Field-scale variability in site conditions explain phenotypic plasticity in response to nitrogen source in *Pinus radiata* D. Don

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ABSTRACT

Aims. Productivity of forest ecosystems is constrained by site resource availability and utilisation at an individual tree level. A better understanding of nitrogen (N) nutrition addition to forest ecosystems is critical for maintaining optimal plantation productivity, given the influence of an environment gradient, genetics, and their interactions.

Methods. We studied the aboveground growth response in a plantation setting of ten commercial *P. radiata* genotypes to N-fertilisation using three different N sources, and also assessed the effect of on-site environmental factors on this response. We compared, on equimolar basis, the effect of N-fertilisation with inorganic N (NH₄NO₃), organic N (L-arginine), and the two N sources combined (L-arginine:NO₃⁻) to that of unfertilised trees on tree height, diameter, descriptors of microsite variability, and climate / seasonal information. After 2.5 years of fertilisation, genotype-specific variation in aboveground growth response to N sources were measured, and these were significantly influenced by field-scale heterogeneity.

Results. Across *P. radiata* genotypes, trees treated with inorganic N forms showed suppressed growth compared to unfertilised trees, while trees fertilised with organic N (either alone or in combination with inorganic N) were not significantly different than the untreated controls. We provide evidence of

significant interactions between N source and genotype, N source and cover as well as genotype and microsite variability affecting temporal trends in tree volume.

Conclusions. We conclude that an understanding of field-scale variability in soil properties and associated environmental variables is essential for understanding genotype performance as they are crucial determinants of intraspecific variation in response to N-fertilisation.

INTRODUCTION

Given the longevity of sessile species, phenotypic plasticity provides an opportunity to sense, change, and adapt their growth and physiological responses to cope with changes in environmental conditions (Bradshaw 1965; Corcuera et al. 2010). An ongoing challenge is to clarify which biological traits are manifested as genotypic variation from evolutionary and breeding history (G), phenotypic response to environmental variability (E) or combinations of both ($G \times E$). Abundant evidence supports the hypothesis that plant species and population responses differ across different environments (e.g. Schlichting and Levin 1984; Valladares et al. 2000). However, genotype-specific variation can be exhibited when grown in comparable conditions, affecting adaptive capacity and, consequently, tree performance (Souza et al. 2017). The flexibility and adaptability of tree genotype requires special attention, not only to understand the complexity of traits in breeding programmes, but also for the greater ecological impact that the increasing inter-seasonal climate changes may have in long-lived organisms.

Pinus radiata D. Don (alt. Monterey pine) is the most common introduced softwood plantation species growing in temperate zones of the southern hemisphere (Richardson 1998). It is also the fastest growing pine species on a global scale (McDonald and Laacke 1990; Mead 2013). Plantations in New Zealand now occupy 1.7 million ha and this is the basis of a major export industry (Forest Owners Association 2016) (Figure 1a). Since the 1960s, the New Zealand forestry industry has undertaken multi-generation breeding programmes using plant material from just a few provenances (Burdon et al. 2008). Recent studies of *P. radiata* families have discovered marked genotype variation in growth rates along nutritional water use efficiency and hydraulic conductance gradients (Hawkins et al. 2010; Xue et al. 2013; Rodríguez-Gamir et al. 2018). This suggests that genotype-specific physiological traits might be advantageous depending on the nutritional characteristics of the site.

47 *In situ* experimental studies enable us to gain an understanding of how plants acclimate to
48 complex environments, including aboveground and belowground community interactions. Traditionally,
49 field trials have typically divided sites into homogeneous units and projected treatment blocks into these.
50 However, this kind of blocking relies on a visual inspection of the site and is often made with relatively
51 little or no information of soil spatial variability within the field. Soil heterogeneity is comprised of
52 changes in soil physicochemical properties and associated microbial processes that can impact tree
53 growth directly (e.g. soil available water) or indirectly (e.g. availability of nutrients for plant uptake). If
54 these physicochemical changes occur within an experimental block, then the experiment results can be
55 unknowingly confounded. Traditional soil sampling methods to systematically quantify the heterogeneity
56 of large field experiments are prohibitively expensive. The recent development of portable, non-invasive
57 instruments, such as the electromagnetic induction meter (EM) has provided low cost methods to quantify
58 the variation of soil physicochemical properties in a timely way (e.g. Williams and Baker 1982; Khakural
59 et al. 1998; Stadler et al. 2015). This technology has been adapted for a number of areas including
60 precision agriculture (e.g. Corwin and Lesch 2005), soil salinity (e.g. Triantafilis et al. 2000), ground
61 water (e.g. Scanlon et al. 1999), and mapping soil physical properties (e.g. Lu et al. 2017), as well as
62 spatial variability in soil moisture (e.g. Brevik et al. 2006; Hossain et al. 2010). The EM method not only
63 provides a tool to lay out experimental blocks to minimise soil heterogeneity, but it also provides an
64 important covariate of underlying variation in sites physicochemical properties (as used in this study).

66 In order to achieve the genetic potential of the tree, intensive forest management incorporates
67 silvicultural operations, which are often reduced to a set of practices that meet basic tree requirements.
68 These include nitrogen (N) fertilisation and the control of competing vegetation (Mead et al. 1984).
69 However, the addition of inorganic N inputs into forest estates, with the intention of achieving increased
70 commercial productivity rates, does not necessarily match the plants capacity to fully utilise the N
71 applied. This provides the potential for nutrient leaching, which may result in both financial loss and
72 negative environmental outcomes such as eutrophication of aquatic ecosystems and a negative impact on
73 biodiversity (Hautier et al. 2009).

75 A large body of research has investigated the effects of N availability on plant development,
76 biomass partitioning and regulation of root nutrient uptake (e.g. Burger 2009). However, little information
77 is available on the ability of different genotypes to acquire, recycle and assimilate nutrients in conifers of
78 commercial interest, such as *P. radiata* (Burdon and Shelbourne 1971; Burdon 1976; Hawkins et al.

2010). More broadly, the relationship between genotype-specific strategies of resource use and growth rates is not well understood (Bradshaw 2006).

Plant species vary strongly in their preferences for different N sources (Hangs et al. 2003; Näsholm et al. 2009; Britto and Kronzucker 2013; Boczulak et al. 2014), yet the effect of different N chemical forms in genotypes of the same species is not well understood (Gallart et al. 2018a). Under controlled conditions, plants supplied with organic N have shown differences in biomass partitioning by increasing the root:shoot ratio compared with those supplied with inorganic N forms (e.g. Cambui et al. 2011; Franklin et al. 2017). However, plant responses to different forms of N in field studies are not so clear, given the highly variable environmental conditions and the potential changes in N mineralisation rates that are due to shifts in rhizosphere microbial activity (Jones and Kielland 2002). In addition, the potential effects on the overall growth and allocation differences in response to N source are unclear, and might ultimately depend on additional genetic and environmental components.

In this study, we undertook an *in situ* field study over 2.5 years in a low rainfall region in New Zealand, in order to investigate long-term differences in growth of ten *P. radiata* genotypes growing under different organic and inorganic N supply. We hypothesised that: (i) there would be genotype variation in aboveground growth responses; (ii) spatial heterogeneity in soil properties and understory vegetation would differently influence genotype response to N source due to the variable distribution in water and nitrogen resources; and (iii) periodical resource limitations associated with inter-annual climate fluctuations would contribute to genotype strategies that were adopted in response to N source.

MATERIALS AND METHODS

Site description

The experimental field site is located in the Canterbury region (Waitaha) of the South Island, New Zealand (43°28'14"S 172°20'13"E) (Figure 1). This region has a temperate climate with cool, wet winters and warm, dry summers with high evaporation rates. The long-term mean annual precipitation is 790 (500 - 1200) mm year⁻¹ and the mean annual air temperature is 12 (6-17°C) (1972-2015 average data obtained from NIWA Darfield station; Table S1). Elevations range from 110 to 115 m above mean sea level across the site (Figure 2b). The soil type is a Lismore Shallow and Stony Silt Loam (30-40% stone), derived

from young fluvial deposits (New Zealand Classification: Pallic Orthic Brown Soil; USDA: Typic Dystrustep)(Hewitt 1993). This is common to the Canterbury plains, characterised by being well-drained, permeable soils, with low water-holding capacity and typically used for pastoral and arable farming and *P. radiata* plantations.

The site was previously used for pasture, followed by a first rotation *P. radiata* plantation, before a wild fire necessitated plantation re-establishment in August 2013. Logging residues from the first rotation were windrowed and ripped along the longitudinal direction. A residual herbicide (terbuthylazine) was initially applied at registered rates to control shrubs and herbaceous weeds. Weed control was no longer maintained after tree establishment, and understory communities progressively developed. This vegetation was mainly composed of grasses, white clover (*Trifolium repens* L.), subterranean clover (*Trifolium subterraneum* L. subsp. *subterraneum*) and other plant species, such as dandelions (*Taraxacum officinale* (L.) Weber ex F.H. Wigg), sheep sorrel (*Rumex acetosella* L.), lamb's-ear (*Stachys byzantina* K.Koch), pigweed (*Polygonum aviculare* L. 1753), fat-hen (*Chenopodium album* L.) and common mallow (*Malva sylvestris* L.). Less abundant species were gorse (*Ulex europaeus* L.) and volunteer radiata pine (*Pinus radiata* D. Don), which germinated from the soil seed bank.

Study design: clonal material and nitrogen treatments

The trial was planted on 26 August 2013 using a planting space of 4 m (between rows) × 2 m (within rows) to give a stocking rate of 1620 ramets ha⁻¹. The trial had a split-plot, randomised block design (Figure 2a), incorporating six replicates of four N treatments applied to the main-plots: (1) NH₄NO₃; (2) L-arginine: NO₃⁻; (3) L-arginine; and (4), Control (no N applied). Each whole-plot contained ten *P. radiata* genotypes (i.e. clones) as individual rows (subplots); i.e. each row was planted with each individual genotype. Seven ramets of each genotype were randomly planted within a row, with each plot having 70 trees in total (Figure 2a). Space between plots comprised at least four buffer trees of reference genotype (GF19).

The ten test genotypes (15, 24, 28, 30, 31, 35, 37, 44, 48 and 50) were clonally propagated and provided by Forest Genetics Limited (Rotorua, New Zealand). These had a narrow genetic background (Figure 3) and were phenotypically selected from progeny trials based on different genetic traits that favoured the New Zealand commercial forestry sector: stem growth rate, corewood stiffness, wood density, resistance to the pathogen *Dothistroma septosporum* ((Dorog.) M. Morelet) and internode length

(branching) (Table S2). *Pinus radiata* is not commercially bred for nitrogen uptake traits. Nitrogen fertiliser application was carried out each summer (December 2013-2015) using equimolar N concentrations. The N concentration of the fertiliser solutions was calculated based on the expected biomass growth requiring 2 g per 100 g dry weight, and assuming a 50% plant uptake. Ammonium nitrate (NH₄NO₃) was used as the inorganic form, L-arginine was used as the organic form, and the L-arginine:NO₃⁻ (M/M) treatment was used as the factorial combination of equimolar N species. For the first two years of N-fertilisation, 1 M N-solutions were used (0.5 M NH₄NO₃, 0.25 M L-arginine, and 0.13 M L-arginine and 0.5 M NO₃⁻ for the N equimolar combination of N forms) applied in 50 ml aliquots to each individual tree using a drench-gun. After two years of N-fertilisation (summer 2015), N-fertiliser solutions were doubled to account for increased tree biomass and the increase in plant N demand.

Growth rate measurements and climate information

Before planting, the average dry weight biomass (mean ± SEM) of the cuttings was 7.0 g ± 0.8 g DW (4.8 g ± 0.3 g shoot DW, 2.4 g ± 0.6 g roots DW), with no significant genotype variation in dry biomass ($p > 0.05$). Nitrogen additions were applied in December 2013 concurrently with initial tree root collar diameter (± 0.1 mm) and tree height (± 0.1 cm) assessments. We repeated measurements in October 2014, May 2015, December 2015 and June 2016.

Estimates of tree volume were calculated assuming a conic stem shape, following the equation $V_{tree} = \frac{1}{3}\pi r^2 h$, in which r (cm) and h (cm) correspond to the radius of the root collar and height of the stem, respectively. Volume increments ($\Delta V_{(t, t+1)} = V_{(t+1)} - V_t$) were used to describe growth over time, t and $t+1$ describe initial and final tree volume after a time period, respectively. The cumulative incremental volume was calculated for the four periods between: December 2013 and October 2014 ($T_1 - T_0$), December 2013 and May 2015 ($T_2 - T_0$), December 2013 and December 2015 ($T_3 - T_0$) December 2013 and June 2016 ($T_4 - T_0$). In October 2014, the proportions of clover, grass and other plant community compositions under the various N treatment conditions were visually determined within a distance of 1 m from each tree.

The climatic data for the years of the study was obtained from the closest NIWA (National Institute of Water and Atmospheric Research, Auckland, New Zealand) weather station 15 km east of the site (agent 41230, 43°29'22"S 172°31'39"E) (Figure 1b). The average of monthly cumulative

rainfall (mm) and mean daily air temperature ($^{\circ}\text{C}$) for each individual time period (T_n) were used to explain differences in volume increment (see subsection 2.5). The historic record of climatic data between 1972 and 2015 was obtained from another meteorological station 16 km west of the site (agent 4836, 43°29'35"S 172°08'13"E) (Figure 1b, Table S1). The relative humidity (%) was calculated using mean vapour pressure (hPa) and the saturation vapour pressure at the given temperature.

Electromagnetic induction apparent soil conductivity survey

In October 2014, an electromagnetic induction sensor (DUALEM-1, Dualem Incorporated, Milton, ON, Canada) took geo-referenced measurements of apparent soil electrical conductivity for the uppermost 160 cm of the soil (EC_a , mS m^{-1}). The DUALEM-1 is a portable ground conductivity sensor that uses a small coil transmitter and two coil receivers to measure the electromagnetic field coupling in the soil (Beamish 2011). DUALEM EC_a is primary influenced by soil texture, moisture, and salinity (Nadler and Frenkel 1980; Cook et al. 1989; Doolittle and Brevik 2014). Rocks and sandy textured soil have lower EC_a , while clay textured soil has higher conductivity. Because the site is known not to have high soil salinity, we considered that differences in EC_a across the site were due to changes in soil texture and soil water holding capacity.

For this study, the instrument measured EC_a beside each tree across the trial using a zig-zag sampling pattern. The entire trial was measured in one day. The DUALEM-1 was laid on the ground directly beside each tree and the electrical conductivity response and geographic positioning system (GPS) coordinates of the measurement was collected by the instrument when triggered manually. The GPS coordinates also provided a measurement of elevation above sea level for each point. This was used to develop the microtopography map (Figure 2). The orientation of the instrument was in the WE and EW directions (following rip lines), depending upon the row being assessed. The instrument was randomly placed on either the left or the right hand side of the tree depending on the operator and muscle fatigue. Approximately halfway through the plot assessments, five soil moisture samples were collected from the first plot in the western side and the instrument batteries were replaced. Because the instrument was placed directly onto the ground for measurements, the influence of atmospheric moisture had no influence on the EC_a signal.

Data were downloaded from the instrument and was subjected to QA/QC analysis and any erratic values were eliminated. Point measurements of EC_a and elevation above sea level were interpolated into spatial maps of EC_a and microtopography by ordinary kriging in ArcMap (Environmental Systems Research Institute, Redlands, CA) and its extension of Geostatistical Analyst (Figures 2b, c). These maps were used as a visual representation of the variation of EC_a and microtopography across the trial only. The point measurements of this data were used for the statistical analysis.

Statistical analyses

Data were analysed in R Studio (Version 0.99.902) (R Core Team, 2012). The effects of plot, N source and genotype on volume increment over the length of the experiment were tested using linear models, followed by one-way analysis of variance (ANOVA) tests. A two-way ANOVA was conducted to test for the effect of genotype, N source and the interaction between the two factors on volume growth response. Given the significant interaction in the previous test, we performed simple main effects by using the volume increment of the ten genotypes across the four levels of N. Tukey's Honest Significant Difference (HSD) test was used to compare means ($p < 0.05$) when significant effects were found.

To test the genetic and environmental factors influencing temporal and spatial trends in volume between December 2013 and June 2016, we constructed a multivariate linear mixed-effects model implemented in statistical package *ASReml-R* (Butler et al. 2009). The model included volume estimated at each different time period (December 2013, October 2014, May 2015, December 2015 and June 2016) as dependent variables and the following predictor variables as fixed effects (together with their interactions): genotype, N source, point EC_a, and percentage understory vegetation. Plot and row were considered to be random effects. Plots were nested in N source and rows nested to genotypes. The Wald statistics was implemented to infer statistical significance of fixed terms. Since the investigated traits are height at different age, all fixed terms should be considered as interaction with time. The volume estimated at each time was normalised with logarithmic transformation in order to meet the assumptions of homoscedasticity and normality. Following analysis, response variables and coefficients were back-transformed for the interpretation.

RESULTS

Genotype-specific growth response to nitrogen source

The study revealed great variability in growth increment throughout the site (Figure 4) with significant differences in volume growth across plots ($F = 10.15$, $p < 0.001$). Maximum growth was observed in plots 6, while the lowest growth was observed in plots 11 and 12. Volume increment was significantly influenced by the effect of N source across replicate blocks ($F = 4.24$, $p = 0.005$) (Table 1). The inorganic N treatment suppressed volume growth compared to untreated trees ($p = 0.002$), while the additions of L-arginine and L-arginine: NO_3^- had not effect. Variation in tree growth was significantly influenced by tree genotype ($F = 15.37$, $p < 0.001$; Table 2). Full-sib genotypes 48 and 31 had the greatest growth increment, with genotype 15 possessing the slowest average growth (over 15% less growth than genotype 48).

The significant interaction effect between N source and tree genotype on the relative tree growth ($F = 2.94$, $p < 0.001$) indicated the presence of genotype-specific growth response to N source (Table 3). Genotypes 30, 48 and 50 showed a significant response to N source, while the remaining seven (i.e. 15, 24, 28, 31, 35, 37 and 44) exhibited a neutral response or non-significant response to N treatment. One-way ANOVA showed that genotypes 30 and 50 were organic N-responsive genotypes, with significantly greater performance in response to L-arginine than to NH_4NO_3 , while genotype 48 responded greater to the factorial combination of organic and inorganic N forms (L-arginine: NO_3^-), than to fertilisation with L-arginine alone.

Genotypes showed apparent heritability of growth rate-related traits and response to N source, especially in those with full-shared parental identity (Figure 3). For instance, full-sibs 31 and 48 had the greatest aboveground growth (Table 2), despite showing different responses to N source, while full-sib genotypes 15, 28 and 24 similarly showed a non-significant response to N source (neutral response), but exhibited significantly different growth increments.

Effect of the climate and microsite variability on the temporal and spatial response of genotype to nitrogen source

The estimate of soil EC_a in proximity to each individual tree range between 3 and 42 $mS\ m^{-1}$ when measured early in spring 2014 (October). The significant differences in electrical conductivity across plots ($F = 39.06$, $p < 0.001$) indicated that topography and soil properties were heterogeneous across the site, both within- and between-experimental plots ($p < 0.001$; Figure 2b, 2c). At the same time, the understory vegetation was dominated by species exhibiting seasonal growth peaks (Figure 5), mostly grasses and clovers, with approximately 20% cover for each. The extent of vegetation cover (clover, grass and other plant species) was significantly increased by the effect of N source ($F = 6.29$, $p < 0.001$) as was grass cover ($F = 7.70$, $p < 0.001$) and other plant species ($F = 2.84$, $p = 0.037$), but N source did not influence the cover of clover ($F = 0.73$, $p = 0.537$) (Figure 5).

Climatic conditions differed seasonally between 2013 and 2016, with a wet cold summer (T_1) preceding a year with low rainfall (T_2) (Figure 6). Monthly cumulative rainfall in autumn 2014 was higher than the average of the last 40 years (1972 – 2015; Table S1). Contrasting conditions were also recorded for minimum temperature, in which the first quarter of years 2015-2016 had higher average temperatures than in 2014. The contrasting inter-annual changes in precipitation and minimum temperature were accompanied by a consistent increase in the monthly maximum temperatures in autumn (2014-2016) over the years of study. Average relative humidity changed in a similar manner to the rainfall, but was within the normal historical ranges (Table S1).

As a result of climate fluctuation, fertilisation with different N sources influenced incremental volume growth over the period between December 2013 and June 2016. The effect of N source significantly influenced the patterns of cumulative incremental growth in tree volume, although this effect varied depending on the time period (Figure 7). Growth increased consistently over time, and about 50% of total growth occurred between December 2015 and June 2016 (T_4). From tree establishment until October 2014 (T_1), the increment in tree volume was greater ($p = 0.046$) for trees growing under L-arginine than in inorganic N (T_1 , $p = 0.050$). After October 2014 and until December 2015 trees reached a similar volume, as shown by the non-significant effect of N source (T_2 , $p = 0.078$; T_3 , $p = 0.173$). From December 2015 towards the end of the experiment, significant differences were found in cumulative growth in response to N source (T_4 , $p = 0.004$), in which untreated trees showed greater incremental growth than those to which inorganic N was applied. Trees fertilised with the combined treatment of

organic and inorganic N forms did not show significant differences in the cumulative incremental volume from trees supplied with either N form alone.

Since the multivariate mixed linear model analysis investigated volume at different time periods, the intercept represents factor time which is shown as statistically significant and all other terms in the model are the interaction with the time. The individual factors tested in the model showed that genotype, and EC_a and cover had statistically significant interactions with time, while N source was not significant factor affecting temporal trends in volume (Table 4). However, there was highly significant interaction between nitrogen source and genotype as well as nitrogen source and cover affecting temporal trends in volume (Table 4). There were separate statistically significant interactions between EC_a and genotype and EC_a and cover (Table 4). The interaction with all five variables, time, genotype, cover, N source, and EC_a, was not significant (Table 4).

DISCUSSION

The study revealed strong effect of genotype in *P. radiata* growth capacity along with a variable degree of phenotypic plasticity in growth responses to the environmental gradients at the site. It also suggests that the legacy nutrients (including N) from previous land use (in this case pasture and a wild fire) had an important, yet unknown effect to which a forestry site will be N responsive. As this study was only located at one site, we were unable to quantify the impact of legacy nutrients in our results. The overall effect of N sources across replicate plots showed that NH₄NO₃ treatment led to a suppression of tree volume growth compared to that in untreated trees, while effects of organic N were similar to the N-free control. The organic plus inorganic treatment (L-arginine: NO₃⁻) was not significantly different from responses to the individual organic and inorganic sources. These unexpected results highlight the plausible microsite effect on the variability in soil properties and the heterogeneous spatial distribution of resources at field-scale, which can strongly impact the availability of N sources. This reveals the potential underestimation of effects when averaging experimental plots in field studies with no information regarding the soil properties of the site. Previous studies of conifers have reported greater growth with NH₄⁺ than with NO₃⁻ treatment (van den Driessche 1971), although others have shown enhanced growth in early successional ecosystems with high soil nitrate content (Parfitt et al. 2003). This suggests that plant species tend to acclimate to the available N source (Kronzucker et al. 1997). Strong interactions between genotype and nutrient availability have been observed among families of *Pinus taeda* (Crawford et al. 1991) and *P. radiata* (Hawkins et al. 2010), sometimes leading to differences in leaf nutrient content

within sites (Burdon 1976). There are very few studies that have reported variation in growth rates of conifers growing in organic and inorganic N forms, and generally the results were inconclusive (Gruffman et al. 2012; Wilson et al. 2013). Nevertheless, fertilisation with organic N is generally associated with differences in biomass allocation, mainly characterised by greater root allocation (root:shoot) compared to inorganic fertilisers (NO_3^-) (Cambui et al. 2011; Franklin et al. 2017). Numerous models have proposed that biomass allocation is regulated by the internal N status, and nutrient limitations on plant growth lead to an increase in allocation to roots and reduction of shoot growth (Thornley 1972). While we could not measure biomass distribution in this non-destructive study, it is likely that limitations in soil N availability led to differences in biomass allocation.

Genotypes with an overall greater biomass were also those that responded strongly to fertilisation with organic N or the combination of N forms (i.e. genotypes 48, 30 and 50). Preference for a N source can be driven by several factors, including an increased affinity of specific transport systems (e.g. amino acid transporters - Näsholm and Persson 2001) or enhanced utilisation and hence growth in response to the form supplied (Britto and Kronzucker 2013). There are two possible scenarios that could explain the apparent genotype preferences for N source and its linkage to growth capacity. The first scenario suggests that the genotypic variation is linked with the capacity for acquisition of different N sources thus leading changes in the overall growth (Harrison et al. 2007; Boczulak et al. 2014). A number of studies have also shown some conifers may take up organic and inorganic N sources at comparable rates (Miller and Cramer 2004; Öhlund and Näsholm 2004). However, Brackin et al. (2015) recently attributed the low N efficiencies of inorganic forms in crop systems, such as sugar cane, and the N loss to the environment, to the capacity of the root to absorb inorganic N being exceeded at certain levels of application, in contrast to organic N that more closely matched the capacity of the root. The second scenario suggests that genotypes with an overall greater performance might be less sensitive to changes in N availability, because of the greater efficiency of N use (Bongarten et al. 1987; Garcia Villacorta et al. 2015). In fact, recent studies have reported that treatments with organic N forms may contribute more efficiently than inorganic forms to a plant N budget, even at lower concentrations, given the lower carbon cost of assimilation (Franklin et al. 2017). In addition to the variation in genotype-specific strategies of uptake and use of nutrients, fertilisation with different N sources may lead to differences in N availability through competitive effects with weeds, thereby influencing tree growth. The greater cover of seasonal grasses observed within organic N treatments (with and without NO_3^-) compared to untreated trees, indicates the potential for greater N retention in the rhizosphere of trees fertilised with L-arginine compared to inorganic N sources.

351

352 Heterogeneity in soil properties influenced the manner in which N source affected plant growth.
353 This likely resulted in direct or indirect changes in the availability of N sources that constrained root
354 absorption. Apparent electrical magnetic conductivity varied across the site from 3 and 42 mS s⁻¹ with the
355 highest EC_a values occurring in the control plots (Figure 2c). Previous research has demonstrated that
356 lower EC_a values are associated with coarser textured soils, more rocks, lower water availability, and the
357 absence of topsoil organic matter (Sudduth et al., 2005). Water retention and availability were likely key
358 determinants of plant N availability, by differentially influencing the retention of N chemical forms with
359 distinct molecular structures (Owen and Jones 2001). Firstly, water is the medium by which nutrient
360 movement and uptake occurs. In this respect, nutrient mobility is important; Öhlund and Näsholm (2002)
361 reported that L-arginine is subject to strong soil immobilisation, due to the negative charge of soil
362 particles, in contrast to the high mobility of NO₃⁻. Secondly, soil water content also affects soil biotic
363 processes and alters the metabolic activity of soil decomposer communities (Manzoni et al. 2012),
364 resulting in changes in heterotrophic respiration and nutrient mineralisation. Therefore, soil moisture
365 content, driven by soil water holding capacity and the variability in rainfall, might contribute to
366 differences in tree growth through a complex interplay of indirect effects involving rates of nutrient
367 supply and interconversion between N forms. These effects are tempered by plant root-associated
368 microbial interactions (i.e. competitive, mutualistic, pathogenic), influencing nutrient availability and the
369 absorption capacity of the plant (Harrison et al. 2008; Näsholm et al. 2013).

370

371 There are four potential issues that would impact the interpretation of our findings. The first issue
372 is that the EC_a values were only measured once during the study (October 2014), thus the EC_a values were
373 presumably influenced by soil water. However, the influenced of soil water to the EC_a signal was likely
374 minimal because the soil has good drainage and measurements were carried out several months after
375 heavy rainfall in the winter months (Figure 6). This is supported by the upper 10 cm average gravimetric
376 water content of 13.1% (SE ±1.8%) that was obtained from five samples at the same time of the EC_a
377 measurements. Therefore, soil physical properties was likely to be the dominant factor influencing EC_a
378 value at each measurement point. The second potential issue is that EC_a values could be impacted by high
379 soil salinity. However, the majority of New Zealand soils, including the region where the study site is
380 located, have low salinity values (McLaren and Cameron 1990). The third potential issue is that soil
381 organic matter, soil nitrogen, cation exchange capacity, and other soil chemistry properties were not
382 measured throughout the trial. If there was large variability of one or more of these factors, they might

have also influenced tree growth and confounded our interpretation of the results. Although this issue cannot be excluded entirely, it is unlikely that the soil chemistry properties varied large enough to confound the results. In addition, previous studies have found that soil organic matter and soil nitrogen can influence soil EC_a values, but soil physical properties have a significantly stronger influence (Doolittle and Brevik 2014). The fourth and final potential issue is that only aboveground biomass was measured in this study. It remains unclear whether differences in tree growth after 2.5-years of N fertilisation with different N source are due to genotype-specific strategies of biomass allocation and root plasticity. However, a parallel study utilising the same field trial and two of ten *P. radiata* genotypes and the organic and inorganic N treatments, found clear evidence that intraspecific variation in response to N form is associated with shifts in composition of the root microbiome (Gallart et al. 2018b).

In addition to the influence of genotype by environmental gradient interactions on plant growth, the seasonal and yearly changes in climatic conditions likely influenced the extent to which genotypes responded to N sources over time. This agrees with previous studies that report a certain degree of phenotypic flexibility in *P. radiata*, which is induced by variable climate conditions (Burdon et al. 2001; Rodríguez-Gamir et al. 2018). The response of cumulative incremental growth volume over time shows that towards the end of the experiment, there is an increase in the overall primary productivity as well as a maximisation of differences between N sources. This suggests the direct and indirect effects of climate were critical for the first two years following tree establishment. Unfertilised trees had accumulative incremental volume that did not significantly differ from the fertilised trees until December 2015, after which trees under L-arginine and L-arginine:NO₃⁻ treatments exhibited greater growth until June 2016. A possible explanation for the greater delayed growth in untreated compared to N-fertilised trees might be the overextension of resources by N-fertilised trees under favourable environmental conditions that became unsustainable under less favourable conditions. Leaf traits were not measured, but fertilisation generally promotes greater investment in needles (Fife and Nambiar 1997). As a result, for a given transpiration rate, fertilisation could potentially increase the total transpiration of the plant, which could overly stress the plant under dry conditions (Irvine et al. 1998; Miyazawa and Lechowicz 2004). As explained above, the combination of porous soil and the presence of periods with very low precipitation, likely accentuated genotype × drought responses in growth. Research on genetic variation in foliar δ¹³C isotopic signature indicates that there may be genotype-specific responses under water stress conditions that determine phenotype adaptability to water tolerance (Cregg and Zhang 2001; Prasolova et al. 2003). Lim et al. (2015) recently proposed that changes in inter-annual precipitation may help explain the reported large variability in growth responses to fertilisation of pine stands. This agrees with our results

that indicate that the effects environmental gradients impacted growth, depending on genotype and the N source. This could imply that different genetic strategies influencing water efficiency might determine tree response to environmental N changes.

CONCLUSIONS

Three patterns emerged from this field experiment. First, our results are consistent with prior findings that growth in temperate conifers is strongly influenced by the genotype by environment interactions, with variation of phenotypic expression of different *P. radiata* genotypes to N-fertilisation, despite the relatively narrow pedigree of our study trees. Second, *P. radiata* showed genotype-specific responses to fertilisation with different N sources (NH₄NO₃, L-arginine:NO₃⁻, L-arginine). Third, the interactive effect of the spatial heterogeneity of soil properties, spatial distribution of understory vegetation and N source determined genotype performance and the susceptibility of trees to climatological fluctuations. The results suggest that the forestry sector requires a better understanding of the flexibility and adaptability of genotypes beyond the capacity for growth and towards efficient strategies of N use. This may have important implications for the establishment of criteria in breeding programmes considering the ecological impact of climate change on tree genetics. N-fertilisation in forest plantations could be optimised by using site-specific N regimes and N sources that adjust to the soil characteristics of the site and to climate in order to increase sustainability, by maximising fertiliser response and minimising N leaching. In conclusion, silvicultural practices would benefit from a more nuanced approach that integrates tree selection and plantation management operations and considers both biotic and abiotic factors as they influence site quality.

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FIGURES

Figure 1: Location of the field site in the South Island of New Zealand. (a) Geographic distribution of planted exotic forest, mostly comprising *P. radiata* plantations, (b) location of the field site and weather stations (yellow triangles) in the Canterbury region, 20 km from Christchurch and (c) map of the field site. Distribution data obtained from 2016 National Exotic Forest Description report (Ministry for Primary Industries of New Zealand 2013) and satellite maps from Google Earth.

Figure 2: Field diagrams synthesising (a) the distribution of whole-plots that contained 10 genotypes as individual rows (subplots), i.e. each row planted with each individual genotype. Seven ramets of each genotype were randomly planted within a row, with each plot having 70 trees in total randomised complete block, arranged as a split-plot factor with six replicates of four N treatments applied to the main-plots, NH_4NO_3 (red), L-arginine: NO_3^- (orange), L-arginine (green) and Control (black). (b) Microtopography map at site-level interpolated from the georeferenced individual tree measures in the EM survey (c) apparent electrical conductivity (EC_a) site-level map interpolated from individuals tree measures of soil electrical conductivity of the top 160 cm of the soil (EC_a , mS m^{-1}).

Figure 3: Genetic background of the *Pinus radiata* genotypes used in the experiment. Capital letters represent the coded series of the ancestors. Grandmothers and parental ancestry were selected by Radiata Pine Breeding Company Ltd (Rotorua, New Zealand) and the resulting progeny was selected by Forest Genetics Ltd.

Figure 4: Volume increment (cm³) across plots between December 2013 and June 2016. Colouring of bars show the six replicates of four N treatments applied to the main-plots, NH₄NO₃ (red), L-arginine:NO₃⁻ (orange), L-arginine (green) and control (black). Data are presented as mean ± SE, after back-transformation from logarithmic scale.

Figure 5: Differences in the percentage of understory vegetation composed by clovers (*Trifolium repens*, *Trifolium subterraneum*), grasses and other plant species (*Taraxicum officinale*, *Rumex acetosa*, *Stachys* sp., *Polygonum aviculare*, *Chenopodium album*, *Malva sylvestris* and *Ulex europaeus*), in response to N source. The assessment was undertaken in October 2014 (spring). Data are presented as mean, after back-transformation from logarithm scale. Letters in bars within the same plant type indicate significant differences across N treatments using Tukey *post hoc* pairwise comparison ($p < 0.05$).

Figure 6: Monthly cumulative rainfall (mm), daily mean maximum (red dots) and minimum temperatures (light blue dots)(°C) between December 2013 and June 2016 obtained from the closest meteorological station, 16 km from the site (agent 41230, 43°48'S 172°52'E). Arrows define the four time periods (T₁, T₂, T₃, T₄) in which tree measures were undertaken (December 2013, October 2014, May 2015, December 2015 and June 2016).

Figure 7: Cumulative incremental volume (cm) between December 2013 (T₀) and October 2014 (T₁ - T₀), May 2015 (T₂ - T₀), December 2015 (T₃ - T₀) and June 2016 (T₄ - T₀). Colour of bars indicate the N treatment supplied, NH₄NO₃ (red), L-arginine: NO₃⁻ (orange), L-arginine (green) and control (black). Letters above bars indicate significant differences between N sources using Tukey *post hoc* pairwise comparison following a one-way ANOVA ($p < 0.05$).

TABLES

Table 1: Effect of N source in the mean volume increment (ΔV , cm³) between December 2013 and June 2016 in *P. radiata*. Results were averaged by N source without considering the genotype effect. Mean $\pm$ SD with different letters are significantly different (Tukey *post hoc* pairwise comparison, $p < 0.05$). Volume values obtained after back-transformation from logarithmic scale. N number of individual replicates.

N source	N	$\Delta V \pm SD$
NH₄NO₃	401	228.65 $\pm$ 2.32 ^b
L-arginine: NO₃⁻	399	249.94 $\pm$ 2.55 ^{ab}
L-arginine	387	257.57 $\pm$ 2.77 ^{ab}
Control	397	286.88 $\pm$ 2.28 ^a

Table 2: Effect of tree genotype in the mean volume increment (ΔV , cm³) between December 2013 and June 2016 in *P. radiata*. Results were averaged by genotype without considering the effect of N source. Mean $\pm$ SD with different letters are significantly different (Tukey *post hoc* pairwise comparison, $p < 0.05$). Volume values obtained after back-transformation from logarithmic scale. N number of individual replicates.

Tree genotype	N	$\Delta V \pm SD$
15	144	172.21 $\pm$ 2.60 ^e
24	155	235.74 $\pm$ 2.27 ^{cde}
28	160	306.11 $\pm$ 2.23 ^{bc}
30	162	218.49 $\pm$ 2.71 ^{de}
31	164	343.56 $\pm$ 2.36 ^{ab}
35	162	258.54 $\pm$ 2.29 ^{bcd}
37	159	207.36 $\pm$ 2.37 ^{de}
44	160	203.38 $\pm$ 2.43 ^{de}
48	160	430.92 $\pm$ 2.27 ^a
50	158	250.89 $\pm$ 2.48 ^{cd}

Table 3: Simple main effects of the ten genotypes at each of the four sources of N, NH₄NO₃, L-arginine:NO₃⁻, L-arginine and control. When significant, the test followed a Tukey *post hoc* pairwise comparison between the volume increment means in each of the N treatments. Mean $\pm$ SD with different letters are significantly different (Tukey *post hoc* pairwise comparison, $p < 0.05$). Volume values obtained after back-transformation from logarithmic scale.

	Volume increment (cm ³)				<i>p</i> -value
	NH ₄ NO ₃	L-arginine:NO ₃ ⁻	L-arginine	Control	
Genotype 15	158.98 $\pm$ 2.35	149.28 $\pm$ 2.77	154.32 $\pm$ 3.24	234.44 $\pm$ 2.09	0.141
Genotype 24	183.10 $\pm$ 2.17	228.16 $\pm$ 2.71	285.86 $\pm$ 2.23	258.29 $\pm$ 1.89	0.100
Genotype 28	280.36 $\pm$ 2.00	352.55 $\pm$ 2.37	252.32 $\pm$ 2.48	350.21 $\pm$ 2.05	0.167
Genotype 30	138.01 $\pm$ 2.65 ^b	227.33 $\pm$ 2.21 ^{ab}	280.16 $\pm$ 3.16 ^a	246.18 $\pm$ 2.53 ^a	0.009
Genotype 31	357.51 $\pm$ 2.02	250.63 $\pm$ 1.96	379.71 $\pm$ 2.94	405.33 $\pm$ 2.34	0.052
Genotype 35	264.09 $\pm$ 2.06	262.75 $\pm$ 2.23	195.95 $\pm$ 2.71	325.92 $\pm$ 2.07	0.060
Genotype 37	265.69 $\pm$ 2.16	188.02 $\pm$ 2.42	161.66 $\pm$ 2.24	228.61 $\pm$ 2.54	0.054
Genotype 44	200.84 $\pm$ 2.03	209.71 $\pm$ 2.80	201.96 $\pm$ 2.58	201.14 $\pm$ 2.40	0.996
Genotype 48	393.26 $\pm$ 1.84 ^{ab}	611.91 $\pm$ 2.35 ^a	341.55 $\pm$ 2.71 ^b	424.29 $\pm$ 2.02 ^{ab}	0.013
Genotype 50	156.36 $\pm$ 2.57 ^c	222.28 $\pm$ 2.15 ^{bc}	436.04 $\pm$ 2.25 ^a	262.03 $\pm$ 2.23 ^b	< 0.001

Table 4: Summary of the analysis of mixed linear model to test the effect of predictor variables genotype, N source, apparent electrical conductivity (EC_a), understory vegetation cover (Cover) - including clover, grass and other plant species - and the interaction of them in the increment of tree volume between December 2013 and June 2016 in *P. radiata*.

	df	SS	Wald stat.	<i>p</i> -value
Time (trait - specific intercept)	5	165917	165917	< 0.001
Time x N	15	11	11	0.730
Time x Genotype	45	748	748	< 0.001
Time x EC_a	5	206	206	< 0.001
Time x Cover	5	70	70	< 0.001
Time x Genotype x N	135	253	253	< 0.001
Time x Genotype x EC_a	45	75	75	0.003
Time x Genotype x Cover	45	44	44	0.527
Time x Genotype x EC_a x N	135	159	159	0.077
Time x Genotype x Cover x N	135	217	217	< 0.001
Time x Genotype x Cover x N x EC_a	180	182	182	0.439

SUPPLEMENTARY MATERIAL

Table S1: Climate data of monthly cumulative precipitation (mm), daily minimum temperature (°C), daily maximum temperature (°C) and relative humidity (%) between 2013 and 2016. The top values represent the average historic record [mean $\pm$ SEM] between 1972 and 2015. Data obtained the Darfield NIWA weather station (agent 4836, 4329°S 17208°E).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Des
Precipitation												
1972-2015	55 $\pm$ 41	55 $\pm$ 32	65 $\pm$ 50	64 $\pm$ 48	67 $\pm$ 40	72 $\pm$ 48	72 $\pm$ 45	81 $\pm$ 52	56 $\pm$ 37	69 $\pm$ 35	58 $\pm$ 30	67 $\pm$ 39
2013	-	-	-	-	-	-	-	-	43.4	72.8	31.8	81.0
2014	15.0	57.0	150.4	216.6	47.2	98.0	0.0	17.0	29.4	20.0	44.2	26.2
2015	17.6	10.8	0.0	59.6	15.6	72.6	44.4	41.4	52.4	11.4	16.2	46.6
2016	87.2	18.6	47.6	12.8	84.0	21.0	-	-	-	-	-	-
Minimum temperature												
1972-2015	11 $\pm$ 1	10 $\pm$ 1	9 $\pm$ 1	7 $\pm$ 1	4 $\pm$ 1	2 $\pm$ 1	1 $\pm$ 1	2 $\pm$ 1	4 $\pm$ 1	6 $\pm$ 1	7 $\pm$ 1	9 $\pm$ 1
2013	-	-	-	-	-	-	-	-	3.6	5.7	9.0	11.1
2014	9.7	11.7	8.7	9.1	3.3	1.8	0.9	1.0	2.6	4.6	6.8	10.3
2015	12.5	10.1	10.5	7.7	2.9	0.5	0.3	1.7	3.2	5.0	7.5	9.2
2016	12.2	13.2	10.1	5.5	6.3	2.2	-	-	-	-	-	-
Maximum temperature												
1972-2015	23 $\pm$ 2	23 $\pm$ 2	21 $\pm$ 2	18 $\pm$ 1	14 $\pm$ 1	11 $\pm$ 1	10 $\pm$ 1	12 $\pm$ 1	15 $\pm$ 1	17 $\pm$ 1	20 $\pm$ 1	22 $\pm$ 2
2013	-	-	-	-	-	-	-	-	14.3	17.7	18.9	21.7
2014	22.2	21.9	18.6	15.6	15.7	13.1	11.2	11.9	14.3	17.3	20.0	21.4
2015	24.0	22.5	20.3	18.2	16.2	12.9	11.4	11.8	13.4	18.2	19.4	21.8
2016	20.8	25.1	21.3	18.9	17.2	13.7	-	-	-	-	-	-
Relative humidity												
1972-2015	64 $\pm$ 6	69 $\pm$ 5	70 $\pm$ 6	74 $\pm$ 5	75 $\pm$ 6	76 $\pm$ 6	74 $\pm$ 5	73 $\pm$ 5	71 $\pm$ 6	70 $\pm$ 5	69 $\pm$ 5	68 $\pm$ 6
2013	-	-	-	-	-	-	-	-	78.4	71.0	76.9	69.2
2014	63.8	72.2	81.0	87.6	74.4	78.5	78.0	77.4	83.2	72.6	60.1	73.4
2015	66.9	69.2	76.1	74.3	70.2	71.3	70.6	78.9	76.2	68.8	63.3	65.9
2016	73.0	67.4	72.9	79.0	69.6	75.0	-	-	-	-	-	-

Table S2: Selection criteria used to analyse genotype production during progeny trials. Above-ground traits measured were stem growth rate, corewood stiffness, wood density, resistance to the pathogen *Dothistroma septosporum* ((Dorog.) M. Morelet), and internode length (branching). Information kindly provided by Forest Genetics Limited.

	Growth rate	Corewood stiffness	Wood density	<i>D. septosporum</i> resistance	Internode length
Genotype 15	✓	✗	✗	✓	✗
Genotype 24	✗	✓	✓	✓	✗
Genotype 28	✓	✗	✗	✗	✓
Genotype 30	✓	✓	✗	✗	✗
Genotype 31	✗	✓	✗	✗	✗
Genotype 35	✗	✓	✗	✗	✗
Genotype 37	✓	✗	✓	✗	✗
Genotype 44	✓	✗	✓	✓	✗
Genotype 48	✓	✓	✓	✓	✗
Genotype 50	✓	✗	✗	✓	✓