Root traits of New Zealand trees:

Community-level responses to a soil fertility gradient,

and plastic responses to nutrient availability

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Kris Rushton Kramer-Walter

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Abstract

The study of plant traits has great application for understanding plant distribution patterns and community assembly at a variety of scales. Roots are a vital component of plant water and nutrient uptake strategy, and yet root traits are not as well understood as leaf or stem traits. In this thesis I aimed to determine the relationships between seedling fine root, leaf, and stem traits of New Zealand tree species, and investigate whether these traits related to species' abundance along a soil fertility gradient. I also investigated how seedling traits compare with adult traits, and whether nutrient availability or method of fertiliser application affected seedling trait expression. To achieve these goals, I measured root, leaf, and stem traits on seedlings of 66 native tree species, and combined this with relative abundance data along a strong soil fertility gradient at Puketī forest. I used principle component analysis and ordination to assess the dimensionality of trait variation across species; and linear regressions to compare community-weighted mean (CWM) traits vs. a summarised axis of soil fertility. I also performed regression analysis between seedling traits and adult traits from the literature. To assess the effects of nutrient availability and application I grew four native tree species under three nutrient treatments: low, pulsed, and slow release. I used two-way ANOVA and co-efficient of variation analyses to determine the strength of responses to nutrient treatments.

Dry matter content was positively associated across leaves, roots, and stems, and negatively associated with root nitrogen concentration and relative growth rate, suggesting that at least as seedlings, traits associated with a fast or slow growth strategy are co-ordinated across organs. Root diameter and SRL were independent from this axis of fast-slow growth, suggesting that SRL does not have a direct effect on seedling growth rates and nutrient foraging. A third axis of variation was also identified, strongly influenced by root phosphorus and nitrogen concentrations, but was difficult to interpret. Regression of CWM traits vs. soil fertility showed that traits which comprised the fast-slow PCA axis co-varied strongly with soil fertility. Neither root diameter nor SRL were significantly related to soil fertility, supporting the conclusion that SRL is not adaptive to nutrient foraging ability. Seedling morphology traits are generally well correlated with adults, but tend to be oriented towards a more "acquisitive" growth strategy, suggesting that species may down-regulate their growth over ontogenetic

development. The absence of correlation between SRL and soil fertility suggests that alternative root traits may be more applicable for understanding species foraging strategy.

Species' responses to the nutrient treatments differed for most traits. Root-to-shoot ratio, growth rate, and root nutrient concentrations responded strongly to nutrient availability, and morphological traits did not respond as strongly. Slow release and pulse treatments were typically similar, and both significantly different to low nutrient treatment. These results suggest that it is practical to compare morphological trait data between studies, provided plants are raised in environments conducive to growth, but that nutrient concentrations and biomass allocation traits can be strongly influenced by soil fertility.

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Chapter 1: Literature review

Ecology and Humanity

> "The highest function of ecology is understanding consequences."
>
> Planetary ecologist Pardot Kynes, from the novel "Dune" (Herbert, 1965)

Never before has the understanding of consequences been of such importance as in the 21st century. Across the globe, billions of people now live in relative comfort, enjoying access to food, clean water, education, modern medicine, and longer lifespans (World Health Organization, 2013). However the technological progress and prosperity of humanity has also enabled exponential population growth, and has come at the price of deforestation, collapse of fisheries, loss of biodiversity, widespread extinction of species, and the perturbation of Earth's physiochemical systems (Vitousek, 1994, Vitousek et al., 1997b, Worm et al., 2009, Barnosky et al., 2011, Schneider et al., 2011, Rogelj et al., 2012, Cardinale et al., 2012). If humanity is to have a long term, sustainable future on this planet, the devastation of ecosystems must be halted, and progress must be made on restoring ecosystem functions and services (Hobbs and Harris, 2001, Sanderson, 2013).

One of the major tasks of modern ecology is to build on our understanding of the consequences of human activity on ecosystem structure and function. We need to refine our knowledge of how ecosystems are affected by climate, biogeochemical cycles, biodiversity, and distributions of species; and how ecosystems in turn affect these factors (Vitousek et al., 1997a, Chapin III et al., 2000). To understand the consequences of our collective actions on Earth's ecology, we must develop models of how ecosystems form, function, and respond to environmental variables, with the hope that such models will allow us to mitigate future ecologically detrimental effects, and to rehabilitate or reconstruct damaged or destroyed ecosystems.

Ecological models

It has long been established in ecology that abiotic gradients are major drivers of spatial changes to regional vegetation types (biomes) (Holdridge, 1947,

Whittaker, 1975). The dominant biome of a region can be predicted accurately from the combination of mean annual temperature (MAT) and mean annual precipitation (MAP) (Figure 1). This general principle has been refined over time (Box, 1996), but this model alone is not sophisticated enough to make predictions about the distributions of individual species, and how distributions may change with alterations to the environment. To better understand the factors which drive individual species' distributions, ecological models need to incorporate data about the properties of individual species, and how these properties are filtered by the environment. Additionally, as MAT and MAP vary over relatively large spatial scales, environmental gradients which vary over smaller scales (e.g. nutrient or light availability) are often more suited to the study of species-level distribution patterns and community assembly processes.
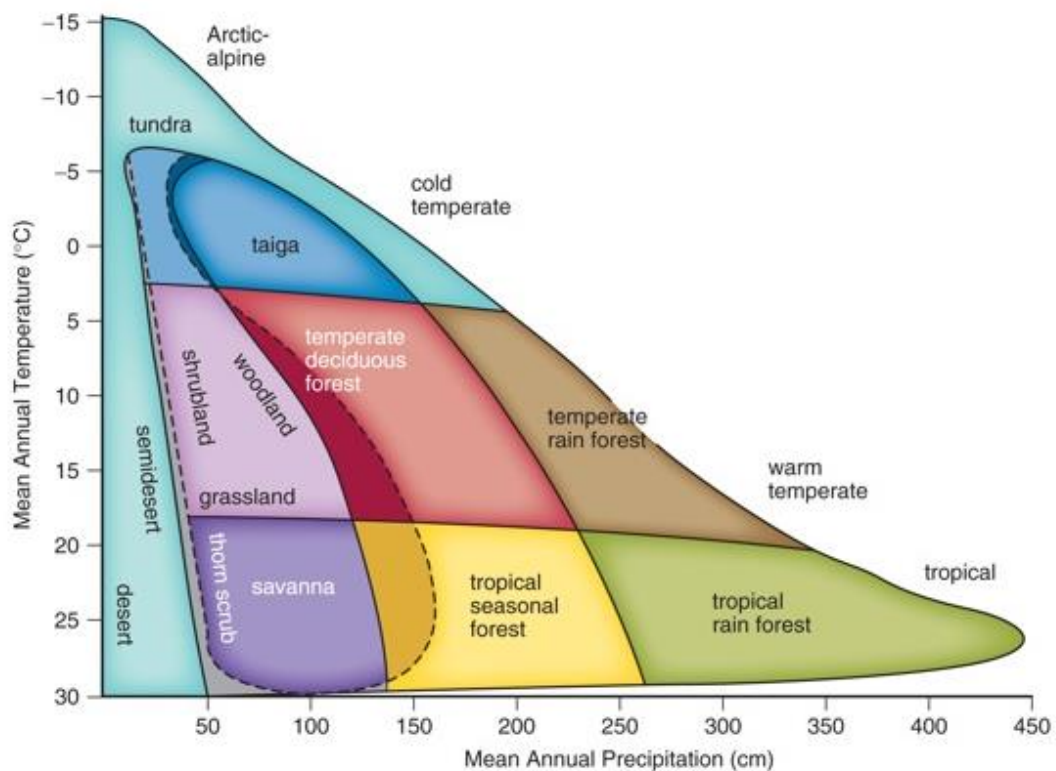
Figure 1. The pattern of global biome types in relation to Mean Annual Temperature and Mean Annual Precipitation (adapted from Whittaker, 1975).

Environmental filtering and limiting similarity

Community assembly can be thought of as resulting from two antagonistic processes: Environmental filtering and limiting similarity (Laughlin et al. 2012). Environmental filtering is the result of differential fitness among species, due to

species' adaptations to the physical, chemical, and biological conditions of a site. For example, higher winds on convex ridges filter for shorter species, as they are better adapted to resist the mechanical and moisture stress on these sites (Lasky et al., 2013). Other environmental filters include disturbance regimes, topography, climate, and soil fertility (Freschet et al., 2011). Harsher and more stressful environments tend to have a stronger filtering effect and offer fewer viable niches, resulting in community clustering around adaptive attributes, with more similarity between species than would be expected by chance.

Limiting similarity is an extension of the competitive exclusion principle, which states that "complete competitors cannot co-exist" (Gause, 1934, Hardin, 1960). As environmental filtering increases functional similarity between species, competition limits functional similarity between species within a community, acting to minimise niche overlap and maximise resource partition (MacArthur and Levins, 1967).

These two processes rely on the concept of similarity or dissimilarity between species, and in order to understand how species' attributes relate to environmental filtering these differences need to be quantified and compared. Functional trait-based ecology represents a method of measuring species' attributes, using these measurements to identify differences between species, and mechanistically linking these to differential fitness under given environments.

Plant functional traits

Traits are heritable features or characteristics of organisms (Darwin, 1859), and functional traits are the morphological, anatomical, biochemical, physiological, or phenological traits which indirectly impact an individual's fitness via their effects on establishment, survival, growth, and reproductive success (Violle et al., 2007, Reich et al., 2003). For ease of reference, both plant functional traits and the trait values measured on individuals will be hereafter referred to simply as 'traits'. Traits can be continuous variables, for instance height at maturity or leaf area; or categorical variables, such as evergreen or deciduous leaf habit (Violle et al., 2007, Pérez-Harguindeguy et al., 2013). Intra-specific trait variation can reveal genotypic diversity within species, as well as species' capacity to adapt to environments via phenotypic plasticity (Hutchings and de Kroon, 1994, Sultan, 2000, DeWitt and Scheiner, 2004). Inter-specific trait variation can be compared

with species' distributions along environmental gradients, to construct mechanistic hypotheses of how physical differences between species ultimately result in different patterns of distribution over environmental gradients (Diaz et al., 1998, Lavorel and Garnier, 2002, Shipley et al., 2006, Ordoñez et al., 2009, Laughlin et al., 2012).

As a mechanistic framework, trait-based ecology may also have potential to predict shifts in vegetation distributions in response to modelled future environmental conditions, including changes to climate, disturbance regimes, and soil fertility (Diaz and Cabido, 1997, Chapin III, 2003, Wright et al., 2005, Swenson and Weiser, 2010). Additional, trait-based approaches may be of use in restoration ecology. By analysing the traits of likely invasive species, and deliberately selecting native species to fill empty niches, restoration planners may be able to construct native plant communities which are resistant to invasion (Pywell et al., 2003, Fukami and Lee, 2006, Funk et al., 2008, Laughlin, 2014a).

Plant strategy

Traits indirectly influence individual fitness by their adaptive value in the current environment, however natural selection does not operate on individuals traits, but rather on the whole organism. Therefore, traits are often considered in combination, as integral components of an overall survival and reproductive "strategy". Craine (2009) defines an individual or species' strategy as "a set of interlinked adaptations that arose as a consequence of natural selection and that promote growth and successful reproduction in a given environment".

Grime (1979) described three major directions of plant strategy which have evolved in response to varying degrees of environmental stress and disturbance: competitors, stress-tolerators, and ruderals (CSR). Competitive species have traits which support an acquisitive growth strategy. Fast growth allows competitive species to rapidly proliferate into high resources patches, and exploit them before slower growing species. Competitors thrive in conditions of low stress and disturbance, but on unproductive or frequently disturbed sites their traits are not conducive to long term fitness. Stress-tolerating species are adapted to stressful and/or low productivity conditions. Stress in this sense refers to conditions which hinder growth, i.e. low availability of vital resources such as light, nutrients, and water. Stress-tolerators are able to survive in unproductive environments by

adopting a conservative growth strategy characterised by traits relating to slow growth and higher resource allocation to storage. Stress tolerators have sacrificed their ability to rapidly exploit resources, in exchange for robust, long-lived organs which are more able withstand damage from grazing or tolerate environmental extremes. Ruderal species are adapted to environments which frequently experience disturbance due to factors such as fire, flooding, landslides, trampling, or intensive animal grazing. To adapt to regularly disturbed habitats, ruderal species are typically small, herbaceous, short-lived and capable of completing their reproductive cycle in a short time period. Like all models, CSR theory is a simplification of reality, and most species fall somewhere in between the three "poles" of strategic directions (Pierce et al., 2013). Despite criticisms of its limitations (Loehle, 1988, Craine, 2005), CSR theory still serves as a valuable and influential model of plant strategy. It highlights the importance of analysing plant traits from a functional point of view, to determine how traits and strategies are environmentally filtered. Subsequent models have also been theoretically based in discussing dimensions of plant strategy, but have developed these strategies in a "bottom-up" approach by studying inter-specific trait variation and inherent trade-offs in organ function (Westoby, 1998, Westoby et al., 2002).

In leaves, there is strong and wide-spread evidence of a trade-off between traits relating to either acquisitive or conservative growth (Reich et al., 1991, Westoby, 1998, Westoby et al., 2002, Wright et al., 2004). This "worldwide leaf economic spectrum" ranges from "cheaply" constructed leaves, characterised by high SLA, low tissue density and fast rates of photosynthesis. Cheap leaves are able to rapidly photosynthesise and return the carbon cost of their construction, but lack defences against damage and thus tend to be relatively short lived. At the other end of the spectrum are "expensive" leaves which tend to have low SLA, high tissue density, and low photosynthetic capacity, but are better adapted to defend against grazing and environmental damage, and therefore can provide photosynthetic returns over a longer time period.

Evidence in support of the world-wide leaf economic spectrum has been documented at a range of scales, from within communities (Reich et al., 1991, Mediavilla et al., 2008, Laughlin et al., 2010, Jager et al., 2015) to across biomes (Reich et al., 1997, Reich et al., 1999, Wright et al., 2004). Leaf economic traits have been demonstrated to relate to multiple independent environmental variables,

including light availability, water availability, soil fertility, and mean annual temperature (Reich et al., 1997, Reich et al., 1998, Reich et al., 1999, Wright et al., 2002, Wright et al., 2004, Wright et al., 2005).

There is some evidence that similar strategic trade-offs between competitive and stress-tolerating growth may be present in other organs. Low density stems cost less carbon to construct, and can therefore grow taller for a given amount of carbon expenditure (King et al., 2005). In contrast, denser stems are more able to withstand mechanical stress, and therefore can facilitate higher maximum growth or survival in environments prone to high winds. Denser stems can sustain higher negative water pressure in the xylem before failure and embolism, conferring drought tolerance, and facilitating increased maximum height (Domec et al., 2008, Tyree and Zimmermann, 2002, Chave et al., 2009). In seeds there is a trade-off between seed mass and seed output. Species which produce larger seeds provide each of their offspring with more energy to establish and grow, which may be advantageous in stressful environments such as shaded forest floors or nutrient-poor soils. In contrast, species which produce smaller seeds can develop more offspring for a given amount of energy, but these offspring may be more reliant on finding canopy gaps or other resource rich sites in which to establish (Henery and Westoby, 2001, Westoby et al., 2002). Seed mass can also influence species' mode of dispersal, with larger seeds typically dispersed by animals, and smaller seeds dispersed by wind (Westoby et al., 1996).

It has also been suggested that plants may coordinate their strategies across multiple organs (Reich, 2014). Some studies support this hypothesis, demonstrating correlations between analogous root and leaf traits such as SRL and SLA (Withington et al., 2006, Laughlin et al., 2010, Laughlin et al., 2011, Fort et al., 2012), root and leaf tissue density or dry matter content (Wahl and Ryser, 2000, Mokany and Ash, 2008, Freschet et al., 2010, Pérez-Ramos et al., 2012, Craine et al., 2001), or root and leaf nutrient concentrations (Craine et al., 2005, Kerkhoff et al., 2006, Freschet et al., 2010, Liu et al., 2010, Holdaway et al., 2011). Other studies have not found correlations between SRL and SLA (Craine et al., 2005, Tjoelker et al., 2005, Markesteijn and Poorter, 2009, Chen et al., 2013), root and leaf tissue density (Kembel and Cahill Jr, 2011), or root and leaf longevity (Withington et al., 2006). Leaf and stem tissue densities have also been demonstrated to be disconnected in woody species (Baraloto et al., 2010,

Fortunel et al., 2012, Richardson et al., 2013, Jager et al., 2015). Therefore it seems that evidence is mixed for above and below ground strategic coordination, and even disconnections between leaf and stem economic traits are common.

The relevance of roots

Roots are vital components of plant function, responsible for absorbing water and nutrients from the soil, and yet compared to leaf traits, the adaptive functionality of root traits is poorly understood. Roots are integral to many soil processes, including nutrient cycling and carbon sequestration (Jackson et al., 1996, Eissenstat et al., 2000, Aerts et al., 1992a). Fine roots represent considerable amounts of global plant biomass (Robinson, 2004), and therefore further investigation of traits relating to fine root lifespan, turnover, and decomposition may be crucial for improving global models of carbon release and sequestration. To date, universal patterns between root morphology and function have yet to be established.

Although leaves and fine roots are both responsible for resource uptake, the air and soil environments in which they function are fundamentally different, as are the physiochemical processes involved in capturing light compared to absorbing water or soil nutrients. Due to these contrasts, the traits relevant to the leaf economic spectrum may not necessarily have analogues in root traits, and instead other traits may be relevant to root foraging strategy (e.g. root-to-shoot ratio, rooting depth, or root branching intensity). If root strategy is not correlated with leaf strategy, then it may represent an independent dimension of plant strategy, which could improve the predictive ability of trait-based models of community assembly (Laughlin, 2014b).

Fine and coarse roots

The root systems of woody plants are often divided into two functionally distinct categories, fine and coarse roots. Fine roots are generally defined as falling under a threshold of diameter, typically 2 mm. An alternative definition is offered by Eshel and Beeckman (2013), defining fine roots as those which break off from the root system during mechanical excavation. For the purposes of this thesis I will use the former definition, however in reality there is a continuum of root development and function, from young, absorptive fine roots, to older, lignified

and hardened roots (Hishi, 2007), which definitions based on branching order or diameter class can fail to appreciate (Guo et al., 2008).

Fine roots function to absorb water and soil nutrients, as well as interface with mycorrhizal symbionts (Brundrett, 1991, Smith and Read, 1996, Ryser, 2006). Newly grown fine roots often have an epidermis with fine root hairs which function to increase absorptive area. Root hairs can account from 43% to 96% of total absorptive length and surface area of woody species, due to their high surface area to volume ratio (Persson et al., 2002, Guo et al., 2008). There are several distinct categories of mycorrhizal symbioses; the majority of land plants form vesicular-arbuscular mycorrhizal symbioses (VAM), and other categories include ectomycorrhizal (EM), ericoid, and orchid type mycorrhizas (Wang and Qiu, 2006). Broadly speaking, mycorrhizal symbioses involve the fungal partner efficiently capturing vital soil nutrients (particularly phosphorus), and providing these to the plant in exchange for photosynthetic carbon (Aerts and Chapin, 1999, Brundrett, 2002). Fine roots lack secondary thickening or other physical defence structures, and are therefore more susceptible to damage from soil pathogens, grazing invertebrates, or physical factors such as frost or water loss (Eissenstat and Yanai, 1997).

Most fine roots do not undergo secondary thickening, but senesce and decay, making them more like leaves than twigs or branches (Eissenstat and Achor, 1999). The fine roots that do undergo secondary thickening and lignification function as robust "pipes", transporting nutrients and water from the fine absorptive roots to the main stem, and anchoring the plant in the soil (Gregory, 2006). Due to these functional requirements, coarse roots typically resemble woody stems or branches, reflected in their functional traits (Fortunel et al., 2012, Fortunel et al., 2014).

The absorptive functions and inherent susceptibilities of fine roots mirror those of leaves, and therefore there may be a similar economic spectrum in roots, relating to investment in root structural integrity and the resulting root longevity. However, research investigating root traits and strategies has lagged behind that of above ground organs, due to the fundamental differences and difficulties between sampling plant material from above vs. below ground.

Root sampling

Studying root traits in the field poses several logistical and methodological difficulties when compared to sampling leaf or stem traits. The primary difficulty is that the surface soils of productive ecosystems are typically densely packed with inter-woven roots, which may originate from several individuals of as many species. Such a density of interwoven roots can make a definitive identification of species and individual plant of origin a tedious task. This can be avoided by sampling in mono-species stands, although these are more typical of herbaceous communities compared to woody communities. Forest root samples are typically taken near to a desired species, and traced back through the soil to confirm the individual of origin. Preparing root samples for analysis typically requires thorough washing in order to remove any attached soil. Depending on the remoteness of the study site, access to sufficient quantities of clean water may be unfeasible. In situations such as these, the best alternative is to store the samples in a cool, air-tight container, keeping them hydrated and fresh until washing can be performed. Trait measurements of fine roots may become unreliable if the sample has deteriorated or decomposed, and therefore fresh measurements are always preferable. The equipment used to measure root length (a digital scanner) is typically fairly bulky, and may be unsuitable for transporting to or operating in remote areas. Despite advances in engineered solutions to root sampling, field sampling equipment can be large and difficult to transport, and still faces difficulties such as cutting through rocky soils and tough, woody roots (Sochacki et al., 2007).

As an alternative to field root sampling, several recent studies have utilised traits of glasshouse grown plants glasshouses (Cornelissen et al., 2003a, Mokany and Ash, 2008, Laughlin et al., 2011, Wishart et al., 2013, Birouste et al., 2014). Growing plants in a glasshouse allows researchers to control environmental factors, including light, water, and nutrient availability, as well as preventing herbivory and monitoring for disease. Glasshouse-based growth can also facilitate the root washing process by allowing the choice of an easily washed soil medium and providing easy access to root washing water, thereby avoiding damage to root systems and maximising the accuracy of root trait measurements.

Trait plasticity

Plants are known to alter their trait expression to better adapt to the local environment, a phenomenon known as trait plasticity (Bradshaw, 1965). By controlling environment factors in the glasshouse environment, researchers can alter factors individually, and assess the plastic responses of species' traits.

SLA has been well documented to respond to light availability (Meziane and Shipley, 1999, Valladares et al., 2000a, Valladares et al., 2000b, Meziane and Shipley, 2001, Rozendaal et al., 2006, Lusk et al., 2008), and leaf traits also respond to nutrient availability (Meziane and Shipley, 1999, Knops and Reinhart, 2000, Navas and Garnier, 2002). SRL has been demonstrated to respond to nutrient availability in some studies (Clemensson-Lindell and Asp, 1995, Hill et al., 2006, Kalliokoski et al., 2010), but not in others (Boot and Mensink, 1990, Cromer and Jarvis, 1990, Borken et al., 2007, Kalliokoski et al., 2010). Root-to-shoot ratio has been widely shown to respond to both light and nutrient limitation, as plants increase their relative biomass allocation to the organ responsible for up-taking the limiting resource, forming a functional equilibrium and effectively co-limiting resources (Brouwer, 1962, Aung, 1974, Chapin III, 1980, McGraw and Chapin, 1989, Cromer and Jarvis, 1990, Garnier, 1991, Schippers and Olff, 2000, Müller et al., 2000, Dyer et al., 2001, Hill et al., 2006, Louw-Gaume et al., 2010, Grassein et al., 2010). Recent research by Freschet et al. (2015) shows that plants typically respond to light limitation by adjusting morphological traits such as SLA, but that responses to nutrient limitation are typically in the form of increased root biomass allocation, rather than modification to SRL.

Understanding and quantifying species' responses to limiting resources has applications for cross-study trait comparisons or pooling data, as it reveals which traits are relatively constant regardless of environmental factors, and which traits can be strongly affected by differences between experimental conditions.

1.1 Chapter overviews

Chapter One:

This chapter introduces the theoretical concepts around which the subsequent chapters are based, including root function, environmental filtering, plant functional traits, plant strategy, and phenotypic plasticity.

Chapter Two: A multi-trait test of the whole-plant economics spectrum

This chapter introduces further literature relevant to root traits and their relationships to plant foraging strategy. The objectives of this chapter involve investigating the relationships between seedling and juvenile traits of the same species, the dimensions of trait variation present across species, and how community weighted mean traits vary along a strong soil fertility gradient. I measured root, leaf, and stem traits measured on seedlings of 66 New Zealand native tree species, and combined this with data from the literature on adult traits and relative abundances along a measured soil fertility gradient at Puketī forest. I used linear regressions to compare seedling and adult traits, PCA and ordination to assess the dimensions of trait variation across species, and linear regressions of community-weighted mean (CWM) traits vs. the principal component of soil fertility attributes.

My results showed that seedling morphology traits are generally well correlated with adults, but tend to be oriented towards a more "acquisitive" growth strategy, suggesting that species may down-regulate their growth as they develop into adults. PCA results showed that dry matter content was positively correlated across leaves, roots, and stems, and negatively associated with root nitrogen concentration and relative growth rate, suggesting that at least as seedlings, traits associated with a fast or slow growth strategy are co-ordinated across leaves, roots, and stems. Root diameter and SRL were independent from this first axis of fast-slow growth, suggesting that SRL does not have a direct effect on seedling growth rates and nutrient foraging. A third axis of variation was also identified, influenced strongly by root phosphorus and nitrogen concentrations, but this axis was difficult to interpret in the context of plant strategy. Regression analysis of CWM traits vs. soil fertility showed that the traits that made up the first "economic" PCA axis were strongly related to the soil fertility gradient, particularly stem dry matter content. Neither root diameter nor SRL were

significantly related to soil fertility, supporting the conclusion that SRL is not adaptive to nutrient foraging ability in these species.

Chapter Three: The effects of soil fertility on intraspecific trait plasticity

This chapter introduces further literature relevant to phenotypic plasticity of plant organs and functional traits in response to nutrient availability. The objectives of this chapter were to investigate whether the availability of nutrients in a potting medium affects trait expression in tree species, and similarly whether the method of fertiliser application affects trait expression. I grew four species representative of native New Zealand trees, under three nutrient availability treatments: low, monthly pulse, and slow release. My results showed that root-to-shoot ratio, relative growth rate, and root nutrient concentrations were the most affected traits, and that morphological traits were not strongly affected by nutrient availability. Slow release and pulse treatments were typically similar, and both were significantly different to low nutrient treatment. The few differences I observed between slow release and pulse were possibly due to differences in total nutrient availability, rather than mode of application. Together these results suggest that it is practical to compare or pool morphological trait data between and among studies, so long as plants were raised in environments conducive to growth. However, care should be taken when comparing organ nutrient concentrations, root-to-shoot ratios, or relative growth rates, as these can be strongly influenced by soil fertility.

Chapter Four: Synthesis

This chapter summarises the main observations of chapters two and three, suggests applications for these findings, and recommends further research to build upon and strengthen these conclusions.

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Chapter Two: A multi-trait test of the whole-plant economics spectrum using fine root, leaf, and stem traits and community-level trait responses to soil fertility

2.1 Abstract

The worldwide leaf economic spectrum has proved to be useful in mechanistically linking community assembly along environmental gradients to the physical properties of individual species. Species with conservative traits are typically associated with low productivity environments, and acquisitive species are typically associated with high productivity sites. It has been hypothesised that root traits may also form an economic spectrum, where the ability to rapidly exploit soil nutrients trades off with defensive ability and root longevity. Previous research has suggested that above and below ground economic strategies may be co-ordinated, but results are inconclusive. As roots are responsible for nutrient and water uptake from the soil, traits relating to a root economic spectrum may be particularly evident along soil fertility gradients. Measuring root traits on glasshouse raised seedlings allows environmental variables to be controlled, and provides an easier method of removing and washing roots for analysis. This chapter has the following three objectives:

(1)   Assess how closely species' seedling traits correlate with adult traits.

(2)   Determine the multivariate relationships within and between the root, leaf, and stem organs of New Zealand tree species.

(3)   Determine how community-level root traits vary along a strong soil fertility gradient.

In this study I measured 14 fine root, stem, and leaf traits on individual seedlings of 66 native New Zealand forest trees grown under controlled conditions. Trait values of seedlings measured in this study were significantly and positively correlated with trait values of adults taken from the literature. However, SLA and SRL were consistently lower in adult individuals, reflecting a down-regulation of growth as trees mature.

Using principal component analysis (PCA), three principle axes of variation were identified. The first axis represented a whole-plant economic spectrum, with high

relative growth rates associated with high specific leaf area (SLA), low tissue densities and low dry matter content in roots, leaves, and stems. This finding supports recent suggestions of a co-ordinated "slow-fast" strategy at the whole plant level. The second axis represented a spectrum of fine root diameter which was inversely related to specific root length (SRL), indicating that fine root diameter and SRL were unrelated to the whole plant "slow-fast" continuum. The third axis was complex, and was strongly influenced by root phosphorus and nitrogen concentration, with lesser influences from SRL, stem tissue density, and root porosity.

Community-weighted mean trait values relating to the whole plant "slow-fast" continuum correlated strongly with soil fertility, particularly SLA, relative growth rate, and leaf, root, and stem dry matter content. This indicates that soil fertility filters for economic traits of leaves, roots, and stems simultaneously. Community-weighted mean SRL showed no significant relationship to soil fertility, contrasting with previous findings. My results suggest that SRL is not directly associated with species' ability to exploit or tolerate high or low nutrient availability. Other traits including root porosity, root branching intensity, and mycorrhizal association may be more adaptive to soil fertility and warrant more detailed investigation.

2.2 Introduction

Understanding the patterns and processes which govern species' distributions has been a long-standing goal of ecology, and the importance of this science increases alongside humanity's impacts to Earth's biological and physiochemical systems. Accurate predictions of how species' distributions could change under future climate scenarios may allow pre-empting of such changes, with the aim of minimising extinctions and losses of biodiversity in natural ecosystems. To understand how species distributions may respond to changes in environmental variables, we must first understand the factors which have resulted in the current distribution of species.

Functional trait-based ecology is a theoretical framework through which ecologists can understand how the characteristics of individuals and species relate to their biological fitness in a given environment. Traits are heritable characteristics of individuals and species (Darwin, 1859), and functional traits are those which indirectly impact an individual's fitness through their effects on establishment, survival, growth, and reproductive success (Violle et al., 2007, Reich et al., 2003). For ease of discussion, individual functional traits and the values measured on individuals or across species will both be hereafter referred to as simply "traits". By comparing and contrasting species' traits and distributions across environmental gradients, plant ecologists can construct mechanistic hypotheses to link species' physical properties to how they are affected by the environment, how they in turn influence the environment, and how species' traits ultimately result in observed species distributions (Diaz et al., 1998, Lavorel and Garnier, 2002, Ordoñez et al., 2009, Shipley et al., 2006, Laughlin et al., 2012). As natural selection acts on the whole organism rather than individual traits, ecologists often consider how species' trait combinations are adaptive to the environment. Collections of an individual's traits can be thought of as a "strategy", which Craine (2009) defines as "a set of interlinked adaptations that arose as a consequence of natural selection and that promotes growth and successful reproduction in a given environment". Grime (1979) outlined three major plant strategies which have evolved in response to varying degrees of environmental stress and disturbance: ruderal species are adapted to frequently disturbed environments, are typically small, short-lived, and prioritise reproductive output over vegetative growth; stress-tolerating species are adapted

to high stress / low productivity conditions, and typically have a conservative growth strategy characterised by slow growth rates, high density tissues, and low specific leaf area (SLA); and competitive species, which thrive in conditions of low stress and disturbance, tend to have an acquisitive growth strategy characterised by lower density tissues, fast growth rates, and high SLA.

Some dimensions of plant strategy can be viewed as "economic spectrums", where photosynthetic carbon is 'invested' in new tissue, with the goal of maximising future 'returns', usually in the form of more photosynthetic carbon or soil nutrients, thereby increasing their fitness in a given environment. However, traits reflective of strategic dimensions tend to be mutually exclusive, and thus aligning to a particular strategy typically precludes the benefits of a different strategy (forming a "trade-off"). For instance, constructing thin, low density leaves allows rapid returns on carbon investment, but precludes long leaf lifespans and investment in defensive chemicals or structures. Tree species tend to be relatively long lived, and thus are not often characterised as having true "ruderal" strategies, instead the main trade-off observed in trees is between stress-tolerating (conservative growth) and competitiveness (acquisitive growth). The "world-wide leaf economic spectrum" (Wright et al., 2004) is the best documented example of this trade-off, in which leaf-lifespan is traded-off against SLA and thus rate of return on carbon investment. Acquisitive species tend to have high SLA leaves, which require relatively less carbon per unit of photosynthetic area, but as a consequence are more vulnerable to herbivory, frost, and mechanical damage, and therefore have a shorter average lifespan. Conversely, conservative species tend to have low SLA leaves, which are more "expensive" to produce, but will provide photosynthetic returns over a longer average lifespan. Evidence for the world-wide leaf economic spectrum has been documented at a range of scales, from within communities (Reich et al., 1991, Mediavilla et al., 2008, Laughlin et al., 2010) to across different biomes (Reich et al., 1999, Wright et al., 2004).

The leaf economic spectrum has been demonstrated to correlate with nutrient availability gradients (Vitousek et al., 1992, Wright et al., 2002, Ordoñez et al., 2009, Jager et al., 2015). Species associated with high fertility environments tend to have an acquisitive, competitive strategy, evidenced by fast growth, high SLA leaves, high leaf nitrogen concentration, and low leaf density; whereas species associated with low fertility environments tend to have the opposite traits. Stem

tissue density has also been linked to soil fertility, with species associated with low fertility soils typically having higher stem tissue densities (Muller-Landau, 2004, ter Steege et al., 2006, Gourlet-Fleury et al., 2011, Jager et al., 2015). These studies have demonstrated the capability of trait-based research to reveal underlying dimensions of plant strategy, and relate them to environmental gradients. Despite the increasing clarity of the role of leaf traits in above-ground strategies, the role of root traits in below-ground and whole plant strategy is not as well understood.

Research on root traits has lagged behind that of above-ground plant organs despite the multiple vital roles that roots perform in plant function, including absorption of both water and nutrients from the soil, physical anchoring, resource storage, and as the point of interface with symbiotic fungi and bacteria (Gregory, 2006). The scarcity of root research is because of the relative difficulty of sampling roots in the field compared to sampling above-ground organs. Extracting roots from the soil without damage is an arduous task, particularly in forests where the soil is often an interwoven matrix of roots, and where woody roots or rocky soils can hinder digging progress. Species identification of root samples can require tracing roots back to the parent tree, particularly for species which lack distinguishing characteristics such as a distinct smell or colour (Holdaway et al., 2011). In addition to the physical difficulties of root sampling, the amount of time between field sampling and laboratory trait analysis can be a logistical concern, particularly for traits which involve fresh weights or volumes. Sampling traits on glasshouse grown plants is a less time and resource consuming alternative to field sampling, and simultaneously offers controlled conditions with which to test hypotheses in the absence of confounding environmental variables. It has been suggested that traits of herbaceous plants raised in a glasshouse environment do not correlate particularly well with field trait data (Mokany and Ash, 2008). However, there is an absence of studies which have compared the traits of glasshouse raised seedlings to those of naturally occurring adult trees. Regardless of close correlations between seedling and adult traits, seedling traits have relevance to adult strategy and community assembly as all tree species must pass through the seedling phase of life, and therefore seedling traits must reflect a viable growth and survival strategy until transitioning to an adult reproductive phase.

Plant economic strategies may be co-ordinated between above and below ground organs. Multiple leaf traits are considered components of the leaf economic spectrum, but as they are aligned along a single axis of variation they can be largely summarised by SLA (Reich et al., 1999, Osnas et al., 2013). Specific root length (SRL) represents absorptive capability (root length) relative to carbon investment (root dry mass), and therefore may summarise root economic strategies, similar to SLA (Lambers et al., 1998, Eissenstat et al., 2000, Cornelissen et al., 2003b). Correlation between SLA and SRL has been investigated as an indicator of above and below ground strategy co-ordination, but results have been inconclusive. Some studies have found positive correlations between SLA and SRL (Withington et al., 2006, Laughlin et al., 2010, Laughlin et al., 2011, Fort et al., 2012), while other studies have found no correlations (Craine et al., 2005, Tjoelker et al., 2005, Markesteijn and Poorter, 2009, Chen et al., 2013). Correlations between leaf and root tissue density or dry matter content have been reported by several studies, (Wahl and Ryser, 2000, Mokany and Ash, 2008, Freschet et al., 2010, Pérez-Ramos et al., 2012, Craine et al., 2001) although these findings are not universal (Kembel and Cahill Jr, 2011). Leaf and root lifespan have also been observed to correlate with each other, as well as with other "economic" traits such as SLA and relative growth rate (Ryser, 1996, Kembel et al., 2008), although again results are not consistent (Withington et al., 2006). Correlations between root and leaf nitrogen and phosphorus concentrations have been observed by several studies (Craine et al., 2005, Kerkhoff et al., 2006, Freschet et al., 2010, Liu et al., 2010, Holdaway et al., 2011). These comparative studies of leaf and root traits suggest that there is some co-ordination of whole-plant economic strategies across leaf and root organs, although evidence is mixed for co-ordination of SLA and SRL.

Regardless of correlations with SLA, SRL may have an economic role in whole plant strategies, facilitating growth by increasing nutrient uptake relative to carbon investment. Studies have observed positive correlations between SRL and relative growth rates (Eissenstat, 1991, Reich et al., 1998, Wright and Westoby, 1999, Comas et al., 2002, Comas and Eissenstat, 2004), and negative correlations between SRL and root lifespan (McCormack et al., 2012). As mentioned earlier, SLA tends to be lower on low fertility sites, facilitating a more conservative growth strategy. However, the relationship between SRL and soil fertility does not

appear to be universal or linear. Studies have found SRL to be highest on low fertility soils, (Fort et al., 2012), high on both fertile and infertile sites but lower on intermediate sites (Holdaway et al., 2011), or not directly correlated with soil fertility (Espeleta and Donovan, 2002). The lack of consistent evidence of a direct adaptive function of SRL in root economics may be due to phylogenetic constraints. SRL is a composite metric of both root tissue density and average fine root diameter, and a number of studies have identified fine root diameter as a highly phylogenetically conserved trait (Pregitzer et al., 2002, Comas and Eissenstat, 2004, Withington et al., 2006, Comas and Eissenstat, 2009, Kembel and Cahill Jr, 2011, Chen et al., 2013, Valverde-Barrantes et al., 2014). It is possible that any adaptive function of SRL may be both determined and limited by alterations to root tissue density, rather than fine root diameter. Also, numerous other root traits may have equal or greater influence on nutrient and water uptake compared to SRL, including root branching intensity, root to shoot ratio, total root biomass, root distribution throughout the soil profile, ability to proliferate into nutrient rich patches, root turnover, and type and extent of mycorrhizal symbiosis (Kembel et al., 2008, Hodge, 2009, Laughlin et al., 2010, Comas et al., 2014). Therefore, it appears that SRL alone cannot be a reliable predictor of root economic strategies.

The overall aim of this study is to test whether a woody flora conforms to the recently proposed whole-plant 'fast-slow' economics spectrum (Reich, 2014) and to test how community-level fine root traits respond to a strong gradient in soil fertility. This chapter has the following three objectives: (1) Assess how closely species' seedling traits correlate with adult traits, (2) determine the multivariate relationships within and between the root, leaf, and stem organs of New Zealand tree species, and (3) determine how community-level root traits vary along a strong soil fertility gradient. These objectives are accomplished by sampling root, leaf, and stem traits from 66 species of angiosperms, conifers, and tree ferns native to New Zealand, combined with data from the literature of species' adult traits and species' relative abundance along a strong soil fertility gradient. I hypothesise that seedling and adult traits will be strongly correlated, due to genotypic constraints on trait expression. I also hypothesise that SRL will be independent of leaf economic traits, due to the different environments and resources these acquisitive organs are adapted to.

2.3 Methods

Species selection

Species were selected from the New Zealand arborescent flora based on their relative abundance and frequency across the country. Based on an analysis of 990 20×20 m plots located on an 8 km grid across all New Zealand islands, i.e., the LUCAS plots (Wiser et al., 2011), species were selected according to the following criteria: (i) the species must attain a diameter at breast height (dbh) of at least 10 cm, (ii) the species must contribute at least 10% to the total basal area of at least one plot, and (iii) the species occurs on at least 1% (9 of 990 plots) of all the plots. These criteria resulted in a list of 68 species, however, no nurseries could be found which stock in seedling size for ten of these species, including tree ferns and montane trees, which are difficult to propagate and have low commercial demand. Eight additional species were also selected due to their common use in restoration planting projects in the Waikato district. In total, trait data were collected from 590 individual seedlings from 66 species in this study.

Sourcing and growing seedlings

Seedlings were sourced from four nurseries across New Zealand: Forest Flora (Ngaruawahia, Waikato), Taupo Native Plant Nursery (Taupo, Waikato), Oratia Native Plant Nursery (Oratia, Auckland), and Pukerau Nursery (Gore, Southland). Whenever possible, plants were ordered at tube stock (~150 mL pot volume) or root trainer (~350 mL) size classes, in order to reduce transplant shock and expedite new growth. For species where smaller plants were unavailable, the largest plants ordered were PB8 size (~4.8 L). Sampled species, number of replicates, and specific abbreviations are presented in Table 2.

Seedlings which were in tube stock or root trainer size were re-potted into 1.5 L pots, and seedlings which were in PB3 (~1.8 L) or larger size bags were re-potted into 9 L pots. The potting medium used was a custom blend made by Daltons (Matamata, New Zealand) consisting of a 5:1 mixture ratio of Daltons potting mix and propagation sand (Appendix Table 12). This mixture has been proven to be suitable for a wide variety of native species. The potting mix contained slow release fertiliser capsules which would ensure growth was not limited by low fertility, and the sand created a more freely draining soil and made washing of roots easier. Once per month, species were rearranged across different tables

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within the glasshouse, in order to minimize the effects of any potential microclimatic gradients within the glasshouse. Plants were hand watered regularly in order to avoid drought stress and facilitate growth. The average daily temperature in the glasshouses was 15.5°C, and the average relative humidity was 76.8%.

Trait measurements

Initial height measurements were taken two weeks after re-potting, to allow plants to re-establish fine roots and root hairs in their new pots. Height was measured from the soil surface to the tallest apical bud using a measuring tape. Height measurements were taken fortnightly from December 2013 to May 2014. Measurements from June 2014 to September 2014 were taken every four weeks, due to slower growth rates over winter. Once a seedling had grown sufficiently for fresh fine roots to be present, a final height measurement was recorded. Relative growth rate (RGR) of individual seedlings was calculated as RGR = ((final height – initial height)/initial height)/days of growth.

Following final height measurements, roots were washed by gently massaging the root balls under water. Mesh sieves were not used for this washing process, as the consistency of the potting medium made root washing easy for most species, and damage to terminal root structure could generally be avoided. Trait measurements for leaves, roots, and stems were done according to protocols published by Cornelissen et al. (2003b) and Pérez-Harguindeguy et al. (2013).

Secateurs were used to cut a short section (approximately 1 – 3 cm) of stem containing mature xylem. Tree ferns and palms did not have true wood tissue, so a section of stipe was removed from tree ferns, and a section of the herbaceous stem of the palm were sampled. Bark from woody species was removed by peeling or scraping. The remaining stem section was rolled in paper towels to remove any residual surface moisture or sap before recording its fresh mass. A Mettler-Toledo MS304S balance (Greifnesee, Switzerland) was used for all measurements of mass. The length and orthogonal diameter dimensions of the stem section were measured using digital Vernier callipers, and the approximate fresh volume was calculated using the standard equation for volume of a cylinder. The stem sections were then dried to constant mass at 60°C for at least 48 hours prior to measuring dry mass. Stem dry matter content (SDMC) was calculated as SDMC = stem dry

mass / stem fresh mass, and stem tissue density (Stem TD) was calculated as Stem TD = stem dry mass / fresh stem volume (Table 1).

Between three to thirty leaves were sampled from each plant, depending on the size per individual leaf. Sufficient numbers of leaves were sampled to total at least 4 cm^2 leaf area per individual. In species with large compound leaves, three leaflets were measured. Phylloclades (i.e. photosynthetic structures on Phyllocladaceae) were treated as operational photosynthetic units and are hereafter referred to as 'leaves'. After cleaning and drying with paper towels, digital Vernier callipers were used to take thickness measurements of three separate leaves, avoiding any prominent leaf veins. Leaf area was measured on a LI-COR Biosciences LI-3100C (Lincoln, NE USA) leaf area meter. Fresh leaf mass was measured on tared scales, and leaves were then dried to constant mass at 60°C for at least 48 hours prior to obtaining dry mass. SLA was calculated as SLA = leaf area / leaf dry mass, leaf dry matter content (LDMC) was calculated as LDMC = leaf dry mass / leaf fresh mass, and leaf tissue density (Leaf TD) was calculated as Leaf TD = leaf dry mass / fresh leaf volume (Table 1).

Subsections of fine roots were removed from the main root ball, and placed into a plastic, water-filled tray for further washing. Once the sections were sufficiently clean, they were transferred into a clear acrylic tray on a flat-bed scanner. Two fine paintbrushes were used to spread the roots out to minimise overlap. Total root length, average root diameter, number of tips, and root volume were calculated using WinRhizo Pro software (Version 2012b, Regent Instruments Inc., Quebec City, Canada) and an Epson Expression 10000XL scanner (Tokyo, Japan). Fresh root mass of each sample was obtained after removing the surface water with paper towels. The root sections were then dried to constant mass at 60°C for at least 48 hours prior to obtaining dry mass. SRL was calculated as SRL = root length / root dry mass, root dry matter content (RDMC) was calculated as RDMC = root dry mass / root fresh mass, root tissue density (Root TD) was calculated as Root TD = root dry mass / fresh root volume, and root branching intensity (RBI was calculated as RBI = number of root tips / root length (Table 1).

To measure root porosity, I used the microbalance method as described by Visser and Bögemann (2003). Ten short sections of fresh root were cut from the root ball using a sharp razor blade. The sections were typically between 5 to 10 mm in length, and were cut at least 10 mm back from the root tip. All sections were taken

only from healthy-looking parts of the root balls, with preference for fresh growth when sufficient suitable root material was available. Roots which appeared to have undergone secondary thickening were not sampled. Whenever possible, sections were taken from first order terminal roots. In species where the terminal roots were too small for this technique, sections were cut from second order roots where no secondary thickening could be visually identified. As each suitable section was identified and cut, they were gently washed with fine paintbrushes to remove any remaining surface dirt, and kept under water to minimise water loss while the remaining sections were cut. Once all 10 sections had been cut, they were transferred using fine paintbrushes onto a piece of dry tissue paper. The paintbrushes were used to gently roll the root sections on the tissue paper, removing any surface moisture. The tissue paper was then folded over to enclose the root sections, and gentle pressure was applied by hand over the root sections. Care was taken to avoid crushing or bursting the cells in the root tissue, effectively forcing out water from the root sections' internal porous spaces.

The root sections were transferred to a weigh boat on a tared balance and weighed. This initial mass was recorded, and the root sections were transferred into a small glass vial which was then filled with water. The cap for this vial was secured loosely to avoid creating a pressure seal, yet tight enough to prevent the lid coming off during rapid pressure changes. The vial was then placed into a near vacuum chamber, which was run for three repetitions of five minutes each. At the end of each five minute period, the vacuum chamber was opened, causing a rapid re-pressurisation of the vial and forcing water into the aerenchyma of the root sections. After low pressure exposure periods were completed, the vial was removed and opened. The root sections were then gently removed from the vial using a fine paintbrush, placed on dry tissue paper, and briefly rolled to remove surface moisture. The root sections were then transferred onto a weigh boat on a tared balance, and had their final mass recorded. The method differed from that presented by Visser and Bögemann (2003) in that it did not use two-piece hard gelatin capsules to hold the root sections between blotting and weighing. This is due to the observation that if the surface moisture was properly blotted, the subsequent mass change from water evaporation was slow and could be negated by minimising the time between blotting and weighing. Root porosity was calculated as (final root mass – initial root mass) / final root mass * 100 (Table 1)

Analysis of root N and P concentration

The remaining root ball from each plant was dried to constant mass at 60°C for at least 48 hours. Individually, the dried root balls were ground to a fine powder using a Retsch MM 2000 grinder (Haan, Germany). Three individual root balls per species were analysed. Most individual replicates had sufficient mass after grinding for chemical analysis (approximately 1 g dry powder mass); however for species where the mass of ground roots was too low per individual, powder from multiple individuals was pooled before analysis. As the data from individual replicates was averaged after analysis, it was assumed that pooling before analysis would not affect the results significantly. Root nitrogen and phosphorus concentration of ground roots was obtained using flow injection analysis on a Lachat QuikChem 8000 series (test number and protocol 206 Landcare Research Environmental Chemistry Laboratory, Palmerston North, New Zealand).

Soil fertility gradient

To investigate the relationships between root functional traits and soil fertility, I used a soil fertility gradient documented at Puketī forest that was recently shown to be correlated with multiple independent functional traits (Jager et al. 2015).

Puketī forest is a warm temperate rain forest in Northland, New Zealand ($35^o\ 13´$ S, $173^o\ 42´$ E), which contains mainly old growth forest (Powlesland, 1987, Best and Bellingham, 1991, Dowding and Murphy, 1994). Mean annual precipitation can be up to 2000 mm, and mean annual temperature is 14 °C. The soils are mostly gravelly silt and clay loams which developed from highly metamorphosed Late Paleozoic argillites, cherts, greywackes, and volcanics (Black, 1994). The dominant driver of compositional turnover at Puketī is topographically-mediated soil fertility, rather than climatic conditions (Jager et al., 2015). The study area features highly dissected topography, with permanent water courses separated by narrow ridges. Distances as small as 150 m between ridge crests and gulley floors can contain large differences in soil properties, such as soil total phosphorus (P) (range = 160–833 mg kg^{-1}) and soil pH (range = 3.6–6.3). Thus, Puketī forest is a model system for testing relationships between soil properties and fine root functional traits.

Community composition was determined using 40 permanent 400 m^2 plots (20 m × 20 m) (Hurst and Allen, 2007) spanning a broad gradient in soil and topographic

variation, with plots located between 70 and 500 m above sea level. Trees within each plot were identified to species, and diameters were measured on all individual stems > 2.5 cm diameter at breast height (dbh; 135 cm). Four soil samples per plot were collected to a depth of 15 cm using a 3 cm diameter corer. Surface litter with botanically recognisable structures (the Oi and Oe layers) was scraped away before taking the cores. Organic and mineral horizons visible within the core were not separated. The four soil samples per plot were pooled for analysis. New Zealand trees are typically very shallow-rooted and have abundant surface roots (Wardle, 1991), thus most nutrients are likely to be acquired from surface litter. Soil samples to 15 cm depth do not comprehensively assess the total inventory of soil nutrients at greater depths, but surface concentrations are indicative of entire soil profile nutrient availability (Stevens, 1968).

An assortment of soil properties were analysed at the Landcare Research Plant and Soil Laboratory in Palmerston North, New Zealand. Soil pH was measured in solution with a 1:2.5 soil-to-distilled water ratio using a Radiometer PHM210 pH meter equipped with a Radiometer pHC2401-8 electrode. Cation exchange capacity (CEC) was measured by pH drop (Brown, 1943), and base saturation was measured as the amount of basic cations that occupied the cation exchange sites divided by the total cation exchange capacity (Bases $\div$ CEC $\times$ 100 = base saturation). Soil total nitrogen (N) and organic carbon (C) were measured using a Leco CNS 2000 Analyser which utilises the Dumas dry combustion principle (Metson et al., 1979). Total phosphorus (P) and organic P were determined using flow injection analysis on a Lachat QuikChem 8000 following ignition at 550 °C for 60 minutes and extraction with 0.5M H_2SO_4 at a soil-to-extractant ratio of 1:200 for 16 hours (Blakemore et al., 1987). Soluble P (i.e. the amount of inorganic P present in soil but not strongly occluded) was measured using flow injection analysis on a Lachat QuikChem 8000. Total K, Ca, Na, Mg were determined using atomic absorption spectroscopy using a Varian SpectrAA-220FS following leaching with molar ammonium acetate buffered to pH 7.

Following Jager et al. (2015), rather than selecting a single variable to reflect soil fertility, I used principal components analysis (PCA) to reduce three highly correlated soil properties (soil pH, soil C:N ratio, and soil total P) down to a single dimension (hereafter, 'Puketī soil PC1') for use as a predictor in the regression analyses. These three soil variables were chosen because pH reflects the

availability of multiple nutrients, soil C:N ratio is widely regarded as a measure of relative N availability, total P is strongly correlated with both soluble P ($r = 0.86$) and organic P ($r = 0.99$) in this system, and these variables are widely used to assess relationships among vegetation structure and soil properties (Ordonez et al. 2009).

Statistical analyses

First, linear regressions using R software were fit to determine the relationships between average seedling and adult trait values. No transformations were applied to the traits for these regression analyses. The following traits were compared between the seedling and adult life stages: SLA, leaf TD, SDMC, stem TD, SRL, root diameter, RBI, root N, and root P. Data for leaf and stem traits of adult tree species were extracted from Jager et al. (2015) and a national trait database that is currently under preparation (D.C. Laughlin, University of Waikato and S.J. Richardson, Landcare Research, Appendix Table 9). Data for root traits of adult tree species were extracted from Holdaway et al. (2011) (Appendix Table 10).

Second, average values were calculated for each trait per species (leaf and stem traits, Table 2; root traits, Table 3). The distribution and skew of each trait was assessed using STATISTICA 12 (Statsoft Inc.). Prior to PCA and ordination, the following trait data were $\log_{10}$ transformed to reduce skew: SRL, root diameter, RDMC, root porosity, RBI, SLA, leaf TD, and RGR.

Given that many functional traits are correlated, it is important to determine which traits represent independent axes of functional specialisation (Grime et al., 1997, Laughlin, 2014b). This PCA was focussed on root traits, and thus included only one leaf and one stem trait. SLA was excluded as it was positively correlated with both leaf dry matter content and tissue density, and LDMC has been shown to be a better predictor of leaf resource acquisition strategy (Wilson et al., 1999). Root, leaf and stem dry matter contents were used over tissue densities, as there were stronger positive correlations between seedling and adult dry matter contents (Figure 2), and root, leaf and stem tissue densities were strongly positively correlated with their respective dry matter contents (Table 4). Furthermore, RDMC has been demonstrated to be a better predictor of root TD as determined by Archimedes principle (i.e. displacement), when compared to root volume as determined by imaging software (Birouste et al., 2014). I used STATISTICA 12

to perform principal components analysis (PCA) and Kaiser's rule (i.e., the number of dimensions with eigenvalues > 1) to determine the intrinsic dimensionality of the plant trait matrix. This analysis of the correlation matrix determines the number of independent axes of functional variation among the 66 tree species in the study. PC-ORD 6 (MjM Software Design) was used to visualise the PCA as a two dimensional ordination, with species grouped by phylogeny. As PCA and ordination require a complete data matrix, missing trait values for porosity, root nitrogen concentration, and root phosphorus concentration for *Cyathea smithii, Elaeocarpus dentatus, Kunzea ericoides, Pittosporum tenuifolium*, and *Podocarpus totara* were assigned the average value across all species for each trait, as this would allow the ordination to operate while also minimising the effects of the missing trait values.

Third, I used the trait data for the 30 species which are present at Puketī forest. Using basal area data from Jager et al. (2015), community-weighted mean (CWM) traits in each plot were calculated as $\sum_{i=1}^{S} t_i\, p_i$, where t_i is the mean trait of species i across all plots, and p_i is the relative basal area of species i, and S is the number of species in the plot. Basal area ($m^2\ ha^{-1}$) was more appropriate than stem density (trees ha^{-1}) as a measure of plant abundance in these forests where a few large individuals could comprise the majority of the stand biomass.

Following Jager et al. (2015), rather than selecting a single variable to reflect soil fertility, PCA was used to reduce three highly correlated soil properties (soil pH, soil C:N ratio, and soil total P) down to a single dimension (hereafter, 'Puketī soil PC1') for use as a predictor in the regression analyses. These three soil variables were chosen because pH reflects the availability of multiple nutrients, soil C:N ratio is widely regarded as a measure of relative N availability, total P is strongly correlated with both soluble P ($r = 0.86$) and organic P ($r = 0.99$) in this system, and these variables are widely used to assess relationships among vegetation structure and soil properties (Ordoñez et al., 2009). Linear and second-order polynomial regression models were used to determine the relationships between CWM functional traits and the first principal component representing soil fertility (Puketī soil PC1).

Mycorrhizal associations

Species' mycorrhizal associations were determined from the literature (McNabb, 1958, McNabb, 1961, Baylis, 1969, Baylis, 1971, Johnson, 1973, Baylis, 1975, Cooper, 1975, Hall, 1975, Cooper, 1976, Baylis, 1980, St John, 1980, Russell et al., 2002, Dickie et al., 2012), and are presented in Appendix Figure 14.

Table 1. Functional trait abbreviations and units.

Trait	Abbreviation	Units
Specific root length	SRL	$m\,g^{-1}$
Root diameter	-	mm
Root tissue density	Root TD	$mg\,mm^{-3}$
Root dry matter content	RDMC	$mg\,g^{-1}$
Root porosity	-	%
Root branching intensity	RBI	cm^{-1}
Root nitrogen conc.	Root N	%
Root phosphorus conc.	Root P	%
Specific leaf area	SLA	$mm^2\,mg^{-1}$
Leaf tissue density	Leaf TD	$mg\,mm^{-3}$
Leaf dry matter content	LDMC	$mg\,g^{-1}$
Stem tissue density	Stem TD	$mg\,mm^{-3}$
Stem dry matter content	SDMC	$mg\,g^{-1}$
Relative height growth rate	RGR	$cm\,cm^{-1}\,day^{-1}$

Table 2. Average leaf traits, stem traits, and relative growth rate values for species measured on seedlings grown in standard conditions

Species	Family	Code	Number sampled	SLA ($mm^2 mg^{-1}$)	LTD ($mg\ mm^{-3}$)	LDMC ($mg\ g^{-1}$)	STD ($mg\ mm^{-3}$)	SDMC ($mg\ mm^{-1}$)	RGR ($cm\ cm^{-1}\ day^{-1}$)
Conifers									
Agathis australis	Araucariaceae	AGAAUS	14	9.1	0.21	311	0.59	544	0.0018
Dacrydium cupressinum	Podocarpaceae	DACCUP	13	3.3	0.75	326	0.59	461	0.0037
Dacrycarpus dacrydioides	Podocarpaceae	DACDAC	10	13.6	0.25	341	0.41	459	0.0045
Halocarpus biformis	Podocarpaceae	HALBIF	5	4.9	0.3	377	0.77	620	0.0003
Halocarpus kirkii	Podocarpaceae	HALKIR	5	7.6	0.28	310	0.69	587	0.001
Phyllocladus alpinus	Podocarpaceae	PHYALP	6	5	0.36	449	0.76	627	0.0002
Phyllocladus trichomanoides	Podocarpaceae	PHYTRI	10	9.4	0.25	347	0.74	539	0.005
Podocarpus cunninghamii	Podocarpaceae	PODCUN	10	4.9	0.26	380	0.56	478	0.0009
Podocarpus totara	Podocarpaceae	PODTOT	6	8.6	*	378	0.61	488	0.0013
Prumnopitys ferruginea	Podocarpaceae	PRUFER	14	12.7	0.26	298	0.7	543	0.0045
Prumnopitys taxifolia	Podocarpaceae	PRUTAX	6	12.1	0.26	376	0.67	586	0.0063
Eudicot angiosperms									
Ackama rosaefolia	Cunoniaceae	ACKROS	12	22.6	0.18	290	0.48	413	0.0127
Alectryon excelsus	Sapindaceae	ALEEXC	7	17	0.26	409	0.49	414	0.0036
Aristotelia serrata	Elaeocarpaceae	ARISER	6	35.7	0.12	277	0.36	366	0.0155
Carpodetus serratus	Rousseaceae	CARSER	6	19	0.2	329	0.52	445	0.0193
Coprosma linariifolia	Rubiaceae	COPLIN	6	10	0.26	378	0.64	537	0.0008
Corynocarpus laevigatus	Corynocarpaceae	CORLAE	9	13.3	0.2	209	0.43	358	0.0089
Dracophyllum longifolium	Ericaceae	DRALON	5	5.8	0.49	440	0.49	499	0.001
Dysoxylum spectabile	Meliaceae	DYSSPE	17	19.9	0.21	248	0.42	360	0.004
Elaeocarpus dentatus	Elaeocarpaceae	ELADEN	12	11.2	*	397	0.53	426	0.0039
Elaeocarpus hookerianus	Elaeocarpaceae	ELAHOO	5	15.7	0.28	397	0.42	438	0.0037
Fuchsia excorticata	Onagraceae	FUCEXC	6	28.1	0.12	174	0.4	352	0.0199
Fuscospora cliffortioides	Nothofagaceae	FUSCLI	9	14	0.3	407	0.59	499	0.0093
Fuscospora fusca	Nothofagaceae	FUSFUS	6	13.6	0.29	417	0.56	488	0.0003
Fuscospora solandri	Nothofagaceae	FUSSOL	9	13.4	0.29	434	0.53	462	0.007
Fuscospora truncata	Nothofagaceae	FUSTRU	9	13.8	0.29	384	0.48	431	0.0071
Griselinia littoralis	Griseliniaceae	GRILIT	10	9.9	0.17	258	0.52	459	0.0083
Hoheria lyallii	Malvaceae	HOHLYA	6	33.8	0.1	190	0.57	493	0.0038
Hoheria sexstylosa	Malvaceae	HOHSEX	5	26.2	0.13	233	0.49	434	0.0024

Table 2. continued

Species	Family	Code	Number sampled	SLA (mm^2 mg^{-1})	LTD (mg mm^{-3})	LDMC (mg g^{-1})	STD (mg mm^{-3})	SDMC (mg mm^{-1})	RGR (cm cm^{-1} day^{-1})
Knightia excelsa	Proteaceae	KNIEXC	13	14.2	0.24	324	0.41	357	0.0064
Kunzea ericoides	Myrtaceae	KUNERI	5	20.4	*	336	0.58	467	0.0183
Leptospermum scoparium	Myrtaceae	LEPSCO	10	19.6	0.2	340	0.53	447	0.0115
Lophozonia menziesii	Nothofagaceae	LOPMEN	12	7.6	0.37	455	0.57	524	0.0007
Melicytus ramiflorus	Violaceae	MELRAM	16	28.9	0.15	224	0.37	307	0.0127
Metrosideros excelsa	Myrtaceae	METEXC	5	6.5	0.29	311	0.55	476	0.0022
Metrosideros robusta	Myrtaceae	METROB	13	9.6	0.29	305	0.55	467	0.0018
Metrosideros umbellata	Myrtaceae	METUMB	10	7.5	0.22	301	0.48	424	0.0021
Myrsine australis	Primulaceae	MYRAUS	6	13.9	0.28	357	0.49	468	0.0064
Myrsine divaricata	Primulaceae	MYRDIV	4	15.5	0.25	338	0.63	487	0.0016
Myrsine salicifolia	Primulaceae	MYRSAL	5	12.8	0.19	244	0.53	479	0.0184
Nestegis lanceolata	Oleaceae	NESLAN	11	10.7	0.26	375	0.6	544	0.0025
Olearia rani	Asteraceae	OLERAN	10	19.6	0.15	235	0.49	407	0.0364
Pennantia corymbosa	Pennantiaceae	PENCOR	6	19.8	0.17	290	0.51	438	0.0188
Pittosporum eugenioides	Pittosporaceae	PITEUG	8	17.6	0.26	313	0.42	377	0.0135
Pittosporum tenuifolium	Pittosporaceae	PITTEN	6	21.1	*	346	0.48	424	0.0075
Plagianthus regius	Malvaceae	PLAREG	5	28.6	0.14	241	0.55	483	0.0015
Pseudopanax arboreus	Araliaceae	PSEARB	6	11.8	0.21	317	0.5	420	0.0114
Pseudopanax crassifolius	Araliaceae	PSECRA	6	4.3	0.36	333	0.5	431	0.0029
Pseudowintera colorata	Winteraceae	PSECOL	7	12.1	0.23	296	0.56	475	0.0065
Quintinia serrata	Paracryphiaceae	QUISER	2	12.2	0.2	286	0.48	442	0.0023
Schefflera digitata	Araliaceae	SCHDIG	6	24.4	0.12	196	0.25	230	0.0258
Sophora microphylla	Fabaceae	SOPMIC	4	20.9	0.2	305	0.71	572	0.0016
Syzygium maire	Myrtaceae	SYZMAI	4	13.9	0.22	320	0.39	351	0.0005
Vitex lucens	Lamiaceae	VITLUC	17	25.3	0.15	237	0.39	333	0.0368
Weinmannia racemosa	Cunoniaceae	WEIRAC	11	11.3	0.27	343	0.46	416	0.0047
Weinmannia silvicola	Cunoniaceae	WEISIL	15	17	0.25	304	0.5	432	0.0016
<u>Magnoliid angiosperms</u>									
Beilschmiedia tarairi	Lauraceae	BEITAR	14	12.3	0.26	324	0.5	444	0.003
Beilschmiedia tawa	Lauraceae	BEITAW	16	12.5	0.4	420	0.54	479	0.002
Hedycarya arborea	Monimiaceae	HEDARB	10	20.6	0.14	206	0.38	322	0.013
Laurelia novae-zelandiae	Atherospermataceae	LAUNOV	13	18.7	0.16	236	0.41	384	0.006
Litsea calicaris	Lauraceae	LITCAL	13	17.7	0.18	300	0.48	403	0.0065

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Table 2. continued

Species	Family	Code	Number sampled	SLA (mm^2 mg^{-1})	LTD (mg mm^{-3})	LDMC (mg g^{-1})	STD (mg mm^{-3})	SDMC (mg mm^{-1})	RGR (cm cm^{-1} day^{-1})
Monocot angiosperm									
Rhopalostylis sapida	Arecaceae	RHOSAP	10	13.7	0.29	282	0.19	148	0.0107
Tree ferns									
Cyathea dealbata	Cyatheaceae	CYADEA	9	17.1	0.29	322	0.54	435	0.0025
Cyathea medullaris	Cyatheaceae	CYAMED	7	29.8	0.13	228	0.22	174	0.0048
Cyathea smithii	Cyatheaceae	CYASMI	5	17.8	*	383	0.32	278	0.0008
Dicksonia squarrosa	Dicksoniaceae	DICSQU	10	19.9	0.22	318	0.43	328	0.001

* Leaf tissue density data unavailable for some species.

Table 3. Average root trait values for sampled species measured on seedlings grown in standard conditions

Code	SRL (m g^{-1})	Diameter (mm)	Root TD (mg mm^{-3})	RDMC (mg g^{-1})	Porosity (%)	RBI (cm$^{\backslash 1}$)	Root N (%)	Root P (%)
Conifers								
AGAAUS	14	0.63	0.24	196	9.48	2.65	1.30	0.26
DACCUP	27.8	0.51	0.21	172	10.41	2.7	1.42	0.63
DACDAC	17.8	0.6	0.21	158	7.81	2.1	1.41	0.20
HALBIF	11	0.64	0.3	254	10.84	2.05	1.21	0.24
HALKIR	14.3	0.65	0.22	189	14.67	3.07	1.81	0.47
PHYALP	12.4	0.6	0.27	262	19.21	2.48	1.42	0.20
PHYTRI	16.5	0.76	0.15	153	13.55	2.76	1.45	0.29
PODCUN	8.4	0.9	0.19	176	19.01	2.35	1.79	0.25
PODTOT	11	0.88	0.15	129	*	1.32	*	*
PRUFER	6.7	0.93	0.23	205	8.08	3.34	1.13	0.44
PRUTAX	11	0.75	0.21	201	12.48	4.67	1.38	0.22
Eudicot angiosperms								
ACKROS	52	0.36	0.2	168	4.83	1.64	1.49	0.45
ALEEXC	44.7	0.43	0.17	109	5.21	2.08	2.17	0.51
ARISER	82.3	0.37	0.12	133	6.64	1.59	1.24	0.32
CARSER	30.8	0.53	0.15	109	6.98	1.61	1.47	0.45
COPLIN	22.3	0.56	0.2	141	6.8	1.9	1.39	0.20
CORLAE	23.8	0.59	0.16	128	8.16	1.11	3.42	0.38
DRALON	130.5	0.22	0.23	227	7.59	6.49	0.97	0.12
DYSSPE	7.1	0.99	0.19	140	4.32	1.42	2.06	0.17
ELADEN	38	0.52	0.15	125	*	1.38	*	*
ELAHOO	33.4	0.42	0.21	158	8.99	1.46	1.53	0.58
FUCEXC	84	0.34	0.13	113	8.91	3.04	1.36	0.42
FUSCLI	78	0.29	0.24	212	10.65	4.33	1.73	0.26
FUSFUS	55.3	0.31	0.27	183	13.4	3.79	1.76	0.26
FUSSOL	103.8	0.24	0.24	191	10.17	4.36	1.45	0.37
FUSTRU	65.8	0.28	0.3	171	3.44	3.94	1.10	0.40
GRILIT	6.7	1.25	0.13	96	8.42	1.76	3.02	0.29
HOHLYA	38	0.54	0.12	95	13.58	1.83	2.51	0.27
HOHSEX	23.9	0.78	0.09	75	15.56	1.33	2.54	0.37

Table 3. continued

Code	SRL (m g^{-1})	Diameter (mm)	Root TD (mg mm^{-3})	RDMC (mg g^{-1})	Porosity (%)	RBI (cm$^{\setminus 1}$)	Root N (%)	Root P (%)
KNIEXC	43.7	0.4	0.19	182	8.79	1.86	1.61	0.33
KUNERI	39.5	0.54	0.13	110	*	1.39	*	*
LEPSCO	97.1	0.37	0.13	120	10.08	1.97	1.65	0.34
LOPMEN	35.4	0.36	0.31	224	7.65	3.21	1.71	0.30
MELRAM	48.9	0.54	0.1	101	3.21	1.87	1.82	0.47
METEXC	24.3	0.49	0.24	133	11.45	2.81	1.15	0.13
METROB	47.6	0.43	0.16	123	7.64	2.55	1.24	0.16
METUMB	42.5	0.4	0.21	130	10.6	2.93	1.53	0.29
MYRAUS	40.3	0.41	0.2	173	8.05	1.1	1.22	0.55
MYRDIV	41.6	0.36	0.25	181	8.06	1.55	1.61	0.17
MYRSAL	29.3	0.48	0.2	172	8.8	1.47	1.20	0.51
NESLAN	11	0.75	0.22	194	10.34	2	1.46	0.50
OLERAN	59.6	0.47	0.11	93	9	1.23	2.24	0.64
PENCOR	34.4	0.5	0.15	120	5.72	1.91	2.06	0.32
PITEUG	49.5	0.42	0.15	135	6.63	1.02	1.39	0.26
PITTEN	51.2	0.43	0.14	134	*	1.42	*	*
PLAREG	58.3	0.44	0.12	94	13.8	1.87	2.32	0.55
PSEARB	17.9	0.78	0.13	111	8.37	1.93	1.26	0.62
PSECRA	10.7	0.93	0.15	119	3.74	1.72	2.85	0.77
PSECOL	28	0.63	0.12	113	10.62	1.59	1.39	0.49
QUISER	38	0.41	0.2	159	4.99	2.43	1.62	0.35
SCHDIG	44.6	0.63	0.07	68	3.3	2.43	2.04	0.53
SOPMIC	31.5	0.49	0.18	150	8.79	1.29	3.27	1.27
SYZMAI	79.3	0.31	0.18	148	17.22	1.8	1.61	0.20
VITLUC	35	0.56	0.13	108	5.42	1.61	2.25	0.25
WEIRAC	44.7	0.37	0.24	149	8.13	1.78	1.43	0.37
WEISIL	39.4	0.38	0.24	169	4.7	1.75	0.98	0.14
Magnoliid angiosperms								
BEITAR	8	0.91	0.21	158	5.23	1.34	1.44	0.26
BEITAW	9.1	0.74	0.27	200	6.75	1.6	1.76	0.21
HEDARB	11.7	1.07	0.1	83	3.46	1.63	2.95	0.38
LAUNOV	11.7	1.02	0.11	93	2.7	1.27	2.32	0.34
LITCAL	11.4	1.02	0.13	85	6.01	1.58	2.95	1.04

Table 3. continued

Code	SRL (m g^{-1})	Diameter mm	Root TD (mg mm^{-3})	RDMC (mg g^{-1})	Porosity (%)	RBI (cm$^{\backslash 1}$)	Root N (%)	Root P (%)
<u>Monocot angiosperm</u>								
RHOSAP	22.3	0.51	0.26	131	6.35	3.41	1.51	0.17
<u>Tree ferns</u>								
CYADEA	19.9	0.58	0.21	174	5.91	1.95	0.95	0.30
CYAMED	28.3	0.47	0.21	172	8.49	1.67	1.00	0.14
CYASMI	14.9	0.62	0.22	158	*	1.39	*	*
DICSQU	15.7	0.69	0.19	187	15.79	2.5	1.32	0.31

* Root porosity and nutrient chemistry data unavailable for some species.

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Table 4. Pearson's *r* correlation matrix derived from 14 traits measured on 66 species.

	SRL	Root Diam.	Root TD	RDMC	Root Poros.	RBI	Root N	Root P	SLA	Leaf TD	LDMC	Stem TD	SDMC
Root Diam.	-0.91 *												
Root TD	-0.07	-0.33 *											
RDMC	-0.07	-0.30 *	0.87 *										
Root Poros.	0.01	-0.12	0.20	0.34 *									
RBI	0.19	-0.34 *	0.46 *	0.43 *	0.23								
Root N	-0.19	0.39 *	-0.50 *	-0.58 *	-0.12	-0.37 *							
Root P	0.00	0.17	-0.40 *	-0.35 *	-0.13	-0.29 *	0.44 *						
SLA	0.28 *	-0.06	-0.50 *	-0.50 *	-0.36 *	-0.37 *	0.28 *	0.17					
Leaf TD	-0.08	-0.19	0.68 *	0.67 *	0.21	0.40 *	-0.43 *	-0.20	-0.80 *				
LDMC	0.04	-0.31 *	0.69 *	0.70 *	0.29 *	0.44 *	-0.38 *	-0.19	-0.57 *	0.77 *			
Stem TD	-0.28 *	0.11	0.35 *	0.46 *	0.44 *	0.22	-0.05	0.09	-0.50 *	0.40 *	0.47 *		
SDMC	-0.22	0.04	0.38 *	0.49 *	0.42 *	0.23	-0.09	0.05	-0.51 *	0.42 *	0.54 *	0.95 *	
RGR	0.19	0.05	-0.55 *	-0.54 *	-0.49 *	-0.25 *	0.19	0.34 *	0.52 *	-0.48 *	-0.53 *	-0.50 *	-0.52 *

Asterisks indicate significant correlations at p <0.05. Root Diam. = Root Diameter; Root Poros. = Root Porosity; all other trait abbreviations as per Table 1.

2.4 Results

2.4.1 Correlations between seedling and adult traits

Across 54 species, leaf and stem traits of seedling and adult plants were significantly and positively correlated (Figure 1). Specific leaf area had the strongest relationship (Figure 2.e), but was typically higher in seedlings compared to adults. Leaf tissue density and LDMC were positively correlated between seedlings and adults, and both tended to be lower in seedlings (Figure 2.c and 1.d). Stem dry matter content was also positively correlated between seedlings and adults (Figure 2.b), and also tended to be lower in seedlings.

Root morphology traits were positively correlated between seedlings and adults across 19 species. Root diameter was the most strongly correlated trait between seedling and adult plants (Figure 3.b). Seedling and adult SRL were also significantly positively correlated (Figure 3.a), although a relatively high seedling SRL value for *Aristotelia serrata* (labelled on figure) pulled the regression line away from the 1:1 line. Root branching intensity was also positively correlated between seedling and adult plants (Figure 3.e). There was no significant relationship between either root N or root P of seedlings and adults (Figure 3.c, d). Root N was typically far higher in seedlings compared with adults (Figure 3.c). In some species root P was over an order of magnitude higher in seedlings compared to adults (Figure 3.d).
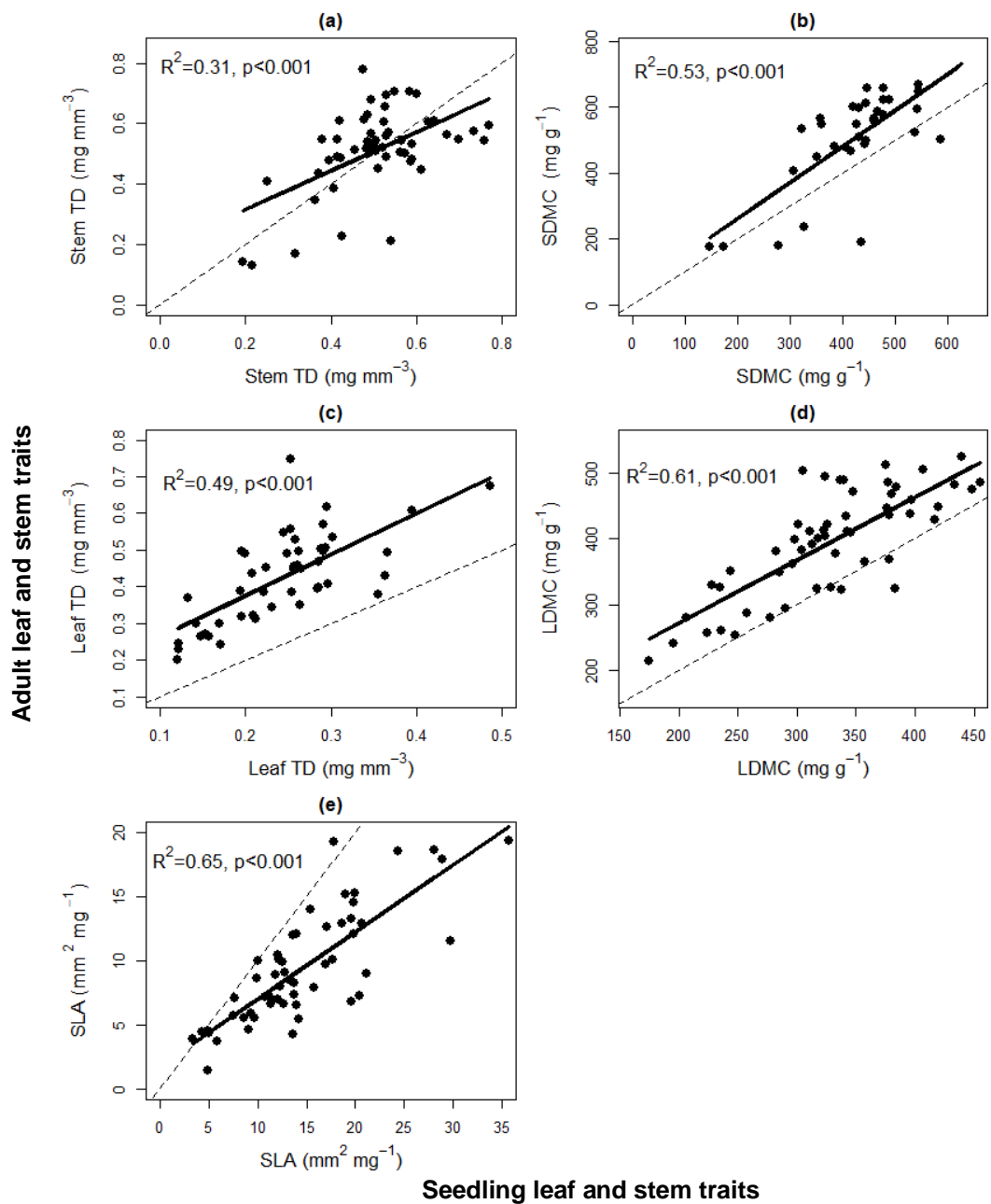
Figure 2. Linear regression between seedling (x axis) and adult (y axis) traits for: a) stem tissue density; b) stem dry matter content; c) leaf tissue density; d) leaf dry matter content; e) specific leaf area. Each point represents a species. The solid line represents the fitted regression line and the dashed line illustrates the 1:1 line.
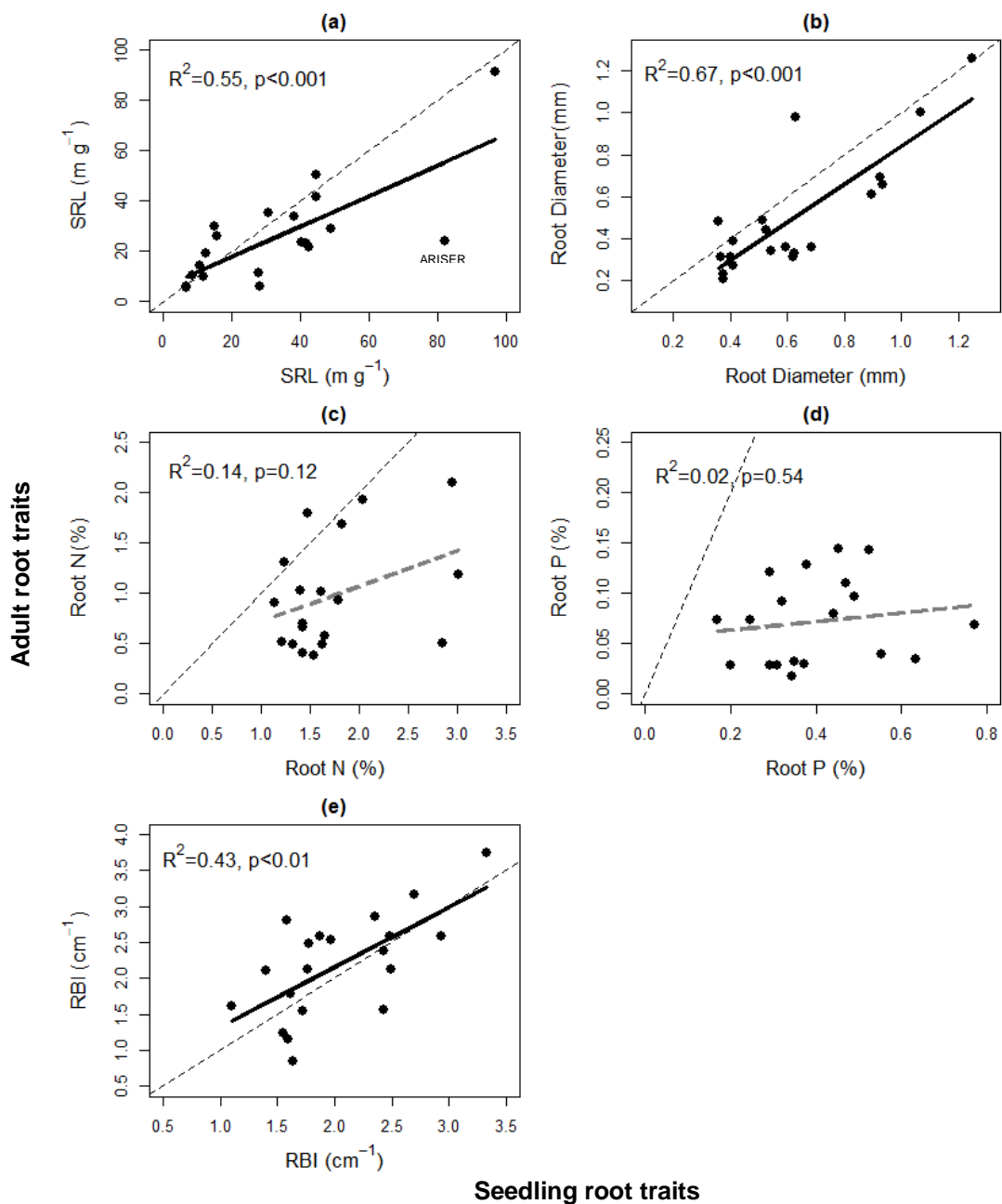
Figure 3. Linear regressions between seedling (x axis) and adult (y axis) traits for: a) specific root length; b) root diameter; c) root nitrogen concentration; d) root phosphorus concentration; e) root branching intensity (RBI). Each point represents a species. The black solid line represents the fitted regression line where significant (p>0.05), the thick grey dashed line (c, d) represents non-significant fits, and the thin dashed line illustrates the 1:1 line. Outlier point labelled on a): ARISER, *Aristotelia serrata*.

2.4.2   Plant trait dimensionality and the whole-plant economic spectrum

Pearson's r correlations between pairs of traits (Table 4) revealed that SRL and SLA were only weakly positively correlated. SRL was strongly negatively correlated with root diameter. Within-organ correlations between tissue density and dry matter content were also very strong and positive, particularly in stems.

The first three eigenvalues derived from the species-trait correlation matrix were >1, indicating that the trait matrix was at least three-dimensional (Table 5). These three dimensions accounted for 70% of the variation and correlation structure of the 10 functional traits including seven root traits, LDMC, SDMC, and RGR (Table 5). The first principle component represents covariation among dry matter content for leaves, stems and roots, root nitrogen concentration, root branching intensity, and relative growth rate. The second principle component represents covariation between specific root length and root diameter, and also suggests that species with larger root diameters tend to have higher stem dry matter content. The third principal component was more complex and was largely driven by root chemistry, with the strongest influence from root phosphorus concentration. Root nitrogen concentration, stem dry matter content, specific root length, and root porosity were also influential on this axis, and all traits on this axis positively correlated with each other.

A two-dimensional ordination of the PCA results (Figure 4) reveals clear groupings of species by phylogeny, which were strongly influenced by SRL and root diameter. The 11 species of conifers measured in this study all grouped in the lower left quadrant of the figure, as they had slow growth rates, high tissue dry matter content, and large fine root diameters. The five Magnoliid species in this study grouped in the lower centre to lower right of the figure, owing to large root diameters, medium to high rates of growth, and medium to low tissue dry matter content. The four species of tree ferns were approximately centred on the figure, although tending towards the lower left quadrant. The one monocot species had low tissue dry matter content and relatively small diameter roots. Eudicot species were distributed across the figure, but tended to have smaller diameter roots and lower tissue dry matter content.

Table 5. Principal components analysis for 10 traits measured on 66 tree species. For each axis, the eigenvalues and proportion of variance explained are provided. Eigenvectors for each of the axes are listed below, and eigenvectors > |0.3| are in bold. The first three axes can be interpreted as dimensions representing variation in whole plant "fast-slow" economics, root diameter, and root chemistry. The fourth axis has an eigenvalue < 1 so is not interpreted (NA).

	PC1	PC2	PC3	PC4
Eigenvalues	3.71	2.15	1.21	0.84
Proportion of variance	37.11	21.48	12.05	8.43
Cumulative proportion	37.11	58.59	70.64	79.07
Interpretation of axis:	Whole plant "fast-slow"	Root diameter	Root phosphorus	NA
SRL	-0.07	**-0.62**	**0.33**	-0.14
Root Diameter	0.24	**0.56**	-0.24	0.05
RDMC	**-0.44**	0.07	-0.11	0.25
Root Porosity	-0.28	0.16	**0.31**	-0.65
RBI	**-0.33**	-0.14	-0.05	0.12
Root N	**0.32**	0.22	**0.39**	-0.20
Root P	0.24	0.08	**0.64**	0.41
LDMC	**-0.42**	0.07	0.14	0.36
SDMC	**-0.30**	**0.35**	**0.37**	0.19
RGR	**0.36**	-0.28	-0.03	0.32

Figure 4. Two-dimensional ordination of PCA results. Each species labelled with a six letter species code as per Table 2. Traits labelled in larger bold text and represented with blue lines. Trait abbreviations as per Table 1.

2.4.3   Community-level root traits along a soil fertility gradient

Soil pH, C:N ratio, and total P were reduced to a single principal component ('Puketī soil PC1'), which captured most of the variation (83%) in these collinear soil properties (Appendix Figure 13) .

Community weighted mean SRL, root diameter, leaf tissue density, and root phosphorus concentration were not significantly correlated with soil fertility (i.e., Puketī soil PC1) (Figure 5.a, b, h; Figure 6.c). The following community-weighted mean traits were negatively correlated with soil fertility: root, leaf, and stem dry matter content (Figure 5.d; Figure 6.d, f); root and stem tissue density (Figure 5.c; Figure 6.e); and root porosity (Figure 5.e). Community-weighted mean root branching intensity had a non-linear relationship with soil fertility, with lowest RBI on intermediate fertility sites, and highest RBI on the lowest fertility sites (Figure 5.f). Community-weighted mean SLA and RGR were strongly positively correlated with soil fertility (Figure 6.a, b), and CWM root nitrogen concentration had a weak positive relationship with soil fertility (Figure 5.g). Stem dry matter content had the strongest correlation with soil fertility of all traits measured (Figure 6.f)
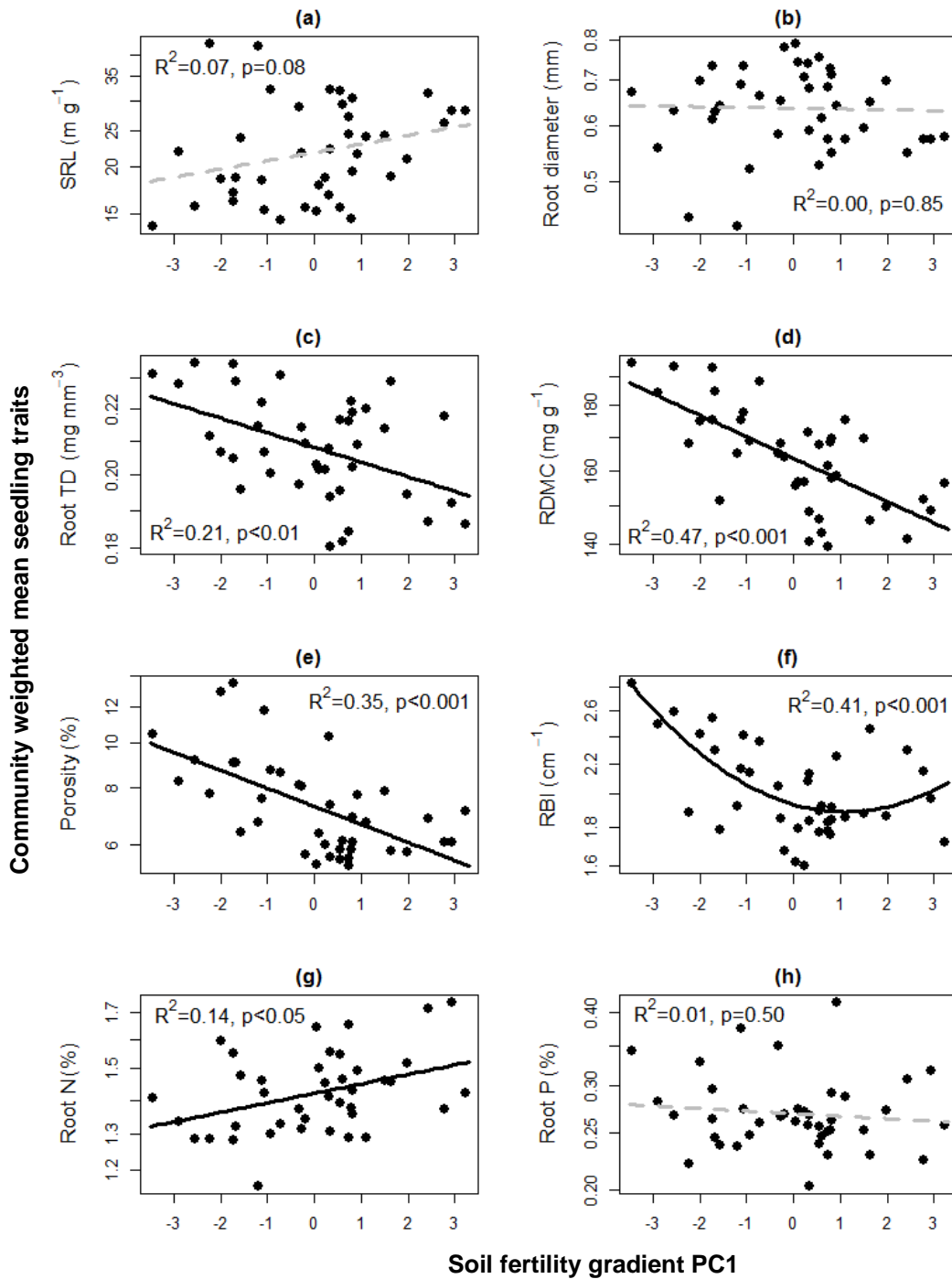
Figure 5. Regression analyses between community-weighted mean (CWM) seedling root traits and Puketī soil fertility PC1. Soil fertility increases from left to right along the x-axis as per Appendix Figure 13. a) specific root length; b) root diameter; c) root tissue density; d) root dry matter content; e) porosity; f) root branching intensity; g) root nitrogen concentration, h) root phosphorus concentration. Each point represents a plot. Y-axis plotted on log scale in all figures.
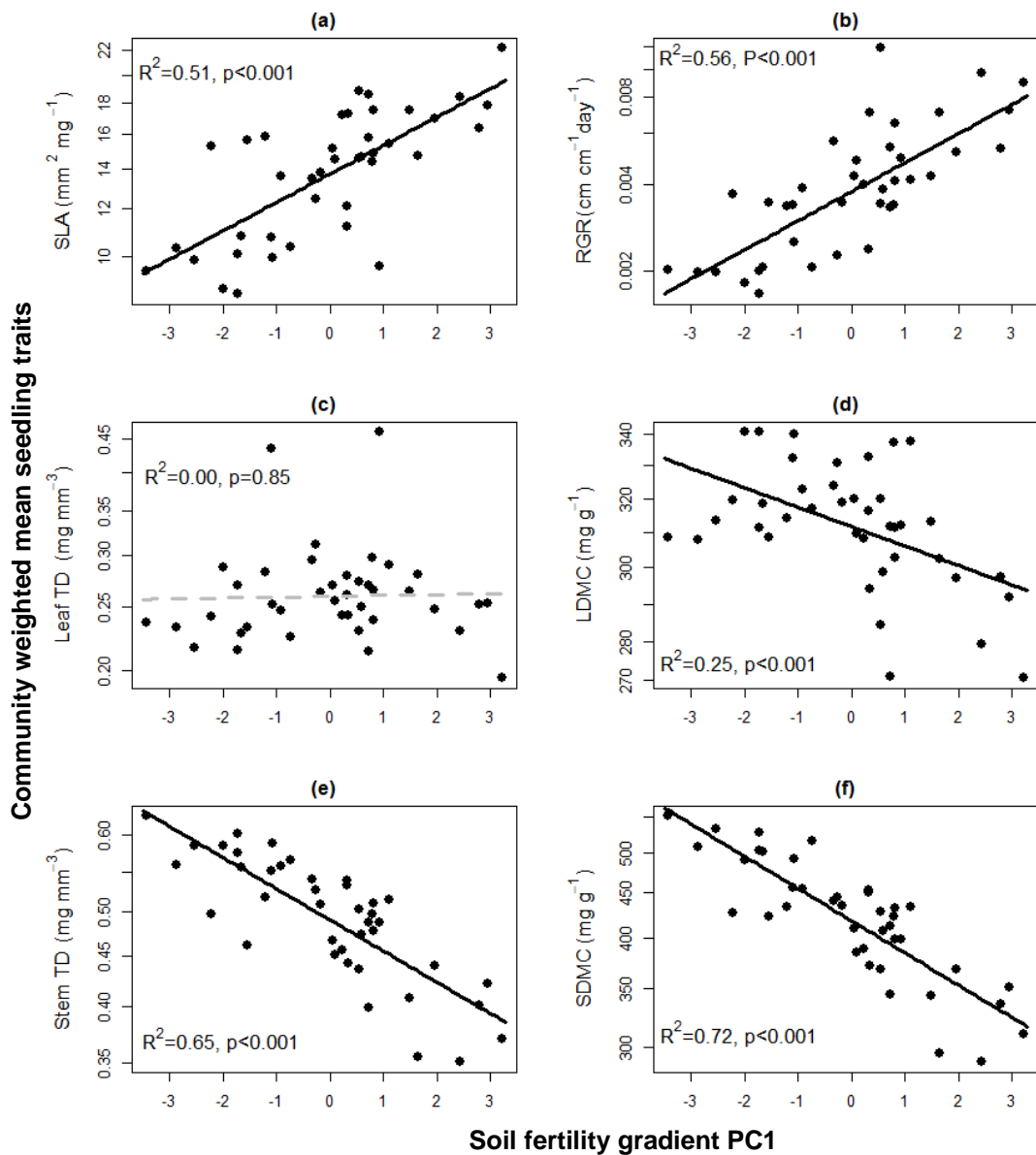
Figure 6. Regression analyses between community-weighted mean (CWM) seedling leaf and stem traits and Puketī soil fertility PC1. Soil fertility increases from left to right along the x-axis as per Appendix Figure 13. a) specific leaf area; b) relative height growth rate; c) leaf tissue density; d) leaf dry matter content; e) stem tissue density; f) stem dry matter content. Y-axis plotted on log scale in all panels.

2.5 Discussion

I found strong positive correlations between seedling and adult morphological traits, but not root nutrient traits. Seedling traits formed three principle component axes, the first is interpreted as a 'whole plant' economic spectrum, indicating co-ordination of plant growth between roots, leaves, and stems. The second axis is interpreted as a phylogenetically conserved root diameter/SRL continuum, which was orthogonal to the first axis. The third axis is complex, consisting primarily of root P, but also associated with root N, porosity, SDMC, and SRL. Traits relating to the whole plant economic spectrum were also strongly related to soil fertility, with "fast" growth traits associated with fertile soils, and more conservative traits associated with low fertility soils. Specific root length and root diameter were not related to soil fertility, but could possibly relate to the type and extent of mycorrhizal symbiosis which plant species form. Porosity was negatively correlated with soil fertility, and may assist species in low fertility soils to proliferate roots more efficiently. Root branching intensity had a non-linear relationship with soil fertility, suggesting that high branching intensity is adaptive at both high and low ends of the fertility gradient.

2.5.1 Relationships between traits of seedling and adult plants

My results demonstrate that morphological traits are strongly correlated between seedlings and adults, but that nutrient concentrations are not (Figure 2, Figure 3). Seedling traits were generally more oriented towards a "faster" or more acquisitive growth strategy than those of adults because seedlings had higher SLA and SRL, and lower leaf and stem dry matter contents. This is consistent with results from Rosenvald et al. (2013), who studied traits in various aged birch stands and found that younger trees tended to have higher SLA, SRL, and leaf nitrogen concentration, suggesting that trees down-regulate growth as they age. Decreases to SLA and SRL with age were observed in herbaceous species (Schippers and Olff, 2000), and decreases to SLA during ontogenetic development were observed in tree seedlings (Thomas and Bazzaz, 1999, Cavender-Bares and Bazzaz, 2000, Lusk, 2004, Niklas and Cobb, 2008). Decreases in SLA are possibly driven by increasing lamina area and/or thickness, requiring proportionally greater investment in supporting structures (Lusk and Warton, 2007). By adopting a "fast" growth strategy as seedlings, tree species

may be able to out-compete their neighbours for crucial light and soil resources. After becoming established on a site, and thus under less intense competition, a "slower" growth strategy may be conducive to long term survival via retention of nutrients and tolerance of stressful or resource limited environmental conditions. However, species vary in their degree of up or down-regulation of growth traits. For example *Aristotelia serrata* had almost two times higher SLA, and nearly four times higher SRL as a seedling than as an adult, whereas on average across all sampled species seedling SLA was only 1.6 times higher than adult SLA, and seedling SRL was on average only 1.5 times higher than adult SRL. This regulation of growth may reflect species' shade tolerance strategy as seedlings, with light demanding species such as *Aristotelia serrata* needing to intercept light gaps early in ontogeny, otherwise they will rapidly reach a negative carbon balance and thus energetically starve (Lusk, 2004).

Field grown plants can also differ from glasshouse plants due to factors other than age. Studies by Poorter and De Jong (1999) and Mokany and Ash (2008) examined traits of herbaceous plants of similar ages grown in both field and glasshouse conditions. Both studies found that glasshouse grown plants had higher SLA than field grown plants. This is consistent with my finding of higher SLA in glasshouse seedlings compared with field adults. However, the lower SLA of herbaceous field grown plants in these studies probably does not reflect ontogenetic change, but is more likely a response to stress from environmental factors such as water scarcity, temperature extremes, wind exposure, competition, or herbivory.

Discrepancies between adult and seedling traits may also arise due to the glasshouse conditions and potting medium that the seedlings were grown in. Roumet et al. (2008) suggest that potted plants may exhibit a higher SRL compared to field grown plants, due to the relative ease of extracting and washing the root system from potting mix compared to extraction of roots from natural soils, where the extraction process may result in the damage or loss of a significant proportion of fine roots. Additionally, in my study seedlings had ample access to light, water, nutrients, and were free from competition with other individuals, while the compared adult root trait data were drawn from a study over a strong phosphorus limitation gradient, where competition for soil resources would be intense. Chapter Three presents results from an experiment which

compared the effects of different nutrient treatments on trait expression for four native tree species. In this experiment, even the lowest nutrient treatment seedlings typically had far higher root nutrient concentrations than naturally grown adults. Although these results are far from conclusive, they suggest that the far lower nutrient concentration observed in adult roots may result from ontogenetic down-regulation of growth, rather than solely due to lower nutrient availability in natural soils.

Therefore, I conclude that morphological traits measured on seedlings are strongly and significantly correlated with those of adults, although tend to be oriented towards a "faster" growth strategy than those of adults. The relationships between seedling and adult root nutrient concentrations are less clear, and require further research to clarify how interactions between soil nutrient availability and ontogenetic changes result in adult root nutrient concentrations.

2.5.2   The whole-plant economics spectrum (PC1)

I found strong evidence for a whole-plant economics spectrum exhibited by co-ordination across above and below ground organ level traits, consistent with the whole-plant economic spectrum proposed by Reich (2014). The strongest axis of variation from my PCA represents a "fast-slow" or acquisitive-conservative spectrum for acquiring light and soil resources, where fast rates of growth are facilitated by low tissue dry matter content in leaves, roots, and stems, as well as low RBI and high root N. The correlation between above and below ground tissue density traits is consistent with results from multiple previous studies. In grasses LDMC was positively correlated with root TD, and both were negatively correlated with RGR (Wahl and Ryser, 2000). Correlations between leaf and root tissue density were observed in 78 grassland species along fertility and disturbance gradients (Craine et al., 2001). Leaf and root dry matter content were moderately correlated across 14 grassland species (Mokany and Ash, 2008). Across 40 species from aquatic, riparian and terrestrial environments, strong correlations were observed between above and below ground traits representing structural investment, including leaf and root dry matter, carbon, and lignin contents (Freschet et al., 2010). Strong correlation between leaf and root dry matter content, was observed in 14 Mediterranean rangeland species along a gradient of nitrogen limitation (Pérez-Ramos et al., 2012). In three New Zealand tree species positive correlations were observed between LDMC and RDMC,

which were both negatively correlated with SRL, SLA, and root and leaf N (Freschet et al., 2013). However, not all studies have observed strong correlations between leaf and root economic traits. Only weak correlations were observed between leaf and root tissue densities in grasses (Craine and Lee, 2003, Craine et al., 2005), and only within, but not between grassland communities (Kembel and Cahill Jr, 2011) . Altogether these results suggest that leaf and roots may co-ordinate their strategies, but that environmental gradients may be important drivers of such co-ordination. Additionally, whether the comparisons are made within or between communities can affect the outcome. Many of these studies have only included grasses or other herbaceous species; therefore more research is required to determine the generality of above and below ground economic co-ordination in woody species.

Multiple studies have shown leaf and stem economic traits to be decoupled in adult trees. No correlations were found between leaf and stem tissue densities across hundreds of neotropical tree species (Baraloto et al., 2010, Fortunel et al., 2012). In a study of New Zealand *Nothofagaceae*, no correlations were found between SLA and stem density, and only limited support for correlations between leaf and stem tissue density (Richardson et al., 2013). Both leaf and stem tissue densities have been found to negatively correlate with soil fertility, but ordinated on separate axes of principle component analysis (Jager et al., 2015). These results contrast with my findings of co-ordination across leaf, root, and stem economic traits, and may be due to the comparison of seedling versus adult traits. As discussed in objective one, compared to adults, seedling traits tend to be oriented to a "faster" growth strategy co-ordinated across leaves, roots and stems. This may arise due to intense competition over limited light and its strong effect on seedling survival, particularly in mature forests (Lusk, 2004). As trees grow, organ-level economic trait strategies may differentiate to reflect organ-specific responses to environmental stresses. For instance, denser stem tissues in adults will slow the rate of height growth, but allow trees to withstand greater mechanical stress and increase resistance to xylem embolism (Chave et al., 2009). Similarly, increased light interception by leaves typically results in different expression of leaf economic traits (Gratani et al., 2006, Lusk et al., 2008). Higher density roots in mature trees can produce higher tensile strength (Bischetti et al., 2005), allowing sturdier anchoring in the soil to support the growing mass of

leaves and stems. Alternatively, roots may need to adopt a more acquisitive strategy in response to local depletion of nutrients (Chapin III, 1980). Complex responses to the selective pressures and interactions of multiple environmental factors could account for the commonly observed differentiation of organ-level economic traits in adult trees.

Other traits have been found to correlate between leaves and roots, and may also be useful as indicators of coupled leaf and stem economics. In a meta-analysis of American grassland species, leaf and root longevity associated with each other, and were negatively associated with relative growth rate, SLA, and leaf and root nitrogen concentrations (Kembel et al., 2008) . In grasses both leaf and root tissue density were positively correlated with leaf and root lifespan respectively, and both leaf and root tissue density were negatively correlated with relative growth rate (Ryser, 1996). While I did not measure leaf chemistry or longevity of leaves and roots, these traits may also be useful for assessing co-ordination of above and below ground economic strategy in woody species.

In conclusion, the use of leaf traits to infer root traits should be approached cautiously, due to the lack of consistent results and the low number of studies of tree root economics. Although my results suggest that there is a "whole-plant economic spectrum" present across species, it is not clear whether this economic spectrum is only present in seedlings, if it is maintained as trees mature, or whether it is a unique feature of the New Zealand flora. Further research would clarify how co-ordination of organ-level economic strategy differs between herbaceous and woody species, between adult and seedling trees, and between ecosystems and regional floras

2.5.3   Root diameter and SRL (PC2)

My results indicate that fine root diameter and SRL were independent from the whole-plant economics spectrum. This contrasts with the hypothesis that SRL can be viewed as a below ground analogue to SLA, with high SRL facilitating growth due to having more absorptive length per unit of dry mass, and thus a more efficient strategy for acquiring soil resources. Multiple studies have found SRL positively correlated with either SLA or relative growth rate (Eissenstat, 1991, Reich et al., 1998, Wright and Westoby, 1999, Comas et al., 2002, Comas and Eissenstat, 2004, Withington et al., 2006, Laughlin et al., 2010, Laughlin et al.,

2011, Fort et al., 2012), although the correlation between SRL and SLA was often only weak, as was found in my study (Table 4). Several studies have reported no correlations between SRL and SLA (Craine et al., 2005, Tjoelker et al., 2005, Markesteijn and Poorter, 2009, Chen et al., 2013). Studies which have observed correlations between SRL and SLA have suggested that SRL is an adaptive, economic trait. However, my results are consistent with studies which demonstrated that SRL and root diameter are phylogenetically conserved (Pregitzer et al., 2002, Comas and Eissenstat, 2004, Withington et al., 2006, Comas and Eissenstat, 2009, Kembel and Cahill Jr, 2011, Chen et al., 2013, Valverde-Barrantes et al., 2014), and not directly related to plant growth strategy or soil nutrient availability (Alvarez-Uria and Körner, 2011, Kembel et al., 2008, Roumet et al., 2008, Boot and Mensink, 1990). In my study, magnoliid angiosperms and conifers both possessed low SRL and large diameter roots, and yet conifers were more aligned with the "slow growth" end of PC1 (Figure 2), probably due to higher tissue dry matter content. Eudicot angiosperms spanned the whole range of root diameters and had both the smallest and largest diameter roots in this study, and yet species with similar root diameters were dissimilar across the PC1 axis (e.g. compare *Dracophyllum longifolium* to *Fuchsia excorticata*).

If SRL does not directly affect species' growth rates, species with low SRL must have some mechanism of effectively competing for soil resources, or else evolution surely would have resulted in an "arms race" of increasing SRL. Several studies have linked lower SRL and higher root diameter to longer root lifespan (McCormack et al., 2012, Tierney and Fahey, 2002, Strand et al., 2008), causing longer term nutrient retention within plant tissue, and thus potentially facilitating tolerance to low fertility soils. However, species with widely different SRL can compete and co-exist on a site (Guo et al., 2008), suggesting that SRL is not being strongly environmentally filtered. The leading hypothesis of how low SRL species compete with high SRL species is that larger root diameters offer mycorrhizal symbionts more root volume to colonize, thus low SRL species can obtain nutrients (particularly phosphorus) through the large surface area of mycorrhizal hyphae, in exchange for photosynthetic carbon (Baylis, 1975, Brundrett, 1991, Eissenstat, 1992, Newsham et al., 1995). By offering a larger root volume to colonize, low SRL species may increase the rate of exchange of resources

between themselves and their mycorrhizae. This hypothesis is supported by some studies, which found root diameter positively correlated with extent of mycorrhizal colonization (St John, 1980, Reinhardt and Miller, 1990, Collier et al., 2003, Comas et al., 2014) or benefit from VAM symbiosis (Graham and Syvertsen, 1985, Manjunath and Habte, 1991, Hetrick et al., 1992), but not by others which found root diameter unrelated to extent of mycorrhizal colonisation (Pregitzer et al., 2002, Zangaro et al., 2007, Roumet et al., 2008, Holdaway et al., 2011) or unrelated to benefit from mycorrhizal infection (Schweiger et al., 1995, Zangaro et al., 2005)

The type of mycorrhizae with which a plant species forms a symbiosis may be as important as the extent of mycorrhizal colonisation for the understanding of the interplay between SRL, root diameter, and soil resource acquisition. Vesicular-arbuscular mycorrhizal symbiosis (VAM) is believed to be the ancestral condition of mycorrhizal symbiosis (Brundrett, 2002), and modern basal angiosperms (particularly Magnoliidae) have retained high degrees of VAM dependency (Baylis, 1975). VAM is the most common category of mycorrhizal symbiosis, both worldwide and across the New Zealand flora (McNabb, 1958, Wang and Qiu, 2006), and the New Zealand Podocarpaceae and *Agathis* species produce specialised root nodules to house VAM fungi (Russell et al., 2002), which facilitate phosphorus uptake (Morrison and English, 1967). Fossil and geological evidence suggests that during the angiosperm radiation of the Cretaceous there was a general trend of more recently diverged lineages having higher SRL, and less dependence on mycorrhizal symbiosis, possibly driven by the prevalence of drier climate (Brundrett, 2002, Comas et al., 2014). This may explain the wide range of SRL/root diameters observed in eudicot species in this study. Ectomycorrhizal type symbioses (EM) also appeared in the Cretaceous (Comas et al., 2014). New Zealand has a small selection of species which form ectomycorrhizal symbioses (all native Nothofagaceae (Baylis, 1980) and *Leptospermum scoparium* (Moyersoen, 1999)), which tended to have highly branched, high SRL roots (Appendix Figure 14) consistent with Comas and Eissenstat (2009) and Comas et al. (2014), although these are not universal traits of all EM species (Pregitzer et al., 2002). Newsham et al. (1995) also theorised that the benefit of VAM symbioses would be greatest to species with low root branching intensity, therefore in plant families with high root branching intensity

it is logical that a transition to a different type of mycorrhizal association would be more likely to evolve. Interestingly, the species with highest branching intensity and SRL in my study was *Dracophyllum traversii*, which forms an ericoid type mycorrhizal symbiosis (McNabb, 1961). High SRL and branching intensity, as well as the absence of root hairs, appear to be characteristic of ericoid mycorrhizal plant roots (Read, 1996). Additionally, some EM and ericoid mycorrhizas have the ability to uptake organic nitrogen sources, which most VAM and non-mycorrhizal species lack (Smith and Read, 1996).

It is apparent that SRL alone does not provide sufficient insight into root strategy. In order to improve the understanding of the relationships between morphological root traits and whole-plant economics, plant ecologists must also understand how root traits other than SRL influence below ground strategy, including branching intensity, porosity, the costs and benefits of different categories and extents of mycorrhizal symbiosis, how these traits scale under differing environmental conditions, and how mycorrhizal symbioses influence plant resource economics.

2.5.4   Root phosphorus (PC3)

The third principle component represents a complex association of traits. Root phosphorus concentration was the predominant driver of PC3, which positively associated with root nitrogen concentration, SRL, SDMC, and porosity. It is intriguing that SRL, but not root diameter, was associated with root phosphorus. This may be due to phenotypically plastic modification of root tissue density in response to phosphorus availability, which would affect SRL, but not root diameter. Paradoxically, PC3 had a positive association between SRL and SDMC, whereas PC2 had a negative association between these two traits. These conflicting associations are probably driven by the contrasting growth rates and tissue dry matter contents of the coniferous and magnoliid species in this study. Both conifers and magnoliids generally had low SRL; however magnoliids typically had fast growth rates and low tissue dry matter content, while in contrast conifers tended to have slow growth rates and high tissue dry matter content. Similarly, PC3 contained a positive association between root nitrogen and SDMC, while in PC1 these traits were negatively associated, although it is not clear which species or groups of species are driving this pair of conflicting associations.

Porosity, or amount of aerenchyma in roots, has been positively correlated with waterlogging tolerance, particularly in wetland plants (Jansen et al., 2005, Visser et al., 2000, Jackson and Armstrong, 1999). Aerenchyma tissue allows for aeration of roots by facilitating gas exchange between submerged roots and the atmosphere. The negative correlation between soil fertility and CWM porosity (Figure 5.e) at first seems counter-intuitive, as the soil fertility gradient in this study was strongly associated with altitude and topography (Jager, 2014) and I would have expected less waterlogging in the ridge-top soils compared with the wetter, high fertility gully soils. However, low fertility sites can also have poor drainage (Jane and Green, 1986, Lusk, 1996), and the higher average porosity values observed in low fertility conifers may represent adaptations to poorly drained, low fertility soils. *Laurelia novae-zelandiae* is a species which is associated with wet, high fertility soils (Jager et al., 2015), yet had particularly low porosity (Table 3); although, this may be explained by the ability of *Laurelia novae-zelandiae* to form pneumatophores (aerial roots), an alternative adaptation to waterlogged soils. However, *Syzygium maire* is also associated with water-logged soils, also produces pneumatophores, and yet had relatively high porosity (Table 3), contrasting with *Laurelia novae-zelandiae*.

In addition to adaptation to water logging, porosity may have other adaptive functions. Studies of aerenchyma formation in maize have suggested that higher porosity may be adaptive in situations other than flood tolerance. (Saengwilai et al., 2014) found that higher porosity is correlated with lower metabolic cost per unit of root length, and thus could be advantageous in soil nutrient acquisition. Zhu et al. (2010) also found higher porosity associated with lower metabolic cost per unit of root length, and demonstrated that plants with higher porosity were more tolerant to drought, due to their relatively lower metabolic cost of soil exploration. These results suggest that my observation of higher porosity in low fertility soils (Figure 5.e) may be an adaptive response which lowers the metabolic cost per unit of root length, therefore increasing the efficiency of soil exploration. Porosity was also moderately negatively correlated with relative growth rate and weakly negatively correlated with SLA, both of which negatively correlated with soil fertility. As gas filled cavities have a very low density, I would expect that that roots with higher porosity should also have lower overall root tissue density, however Pearson's *r* correlations between the two traits did

not support this (Table 4). There was a weak positive correlation between porosity and root dry matter content, therefore it is possible that higher porosity roots also have relatively high dry matter content in root cells (cortex, dermal layers, vascular tissue etc.), which would account for both the lack of correlation between porosity and overall root density, and the positive correlation with root dry matter content. These results suggest that the costs and benefits of differing extents of porosity may be worth investigating in relation to whole plant strategy.

2.5.5 Root traits selection along a soil fertility gradient

Regression analyses demonstrated that traits relating to the "whole-plant economic spectrum" were strongly correlated with the Puketī soil fertility gradient (Figure 5, Figure 6). Species with traits associated with an acquisitive growth strategy were more common on high fertility sites, and species with traits associated with a conservative growth strategy were more common on low fertility sites, consistent with CSR theory (Grime, 1979). These results are also consistent with previous studies which have observed high soil fertility filtering for species with acquisitive leaf and stem traits, and low fertility soils filtering for species with conservative leaf and stem traits (Muller-Landau, 2004, ter Steege et al., 2006, Gourlet-Fleury et al., 2011, Jager et al., 2015). The co-ordination of economic trait strategy across leaves, roots, and stems is consistent with results from Freschet et al. (2010), but to my knowledge this is the first time this "whole-plant economic spectrum" has been demonstrated to correlate at the community level with a strong soil fertility gradient.

The absence of correlation between soil fertility and community-level SRL (Figure 5.a) contrasts with results from multiple studies (Holdaway et al., 2011, Fort et al., 2012, Prieto et al., 2015), although the relationship between SRL and soil fertility in these studies was not always linear. Intra-specific SRL has been demonstrated to increase in response to nutrient limitation (Clemensson-Lindell and Asp, 1995, Hill et al., 2006, Kalliokoski et al., 2010), or to not respond to nutrient limitation (Boot and Mensink, 1990, Cromer and Jarvis, 1990, Borken et al., 2007, Kalliokoski et al., 2010); however, species' responses were influenced by their phylogeny and the particular limiting nutrient. Clearly these contrasting results illustrate the need for further research of how community level SRL relates to soil fertility gradients, and how the discrete components of SRL (fine root diameter and root tissue density) vary both within and between species across

such gradients. Determining how the resulting variation in SRL is driven by changes to either root diameter or root tissue density will help to test the hypothesis that fine root diameter is highly phylogenetically constrained. Under this hypothesis, any adaptive capacity of SRL is likely to be driven by and limited to alterations of root tissue density, rather than root diameter.

Although SRL has been proposed as a below ground equivalent of SLA (Eissenstat et al., 2000) and thus hypothesised to correlate to soil fertility, other root traits may have equal or greater influence on nutrient and water uptake. I observed CWM root porosity to decrease with soil fertility (Figure 5.e), and as discussed earlier some studies have found that higher porosity decreases the metabolic and dry matter costs of root proliferation (Saengwilai et al., 2014, Zhu et al., 2010), and thus may be beneficial on water or nutrient limited sites. I also observed a non-linear relationship between soil fertility and CWM root branching intensity (Figure 5.f), with low fertility sites associated with species with high RBI, and intermediate fertility sites associated with species of lowest RBI. These results are somewhat consistent with those of Holdaway et al. (2011), who observed a linear negative relationship between RBI and phosphorus availability. As mentioned earlier, RBI may relate to species' mycorrhizal association, with EM species typically having high RBI compared to obligate VAM species (Comas and Eissenstat, 2009, Comas et al., 2014) (Appendix Figure 14). Other root traits which may be important to below ground strategy include root-to-shoot ratio, total root biomass, root distribution throughout the soil profile, ability to proliferate into nutrient rich patches, root turnover, and type of mycorrhizal symbiosis (Kembel et al., 2008, Hodge, 2009, Laughlin et al., 2010, Comas et al., 2014). The importance of these traits in determining community assembly along soil fertility gradients could be clarified by their inclusion in future studies. For now, it appears that SRL alone cannot be a reliable predictor of below ground strategy or species' adaptations to nutrient availability.

2.6 Recommendations

The conclusions I reached regarding the relationships between seedling and adult traits could be strengthened by measuring a wider range of species, particularly those which were not able to be sourced from nurseries due to their low commercial demand or propagation difficulties. These species were typically associated with low fertility soils, and while I was able to obtain some data for these species, more replicates would encompass a greater degree of natural variability and increase confidence in the trait averages for these species. My comparison between seedling and adult roots was also limited to less than 20 species due to lack of adult field root data, and thus the relationships between adult and seedling root traits could be clarified by gathering adult root trait data for additional species. To improve the understanding of the relationships between seedling and adult root nutrient concentration traits, it would be valuable to gather adult root chemistry data for a representative sample of species, growing in a variety of soil conditions. This would improve our understanding of how local soil conditions influence species' root nutrient concentrations. It may also be valuable to sample both morphological and nutrient root traits within a narrow soil fertility gradient, from a range of tree ontogenetic stages, including seedlings, saplings, young adults, and mature canopy dominants. This would provide valuable data on how ontogeny influences trait expression over tree lifespans.

Similarly, the use of seedlings to infer adult root traits is far more logistically convenient that field sampling and offers controlled conditions for hypothesis testing; however more experiments are needed to examine how trait expression is influenced by both abiotic and biotic factors. There is a call from the literature to conduct controlled experiments which investigate the effects of root competition on root foraging abilities, resource uptake, low fertility tolerance, and trait expression. In chapter three, I present result of an experiment which examined four species responses to differing nutrient treatments, although these experiments were performed on isolated plants rather than in competitive conditions, the results are still of relevance to seedling trait research. Interpretation of trait data from controlled conditions would be improved by comparative research between glasshouse grown plants and those from a variety of natural field conditions, particularly varying degrees of light, fertility, and competition.

SRL does not appear to be the below ground equivalent of SLA as has been theorised, correlating neither with growth related traits or soil fertility. However, SRL may still be an important factor in understanding species' distributions, possibly related to the category and extent of mycorrhizal associations, which represents a promising and relatively unexplored area of root trait research. There are a plethora of questions regarding mycorrhizal symbiosis that could be investigated. For example, do high SRL species have a greater degree of control over their mycorrhizal associations compared to low SRL species? How does the uptake of soil water by mycorrhizal hyphae compare to that of fine roots of either small or large diameter? Does total water uptake ability differ between a low SRL species with high mycorrhizal infection, and a high SRL species with low or no mycorrhizal infection? Are certain species of mycorrhizal fungi more effective at extracting soil nutrients than others? SRL has also been linked to root longevity by several recent studies, and despite the logistical difficulties of obtaining root lifespan data, it may be useful in understanding how SRL influences below-ground competitive strategy. Both mycorrhizal associations and root lifespan data have great potential for improving models of soil carbon and nutrient cycling.

Porosity and root branching intensity both had a weak relationship with soil fertility, and root branching intensity was associated with the "whole plant strategy" PC1, while porosity was associated with the "root phosphorus" PC3. These traits may have some potential in future root trait research, porosity particularly may be useful in wetland or regularly flooded ecosystems, while the relationships between root branching intensity, soil fertility, and SRL are not yet well understood.

Objective three of this study investigated seedling traits and their relationship to a strong fertility gradient present in Puketī forest. It would be interesting to see if the relationships I found between whole plant-economic traits and soil fertility are consistent on a wider scale. Soil fertility and species' relative abundance data are available on a nationwide scale in New Zealand (LUCAS plots), and would be a perfect dataset to investigate how root traits vary along both soil fertility and climatic gradients at a national scale.

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Chapter Three: Intraspecific morphological root trait variation is less affected by soil fertility than biomass allocation or root nutrient concentrations

3.1 Abstract

Measuring traits on glasshouse grown seedlings allows researchers to provide standardised growing conditions, and is a less time and resource consuming alternative to field trait sampling, particularly for root traits. However, seedlings propagated in glasshouse conditions are also typically grown in a common high fertility potting medium, despite potential differences in species' natural soil fertility associations. As plants are known to alter their phenotypes in response to environmental variables ("phenotypic plasticity"), it is crucial to understand how traits are affected by environmental variables, particularly soil fertility. Previous research suggests that biomass allocation responds strongly to soil nutrient availability, although results from the literature are mixed regarding how soil nutrient availability affects morphological root traits such as tissue density or specific root length. In this study I investigated the effects of nutrient availability on trait expression in a selection of New Zealand native woody species. I also investigated whether the method of fertiliser application (liquid pulses vs. slow release granules) affected trait expression. Species were selected to represent a wide range of forest tree species, including two major phylogenies (conifers and angiosperms) and both high and low soil fertility associations. I measured fifteen traits on four species in response to three soil nutrient treatments. Plants received either zero fertiliser addition ("low"), monthly doses of liquid fertiliser ("pulse"), or slow release fertiliser granules ("slow release). Relative growth rate responded strongly to nutrient treatments, as did root-to-shoot ratio, root nitrogen concentration, and root phosphorus concentration. Morphological traits relating to the "whole-plant economic spectrum" were not strongly plastic in response to nutrient availability. Specific root length (SRL) had a weak response to the nutrient treatments, which was driven by alterations to root tissue density rather than root diameter. Across all species, the strongest differences to trait expression were between low and slow release treatments, and trait expression in pulse treatments were typically more similar to slow release than to low treatment trait expression. These results suggest that fertiliser application method does not

influence trait expression, and differences between slow release and pulse treatments may have been driven by differences in total nutrient availability. Species' plasticity may be influenced by phylogeny, as angiosperms were on average more plastic than conifers. Species' soil fertility preference did not appear to strongly affect phenotypic plasticity. However, these trends were strongly driven by individual species' responses and therefore further research on a wider range of species, soil fertilities, and phylogenetic groups is required to confirm these generalisations. Altogether, these results provide support for comparing and pooling morphological trait data between studies, provided that plants were raised in conditions conducive to growth. However, traits relating to organ-level chemistry, biomass allocation, and growth rates are more strongly affected by nutrient availability, and thus care should be taken when comparing these traits between studies.

3.2 Introduction

The developing field of trait-based plant ecology has tended to focus on leaf, stem, and reproductive traits, but the role of root traits in plant strategy has not received comparable attention. Recent studies have measured root traits of plants grown in glasshouses (e.g. Cornelissen et al., 2003a, Mokany and Ash, 2008, Laughlin et al., 2011, Wishart et al., 2013, Birouste et al., 2014, Chapter Two of this thesis) as the glasshouse environment allows controlled and standardised conditions, and is a logistically simpler method compared to root sampling in the field. High fertility potting mix is used often as a growing medium in glasshouse studies, providing optimal nutritional conditions and expediting growth. In contrast, natural soils encompass a range of conditions, including concentrations of vital mineral nutrients such as nitrogen (N) and phosphorus (P), pH, and particle size. Plants are known to alter their morphology and physiology in response to varying environmental conditions, a phenomenon known as phenotypic plasticity (Bradshaw, 1965, Schlichting and Levin, 1986, Sultan, 2000). Due to the contrast between natural and glasshouse conditions, it is important to clarify how soil variables affect trait expression, particularly nutrient availability. Quantifying trait plasticity in response to nutrient availability will also provide context for pooling trait data across studies, particularly where soil conditions are not identical.

Some traits are more plastic than others (Schlichting and Levin, 1986), which may be related to species' overall growth strategy and soil fertility associations. Species from low fertility sites typically have slower growth rates and traits which are supportive of a conservative growth strategy, while species associated with high fertility sites typically have traits which support an acquisitive and competitive growth strategy (Figure 4) (Schläpfer and Ryser, 1996, Chapin III, 1980, Aerts and Chapin, 1999). This is consistent with CSR theory (Grime, 1979), which also predicts that acquisitive species from high fertility soils will be more plastic in their phenotypic responses to nutrient enrichment than conservative species from low fertility soils (Grime et al., 1991). This prediction has been tested in several studies. Hodge (2004) reported that acquisitive species demonstrate more morphological plasticity, while conservative species exhibit greater physiological plasticity as it is "less expensive" than morphological plasticity. Grassein et al. (2010) observed higher phenotypic plasticity in

acquisitive versus conservative grass species, and Freschet et al. (2013) also observed that species with acquisitive growth traits responded more plastically than conservative species to resource limitation, primarily evident in leaf and root dry matter contents. Other morphological traits related to nutrient acquisition may also respond strongly to nutrient availability.

Specific root length (SRL) is a measure of root length divided by root dry mass, and has been theorised to represent resource uptake potential relative to carbon investment in roots (Eissenstat, 1992). Some studies have supported this hypothesis, documenting phenotypic changes to SRL in response to soil fertility gradients. Berntson et al. (1995) observed higher SRL in response to nutrient limitation in *Betula alleghanienis*. Hill et al. (2006) studied responses of pasture species to low soil concentrations of N and P, and documented decreases to root diameter and increases to SRL under P- limited conditions, although not all species responded to N limitation in this way. Kalliokoski et al. (2010) documented that an angiosperm (*Betula pendula*) produced higher SRL on lower fertility sites, but also that over the same fertility gradient there was no trend in SRL for two species of conifers (*Picea* sp.). While studying the effects of fertility treatments on *Picea abies*, Clemensson-Lindell and Asp (1995) observed that SRL decreased in response to ammonium sulphate fertilisation, but increased in response to a complete nutrient fertiliser. These studies suggest that SRL may exhibit idiosyncratic responses to soil nutrient availability, with responses depending on both the limiting nutrient and species' phylogeny.

However, not all studies have observed correlations between SRL and soil fertility or growth strategy. Boot and Mensink (1990) observed that both specific root length and fine root diameter were not affected significantly by alterations in soil nitrogen availability. Cromer and Jarvis (1990) did not observe an increase in SRL under low nitrogen availability in *Eucalpytus grandis*. Hetrick et al. (1991) observed no changes to SRL under P limitation for five species of cool-season grasses. Aerts et al. (1992b) reported no changes to SRL under differing N availabilities for four species of *Carex*. Both Borken et al. (2007) and Kalliokoski et al. (2010) did not observe changes to SRL of *Picea* sp. in response to soil fertility gradients. Furthermore, multiple studies have concluded that SRL and fine root diameter are strongly influenced by phylogeny (Withington et al., 2006, Kembel and Cahill Jr, 2011, Chen et al., 2013, Valverde-Barrantes et al., 2014,

Pregitzer et al., 2002, Comas and Eissenstat, 2004, Alvarez-Uria and Körner, 2011), with conifers and magnoliid angiosperms typically having far lower SRL and larger fine root diameter compared to eudicot angiosperms. Therefore it is not clear exactly how SRL and root diameter relate to species' abilities to compete for and uptake nutrients.

Relative biomass allocation to above and below ground organs (root-to-shoot ratio) has been repeatedly demonstrated to be highly plastic in response to nutrient availability, particularly in fast-growing species (Cromer and Jarvis, 1990, Dyer et al., 2001, Hill et al., 2006, Louw-Gaume et al., 2010, Grassein et al., 2010). In a review of biomass allocation studies, Poorter et al. (2012) found that relative root biomass is negatively correlated with soil fertility, while relative leaf biomass investment is positively correlated with soil fertility. Furthermore, phylogeny has a clear effect on biomass allocation; for example, gymnosperm seedlings allocate more biomass to leaves and stems and less to roots in comparison with angiosperm seedlings (Poorter et al., 2012). In contrast, Hodge (2004) found that neither SRL, root demography, or biomass allocation alone were definitive criteria for assessing plasticity among studies.

In Chapter Two of this thesis, I measured trait data on seedlings grown in controlled glasshouse conditions and a potting mix containing slow release fertiliser granules. The glasshouse environment provides controlled growing conditions (cf. Integrated Screening Programme, Grime et al. 1997) and, compared to sampling roots from natural environments, is a less time consuming and overall easier method of obtaining root trait data. However, it is important to recognise that glasshouse grown seedlings can express different traits compared to natural field grown seedlings (Mokany and Ash, 2008). My comparisons between traits of glasshouse-grown seedlings and adults from natural soils revealed close correlations for morphological traits, but not for concentrations of root nitrogen or phosphorus (Figure 2, Figure 3). It is not clear whether the poor correlations between seedling and adult root nutrient concentrations were due to ontogenetic development, phenotypic plasticity, or the contrasting fertility of natural soils versus slow release potting mix. Also, as Chapter Two studied traits of species from across natural soil fertility spectrums, it is not clear whether the relatively high fertility of the potting mix which I used resulted in phenotypic plasticity of morphological traits, particularly in plants which are adapted to low fertility soils.

However, "high" and "low" fertility soils are simplified concepts of what are in reality highly dynamic soils, in which nutrients can be distributed heterogeneously in both space and time (Campbell and Grime, 1989, Campbell and Grime, 1992, Craine, 2009).

The first objective of this chapter is to determine which traits are most affected by soil nutrient availability. I hypothesise that biomass allocation (root-to-shoot ratio) will be strongly affected by nutrient availability, where individual plants grown in low fertility will have the highest relative allocation to roots, consistent with Cromer and Jarvis (1990), Dyer et al. (2001), Hill et al. (2006), Louw-Gaume et al. (2010), and Grassein et al. (2010). Relative growth rate is also likely to be lowest in low fertility treatments, as low nutrient availability strongly limits growth (Epstein, 1972, Chapin III, 1980, Porter and Lawlor, 1991, Gregory, 2006, Aerts and Chapin, 1999). SRL and root diameter did not correlate with soil fertility at the community level (Figure 5.a, b) or at the species level (Cromer and Jarvis, 1990, Borken et al., 2007, Kalliokoski et al., 2010), and therefore I predict they will not respond plastically in my experiment.

The second objective is to determine whether the method of nutrient application produces strong plasticity. Slow release fertiliser granules are common in commercial potting mixes and provide nutrients relatively homogenously across both space and time. However, applying pulses of liquid fertiliser to an otherwise infertile potting mix will make nutrient availability heterogeneous across time, more closely approximating the nutrient availability of some natural soils (Campbell and Grime, 1989, Campbell and Grime, 1992, Craine, 2009). In response to more heterogeneous soil fertility, species' may increase their soil foraging effort, which would be evident in increased SRL or root-to-shoot ratio.

To meet these objectives, I grew four species representing a range of soil fertility associations and phylogenies under three soil nutrient treatments and measured the responses of 15 traits from leaf, stem, and root organs, as well as relative growth rate and biomass allocation. I used ANOVA and co-efficient of variation analyses to determine the significance and relative plasticity of traits and species in response to the three nutrient availability treatments.

3.3   Methods

Selecting species

This experiment was designed to observe the effects of soil nutrient availability on phenotypic expression. I selected four tree species that reflected a broad range of functional types and phylogenies within the New Zealand flora. To encompass a wide range of species' responses to nutrient availability I selected one conifer (*Dacrycarpus dacrydioides* (A.Rich.) de Laub.) and one angiosperm (*Laurelia novae-zelandiae* A.Cunn.) associated with high soil fertility, and I selected one conifer (*Agathis australis* (D.Don) Loudon) and one angiosperm (*Knightia excelsa* R.Br.) associated with low soil fertility (Jager et al., 2015). Species selection was also restricted by the commercial availability of the species in a small size class and in sufficient quantity for replication. All seedlings were sourced from Taupo Native Plant Nursery (Taupo, Waikato), with the exception of 20 seedlings of *Knightia excelsa* that were sourced from Oratia Native Plant Nursery (Oratia, Auckland). Plants were ordered at tube stock (~150 mL pot volume) or root trainer (~350 mL) size classes, as smaller root systems would suffer less damage from repotting, therefore reducing transplant shock and expediting new growth. From this point forward, the four species used in this experiment will be referred to by their genus (*Agathis, Dacrycarpus, Knightia,* and *Laurelia*).

Nutrient treatments

Seedlings were re-potted into 1.5 litre pots, and randomly assigned to one of three treatments. Replicates consisted of 15 or 16 seedlings per species per treatment, although some seedlings did not survive until the end of the experiment (Appendix Table 11). Plants assigned to the slow release nutrient treatment (SR) were grown in a standard commercial potting mix, blended in a 5:1 ratio with propagation sand (identical to the potting mix used for all plants in Chapter Two). This potting mix contained dolomite and lime additives and slow release fertiliser granules (Quantities of nutrient additives as per Table 12 , Everris Osmocote™ Exact Standard 3-4 Month, nutrient contents as per Appendix Table 13; Everris Osmoform™ NXT 22N, nutrient concentrations as per Appendix Table 14). The two other treatments were low nutrient (L) and pulsed nutrient (P). The potting mix for these two treatments was initially identical and was assembled from the

constituent components of the commercial potting mix, without the addition of additives or slow release fertiliser granules (Appendix Table 15). The low nutrient treatment did not receive any nutrient addition for the extent of the experiment. Pulse treatment plants were given a dose of dilute liquid fertiliser once every four weeks. The liquid fertiliser used was Yates Thrive™ Concentrate All-Purpose, diluted with tap water at a ratio of 1:400 (undiluted fertiliser nutrient concentrations in Appendix Table 16). I applied 250mL of dilute solution to the potting mix surface at each 4-weekly dosing. Yates Thrive™ Concentrate All-Purpose was selected as it did not contain any plant growth hormones which may otherwise have affected trait expression and plant growth.

Seedlings were randomly arranged on tables in a glasshouse, and were randomly re-arranged once per month in order to minimize the effects of any potential microclimatic gradients. Average daily glasshouse temperature was 16.1°C, and relative humidity averaged 76.8%. Plants were hand watered evenly to avoid drought stress and to facilitate growth. Timed halogen lamps were also used to promote growth. Lamps were initially on from 07:00 to 18:00 hrs, and gradually increased to 06:00 to 20:00 hrs over the course of a month. Pest insects were controlled using insecticide sprays (pyrethrum based and Imidacloprid), as well as hanging adhesive strips. An outbreak of thrips (Order Thysanoptera) occurred during the second month of the experiment, particularly affecting the smaller *Knightia* seedlings. Seedlings badly affected by thrip damage were excluded from the final data.

Trait measurements

Initial height measurements were taken two weeks after re-potting to allow plants to re-establish in their new pots. Height was measured from the soil surface to the tallest apical bud using a measuring tape. Height measurements were taken monthly from May to November 2014. Once a seedling had grown sufficiently for freshly grown fine roots to be present, a final height measurement was recorded. Relative growth rate (RGR) of individual seedlings was calculated as RGR = ((final height − initial height)/initial height)/days of growth. Following final height measurements, roots were washed by gently massaging the root balls under water. Loose potting mix and wash water were passed through a 5 mm mesh. Fine roots were extracted from the sieve and retained with the main root ball,

permitting the vast majority of the root biomass to be utilised for total biomass measurement. Root-to-shoot ratio was calculated as total root dry mass divided by total shoot dry mass. Trait measurements for leaves, roots, and stems were conducted according to protocols published by Cornelissen et al. (2003b) and Pérez-Harguindeguy et al. (2013).

Secateurs were used to cut a short section of stem (approximately 1 – 3 cm in length) containing mature xylem. Bark was removed by peeling or scraping. The remaining stem section was rolled in paper towels to remove any residual surface moisture or sap before recording its fresh mass. A Mettler-Toledo MS304S balance (Greifnesee, Switzerland) was used for all measurements of mass. The length and orthogonal diameter dimensions of the stem section were measured using digital Vernier calipers (Measuremax IP54, Peterborough, Canada) and the fresh volume of the stem was calculated using the standard equation for volume of a cylinder. The stem sections were then dried to constant mass at 60°C for at least 48 hours prior to measuring dry mass. Stem dry matter content (SDMC) was calculated as SDMC = stem dry mass / stem fresh mass, and stem tissue density (Stem TD) was calculated as Stem TD = stem dry mass / fresh stem volume (Table 1).

Three leaves were sampled from each plant. Instead of individual leaves of *Dacrycarpus*, I sampled three small non-woody branches with numerous leaves attached. After cleaning and patting dry, digital Vernier callipers were used to take thickness measurements of three separate leaves, avoiding any prominent leaf veins. Leaf area was measured on a LI-COR Biosciences LI-3100C (Lincoln, NE USA) leaf area meter. After measuring fresh mass, leaves were dried to constant mass at 60°C for at least 48 hours prior to obtaining dry mass. SLA was calculated as SLA = leaf area / leaf dry mass, leaf dry matter content (LDMC) was calculated as LDMC = leaf dry mass / leaf fresh mass, and leaf tissue density (Leaf TD) was calculated as Leaf TD = leaf dry mass / fresh leaf volume (Table 1).

Subsections of fine roots were removed from the main root ball and transferred into a clear acrylic tray on a flat-bed scanner. Two fine paintbrushes were used to spread the roots out to minimise overlap. Total root length, average root diameter, number of tips, and root volume were calculated using WinRhizo Pro software (Version 2012b, Regent Instruments Inc., Quebec City, Canada) and an Epson

Expression 10000XL scanner (Tokyo, Japan). Fresh root mass of each sample was obtained after drying excess surface moisture with paper towels. The root sections were then dried to constant mass at 60°C for at least 48 hours prior to obtaining dry mass. SRL was calculated as SRL = root length / root dry mass, root dry matter content (RDMC) was calculated as RDMC = root dry mass / root fresh mass, root tissue density (Root TD) was calculated as Root TD = root dry mass / fresh root volume, and root branching intensity (RBI was calculated as RBI = number of root tips / root length (Comas and Eissenstat, 2009) (Table 1).

To measure root porosity, I used the microbalance method as described by Visser and Bögemann (2003). Ten short sections of fresh root were cut from the root ball using a sharp razor blade. The sections were typically between 5 to 10 mm in length, and were cut at least 10 mm back from the root tip. Roots which appeared to have undergone secondary thickening were not sampled. Whenever possible, sections were taken from first order terminal roots. In species where the terminal roots were too fine for this technique, sections were cut from second order roots where no secondary thickening was apparent. As each suitable section was identified and cut, they were gently washed with fine paintbrushes to remove any remaining surface soil, and kept under water to minimise water loss while the remaining sections were cut. Once all ten sections had been cut, they were transferred using fine paintbrushes onto a piece of dry tissue paper. The paintbrushes were used to gently roll the root sections on the tissue paper, removing any surface moisture. The tissue paper was folded over to enclose the root sections and gentle pressure was applied by hand over the root sections. Care was taken to avoid crushing or bursting the cells in the root tissue, but effectively forcing out water from the root sections' internal porous spaces.

The root sections were transferred to a weigh boat on a tared balance and weighed. This initial mass was recorded, and the root sections were transferred into a small glass vial which was then filled with water. The cap for this vial was secured loosely to avoid creating a pressure seal, yet tight enough to prevent the lid coming off during rapid pressure changes. The vial was then placed into a near vacuum chamber, which was run for three repetitions of five minutes each. At the end of each five minute period, the vacuum chamber was opened, causing a rapid re-pressurisation of the vial and forcing water into the aerenchyma of the root sections. After the low pressure exposure periods were completed, the vial was

removed from the chamber and opened. The root sections were gently removed from the vial using a fine paintbrush, placed on dry tissue paper and briefly rolled to remove surface moisture. The root sections were transferred onto a weigh boat on a tared balance, and had their final mass recorded. The method differed from that presented by Visser and Bögemann (2003) in that it did not use two-piece hard gelatin capsules to hold the root sections between blotting and weighing. This is due to the observation that if the surface moisture was properly blotted, the subsequent mass change from water evaporation was slow and could be negated by minimising the time between blotting and weighing. Root porosity was calculated as (final root mass − initial root mass) / final root mass * 100 (Table 1).

Knightia roots produced a unique response to the different nutrient treatments in the form of proteoid cluster root development (Dinkelaker et al., 1995, Skene, 1998, Neumann and Martinoia, 2002). For each *Knightia* root ball, discrete clusters of proteoid roots were counted, with the total number then divided by total dry root mass.

Species' average trait values under each nutrient treatment are presented in Appendix Table 17.

Analysis of root N and P concentration

The remaining root ball from each plant was dried to constant mass at 60°C for at least 48 hours. Individually, fine roots from the dried root balls were ground to a fine powder using a Retsch MM 2000 grinder (Haan, Germany). Root nitrogen and phosphorus content of ground roots was obtained using flow injection analysis on a Lachat QuikChem 8000 series (test number and protocol 206 Landcare Research Environmental Chemistry Laboratory, Palmerston North, New Zealand).

Statistical Analysis

Prior to ANOVA all traits were assessed for normal distribution using Statistica 12 (© Statsoft Inc, 1984 – 2014). As there were insufficient replicates to test normality for each trait by species and treatment combination, normality was instead assessed for each trait for the entire species and treatment dataset. Log_{10} transformations were applied to reduce skew on the following traits: SRL, Root diameter, SLA, Leaf TD, LDMC, Stem TD, and root-to-shoot ratio. Two-way

factorial ANOVA was performed in Statistica 12, using type III sum of squares. F-test statistics and associated significance levels were recorded. To determine whether treatment means were significantly different following a significant treatment effect, I used Tukey's procedure post-hoc analysis between treatment means for each species and trait. *Knightia* cluster roots were only comparable between *Knightia* treatments; therefore I used one-way ANOVA with type III sum of squares.

Co-efficient of variation (CV) scores were used as a relative index of plasticity (Schlichting and Levin, 1986). For each trait, average CV scores were calculated by species using the formula: CV = standard deviation of treatment means ÷ grand mean of treatment means × 100. CV scores were calculated using raw trait data, as opposed to $\log_{10}$ transformations. All four species' CV scores were averaged for each trait, and then ranked to compare the relative plasticity. Comparisons of average CV values were also made between angiosperms and conifers, or 'high fertility' and 'low fertility' associated species (Appendix Table 18).

Mycorrhizal associations

Species' mycorrhizal associations were determined from the literature (McNabb, 1958, McNabb, 1961, Baylis, 1969, Baylis, 1971, Johnson, 1973, Baylis, 1975, Cooper, 1975, Hall, 1975, Cooper, 1976, Baylis, 1980, St John, 1980, Russell et al., 2002, Dickie et al., 2012), and are presented in Appendix Figure 14.

Table 6. Functional trait abbreviations and units.

Trait	Abbreviation	Units
Knightia cluster roots	-	clusters mg^{-1}
Relative growth rate	RGR	cm cm^{-1} day^{-1}
Root-to-shoot ratio	R:S	ratio
Root phosphorus content	Root P	%
Root nitrogen content	Root N	%
Root branching intensity	RBI	cm^{-1}
Root porosity	-	%
Root dry matter content	RDMC	mg g^{-1}
Root tissue density	Root TD	mg mm^{-3}
Specific root length	SRL	m g^{-1}
Specific leaf area	SLA	mm^2 mg^{-1}
Leaf tissue density	Leaf TD	mg mm^{-3}
Stem dry matter content	SDMC	mg g^{-1}
Leaf dry matter content	LDMC	mg g^{-1}
Stem tissue density	Stem TD	mg mm^{-3}
Root diameter	-	mm

3.4 Results

3.4.1 Trait plasticity in response to soil nutrients

At the end of the experiment, soil N and P concentrations were highest in the slow release treatment and lowest in the low and pulse treatments, and final nutrient concentrations within each treatment did not differ significantly between species (Figure 7).
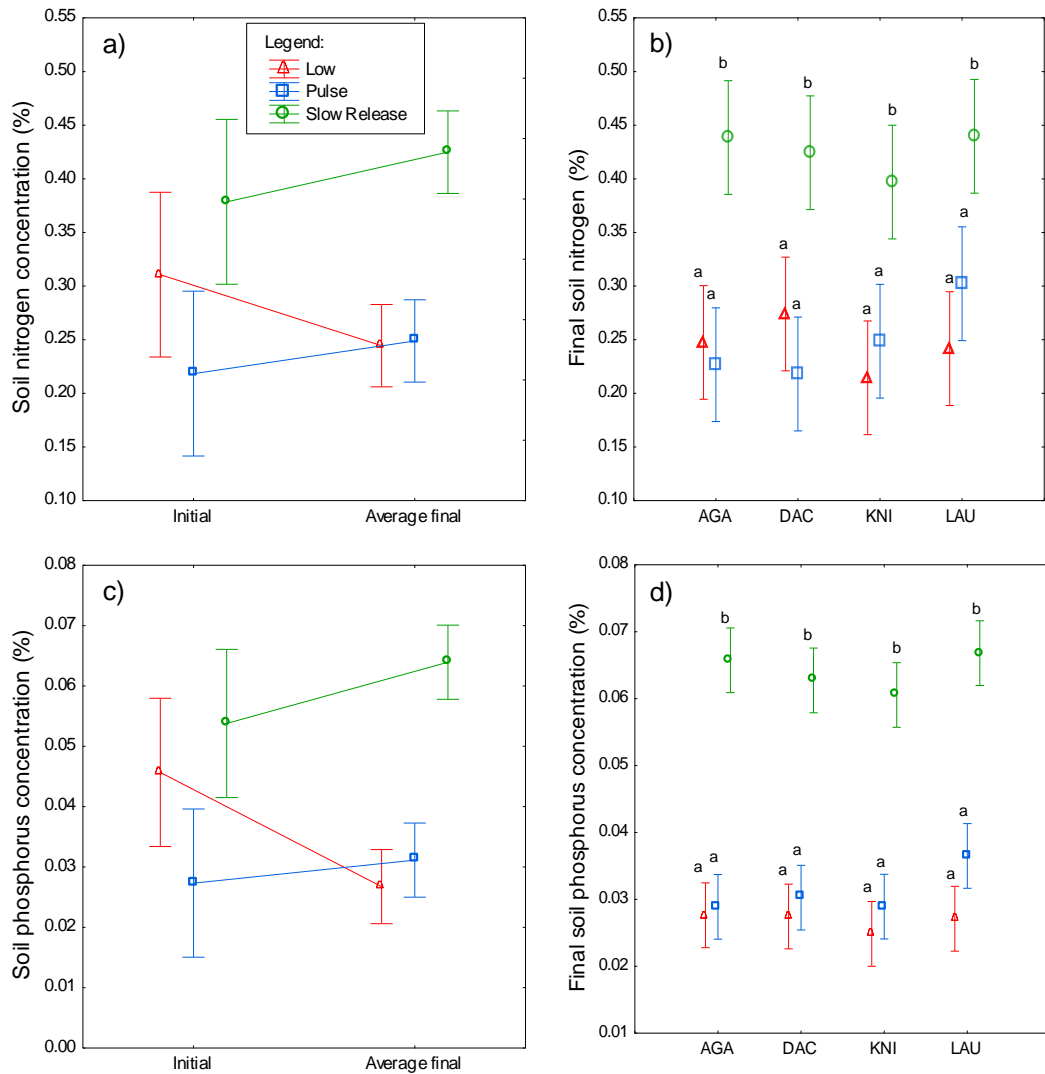
Figure 7. Mean and 95% confidence intervals for soil concentrations of nitrogen and phosphorus. a) Initial and average final soil nitrogen concentrations; b) final soil nitrogen concentration by species; c) Initial and average final soil phosphorus concentrations; d) final soil phosphorus concentration by species. Final concentrations in a) and c) are averages of all four species' final concentrations as per b) and d). Lower case letters above whiskers on b) and d) represent significant differences ($p < 0.05$) between treatment means as determined by Tukey's procedure post-hoc analysis.

All functional traits differed strongly among species, and the main effect of fertiliser treatment was significant for all traits except SLA (Table 7). There were also many significant species × treatment interactions indicating that the response of some traits to soil nutrients differed by species (Table 7). SRL, porosity, leaf tissue density, and stem tissue density did not exhibit significant species × treatment interactions (Table 7).

Table 7. F statistics and significance levels for species, treatment, and species × treatment interaction for each functional trait.

Trait	Species (d.f. = 3)		Treatment (d.f. = 2)		Species × Treatment interaction (d.f. = 6)	
SRL	307.92	***	3.19	*	2.12	
Root Diameter	560.83	***	6.73	**	3.24	**
Root TD	179.25	***	19.44	***	2.75	*
RDMC	228.13	***	19.89	***	5.00	***
Porosity	26.4	***	4.56	*	0.90	
RBI	35.82	***	15.55	***	3.26	**
Root N	215.57	***	163.84	***	4.64	***
Root P	64.87	***	84.72	***	11.32	***
SLA	76.44	***	2.03		3.82	**
Leaf TD	151.38	***	10.97	***	1.53	
LDMC	103.74	***	7.72	***	2.23	*
Stem TD	296.05	***	14.25	***	2.09	
SDMC	311.64	***	25.63	***	2.80	*
RGR	38.95	***	33.79	***	3.87	**
R:S	58.94	***	84.89	***	7.55	***
Knightia cluster roots			7.20	**		

Significance level represent by asterisks. * < 0.05, ** < 0.01, *** < 0.001

Across all species, relative growth rate was the most plastic trait, followed by root-to-shoot ratio, and root N and P concentrations (Table 8). The most plastic trait was cluster roots per dry mass, but this was only relevant to *Knightia*. All other traits exhibited much weaker plastic responses. Root branching intensity was the most plastic root morphology trait, while fine root diameter was the least plastic trait overall. Tissue densities and dry matter contents for leaves, roots, and stems had relatively low levels of plasticity, although of the three organs, root tissue density and root dry matter content had the most plastic response to the nutrient treatments.

Table 8. Co-efficient of variation (CV) scores by species and trait. Traits sorted by descending average CV score. Trait abbreviations as per Table 6.

Trait	*Agathis*	*Dacrycarpus*	*Knightia*	*Laurelia*	**Average**
Cluster roots	-	-	70.3	-	**70.3**
RGR	52.8	53.5	58.8	36.3	**50.4**
R:S	13.4	56.9	39.4	29.8	**34.9**
Root P	44.6	12.8	52.4	24.6	**33.6**
Root N	26.2	23.6	39.6	28.6	**29.5**
RBI	6.7	20.5	22.3	16.6	**16.5**
Porosity	4.3	15.0	11.5	11.3	**10.5**
RDMC	2.6	9.6	19.5	10.1	**10.5**
Root TD	2.7	9.4	17.8	11.5	**10.3**
SRL	7.4	6.7	14.0	7.3	**8.9**
SLA	10.3	7.2	9.3	8.7	**8.9**
Leaf TD	1.4	13.2	10.2	7.0	**8.0**
SDMC	2.4	9.5	3.2	10.6	**6.4**
LDMC	3.3	7.3	6.7	8.0	**6.3**
Stem TD	2.7	5.4	4.9	9.6	**5.7**
Root diameter	2.6	1.7	8.3	9.1	**5.4**
Species average	**12.2**	**16.8**	**21.2***	**15.3**	

* average CV value for *Knightia* excludes CV of cluster roots.

Responses to nutrient treatments for each trait were generally similar between species, but the only trait where all four species did not exhibit any significant difference between treatments was porosity (Figure 9.a). For most traits, slow release treatment was significantly different to low treatment in at least one species (Figure 8.a-e; Figure 9.b, c, d, f; Figure 9.a, b, c, d). In instances where slow release and low treatments were significantly different, pulse treatment was typically also significantly different from low treatment (Figure 8.a-e; Figure 9.b, c, d, f; Figure 10.b, c, d). Also, in most instances pulse treatment was not significantly different from slow release treatment, except for three traits where a single species exhibited a significantly different response to each of the three treatments (*Knightia* root P (Figure 8.c); *Dacrycarpus* root N (Figure 8.d); and *Knightia* RDMC (Figure 9.d))
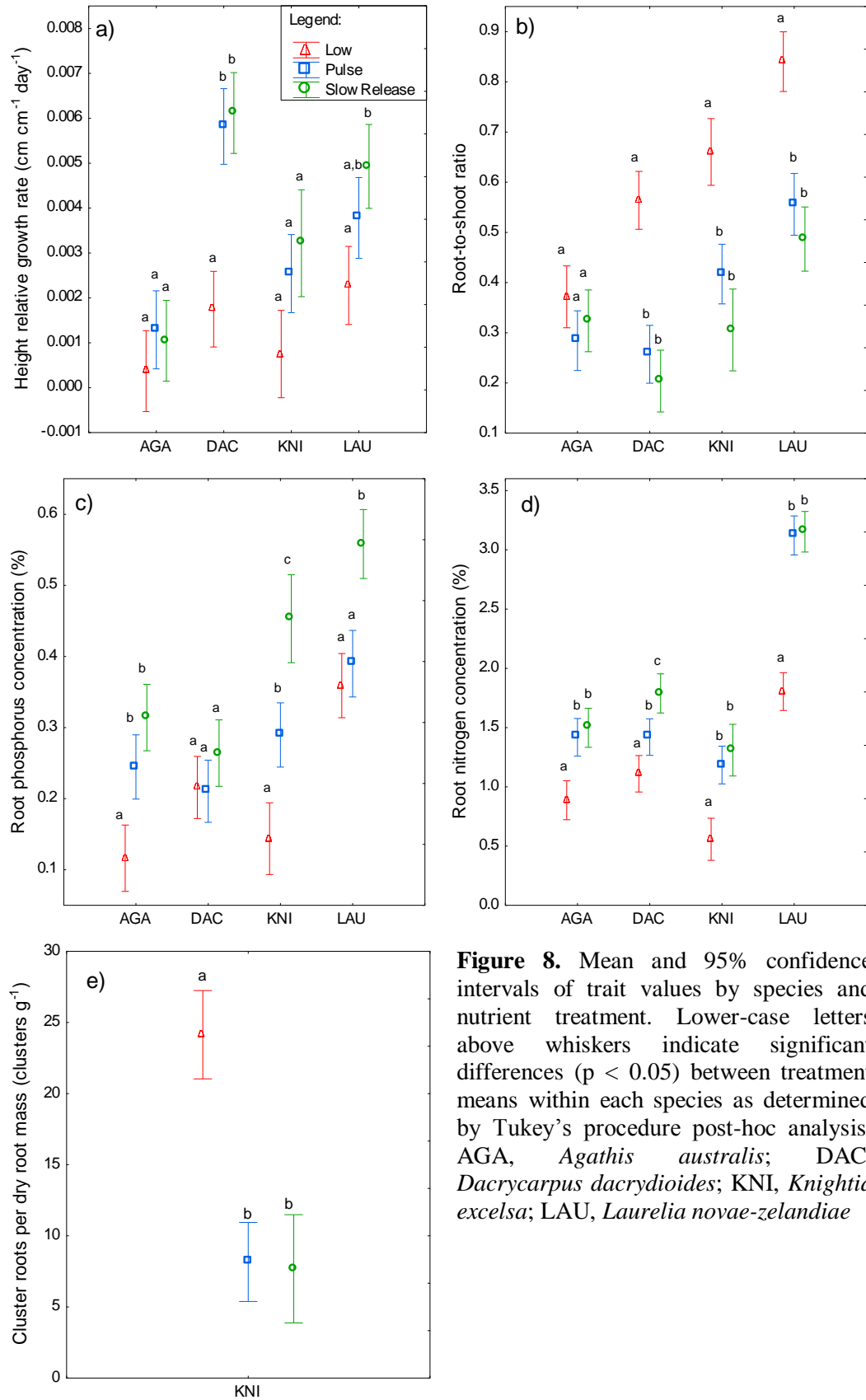
Figure 8. Mean and 95% confidence intervals of trait values by species and nutrient treatment. Lower-case letters above whiskers indicate significant differences ($p < 0.05$) between treatment means within each species as determined by Tukey's procedure post-hoc analysis. AGA, *Agathis australis*; DAC, *Dacrycarpus dacrydioides*; KNI, *Knightia excelsa*; LAU, *Laurelia novae-zelandiae*
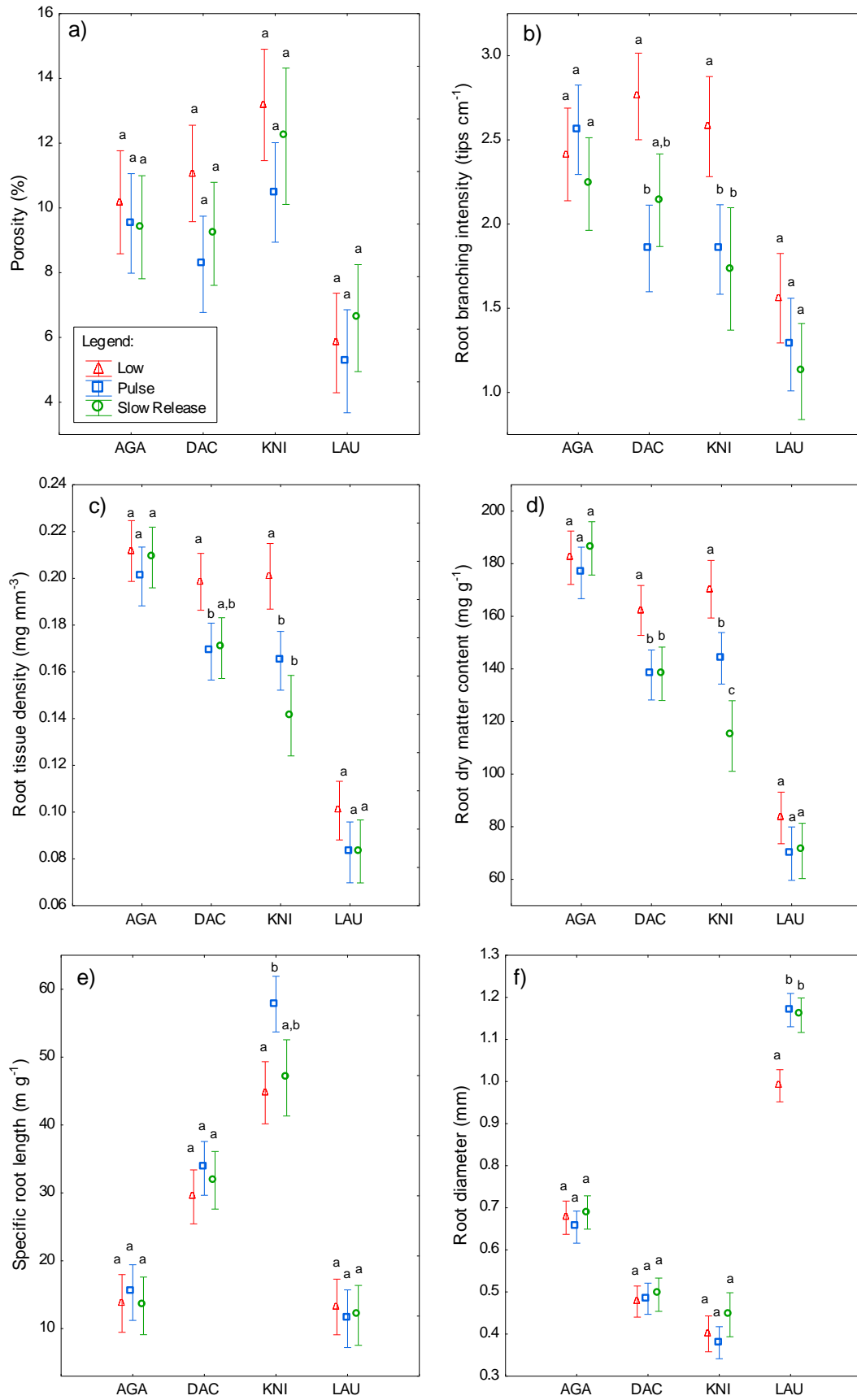
Figure 9. Mean and 95% confidence intervals of trait values by species and nutrient. Lower-case letters above whiskers indicate significant differences (p < 0.05) between treatment means within each species as determined by Tukey's procedure post-hoc analysis. AGA, *Agathis australis*; DAC, *Dacrycarpus dacrydioides*; KNI, *Knightia excelsa*; LAU, *Laurelia novae-zelandiae*

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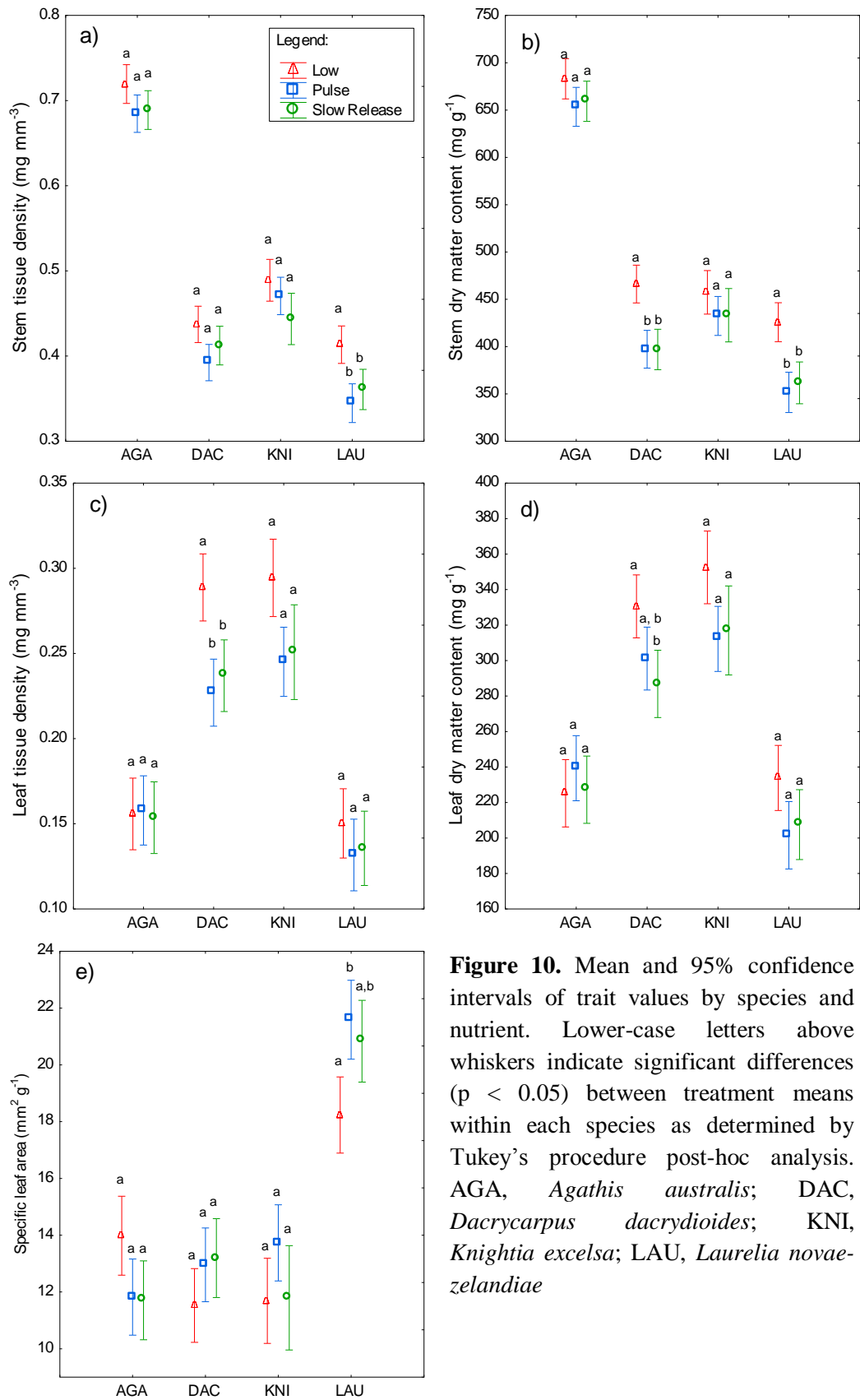
Figure 10. Mean and 95% confidence intervals of trait values by species and nutrient. Lower-case letters above whiskers indicate significant differences (p < 0.05) between treatment means within each species as determined by Tukey's procedure post-hoc analysis. AGA, *Agathis australis*; DAC, *Dacrycarpus dacrydioides*; KNI, *Knightia excelsa*; LAU, *Laurelia novae-zelandiae*

3.4.2 Species' unique responses

The two conifers exhibited very different responses to soil fertility. *Agathis* was the least plastic species on average across all traits (Table 8), and had the lowest total number of traits which responded to soil nutrient availability. For 13 out of 15 traits, mean values for *Agathis* were not significantly different between treatments. The two traits which did show significant differences between treatments were both related to root chemistry rather than morphology, with low nutrient treatment resulting in significantly lower root nitrogen and phosphorus content compared with both pulse and slow release treatments (Figure 8.c,d). In contrast, *Dacrycarpus* was moderately plastic across all traits (Table 8), and had significantly different responses between nutrient treatments for nine traits (RGR, R:S, root N, RBI, root TD, RDMC, SDMC, leaf TD, and LDMC). In contrast with the other species, there were no significant differences between treatments for *Dacrycarpus* root phosphorus concentration (Figure 8.c). *Dacrycarpus* also had the largest difference in relative growth rate, with both pulse and slow release treatments resulting in a highly significant increase in growth (Figure 8.a).

The two angiosperm species were on average more plastic than the two conifer species (appendix table). *Knightia* was the most plastic species overall (Table 8), and had significant differences between treatments for seven out of 15 traits (R:S, root P, root N, RBI, root TD, RDMC, and SRL). *Knightia* also exhibited a unique morphological response in the form of proteoid cluster roots, producing significantly more cluster roots per unit dry root mass under low nutrient treatment compared to both pulse or slow release nutrient treatments (Figure 8.e). *Laurelia* was also moderately plastic across all traits (Table 8), and had significant differences between treatments for eight out of 15 traits (RGR, R:S, root P, root N, root diameter, stem TD, SDMC, and SLA). *Laurelia* was the only species which had a significantly smaller root diameter under low treatment compared to pulse and slow release treatments (Figure 9.f), although this did not also result in a significant difference to SRL between treatments (Figure 9.e).

3.5 Discussion

This experiment yielded two results that have broad implications for functional ecology. First, functional traits differ in their intraspecific response to soil nutrient treatments, but morphological traits related to the 'whole-plant economic spectrum' are relatively stable among soil fertility gradients compared to traits such as biomass allocation and tissue nutrient concentrations. Second, the method of nutrient delivery (i.e. slow release or pulses) does not consistently affect trait expression. These results imply that morphological root traits can be justifiably be pooled among studies to examine broad scale global patterns. However, caution must be made when pooling data on biomass allocation or tissue nutrient concentrations given their plastic response to soil nutrient availability.

3.5.1   Phenotypic plasticity of traits

Relative growth rate, root-to-shoot ratio, and both root N and P concentrations were the most plastic traits that were measured on all four species (Table 8). It is no surprise that growth rate increased as a result of higher availability of soil nutrients, as nutrient limitation has a widely observed negative effect on growth (Epstein, 1972, Chapin III, 1980, Cromer and Jarvis, 1990, Porter and Lawlor, 1991, Gregory, 2006). It is logical that root N and P concentrations were also strongly plastic in response to nutrient availability, as higher nutrient availability will result in faster rates of diffusion or active uptake of nutrient ions from the soil into the roots (Aerts and Chapin, 1999). Root-to-shoot ratio is perhaps the most interesting trait which responded strongly to soil fertility, as it represents a deliberate adjustment of growth by the plant, acting to maximize resource uptake in response to limiting resources. Root-to-shoot ratio is perhaps the most well documented and understood plastic response to resource limitation, with numerous previous studies observing adjustments to biomass allocation in response to limiting nutrients and/or light (Brouwer, 1962, Aung, 1974, Chapin III, 1980, McGraw and Chapin, 1989, Cromer and Jarvis, 1990, Garnier, 1991, Schippers and Olff, 2000, Müller et al., 2000, Dyer et al., 2001, Hill et al., 2006, Louw-Gaume et al., 2010, Grassein et al., 2010, Freschet et al., 2015). In resource-limited conditions, root-to-shoot ratio forms a "functional equilibrium" to maximise the uptake of growth-limiting resources, although light limitation tends to induce alterations of leaf morphology (i.e. SLA), while nutrient limitation

tends to induce higher relative biomass allocation to roots, rather than changes to root morphology (i.e. SRL) (Freschet et al., 2015).

Neither root diameter nor SRL were strongly responsive to nutrient availability. While previous studies have shown SRL to respond to soil nutrient availability in some species (Clemensson-Lindell and Asp, 1995, Hill et al., 2006, Kalliokoski et al., 2010), my results agreed with other studies that have shown that intraspecific SRL does not universally respond to nutrient availability (Boot and Mensink, 1990, Cromer and Jarvis, 1990, Borken et al., 2007, Kalliokoski et al., 2010). Furthermore, average fine root diameter was the least plastic trait in this study, and only differed among treatments within *Laurelia*. This supports previous conclusions that root diameter is strongly phylogenetically conserved (Pregitzer et al., 2002, Comas and Eissenstat, 2004, Withington et al., 2006, Kembel and Cahill Jr, 2011, Alvarez-Uria and Körner, 2011, Chen et al., 2013, Valverde-Barrantes et al., 2014). In contrast, Zobel et al. (2007) observed both increases and decreases to 12 species' root diameters in response to nutrient availabilities, but noted that the availability of different nutrients (including nitrate, phosphorus, aluminium, ammonium and tannic acid) triggered different responses, and that the interaction of species and nutrient was significant. Both root tissue density and root dry matter content were moderately plastic, increasing in the low nutrient treatments. Schläpfer and Ryser (1996) did not observe grass species' root tissue density significantly changing across three sites ranging in fertility, and few other studies have directly measured plasticity of root tissue density or dry matter content in response to soil fertility. However, higher tissue density and dry matter content are traits associated with slow growing species and low fertility sites (Jager et al., 2015, Pérez-Ramos et al., 2012, Craine and Lee, 2003, Ryser, 1996). In my experiment, the single instance of SRL significantly differing between treatments (Figure 9.e) was accompanied by a significant difference in root tissue density (Figure 9.c), but not in root diameter (Figure 9.f). This is consistent with an interpretation by Craine et al. (2001), which suggests that plastic responses of SRL are due to modifications root tissue density and dry matter content, rather than root diameter.

Root branching intensity, porosity, root tissue density, and root dry matter content were moderately plastic (Table 8). Root branching intensity in herbaceous species has been shown to increase in localised patches of soil nutrients (Drew et al.,

1973, Drew, 1975, Gross et al., 1993, Hutchings and de Kroon, 1994, Larigauderie and Richards, 1994, de Kroon and Hutchings, 1995). In contrast, my results show root branching intensity was significantly higher in the low nutrient treatments for two species (Figure 9.b). It is not clear whether woody species generally respond to low nutrient availability by increasing RBI, however at the community level RBI has been shown to negatively correlate with phosphorus availability (Holdaway et al., 2011), and to have a non-linear relationship with soil fertility, highest on low fertility soils, and lowest on intermediate soil fertility (Chapter Two, Figure 5.f). Therefore, while RBI in woody species may correlate with soil fertility at the community level, more experimental research is required to determine if increased RBI in response to low nutrient availability is a common plastic response among woody species. Root branching intensity has also been demonstrated to be influenced by mycorrhizal association (Berta et al., 1995, Comas and Eissenstat, 2009, Comas et al., 2014) and phylogeny (Roumet et al., 2006, Roumet et al., 2008). It is not apparent how interactions between soil nutrient availability, mycorrhizal associations, and species' phylogeny combine to influence the extent of phenotypic plasticity of root branching intensity.

Porosity (or formation of aerenchyma) is typically associated with waterlogging and flood tolerance, as aerenchyma can facilitate gas exchange between roots and air spaces in the soil (Laan et al., 1989, Jackson and Armstrong, 1999, Visser et al., 2000). However, studies in crop species have demonstrated that higher porosity can be beneficial in both drought tolerance (Zhu et al., 2010, Jaramillo et al., 2013) and both nitrogen and phosphorus acquisition (Fan et al., 2003, Saengwilai et al., 2014). The trade-offs of increased porosity are unconfirmed, but may include reduced mycorrhizal colonisation, vulnerability to the longitudinal spread of pathogens, reduced storage capability, and decreased ability to transport water and nutrients (Fan et al., 2003). Whether porosity serves these functions in woody species has not been experimentally trialled. Interestingly, porosity was typically highest in the low and slow release nutrient treatments, and lowest in the pulsed treatments (Figure 9.a), although what drives this pattern is unknown. Porosity is not a commonly measured root trait, but my results suggest that further research into the advantages and disadvantages of porosity may help to understand its role in plant strategies.

Leaf traits were weakly responsive to nutrient availability. Most species are capable of altering leaf morphology under contrasting light availabilities (Meziane and Shipley, 1999, Valladares et al., 2000a, Valladares et al., 2000b, Meziane and Shipley, 2001, Rozendaal et al., 2006), although species' responses to shading are not universal (Lusk et al., 2008). SLA has been demonstrated to decrease under low nutrient availability conditions in herbaceous angiosperms (Meziane and Shipley, 1999, Navas and Garnier, 2002) and grasses (Knops and Reinhart, 2000), although other studies have not observed strong responses of SLA or LDMC to nutrient availability (Valladares et al., 2000a, Grassein et al., 2010). The effect of low soil N availability may be more pronounced on physiological or leaf chemistry traits such as net assimilation rate or leaf nitrogen content (Meziane and Shipley, 1999). In my experiment, SLA, leaf TD and LDMC were each only significantly different between treatments for one out of four species, suggesting that the leaf traits of woody seedlings do not respond strongly to nutrient limitation, although more data would help to confirm the generality of this observation.

Stem tissue density and stem dry matter content are highly correlated (Table 4) and neither strongly responded to nutrient availability. It has been suggested that plastic responses may be limited for these traits due to high heritability and phylogenetic constraints (Chave et al., 2006, Swenson and Enquist, 2007). However, some degree of phenotypic plasticity is known to occur in stem tissue density, resulting from mechanical stress (Woodcock and Shier, 2003) or as a function of ontogenetic development and height (Chave et al., 2009, Hietz et al., 2013). Stem tissue density is also strongly associated with water availability and drought tolerance, as denser stems are indicative of anatomical traits which confer cavitation resistance (Hacke et al., 2001, Pratt et al., 2007, Martinez-Meier et al., 2008). There are few studies which have investigated the effects of soil nutrient availability on stem tissue density. Soil phosphorus availability was negatively correlated with stem tissue density for only one out of four *Nothofagus* species (Richardson et al., 2013). In contrast, studies of forestry trees have demonstrated that long-term nutrient addition can result in a 20% decrease to stem tissue density (Cown and McConchie, 1981, Mäkinen et al., 2002). Only long-term differences in nutrient concentrations are likely to result in plastic responses to tissue density, with year-to-year differences in stem tissue density forming radial gradients

(Hietz et al., 2013). As the plants in my experiment were only grown for three to four months under the differing nutrient treatments, it is unlikely that they produced enough new wood to result in plastic changes to stem density. Therefore, while leaf and stem traits can exhibit phenotypic plasticity in response to environmental variables, my experimental design controlled for the variables to which these traits respond strongly (i.e. light and water availability, ontogenetic development). The weak plasticity I observed in stem and leaf traits demonstrates that nutrient availability is not a strong driver of phenotypic plasticity in the leaves and stems of tree seedlings.

Altogether, my results suggest that plant morphological traits are not strongly affected by nutrient availability, but that relative growth rate, biomass allocation, and root chemistry traits are more strongly affected nutrient availability. This has implications for pooling trait data among studies where plants were not grown in identical soil conditions, as is often done in large trait databases (Kattge et al., 2011). Particularly, morphological traits relating to the whole-plant economic spectrum (as discussed in Chapter Two) are broadly transferable between studies, so long as the plants are raised in conditions conducive to growth. If resources such as nutrients, water, or light are limited, then phenotypic plasticity may result in altered trait expressions to optimize growth. However, as these resources are often limited in natural ecosystems, more studies are needed to compare the traits of natural sourced plants with those raised in glasshouses. Also, it was beyond the scope of this study to determine how traits respond to deficiencies of any particular nutrient, and as natural soils can vary widely in their availabilities of vital nutrients, these types of experiments could also have valuable application for comparisons of natural sourced versus glasshouse raised plants.

3.5.2 Pulse vs. slow release treatments

Despite the difference in final nutrient concentrations between the pulse and slow release treatments (Figure 7.a-d), traits responded similarly to these two different methods of nutrient delivery. Species typically exhibited significantly different responses to low and pulse nutrient treatments, but responded similarly to slow release and pulse nutrient treatments (Figure 8, Figure 9, Figure 10). I hypothesised that pulse treatment would be a better proxy for natural low fertility soils than slow release treatment, as nutrient availabilities in natural soils can fluctuate as a result of natural processes including fire, freezing, nutrient

mobilisation after rain, and animal excretions (Hobbs et al., 1991, Smith et al., 1998, Schimel and Bennett, 2004, Reynolds et al., 2004). In low fertility soils, pulses may be more crucial to plant productivity as they represent a short term flux of nutrients into an otherwise poor soil, facilitating the growth of species able to exploit them. Craine (2009) theorised that in many low fertility systems, pulses are of low importance to plant growth, as pulses tend to occur at low productivity times of the year, and low-fertility adapted species tend to lack root adaptations for rapid proliferation and exploitation of nutrient pulses. Species which are adapted to low fertility sites may have reduced or lost the sensory and regulatory mechanisms required to detect and exploit nutrient pulses, in order to reduce the energetic cost of growth and metabolism (DeWitt et al., 1998).

However, these lines of reasoning are not consistent with observational or experimental evidence. Deserts are typically characterised by low productivity and low soil fertility; however, sporadic rainfall mobilises nutrients and results in dramatic increases to productivity due to species' exploitation of both water and nutrient resources (Austin et al., 2004). Furthermore, experimental results suggest that species with conservative growth are able to exploit short term nutrient pulses (<10 hours) more efficiently than faster growing species (Campbell and Grime, 1989). Species adapted to low soil fertility have been demonstrated to have a relatively low capacity to absorb immobile ions such as phosphate (Raab et al., 1999), but a relatively high capacity to absorb mobile ions such as potassium (Veerkamp and Kuiper, 1982). In my experiment, the only species which responded consistently with Craine's theory was *Agathis*, which did not respond strongly to either pulsed or slow release treatments for the majority of traits. *Agathis* was the species which exhibited the lowest average plasticity, and also the species most strongly associated with low fertility soils. *Knightia* is also associated with low fertility soils, although not as strongly as *Agathis*, and yet it responded to both pulse and slow release nutrient treatments. This may be due to the ability of *Knightia* to form cluster roots (Figure 11), which are highly efficient at phosphorus uptake and may also be an adaptation to exploit patches and pulses of nutrients (Dinkelaker et al., 1995, Skene et al., 1996, Skene, 1998).

The general similarities between traits of both slow release and pulse treatment plants across all four species suggest that the method of fertiliser application does not significantly alter morphological trait expression. The differences in tissue

nutrient concentrations between slow release and pulse treatment were likely due to differences in the total amount of nutrients available in each treatment. Future experiments should examine the effects of different pulse regimes, varying both the amount of fertiliser applied and the length of time between pulses. This will reveal whether differences between pulse and slow release treatments are due to the pulse itself, or due to total nutrient availabilities.

3.5.3 Group responses

In contrast with predictions from CSR theory (Grime, 1979), I did not observe higher plasticity in high fertility associated species when compared to low fertility associated species (Appendix Table 17). This is at least partially due to my experimental design, which was limited to only four species, and also included a separate grouping by phylogenetic group. Phylogeny was a better predictor of species' plasticity, with conifer species being less plastic on average than angiosperm species (Appendix Table 17). However these results should be interpreted tentatively, as the phylogenetic trend was largely driven by two species: the conifer *Agathis*, which was the least plastic species in my experiment; and the angiosperm *Knightia*, which was the most plastic species. The other two species, *Dacrycarpus* and *Laurelia,* were both of similar intermediate plasticity despite their contrasting phylogenetic groupings. These phylogenetic groupings were perhaps too broad, as the four species were members of four different families, being Araucariaceae (*Agathis*), Podocarpaceae (*Dacrycarpus*), Proteaceae (*Knightia*), and Atherospermataceae (*Laurelia*). In particular, the pairing of both *Laurelia* and *Knightia* was not an ideal representation of angiosperms. As a magnoliid, *Laurelia* has large fine root diameter and a high dependence on mycorrhizal symbiosis (Baylis, 1975), while the eudicot *Knightia* is non-mycorrhizal (Tozer, 2006), and produced cluster roots in response to low nutrient availability (Figure 10.e). Ideally, future plasticity experiments should include multiple species from within a single family or genus, while also spanning a range of soil fertility associations. A good example of this would be the New Zealand Podocarpaceae, which span from low fertility associated *Halocarpus kirkii* to the high fertility associated *Podocarpus totara*, as well as including con-generics such as the multiple *Podocarpus*, *Halocarpus*, and *Prumnopitys* species. By limiting the amount of phylogenetic variation in the experimental species pool, stronger conclusions could be reached about the influence of species' soil fertility

associations on phenotypic plasticity. Similarly, to more robustly test the effects of phylogeny on phenotypic plasticity a larger range of species should be included, ideally incorporating phylogenetically diverse species from within naturally occurring plant communities. In summary, my results show preliminary support for conifers being in general less plastic than angiosperms, and little difference in plasticity between high vs. low fertility associated species. However, due to low numbers of species sampled, and strong influence from individual species, additional data is needed in order to conclude upon the generality of these group plasticity trends.

3.5.4   Cluster roots

Knightia significantly increased its production of cluster roots in response to low nutrient availability (Figure 11). Cluster roots represent   "the third great adaptation for improved nutrient uptake in plants, the others being nitrogen fixation in root nodules and mycorrhizas" (Skene, 1998), and are phylogenetically restricted to members of two monocotyledonous and eight dicotyledonous families (Lambers et al., 2006). Cluster roots are "bottlebrush-like clusters of rootlets with limited growth" that form on lateral roots (Neumann and Martinoia, 2002), and function to uptake phosphorus with high efficiency (Dinkelaker et al., 1995). Across all traits which I measured in this experiment, cluster root formation was the most plastic in response to nutrient availability, with significantly more clusters formed in low treatment plants than either pulse or slow release treatments. Cluster root formation and lack of mycorrhizal symbiosis are characteristics of most Proteaceae, and therefore New Zealand's only other Proteaceae member, *Toronia toru* is also likely to possess these traits. Due to the high efficiency of phosphorus uptake by cluster roots, Proteaceae species can suffer from phosphorus toxicity in high fertility soils, with symptoms including leaf necrosis, chlorosis, and leaf rosetting (Hawkins et al., 2008). While some necrosis and chlorosis were observed in my experimental plants, these symptoms occurred in all three treatments, and both slow release and pulse plants generally looked 'healthier' than pulse treatment plants (Figure 12, additional high resolution images on appendix DVD). Therefore while phosphorus toxicity does not appear to have negatively affected my experimental plants, care should be taken in future experiments to avoid supplying excessive phosphorus to Proteaceae species.
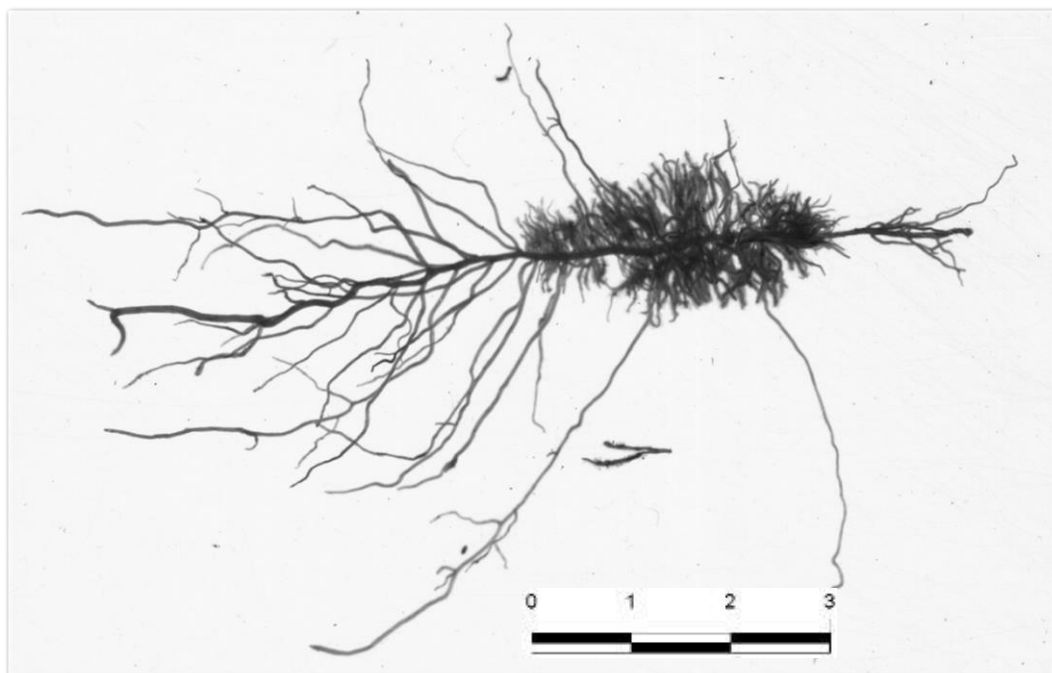
Figure 11. Scanned *Knightia* root section showing cluster root formation and normal lateral roots. Scale bar in centimeters.

Figure 12. Comparison of *Knightia* seedlings in three nutrient treatments. Treatments indicated by capital letters: SR, slow release; P, pulse; L, low. Some necrosis is visible on both the slow release and pulse treatment plants, and severe chlorosis is visible on the low treatment plant.

3.5.5   Mycorrhizal influence

The presence or absence of mycorrhizal symbionts may also affect plant growth and responses to soil nutrient availability. *Agathis* and *Dacrycarpus* are known to form vesicular-arbuscular mycorrhizal symbioses (VAM) (McNabb, 1958, Morrison and English, 1967, Baylis, 1969, Russell et al., 2002), *Laurelia* is

suspected to be highly dependent on mycorrhizae as is typical of Magnoliidae species (Baylis, 1975), and *Knightia* is suspected to be non-mycorrhizal as is typical among Proteaceae species (Tozer, 2006). I did not examine the mycorrhizal infection status of my experimental plants, although this would have been valuable information. A study by Zangaro et al. (2007) measured the traits of 12 species in response to combinations of high and low fertility soils and the presence or absence of mycorrhizal colonisation. Their results show that in infertile soils, the absence of mycorrhizal infection can significantly alter trait expression, particularly in early successional species. Within each species, plants with mycorrhizal colonisation in low fertility soils displayed similar traits to plants which lacked mycorrhizal colonisation but were grown in high fertility soil. The combination of both mycorrhizal colonisation and high fertility soils altered the expression of some traits when compared to the three other treatment groups, particularly for total biomass, shoot phosphorus concentration, and root tissue density in early successional species. Hayman and Mosse (1971) observed similar responses in both onion (*Allium cepa*) and *Coprosma robusta*, where either phosphorus addition or VAM infection augmented growth of both species compared to un-inoculated control plants in low phosphorus availability soil. Mosse (1973) demonstrated that mycorrhizal colonisation can both help and hinder growth, depending on the soil nutrient availabilities. Mosse also observed VAM infection decreasing at very high availabilities of phosphorus, and disappearing altogether at the highest phosphorus dosage. Johnson (1976) demonstrated this hindrance effect in *Fuchsia excorticata*, where VAM inoculation augmented growth below 11 ppm of phosphorus, but negatively affected growth at and above 25 ppm of phosphorus. The role of mycorrhizae in plant nutrition may be more relevant in soils of highly heterogeneous fertility. Both Tibbett (2000) and Hodge (2006) suggest that proliferation of mycorrhizal hyphae into nutrient rich patches may be of equal or greater importance than root proliferation. Altogether, these studies suggest that while the absence of mycorrhizal symbiosis may negatively affect species in low fertility soils, these effects can be mitigated by nutrient addition. However, the role of mycorrhizae in exploiting nutrient patches and pulses requires further investigation. While it would still be useful to investigate mycorrhizal infection in experimental plants, phenotypic plasticity caused by the absence of mycorrhizal symbionts can therefore be mostly mitigated by providing plants with sufficient fertiliser.

3.5.6   Additional factors which may influence phenotypic plasticity

Leaf traits such as SLA, leaf nitrogen content, and leaf area are known to be strongly influenced by light availability (Meziane and Shipley, 1999, Gratani et al., 2006, Valladares et al., 2000a, Rozendaal et al., 2006, Freschet et al., 2015). Shading also affects root-to-shoot ratio, prompting increased biomass allocation to leaves (Aerts and Chapin, 1999, Aikio and Mari Markkola, 2002, Poorter et al., 2012). In my experiment, all species and treatments were grown in a common, high light environment, thereby minimizing plastic effects in response to low light availability. However, patterns of biomass allocation may be more complex when both light and nutrients are limited, and be more contrasted between species compared to single resource limitation (Freschet et al., 2013). Therefore there is still a need to study the responses of a wide range of species to combinations of light and soil nutrient availability, in order to understand whether such responses can be generalised across species.

Competition may strongly influence root trait expression, although research on competitive effects on trait plasticity is limited. de Kroon (2007) observed greater proliferation of fine roots in proximity with competitor roots, and noted that plants seem to be able to differentiate between their own roots and those of a competitor. Semchenko et al. (2007) observed contrasting responses to competitor roots in two grass species, with one species consistently avoiding competitor roots, while the other species demonstrated both avoidance and attraction to competitor roots. Studying the effects of competition on experimental plants will provide much needed context for how trait data from glasshouse trials compares to field data.

Soil moisture availability can cause phenotypic plasticity. Aspelmeier and Leuschner (2006) performed a glasshouse experiment on seedlings of European birch (*Betula pendula*), and observed that under drought conditions SRL did not significantly change, while root diameter increased and total root surface area decreased. A similar drought experiment on European birch saplings by Meier and Leuschner (2008) did not observe any significant changes to root morphology or relative root-to-shoot ratio in response to drought conditions, however drought treatment saplings had 30-40% less total fine root biomass, and had an increase in fine root turnover. Low soil water availability may not produce a particularly strong effect on trait expression in New Zealand forest species; however, plastic

response to drought may be far more important for species from arid or semi-arid biomes.

Ontogenetic change may result in differences to trait expression as plants age. Over increasing mean-aged stands of *Betula pendula,* Rosenvald et al. (2013) observed decreased SLA, root nitrogen content, and SRL and increased fine root diameter and tissue density. The authors suggest that trait differences between young and old stands may result from higher physiological activity necessary for fast growth in younger trees. Stem tissue density also increases over ontogeny, as more mechanical strength is needed to support growing leaves and stems (Woodcock and Shier, 2003, Chave et al., 2009). Therefore, considerations of age and the potential effects on trait expression are important, and trait comparisons should be made between similar aged individuals wherever possible.

Soil compaction has been shown to affect root traits in *Fraxinus* seedlings, with higher compaction resulting in a lower proportion of fine roots, lower SRL, and lower root xylem vessel proportion (Alameda and Villar, 2012). The authors suggest that *Fraxinus angustifolia* seedlings are more sensitive to physical properties of the soil than to chemical properties; however it is not clear if this is a general response in woody plants. The comparatively loose structure of potting mix compared to natural soils may influence trait expression, and therefore may be worth studying.

3.6 Conclusion

My results suggest that plant traits such as root-to-shoot ratio, relative growth rate, and root nutrient concentrations respond strongly to differing nutrient availabilities, but that morphological traits, particularly those relating to the 'whole-plant economic spectrum' are not strongly plastic in response to nutrient availability. These results justify the use of traits from glasshouse raised plants as a proxy for field measured traits, particularly for morphological traits. Plant traits under pulse and slow release treatments were typically not significantly different, and the few differences I observed may be due to total nutrient availability rather than method of fertiliser application. The combination of these two observations has great utility for pooling trait data from multiple studies, provided that environmental conditions were conducive to growth. Preliminary comparisons suggest that in general, conifers may be less plastic in response to soil fertility than angiosperms; however the responses of a wider range of species need to be studied before these generalisations can be concluded upon. My experiment only examined trait responses to a complete nutrient solution, and more research is needed to determine how deficiencies of individual nutrients can affect trait expression. Other variables such as presence of mycorrhizae, shading, competition, water availability, plant age, and soil compaction can also alter trait expression, and inferences from glasshouse trait data to natural ecosystems will be strengthened by studying the effects of these variables both individually and in combination.

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Chapter Four: Synthesis

4.1 Conclusions

The primary objectives of this study were to determine the relationships between the leaf, stem, and fine root traits of New Zealand tree species, and to assess how these traits vary at the community level over a strong soil fertility gradient. I also aimed to assess how traits of glasshouse grown seedlings relate to those of adults, and whether the growth medium or fertilisation method used in the glasshouse environment would affect trait expression.

In Chapter One, I demonstrated that in seedlings there is a strategic dimension coordinated across multiple organs, relating to growth rate and the acquisitive-conservative strategic dimension which has been well documented in leaves. Independent from this growth strategy dimension was a second strong axis of variation, which was strongly influenced by root diameter and specific root length (SRL). This finding casts doubt on the notion that SRL is a below ground-analogue of specific leaf area (SLA), and is consistent with studies which propose that fine root diameter is a strongly phylogenetically conserved trait. When community-weighted mean traits were assessed across a strong fertility gradient, traits related to the conservative-acquisitive strategic dimension were strongly related to soil fertility, particularly SLA, and leaf, stem, and root dry matter content. Both SRL and root diameter were not significantly related to soil fertility, further reinforcing the notion that SRL does not mirror the adaptive function of SLA. Comparison of glasshouse grown seedling traits to those of field surveyed adults revealed moderate correlations for morphological traits, which tended to be skewed towards an acquisitive growth strategy in seedlings. Root nutrient concentrations were not significantly correlated between seedlings and adults.

In Chapter Two I assessed the variation of seedling traits in response to soil nutrient availability, and found that in general, the expression of morphological traits was not strongly affected by soil nutrient availability. This suggests that these traits may be broadly comparable among studies, despite differences in soil nutrient availability. However, root-to-shoot ratio, growth rate, and root nutrient concentrations were strongly influenced by nutrient availability, suggesting that care should be taken when comparing these traits among studies. Trait expression

was typically not significantly different between pulse and slow release treatments, but both were typically significantly different compared to low nutrient treatment in traits which responded strongly to nutrient availability. These results suggest that as long as adequate nutrients are provided, there is little difference on trait expression between pulsed or slow release fertiliser applications.

4.2 Applications and recommendations

Despite the correlations observed in this study, the inference of adult traits from seedling traits should be approached with caution until a larger range of species from diverse floras have been compared. Similarly, the co-ordination of growth strategy across multiple organs may appear suitable for inferring root traits from leaves or stems; this should also be approached cautiously until research from diverse floras can confirm the generality of this multi-organ coordination of strategy.

In the absence of trait correlations between seedling and adults, seedling traits are still be relevant to community assembly processes, as they reflect the selective pressures of environmental filtering during the seedling phase of regeneration. My observation that nutrient availability does not strongly affect species' trait expression suggests that there may also be strong correlations between glasshouse raised seedlings and those from natural soils. However, factors other than soil fertility can differ between the glasshouse and natural environments, and therefore may also affect trait expression. By comparing trait data between glasshouse grown and field sourced seedlings, stronger conclusions could be drawn about the relationships between the two. If glasshouse grown and field sourced seedlings are found to be strongly correlated, glasshouse grown seedlings may have great application as substitutes for natural seedling trait data. This would allow researchers to carefully control growth conditions and thereby minimise trait variation caused by unmeasured and uncontrolled factors.

SRL appears to have no direct functional effects on plant growth strategy or environmental filtering along a soil fertility gradient. This may be explained by a potential role of root diameter in facilitating mycorrhizal symbiosis, effectively allowing highly mycorrhizal species with low SRL to effectively compete for nutrients with less mycorrhizal species with higher SRL. While some evidence

exists to suggest that fine root diameter may be linked to benefit from mycorrhizal symbiosis or degree of infection, more research into the benefits and extents of mycorrhizal infection in naturally grown plants would provide data needed to test this hypothesis.

This study investigated community-weighted mean traits along a soil fertility gradient in a single forest. It would be valuable to see if my observations of strong correlations between soil fertility and growth rate traits, and the non-significance relationship of SRL to soil fertility are upheld over a nationwide scale of soil fertility, as this would confirm the generality of my observations.

At the community level, both porosity and root branching intensity were moderately correlated with soil fertility. High porosity is traditionally associated with waterlogging tolerance, but may also function to reduce root construction and metabolic costs. Root branching intensity may represent species' ability to proliferate roots into nutrient rich patches, and may also be influenced by mycorrhizal associations and infection rates. These traits may be valuable for understanding root foraging strategy, and warrant further study.

Appendix:

This appendix contains figures and tables not included in the main body of the text. Additional high resolution images of washed root balls and scanned root sections are available on the attached DVD.
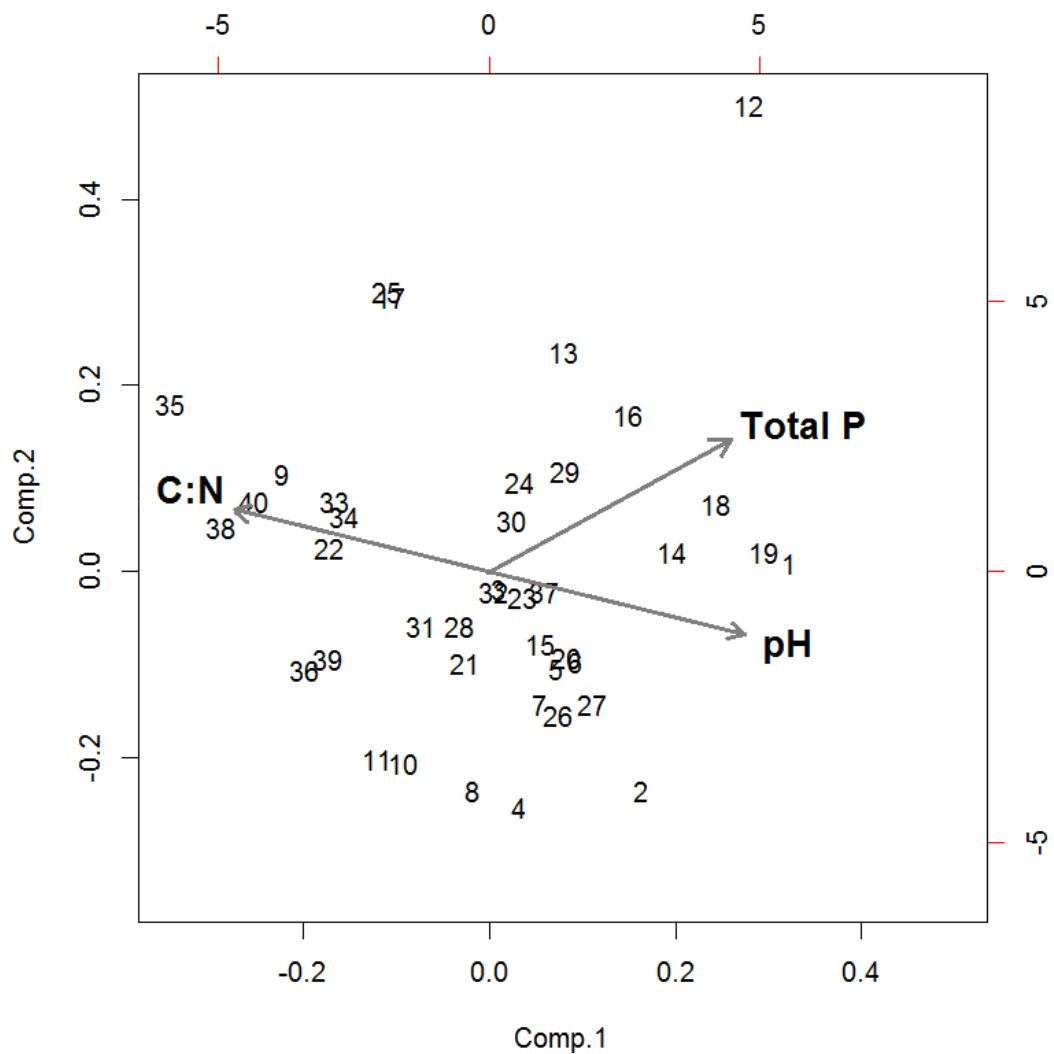
Figure 13. 40 vegetation plots from Puketī forest ordinated by soil fertility principle component 1, consisting of carbon to nitrogen ratio negatively correlated with pH and total available phosphorus

Table 9. Seedling and adult stem and leaf traits for 54 species. Adult traits sourced from Jager et al. (2015).

Species	SLA (mm² mg⁻¹)		Leaf TD (mg mm⁻³)		LDMC (mg g⁻¹)		Stem TD (mg mm⁻³)		SDMC (mg g⁻¹)	
	Adult	Seedling	Adult	Seedling	Adult	Seedling	Adult	Seedling	Adult	Seedling
Conifers										
AGAAUS	4.6	9.1	0.436	0.208	411	311	0.472	0.587	649	544
DACCUP	3.9	3.3			421	326	0.481	0.591	565	461
DACDAC	4.3	13.6	0.748	0.252	434	341	0.386	0.406	560	459
HALBIF	1.4	4.9	0.408	0.297	486	377	0.593	0.771		620
PHYALP	4.4	5.0	0.428	0.364	474	449	0.543	0.760		627
PHYTRI	5.9	9.4	0.555	0.253	472	347	0.574	0.735	524	539
PODHAL	4.5	4.9	0.451	0.258	468	380	0.486		658	478
PODTOT	5.6	8.6	0.416		437	378	0.446	0.611	624	488
PRUFER	6.7	12.7	0.457	0.261	399	298	0.547	0.699	593	543
PRUTAX	6.9	12.1	0.454	0.258	446	376	0.564	0.672	503	586
Eudicot angiosperms										
ARISER	19.4	35.7	0.231	0.122	281	277	0.348	0.364		366
CARSER	15.2	19.0	0.319	0.196	326	329	0.519	0.521	497	445
COPLIN	9.9	10.0	0.349	0.264	369	378	0.607	0.641		537
DRALON	3.7	5.8	0.675	0.486	525	440	0.504	0.491		499
DYSSPE	15.3	19.9	0.314	0.211	254	248	0.490	0.416	550	360
ELADEN	7.1	11.2	0.582		459	397	0.489	0.530	550	426
ELAHOO	7.9	15.7	0.469	0.285	438	397	0.487	0.422		438
FUCEXC	18.6	28.1	0.201	0.120	215	174	0.476	0.395	450	352
GRILIT	8.6	9.9	0.241	0.171	288	258	0.604	0.524		459
KNIEXC	5.4	14.2	0.547	0.245	493	324	0.548	0.415	566	357
KUNERI	7.2	20.4	0.593		489	336	0.704	0.585	586	467
LEPSCO	6.8	19.6	0.498	0.195	490	340	0.695	0.530	659	447
MELRAM	17.9	28.9	0.264	0.147	257	224	0.436	0.372	405	307
METROB	5.6	9.6	0.618	0.295	503	305	0.706	0.549	588	467
METUMB	5.7	7.5	0.387	0.221	421	301	0.780	0.476		424
MYRAUS	12.1	13.9	0.394	0.285	364	357	0.679	0.494		468
MYRDIV	13.9	15.5	0.387	0.254	322	338	0.605	0.629		487

Table 9. continued

Species	SLA (mm² mg⁻¹) Adult	SLA (mm² mg⁻¹) Seedling	Leaf TD (mg mm⁻³) Adult	Leaf TD (mg mm⁻³) Seedling	LDMC (mg g⁻¹) Adult	LDMC (mg g⁻¹) Seedling	Stem TD (mg mm⁻³) Adult	Stem TD (mg mm⁻³) Seedling	SDMC (mg g⁻¹) Adult	SDMC (mg g⁻¹) Seedling
MYRSAL	9.1	12.8	0.388	0.194	351	244	0.655	0.527	576	479
NESLAN	7.2	10.7	0.528	0.258	511	375	0.698	0.601	669	544
NOTCLI	6.5	14.0	0.533	0.301	505	407	0.532	0.591		499
NOTFUS	12.0	13.6	0.398	0.286	430	417	0.542	0.565		488
NOTMEN	7.0	7.6	0.493	0.367	485	455	0.499	0.574		524
NOTSOL	8.4	13.4	0.507	0.293	483	434	0.559	0.530		462
NOTTRU	8.2	13.8	0.504	0.289	478	384	0.629	0.485		431
OLERAN	13.2	19.6	0.272	0.153	327	235	0.538	0.487	478	407
PENCOR	14.5	19.8	0.298	0.170	295	290	0.452	0.511		438
PITEUG	10.1	17.6	0.444	0.257	392	313	0.609	0.420		377
PITTEN	9.0	21.1	0.460		409	346	0.614	0.479		424
PSEARB	8.9	11.8	0.321	0.209	324	317	0.507	0.504	602	420
PSECOL	10.4	12.1	0.343	0.230	362	296	0.505	0.563		475
PSECRA	4.4	4.3	0.379	0.356	378	333	0.565	0.495	599	431
QUISER	10.0	12.2	0.489	0.199	349	286	0.516	0.482	488	442
SCHDIG	18.5	24.4	0.244	0.122	241	196	0.408	0.252		230
WEIRAC	6.6	11.3	0.450	0.266	412	343	0.512	0.455	466	416
WEISIL	9.7	17.0	0.489	0.249	383	304	0.525	0.503	510	432
Magnoliid angiosperms										
BEITAR	8.0	12.3	0.495	0.263	404	324	0.544	0.505	612	444
BEITAW	9.9	12.5	0.607	0.396	447	420	0.572	0.536	623	479
HEDARB	12.9	20.6	0.300	0.143	279	206	0.547	0.378	535	322
LAUNOV	12.9	18.7	0.265	0.157	260	236	0.387	0.408	482	384
Monocot angiosperm										
RHOSAP	7.4	13.7	0.570	0.292	381	282	0.140	0.194	176	148
Tree ferns										
CYADEA	12.6	17.1	0.498	0.291	413	322	0.213	0.541	190	435
CYAMED	11.5	29.8	0.369	0.133	330	228	0.132	0.216	178	174
CYASMI	19.2	17.8	0.343		324	383	0.169	0.316	180	278
DICSQU	12.1	19.9	0.451	0.225	400	318	0.226	0.427	238	328

Table 10. Seedling and adult root traits for 20 species. Adult traits sourced from Holdaway et al. (2011)

Species	SRL (m g^{-1}) Adult	SRL (m g^{-1}) Seedling	Root diameter (mm) Adult	Root diameter (mm) Seedling	RBI Adult	RBI Seedling	Root N (%) Adult	Root N (%) Seedling	Root P (%) Adult	Root P (%) Seedling
Conifers										
DACCUP	11.3	27.8	0.49	0.51	3.16	2.70	0.66	1.42	0.034	0.634
PHYALP	19.1	12.4	0.36	0.60	2.58	2.48	0.70	1.42	0.028	0.200
PODCUN	10.3	8.4	0.61	0.90	2.85	2.35	0.93	1.79	0.073	0.246
PRUFER	5.9	6.7	0.69	0.93	3.74	3.34	0.90	1.13	0.080	0.441
Eudicot angiosperms										
ARISER	23.8	82.3	0.31	0.37	2.81	1.59	1.30	1.24	0.092	0.321
CARSER	35.1	30.8	0.44	0.53	1.79	1.61	1.79	1.47	0.144	0.453
GRILIT	5.4	6.7	1.26	1.25	2.13	1.76	1.19	3.02	0.121	0.294
LEPSCO	91.3	97.1	0.21	0.37	2.53	1.97	0.57	1.65	0.018	0.343
MELRAM	29.0	48.9	0.34	0.54	2.58	1.87	1.68	1.82	0.110	0.470
METROB	21.5	42.5	0.31	0.40	2.58	2.93	0.38	1.53	0.028	0.292
MYRAUS	23.6	40.3	0.39	0.41	1.62	1.10	0.51	1.22	0.039	0.554
MYRDIV	22.9	41.6	0.48	0.36	1.24	1.55	1.01	1.61	0.074	0.169
PSECOL	14.1	10.7	0.66	0.93	1.54	1.72	0.50	2.85	0.068	0.771
PSECRA	6.2	28.0	0.98	0.63	1.15	1.59	1.03	1.39	0.096	0.490
QUISER	33.7	38.0	0.27	0.41	1.56	2.43	0.50	1.62	0.032	0.350
SCHDIG	50.3	44.6	0.33	0.63	2.38	2.43	1.93	2.04	0.143	0.526
WEIRAC	41.5	44.7	0.23	0.37	2.49	1.78	0.41	1.43	0.030	0.373
Magnoliid angiosperm										
HEDARB	9.9	11.7	1.00	1.07	0.84	1.63	2.09	2.95	0.128	0.379
Tree ferns										
CYASMI	30.1	14.9	0.31	0.62	2.10	1.39	0.53		0.039	
DICSQU	25.8	15.7	0.36	0.69	2.12	2.50	0.49	1.32	0.029	0.310
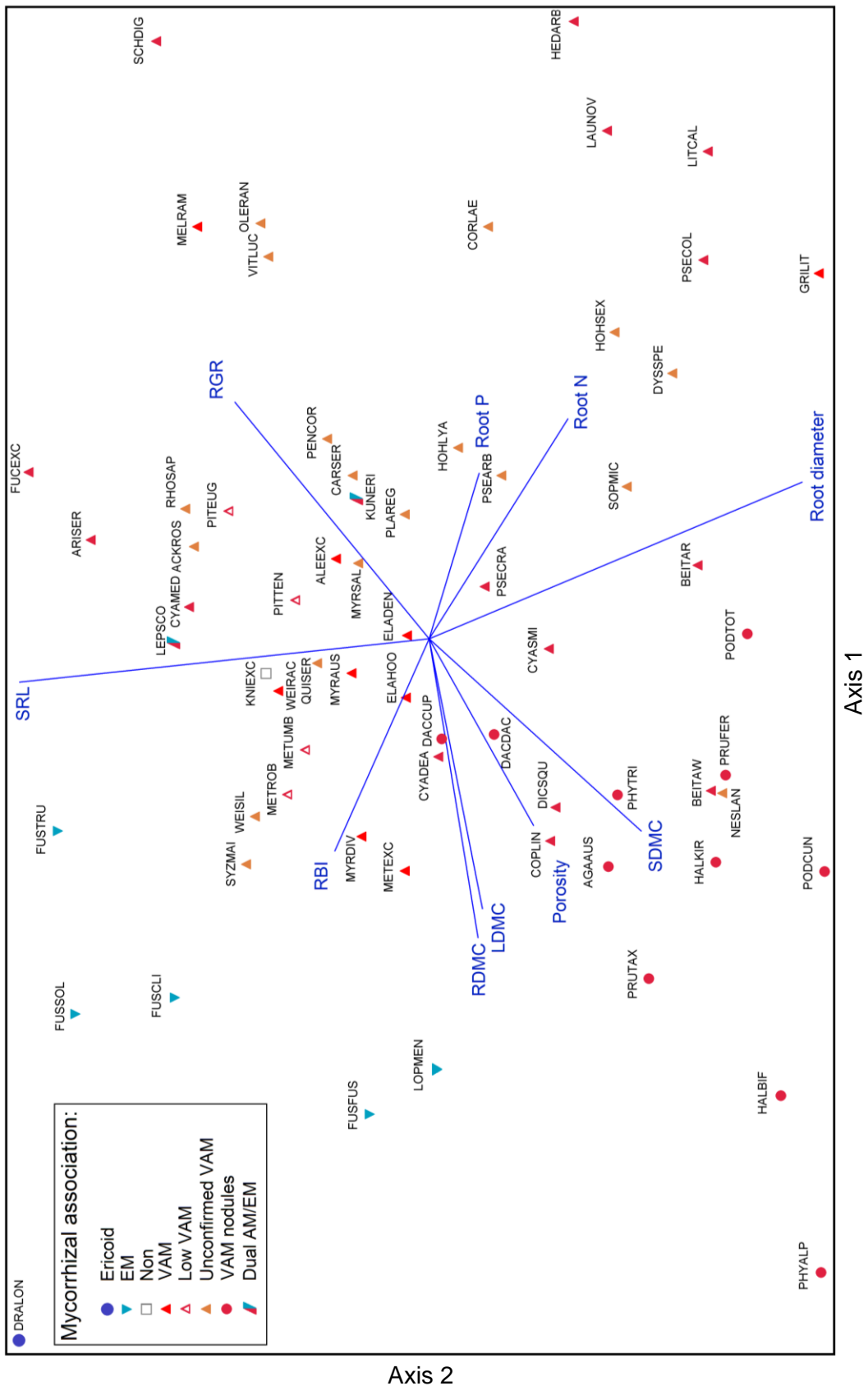
Figure 14. Two-dimensional ordination of PCA results as per Table 5. Species are grouped by mycorrhizal association. Trait abbreviations as per Table 1. Traits labelled in larger bold text and represented with blue lines. Each species labelled with a six letter species code as per Table 2.

Table 11. Number of replicates by species and treatment in Chapter Three.

Species	Treatment	Replicates
Agathis	Low	14
Agathis	Pulse	15
Agathis	Slow Release	14
Dacrycarpus	Low	16
Dacrycarpus	Pulse	16
Dacrycarpus	Slow Release	14
Knightia	Low	12
Knightia	Pulse	15
Knightia	Slow Release	8
Laurelia	Low	15
Laurelia	Pulse	14
Laurelia	Slow Release	13

Table 12. Quantities of nutrient additives in slow release potting mix.

Additives	kg/m³
Dolomite	2.00
Growers Choice Granular Wetting Agent	0.75
Gypsum Fine	2.00
Lime – Agricultural Grade	2.00
Everris Osmocote Exact Standard 3-4 Month	3.00
Everris Osmoform NXT 22N	0.65

Table 13. Nutrient concentrations for Everris Osmocote Exact Standard 3-4 Month slow release granules.

Nutrient	Percentage
Nitrogen Total (N)	16.0%
Phosphorus pentoxide (P_2O_5)	9.0%
Potassium oxide (K_2O)	12.0%
Magnesium oxide (MgO)	2.0%
Iron (Fe)	0.45%
Manganese (Mn)	0.06%
Boron (B)	0.03%
Copper (Cu)	0.050%
Molybdenum (Mo)	0.020%
Zinc (Zn)	0.015%

Table 14. Nutrient concentrations for Everris Osmoform NXT 22N slow release granules.

Nutrient	Percentage
Nitrogen total (N)	22.0%
Phosphorus pentoxide (P_2O_5)	5.0%
Potassium oxide (K_2O)	11.0%
Magnesium oxide (MgO)	2.0%
Iron (Fe)	0.50%
Manganese (Mn)	0.10%
Copper (Cu)	0.020%
Molybdenum (Mo)	0.001%
Zinc (Zn)	0.020%

Table 15. Coarse structural components of low and pulse treatment potting mixes.

Basic Structural Ingredients	Percentage
Bark Fibre	30%
Daltons C.A.N Fines A Grade	55%
Daltons Propagation (No 2) Sand	15%

Table 16. Nutrient concentrations of Yates Thrive™ Concentrate All Purpose.

Nutrient	Percentage
Nitrogen	12.4%
Phosphorus	3.0%
Potassium	6.2%
Magnesium	0.01%
Iron	0.008%
Manganese	0.008%

Table 17. Average trait values by species and nutrient treatment.

Species	Treatment	SRL	Root Diameter	Root TD	RDMC	Porosity	RBI	Root N	Root P	SLA	Leaf TD	LDMC	Stem TD	SDMC	RGR	R:S	Cluster roots
AGA	L	13.7	0.68	0.212	182	10.2	2.41	0.89	0.12	13.98	0.156	225	0.719	683	0.0004	2.88	
AGA	P	15.3	0.65	0.201	176	9.5	2.56	1.42	0.24	11.82	0.158	239	0.685	653	0.0013	3.62	
AGA	SR	13.3	0.69	0.209	186	9.4	2.24	1.50	0.31	11.71	0.154	227	0.689	659	0.0010	3.22	
DAC	L	29.4	0.48	0.199	162	11.1	2.76	1.11	0.22	11.53	0.289	331	0.437	466	0.0017	1.87	
DAC	P	33.6	0.48	0.169	138	8.3	1.86	1.42	0.21	12.96	0.227	301	0.392	397	0.0058	3.96	
DAC	SR	31.4	0.50	0.171	140	9.1	2.14	1.79	0.26	13.17	0.232	288	0.413	394	0.0062	5.09	
KNI	L	41.8	0.39	0.246	202	12.6	2.68	0.56	0.14	10.66	0.335	382	0.509	476	0.0007	1.67	38.1
KNI	P	56.6	0.38	0.164	156	10.5	1.87	1.18	0.29	13.72	0.249	310	0.467	432	0.0027	2.97	21.1
KNI	SR	46.9	0.45	0.141	114	12.2	1.73	1.31	0.45	11.80	0.251	317	0.444	433	0.0032	4.01	9.4
LAU	L	13.2	0.99	0.101	83	5.8	1.56	1.80	0.36	18.23	0.150	234	0.413	426	0.0023	1.21	
LAU	P	11.5	1.17	0.083	70	5.3	1.28	3.12	0.39	21.59	0.132	202	0.345	352	0.0038	1.91	
LAU	SR	12.0	1.16	0.083	71	6.6	1.12	3.15	0.56	20.83	0.136	208	0.361	362	0.0049	2.17	

Treatment codes: L, Low; P, Pulse; SR, Slow Release.
Species codes: AGA, *Agathis australis*; DAC, *Dacrycarpus dacrydioides*; KNI, *Knightia excelsa*; LAU, *Laurelia novae-zelandiae*

Table 18. Co-efficient of variation (CV) averages for groupings by phylogeny and soil fertility associations.

Trait	High fertility	vs.	Low fertility	Conifers	vs.	Angiosperms
Cluster roots	-		-	-		-
RGR	44.9	<	55.8	53.2	>	47.6
Root : Shoot	43.4	>	26.4	35.2	≈	34.6
Root P	18.7	<	48.5	28.7	<	38.5
Root N	26.1	<	32.9	24.9	<	34.1
RBI	18.6	>	14.5	13.6	<	19.5
Porosity	13.2	>	7.9	9.7	<	11.4
RDMC	9.9	≈	11.1	6.1	<	14.8
Root TD	10.5	≈	10.3	6.1	<	14.7
SRL	7	<	10.7	7.05	<	10.7
SLA	8.0	≈	9.8	8.8	≈	9
Leaf TD	10.1	>	5.8	7.3	≈	8.6
SDMC	10.1	>	2.8	6.0	≈	6.9
LDMC	7.7	>	5	5.3	<	7.4
Stem TD	7.5	>	3.8	4.1	<	7.3
Root diameter	5.4	≈	5.45	2.2	<	8.7
Species average	**16.1**	≈	**16.7**	**14.5**	<	**18.2**

CV values considered ≈ where the difference between the compared values was < 2.