previously been observed at sites with higher rainfall (Stape *et al.*, 2006); however, this trend is often specific to particular soil profiles (Turner *et al.*, 2001).

### **Predicting volume growth response to mid-rotation fertiliser**

Responses to mid-rotation fertiliser differed between the two available datasets (MID and EST+MID); however, when relative responses to mid-rotation fertiliser were plotted against single and multiple predictor variables, the two datasets followed the same trajectory. Unfortunately, the accuracy of the models predicting response to mid-rotation fertiliser was much lower compared with those developed for establishment fertiliser. The best model predicted volume growth response one year post-application from the foliar N/P ratio and long-term mean annual rainfall sourced from SILO with only 43% accuracy. Regardless of accuracy, this is to our knowledge the first model predicting volume growth response to mid-rotation fertiliser. The accuracy of this model is similar to the best foliar-based model predicting response to establishment fertiliser which used FOL N:P and N:S (49% accuracy). Unfortunately 0-10 cm pre-treatment soil variables were not available for all MID and EST+MID sites, therefore we could not develop models similar to the most successful establishment fertiliser models described above. However, given that (i) the accuracy of the foliar models is similar for both establishment and mid-rotation applications and (ii) the best foliar models for both application ages use similar predictor variables; it gives us the confidence to suggest that soil-based models may also have the ability to predict volume growth responses to mid-rotation fertiliser.

## How to apply the outputs of this project in practice

Forest managers can now utilise the models developed in this project to predict the magnitude of response to fertiliser, particularly one year post-application. Some models have better predictive capacity than others, but all involve inaccuracy. In practice, we expect that at this stage, these models can be used simply to rank sites in descending order of responsiveness; allowing managers to deploy fertiliser resources to sites more likely to show a significant (i.e. >10%) volume growth response and avoid applying fertiliser where it is not needed. To run any of our models, plantation managers will need to first select the model they wish to use, acquire the appropriate site data (climate variables), then collect soil or foliar samples and send them to a laboratory for analysis. When the analytical results become available, the required site data can be entered into our models, with the output being a predicted volume growth response in percentage terms. Each site and management unit within each site can then be ranked according to magnitude of response and fertiliser resources allocated accordingly. Our best models only predict short-term growth response (i.e. one year post-application) with any level of accuracy and therefore there remains the strong possibility that sites identified as more responsive will not show significantly higher standing volume at harvest. However, in the absence of any other predictive diagnostic strongly related to volume growth response, our models have significant value, particularly in identifying sites highly unlikely to respond to fertiliser. From this study, it was not possible to describe model performance where significant limiting factors such as micronutrient deficiencies were present; therefore due caution should be taken to firstly correct for any suspected trace element deficiencies. Diagnostic foliar sampling remains one of the best methods of identifying micronutrient deficiencies likely to limit response to N and P fertilisers (Dell *et al.*, 2001). Our models require validation and we provide a means of doing so in the section: ‘Suggested approach for validation of project outputs and direction of future research’ below.

Using and testing the performance of the models presented here is the best way to use and improve on the outputs of this project. From our analysis, we are also able to provide some approximate threshold values for individual parameters which can also be used to distinguish sites more likely to respond to fertiliser. These threshold values are summarised in Table 16 and are intended only as an approximate guide. Sites with Hot KCl  $\text{NH}_4\text{+NO}_3\text{-N}$  (0-10 cm) concentrations of  $>30 \text{ mg kg}^{-1}$  were less likely to show large responses to fertiliser; as were sites with  $>30 \text{ g kg}^{-1}$  of Total C. We also suggest a lower threshold for Total N,  $3 \text{ g kg}^{-1}$  compared with  $6 \text{ g kg}^{-1}$  suggested by Smethurst *et al.* (2004) and a similar Bray2 P concentration of  $30 \text{ mg kg}^{-1}$  to that suggested by Mendham *et al.* (2002). Several sources suggest a critical foliar N threshold of  $25 \text{ g kg}^{-1}$ , also supported by our study (Judd *et al.*, 1996; Dell *et al.*, 2001). Foliar nutrient concentrations including Ca and Mg were negatively related to volume growth response in our study and have previously been correlated with growth (Bennett *et al.*, 1996). In our study, response to fertiliser was less likely at foliar Ca concentrations less than  $7 \text{ g kg}^{-1}$  and foliar Mg less than  $2 \text{ g kg}^{-1}$ ; which also correlates well with previous studies (Dell *et al.*, 2001).

## Complications of multi-experiment analysis: a word of caution

Despite commercial plantings of *Eucalyptus globulus* having been managed across Mediterranean and temperate regions of southern Australia for more than 30 years (May *et al.*, 2009b); no studies have quantified the effects of fertiliser on productivity at regional scales using multiple sites (see Stape *et al.*, 2006; Watt *et al.*, 2008 for international examples). To retrospectively address this lack of quantitative information, plantation managers across southern Australia volunteered individual datasets for this study. An

unfortunate complication of compiling experiments from different sources was that experimental design, although while similar, was not identical for each age of application. Further, the results we report are entirely empirical and therefore dependent on the range of sites used. In compiling any multi-experiment dataset from different sources, a number of factors could not be controlled and therefore more than likely introduced error which could affect fertiliser response. For example, the majority of the experiments from south-eastern Australia compiled in this study occurred during a period of extended drought (McGrath *et al.*, 2012). Given the experiments used here span almost 20 years, we also cannot be certain that methods of sample collection and analysis were identical. We acknowledge the complexities these factors contribute to our analysis and they should be considered when interpreting our results, particularly with regard to the magnitude and duration of response to fertiliser. While the relatively large number of experiments compiled here represented multiple growing regions over a 20-year period, the models derived from this dataset must be used with caution and validated by both researchers and plantation managers.

**Table 16.** Approximate values for single predictor variables, above (>) or below (<) which large responses to fertiliser are less likely. Values were determined by visual interpretation of relationships between significant single predictors and volume growth response determined one year post-fertiliser application for both application ages (establishment and mid-rotation). Dashes indicate either (i) variables were not available (e.g. 0-10 cm soil variables for mid-rotation responses) or (ii) that the variable was not significantly related with volume growth response one year post-application.

<b>Variable</b>	<b>Establishment</b>	<b>Mid-rotation</b>
<i>0-10 cm soil variables</i>		
Min-N	>30 mg kg <sup>-1</sup>	-
Total C	>30 g kg <sup>-1</sup>	-
Total N	>3 g kg <sup>-1</sup>	-
Bray2 P	>30 mg kg <sup>-1</sup>	-
<i>Foliar variables</i>		
FOL N	>25 g kg <sup>-1</sup>	-
FOL Ca	<7 g kg <sup>-1</sup>	-
FOL Mg	<2 g kg <sup>-1</sup>	-
FOL N:P	-	>14
FOL N:S	>12	-
FOL N:MAI	-	>1

### **Suggested approach for validation of project outputs and direction of future research**

In reviewing this report, our industry project partners requested advice with regard to the best direction to take in (i) validating the models presented and (ii) advancing understanding of management of nutrition over multiple rotations. We present the following as an opinion only in answer to this request, but acknowledge that (i) this is not the only way to move forward and (ii) at times it represents a divergence from ‘traditional’ research methodologies and therefore should only be acted upon with due diligence and appropriate expert advice. As evidenced by this project, research collaboration in plantation forestry is strong and ideally what we suggest here should be carried out by the industry as a whole.

Empirical modelling based on field experiments remains one of the best methods available for quantifying growth responses to fertiliser, despite fluctuations in site and climate variables. Empirical datasets must, however, be of high-quality and comprehensive to represent plantations at a regional level, as well as to absorb variations in growing conditions. Several fertiliser response studies in Australia have significantly contributed to our understanding of eucalypt plantation nutrition management; many of which are cited in our report. Experimental networks are often designed such that the number of study sites is small in order to maximise the number of treatments and replicates installed at each site. This is often the most favoured approach as it maximises the number of silvicultural options tested (i.e. fertiliser rates, types and/or timings); as well as the ability to detect statistical differences between treatments. Despite rigorous experimental design, these studies and therefore their recommendations are often limited to the small number of soil profiles and climatic zones where they are carried out.

Stape *et al.*, (2006) successfully demonstrated an alternative ‘twin-plot’ approach to silvicultural research, where only a single treatment and control plot were installed at any one location; maximising spatial coverage and therefore application of results across regions. Paired-plot networks typically rely on installing an alternative treatment plot alongside existing permanent sample plots (PSPs). Paired-plot networks can potentially leverage the value of existing PSP networks to improve estate-wide silvicultural management. We suggest that the installation of paired-plot networks across major hardwood producing regions would (i) provide the data required for widespread validation of the models presented in our report and (ii) with additional collection of explanatory variables from each site, allow the construction of new, better models. This approach has been successfully applied to develop estate-wide network of experiments which have identified the drivers of site productivity in both Brazil and New Zealand (Watt *et al.*, 2008; Stape *et al.*, 2010).

The fundamental difference in a twin or ‘paired-plot’ approach compared with more ‘traditional’ experimental designs lies in the statistical techniques used. Paired-plot experimental networks replace standard treatment comparisons using ANOVA in favour of model development, using methods such as multiple linear regression analysis as used in this study. Whenever the results of field experiments installed at a small number of sites are presented, the audience inevitably first critiques the sites used, to look for artefacts which explain the results, rather than instantly believing that responses are the result of the treatments. We instinctively do this as we are aware of the dangers of extrapolating results from a limited number of sites. As a consequence, the value of field research is often downgraded due to perceived limited application across all soil types and climatic zones. The great advantage of a paired-plot network approach is that it covers as many growing conditions as possible, such that we are always interpolating, rather than extrapolating results. Replication is still a critical component of paired-plot experimental designs; however, replicates are spatially distributed throughout the landscape to cover a wider range of conditions, as opposed to closely grouped at a single location to minimise variation.

Several factors are critical in designing a paired-plot network: (i) treatment selection, (ii) control plot management, (iii) plot stratification, (iv) collection of appropriate explanatory variables and (v) willingness and ability of industry to participate. In traditional experiments, many treatments are often installed to determine the optimum; however, this is not possible in a paired-plot network where only one or two treatments can be installed alongside a control. The paired-plot approach can potentially be applied to any silvicultural research question; however, like any experimental design, it assumes that the treatment selected is relevant to the question being asked. A luxury treatment with repeated high rates of fertiliser will indicate

the maximum productivity of a site under a given climatic regime, but not show what standard operational prescriptions will achieve. One of the greatest challenges in plantation silviculture at present is maintenance of productivity over multiple rotations (Nambiar, 2010). Large areas of the national plantation estate have moved into the second rotation and productivity appears to be significantly lower than the first; referred to as '2R Decline', a term borrowed from a similar experience in pine plantations in western Victoria and eastern South Australia during the 1960's. The likely causes for this decline are complex; most likely with compounding interactions and will undoubtedly vary both within and between growing regions. In this situation, it may be advisable to install a network of luxury treatment plots and conduct an analysis as used by Stape *et al.* (2006; 2010) and Watt *et al.* (2008), to identify the key factors limiting productivity. In parallel, more intensive experiments can be installed on a small number of sites where nutrition is deemed to be limiting productivity in order to determine optimum levels of treatment.

As for any experimental design, treatment selection in paired-plot studies should be done with an appropriate control in mind. Ideally a paired-plot network will span multiple forest managers and therefore involve alternative silvicultural regimes. Stape *et al.* (2006) and Watt (2008) used a control plot which received the same treatment as the site, with each site receiving operations as required. This approach can work well when the comparative treatment plot receives luxury treatments far above those applied to the control. It is also much easier to manage this type of paired-plot network, as less vigilance is required in excluding small experimental plots from silvicultural operations. Both the control and treatment can receive the operational prescription of the site, then additional treatments can be applied to the treatment plot. There are two drawbacks of this approach: (i) if operational prescriptions differ substantially between sites, the control is not standardised and therefore variation in responses to treatment will increase and (ii) this type of control will obviously be of no use in determining the effect of standard operational prescriptions. The alternative approach is a control which is excluded from operations of interest, for example, when quantifying responses to fertiliser, control plots should receive all operations except fertiliser. Since many plantations have moved into second rotation, silvicultural management has diversified, such that forests are now managed as either coppice or replanted seedlings under a range of fertiliser, pesticide and herbicide regimes. As such, it would appear that (i) paired-plot networks of coppice and seedling plantations should be treated separately and (ii) a standardised control is necessary with regard to other silvicultural practices. It can be very difficult to exclude small plots from operations at a site, but options such as exclusion of entire (short) rows, adequate signage and additional management of contractors can prevent plots from being compromised.

Placement of plots should ideally be stratified according to growing conditions, that is, the combination of soil type/depth and climatic zones, as well as other local factors which are likely to affect productivity and response to fertiliser. PSP networks are typically installed to capture this variation; therefore it is logical that treatment plots are installed adjacent to existing and future PSPs. Maintenance and monitoring of paired-plots can therefore be integrated with standard PSP measurements. Placement of paired-plots within an estate will determine how the data collected can be used. For instance, stratifying plots according to the total area of each soil type within an estate will allow analysis of responses to fertiliser in proportion to the area managed. In this case, some minor soil types will only be represented by a small number of plots; potentially not enough to build predictive relationships such as those presented in this report. This will result in insufficient data to build predictive relationships for that specific soil type. Continuing this example, an alternative approach is to install a minimum number of paired plots on each soil type, therefore allowing analysis within and between each soil type. The best means of grouping sites for analysis is often unclear

until after data has been collected, therefore it is critical that each site is described in as much detail as possible.

By maximising the information collected from each plot pair across a network, the number of potential explanatory variables captured is large which increases the likelihood of identifying high-quality empirical models describing response to fertiliser. The most critical aspect of collecting site information across a network of plots is consistency in sampling methodology. Indeed, consistency in methods of data collection is one of the major reasons for strong collaboration between plantation growers. After consultation with all participants, a standardised methodology for all potential explanatory variables should be developed and rigorously followed. This may mean that an individual partner needs to deviate from their own standard methods, creating concerns as to how applicable any models developed from the network are to their estate. This need not be a major issue, as individual participants can use their own internal procedures to collect and assess explanatory variables of interest in addition to the standard methodology. The list of potential explanatory variables is vast and we mention only a few. The models presented in this study suggest that diagnostic N-status indicators including soil (0-10 cm depth) and foliar samples (youngest fully expanded leaves) are related to response fertiliser and therefore their use should be continued. Additional information on soil characteristics at depth, including water storage and groundwater table depth, would contribute significantly to understanding fertiliser responses.

Leaf area index shows great potential to indicate nutrient requirement and response to fertiliser (Smethurst *et al.*, 2003) and should therefore be integrated as a key explanatory variable. Leaf area index methods have typically been time-consuming and difficult; however, recent development of LAI applications for smart phones (Fuentes *et al.*, 2012; Confalonieri *et al.*, 2013), including fisheye applications and lenses (e.g. <http://photojojo.com/store/awesomeness/cell-phone-lenses/> and <http://snappr.us/>); combined with image analysis software such as CAN\_EYE (e.g. Adamek *et al.*, 2009) will hopefully soon translate to rapid capture of LAI and its use as a plantation management tool. Further, use of satellite imagery to measure LAI and detect nutrient deficiencies shows great promise (Coops *et al.*, 2003; Coops *et al.*, 2004; Flores *et al.*, 2006) and should be pursued as a diagnostic tool for plantation management. High-quality process-based models such as 3-PG, CABALA (Landsberg and Waring, 1997; Battaglia *et al.*, 2004) have the functionality to predict fertiliser response; but like the empirical models in our study, they require significant validation, with regard to fertiliser response. Both empirical and process-based approaches to silvicultural research should be pursued, therefore every effort should be made to collect the explanatory variables required (i) to validate existing empirical models, (ii) to build new empirical models and (iii) to validate existing process-based models. This is not a daunting task, as many of the explanatory variables required for both empirical and process-based models are the same. The developers of existing models are typically interested in seeing them independently tested; therefore it may be beneficial to make contact when starting a new research program; where interest may translate into assistance in data collection.

Lastly and most importantly, industry must have both the willingness to work through all of the above decisions in a collaborative way; as well as the ability to manage and fund the installation, sampling and measurement of paired-plots on their estate. The four considerations of designing a paired-plot network listed above barely scratch the surface with regard to the experimental design and the practical challenges which need to be overcome. Engagement of research providers to lead the installation of a paired-plot network has some advantages, with regard to providing expertise in experimental design and project management. A critical role of the project manager, be they a research provider or industry leader, will be in the development of standardised methodologies in the installation and

monitoring of the network. Ideally, the cost of a new paired-plot network should be borne, at least in part, through competitive grant schemes, such that the burden does not fall completely on the industry.

The paired-plot network approach is not the only means to increase silvicultural knowledge and several new research questions requiring an alternative approach have also emerged from our study. To advance knowledge, it is important to not merely describe which explanatory variables best describe response to fertiliser, but to take the next step and understand how or why they do it. For instance, our study found that the N/P ratio was related to fertiliser response, but the underlying mechanism is not clear. This study also failed to satisfactorily predict mid-rotation fertiliser responses and therefore there exists significant research potential to determine under what conditions productivity can be increased through later age fertiliser applications. It has been shown that responses to fertiliser are lower after mid-rotation, with a decrease in resource use efficiency, i.e. conversion of water and nutrients into merchantable yield, rather than a reduction in resource use, the primary cause (Binkley *et al.*, 2002). However, responses to fertiliser applied after canopy closure should be investigated further to determine their potential across a range of growing conditions.

## Conclusions and Recommendations

Relative volume growth responses can last for ~4 years after application of high rates of nitrogen fertiliser in the order of 200-250 kg N ha<sup>-1</sup>. Significant gains in final standing volume can be achieved through single or multiple applications of N-fertiliser, particularly when applied at responsive mid-rotation sites. Central to realising this growth gain is the ability to confidently identify sites most likely to show growth responses to fertiliser; an ability which has eluded plantation managers and silvicultural researchers alike. We provide several alternative soil- and foliar-based models which predicted growth response to establishment fertiliser from 2-3 explanatory variables with a minimum of 70% accuracy for a short-term response (2 years) and 60% accuracy for a longer response (4 years). The simplest and most reliable model ( $R^2=0.70$ ) predicting volume growth response to establishment fertiliser relied on a single test on a topsoil (0-10 cm) sample (Hot-KCl NH<sub>4</sub>+NO<sub>3</sub>-N; or 'Min-N'), combined with long-term annual rainfall for a given site (sourced from ESOCCLIM). We also provide a foliar-based model which predicted a short-term (1-year) response to mid-rotation fertiliser; however accuracy was only 43%. Low accuracy of the mid-rotation model, combined with complexities in the underlying data used to build it, suggest that the establishment models have a higher likelihood of identifying sites more likely to show a significant response to fertiliser.

With regard to the practical application of these findings to plantation management, we suggest using the best soil-based establishment model to rank sites according to predicted magnitude of initial growth response, i.e. one year post-application. In this way, fertiliser resources can be directed only to those sites likely to show a significant response to fertiliser. We hope that the outcomes of this study will re-enforce the value of regional-scale experimental networks and that it will contribute to the co-ordinated design and installation of an estate-wide network of paired-plots to both validate and improve upon the models presented here.

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